Author comment on bg-2010-90

We are very thankful for the constructive comments from the four referees and are pleased to present our point-to-point response which also shows our changes according to the referees' comments and suggestions.

Response to Referee #1:

"Although mesocosm experiment gave similar results as those observed for the day 2, with slick formation and lowest wind, the authors should be aware that mesocosm prevent horizontal OM dilution or lose, leading to higher OM enrichment. I assume that simulation of moderate wind in the mesocosm would not disrupt SSM."

We certainly agree that the employment of mesocosms generally causes problems in their extrapolation to natural systems. Our aim for the employment of mesocosms in the present study was to "conserve" calm wind conditions. The mesocosms were open at the bottom and reached about 70 cm into the water column in order to have comparable conditions in the ULW outside and inside the mesocosms. We think that the potential, artificial accumulation of organic material in the SML due to prevention of OM loss is acceptable for the present study because we did not want to determine absolute values for changes of OM enrichments in relation to wind speed, but were rather interested in the relative changes of the bacterioneuston to highly calmed SMLs. Therefore, we think that the reactions of the bacterioneuston to increased organic matter in the SML are still valid even though concentrations of OM might be overestimated. We now acknowledge the problem of artificial OM accumulation in the mesocosms in the discussion section as follows: "*Field studies and mesocosm experiments in the coastal zone of the Baltic Sea were employed in the present study to examine the effects of minimized wind-induced turbulence on the SML. Generally, mesocosms provide valuable experimental setups to study biological responses to perturbations in aquatic systems (Riebesell et al., 2008). There are, however, potential drawbacks that complicate the extrapolation of the results obtained to unperturbed, natural systems (Riebesell et al., 2008). In the present study, the mesocosms might have e.g. artificially enhanced the enrichment of organic matter in the SML by preventing horizontal dilution or dispersion. However, the characteristics of the organic matter and its enrichment are comparable to the extensive slick formation in the same area (see above). Furthermore, as this study aimed to investigate the principal mechanisms of organic matter enrichment and the bacterioneuston response, we think that the potential overestimation of organic matter concentrations caused by the mesocosms can be neglected. Such artefacts, however, have to be considered when future studies might aim to balance fluxes of organic matter into, away or across the SML under varying low wind conditions.*"

"P5, lines 6-8. The authors cite: "However, there is evidence that glass-plate samples underestimate concentrations of parameters in the SML due to dilution with bulk water (Cunliffe et al., 2009a)." However I do not agree with that, it is more question of selectivity than dilution."

The employment of different sampling devices used in SML studies has certainly hampered a better understanding of SML properties due to their different modes to collect the samples. Thus, SML samples are influenced by the thickness to be collected as well as potential, selective bias. The selectivity of the glass-plate sampler was one big concern in our last study (Stolle et al., Aquatic Microbial Ecology, 2009) and we have shown that the glass-plate technique, we used in the former and the present study, did not selectively influence bacterial parameters of interest (abundance, community fingerprints, TdR-incorporation) which is also in accordance with one other study (Agogué et al, L&O Methods, 2004). Moreover, Momzikoff et al. (L&O Methods, 2004) have demonstrated the suitability of glass-plate

samplers for the chemical characterisation of the SML. As we agree that this topic is always one of the major challenges in SML studies, we have extended the methods section "Field study and sampling" to discuss this topic in more detail: *"SML samples were obtained using the glass-plate technique (Harvey and Burzell, 1972) and a framed wiping device which collects layer thicknesses of about 50 µm (Stolle et al., 2009) and is in the range of measured SML thicknesses by pH microelectrodes (Zhang et al., 2003). However, there is evidence that glass-plate samples bias measurements of bacterial parameters in the SML due to dilution with bulk water (Cunliffe et al., 2009a) or due to the glass-plates' mode of operation (Hühnerfuss 1981). Nonetheless, this sampling method was chosen in order to obtain sufficient quantities of the SML. Furthermore, it was previously shown that no bias is introduced into measurements of many bacterial parameters (Agogué et al., 2004; Stolle et al., 2009)."* Please also see comments by referee #3.

"Para 2.3 Line 15: bracket is missing after: 0.05% ïnˇA¸nal concentration)"
In the version of the manuscript on the bgd homepage this is not the case.

"Para 2.5. P9, line 14: which pore size?"
According to the manufacturer GF/F filters have a pore size of approximately 0.7 µm. We added this to the text.

"P 10, line 24-p11, lines 1-2: Authors obviously took PN as organic N form i.e. PON, which is small part of POC. Therefore contribution of PN to total organic matter pool may not exceed contribution of POC to TOC. The same is for dissolved fraction. Aren't there dissolved N nutrients (inorganic N) measured in DN fraction? If not, it is not clear from the methods section 2.5."
The referee is absolutely right in his/her assumption that PN of the present study can be more or less considered as POC. Moreover, it is correct that DN (and also TN) measurements include concentrations of inorganic nitrogen. The contributions of the particulate fractions to the total fractions, which the referee refers to, are related to the total fraction of each element. As this misunderstanding was probably due to our misleading formulation we rewrote the sentence as follows: *"In the slick, POC and PN concentrations contributed 70% and 79% to TOC and TN, respectively, compared to only 11% POC and 14% PN during pre-slick conditions and 6–9% POC and 14–18% PN in the ULW on all sampling days."*

"For the no specialist please define better: 16S rRNA and 16S rRNA gene fingerprints are written in the text while in the Fig 2a it is written 16S rRNA and 16S rDNA"
It is now defined in the methods section "Extraction of nucleic acids and fingerprint analyses" and in section 3.1.2 that the 16S rRNA fingerprints represent the presumably active bacteria and the 16S rRNA gene fingerprints represent the total bacterial community. Throughout the whole text and figures it is now uniformely written 16S rRNA / 16S rRNA gene fingerprints.

"P12, lines 3-4. The sentence "(1) the dissimilarities in the 16S rRNA gene ïnˇA¸ngerprints were higher than in the corresponding 16S rRNA ïnˇA¸ngerprints, except on day 1;" is not clearly written."
We inserted "between the bacterioneuston and bacterioplankton community" which hopefully helps to better understand that the dissimilarities refer to the comparison between the neuston and plankton fingerprints.

"The authors did not present data on the TOC, POC and DOC from the mesocosm experiments, apart from the discussion on EF. I think those data are important. Ranges of the concentrations would be fine."

We added the Supplement Table 1, in which the range (as suggested by the referee) and the mean concentrations of TOC, DOC, POC, TN, DN and PN are given. We furthermore provided the link to this table in the results section "Dynamics of organic carbon and nitrogen" as follows: *"Absolute concentrations of TOC and TN in all samples as well as DOC, DN, POC and PN in a subset of samples (see below) were in the range of previously reported values from the southern Baltic Sea (Nausch et al., 2009; Supplement Table 1)."*

"Discussion First paragraph should be removed as it is already conclusion."
The first paragraph was now removed.

"Para 4.1. First sentence is not enough accurate. Maybe instead of "living cells as well as detritus" (what about exudates?) authors may say OM "is mainly autochthonous"."
We now deleted this part of the sentence as we got aware that discussion of the origin of the OM was not the main intent to discuss here, but rather the diversity of substances. The question of the origin of the OM in the SML is discussed later in the same section.

"P16, line 21 – Please remove word "finally""
The word is now removed

"P16, para 3 – Whole discussion on OM contribution would be much stronger if authors would present Chl a data."
Data of Chl a of the mesocosm experiments is now given in the Supplement Figure 1B.

"Para 4.2. P18, lines 10-12. I assume that cell-specific activity in mesocosm experiments might be lower also due to the fact that these experiments were performed in November-December during much lower sun irradiance than in May."
Our sentence *"This might have been at least partially due to a smaller inhibitory influence of UV radiation in the mesocosm experiments, as samples were taken shortly after sunrise."* refers to the fact that EFs of cell-specific activity were higher on the third day. As this misunderstanding might be due to a unclear phrasing and also to include the "seasonality"-assumption of the referee, we now changed the sentence into *"This exception might have been at least partially due to a smaller inhibitory influence of UV radiation in the mesocosm experiments due to the sampling season as well as the sampling time shortly after sunrise."*
In fact, cell-specific activites were lowest in the slick study and not – as assumed by the referee – in the mesocosms. However, this still makes an influence of UV-radiation on the bacterioneuston as suggested by us, as well as the referee, a reasonable assumption.

"*Conclusions should not have referencing."*
The references have been deleted.

"Table 2: type mistakes in column 4, rows 7 and 9 (pj001 and pj0.001)"
This has been already corrected in the version at the bgd-homepage.

"Table 3. Sentence "values in parentheses indicate contribution of the particulate matter to the total organic matter pool (%)." Should be moved to the upper part of table caption, after: ": : :., samples were only taken on the last day of each experiment."
The sentence has been moved according to the referees' suggestion.

"Figures: I strongly suggest symbols to be coloured. Font size at all the figs should be increased as much as possible."
We have now coloured the figures and changed font sizes according to the referees' suggestion.

"Please uniform letter "a" in the text and in figs, Upper or lower-case."
This is now uniformly upper-case.

"Fig. 4. The bands are not mentioned in the fig caption. Please add appropriate text."
The caption of Fig. 4 has been changed into: *"(B) Cluster analysis of all communities as revealed by their single-strand conformation polymorphism (SSCP) band patterns over the course of four days during the second experiment. The day of the experiment as well as the origin of the samples are indicated (SML = sea-surface microlayer, ULW = underlying water, control = outside mesocosms)."*

"Authors too many times cite "data not shown""
By our implementation of supplemental material, we now reduced the times, when it cites "data is not shown".

Response to Referee #2:

„As the biotic and abioic parameters of the SML and ULW were analyzed and discussed in the ms, they need to be included in supplementary information."
We now provide data of the underlying water taken during the mesocosm experiments. Following parameters are shown: salinity, chlorophyll a, TOC, TN, bacterial abundance and TdR-incorporation. Please see Supplement Figure 1.

„Methods P 5, lines 124-125. Not clear, if you write this sentence you need to explain why only one mesocosm remained intact during the 4 days period, but I don't think that this need to be mentioned."
We rewrote the sentence as follows: *"The other 2 mesocosms in each experiment lost air pressure in the floatation rim and, thus, had to be rejected."* We would not like to remove it, as this is, in our opinion, an important note and explains why we present no replicates as we do for the measurements outside the mesocosms; which is also important in the light of the comments of referee #3 who underlines the necessity of improvement in the mesocosm design for future studies.

"P 6, lines 133-136. Same remark as above, you cut it down or, if not, you need to explain which king of "material" was adhering to the mesocosm."
We now have shortened this section as follows: *„In order to avoid misleading results of chemical and biological parameters in the SML due to initial disturbance upon installation of the mesocosms, SML samples from the first day inside the mesocosms were not included in the analysis."*

"P 8, line 190. 16S rRNA and 16S rRNA gene fingerprintings : not clear, please rewrite."
The sentence was rewritten as follows: *"The 16S rRNA (presumably active bacteria) and 16S rRNA gene (total bacteria) fingerprints of the bacterial communities were analyzed using single-strand-conformation polymorphism (SSCP)."* Please also see suggestions by referee #1.

"P12, line 282-283, same remark as above, not clear, please precise that you want to compare the total and the active community."
The sentence was completed to: *"A comparison of bacterioplankton and bacterioneuston community composition, using 16S rRNA (presumably active bacteria) and 16S rRNA gene*

(total bacteria) fingerprints, revealed strong changes throughout the sampling period." Please also see suggestions by referee #1.

"Figure 2 and figure 4 : be coherent, if you put the photo of the DGGE gel for the dendrogram in fig 4 you need to do it also for fig 2."
We have now removed the gel-photo from Fig.4 as it does not provide any further information than can be seen by the cluster analysis.

„Technical corrections"
The errors were corrected according to the referees' suggestions.

Response to Referee #3:

„1) Sampling strategy"
The selectivity of SML sampling devices – especially the glass-plate – as well as their collection of different SML thicknesses is a major challenge (please also see comments from referee #1 and our reply).
Concerning the thickness of the SML:
As already mentioned by the referee (and also referee #1) there is a series of publications by Zhang and colleagues (**1998**, J. Colloid Interf. Sci, 204:249-299; **2003**, J. Colloid Interf. Sci, 264:148-159; **2003**, Sci. in China (B), 46:339-351) who have shown that there is "a layer of sudden change" at about 50 µm water depth, indicating that the SML thickness is determined by the more or less stable uppermost 50 µm of the water column. Therefore, we certainly agree that SML samples taken with the glass-plate technique likely represent what can be considered as the 'actual' SML – one of the reasons we employed this technique continuously. However, concerning the depth distribution of the bacterioneuston, this clear picture can not be drawn. Certainly, the physico-chemical properties of the SML determine bacterial responses in this habitat, but whether this coincides with the 50-µm-model of the SML has to be proven yet. In this light, the study of Cunliffe et al. (2009) indicates that the bacterioneuston community structure in membrane samples is different from the communities obtained by the glass-plate. That is the reason why we mentioned the potential dilution problems of glass-plate samples. As this might be also a matter of unclear formulation in the present study, we now underlined that this refers to bacterial parameters in the SML as also suggested by the referee (see methods section 2.1 "field study site and sampling": *"However, there is evidence that glass-plate samples bias bacterial parameters in the SML due to dilution with bulk water (Cunliffe et al., 2009a) or due to the glass-plates' mode of operation (Hühnerfuss 1981)."*).
Concerning the selectivity of the sampling devices:
To determine potential bias introduced by the sampling techniques, we have undertaken a comparison of sampling techniques in our previous study (Stolle et al., 2009), which has been mentioned by the referee #3, who states: *"Then they claim that glass plate sampler introduce no bias in the measurement of biological parameters, even so in their earlier paper (Stolle et al. 2009) they report inhibition of bacterial productivity by 90% in samples collected with the glass plate and metal screen sampler."* We think that there might be a misunderstanding of the results from our 2009-paper: Indeed, we have found an inhibition of bacterial productivity in glass-plate and screen samples. However, we have further evaluated the glass-plate sampler and established a setup with a framed wiper, where this inhibition was not observed anymore. To underline the usage of this framed wiping device in the present study, and, thus, to stress our conclusion that this sampling device introduces no selectivity into the measurements of bacterial parameters, we now mention it in the methods section 2.1 "field study site and sampling" as follows: *SML samples were obtained using the glass-plate technique (Harvey*

and Burzell, 1972) and a framed wiping device which collects layer thicknesses of about 50 µm (Stolle et al., 2009) and is in the range of measured SML thicknesses by pH microelectrodes (Zhang et al., 2003).

"However, that needs to be addressed in more details in the discussion, in particular in regards of the author's earlier findings in Stolle et al. (2009)"
As we hope that this topic is now sufficiently discussed in the methods section (see above), we have the impression that it does not need to be discussed again in the discussions section. Please also see comments of referee#1 and our responses regarding SML sampling techniques.

„2) Observation period"
We definitively agree on the referees' opinion that extending the time period for the experiments "*may prove to be the best way to study this complex system."* This would be especially helpful to better understand the dynamics under which the bacterioneuston community might increasingly develop to be a specific community (as indicated from studies in limnic systems) or at least to be increasingly distinguishable from the bacterioplankton. The referees' suggestion to increase the time for observation is, thus, a very interesting approach for future studies, although one has to be aware of increasing potential drawbacks as already mentioned by the referee and also referee#1 (artificial accumulation of organic material inside the mesocosms). We furthermore think that increasing the number of samples per time unit would also help to better understand dynamic changes of biotic and abiotic properties of the SML (e.g. day-night cycles to investigate the influence of photochemical transformations). However, sampling of the SML heavily depends on the meteorological conditions and an investigation of a 4-day period with low wind speed is rather an exception than the rule. Additionally, the mesocosm experiments were finished after 4 days as we wanted to avoid potential problems of sampling the whole sea-surface due to limited surface area of the mesocosms. As, to our knowledge, this type of experiment has not been published before we still think that besides the limited time period, the results provide a valuable basis to further investigate succession mechanisms in the SML, although – as already mentioned by the referee – improvements (e.g. mesocosm design) have to be done in future studies. To appreciate the important aspect of 'time' in SML characteristics, we now state: p. 3172, l.13: *"Although the present study has some limitations due to the limited observation period, it can be speculated that very low wind speed induces a succession of […]."*

"I am particular concern about the model the authors describe with this limited observation in section 4.3/Figure 5. I recommend deleting the description of the model and Figure 5 due to the limited observation made."
As explained above, we certainly agree that the limited observation period is one limitation in the present work. However, we would not like to retract the model (Fig.5) and its description in the manuscript for the following reasons: the model summarizes the results of the present study as well results from our previous paper (Stolle et al. 2009). The latter paper supports the model description of bacterioneuston characteristics during moderate wind speeds. The importance of particulate matter during low wind conditions in the models description is supported by Obernosterer et al. (2008) as discussed in the discussion section of the present manuscript.
We thus, agree with the referee that the model has limitations, but we did not intend to describe a universally valid model, but summarize our findings in coastal zones of the Baltic Sea. This has now been made clear in the capture of Fig.5 as well as in the discussion section and we hope that this finally justifies to keep the model in the manuscript.

3) Limitation to coastal waters

We agree that the results from the present study have to be focussed on coastal SMLs. We therefore changed the title according to the referees' suggestion and implemented this point to all sections throughout the manuscript by always stating that the SML studied was from the coastal zone of the Baltic Sea. We also added the GPS coordinates of the field sampling site as well as the study site of the mesocosms to show that although the mesocosm site was located in the harbour, both sites are quite well comparable. For that reason we decided to remove the word offshore as this may cause a misleading interpretation of the sampling site and changed the sentence as follows: *"Samples from the SML were taken in the coastal zone near Warnemuende in the southern Baltic Sea in proximity to position N 54°19' E 12° 05' from May 6th to 9th 2008."*

"P.3161, L21 I do not understand the last sentence here. The authors need to clarify if they have measured TOC/TON or DOC/DON?"
We have changed the sentence according to the referees' suggestions: *"The filtrate, i.e. dissolved organic carbon (DOC) and dissolved nitrogen (DN) was analyzed as described for TOC and TN (see above)."*

"Or was DOC/DON measured in addition to TOC/TON? If so, why since TOC=POC+DOC."
We measured TOC/TN in all mesocosm samples, because we were not able to measure the dissolved and particulate fraction of each sample separately due to sample volume limitations (see results section). Therefore, only from a subset of all mesocosm samples also POC/PN and DOC/DN were measured. From the field study the particulate and dissolved, but not the total fraction were analysed. We make this now clear in the methods section "Analysis of organic carbon and nitrogen": *"Ten-ml water samples from the mesocosm experiments were sealed in HCl-precleaned and precombusted (450°C, 6 h) glass ampoules using a portable propane torch.* […] *For the analysis of particulate organic carbon (POC) and nitrogen (PN), 50–500 ml (field study) and 95–250 ml (subset of samples from the mesocosm experiments) of sample were filtered through* […]"

"Analyzing unfiltered coastal waters in a HTCO analyzer is prone for high errors due to particulates accumulating on the catalyst affecting recovery. Using an autosampler, particles tend to settle to the bottom of the sample vial. Report relative errors and blanks for those measurements."
In order to check the potentially erroneous measurements of TOC/TN using the autosampler, as highlighted by the referee, we measured a MilliQ-water sample as an internal control after every third sample. This analysis showed that we could not see any matrix effects due to particle retention on the catalyst. Concerning the settlement of particles within the vials, all samples were bubbled for 10 min directly prior analysis with carbon-free air in order to purge inorganic carbon out of the samples. As a side-effect, this bubbling also mixes the sample and, thus, a strong settlement of particles is expected to be minimized during the analysis. Figure A shows that except for the samples with very high concentrations of TOC/TN, the measured and calculated (TOC=POC+DOC) values for TOC/TN were indeed similar, supporting that expectation. All samples showing an underestimation of measured TOC/TN compared to the calculated TOC/TN were samples with concentrations of >500 µmol TOC l^{-1} and >50 µmol TN l^{-1} (see FigA). All these samples were from the SML and showed the highest EFs for POC/PN, indicating that the referees' suggestions are valid for samples with very strong particle loads. This, however, does not effect the conclusions from our study, where the importance of particles in the coastal SML is one of the main conclusions to be drawn.
Relative errors have now been added to the methods section "Analysis of organic carbon and nitrogen" as follows: *"Measurements uncertainties for DOC, DN, POC and PN were < 5 µM l^{-1}, < 3 µM l^{-1}, < 1.3 µM and < 0.5 µM, respectively."*

"P. 3163, L13 and L15 Did the bacterial abundance and activity decrease to the value from the first day?"

As one can see in Fig.1, bacterial productivity decreased to values of day 1, abundance decreased even below the value of day 1. We have implemented this in the text: *"On day 2, $4.6x10^6$ bacterial cells ml^{-1} were measured in the SML, which was nearly twice as high as on day 1; however, with dissolution of the slick on days 3 and 4 the number of cells decreased even below the value of day 1 (Fig. 1B). A similar pattern was observed for bacterioneuston productivity, which peaked in the slick and subsequently decreased over the following days to the initial value of day 1 (Fig. 1C)."*

"…the authors should investigate for a similar relationship to add further evidences that the enrichment of particulates depends on wind stress."

As one can see in Figure B, there are indication of a relationship between lowest enrichments of POC and PN and the lowest wind speed. However, we did not include this graph into the manuscript as there are some drawbacks: (1) the field study has only four data points which is apparently not enough for a robust statistical analysis (see also referees' comments about "observation period") and (2) the values from outside the mesocosms cannot be directly linked to the statistical analysis, as the wind data was not directly measured at the site where the experiments were done. This inexact wind speed measurements might have been caused the high variability of enrichments at a certain wind speed (see Figure B – especially at wind speeds of 10 m s^{-1}).

Nevertheless, based on the indication of the relationship between POC/PN enrichment and wind speed in the field experiment, our aim was to 'simulate' very low wind conditions inside the mesocosms and not a succession of decreasing wind regimes. This again, is certainly needed in future studies, but was not within the scope of the present study.

"P.3164, L1 Rewrite this sentence as it is somewhat contradictory."

Please see comments of referees #1 and #2. This sentence should be more clear now after correcting the paragraph before and after it.

"P.3165, L15 Were the enrichments of DOC/DON similar between outside and inside mesocom. Have depletion of DOC/DON (EF<1) observed?"

The dynamic enrichment of DOC and DN were similar in- and outside the mesocosms, whereby the enrichment of DN was much more variable within the replicates (please see Fig. C). As one can also see in Fig. C we did not observe any depletion of DOC or DN in all SML samples (both inside and outside the mesocosms).

"P.3165, L23 Discuss inhibition of bacterial activity using glass plate sampler. That may have affected the observation of lower bbacterial activity in the SML."

Please see the referees' comments about sampling strategy and our reply (above).

"In Figure 3e, during experiment 3 high bacterial activities has been observed in the ULW during the last two days. Those observation seem to be exceptionally high, and affect the statistical conclusion that bacterial activity was significant greater in the ULW than in the SML."

Fig 3E shows that the bacterial productivity was higher in the SML and not in the ULW (please note that the values given show the enrichment factors). Furthermore, our conclusion from the statistical analysis states that *"^{3}H-TdR incorporation in the SML outside the mesocosms was slightly decreased compared to the ULW (Wilcoxon test; p = 0.010, n = 36; Table 2, Fig. 3e) whereas in the SML inside the mesocosms it was highly variable and not significantly different from ^{3}H-TdR incorporation in the ULW (Wilcoxon test; p = 0.347, n = 9;*

Table 2, Fig. 3e)." (p.3165, l.23ff). Nevertheless, these high enrichments most do not effect the statistical analysis as EFs in the first 2 experiments are already highly variable (Wilcoxon test, p=0.917, n=6).

"Regarding the measurement of bacterial activity, it seems to me from p.3159, L 9 that only a single sample of 2.5 mL has been measured, e.g. no triplicate. Is that correct? The authors need to discuss those two observation during the last two days of the third experiment."
This was indeed a misleading formulation. We now changed the sentence into: *"Due to sample-volume limitations, triplicates of only 2.5 ml of each sample were incubated in the mesocosm experiments."*

"P.3166, L1-7 In L4 it says that the enrichment were different (p<0.04) but the authors claim then that the difference were not statistically different using Bonferroni correction. It is a confusing sentence."
The software SPSS does not provide a post-hoc test for the Friedman-test which, however, is needed to evaluate whether the significance level p is really significant for each single comparison within the test. For that reason the Bonferroni correction was applied. Due to the confusing formulation as stated by the referee we now reduced the last two sentences as follows: *"The results implied that the enrichment of CTC-positive cells and ^{3}H-TdR incorporation were not statistically different inside vs. outside the mesocosms (Table 2)."* Moreover, we changed the Table 2 in a way that the bold values, indicating significant differences, are not bold anymore in the last column.

"P.3166, L21 That is the reason why SML studies requires observation over a longer period. Contradictory"
Please see the "observation period" discussion above and their implementation in the discussion section.

"P.3166, L25-27 SML is spatially very heterogenous due to dispersion processes by wind and surface currents. I believe the observation that enrichments are always higher inside the mesocom is not surprising as no dispersion occurs within the mesocom. This observation could be an artifact of the experimental design."
Please see first comment from referee#1 and our reply including the newly added paragraph in the discussion section, where we discuss potential problems associated to the employments of mesocosms.

"P.3170, L7 Low cell-specific activity in the SML may have been caused by the glass plate sampling technique using a hand wiper as the authors reported in an earlier paper (Stolle et al. 2009, p71, section "tank experiments"). The authors seem to ignore their earlier findings on the effect of sampling technique on 3G-TdR incorporation activity."
Please see our reply to the referees' comments about selectivity of the sampling device used in the present study ('sampling strategy'). As indicated there, we have now specified that we used the glass-plate sampler and the framed wiping technique which was proven not to selectively inhibit bacterial incorporation of ^{3}H-TdR in our earlier study (Stolle et al. 2009). Therefore, we exclude the possibility that the observed reduction of ^{3}H-TdR-incorporation found in the present study was caused by the sampling device.

"P.3170, L13 It may be worthwhile to mention a study (Elasri and Miller, 1999; Appl. Environ. Microbiol. 65, 2025-2031) showing that biofilms offer considerable protection from UV radiation to bacteria. The SML with its hydrated gel matrix is certainly a biofilm habitat."
We have integrated that point into the discussion section as follows: *"Thereby, the matrix-like structure of the SML might on the one hand protect the bacterioneuston from UV-radiation*

comparable to biofilm habitats (Elasri and Miller, 1999) or on the other hand increase stress by photodegradation products such as H_2O_2 (Anesio et al., 2005)."

P. 3172, L15 Based on the limited observation period for each experiment (3-4 days) and the heterogenous nature of the SML I feel that this statement is rather speculative. The experimental design of mesocom studies may have also caused some artificial enrichment.

As discussed above (please see 'observation period' and comments by referee #1) we are certainly aware that the present study has some limitations due to the short period of observations during the experiments. We included this aspect according to the referees' suggestion in the discussion section "succession of the SML" p. 3172, l.13: *"Although the present study has some limitations due to the limited observation period, it can be speculated that very low wind speed induces a succession of […]."*

"P.3173, L6 Some important references are missing here reporting that the bacterioneuston is a different microbial ecosystem compared to the community in the underlying water, at least in estuarine and coastal waters (Fehon and Oliver, 1979, Estuaries 2, 194-197; Cuncliffe et al., 2008, ISME 2, 776-789; Franklin et al. 2005, Environ. Microbiol. 7, 723-736). As those studies refer to estuarine/coastal SML, I suggest to include these references in the discussion. The authors cite the excellent work by Obernosterer et al. 2008. However Obernosterer et al's work refers to an oligotrophic area in the South Pacific: : :very different to the conditions of the presented study."

We have now extended the discussion according to the referees' suggestions: *"Several studies from coastal and estuarine habitats have shown distinct bacterioneuston and bacterioplankton communities (Fehon and Oliver, 1979, Franklin et al., 2008, Cunliffe et al., 2008)."*

Response to Referee #4:

"End of page 3156: Is there reason to expect the stability or dynamics of the SML to change with climate change (more or less wind)? May be another good reason for the study."

Alcamo, J., et al., (2007: Europe. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, M.L. Parry et al., Eds., Cambridge University Press, Cambridge, UK, 541-580.) report changing mean wind speeds in several climate projections models. These changes (increasing or decreasing wind speed) seem to be regionally and seasonally different and, thus, no general picture can be drawn. This, however, might still indicate additional importance for SML studies as suggested by the referee. Increasing mean wind speed could reduce events of calmed sea surfaces and therefore heavy slick situations as investigated in the present study. On the other hand, a recent paper in this forum (Wurl et al., Biogeosciences Discuss., 7, 5719-5755, 2010) suggests that SMLs are stable up to wind speeds of 10 m s^{-1}. These findings make a discussion of climate change on SML dynamics and possible indications for biogeochemical cycling across the air-water interface a very interesting topic. However, we think that the influence of changing wind speed is not the only factor, which has to be investigated to fully appreciate the SML's reaction to climate change, as also changing autotrophic and heterotrophic production (i.e. changing concentrations of surfactants, TEPs, etc.) will effect the SML as a habitat as well as a barrier for air-water exchange processes. As the combinatory analyses of these factors were beyond the scope of the present study we would not like to integrate this topic in the present discussion.

"Methods 2.1: Any further discussion on why the glass plate method was chosen or is ok? Is a glass collection tube whose ends were closed by a drop-weight mechanism a Van Dorn

sampler? In explanation of enrichment factor replace _ and b subscripts with SML and ULW the additional symbols unnecessarily complicate the description."
For the discussion on SML sampling devices please see comments by referees#1 and #3 as well as our replies and the changes following that discussion. We hope that this topic is now discussed sufficiently.
The device to sample the ULW looks very much like a VanDorn sampler; it has, however, a different closing mechanism as the classical VanDorn sampler and therefore we would like to leave our description of the sampling device as it is in order to avoid misunderstanding of the instrumental setup.
We have now replaced the subscripts in the text according top the referees' suggestions.

"Methods 2.2: Does sampling cause mixing between the SML and ULW?"
Sampling efficiencies of the GP were shown to be up to 86% (Hatcher and Parker, 1973, L&O), although also lower efficiencies have been measured (VanVleet and Williams, 1980, L&O). This depends partly on the material used in these investigations, but also a mixing between the SML and the ULW caused by insertion of the GP into the water column can not be excluded. We tried to minimize this effect by a careful handling, i.e. slow sampling. We furthermore did the observation during sampling of a thick SML that movement of visible particles at the sea surface was much stronger when pulling the GP out of the water column (i.e. the actual sampling) compared to insertion of the GP – however, we can not present supporting data. Nonetheless, if mixing occurs, a fast re-formation of the SML can be expected within seconds (see Hardy 1982 in discussion section "succession of the SML"). Taken together, mixing would – if it does at all – cause an underestimation of enrichments in the SML. The EFs presented here are well comparable to previous studies and thus, we are confident that (a) we sampled the actual SML (as could be seen by particle-movement towards the GP during sampling) and (b) ULW samples were not "contaminated" by SML samples as the GP reached not as deep into the water column (approx. 20 cm) as ULW samples were taken from (30-50 cm).

"Methods 2.6: More detail on community composition analysis should be provided. Citing manufacturer determined default methodologies isn't useful unless you briefly describe them."
We have extended the description of the fingerprint analysis according to the referees' suggestions as follows: *"Cluster analysis and pairwise comparisons of the band patterns in digitised images were done with GelCompare II (Applied Maths NV) based on their densitometric profiles (Pearson correlation coefficient) after background subtraction, least-square filtering, and optimization which were used for background removal, smoothing of the profiles and to optimize the settings for the analyses, respectively, according to the manufacturers' instructions."*

"Results 3.1: How is the depth of the SML determined? Didn't find this in the methods. Was this measured on the other days?"
We implemented the answer to the referees' questions into the results section, where it is now stated that: *"SML thickness, determined by the sample volume per area of the sampling device, was only measured on day 2 and was 46 µm. This was in the range of previously reported values (Stolle et al., 2009)."*

"Results 3.1.2, line 2: "4.6 106" should be "4.6x106""
This was now changed according to the referees' suggestion.

"Results 3.2: I think the comparison of ULW conditions in and out of mesocosm should be shown."

Please see comments by referee#1 and our reply. We have now added a Supplement Figure as well as a Supplement Table which shows absolute concentrations of most parameters in the ULW outside and inside the mesocosms.

Additional changes:

Harvey 1972 was changed to Harvey&Burzell 1972

Following references were added to the bibliography:
Hühnerfuss 1981
Zhang et al., 2003
Riebesell et al., 2008
Elasri and Miller, 1999
Anesio et al., 2005
Fehon and Oliver, 1979
Franklin et al. 2005
Cunliffe et al. 2008
Nausch et al., 2009

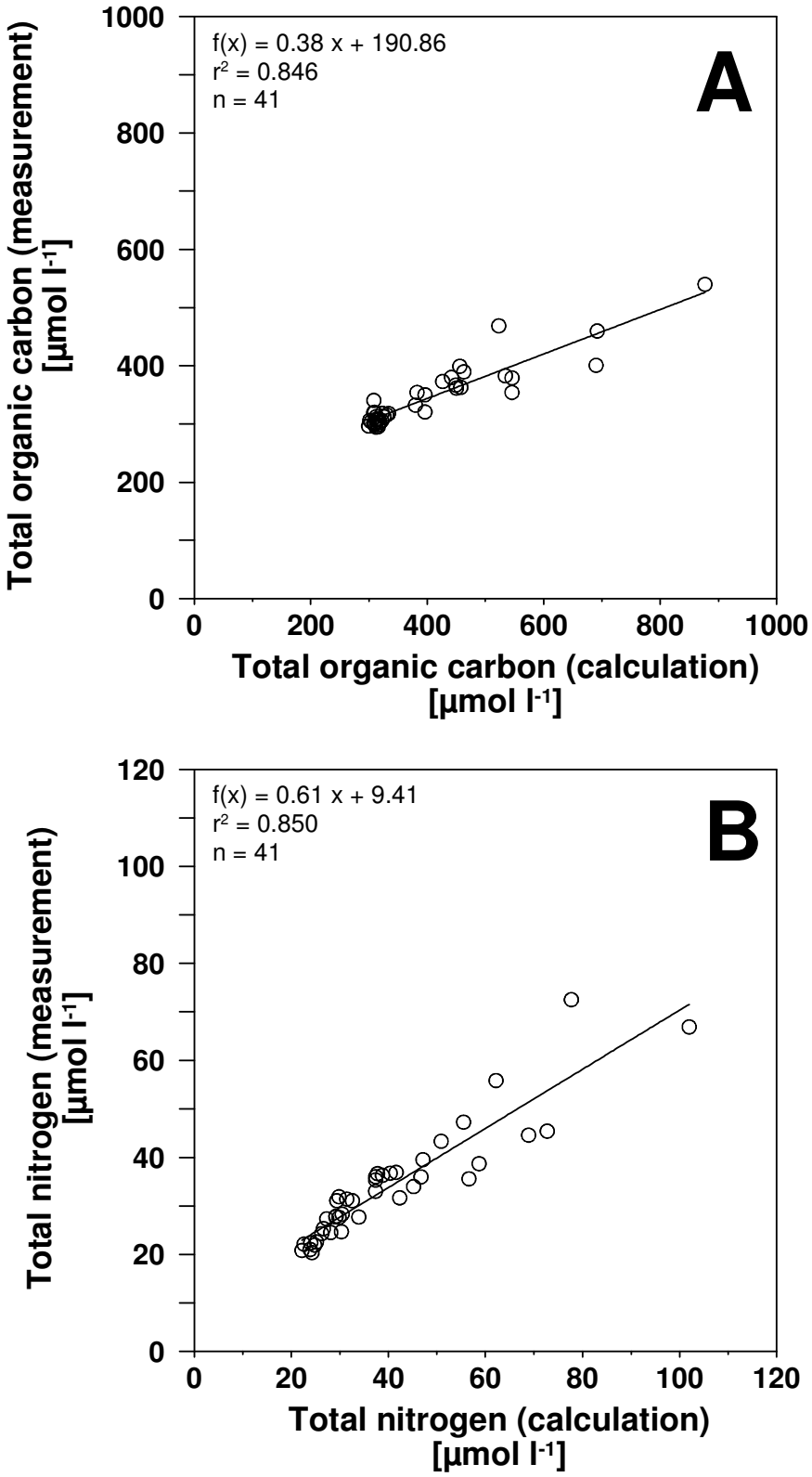


Figure A

The relationship between calculated (i.e. the sum of separately measured dissolved and particulate fractions) and measured concentrations of total organic carbon (A) and total nitrogen (B).

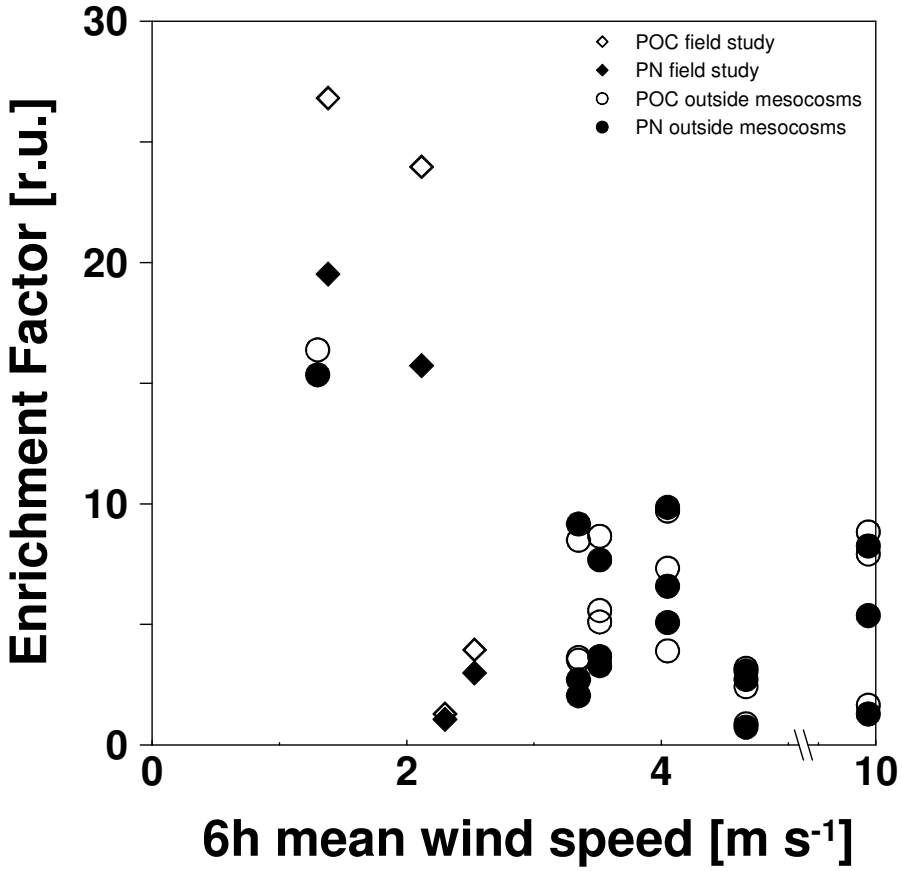


Figure B

The relationship between 6h mean wind speed and the enrichment factor of particulate organic carbon (POC, white symbols) and nitrogen (PN, filled symbols). Enrichment factors are from the field experiment (diamonds) as well as from outside the mesocosms (circles).

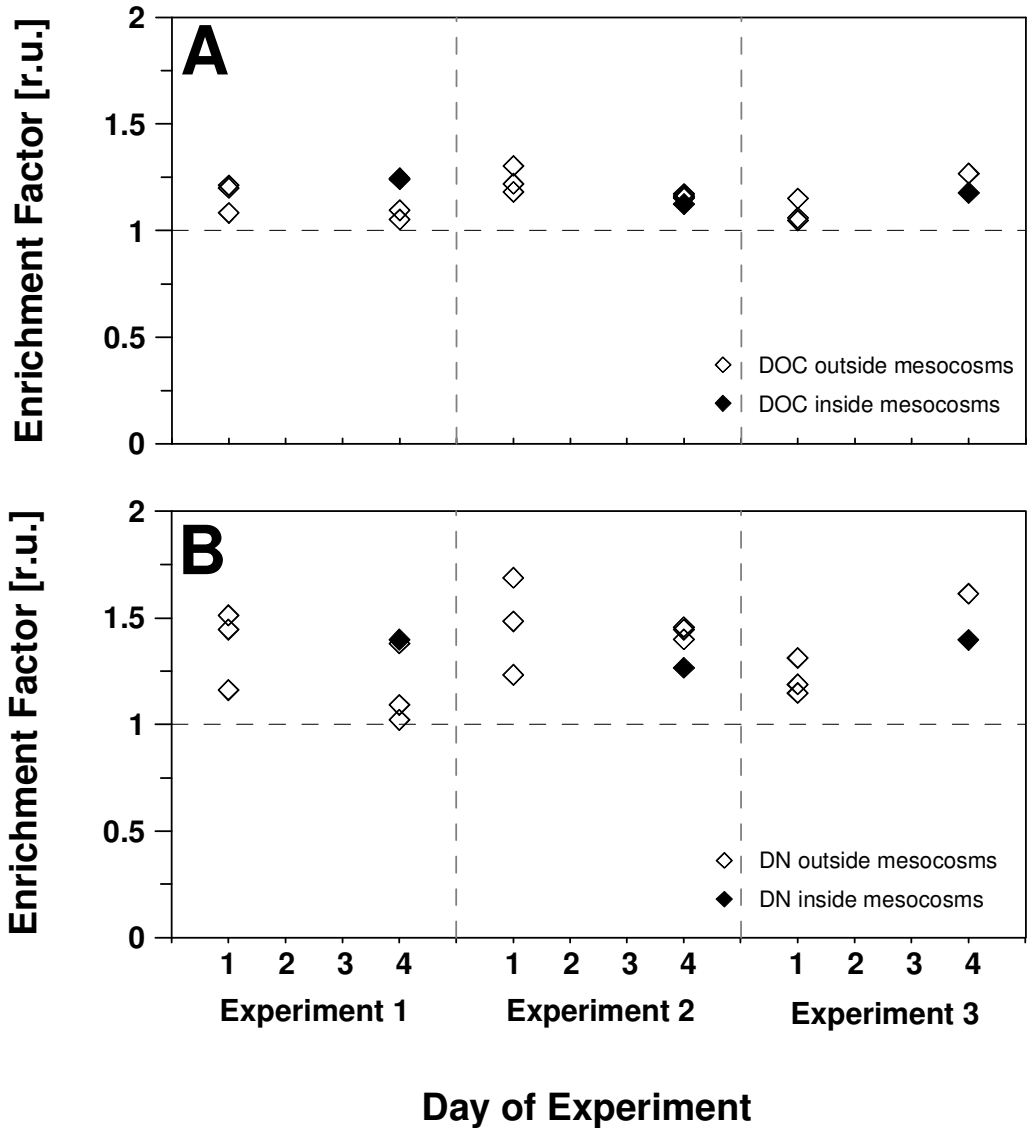
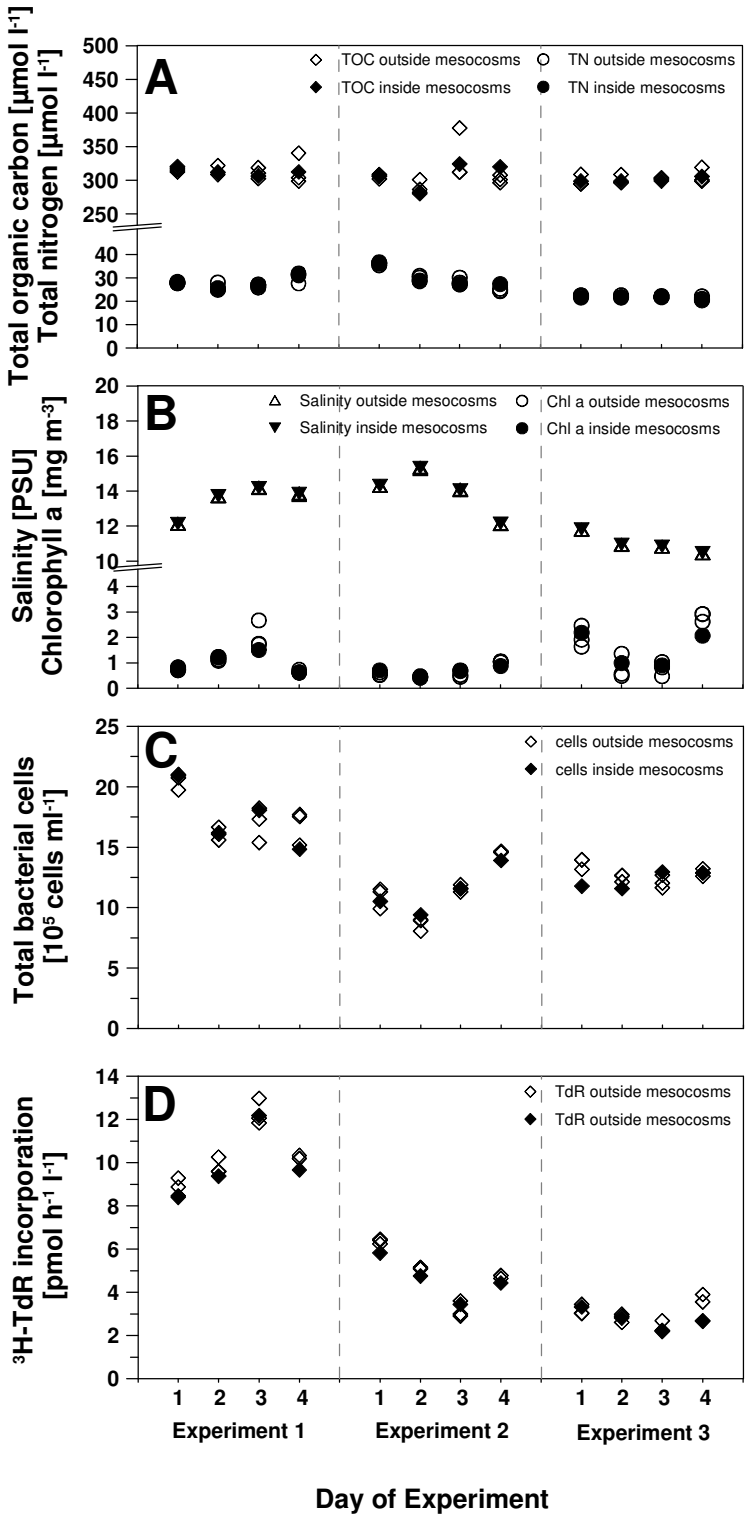


Figure C

The enrichment of (A) dissolved organic carbon (DOC) and (B) dissolved nitrogen (DN) over the course of three mesocosm experiments. White and black diamonds show results from inside and outside the mesocosms, respectively.



Supplement Figure 1

Absolute concentrations of (A) total organic carbon and nitrogen, (B) salinity and chlorophyll a, (C) total bacterial cells and (D) ^{3}H-TdR incorporation measured in the underlying water inside (filled symbols) and outside (white symbols) the mesocosms.

Supplement Table 1 Absolute concentrations of total organic carbon (TOC) and nitrogen (TN), dissolved organic carbon (DOC) and nitrogen (DN) as well as particulate organic carbon (POC) and nitrogen (PN) measured during the mesocosm experiments. Results are given for the underlying water (ULW) and the sea-surface microlayer (SML) in- and outside the mesocosms. The minimum and maximum values as well as the mean ± standard deviation (SD) are given. * values for TOC and TN are from separate measurements and not from addition of the dissolved and particulate fractions, values in parentheses indicate number of samples (n).

	TOC [µmol l^{-1}] *		**DOC [µmol l^{-1}]**		**POC [µmol l^{-1}]**		**TN [µmol l^{-1}] ***		**DN [µmol l^{-1}]**		**PN [µmol l^{-1}]**	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
ULW outside mesocosms	282.5 – 377.9	307.3 ± 11.3 (35)	280.2 – 301.2	291.7 ± 6.0 (18)	12.5 – 29.0	21,5 ± 4.8 (18)	20.8 – 36.7	27.1 ± 5.3 (35)	19.5 – 37.3	26.2 ± 5.3 (18)	1.4 – 3.1	2.4 ± 0.5 (18)
SML outside mesocosms	317.9 – 548.5	404.5 ± 67.7 (36)	294.6 – 388.6	339.1 ± 25.9 (16)	23.5 – 323.9	124.2 ± 78.0 (16)	24.7 – 72.5	42.1 ± 12.1 (36)	23.9 – 60.8	36.4 ± 10.0 (16)	1.9 – 41.3	12.7 ± 10.1 (16)
ULW inside mesocosms	280.5 – 320.5	310.7 ± 8.0 (12)	281.7 – 299.7	292.1 ± 7.6 (4)	13.2 – 26.4	19.7 ± 6.2 (4)	21.6 – 36.0	27.9 ± 7.8 (12)	21.4 – 27.7	27.7 ± 6.5 (4)	1.7 – 3.1	2.2 ± 0.6 (4)
SML inside mesocosms	363.0 – 876.1	434.6 ± 93.2 (9)	328.8 – 349.5	341.7 ± 11.3 (3)	125.3 – 526.4	329.3 ± 200.6 (3)	34.0 – 86.5	48.5 ± 16.8 (9)	29.8 – 38.8	33.4 ± 4.7 (3)	13.5 – 72.1	38.4 ± 30.3 (3)