

***Interactive comment on* “Technical Note: Sampling and processing of mesocosm sediment trap material for quantitative biogeochemical analysis” by T. Boxhammer et al.**

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Response to Reviewer #2 (Anonymous Reviewer)

We thank the reviewer for the constructive comments on this technical note, which were very helpful to refine the manuscript. Our responses to reviewer comments, including modifications to the manuscript, are detailed in the following:

Comment 1 by Reviewer #2: The first and most obvious issue is how the authors have dealt with growth on the sides of the mesocosms? This is not mentioned in the manuscript and may have a large impact on the estimates of export from the meso-

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cosms. Were the sides cleaned or the material left to grow – did the authors estimate that growth at the end of the experiments or were any measures taken to avoid such growth?

Author response: As the reviewer correctly points out, wall growth is be a big issue in mesocosm experiments leading to biomass build-up not represented in water column or vertical flux measurements. To prevent biofilm formation on the inside walls of the cylindrical KOSMOS mesocosms we developed a ring-shaped, double-bladed wiper used since 2011 for regular cleaning as once per week (Riebesell et al., 2013). To provide a clear reference for the reader we will rephrase page 18696, line 15 – 18 to read in revised manuscript: ‘The sediment trap design of KOSMOS used since 2011 consists of a flexible thermoplastic polyurethane (TPU) funnel of 2 m in diameter, connected to the cylindrical mesocosm bag by a silicon-rubber-sealed glass fibre flange (Fig. 1a). A detailed description of the KOSMOS setup and maintenance requirements such as wall cleaning can be found in Riebesell et al. (2013).’

Comment 2 by Reviewer #2: Have the authors made any tests of the oxygen consumption of material captured in the collection cylinders at the bottom of the mesocosm? Would this material go anoxic before sampling on either daily or every second day? Anoxic conditions could have important implications for the biogeochemical measurements of the settled material, e.g. a build-up of CO₂ could cause dissolution of calcium carbonate and other nutrient cycling could take place (e.g. anoxic steps of the nitrogen cycle).

Author response: So far we have not measured the oxygen level or consumption rate inside the collection cylinders. Sampling the sediment traps in a 24 or 48 hours routine we only once observed anoxic conditions by the smell of hydrogen sulfide in collected samples from a single KOSMOS unit. The outlet of the collection cylinder of this specific unit was partly blocked for several days by a Plexiglass[®] pipe that got lost from a manipulation device. This pipe had the effect of a partial bypass inside the collection cylinder. Surprisingly no change in the carbon to nitrogen to phosphorus ratio of the

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collected material was found in comparison to the other nine mesocosm units used in that specific study (author's unpublished data). However, we clearly agree with Reviewer #2 that the formation of anoxic conditions could potentially alter the collected particle stoichiometry and therefore has to be avoided by high sampling frequency. To account for this we will change page 18705 (line 6 – 8) and page 18705 (line 4 – 8) in the revised manuscript to read: 'Sampling intervals of the traps should be kept short – two days or less – to limit bacterial- and zooplankton-mediated remineralisation of the settled material and to avoid or minimize the time of possible carbonate undersaturation or anoxic conditions.' and 'High sampling frequency limits organic matter degradation and potential carbonate undersaturation or anoxia in the traps.'

Comment 3 by Reviewer #2: These are issues of particles sticking to the sides of the funnel as they slide down to the collecting cylinder at the bottom of the mesocosm and if the flow rate of the water in the silicone tube connecting the collecting cylinder to the sample bottle is high enough to ensure collection of particles with high sinking velocities (see specific comments).

Author response: Please see Author response to specific Comment 6 and 9 by Reviewer #2.

Comment 4 by Reviewer #2: Finally, why did you decide not to poison the sampled material during the sedimentation and centrifugation procedure, would this not have limited further degradation and allowed for longer sedimentation periods?

Author response: Several reasons convinced us not to poison the sampled material. As we point out on page 18695, line 26 – 29, the number of samples recovered per day and their volume can be very high depending on the experimental mesocosm setup. Including ten KOSMOS units, which are sampled on a daily basis for a two-month period, one has to process up to 30 L per day and easily more than 1000 L in total. First, the limited time for sample processing in the field, restricted by the sampling frequency, limits the need of poisoning of the samples. Second issue would be the

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storage of such big sample volumes and the disposal of the toxic water after particle concentration. Finally we wanted to avoid the exposure to toxins during the grinding process.

Comment 5 by Reviewer #2: Page 2, Line 4: With “these two processes”, I guess you refer to particle flux and particle formation, maybe write the processes out to avoid confusion.

Author response: We thank the reviewer for pointing this out. To avoid confusion we will change the sentence in a revised version of this manuscript to read (page 18694, line 3 – 4): ‘However, the spatial decoupling between particle formation in the surface ocean and the collection in sediment traps often handicaps reconciliation even within the euphotic zone.’

Comment 6 by Reviewer #2: Page 4, Line 18-19: Often marine snow and other aggregates are very ‘sticky’ and adhere to surfaces, did you test if the aggregates did slide down the funnel surface.

Author response: We frequently lowered down a camera system inside the mesocosms to get a snapshot of the material accumulating in the sediment traps. A short sequence of one of these videos can be seen in a published video of the sampling strategy to empty sediment traps of the KOSMOS setup, cited in the manuscript on page 18696, line 26 (Boxhammer et al., (2015)). The funnel of the sediment traps is made of a flexible 1 mm thick thermoplastic polyurethane foil where the particles can slide down in a 63° angle as shown in Figure 1b of the manuscript. The vertical movement of the mesocosms due to wave action also generates slight movement of the funnel, which seems to promote the movement of particles on the funnel surface. From our frequent observations with camera systems we can state that particle aggregates do slide down the funnel surface.

Comment 7 by Reviewer #2: Page 4, Line 20: Do you mean the tip or the bottom of the collecting cylinder?

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Author response: The collecting cylinder has a conical bottom end (Fig. 1b) with a hose connector at its tip to attach the silicon tube for sampling. This detail will be added to page 18696, line 20 – 23 in the revised manuscript so that it reads: ‘A silicon tube of 1 cm inner diameter reaches down to the collecting cylinder outside of the mesocosm bag (Fig. 1a). A hose connector links the silicon tube to the conical bottom end of the collector while a wire helix hose coating the first 1.5m prevents current related bending of the tube (Fig. 1b).’

Comment 8 by Reviewer #2: Page 4, Line 23: How long was the tube that connected the bottom of the mesocosm to the Schott Duran glass bottle? The KOSMOS mesocosms vary in depth between 15 and 25 m, is that including the funnel below the mesocosm? If the tube was 25 m plus a bit extra so likely around 30 m long? This means that there was around 3 L of seawater in the tube itself. In addition, the collecting cylinder contained 3.1 L of seawater, which means that the total water volume in the tube and in the collecting cylinder made up 6.1 L while the Duran Schott bottle only collected 5 L of water. Was this enough to ensure that all aggregates were collected?

Author response: The KOSMOS mesocosms deployed so far reached a water depth of 15 to 25 m including the funnel of the sediment traps. The silicon tubes used for vacuum sampling of the traps were indeed up to 30 m long resulting in a maximum volume of about 2.4 L. To keep the sample volume low and not to dilute the relatively dense particle suspensions originating from the collecting cylinders we separated and discarded the water originating from the tubes if clear, as described on page 18697, line 3 – 5. Only in the case of particles being present in the water originating from inside the tubes in combination with a collapsing phytoplankton bloom in the mesocosms one could exceed the volume of 5 L, but this was usually not the case. To ensure that we sampled all collected aggregates from the collecting cylinders we visually observed the fluent passing through the Plexiglas[®] pipe. The sampling procedure was only terminated when no more particle were visible in a water volume of about half a litre passing through the pipe (see Author response to Comment 13 by Reviewer #2).

Comment 9 by Reviewer #2: Page 5, Line 1-3: Have you calculated or measured the water flow in the tube? This needs to be more than the settling velocity of the collected aggregates. These can potentially sink with several hundred meters per day. Some ballasted aggregates and fecal pellets have quite high sinking velocities (e.g. Bruland and Silver 1981, Iversen and Robert 2015, Ploug et al. 2008), though most are likely around 100 m d⁻¹. Did you calculate what your theoretical flow rate was and have you considered if any potential boundary effects potentially would make you lose some particles?

Author response: Unfortunately we have never measured the water flow inside the silicon tube during the sampling process. Assuming a realistic water flow rate of 0.5 L per minute at the given inner diameter of the silicon tube of 1 cm, we calculate a water flow velocity of 9167.32 m d⁻¹ according to equation (1).

$$v = Q / (3600 * \pi * (d/2)^2) \quad (1)$$

v = water flow velocity (m s⁻¹)

Q = water flow rate (m³ h⁻¹)

d = silicon tube inner diameter (m)

As the water flow velocity exceeds the maximum sinking velocity of even dense particles by far, we are sure to be able to recover all settled material from the sediment traps. This also correlates with our observations during sampling. Particles in the collecting cylinder are stratified after their density and grain size which becomes obvious when observing the particle suspension passing through the Plexiglas[®] pipe, please see Boxhammer et al., (2015). Even small screws that got lost from sampling devices inside the mesocosms were brought up within the dense particle suspensions.

Comment 10 by Reviewer #2: Page 5, Line 3-5: Did you typically discard the volume contained within the silicon tube before sampling, e.g. 3 L for a 30 m long tube?

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Author response: Please see Author response to specific Comment 8 by Reviewer #2.

Comment 11 by Reviewer #2: Page 5, Line 7-10: It seems unlikely that the integrity of the particles were preserved during the sampling. First you collected the particles in the collection cylinder where they would land on top of each other after rolling down the sides of the funnel. Already here you have changed their size and structure. Thereafter they are pumped up a long tube and finally flushed into a Duran Schott bottle. Even if this is gently done, it will still affect the aggregates, especially marine snow, fecal pellets might survive the procedure. However, it is not important for your study to preserve the size, shape, and structure of the aggregates, since you are interested in chemical analysis, so I would suggest to remove this sentence from the manuscript.

Author response: We agree with this statement of Reviewer #2 and will remove this sentence (page 18697, line 7 – 10) in a revised manuscript.

Comment 12 by Reviewer #2: Page 5, Line 11-14: There would be several issues by using the particles collected in this way to measure the particle sinking velocity and microbial respiration rates if you assume that the particles are the same as those formed and settling within the mesocosm. This would need some direct comparisons of aggregates collected within the mesocosm to the ones collected with the method described in this manuscript. However, as long as we are aware of the differences and changes made to the particles collected here, there is still much valuable information to be made from measurements of the particles collected here, as long as they are well characterized at each sampling point in terms of composition and type for instance.

Author response: This is an important point that Reviewer #2 has highlighted here. The particles that were sinking in-situ passed several steps of potential disintegration and re-aggregation when (1) accumulating in the sediment traps, (2) being sampled through the silicon tube and (3) being subsampled in the lab. We agree that the aggregates in the subsamples used for measurements of sinking velocity and microbial respiration rate were not exactly the same as those settling in the mesocosms, but they

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consisted of the same primary building blocks. The potential of sinking velocity measurements from particles originating from KOSMOS mesocosm sediment traps and its limitations are discussed in (Bach et al., 2012). The detailed information about particle stoichiometry (e.g. inorganic carbon or biogenic silica load) gained by following the protocol described in the present paper can then be combined with measurements as particle sinking velocity or microbial respiration measurements.

Comment 13 by Reviewer #2: Page 5, Line 15: When did you decide that you had collected all the aggregates? 1-4 L of particle suspensions seems rather low for the 1 L, but maybe you stopped when no more particles were observed after a certain time or a certain water volume?

Author response: We thank the reviewer for pointing out this missing information. The samples that have been pumped up to the sea surface usually had a very high particle density. During phases of low vertical particle flux the sample volume (not including the separated clear water originating from the silicon tube) can easily go down to about 0.5 L. We permanently observed the water passing through the Plexiglas[®] pipe and terminated the sampling procedure after about 0.5 L of clear water being sampled after the dense particle suspension. We will add this information so that it reads on page 18697, line 5 – 8: ‘The dense particle suspensions originating from the collecting cylinders were then vacuum-pumped into the sampling flasks until no more particles were visible in the Plexiglas[®] pipe in a sampled extra volume of about 0.5 L (Boxhammer et al., 2015 (video)).’

Comment 14 by Reviewer #2: Page 5, Line 16-18: Consider to point out that this subsampling is not the one used to do the biogeochemical parameters, but subsampling for other measurements and that you are keeping this low in order to be able to have reliable chemical measurements from the total flux of particles. Did you measure the precise volume of the ‘pre-samples’?

Author response: As suggested by Reviewer #2 and in addition to the statement that

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the bulk sample is used for biogeochemical analysis (page 18697, line 14) we will emphasise this point in line 16 – 18 (page 18697) so that it reads in the revised manuscript: ‘Total volume of all subsamples should be kept small (ideally below 5 %) in order to limit the subsampling bias on the remaining sample that is processed for the quantitative biogeochemical analysis.’

We determined the quantities of each sample (‘pre-subsample’) and all subsamples gravimetrically with an accuracy of 0.1 g for individual share calculations. We will add this information (page 18697, line 21 – 22) in a revised version of the manuscript to read: ‘Quantities of the main sample and all subsamples were gravimetrically determined with an accuracy of 0.1 g for individual share calculations.’

Comment 15 by Reviewer #2: Page 6, Line 4: Why do you use the term total particulate carbon? Was this because you did not remove inorganic carbon (calcium carbonate) with hydrochloric acid?

Author response: We used total particulate carbon as a proxy as this parameter was measured of all samples we used for analysis while particulate organic carbon was only measured of samples from mesocosm studies which enclosed a substantial amount of calcifying organisms in the water columns, e.g. coccolithophores.

Comment 16 by Reviewer #2: Page 6, Line 9-10: How did you know that the copepods were alive if they were on the filter? Did you do this step immediately after filtration or after freezing?

Author response: We thank Reviewer #2 for pointing this out. We will change page 18698, line 9 – 10 in the revised manuscript to read: ‘Copepods, which could occasionally be found in the liquid, were carefully removed from the filters right after filtration.’

Comment 17 by Reviewer #2: Page 6, Line 22: Did you calculate what the slowest sinking velocity would be for the settling particles reaching the bottom of the bottle? If the bottle was 20 cm tall, then particles sinking with velocities slower than 2.4 m d⁻¹

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would not make it from the top of the bottle to the bottom within 2 hours, assuming that the bottle was full. Try to calculate this and see what the slowest velocity would be, some single phytoplankton cells settle with around 1 m d^{-1} . This might give you an idea of what the carbon sources for the supernatant could be.

Author response: It is an interesting point that Reviewer #2 has highlighted here. With about 4 L of particle suspension being sampled and the glass bottles stored in a 60° angle, we measured a maximum settling distance for particles of 0.18 m. Thus particles with a sinking velocity of less than 2.16 m d^{-1} would not have been able to reach the bottom of the sampling bottles within 2 hours. Sinking velocities of marine particles are determined by their size and density, which is a result of their individual origin. To enable even single cells to settle down to the bottom of the glass bottles, settling times of greater than 48 h would be required, which is not practicable at high sampling frequencies of a set of mesocosms and would require poisoning of the samples to inhibit microbial degradation of the organic matter. To include this information in the revised manuscript we will add the following sentence to section 2.2.1 (page 18699, line 13): 'To increase the concentration efficiency of passive settling, longer sedimentation periods of up to 48 hours for single plankton cells would be required. However, this is not practical at high sampling frequencies of a set of several mesocosms and would require poisoning of the samples to inhibit microbial degradation of organic matter.'

Comment 18 by Reviewer #2: Page 9, Line 27-29: Do you think the improved concentration efficiency of the FeCl_3 in comparison to the passive settling and the centrifugation was due to loss of CaCO_3 from both the sediment and the supernatant?

Author response: A loss of calcium carbonate (CaCO_3) would most likely decrease the concentration efficiency, as CaCO_3 should contribute more to sedimented carbon than to residual carbon in the supernatant even after a relatively short time of sedimentation (1 h). During the study where FeCl_3 was used for particle concentration there was only a negligible number of calcifying organisms present inside the mesocosms not able to build up a considerable amount of CaCO_3 . Additionally, the number of undersaturated

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samples after precipitation with FeCl₃ was reduced by 2 and 6 samples with respect to aragonite and calcite (page 18701, line 24 – 26), why we do not think that the sample pH had an effect on particle concentration. The efficiency of chemical precipitation of particles with FeCl₃ is visualised in the supplementary video S1.

Comment 19 by Reviewer #2: Page 12, Line 8: Change to “. . .macroscopic structures were visible after. . .”.

Author response: We thank the reviewer for pointing this out and will change the time to past tense for p. 18704, line 8 in the revised manuscript.

Comment 20 by Reviewer #2: Section 2.4: How did you do the quantitative measurements of the chemical parameters of the grounded material? Did you weigh the total mass of all the grounded material before taking subsamples from it?

Author response: We weighed the freeze-dried samples before and after the grinding procedure to determine the dry-weight of each sample. The dry-weight was then corrected for the subsamples taken previously to the concentration procedure. The quantitative values of biogeochemical parameters were then calculated from the individual subsample weight used for analysis and the corrected dry-weight of the main samples.

Comment 21 by Reviewer #2: Page 12, Line 9: Change to: “. . .diatom frustules became detectable. . .”.

Author response: We thank the reviewer for pointing this out and the time will be changed to past tense for p. 18704, line 9 in the revised manuscript.

Comment 22 by Reviewer #2: Page 12, Line 15-18: It still remains to show that particles are not stuck to the sides of the funnel when they are sliding down inside the mesocosm. In addition, it would be good to estimate the flow rate of the water within the tube leading from the bottom of the mesocosm to the collection Duran Schott bottle at the water surface and test if there are shear or boundary effects affecting the trans-

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port of particles through the silicon tube. Finally, was the water volume collected in the Duran bottle enough to sample all the particles in the collecting cylinder at the bottom of the mesocosm?

Author response: Concerning the issue of particles interacting with the funnel of the sediment traps please see Author response to comment 6 by Reviewer #2. The water flow rate has never been measured, but we estimate a realistic rate of 0.5 L per minute, which leads to a corresponding water flow velocity of 9167.33 m d^{-1} inside the silicon tube of 1 cm inner diameter. For details please see Author response to comment 9 by Reviewer #2. We have no doubt that even particles of high density are transported in the water flow inside the silicon tube during sampling, but it is likely that shear or boundary effects modify the particle's size and structure. To clarify any concerns about the sampled water volume please see Author response to comment 8 by Reviewer #2.

Comment 23 by Reviewer #2: Page 13, Line 17-18: Would the simplest method to use in the field not be the passive settling? It seems that a longer settling period would increase the efficiency of collecting the settling material at the bottom of the bottle?

Author response: Passive settling would be the simplest method to use, if time and space would not be limiting factors and if samples would be poisoned to stop microbial degradation of the organic matter. As whole sample centrifugation speeds up the gravitational settling of particles we recommend using this method in the field to deal with a realistic sample volume of up to 30 L per day (10 mesocosm units, daily sampling frequency, ≤ 3 L of individual sample volume). To speed up the process or in case of larger sample volumes, FeCl_3 can be used as a pre-treatment.

Comment 24 by Reviewer #2: Page 13, Line 25-26: Do you have a reference or some tests showing that the precipitation of phosphate to particulate phosphorous is negligible?

Author response: In fact it is likely that most of the inorganic phosphate being present in the water fraction of the samples (particle suspensions) has been precipitated in

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the form of ferric phosphate (FePO_4). However, the amount of potentially precipitated phosphate is negligible compared to the phosphorus incorporated into the biogenic particles in the samples. Assuming 100% of the taken sample volumes to be particle free seawater and using the phosphate concentrations measured in the mesocosm water columns we calculated a theoretical contribution of precipitated phosphate of in average 0.5%. At high phosphate concentrations of about $0.8 \mu\text{mol L}^{-1}$ and very low particle flux (pre-bloom phase of the phytoplankton) we got only 4 out of 477 samples (0.8%) with a potentially higher contribution than 5% to particulate phosphorus in the samples. A significant contribution of precipitated phosphate to particulate phosphorus in samples with low organically bound phosphorus would also be visible in the ratio of nitrogen to phosphorus, which was not the case in 540 samples that were precipitated with ferric chloride (unpublished data by authors).

Comment 25 by Reviewer #2: Page 14, Line 3: For me it seems that there are many issues with the addition of FeCl_3 to the sediment sample? Decrease of pH, precipitation of phosphate, addition of iron, and interference with spectrophotometric analysis?!? Would this method not be best to avoid?!?

Author response: The pH of sediment trap samples from mesocosm systems is generally a critical point as the traps cannot be poisoned and CO_2 released by microbial degradation can decrease the sample pH. Using FeCl_3 for particle precipitation the individual sample pH needs to be adjusted with sodium hydroxide (NaOH) also allowing for compensation of the biologically driven pH reduction. Thus this can also be seen as an advantage of using this method. The contribution of precipitated inorganic phosphate (FePO_4) to particulate phosphorus is negligible as shown in the Author response to comment 24 by Reviewer #2. As we stated on page 18705, line 26 and following, iron ions have the potential to interfere with spectrophotometric analysis but require very high concentrations. According to Hansen and Koroleff, (2007), the colour intensity when measuring phosphate is only increased by about 1% with $180 \mu\text{mol L}^{-1}$ of iron ions being present in a measured sample. Even though each of our 540 samples

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contained varying amounts of organic material and iron ions we have not observed any influence on measurements, which should be visible in the elemental stoichiometry (increased silicate or phosphorus values, relative to carbon or nitrogen). Being aware of the advantages and pitfalls of this method we recommend using FeCl₃ for particle precipitation when enclosing highly productive ecosystems in pelagic mesocosms with sediment trap samples of larger than three litres.

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