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Interactive comment on "A refinement of coccolith separation methods: Measuring the sinking characters of coccoliths" *by* Hongrui Zhang et al.

Anonymous Referee #2

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In this manuscript Zhang et al. investigate empirically the settling rates of sedimentary coccoliths of different sizes and shapes in the laboratory. Repeated settling is a technique that is widely used to separate natural sediment assemblages into near mono-specific fractions, but as far as I am aware this is the first attempt to calibrate this approach quantitatively over a range of species. The dataset presented by Zhang et al. will be a useful contribution to the sedimentary coccolith literature, and I believe Biogeosciences is a good place to present this work. However I do have a number of comments.

In general the manuscript is well-written, although there are parts that feel muddled and are difficult to understand - especially where equations are derived. The motivation for the research is not particularly well laid out (why do we care about obtaining mono-

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Discussion paper

specific fractions?). The dataset is good, but is not presented in a particularly helpful way for anyone who wants to use their results.

The importance of this work is in the practical laboratory application of separating coccoliths, rather an advance in scientific understanding. In this manuscript in its current form, this is lost behind the emphasis of settling velocities. The importance of settling velocities of individual suspended coccoliths in the lab is hard to appreciate; this isn't a quantity that can be used directly for the purpose of species separation in the lab, and its biogeochemical importance in the natural environment is questionable. I would suggest that the authors consider reframing this work as a tool for subsequent authors to separate mixed coccolith samples into monotaxonomic fractions: Specifically to describe their suggested protocol, and how to calculate ideal combination of settling times and (e.g. as a trade off between yield and quality of separation?). If they do wish to comment on a comparison between settling rates observed in the laboratory, and those observed in sediment traps, I think this needs at least a full paragraph in the discussion.

The assumptions underpinning this work should be more clearly stated in the main text. Firstly, all coccoliths belong to a particular species are assumed to sink at exactly the same rate. Secondly, they are assumed to sink at a constant velocity from the instant that the suspension is left. I would like to see a calculation in the appendix estimating the time and distance that a particle falls before it reaches terminal sinking velocity, to show whether or not it is justifiable to ignore the accelerating phase for all of the particle sizes considered here. Intuitively I imagine this is a fair assumption, but it would be nice to see in numbers. Furthermore, I think it would be useful for the authors to test their approach with spheres (such as spherical glass beads) of similar size to coccoliths such as those used to calibrate Coulter counters. The authors have made theoretical calculations based on idealized spheres (and compared the differences between the observed values in lines 211-212), so this sort of approach would be a good test of their proposed protocol - elucidating the degree to which differences between an idealized

scenario in theory and observations of coccoliths is due to shape, the experimental set up, or assumptions made in the calculation of their parameter, R. They could also spike a sample of sedimentary coccoliths with these beads in order to test the density of suspension that leads to hindered settling.

The authors justify the assumption that settling rates are approximately constant with a time course analysis of *Gephyrocapsa oceanica*, concluding that for the first 4 hours, settling velocities do indeed appear to be constant. Is this period of 4 hours applicable across coccoliths of other size and shape? What causes the deviation from the ideal stokes law behaviour after 4 hours? If this were an ideal scenario, the top part of the vessel should be completely devoid of coccoiliths of a given size after a period of time T, where $T = \frac{D}{sv}$. I would like a more in depth discussion of these features and other factors affecting sinking velocities in the lab - for example - temperature gradients leading to convection, entrainment of small particles by larger ones (i.e. do smaller coccoliths sink faster when there are large coccoliths present?).

8-9 I suggest that the authors remove the reference to CaCO3 export from the surface ocean. In the ocean, sinking velocities are greatly complicated by flocculation with organic matter, and through grazing - as mentioned in line $\sim$ 178, most coccoliths probably ended up in sediment packaged up in larger aggregates such as faecal pellets.
It would be useful however to have the complexities of the real ocean alluded to much more clearly and earlier in the manuscript, so that readers are not tempted to use these calculations to estimate export rates directly from individual coccoliths in sediment.

24-38 From a non-specialist point of view it is not clear from the first paragraph why it is desirable to obtain monospecific fractions.

Eq. 2-2 test this equation in an ideal scenario using glass spheres?

150 This doesn't make sense

89-119 I think this section would benefit from being slightly more thorough and clear about how the proposed protocol is actually implemented. For example: I assume that when counting coccoliths in the lower part of the settling vessel, that the remaining suspension must be homogenized, including re-suspending any coccoliths that have settled out, before counting. If so, this should be stated explicitly.

162-164 "sediments accumulating in the lower suspension, the particle concentration can be more than 4 times higher than the initial homogenous concentration" – This is important and should be discussed thoroughly. How do these higher concentrations arise? Presumably due to the size range of coccoliths in the sample. Can this effect be described quantitatively as a function of the standard deviation of coccoliths sizes in the initial sample?

179 "confirming the fact" is far too strong. It is true that these numbers are consistent.

189 Why is H. carteri excluded?

Table. 2 I assume that the asymmetrical uncertainties on sinking velocity may arise due to an assumed normal distribution of coccolith size via the quadratic relationship? If so, this should be stated.

Fig.2 This figure doesn't really represent the assumptions made by the authors. For coccoliths of a given size, the boundary between the suspension and the supernatant is infinitely sharp, and the suspension does not change in density - but rather there is a build up of coccoliths deposited on the bottom of the vessel. In a mixed species assemblage, or where coccoliths are a range of sizes, then the suspension will become more dense towards the bottom over time as shown here, but this isn't currently represented in the equations (or at least not clearly!).

For this reason, these coccolith images are fairly unhelpful. A schematic figure that more clearly shows the change in coccolith density might be better, with a more obvious range in sizes (or not).

Fig.2   If the authors are using the volume and sinking distance to estimate the average vessel diameter, the equation given in the caption doesn't look right. I think it should be: $\phi = 2 \times \sqrt{\frac{V_1}{\pi D}}$.

Appendix D   While the math seems sensible, I found it difficult to follow this derivation despite its simplicity. Nevertheless, the way of measuring sinking velocity proposed here is interesting, and I would personally prefer to see its derivation in the main text rather than the appendix. Specific points:

- Each variable should be defined after it is first used throughout the text, and again within the appendix if this is to constitute a stand alone derivation.
- A single symbol would be better for sinking velocity unless either 's' or 'v' is subscripted.
- If sv is a function of t, show this. If not, and you're interested in the average sv, I think
- The ratio given in line 458 is not the number of coccoliths in a thickness dD as stated - as the authors have defined here, it is the number of coccoliths per unit unit thickness.
- Figure D1: What does Monte Carlo mean in b) here? Have the parameters of the model been fitted to the data points multiple times, resampling their values from an assumed distribution? If so, the spread of constrained these values rather than just the average needs to be plotted to show how uncertain this relationship is. I assume that the early, straight part of the line in b) is the part that is described by equation 2-2, before the settling velocities

decrease when the suspension is left for 4 hours (d) - if so, it would be helpful to plot this straight line on here too and label it as the fit to equation 2-2 in the valid region. I don't understand how the authors obtain the shape of the relationship in b), so would benefit from further explanation. Why are there more data points in d) than in b)?

482 equation 2-6 doesn't exist. Should this be D-6?

eq. D-6 This is difficult to follow. Keep equation in symbol format before introducing numbers.

eq. D-7 What is -10, and what is k?

Appendix E It's not clear to me how a Monte Carlo approach has been used here, nor the benefits of using such an approach over propagation of uncertainty equations. As far I understand it, the authors have simply calculated the uncertainty associated with equation 2-1, for a range of explicit values of N1 and N2.