We greatly appreciate the valuable comments made by J. Henderiks and a second reviewer to improve the scientific quality of the manuscript. Please find below our answers (red font) to the reviewers comments (black font) and the changes made in the manuscript (red italics).

General re-evaluation of data:
Apart from the changes made to address the reviewers' comments, we also slightly improved data analysis by re-evaluating the Coulter Multisizer measurements and analysing the data assuming a normal distribution. This resulted in slightly lower average coccolith volumes. The results for coccosphere and cell diameter were not influenced by the re-evaluation. This analysis (assuming normal distribution) has the advantage that signals of coccoliths which are attached/sticked to each other (therefore measured as one particle with a double volume) have a minor influence on the average coccolith volume. This is stated in the Method section of the manuscript.

Reviewer 1 (J. Henderiks)
General comments
The authors report on experimental results of the coccolithophore Emiliania huxleyi that was grown under nutrient replete (+N) and nitrogen limited (-N) conditions, both under a similar range of pCO2 levels / CO2 concentrations in the culture media. Each set of experiments was subjected to three concentrations of CO2 (corresponding to a "preindustrial" lower-than-modern pCO2 of _200-280 _atm, and two higher-than-modern levels, _450 and 1000 _atm). In their Introduction, the authors mention the fact that most "ocean acidification" experiments to date have been done under nutrient-replete conditions, and that E. huxleyi's physiological response is rather uniform, despite strain-specific differences (e.g. Langer et al., 2009; Findlay et al., 2011). Fewer physiology studies exist on the same species (and strains) grown under nutrient-limitation in combination with variable/ elevated CO2. Here, we are thus offered a "direct" comparison between the two (or, rather, 6) scenarios - on one single strain. Still, some fundamental differences are inherent to comparing batch and chemostat cultures, both methodologically and in terms of the physiological state of the algae.

The reviewer is right mentioning the differences between batch and chemostat experiments. Therefore, we added a more detailed description of the experimental set up and a paragraph in the discussion section about the different growth conditions in batch and chemostat experiments. Please see details at the 'specific comments' section.

Another interesting aspect of this study is the data on coccosphere/cell diameter (_m) and individual coccolith volumes (_m3), as calculated from Coulter Counter analysis. The rationale to look at these parameters appears fuelled by the fact that paleoceanographic studies, that aim to reconstruct past changes in coccolithophore productivity and calcification, primarily rely on the calcite fossils of this group, which are generally single coccoliths and rarely fossilized intact coccospheres. Since coccolith size and its volume (and thus weight, if multiplied by the density of calcite) does not necessarily reflect calcification rate (which can only be estimated through growth experiments; PIC production rates), valuable insights should be gained by the presented approach. The authors conclude that coccolith volume is best correlated with the coccosphere/cell diameter, but that coccolith volume is not correlated to PIC production rate. To explain variations in sphere/cell diameter, the authors argue that these variations are "presumably related to lengthening and/or compression of certain phases in the cellular division cycle (Müller et al., 2008)." But it's rather disappointing that this is not further explained or discussed - in light of the different phyisological states that the batch (exponential growth) and chemostat (stationary growth) cultures represent.

We removed the statement about “lengthening and/or compression of certain phases in the cellular division cycle” for two reasons. First, the statement was too speculative and not supported by experimental data of this study. Second, a comprehensive discussion on the possible mechanisms linking the cell diameter to the cellular division cycle might be too complex for the present manuscript. However, we added an extensive discussion on the connection of nutrient limitation (N+P), CO2, pH to cell diameter and coccolith volume. Please see details at the ‘specific comments’ section.

Specific comments

1. Batch vs. chemostat cultures

If I understand correctly, the batch and chemostat culture vessels were identical (filled to 1.8 litre) (p. 4983, l. 13-14), and the culture media prepared in the same way to attain the three different CO2 target values (aeration or "bubbling" with mixtures of pure CO2 and CO2-free air during 4 days, prior to inoculation of E. huxleyi cultures). The pre-cultures were not acclimated to the CO2 concentrations before inoculation; but the authors are quick to state that this is not an issue, that "over the course of 5 or more generations" the algae would be fully acclimated to the conditions at the time of sampling (p. 4983, l.5-11). It is not made clear if, nor how, the pre-cultures for the N-limited chemostats were treated (did acclimation also start first after inoculation? If so, the algae has to acclimate to both -N and different CO2, any comments on the rationale here would be informative).

Yes, pre-cultures for the –N treatment had to acclimate both to –N and CO2 conditions. Basically, chemostat experiments started similar to the batch experiments. Nitrogen acclimation occurred after exponential growth and maximum cell number was reached. When the dilution of the chemostats was started the culture needed about 7 to 10 days to reach acclimated conditions (constant cell number for 10 days). Therefore, cultures were growing under nitrogen limitation for 17 to 20 days.

We described the experimental set up for the chemostat experiments in more detail:
“When the chemostats were filled with 1800ml of culture medium the supply was stopped and a preculture of E. huxleyi was inoculated. Emiliania huxleyi (1000cellsml−1) was allowed to grow exponentially to maximum population density (limited by the nitrate concentration of 9.0±1.4µmoll−1) and the medium inflow from the supply tanks to the chemostats was restarted. The chemostats were operated at a constant dilution rate (D=0.49±0.01d−1) which was periodically checked by weighting the incoming medium.
After growing for 7 to 10 days under nitrogen limitation (acclimation period), E. huxleyi reached equilibrium state conditions (constant cell number) and was allowed to grow for another 10 days before the dilution was stopped and the chemostat culture was sampled. Cell number, coccosphere/cell diameter and coccolith volume were checked daily with a Coulter Multisizer™ 3. Samples were taken for DIC….”

Nutrient-replete batch cultures were conducted in triplicate, whereas the nitrogen limited continuous/chemostat experiments appear to not have been replicated (as indicated by lack of SD values in Tables 1-3). Therefore, statistical power is much weaker for the chemostat series. Also, some cellular production rates and ratios data are lacking for C1, but why is not mentioned in Table 2 - what happened?

p. 4990, lines 6-8; Given Fig. 3 and Table 4, I cannot judge whether the Coulter Counter data reported for the chemostats reveal significant "trends" within the range of tested CO2, or not.

Without replicates, I'd be very reluctant to state, as the authors do, that such trends exist under N-limitations.

Correct, chemostat experiments were not replicated whereas batch experiments were conducted in triplicates. However, chemostats allow an averaging over time (repeated daily measurements during the 10 days of equilibrium state) and therefore we are confident that the observed increase in coccolith volume from 0.5 to 0.63 µm^3 (>20%) with elevated CO2 (Table 4) is worth to be mentioned as a trend. Nevertheless, the conducted repeated daily measurements can also be interpreted as pseudo-replication (multiple measurements under equilibrium state) and thus, we rephrased the statements about the CO2 effect on coccolith volume under nitrogen limitation.

Results section:
"Coccolith volume was substantially reduced under nitrogen limitation compared to nutrient replete conditions (Fig. 3, Table 4) and increased significantly from low to high pCO2 under nutrient replete conditions. Repeated daily measurements (n=10) of coccolith volume during equilibrium conditions (nitrogen limitation) revealed an increasing trend from low/intermediate to high pCO2 (Table 4). "

Discussion section:
"A significant positive increase in coccolith volume was detected with elevated pCO2 under nutrient replete conditions and a similar trend was observed from low/intermediate to high pCO2 under nitrogen limitation (Fig. 3, Table 4)."

Unfortunately, the filter for TPC analysis of the chemostat experiment C1 was lost during analyses procedure (mishandling of the filter while packing it into the tin capsules). This is now stated in the Methods section:
"The TPC/TPN filter from chemostat experiment C1 was lost during the preparation and measurement procedure."

2. Size, volume, weight, statistical treatment
The authors conclude that "coccolith volume was found to be primarily a function of the coccosphere/cell diameter both under nutrient replete and nitrogen limited conditions". Given the data in Table 4 and Figure 4, again, I would argue that the relationship is not convincingly demonstrated under N-limitation in the chemostats: Table 4 lacks any significance statistics (because of no replicate experiments) - assuming the p- and Fvalues given for the batch set is from a one-way ANOVA (as in Table 2)?

Correct, p-and F-values in Table 4 are from a one-way-ANOVA. We stated this now clearly in Table 4.

The lith volume to sphere/cell diameter chemostat triangles look like a flat-liner, non-relationship. (see also p. 4990, lines 6-8, for similar question marks re. the pCO2 and coccolith volume in chemostats). A relationship between sphere/cell diameter and coccolith volume seems evident for the batch series (Fig. 4), but by definition it cannot be a linear relationship, since you compare _m-units to _m3-units. Coccoliths are not spherical, but flat elliptical discs, and in case of E. huxleyi the volume will even be less than a "full disc" due to

the space between individual coccolith elements - so that the data presented in Figure 4 indeed appear to "fit" a linear regression (as shown). The authors should discuss this (and why it may be the best one can do for a first assessment of the data), and highlight that this relationship is expected to (drastically) change given more data on additional E. huxleyi strains / morphotypes and other species (as, for example, suggested by the data in Beaufort et al., 2011).

1) We added a more detailed description on the Coulter measurement principle and the spaces between the individual coccolith elements in the method section:

"Coccoliths of E. huxleyi morphotype A have a complex geometrical structure with a distal and proximal elliptical shield or plate connected by a central tube. The distal shield has a grid like structure with small 'gaps' between the coccolith elements whereas the proximal shield is solid (see Fig. 5). The Beckman Coulter Multisizer™3 is able to recognise these gaps if they are soaked and filled with the electrolyte (in this case: sterile filtered seawater)."

2) We added discussion about the relationship between coccosphere/cell diameter and coccolith volume:

"In general, the coccolith volume was found to correlate with the coccosphere/cell diameter (Fig. 4). However, analysing the batch and chemostat experiments separately, the correlation is only applicable under nutrient replete conditions (batch experiments). This is presumably caused by the absence of additional data points to support such a relationship under nitrogen limited conditions. Therefore, the overall linear relationship between coccosphere/cell diameter and coccolith volume (Fig. 4) should be interpreted as a first approximation. A power function relationship was recently indicated for the coccolith distal shield length (DSL) and the coccolith weight of Coccolithus pelagicus (Cubillos et al., 2012). If a similar correlation exists for the coccosphere/cell diameter and the coccolith volume has to be validated in future studies. Experiments investigating the effect of various parameters (e.g. light, temperature and salinity) on different morphotypes and species will provide a suitable basis."

Clearly, future studies should also provide biometric data of individual coccoliths (SEM or LM) - so that we can truly test the Equation (2) (recast from that in Young & Ziveri, 2000), in similar fashion as was recently done by Cubillos et al. (2012) on fossil and modern Coccolithus pelagicus (sensu lato). In other words, is coccolith volume primarily affected by changes in size (maximum diameter), or by changes in the morphology/ thickness of individual elements? Therefore it's a real pity that there's not more SEM evidence - not only to verify whether the batch cultures rendered lith sizes
>4.5_m, but also to verify if the chemostat rendered smaller liths (according to the Young & Ziveri 2000 recast equation, given same shape factor of 0.2 and Table 4 CC volume, they would have been _3.5_m).

We used recent laboratory and field data from the literature to compare the relationship of coccosphere/cell diameter and coccolith geometry (DSL, Fig. 6) and added a discussion on this in the manuscript:

"This study presents direct coccolith volume measurements from culture experiments and data for comparison are rare. However, coccolith distal shield length (DSL) has been described to correlate with the coccosphere diameter in field and fossil samples (Henderiks et

al., 2012; Henderiks, 2008). Converting the measured coccolith volume (V) to distal shield length (DSL) by applying equation (2) with the species specific constant ks = 0.02 (as given for normal calcified coccoliths of E. huxleyi morphotype A, Young and Ziveri (2000)) results in an average coccolith DSL ranging from 2.9 to 3.2µm and 4.1 to 5.5µm for the chemostat and batch experiments, respectively.

Equation (3) – please refer to the manuscript.

Corresponding to the estimates for coccolith mass the calculated DSLs from the batch experiments (nutrient replete) are higher than average DSL of ≈ 3.5µm derived from field samples of E. huxleyi morphotype A (Henderiks et al., 2012; Poulton et al., 2011; Triantaphyllou et al., 2010; Young and Ziveri, 2000). Visual inspection of coccoliths from the batch culture experiments via scanning electron microscopy confirmed the presence of coccoliths with DSL > 4.1µm (Fig. 5A-C) while coccoliths from the nitrogen limitation experiments (C1-C3) were found to be partly or completely disintegrated due to preservation problem (Fig. 5D).

Previous observations of E. huxleyi morphotype A with DSL > 4.1µm (Cubillos et al., 2007) and the present SEM pictures let us assume that the calculated DSL (and consequently the coccolith volume) from the batch and chemostat experiments is valid and comparable to previous applied methods measuring the DSL of coccoliths.

A comparison of field and laboratory data on the relationship between coccosphere diameter and coccolith DSL of E. huxleyi (Fig. 6) reveals that results from laboratory experiments (Bach et al. (2012) and this study) have a distinct pattern from field data (Henderiks et al., 2012; Triantaphyllou et al., 2010). The difference between laboratory and field data is not surprising. Laboratory studies are commonly conducted with one single strain of E. huxleyi and environmental parameters are kept constant and optimised, except for one variable parameter (e.g. carbonate system or nutrient concentration). Field studies, on the other hand, are investigating whole E. huxleyi populations (assemblages of multiple strains) and several environmental parameters can change with time and space, amplifying or balancing their effect on physiology and coccolith formation. Additionally, environmental parameters can either influence directly physiology and coccolith formation or alter the strain distribution in one population towards a strain with different coccolith geometry/morphology."

Fig. 5: May we see some SEM images from the chemostat experiments as well? In the debate of size vs. volume (weight), it would be instructive to see how well- or malformed the individual coccoliths were under these growth conditions.

As mentioned earlier, we experienced unusual preservation problems but we added now an additional SEM image from the chemostat experiment (Fig. 5D). The picture indicates some coccoliths which are partly or nearly completely disintegrated (please see text for details).

3. Process-based interpretations

As mentioned above, it is a bit disappointing that the physiological reasons behind the observations remain unexplained. The obvious differences in cell size and growth rate between the batch (+N) and chemostat (-N) would give some hints as to whether "certain

phases in the cellular division cycle are lenghtened and/or compressed", or if not, why not? Please expand this discussion.

That said, maybe we should accept these experimental results as what they are: an interesting data set that raises important questions and offers a good starting point to discuss what parameters should be routinely measured, and how, in future. In my view, it's a real shame that the "wrong" filters and storage problems apparently precluded any detailed microscopy (SEM or LM) which is clearly the common methodology that (experimental) marine biologists and paleoceanographers share in their quest to better understand calcification in coccolithophores and other prominent marine calcifiers.

As said above, we added discussion on possible cellular mechanisms behind the relationship of coccosphere/cell diameter and coccolith volume (geometry):

"Figure 4 and 6 indicate the link between coccosphere/cell diameter and coccolith geometry (volume and DSL). It remains an open and interesting question if environmental parameters influence coccosphere/cell diameter and coccolith geometry separately or if coccolith geometry is indirectly influenced due to variations in coccosphere/cell diameter. Even an interaction of the two possibilities cannot be excluded.
Coccoliths of E. huxleyi are produced intracellular in the coccolith vesicle, a special cellular compartment, derived from the Golgi apparatus. After formation of the protococcolith ring, the coccolith matures inside the coccolith vesicle while transported to the cells surface. The time the coccolith matures inside the coccolith vesicle is presumably mainly influenced by the responsible metabolic rates and the distance to the cells surface. Therefore, an increase/decrease in cell diameter would provide additional/less time for coccolith growth and formation. Nitrogen limitation, for example, induces cell and coccolith shrinkage (Table 4, Paasche (2002)). Cell shrinkage increases the surface to volume ratio and hence the nutrient uptake efficiency but the cell has to maintain a certain cell diameter to pass on sufficient biomass and genetic material to the two daughter cells assuring their survival. Contrary, phosphorus depletion inhibits DNA synthesis while biomass buildup continuous resulting in an increase of coccosphere/cell diameter and coccolith size (Paasche, 2002; Müller et al., 2008). Indications are given that carbonate system parameters (e.g. pCO2 and pH) have diverging effects on the coccosphere/cell diameter of E. huxleyi. Elevated pCO2 conditions positively affects the cell diameter till a saturated level (reached at ≈ 1200µatm), presumably due to an overconsumption of carbon (Bach et al., 2011; Engel et al., 2008). Increasing acidity (pH<7.5 or pCO2 > 1500µatm), on the contrary, negatively affects the cell diameter (Bach et al., 2011). The combination of both effects results in an optimum curve response of the cell diameter to ocean acidification/carbonation (Bach et al., 2011, 2012). It remains to be tested how changes in coccosphere/cell diameter induced by other environmental parameters (e.g. temperature, irradiance, salinity, trace metal availability) will influence coccolith volume or size. The complexity in coccolith size variability of natural observations as a result of various environmental parameters has been indicated (Herrmann et al., 2012; Poulton et al., 2011). A comparison of the different methods to estimate coccolith volumes and mass (birefringence-based, SEM and resistive method) is urgently needed to validate and confirm results on coccolith geometrics as previously mentioned by Poulton et al. (2011). The Coulter Multisizer™ method is an efficient and precise way to easily estimate the mean coccolith volume of culture experiments by counting thousands of coccoliths within seconds. Controlled laboratory experiments will provide a suitable basis for method comparison because sufficient sample material can be produced and experimental parameters are regulated and monitored."

Technical corrections
2.2. Experimental setup You mention "The target pCO2 value (see Table 1)" – where Table 1 gives the final values as attained in each experimental set up. The final pCO2 value may

deviate from the target value for several reasons (e.g. the low pCO2 in the chemostat reservoir tanks and C1 appears much lower than that in the batch B1), so I'd suggest to mention the 3 target values in this paragraph (also because it reads nicer), and then report Table 1 under the results section.

Target CO2 values are now mentioned in the Method section.

The language has certainly improved since the earlier version of this ms. Some textual issues remain, and I recommend a careful read by the authors and a native English speaker. This list of examples is not exhaustive:

We improved overall wording and structure of the manuscript.

p.4980, l. 25: replace "are subsequently" by "have since been" (or similar phrasing)
Done.

p. 4981, l. 4: referred to "as" ocean carbonation/acidification .

Done.
p. 4986, l.1: "f/20, excepting the nitrate concentration" - "except for" or rewrite into two sentences.

Done.

p.4990, l.9/10: reshuffle sentence structure to "Production rates ... were decreased byover 50%, under equilibrium .... compared to ..."

Done, results section was restructured for a better reading flow.

Table 3 caption: "phosphor" = "phosphorus"

Done.

References
Cubillos, J.C., Henderiks, J., Beaufort, L., Howard, W.R., and Hallegraeff, G.M., 2012, Reconstructing calcification in ancient coccolithophores: Individual coccolith weight and morphology of Coccolithus pelagicus (sensu lato): Marine Micropaleontology, v. 92-93, p. 29-29.
Other cited references as in reviewed manuscript.