Referee#1 (Anonymous)

The study: "Vanishing coccolith vital effects with alleviated CO2 limitation“ by Hermoso and co-workers contains highly interesting results on growth, and stable carbon/oxygen isotope fractionation in coccoliths of four different coccolithophore species. The laboratory work makes a very good impression although I cannot really comment on the isotope methodology. I have, however, one major and one minor concern with the data interpretation. I will try to explain these concerns in the following.

Major concern: A core parameter in your study is DCUt. To calculate this parameter you assume that “passive influx of CO2 constitutes the only source of carbon to the cell”. I have very strong concerns with this assumption (which seems to be central to many of your interpretations and hypothesis) and worry that it is not valid. You underline this assumption with studies by Sekino and Shiraiwa (1994) and Kottmeier et al., (2014).

However, Sekino and Shiraiwa (1994) stated in the abstract that “HCO3- was utilized mainly for production of CaCO3 and accumulation of internal inorganic carbon” which contradicts your assumption. Kottmeier et al. (2014) indeed showed that CO2 is the dominant DIC source under high DIC but this finding is only true for photosynthesis. Kottmeier et al., (2014) did not investigate the carbon source for calcification.

Furthermore, there are a large number of studies with different methodological approaches which have shown that HCO3- is a (or even the) key source ion for photosynthesis (e.g. Rost et al., 2003, 2006; Schulz et al., 2007) and calcification (e.g. Sikes et al., 1980, Nimer et al., 1993, Buitenhuis et al., 1999, Bach et al., 2013).

Please clarify this issue because if this assumption is not true then DCUt cannot be interpreted in the way you do in this paper.

(Please have a special look on lines 22-27 on page 15846, lines 23-29 on page 15849, and lines 17-18 on page 15855.)

Authors’s response: See previous answers on this specific point in the Interactive Discussion, and also how we have dealt with this problem in our introductory Revision Notes. We do not state that calcification originates from a CO_2 substrate (anymore). We have removed references to the *DCUt* index (explicitly using ambient [$CO_{2\ aq}$] concentrations) from the revised manuscript. In particular, the paragraphs mentioned by the Referee have been totally reformulated to avoid any confusion in the use of this “index”.

- Page 15846 lines 22-27

“*Assuming that the passive influx of CO_2 constitutes the only source of carbon to the cell,…*” has been **removed**.

“*We are aware of no evidence for increased HCO_3^- assimilation in coccolithophores under high CO_2 environments, so we assume prominent CO_2 influx at high DIC, consistent with the work of Kottmeier et al. (2014) and Hermoso (2015).*” has been **removed**.

- Page 15849 lines 23-29

The sentences "*We emphasise that our understanding of the internal carbon pool build-up favours a preponderant CO_2 assimilation by phytoplanktonic calcifiers and that both pathways use a common internal carbon pool (Sekino and Shiraiwa, 1994; Bolton and Stoll, 2013; Hermoso et al., 2014; Kottmeier et al., 2014). Hence, the assumption that calcification utilises bicarbonate ions transported from the extracellular environment to the coccolith vesicle with no influence from photosynthetic carbon fixation conflicts with many physiological and isotopic evidence.*" have been **removed**.

- Page 15855 lines 17-18

"*A "reverse" approach using the present calibration utilising the magnitude of the vital effect, appears possible to derive DCUt estimates that can be, in turn, linked to [$CO_{2\ aq}$] concentrations*". – sentence **deleted**.

Minor concern: DIC concentrations in the highest treatment were ~12000 µmol/kg. When I calculate Omega_calcite for this concentration (assuming pH 8.2 (pH scale missing! See comment 4), S=35 (not given, why?), T=15, K1/K2 by Mehrbach et al.1973 refitted by Dickson and Millero 1987) I get values of 26 (pH on free scale) or even 30 (pH on total scale). At such high Omega_calcite values there is a high potential of inorganic CaCO3 precipitation. Could this interfere with your results? And to some extent explain the absence of vital effects at high DIC? I noticed that you seem to address this issue at the beginning of section 4.1 but I did not understand your argumentation here.

Authors's response: The pH scale used is the Total Scale (now added in the ms). Salinity was 33 (information now given). In our medium, the addition of chelators such as EDTA and the presence of relatively elevated phosphate content prevent spontaneous precipitation in the culture medium We did not observe any evidence of inorganic calcite / aragonite precipitation either in the form of spines, orthorhombic crystals or overgrowths on coccolith calcite under the SEM. The mass of such inorganic calcite would need to be substantial to "homogenise" the isotopic signatures of coccoliths of the heavy (as *E. huxleyi*) and light (as *C. leptoporus*) groups.

Specific comments:

1) Page 15838 line 13: What do you mean by "primarily CO2"? Changing DIC at pH 8.2 primarily affects HCO3-.

Authors's response: "*primarily CO2*" has been **removed**.

2) Page 15838 line 17: The term "static vs. dynamic" is unclear in this context (at least for the reader not experienced with isotope geochemistry and vital effects).

Authors's response: This now reads: "…whether the vital effect in coccolith calcite remains constant for a given species or changes with the environment".

3) Page 15840 line 6: Perhaps a question which is a bit difficult to answer but do you expect that there is an effect of N2 purging on cell physiology? I mean, you effectively removed O2 and all other trace gases as well. I wonder if this makes a difference to the cells. (Since your growth rates are fine I don't think it does but I am just wondering.)

Authors's response: This is indeed a tricky question, but considering the very fast dynamics of photosynthetic carbon fixation in the cell and consecutive oxygen liberation, it is unlikely that such effects have influenced our results beyond the first minutes of the bioassays.

4) Page 15840 line 11. Please give the pH scale. This is absolutely essential in carbonate chemistry experiments.

Authors's response: This has been done (Total Scale).

5) Page 15840 lines 14-15. What do you mean by successive alterations of the carbonate chemistry. Please try to be less cryptic.

Authors's response: We refer to successive additions of HCl, $NaHCO_3$ and NaOH used to reach targeted DIC and pH values. This has been **added in text**.

6) Page 15846 line 25. Bach et al., 2014 does not exist. Do you mean 2013 or 2015?

Authors's response: We apologise for the confusion: "2014" has been **replaced** by "2013".

7) Page 15850 lines 13ff. Langer et al., (2009) only showed this for a much narrower range of carbonate chemistry conditions. I doubt that no changes in PIC/POC would occur in your experiment because your DIC range is huge.

Authors's response: According to Referee#2's suggestion the analogy with the work by Langer has been removed, and **replaced** by the more suitable study by Bach et al. (2013).

8) Page 15851 line 11ff. More recent results showed that another strain of C. pelagicus changes PIC/POC in response to changing carbonate chemistry (Bach et al., 2015).

Authors's response: Reference added.

9) Page 1854 Lines 18-21. I wonder: Isn't it a bit too optimistic to make this suggestion based on the current evidence?

Authors's response: We regard this as a fact, and this research avenue is also illustrated by the ongoing effort to explore the geochemistry of the coccoliths in the geological record (e.g. Bolton et al., 2012, amongst others). Nevertheless, we have **replaced** *levels* by *estimates* to slightly tone this down.

10) Page 15855 Lines 1f. This would only work if coccolith size is bound to cell size. However, there are also very large species with very small coccoliths (e.g. Pleurochrysis carterae).

Authors's response: This is true. *Pleurochrysis* spp. has no existence in the geological record, and for other species, the works by Henderiks provided reliable correlation between coccolith, coccosphere and cell size (Henderiks and Rickaby, 2007; Henderiks, 2008; Henderiks and Pagani 2008). Therefore, this possibility is feasible and ought to be further explored combining geochemical and morphological analyses of sedimentary coccoliths in the geological record.

I hope my comments help to improve the manuscript further.

Referee#2 (Lena-Maria Holtz)

General comments

Summary: The authors of the study cultured four different coccolithophore strains, each at six different carbonate systems, and measured the corresponding $\delta^{13}C$ and δ18O values of the coccoliths. Cells were cultured at densities that were too low to determine particulate inorganic and organic carbon (PIC and POC, respectively) per cell. All six carbonate systems have the same pH value, but different dissolved inorganic carbon concentrations (DIC): 2, 4, 6, 8, 10, and 12mmol·L−1. Measured δ13C and δ18O values are then plotted over DIC (or [CO_2]). A so-called carbon usage index (DCUt) is introduced (after Rau et al. [1] and Bidigare et al. [2]) and correlated to δ13C and δ18O values. Then, the authors try to interpret found correlations for δ13C and δ18O data from a mechanistic viewpoint.
One of my major concerns (complete list below) with the presented work is that the authors persistently argue that external CO_2 was the prime carbon source of calcite. This is against all experimental evidence (and also against their own presented data set). The authors know the relevant literature (I listet some of it in an earlier review for them and they cite some of it – for different aspects though). After reading the passage on page 15849 lines 23-end[1], I understood why they think that way.
1 *"We emphasise that our understanding of the internal carbon pool build-up favours a preponderant CO2 assimilation by phytoplanktonic calcifiers and that both pathways use a common internal carbon pool (Sekino and Shiraiwa, 1994; Bolton and Stoll, 2013; Hermoso et al., 2014; Kottmeier et al., 2014). Hence, the assumption that calcification utilises bicarbonate ions transported from the extracellular environment to the coccolith vesicle with no influence from photosynthetic carbon fixation conflicts with many physiological and isotopic evidence."*

They do not believe that the prime carbon source for calcite is HCO_3^- and that the isotopic signal in calcite is influenced by photosynthetic carbon fixation at the same time. I admit that this apparent controversity is difficult to think through just by means of a human brain. A computer-based model can help here. The symbiont-bearing foram model of Zeebe et al. [6] for instance faces a similar issue – with external (not internal) symbionts though – and also finds the carbon signal in calcite influenced by symbiont activity, although symbionts use CO2 and calcite is precipitated from CO32−. One thing that is essential to have in mind when aiming at understanding measured carbon isotopic signals is that internal carbonate systems of living cells are out of chemical equilibrium [8]. Furthermore, it is important to have in mind that there are "two carbonate systems" (12C and 13C) the reactions of which occur in parallel [7].
The presented data sets, however, show that HCO_3^- is the prime external carbon source of calcite in all four cocco species: The authors present the isotopic data as if HCO_3^- was presumed to be the prime carbon source of calcite:
$\delta^{13}C_{calcite}$ - $\delta^{13}C_{DIC}$ (where $\delta^{13}C_{DIC}$ d δ13C HCO_3^-) is presented and not $\delta^{13}C_{calcite}$ - $\delta^{13}C_{CO2}$.

Authors's response: The expression of this offset is justified by: i/ the fact that the inorganic calcite is precipitated from the relative proportion of the DIC species (the "S" of Zeebe), therefore primarily from HCO_3^- at the considered pH; and ii/ to conform to the palaeoceanographic usage (e.g. works by Spero; Ziveri and others). This is now even more

justified, as we do not make any *a priori* hypothesis on which DIC substrate is incorporated by the cell in the revised ms.

δ13Ccalcite - $\delta 13C_{DIC}$ does not deviate strongly from 0. $\delta 13C_{calcite}$ - $\delta 13C_{CO2}$, in contrast, would (not shown) strongly exceed zero. It hence follows that calcite was most likely built from external HCO_3^- and not CO2.

Authors's response: The difficulty here is to detangle true fractionation and apparent fractionation in coccolith calcite. *E. huxleyi* (the cell with the highest photosynthetic rates relative to calcification rates) calcite $\delta^{13}C$ values can be substantially high relative to inorganic calcite (2-3 ‰ higher than a HCO_3^- source). Indeed, this implies that $\delta^{13}C$ coccolith values are even more significantly shifted towards positive composition relative to CO_2. Once again in our newly revised manuscript, we do not assume a sole assimilation of CO_2 in our revisions, but rather develop an empirical calibration, hence we use $\delta^{13}C_{DIC}$ as the baseline from which we express the coccolith ^{13}C vital effect.

Major concerns

The heart piece of the work, i.e. the data set, is not presented.

Authors's response: A table presenting the collected data has been added within the ms.

Furthermore, no error bars (how many replicates?) are presented in the figures.

Authors's response: The error bars on coccolith stable isotope compositions would be smaller than the symbols on the figures. Two culture replicates were performed (information added in text).

Tables that list carbonate systems and results should be given.

Authors's response: Done.

Carbonate systems are manipulated unconventionally, why a presentation of the carbonate systems is even more important than usually. The authors mention a publication on their own website (data are not yet accessible). Since the data are the actual output of the work, I am of the opinion that they belong to the article.

Authors's response: Once again, the table will be fully part of the paper.

An (in my opinion) odd interpretation of a carbon usage index DCUt.

Authors's response: See previous discussion with Referee#1 and 2 during the Interactive Discussion Phase on this matter, and our revision notes at the beginning of this rebuttal letter. This index has been removed from our revisions.

Misinterpretation of some cited literature (see also referee 1).

Authors's response: This has been corrected, as e.g. the Sekino and Kottmeier papers now do not indicate the use of CO_2 for calcification.

I am not sure, but the correlation between δ13C and δ18O and this DCUt index (calculated from [CO2]) might originate from the correlation between δ13C and δ18O and [CO2] (latter correlation in most cases higher). This should be tested statistically.

Authors's response: See graphs provided during the Interactive Discussion. Most of the correlations indeed came from DIC (CO2) concentration. The old figures 3 have been **removed**.

I am of the opinion that the title statement "vanishing coccolith vital effects with alleviated CO2" is misleading and does not follow from the presented work.

Authors's response: We have change "CO_2" for "carbon" to account for the change made in the manuscript.

I am of the opinion that a profound discussion on cellular carbon fluxes when just having δ13C and δ18O data (without the corresponding data of the organic phase or particulate organic and inorganic carbon fixation rates) is not possible.

Authors's response: Yes, we fully agree. See response in the Interactive Discussion, and response on this point already made above.

Some specific comments

p. 15836
l. 5/6: "Under high DIC ...": (i) inorganic values were not measured, just calculated and may deviate from the calculation. However, this is not the point. (ii) "lacked any offset" is a very optimistic expression (compare data at 12mmol·L−1 with calculated inorganic value). (iii) since your regression lines (Fig. 2) are linear and do not approach the calculated value for inorganic calcite asymptotically, I am of the opinion that you cannot conclude that it "lacks any offset with inorganic calcite". What would, for instance, be the corresponding values at 14mmol·L−1 for E. huxleyi or C. pelagicus?

Authors's response: This is true. This sentence has been reformulated: "Under high DIC levels, all the examined coccoliths exhibit significantly *reduced* isotopic offsets from inorganic calcite compared to the substantial vital effects expressed at low (present-day) DIC concentrations."

l. 15-18: I think you should discuss in which respect the established carbonate systems resemble those of (which?) geological times.

Authors's response: This would require precise knowledge of many natural environment parameters (temperature, salinity, pH, omega calcite etc….) that nobody knows with enough accuracy. These parameters are the quest of palaeoceanographers! We performed our experiments at one temperature, one pH, hence, it is just impossible to draw a parallel (especially at a global scale) between experimental conditions and the ocean taken as a whole over the Earth's history.

The way you manipulated your carbonate systems deviates from common approaches applied in coccolithophore physiology, where O(cean) A(cidification) effects are often investigated (thus, pH values are altered in these approaches – which is an important difference to your

data set). Similar to your approach: data at pH 8.3 of Bach et al. [9]. It is in my opinion important to note differences between these deviating approaches.

Authors's response: We did not aim to contribute to the OA research effort here, although the data can be subsequently explore in this way. As done in the work by Rickaby et al. (2010 Clim. Past), pH was kept constant to avoid a superposition of effects in our study. We only aimed to determine how coccolith stable isotope compositions evolve with higher carbon availability for the cells with a geological perspective.

l. 24: Introduction of "vital effect". Since the term is not uniformly used in literature (and not (yet :-)?) common in cocco community), I think a more precise definition of the term "vital effect" (I mean how you will refer to it throughout the MS, cf. eqn. (3)) would be beneficial for the reader.

Authors's response: This has been done, and was indeed useful for clarity. "As a consequence of the biological controls on chemical signals in algae, most biominerals do not precipitate at equilibrium conditions and the compositional departure between biocarbonates and an inorganic reference is commonly referred to as the vital effect."

On this note, we have also homogenised the use of the vital effect for the carbon isotopes ($\delta^{13}C_c - \delta^{13}C_{DIC}$ vs. $\delta^{13}C_c - \delta^{13}C_{inorg}$ throughout the ms).

p. 15838
l. 11f.: You should clearly state here that the pH was set to a fixed value of 8.2. "A wide range of pCO2" could otherwise be misunderstood.

Authors's response: Done.

"As varying the availability of ambient DIC (primarily CO2)": As mentioned in a previous review for you (and also mentioned by referee 1), you need to clearly state that the prime carbon source of calcite is external HCO3− (you can see that in your data)!!! The carbon source of photosynthesis can be CO2 and/or HCO3− (in E. hux)!!! This is very important!!! This is for instance (indirectly though) shown in the study of Sekino and Shiraiwa [3] who you cite in line 15 (but see also (for instance): Paasche [10], Burkhardt et al. [11], Sikes et al. [12], and isotopic data in Rost et al. [13] and here in your study). CO2 limitation (in E. hux) occurs at CO2 concentrations below 10 µmol·L−1 (cf. e.g. Bach et al. [9]).

Authors's response: "primarily CO2" has been removed, also as per Referee#1 advice. In the revised ms, we do not favour one source over the other, as we do not want to enter into this controversy, and our dataset cannot tackle it.

You should compare your carbonate system ([CO2], [HCO_3^-], DIC, and total alkalinity (TA)) to ancient conditions here.

Authors's response: Same response as above. This is not feasible without making huge assumptions unfortunately.

p. 15840
l. 1f.: Why do you first remove all DIC from the water? You measured (and calculated) δ13C...

Authors's response: This method allows us to attain our targeted DIC more accurately, and more importantly to obtain the same $\delta^{13}C_{DIC}$ composition in all bioassays.

How much (mol·L−1) Cl− (and Na+) did you add? You should probably mention the increase (from ... to ...) in [Cl−]?

Authors's response: We see no change in TA before and after treatment. Changes in Na^+ and Cl^- are very minor compared to the original seawater concentrations (33 psu).

You should really add a table with the carbonate systems (incl. TA, DIC, CO2, HCO3−, CO32−, and maybe [Ca2+]) and also mention salinity. If you do not want to put it into the main document, you should definitely provide it as appendix.

Authors's response: Done.

l. 19f.: You should mention the cell densities. It is really a pitty that you could not measure PIC and POC per cell. Why did you not sample at higher cell densities then? This is probably how it is usually done, I suppose. As far as I know (I am not an experimentalist), the cell densities up to which you can sample (without signigicant carbonate system shifts) are ± known.

Authors's response: Growing more cells in each flask, especially for the low DIC levels, would have resulted in a shift in the composition and pH of the medium that may have altered the validity of our experiments.

Since Bach et al. (2013) used a similar carbonate system (the one at pH 8.3) at the same irradiance level (though different diurnal cycles), you may use their results to estimate the behaviour of daily POC and PIC production rates (e.g. POC/cell · dision rate), even though the strain deviates from the one you used...

Authors's response: Thanks. We have added this information in place of the reference to the work by Langer et al. (2006). "In the culture experiments on *E. huxleyi* by Bach et al. (2013), PIC/POC ratios increased in response to increasing [DIC], which was explained by a decrease in the production of POC."

At which time point (after onset of light) did you take samples to measure cell densities and cell sizes ...?

Authors's response: Approximately 3 hours after the beginning of the photoperiod (added in text).

p. 15841
l. 1ff: calculated specific growth rates should be listed in a table. How many replicates do you have?

Authors's response: Done. We did two replicates for each species and each DIC level (information added in text).

p. 15842

eqn. (2): How reliable are the calculations of δ18Oinorg and δ13Cinorg? It would make more sense to me to calculate " values that relate the calculated isotopic calcite signal to the isotope value of the external carbon source (i.e. relationship between δ13CC and δ13C HCO_3^- in terms of calcite, " is close to 0 (Rost et al. (2002)) ! almost no fractionation between ext. HCO_3^- and calcite ! HCO_3^- seems to be the carbon source of calcite ...).

Authors's response: The use of these inorganic references present the massive advantage to be independent of temperature, and hence eases comparison with other works.

We reiterate here that we only measure apparent fractionation in coccolith calcite. That coccolith $\delta^{13}C_c$ resembles values of bicarbonate ions does not represent a formal proof for the unique use of this DIC substrate from an isotopic point of view. But, once again, we do not want to revisit this controversy here or in our manuscript.

Well, in fig. 2 you give the d between δ13CC and δ13CDIC...

Authors's response: As explained above, we stick to an empirical calibration and conform to previous studies (as for the "$\delta^{18}O_c - \delta^{18}O_w$").

I don't understand why you give the difference between δ13CC and δ13CDIC (which is close to δ13C HCO_3^-) and not the difference between δ13CC and δ13CCO2 , when you assume that CO2 is the external carbon source. When plotting the latter value, you could see that (δ13CC - δ13CCO2) becomes much higher, i.e. the offset to zero increases strongly (δ13CCO2 d δ13C HCO_3^-)! This shows that HCO_3^- is the prime carbon source of calcite, not CO2.

Authors's response: See responses above (and also the first made to this Referee).

Calculating the ε value for oxygen would give evidence about how well the oxygen signal is correlated with the carbon source. Oxygen isotope effects are (even) more complicated than carbon isotope effects, because of their more complex interactions with other oxygen containing molecules, such as the ubiquitous H2O molecule.

Authors's response: $\delta^{18}O_c - \delta^{18}O_w$ values and $\varepsilon^{18}_{c\text{-}w}$ $\varepsilon^{13}_{c\text{-}DIC}$ values (now added in Table) are linearly correlated, hence we do not loose any information by adopting the well established δ – δ notation applied in foraminifera and coccolith researches.

p. 15842
l. 15f.: I miss the table with the corresponding values :-).

Authors's response: Done.

p. 15844
l. 17: why should growth rates (in the cited paper's title "metabolic rates") and cell sizes covary? "Metabolic rates" should be ± proportional to cell volume or POC/cell. Same for humans: 100 kg people exhibit higher respiration rates per individuum than 40 kg people... However, in respect to growth rates and cell sizes, I would have expected a negative correlation under nutrient limitation and maybe high light intensities where maximum speed of cell cycle cannot be increased any further... But that is another story. I should simply read the cited paper.

Authors's response: We are not sure we follow the argument made by the Referee here. This is indeed an excellent contribution to the field.

p. 15845
l. 5f.: Either, I misunderstand something, or: The regression lines are linear (Fig. 2 by the way), I cannot see an approximation towards the inorganic value. At 12mmol DIC·L−1, the value of E. hux is below the inorganic one.

Authors's response: Fig. Number corrected. *E. huxleyi* $\delta^{13}C_c$ values decrease with more external DIC. Indeed, at 12 mM, the values are slightly below inorganic, but only by 0.2 – 0.3 ‰. Then we feel that we can maintain our statement. These are biological data, and a spread exists between replicates.

p. 15846
DCUt as index for "internal carbon pool": Who's interpretation is that? CO2 cannot be the only carbon source of the cell! cf. e.g. your own data set. ...

Authors's response: Sentence (and concept) **removed**.

- Your interpretation of DCUt is strange, in my opinion.

Authors's response: See response above, in our previous ACs, and at the beginning of this letter.

- My interpretation of DCUt is that it indicates how much CO2 may be used for photosynthetic carbon fixation. The remaining carbon demand (of photosynthesis) would have to be covered by HCO_3^-. "μ · volume" gives an indicator for the photosynthetic (!!) carbon demand of the cell.

Authors's response: We agree with this statement, as it was clearly expressed in our original submission (reflecting the whole internal pool dynamics, and not specific to calcification).

"Growth rate (μ), cell size and ambient DIC (or CO_2) concentrations can be combined to generate an index accounting for the supply and photosynthetic utilisation of carbon by the cells (Rau et al., 1996; Bidigare et al., 1997; Burkhardt et al., 1999; Laws et al., 2002)." Sentence **removed**.

"We emphasise that this index is not specific to calcification, but rather, gives an estimates of the dynamics of the whole carbon inventory (pool), regardless its subsequent partitioning into photosynthetic ($CO_{2\ aq}$) or calcification (HCO_3^-) pathways." Anyway: Sentence **removed**.

"[CO2] · surface-area", in contrast, is used as indicator for the potential to take up CO2 via the surface-area (diffusive CO2 uptake is dependent on external CO2 concentration and the surface-area). Depending on which carbon species is used for photosynthesis, external CO2 or HCO3−, the internal isotopic carbon signal changes. As far as I know, Rau et al. and Bidigare et al. both worked with the isotopic carbon data of the organic phase (not calcite!). However, I do not doubt at all that the isotopic signal in calcite is influenced by photosynthetic activity.

Authors's response: We feel that we have already answered this above, and changed the ms accordingly, but we agree with the Referee's reasoning.

I think that μ · volume · surface area−1 does not change much in comparison to [CO2]. When altering light or nutrient conditions this may be different though. However, you should test the significance of the correlation between d and DCUt cs. d and [CO2].

Authors's response: We now clearly state that the primary driver is [DIC], division rates and cell geometry, themselves influenced by DIC have only a secondary role, as now explained in the discussion.

l. 10 CO2 is not the carbon source of calcite E. hux is not the only alga that can use CO2 as well as HCO3− for photosynthesis. This is a common feature, although it may be the only coccolithophore species for which a shift towards CO2 usage at high [CO2] has been measured.

Authors's response: We would have welcomed some references supporting this statement for other coccolithophores apart from *E. huxleyi* as to our knowledge there is almost no data on this point for species other than *E. huxleyi*. Regardless, this paragraph has been removed and we do not suggest that the *Ci* comes from ambient $CO_{2\ aq}$ assimilation by the cell anymore.

l. 25: Why should cells take up more HCO3− at high [CO2]? I don't understand this. You should also have in mind that you increased the concentration of HCO_3^- by a factor of 6, not only CO2. Kottmeier et al., in contrast, rarely changed HCO_3^-.

Authors's response: This paragraph was only an attempt to compare the DIC fluxes of the work by Kottmeier et al. (2014) with our isotopic measurements on coccolith calcite – this has been **removed**.

"carbon pool": this is in my opinion a "black box word". What is your (precise) interpretation of it? Do you simply mean the sum of all carbon species within the cell comprising all cellular compartments? Or is it rather a pool in the cytosol with locally enriched DIC, where the carbonate system is in chemical equilibrium? Why should a cell have such a pool? It is expensive to maintain, because the import of DIC (HCO_3^- ?) into this pool would have to function against a strong concentrational gradient (+ CO2 may leak out) and the import rates of DIC into the cell would have to be the same rates as without such a pool (because C fixation rate (C sink term) = C uptake rate (C source term), if DIC pool remains constant over time) .... i.e. high C fixation rates = high C uptake rates and vice versa [cf. 14]. Or may the pool even comprise organic components? However, the type of "pool" would have a major impact on the isotopic
signal ...

Authors's response: We now clearly indicate: "**inventory** of internal DIC species" in the text to explain this idea of a carbon pool (see e.g. Nimer et al., 1992 – ref now added in the ms). We are not sure about the arguments presented here. To achieve the photosynthesis and calcification rates under ambient carbon conditions, coccolithophores *must* have an intracellular reservoir of carbon. In some cases, this pool can be measured using silicone oil centrifugation, or ^{14}C pulse chase experiments which probe the acid labile carbon contents of the cells or the "internal pool" see Isensee et al., 2014; Sekino and Shiraiwa, 2008, Nimer et al., 1992.

p. 15847
l. 1/2: Where are the data that tell you that? Do you think C limited cells store C internally? Why should they do such a thing?

Authors's response: This feature is evidenced by the overall fertilising conditions induced by the addition of DIC around the cells, with increased growth rate from 2 to 4 mM.

DIC is the sum of CO2, HCO3− , and CO32−(+ H2CO3) and I suppose the cell reacts to all C species differently, depending on the type of import mechanism for instance.

Authors's response: Yes.

l. 12: reference to Fig.

Authors's response: Done.

secs. 3.4.1 and 3.4.2: As mentioned above already, I think the correlations may be better with CO2 than with DCUt.

Authors's response: See Interactive Discussion and responses above. We do not refer to this index anymore.

p. 15848
sec. 4.1: How do you explain that δ13C and δ18O both increase or both descrease for one species? Isn't that counter-intuitive when considering CO2 in the external medium equilibrium) being depleted in 13C, but enriched in 18O compared to HCO−
3 ?

Authors's response: This is the point of section 4.2.

p. 15849
l. 2/3: Inorganic or organic precipitation? Did Anning et al. do precipitation experiments? Watkins and Hermoso: O isotopes, not C isotopes?

Authors's response: Inorganic. The references Anning et al., 1996; Watkins et al., 2014; Hermoso, 2015 have been deleted.

l. 5: "co-evolution of DIC": I don't understand that. A reference is missing

Authors's response: This sentence now reads: "Thermodynamically, the mechanisms and the dynamics of $^{18}O/^{16}O$ fractionation are very different to those described for the carbon isotopes (Zeebe and Wolf-Gladrow, 2001)".

l. 22: No. We did not separate photosynthesis and calcite precipitation for DIC sourcing in our early coccolithophore model Holtz et al. [8]. As a result, we find that CO2 and HCO_3^- interconversion inside the cytosol is low, leading to a separation between CO2 and HCO_3^- fluxes through the cytosol. In a refined model version [15], we even explain the observed

increase in PIC production rates at low [CO2] with the elevated uptake of HCO_3^- for photosynthesis – which strongly opposes the idea of separated DIC sources for photosynthesis and calcite precipitation!

Authors's response: Noted. The corresponding paragraph has been **removed**.

Bach et al. (2015) did not intend to describe carbon fluxes mechanistically. They used correlations between seawater chemistry and POC and PIC production for their model. This model is neither based on internal pools nor on a mechanistic explanation of cellular carbon fluxes.

Authors's response: Same here.

l. 23-29: HCO_3^- is the prime external C source of calcite (cf. references I listed before). There is no well-grounded experimental evidence (known to me) arguing against this (at least not for E. hux – and you just presented evidence for HCO_3^- usage for three more cocco species in your data set). In case you know something else please let me know. This (HCO_3^- as carbon source for calcite) does, however, not imply that the isotopic carbon signal of calcite is not altered by photosynthetic activity...

Authors's response: As pointed by the Referee, only a comprehensive model requiring coeval $\delta^{13}C_{org}$ values and PIC/POC ratios could formalise this HCO_3^- / $CO_{2\,aq}$ "controversy". These lines have been **removed**.

p. 15850
l. 1-5: The RubisCO effect (Spero et al. 1997) is one more argument for the potential of an isotopic signal to spread from the symbionts through the host and into the calcifying space (cf. model of Zeebe et al. (1999))

Authors's response: We agree with this statement but we are unsure how (*if?*) this information ought to be mentioned in our ms.

l. 2: To me, "12C depletion" sounds as if [13C]/[12C] > 1

Authors's response: This has been reformulated using inorganic calcite as a reference.

l. 13: Langer et al. (2009) used carbonate systems with DIC d 2mM and changing pH values. You may rather use the data set of Bach et al. (2013) (cf. above) who used a similar carbonate system set up as you (the one at pH 8.3). PIC/POC increases with increasing DIC due to a decrease in POC.

Authors's response: This is true. The work by Bach is more suitable for comparison with our own study. We have removed here the reference to the paper by Langer, and replaced it with reference to the bach's paper with the observation of the data made by the Referee.

l. 16: wrong figure reference? By the way, units in figures 1 and 2 are not correct: mM/kg. I guess you mean mmol/kg or mmol/L? Previously you used mM, so mmol/L ... it may be good to stick to one unit?

Authors's response: Reference to Figure has been corrected and units corrected. Thanks for spotting this.

l. 16: what is a lower degree of carbon utilisation? Do you mean lower carbon fixation? Or DCUt?

Authors's response: We mean a relatively lower utilisation of the internal DIC compared to the supply (hence a lower DCUt when we were referring to this index in the original version of the ms).

It would be so much easier if you would give your data in ε values and would have defined "internal carbon pool" precisely...

Authors's response: One again, we conform here to palaeoceanographic usage. All previously reported culture fractionation coefficients have been given via the "δ – δ" notation (coccoliths and beyond). We have nevertheless added the corresponding ε values in the table. By internal carbon pool, we mean the inventory of DIC in the cell (see above - now specified in text).

I miss the statement that CO2 is isotopically depleted in 13C compared to HCO_3^-. My interpretation of all data HCO_3^- is the prime carbon source of calcite. For E. hux and P. placo data CO2 diffuses across membranes. Thus, at high DIC, where [CO2] is very high also, CO2 enters the cells and brings in a lot of 12C (comp. to 13C). Most organic carbon might be built from CO2 at these conditions. But this is unfortunately not measured. Would have been very interesting to see the corresponding values for the organic phase ... For the other two species, this effect seems to be superimposed by other effects. Cellular structure, PIC and POC production rates, as well as the isotopic signal in biomass may give further evidence ... Concerning the data of leptoporus and pelagicus: are MIMS etc. data available here? What do they say?

Authors's response: To our best knowledge, there is no MIMS data available for *C. leptoporus*. We wonder why this ^{12}C-rich influx would be restricted to *E. huxleyi* and *P. placolithoides* since CO_2 can diffuse across all membranes. This explanation therefore does not reconcile the interspecies response. Instead, and as pointed by the same Referee in her review quoting the data by Bach et al. (2013), reduced POC production may better account for decreased $\delta^{13}C$ values in these two species, as now explained in text.

p. 15851
l. 3: H+ hypothesis: would you not first expect a reduction in the precipitation rate? ok, you don't have PIC production data ...

Authors's response: We are indeed unable to answer this.

l. 13: The data of Langer et al. (2006) are not comparable to your data set. DIC varies around 2mmol·L−1, pH is lower at high [CO2] which is thought to reduce PIC, I suppose (I right now did not have another look at the data)... thus, a completely different set-up

Authors's response: We have removed the reference to the work by Langer.

l. 22: Changes of δ18O (changes in which which direction?) may originate from increased proportion of HCO_3^- over ....

Authors's response: Sentence deleted.

sec. 4.2.2: This section is even more difficult to follow than previous deiscussion.

Authors's response: We have shortened (as per Referee#3's suggestion) and reformulated this paragraph, which is now easier to read (see changes track version attached).

Do you have any experimental evidence (apart from your interpretation of the carbon isotopic signals which should in my opinion rather be discussed in terms of the carbon source) for an increase/decrease in the residence time/overturning rate? Values such as PIC or POC production (C fixation) rates versus C uptake rates? Nevertheless, I do not doubt that residence time (separation between 12C and 13C necesary) can alter the isotopic signal...

Authors's response: We were trying to parallel the approach used by Bidigare et al. (1997) and many others, and as stated in text, to generate a view of the degree of utilization of an internal pool and how it matched with the degree of vital effect expressed, so using the index $\mu \times$ volume / $Ce \times$ surface-area to approach a carbon supply-to-demand balance by the cells (see Hermoso, 2015 – Paleoceanography). However this is now **removed** from the manuscript.

You seem to discuss different carbon sources here. I am wondering why you did not discuss this issue for carbon isotopes which would be more obvious to me than with oxygen isotopes, whose reactions comprise much more reactions than the carbonate system ... but maybe I got you wrong here.

Authors's response: This section has been shortened as also recommended by Referee#3. We do not discuss potential sources used for calcification in the revised ms. As pointed by Referee#2 earlier, carbon and oxygen isotopic systems are way different so that it is difficult to draw a parallel discussion on the two elements (time dependent isotopic exchange between DIC and H_2O for O does not apply on C). Furthermore, existing literature on coccolith (and foraminifera) show a definite focus on oxygen isotopes, hence there are more hypotheses open for discussion. This is due to the widely used $\delta^{18}O$ temperature proxy.

I stop here, since I have too many open questions ...
I hope my comments are helpful for you,
Lena Holtz

Technical comments

p. 15840
l. 9: To obtain

Authors's response: Done.

l. 10 mMk−1
sw?

Authors's response: mmol kg_{sw}^{-1}.

p. 15841
15-19: Which standard did you use for dO? L. 15: V-PDB; l. 19: V-SMOW –
I am confused. Cf. next page, l. 6-8.

Authors's response: The same standard (derived from the V-PDB) is used for both carbon and oxygen isotope ratios. A different thing is the scale: oxygen isotope of calcite is expressed in V-PDB, whereas seawater composition is expressed against V-SMOW.

l. 25: Here, you should first introduce the calculations of δ13CC and d8OC. –
where are the values?

Authors's response: We feel that this is common knowledge and would disrupt the flow of the Methods section.

l. 26: "offset of coccolith calcite from inorganic" (add) calcite.

Authors's response: Done.

p. 15842
l. 19: "per day (μ d 0.7 d−1)": Not everyone is familiar with the difference between specific growth rate and division rate. You should hence say that this μ value corresponds to 1 divion per day. E. hux actually reaches values μ > 1.

Authors's response: A value of μ of 0.7 corresponds to one division per day (population doubling everyday).

l. 21: for reasons of comparability, you should mention cell diameter/radius of E. hux also.

Authors's response: Done.

p. 15843
l. 1: δ13C = δ13CC?

Authors's response: Yes, a "$_c$" has been added.

l. 2: 280 is the pre-industrial value :-)

Authors's response: "(pre-industrial)" has been added.

l. 2: where is this shown? Reference is missing. Table? Figure?

Authors's response: We have added reference to Fig. 2a here.

l. 1-3: do you speak about the vital effect or δ13CC?

Authors's response: This has been clarified: "(hence, a "positive" ^{13}C vital effect)".

l. 3: "very large": is this very large?

Authors's response: We regard an interspecies offset of 3 ‰ as very large indeed.

l. 6: You cite Fig. 2 before Fig. 1. Description in text and figure do not belong together.

Authors's response: This has been corrected.

l. 12f.: what do you mean with "cultures were implemented"? inoculated? Grown? Kept at? Or did you implement a model?

Authors's response: We have changed "implemented" by "grown" to avoid confusion.

p. 15844
l. 1: it can be, not been

Authors's response: Changed.

3.2 "Effect of increased DIC" (at constant pH) " on growth .... "

Authors's response: "at constant pH" has been added.

l. 17-18: is this a sentence?

Authors's response: This long sentence has been divided into two shorter ones for clarity and now reads: "Overall, there is no covariation between growth rates and coccosphere and cell sizes for the species examined here (Fig. 1a; Fig. 1b). One may expect decreased μ to be accompanied by longer generation time, and hence larger cell sizes (Aloisi, 2015). Nevertheless, the data indicate that both *E. huxleyi* and *P. placolithoides* cells become relatively larger with elevated DIC levels, as observed for the former in the work by Müller et al. (2012)".

p. 15844
l. 20: "become relatively larger"

Authors's response: We have changed "species" for "cells".

p. 15845
l. 3: wrong Fig. cited?

Authors's response: Corrected.

l. 19: vital(skip s) effect: is defined differently in brackets here and eqns. (2) and (3) ...

Authors's response: Done.

p. 15848
l. 19: "such contrasting responses": reference to which responses?

Authors's response: We have added "interspecific" response.

p. 15850

l. 1: "Specific to photosynthetic": I do not understand what you mean here.

Authors's response: The sentence now reads: "In photosynthetic, or photosynthetic-associated biomineralisers such as the foraminifera, corals and coccolithophores a ^{12}C-DIC depletion of the internal carbon pool due to photosynthetic fractionation by the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) may imprint the whole *Ci* leading to substantial isotopic consequences on the stable isotope composition of biominerals (McConnaughey, 1989; Spero et al., 1997; Hermoso et al., 2014)".

l. 1/2: The sentence is weird.

Authors's response: See previous answer.

l. 2: "coccoliths" should read coccolithophores

Authors's response: Done.

l. 3: you should introduce RubisCO here (I mean the full name)

Authors's response: Done.Although it should actually be RuBisCO

Referee#3 (Anonymous)

The work by Hermoso et al. is a valuable contribution to the complex field of carbon and oxygen isotope fractionation in coccolithophores. The multi-species approach is particularly helpful and besides providing much needed insights, raises, as is so often the case, many additional questions. This is not a bad thing, of course, and I appreciate the author's attempt to tackle some of them in considerable detail.

Authors's response: We are grateful to this Referee for acknowledging the relevance of our work, and pinpointing the challenge to undertake a biogeochemical study for palaeoceanographic purposes, as the concepts are really different leading to misunderstanding between the two worlds.

However, I think that sections 4.2.1 and 4.2.2, although a nice exercise, bring little to the table in terms of the central section on proxy development (4.3). Moreover, section 4.2.1 for instance uses the PIC/POC ratio as one important parameter in the argument. But in the present study PIC/POC was not determined. So I feel that such highly conjectural sections (4.2.1 and 4.2.2) take too much space and could be shortened considerably.

Authors's response: Some sentences of these two sections have been shortened to avoid vagueness, in particular we removed two paragraphs from the oxygen isotope section. However that the elements of discussion presented here are necessary to bridge biogeochemical concepts to palaeoceanographical implications (indeed presented in section 4.3). Thanks to Referee#2, a more suitable study allows more meaningful comparison of data (PIC/POC) published literature data (Bach et al., 2013) for *E. huxleyi*.

Concerning DCUt, I would suggest keeping it as it is used in section 4.3.

Authors's response: As discussed with Referee#2 during the discussion phase, as following the AE's guidance, we have removed the DCUt index. We will show the correlation with external total DIC in the ms.

Thanks to the thorough comments by the other reviewers there is not much left to say, from my point of view. I will merely highlight a few technical points which might even have been mentioned by the others (if so sorry for that).

1 Please state explicitly which parameters of the carbonate system were used to calculate it, and how it was calculated.

Authors's response: Done (in new Table 1).

2 Give the full carbonate chemistry in a table (and preferably the other data as well)

Authors's response: A Table with all numerical data has been prepared.

3 How many replicates were run?

Authors's response: Two replicates for each species and each DIC conditions were done. This is now stated in the body text.

4 It would be helpful to have a figure showing cell density on y and time on x as an illustration of the semi-continuous approach

Authors's response: Unfortunately, we are unable to produce such graphs with the data we have.

5 In section 3.3 you say that you used coccosphere size as opposed to (naked) cell size. Actually, your Coulter Counter measurements are probably much closer to naked cell size than to coccosphere size. This type of machines is hardly capable of "seeing" the coccosphere.

Authors's response: In other studies, we obtained two distinct peaks on the Counter Counter by measuring coccospheres and decalcified cells. The difference for each species is above the sensitivity of the instrument, and the standard deviation of the size distribution of the latex beads (5 and 10 microns in diameters) used to calibrate the Coulter Counter. Therefore, we prefer indicating coccosphere sizes rather than cell sizes, as we effectively measured untreated (unacidified) coccolithophores.