Response to Howard Spero (Reviewer#1)

We are very grateful to the reviewer Dr. Howard Spero for his positive and valuable comments on our manuscript. The issues raised by the reviewer are taken into consideration and in the following paragraphs, we present our reply to each of them.

Haruka Takagi
(on behalf of all co-authors)

Line 140 – please add to this sentence that 'non functional' chlorophyll could come from phytoplankton in the guts of zooplankton prey. This caveat eliminates the potential that a reader of your paper concludes that your data indicates that all foraminifera species ingest phytoplankton prey directly which is not the case for species such as *sacculifer*, *ruber* and *Orbulina*.

Reply 1-1: Thank you for the suggestion. We will add the sentence as suggested. We agree that it will avoid readers to misunderstand the trophic activity of foraminifera.

Line 145 – The chl content of a dinoflagellate symbiont cell is » than that in a pelagophyte or chrysophyte symbiont from thermocline dwellers. How do you determine symbiont 'density', which I interpret to mean number of symbionts, from Chl a content? Certainly a single dinoflagellate cell has » chl a than a very small chrysophyte cell. Hence there is little connection between chl and symbiont 'density'.

Reply 1-2: Thank you for the comment. In this part, we used the word 'density' for expressing 'per unit mass', which we admit that it is not a good wording. In order to state it precisely, we will change "As an indicator of symbiont density of an individual, …" to "To normalize by the size of an individual, …".

Line 200 – please provide a conversion for the fluorescence units you use - 10-20 m2 quanta-1 to the more generally used units - ïA¸ mol photons m2 s-1

Reply 1-3: The unit for σ_{PSII} is often '$Å^2$ $quanta^{-1}$' (Å = 10^{-10} m). Since Å is not an SI-unit, we used 'm' instead. We will add '$Å^2$ $quanta^{-1}$' next to '$\times 10^{-20}$ m^{-2} $quanta^{-1}$', in the definition table in Figure 3.

I am having trouble understanding the relationship between σPSII and photosynthetic saturation. For the readers, would it be possible to explain this light absorption efficiency term in a way that one can interpret it relative to the light field in the ocean. I observe that the results seem to be inverted relative to photosynthetic light saturation – a concept that many researchers understand. This should be explained better in the discussion (line 345). In this regard, on line 352 you note that this parameter indicates a higher acclimation potential to a low-light environment. How does this relate to Ik in a P/I curve for symbiont photosynthesis? Note that Jorgensen et al (1985), Spero and Parker (1985) and Rink et al. (2005; 1998) show P/I curves that could easily be related to the photosynthetic efficiency term here. Such a link would go a long way to relate previous research on symbiont photosynthesis with the new data you present here and in your other papers.

Reply 1-4: We appreciate your comment. We agree that this point is important when comparing our results to the previous studies.

Saturating irradiance, I_k, is defined as the point where the extrapolated initial slope (α) of the photosynthesis–irradiance curve (P-I curve) crosses the saturation level of photosynthetic rate (P_{max}), thus $I_k = P_{max}/\alpha$. α takes into account that the light absorbed by the algal cell is proportional to the functional absorption cross-section (σ_{PSII}) of the photosystem II (the effective area that a molecule presents to an incoming photon and that is proportional to the probability of absorption) and to the number of photosynthetic units (n), $\alpha = n * \sigma_{PSII}$ (Falkowski and Raven, 1997). Therefore, theoretically, I_k is inversely proportional to σ_{PSII}. In general, low-light acclimated algae shows low I_k, low P_{max}, and high α (thus high σ_{PSII}). Jorgensen et al. (1985), Spero and Parker (1985), and Rink et al. (1998) all showed that the I_k of dinoflagellate-bearing species was high, which is consistent to the low σ_{PSII} of dinoflagellate-bearing species in our results. Although I_k or α of pelagophyte-bearing species has not been reported so far, the high σ_{PSII} of pelagophyte-bearers indicates low-light acclimated photophysiology (Babin et al., 1996; Bouman et al., 2018). We will add this discussion in the revised version.

Line 190 – Does your *O. universa* data use pre-sphere *O. universa* or just spherical *O. universa*? Are the size measurements for *Orbulina* on the inner trochospiral test or the diameter of the sphere? If the latter, then the measurements are not that valuable as the sphere is seldom filled with cytoplasm in a plankton tow. Please indicate this in the text and tables.

Reply 1-5: The measured *Orbulina universa* specimens smaller than 400 μm were all trochospired (pre-sphere stage). The rest were spherical adult, and we measured their sphere diameter since the inner trochospired test was not always visible depending on the thickness/transparency of the sphere. We have confirmed that when the spherical adult specimens alone were used for the regression analysis, it also showed a significant positive correlation between Chl *a* content and the spherical diameter ($p << 0.01$, $R = 0.419$, $y = -5.63 + 2.51x$, $N = 69$). As you mentioned, and as is shown in Spero and Parker (1985), the symbiont content should be better correlated with

juvenile trochospired test size than with spherical diameter of *O. universa*. However, it may be the case for other species as well; e.g., the final sac chamber of *G. sacculifer* is seldom filled with cytoplasm, and the symbiont content may have a higher correlation with test size without a final chamber. In our study, we consistently used the maximum diameter of the test as the 'test size' whatever the growth stage is. We will explain it in the text, Figure 6, and Table S1. In addition, in Table S1, the juvenile specimens of *O. universa* will be marked with *.

Line 235. Please mention/discuss the results from Fehrenbacher et al (2018) that support a microhabitat for non-spinose species on organic aggregates. Marine snow is the primary organic particulate that transports phytoplankton cells from the surface to deep ocean. *G. scitula* and *crassiformis* may obtain chlorophyll from such material. Alternatively, many of the zooplankton prey of these foraminifera could participate in the nightly diurnal migration of the deep scattering layer where the zooplankton could feed on surface phytoplankton at night and migrate back to depth during the day where the forams could capture/ingest them.

Reply 1-6: Thank you for the insightful comment. We agree that their lifestyle (attaching to organic aggregates) is one of the factors they incorporate non-functional chlorophyll. We will include the possibility of marine snow grazing of non-spinose species citing Fehrenbacher et al. (2018). As we replied in Reply 1-1, phytoplankton in the gut of zooplankton prey is also an important path that indirectly incorporates non-functional chlorophyll. We will include this possibility as well.

Line 248 – contact Barbel Hoenisch at LDEO. She has unpublished observations on Sphaeroidinella dehiscens from Puerto Rico culture experiments that supports your observations on the 7 dehiscens you observed. She collected dozens of specimens using scuba and had them in culture until gametogenesis when they put on a cortex. All looked like sacculifer and contained dinoflagellate symbionts. You could ask for details and permission to provide Barbel's 'unpublished data' for the observations you describe.

Reply 1-7: We appreciate your suggestion and are happy to know that her observation supports ours. However, since informal references such as personal communication should be avoided in this journal, we would like to refrain from including such unpublished data.

Line 248 – are you 100% certain that the *G. tenella* and *G. rubescens* you claim to have collected have dinoflagellate symbionts and were not early/juvenile *ruber* or *sacculifer*? The latter look very different than the adult stages when the shells are only 100 um in size.

Reply 1-8: Since *G. ruber* pink is absent in the Pacific, foraminifera with pink pigmentation collected from the Pacific were 100% *G. rubescens*. They had dinoflagellate-like symbionts. For specimens collected from the Atlantic, we identified them based on the key taxonomic features such as four globular chambers in the last whorl, high arched umbilical aperture, and lack of supplementary aperture, in addition to the typical pink pigmentation and small test size. Likewise, *G. tenella* was identified based on its key features; four globular chambers in the last whorl, high arched umbilical aperture, single small supplementary aperture, and small test size. Most of the specimens we analyzed were larger than 100 μm, and can be distinguished from small *G. ruber* (s.s.) or *G. sacculifer* based on the above features. We recognize that confirmation of their molecular taxonomic position should be needed and this should be the next step.

Line 253 – add that the relationship observed by Spero and Parker was a logarithmic relationship. Again – is the relationship in Figure 6 for *Orbulina* comparing sphere diameter or trochospiral shell length? You may be able to compare your chl data with the regression in Spero and Parker to generate a true chl vs symbiont density relationship for the dinoflagellate symbionts in other species.

Reply 1-9: We will add the statement of the logarithmic relationship between test size and symbiont density. As we have explained in the above (Reply 1-5), we used the maximum test diameter regardless of the growth stage of foraminifera; i.e., trochospiral diameter for prespherical *O. universa* and sphere diameter for spherical *O. universa*. Using the relationship of Spero and Parker (1985), we can show the Chl *a* vs symbiont density. Since the linear regression of Spero and Parker (1985) was performed on half-log scaled cross-plot (test size is in linear scale and symbiont number is in log scale) whereas ours is double-log scaled (both test size and Chl *a* are in log scale), the relation between Chl *a* vs symbiont density is expressed as an exponential function in double-log scale (Fig. A). In this relationship, the Chl *a* content per symbiont cell varies significantly.

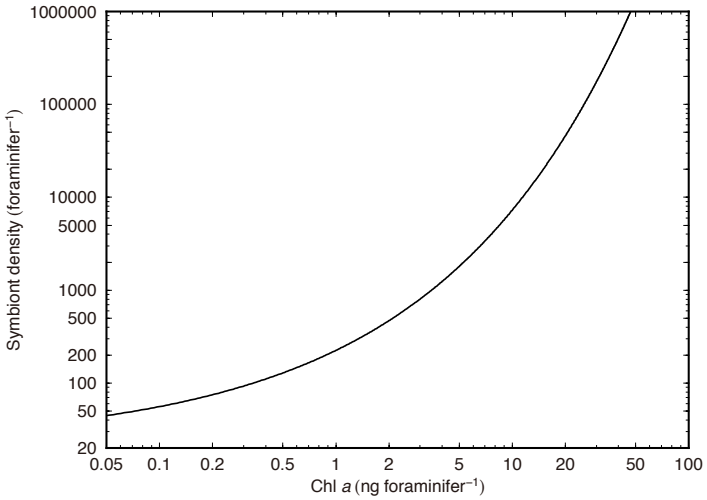

Figure A. Relationship between Chl *a* content and symbiont density derived from test size-Chl a relationship (this study) and test size- symbiont density relationship (Spero and Parker, 1985).

Alternatively, when we use a certain Chl *a* content of symbiont, e.g., 1-5 pg cell^{-1} (cf. Fitt et al., 2000, for *Symbiodinium* in corals), we can show a test size-Chl *a* content relationship derived from Spero and Parker (1985) and can compare it to ours (Fig. B). We will include the figure and a short discussion about it in the supplementary material.

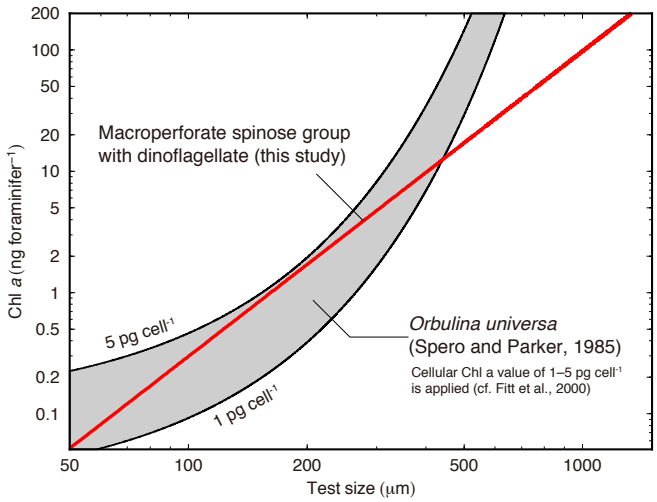

Figure B. Relationship between test size and Chl *a* content. Red line; relationship in dinoflagellate-bearing group (regression line for macroperforate spinose group with dinoflagellate in Figure 9). Gray area; relationship in *Orbulina universa* derived from Spero and Parker (1985) using a range of Chl *a* content per symbiont cell (cf. Fitt et al., 2000).

Section 4.2. This section discusses chlorophyll content in terms of host size, photosynthetic characteristics relative to chamber morphology or spinose vs non-spinose species. It is the opinion of this reviewer that this section fails to discuss the two most important parameters – differences in symbiont type (dinoflagellates have » more chl a per symbiont cell than does chrysophyte/pelagophyte symbionts) and depth habitat (the ambient light regime as a function of water depth controls light availability for the symbionts. Self-shading due to internal vs external symbiont distribution has little to no effect on available light as the shells are virtually transparent to light penetration given their thickness and the size of the foraminifera. Rather, the internal/external location difference will have an effect on nutrient availability or DIC supply for photosynthesis. Unlike the smaller symbionts in the deeper dwellers, the dinoflagellate symbionts in the mixed layer species would quickly exhaust their DIC supply if they were inside the foram test during the day rather than on the spines where DIC availability is only diffusion limited. This section should be modified accordingly.

Reply 1-10: Thank you for the insightful comment and discussion. In the second paragraph, we discussed the higher Chl *a* content in the spinose group than the non-spinose group from a morphological perspective (i.e., possession of spines). In fact, we think the difference cannot be simply related to the symbiont type because the

spinose group includes several types of symbionts (dinoflagellate for globigerinoidids, pelagophyte for *G. siphonifera* Type II, and prymnesiophyte for *G. siphonifera* Type I). Moreover, pelagophyte symbiont is possessed in the non-spinose group as well (*N. dutertrei*). As shown in Fig. 10, spinose group with dinoflagellate symbiont (red) and spinose group with non-dinoflagellate symbiont (orange) are similarly distributed, and both show higher Chl *a* content than the non-spinose groups. It indicates that the symbiont type is not the primary factor to make the difference. We believe that the symbiont type would affect the relationship to some extent, but considering our data, the effect is not apparent. In terms of the effect of depth (light environment relating to depth), it is hard to discuss here because specimens collected from various depth (< 100 m) are mingled in the test size-Chl *a* relationship. As we will comment in the following reply (Reply 1-11), statistical modeling such as GLMM or GAMM will be suitable approaches to reveal the effect of depth or symbiont taxonomy. In this section, we will not include a detail discussion on taxonomy or depth because of the above reason, but will mention the possibility of their effect.

We totally agree that the presence of spines and symbionts distribution on them have to do with nutrient availability and DIC supply (so we will include this point in the text). Likewise, it is our opinion that this does affect the illumination on each cell as well. We believe that the spherical distribution of symbionts on spines does affect the exposure to light, hence affect photosynthesis. As you pointed out, test wall characteristics such as macroperforate or microperforate may make little difference to the light penetration when they are sequestered inside the test. We will delete this point, but leave the effect of spines on illumination as it is.

Line 375 – do you see any differences in photophysiology when comparing specimens from oligotrophic environments with a deep mixed layer and clear water (deep light penetration) vs locations with a shallower chlorophyll maximum? This basic difference in light field in the water could explain some of the photophysiological differences between species and locations.

Reply 1-11: Overall, stations in the Atlantic (M140 stations) were more productive than those in the Pacific subtropical gyre (KH-17-4 stations). When we compare the data of these two cruises, the former tended to show higher F_v/F_m and lower σ_{PSII}. However, this tendency was not necessarily true for all species, thus we hesitate to discuss this possibility in the text. In our opinion, factors determining photophysiology is various, and we need further detailed analysis to relate the obtained data of photophysiology and controlling factors such as light penetration, nutrient, symbiont taxonomy, etc…. We believe that using statistical models such as GLMM or GAMM to see the relationship between photophysiology and environmental factors is the future step to better understanding on photosymbiosis.

Besides, in order to discuss more detail on interspecific photophysiological differences, comparison of the photophysiological parameters for specimens cultured under controlled condition, or the compilation of individual data collected from the similar environmental condition would be useful.

Figure 11 is very interesting, but is not discussed at all in the text of the manuscript. Nevertheless, I would like to point out that the spectrum of endosymbiosis concept drawn up in this figure does not take into consideration that the foraminifera lose their symbionts every generation and must reestablish the symbiosis every new generation. Also, I have been culturing planktic foraminifera for over 40 years and have never observed a *sacculifer*, *Orbulina* or *G. ruber* without symbionts. LeKieffre et al (2018) shows an amazingly tight inter relationship between symbionts and host foraminifera in Orbulina. The dinoflagellate bearing foraminifera species are incapable of surviving without their symbionts – The horizontal arrow that you have drawn in Fig. 11 does not reflect this 'all or nothing' symbiotic association which must be as necessary as zooxanthellate in reef building hermatypic corals.

Reply 1-12: Thank you for the comment and valuable information based on your years of observation. In the figure, we used the word "acquired phototrophy" for foraminifera, which we intended to show that the symbiotic relationship must be acquired at every new generation. To make it clearer, we will explain it both in the text and the caption of Figure 11 with saying 'sexually reproduced new generation must acquire symbionts from the environment'.

In fact, in the conceptual diagram of photosymbiosis, we wanted to draw a line dividing "all-or-nothing" (so-called obligate) relationship and flexible (so-called facultative) relationship. As shown in LeKieffre et al. (2018), the relationship between *Orbulina* and its symbionts must be strong and their trophic interaction should be called "obligate". However, we have no information on such interaction for the other species. In our method, we cannot go into such detailed interactional relationship. Since our knowledge of foraminiferal photosymbiosis is based on a set of snapshot information of algal possession at a certain time of their lifecycle, whether the observed phenomena is truly essential for survival cannot be concluded. Therefore, at this moment, we thought it is inappropriate to categorize the foraminiferal photosymbiosis into the criteria of obligate or facultative. That is why we simply mapped them based on the statistical result alone (i.e., the PCA results).

In the revised version, we will add a discussion about the perspectives of the necessity of symbiosis citing the result of LeKieffre et al. (2018) and other related studies. We believe future works will reveal the interrelationship between the host and symbionts for the other species, and will make the diagram more elaborate and sophisticated.

References:

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