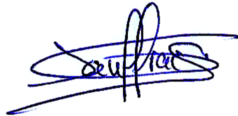
Dear Dr. Middelburg,

Please find enclosed our revised manuscript entitled "**Effect of light on photosynthetic efficiency of intertidal benthic foraminifera**" by Thierry Jauffrais, Bruno Jesus, Edouard Metzger, Jean-Luc Mouget, Frans Jorissen, Emmanuelle Geslin, for submission to Biogeosciences as an original research paper.

We replied below to the different comments done by the reviewers, we improved the material and methods section and reduced the discussion as requested.

We hope that these revisions will fulfill all the requests and will make the manuscript acceptable for publication.

Thank you for your work,
Best regards,



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Thierry Jauffrais

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Anonymous Referee #1

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The manuscript reported about the effect of different light intensities on chlorophyll concentrations, photosynthetic capabilities, and oxygen production/consumption rates during 7 days incubation experiments. They found that chlorophyll concentrations and photosynthetic capabilities differ with light intensity, even between low level of light intensities. The authors also reported that A. tepida did not show such long retaining of chloroplast, suggesting H. germanica should have some way to keep chloroplast, not just digesting them. Some of the findings are new (and the method they used is probably new for foraminiferal kleptoplasty study), but the present manuscript should be re-organized before its publication.

Introduction
Comment: In the introduction, the authors need to specify (or concentrate) more on precise objective of the authors study: i.e. what is known about the light intensity effects on kleptoplasty (only dark and light comparison before?), and why the authors need to clarify light intensity effects, not a function of chloroplast etc.

Reply: We politely disagree, we clearly state the objective in P4L12 and the introduction already contains the existing information about our topic, e.g. P3L20-27, P3L30

P3 line 21-27: "Foraminiferal kleptoplast retention times can vary from days to months (Lopez 1979; Lee et al. 1988; Correia and Lee 2002b; Grzymski et al. 2002). The source of this variation is poorly known but longer kleptoplast retention times were found in dark treatments (Lopez 1979; Correia and Lee 2002b), thus suggesting an effect of light exposure, similar to what is observed in kleptoplastic sacoglossans (Trench et al. 1972; Clark et al. 1990; Evertsen et al. 2007; Vieira et al. 2009), possibly related to the absence of some components of the kleptoplast photosynthetic protein complexes in the host (Eberhard et al. 2008)."
P3 Line 30: "To our knowledge little is known about the effects of abiotic factors on photosynthetic efficiency of sequestered chloroplasts in benthic foraminifera, particularly on the effect of light intensity on kleptoplast functionality."
P4 Line 12:" The objective of the current work was to investigate the effect of irradiance levels on photosynthetic efficiency and chloroplast functional times of two benthic foraminifera feeding in the same brackish areas, *H. germanica*, which is known to sequester chloroplasts and *A. tepida*, not known to sequester chloroplasts."

Discussion
Comment: 4.2. Most of this section, in particular 2nd and 3rd paragraphs, the discussions are stretchs from the current manuscript. I am sure that the ecological role of kleptoplasty is very important topic and the authors' future goal would be this scope, however, the current manuscript reported about the effect of light intensity on the chlorophyll intensity (chloroplast abundances) and its photosynthetic efficiency. If the authors want to keep these discussions, they must discuss by incorporating their findings in this manuscript. I rather suggest to discuss about the meaning of the authors findings that the chlorophyll retention times and photosynthetic capabilities differ greatly between LL and HL, "although HL is still far below the natural photon radiation levels".

Reply: We agree, to make the manuscript more focused and within the scope of our experimental work we deleted from the reviewed manuscript the 2nd and 3rd paragraphs of the section 4.2:
"Using kleptoplasts, *H. germanica,* like other kleptoplastic organisms (e.g. *Elysia viridis* (Teugels et al. 2008)), is also theoretically capable of assimilating inorganic nitrogen via the glutamine synthetase and glutamate 2-oxo-glutarate aminotransferase (GS-GOGAT) pathways to produce glutamate and glutamine after the successive reduction of nitrate to nitrite and nitrite to ammonia or directly through

ammonium uptake (Zehr and Falkowski 1988). However, the first reduction occurs in the diatom cytoplasm via the enzyme nitrate reductase (NR) and not inside the chloroplast. It is not known if *H. germanica* has this enzyme but it is present in *N. stella* (Grzymski et al. 2002). Interestingly, nitrogen (i.e. nitrite and ammonium) assimilation by sacoglossans (e.g. *Elysia viridis*) was observed under light and dark conditions with significantly higher nitrogen assimilation observed under light condition (Teugels et al. 2008). The uptake of ammonium and nitrite are light dependent as their relevant enzymes require kleptoplast electron donors (NiR and GOGAT); i.e. reduced ferredoxin formed in the photosynthetic electron transport chain are used as electron donors in the reaction involving the nitrite reductase [NiR]. Furthermore, the GS metabolic reaction is ATP-dependent, and gene expression of some key enzymes (NiR, GS and GOGAT) is light regulated (Grossman and Takahashi 2001). This suggests that kleptoplasts might also have an added value in providing extra nitrogen source to metabolic pathways in foraminifera under light exposure and also possibly over short periods under dark conditions. It is also noteworthy that ammonium incorporation might take place through the glutamine dehydrogenase (GDH) pathway in the mitochondria that converts glutamate to α-ketoglutarate, which can subsequently be assimilated in the kleptoplast via the GOGAT pathway (Teugels et al. 2008).
Diatoms are also known to assimilate organic nitrogen (Antia et al. 1991), to use their ornithine-urea cycle for anaplerotic carbon fixation into nitrogenous compounds (Allen et al. 2012) and some of the benthic species present on mudflats are also able to assimilate organic carbon (Admiraal and Peletier 1979). Apparently some benthic diatoms can alternate between an auto- or heterotrophic metabolism in function of the environment. Analysing the kleptoplast DNA would provide interesting data to determine if foraminifera are capable of selecting facultative heterotrophic diatoms to improve their ability to assimilate dissolved organic compounds. Finally, another possible added value of incorporating kleptoplasts is the possibility of using them as an energy stock to be digested during food-impoverished periods particularly when foraminifera are transported below the photic zone of the sediment by macrofaunal bioturbation."

Other minor comments or corrections

Page 2
Comment: Line 12 If the authors mention "secondary role" here, then the authors need to mention about that bacteria play primary roles on carbon cycling in aerobic sediments.
Reply: We agree with the reviewer and modified the sentence to clarify what we wanted to say:
Line 13-16: **"**Their minor role in organic carbon cycling in aerobic sediments, compared to bacteria, contrasts with their strong contribution to anaerobic organic matter mineralisation (Geslin et al. 2011) and they can be responsible for up to 80% of benthic denitrification (Pina-Ochoa et al. 2010; Risgaard-Petersen et al. 2006)."

Comment: Line 16 The sentence starting with "Some benthic foraminifera: "seems appeared abruptly. Is kleptoplasty related to carbon cycling or anoxic adaptation of the foraminifera? If so, please add relevant connections from the former sentences.
Reply: Agreed, since it is a separate topic and to clarify this section we changed it to a new paragraph.

Page 3
Comment: Line 13 Costal > coastal
Reply: corrected

Page 4
Comment: Line 21 What is the "-" before 2.019W? Does this mean 2.019E?
Reply: Corrected, the "-" was a mistake

Comment: Line 23 ~20 kg
Reply: Corrected as suggested, "±20 kg" was changed to "~20 kg"

Comment: Line 27 Please note the filter size
Reply: Added as suggested: "filtered (GF/C, 1.2 µm, Whatman) autoclaved sea-water"

Page 5
Comment: Line 11 Please explain shortly about the methods described in Jesus et al. (2008).
Reply: The short explanation was already in the manuscript (type of machine, sensor position…), however to clarify the paragraph we added the word "concisely" to link the two sentences. "Concisely, a USB2000 (Ocean Optics, Dunedin, FL, USA) spectroradiometer with a VIS-NIR optical configuration controlled by OObase32 software (Ocean Optics B.V., Duiven, the Netherlands) was used. The spectroradiometer sensor was positioned so that the surface was always viewed from the nadir position. Foraminiferal reflectance spectra were calculated by dividing the upwelling spectral radiance from the foraminifera (Lu) by the reflectance of a clean polystyrene plate (Ld) for both of which the machine dark noise (Dn) was subtracted (eq. 1)."

Comment: Line 12 50 specimens of H. germanica and :
Reply: Agreed and modified : "Pigment spectral reflectance was measured non-invasively to determine the relative pigment composition on 50 fresh specimens of *H. germanica*, on 50 fresh specimens of *A. tepida* and on benthic diatom as explained in Jesus et al. (2008)."

Comment: Line 25 This is the first place appearing RLC, so please explain.
Reply: Agreed, we slightly modified a sentence in the introduction to introduce the term RLC, Page 4 Line 7-9: "This non-invasive technique has the advantage of estimating relative electron transport rates (rETR) using rapid light curves (RLC) and photosystem II (PSII) maximum quantum efficiencies (*Fv/Fm*) very quickly and without incubation periods."

Comment: 3 X 10 specimens (or individuals)
Reply: Agreed, we clarified this sentence: Page 7 line 20 "For each condition, ten specimens were used per replicate and three replicates per light treatment; furthermore all plastic Petri dishes were filled with Bourgneuf bay filtered-autoclaved seawater."

Page 6
Comment: Line 11 How long did the authors wait till the oxygen microprofiling after putting foraminifera into the tube?
Reply: We added a sentence in this section to clarify this point: "Measurements were registered when the oxygen micro-profiles were stable; they were then repeated five time in the centre of the micro-tube"

Comment: Line 19 Which position of oxygen gradients were used to calculate diffusion flux? Near foraminifera? Maximum slope? Or did authors proximate in some way? Please specify and describe.
Reply: We used the R^2 to determine the best slope and to avoid the small O2 turbulences that often occur close to the foraminifera, therefore we modified a sentence in this section to clarify this point "The O_2 concentration gradients were calculated with the oxygen profiles and using the R^2 of the regression line to determine the best gradient."

Page 7
Comment: Line 20 I guess Ammonia tepida exhibited chlorophyll at the start of the experiment because they still have some diatoms in food vacuoles. It may be interesting to compare the

concentration of chloroplast at the begging (perhaps reflecting selective ingestion?) or reduction of chlorophyll in A. tepida as an index of degradation of chloroplast and that of H. germanica, which retain chloroplast.
Reply: Thank you for the suggestion, we agree that it would be an interesting measurement but we chose to use the Fv/Fm to follow the chloroplast degradation. Although it is an indirect measurement it has the advantage of being less invasive than using microscopy, where exposure to light during the time necessary to produce an image would automatically have an impact on the chloroplast due to the microscope light.

Comment: Line 25 Please note wave length
Reply: added (, excitation wave length 485 nm)

Page 8
Comment: Line 5 Triplicate measurement for each specimen? or just using 3 specimens as triplicate? Please specify.
Reply: We clarified it in Page 7 line 20 "For each condition, ten specimens were used per replicate and three replicates per light treatment; furthermore all plastic Petri dishes were filled with Bourgneuf bay filtered-autoclaved seawater."

Comment: Line 9 Again, individuals or specimens are better than using "foraminifera"
Reply: Agreed and replaced the word "foraminifera" by "specimens" as suggested.

Comment: Line 12 To compare the foraminifera test mean maximal elongation "between what"
Reply: We clarified it in P5 line 4: "the length of the axes going from the last chamber to the other side of the test and passing by the umbilicus".

Comment: Line 23 "390 +- 42 um (SD, n = 34)" is better
Reply: Agreed and modified in the text accordingly.

Comment: Line 29 Absorption at 435 and 585 nm are not "deep absorption feature".
Page 9
Comment: Line 1 In the figure 1, there is no indication of Chla at 435 nm wavelength.
Reply: For the two last comments, we agree and modified the text in P9 line 20-26:
"Fresh *Haynesina germanica* showed a typical diatom spectral signature with high reflectance in the infrared region (>740 nm) and clear absorption features around 585, 630 and 675 nm; the absorption feature around 675 nm correspond to the presence of chlorophyll *a*; the 585 nm feature is the result of fucoxanthin and the 630 nm absorption feature is the result of chlorophyll *c* (arrows, Figure 1). *Ammonia tepida* showed no obvious pigment absorption features apart from 430 nm (Figure 1)."

Comment: Line 3 Please note which sample (starved and kept dark under 7 days?) was used forthis spectral signatures in Figure 1.
Reply: This has been clarified in P5 line 12-14: "Pigment spectral reflectance was measured non-invasively to determine and compare the relative pigment composition on 50 fresh specimens of *H. germanica*, on 50 fresh specimens of *A. tepida* and on a benthic diatom as explained in Jesus et al. (2008)."

Comment: Line 12 There is no statistical indication in Figure 3. Also, Kruskal Wallis can detect differences between several samples, but cannot say anything about the difference between s pecific two samples. Therefore, if the authors describe "Samples kept in the dark did not show an obvious decrease", then the authors need to perform another statistical analysis on this.
Reply: This part is addressed in the description of fig 2 and not fig 3, there is no statistical test associated to it.

Comment: Regarding figure 3, did the authors perform any kind of "calibration" between pixel values and chlorophyll concentration? If not, the vertical axis (pixel values) does not have any numerical meaning. I therefore suggest to present as relative chlorophyll fluorescence as T0=100%.
Reply: This has been clarified in the material and method P6 line 3-8: "In a RGB image each channel contains pixels between 0 and 255 values. The majority of the information regarding chlorophyll fluorescence is encoded in the red channel, therefore the green and blue channel were discarded and only the red channel was kept. The images from the different treatments were directly comparable as all images were taken using the same acquisition settings. Thus, the mean red pixel values were used as a proxy for chlorophyll fluorescence."

Comment: Line 21 No evidence of photoinhibition "of this measured range" or something
Reply: Agreed and modified accordingly: **"**showing no evidence of photoinhibition within the light range used (Figure 4)

Comment: Line 30 "light respiration being lower than dark respiration" Based on the Table 1, LL respiration was higher than dark respiration. Does "light respiration" mean the average of LL and HL? Please specify.
Reply: This has been clarified in the material and methods P8 lines 21-24**: "**Oxygen measurements were repeated at 300 µmol photons m^{-2} s^{-1} and in the dark at the end of the experiment (7 days of incubation) for all different light treatments (D, LL, HL) to assess the production or consumption of oxygen at these two light levels in all treatments.*"*

Comment: Page 10 Line 2 LSD test ")".
Reply: The parenthesis has been added.

Comment: Line 26 Clearly show "that"?
Reply: Changed.

Comment: Page 11 Line 8 "24" umol photons?
Reply: Corrected to "24".

Comment: Line 11 Do the authors have any idea on the in situ light intensity?
Reply: Added "very high irradiance levels (>1000 µmol photons m^{-2} s^{-1}) at the surface of the sediment during low tide". Please also note that irradiance levels are very quickly attenuated in muddy sediments. For example, at an ambient light of 1500 µmol photons m^{-2} s^{-1}, light levels at 500 µm deep will be reduced to 75 µmol photons m^{-2} s^{-1} in muddy sediments with a light attenuation coefficient of 8 mm^{-1}.

Comment: Page 12 Line 7 It seems the authors want to say "modestly" or something instead "little".
Reply: "little" has been replaced by "not much"

Anonymous Referee #2

Received and published: 17 March 2016

Comment: Author analysed the functionally of chloroplast retained by some species of benthic foraminifera. Study conducted is very interesting, and the techniques used are new and applicable to other organisms, which makes the manuscript relevant to a broad readership. However, methods section needs to be carefully revised as it does not follow a logical sequence, and experimental design needs to be explained in more detail. Moreover, manuscript needs to be proofread and revised by a native English speaker. Many problems with punctuation throughout the text.

Reply: The Methods section has been carefully revised, clarified and completed.

Introduction
Page 2
Comment: Ln. 19-24: Kleptoplasty is also very common in carbonate reef environments when conditions are favourable (i.e, oligotrophy; e.g., Ziegler and Uthicke 2011).
Reply: We disagree with the species identification in this publication, i.e. the only kleptoplastic foraminifera mentioned, an *Elphidium* sp., is clearly not an *Elphidium*, and, therefore we prefer not to cite the publication.

Comment: Ln. 25-28: Studies by Correia and Lee need to be acknowledged and cited here as they represent a good contribution to this research field.
Reply: Agreed, the studies of Correia and Lee were added at the end of this sentence.

Methods

Page 4
Comment: Ln. 11-16: Please provide a rationale for only exposing the specimens to different light levels for one week only.
Reply: Agreed and clarified P7 L18-20: "A short term experiment was thus carried out (7 days) to study the effect of light on healthy specimens rather than the effect of starvation."

Page 5
Comment: Ln. 27-28: Clarify why A. tepida specimens were not starved under light conditions, and if A. tepida was exposed to different light conditions at all.
Reply: Because after one day Fv/Fm was already very close to zero. Thus all posterior measurements would be zero and meaningless.

Comment: Experimental design: Please, clarify the total number of individual used per replicate and number of replicates per treatment.
Also, please clarify the experimental design. Was A. tepida exposed to different light treatments? There is no information in the methods (where it should be). It is surprising that the authors only used one paragraph to explain their experimental design, which is the most important part of the study. There is no way for the reader to know number of replicates, total number of specimens, why conditions were chosen, how light levels were reached, temperature, static or flow-through system? Detail explanation of the experimental design is necessary.
Comment: Methods section does not follow a logic sequence when explaining each parameter analysed. This section needs to be carefully revised. Were all specimens used in the experiment tested for all parameters analysed? Please, clarify.
Page 6
Comment: Ln. 10-11: Was one individual used at a time or all at once? Please, clarify.

Comment: Ln. 20-22: What about inter specific differences? Did the authors use a pool of 7- 10 individuals for O2 consumption measurements? Or the measurements were done individually?
Comment: Ln. 24: Authors stated that seven specimens were used, but previously (ln. 10) mentioned "7 to 10 foraminifera". Please, be consistent.
Comment: Ln. 26: Please clarify why only two steps were used for A. tepida.
Page 7
Comment: Fluoresce measurements: What light was used to measure Fo? Please, clarify
Page 8
Comment: Ln. 15-16: It seems that the authors have a blocked design, but it hard to tell based on the current description of the experimental design. For example, if both species were put in the same experimental petri dish or not. That requires a more detailed description of the methods. Therefore, it is impossible to judge if authors conducted the appropriate statistical analyses. Throughout the methods section author put in brackets "3x10 foraminifera". Please, clarify if this means replicates or trials per parameter analysed.

Reply: to answer to the previous 9 comments, the Material and Methods section has been carefully revised and modified to address the different points mentioned by reviewer 2 from section 2.3 to 2.7:

2.3 Spectral reflectance

Pigment spectral reflectance was measured non-invasively to determine and compare the relative pigment composition on 50 fresh specimens of *H. germanica*, on 50 fresh specimens of *A. tepida* and on a benthic diatom as explained in Jesus et al. (2008). Concisely, a USB2000 (Ocean Optics, Dunedin, FL, USA) spectroradiometer with a VIS-NIR optical configuration controlled by OObase32 software (Ocean Optics B.V., Duiven, the Netherlands) was used. The spectroradiometer sensor was positioned so that the surface was always viewed from the nadir position. Foraminiferal reflectance spectra were calculated by dividing the upwelling spectral radiance from the foraminifera (Lu) by the reflectance of a clean polystyrene plate (Ld) for both of which the machine dark noise (Dn) was subtracted (eq. 1).

$$\rho = \frac{(Lu - Dn)}{(Ld - Dn)} \qquad \text{(eq.1)}$$

2.4 Image analysis

Foraminifera kleptoplast fluorescence was measured using epifluorescence microscopy (×200, Olympus Ax70 with Olympus U-RFL-T, excitation wave length 485 nm). Two Tif images (1232 × 964 px) of each foraminifer were taken (one bright field photography and one epifluorescence photography) using LUCIA G^{TM} software. The bright field photography was used to trace the contours of the foraminifer and an ImageJ macro was used to extract the mean pixel values of the corresponding epifluorescence photography. Higher mean pixel values corresponded to foraminifera emitting more fluorescence and thus, as a proxy, contain more chlorophyll. In a RGB image each channel contains pixels between 0 and 255 values. The majority of the information regarding chlorophyll fluorescence is encoded in the red channel, therefore the green and blue channel were discarded and only the red channel was kept. The images from the different treatments were directly comparable as all images were taken using the same acquisition settings. Thus, the mean red pixel values were used as a proxy for chlorophyll fluorescence.

2.5 Oxygen measurements

Oxygen was measured using advanced Clark type oxygen microelectrodes of 50 µm in diameter (Revsbech, 1989) (OXI50 - Unisense, Denmark). Electrodes were calibrated with a solution of sodium ascorbate at 0.1 M (0%) and with seawater saturated with oxygen by bubbling air (100%). Foraminiferal photosynthesis and oxygen respiration rates were measured following Høgslund et al. (2008) and Geslin et al. (2011). Measurements were carried out in a micro-tube made from glass Pasteur pipette tips with an inner diameter of 1 mm. The micro-tube was fixed to a small vial, filled with filtered autoclaved seawater from Bourgneuf Bay. The vial was placed in an aquarium with water kept at room temperature (18°C). A small brush was used to position a pool of 7 to 10 foraminifera in the glass micro-tube after removing air bubbles. Oxygen micro-profiles started at a distance of 200 µm above the foraminifers to avoid oxygen turbulences often observed around the foraminifers. Measurements were registered when the oxygen micro-profiles were stable; they were then repeated five time in the centre of the micro-tube, using 50 µm steps until 1000 µm away from the foraminifers (Geslin et al. 2011). The oxygen flux (J) was calculated using the first law of Fick:

$$J = -D \times \frac{dC}{dx} \qquad \text{(eq. 2)}$$

Where D is the oxygen diffusion coefficient ($cm^2\ s^{-1}$) at experimental temperature (18°C) and salinity (32) (Li and Gregory, 1974), and dC/dx is the oxygen concentration gradient (pmol O_2 cm^{-1}). The O_2 concentration gradients were calculated with the oxygen profiles and using the R^2 of the regression line to determine the best gradient. Total O_2 consumption and production rates were calculated as the product of O_2 fluxes by the surface area of the micro-tube and subsequently divided by the foraminifera number to finally obtain the cell specific rate (pmol O_2 $cell^{-1}$ d^{-1}) (Geslin et al. 2011).

2.6 Fluorescence

All pulse amplitude modulated fluorescence measurements were carried out with a Water PAM fluorometer (Walz, Germany) using a blue measuring light. Chloroplast functionality was estimated by monitoring PSII maximum quantum efficiency (*Fv/Fm*) and by using P-I rapid light curves (RLC, e.g., Perkins et al. (2006)) parameters (α, initial slope of the RLC at limiting irradiance; rETRmax, maximum relative electron transport rate; Ek, light saturation coefficient; and Eopt, optimum light) (Platt et al. 1980). Rapid light curves were constructed using eight incremental light steps (0, 4, 15, 20, 36, 48, 64, 90 and 128 µmol photons m^{-2} s^{-1}), each lasting 30 seconds. The PAM probe was set up on a stand holder at a 2 mm distance from a group of 10 foraminifera.

2.7 Experimental design

Haynesina germanica, a species known to sequester chloroplasts, were placed in plastic Petri dishes and starved during 7 days under three different light conditions: dark (D and Dark-RLC), low light (LL, 25 µmol photons m^{-2} s^{-1}) and high light (HL, 70 µmol photons m^{-2} s^{-1}); whereas for comparison, *A. tepida*, a foraminifer not known to sequester chloroplasts was starved but only exposed to the dark condition. A short term experiment was thus carried out (7 days) to study the effect of light on healthy specimens rather than the effect of starvation. For each condition, ten specimens were used per replicate and three replicates per light treatment; furthermore all plastic Petri dishes were filled with Bourgneuf bay filtered-autoclaved seawater. This experiment was carried out in a thermo-regulated culture room at 18° C, equipped with cool light fluorescent lamp (Lumix day light, L30W/865, Osram) and using a 14:10 h (Light:Dark) photoperiod. The distances between the light and the experimental

conditions were assessed using a light-meter and a quantum sensor (ULM-500 and MQS-B of Walz) to obtain the desirable light intensities. Concerning the dark condition, the Petri dishes were place in a box covered with aluminium foil.

Haynesina germanica kleptoplast fluorescence was measured using epifluorescence microscopy, as explain above, before and after the different light treatments. At the beginning of the experiment it was done on 30 independent specimens to assess the natural and initial variation of *Haynesina germanica* kleptoplast fluorescence. At the end of the experiment, the measurement were done on all foraminifera exposed to the different light condition (a total of 30 specimens per condition). This was also measured on *A. tepida*, but results are not presented because no chlorophyll fluorescence was observed at the end of the experiment.

Haynesina germanica and *A. tepida* oxygen production and consumption were measured at the beginning of the experiment on three independent replicates with 7 specimens in each replicate. Six different light steps were used to measure O_2 production (0, 25, 50, 100, 200 and 300 µmol photons m^{-2} s^{-1}) for *H. germanica* and only two light steps (0 and 300 µmol photons m^{-2} s^{-1}) for *A. tepida*. Photosynthetic activity (P) data of *H. germanica* were fitted with a Haldane model, as modified by Papacek et al. (2010) and Marchetti et al. (2013) but without photoinhibition (eq. 3).

$$P(I) = \frac{Pm \times I}{I + Ek} - Rd \qquad \text{(eq. 3)}$$

Where Pm is the maximum photosynthetic capacity (pmol O_2 $cell^{-1}$ d^{-1}), I the photon flux density (µmol photons m^{-2} s^{-1}), Ek the half-saturation constant (µmol photons m^{-2} s^{-1}) and Rd the dark respiration, expressed as an oxygen consumption (pmol O_2 $cell^{-1}$ d^{-1}). The initial slope of the P–I (Photosynthesis –Irradiance) curve at limiting irradiance α (pmol O_2 $cell^{-1}$ day^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$)) and the compensation irradiance Ic were calculated according to equations 4 and 5.

$$Ic = \frac{Ek \times Rd}{Pm - Rd} \qquad \text{(eq. 4)}$$

$$\alpha = \frac{Rd}{Ic} \qquad \text{(eq. 5)}$$

Oxygen measurements were repeated at 300 µmol photons m^{-2} s^{-1} and in the dark at the end of the experiment (7 days of incubation) for all different light treatments (D, LL, HL) using 10 specimens, to assess their production or consumption of oxygen at these two light levels (300 µmol photons m^{-2} s^{-1} and in the dark) in all treatments.

For All conditions (D, LL, HL and Dark-RLC) *Fv/Fm* were measured daily at early afternoon, after a one-hour dark adaptation period and were done in triplicate for each Petri Dish.

Rapid light curves were also carried out in all light treatments at the beginning and end of the experiment, after one-hour dark adaptation for the 2 tested species. Additionally, RLC were carried out daily in an extra triplicate kept in the dark (Dark-RLC) throughout the duration of the experiment.

Results

Comment :Ln. 24-25 Please add ", respectively", after "This resulted in cytoplasmic biovolumes equal to 1.20 _ 107 _m3 (SD = 25 3.9 _ 106 _m3) and 1.01 _ 107 _m3 (SD = 3.65 _

106 _m3)"
Reply: Changed as suggested.

Page 9
Comment: Ln. 5-6: Figure 2 only shows data on H. germanica fluorescence. Please, amend the sentence accordingly.
Reply: Corrected as requested**,** "Foraminiferal" was replaced by "*H. germanica*"

Comment: Ln. 15-19: The manuscript would improve if all these numbers were put in a table or graph.
Reply: We believe it would take excessive place to include a table for only 2 values but would be happy to do it if the editor wishes.

Page 10
Comment: Ln. 20-22: Please, clarify why data is not shown. Maybe authors could add these results to supplementary material, if possible. The manuscript would benefit from a figure plotting the relative difference of Fv/Fm between light treatments, specially low and high light levels.
Reply: Figure 7 already plots *Fv/Fm* differences between treatments; since all *H. germanica* treatments start from the same *Fv/Fm* values it is easy to compare differences between light treatments.
Information about the light effect on *A. tepida* comes from a preliminary experiment we carried out where we saw that even low light levels would have a very strong effect on *A. tepida Fv/Fm* values. We decided to run the real experiment with just *A. tepida* in the dark because it would gives a better idea of how long the chloroplasts would be stable without any light effect, i.e. in their optimal conditions. We could repeat the experiment with just *A. tepida* but it seems a bit out of scope since our main objective was to investigate *H. germanica* and a similar experiment (i.e. with light levels) with just *A. tepida* would be a collection of zeros after 1-2 days.

Page 11
Comment: Ln. 7-12: Figure 4 does not show this result.
Reply: The words "capture photons" were deleted from the sentence to fit better the data presented in Fig. 4:
"Furthermore, *H. germanica* has the ability to produce oxygen from low to relatively high irradiance, as shown by the low compensation point (Ic) of 24 µmol photons m^{-2} s^{-1} and the high onset of light saturation (>300 µmol photons m^{-2} s^{-1}) (Figure 4)"

Page 12
Comment: Ln. 21-23: This is expected, given that exposure to high light levels generates a lot of reactive oxygen species inside the chloroplasts. This should be mentioned and discussed.
Reply: Line 21-23 concern A. tepida which was maintained in the dark, so we do not understand this comment.
However, we agree that ROS could have an impact on both kleptoplasts and foramninifera.
Therefore we added a sentence in the discussion P14 L26: " In *H. germanica* exposed to HL it is also possible that reactive oxygen species (ROS) production rates of the sequestered chloroplasts might exceed the foraminifera capacity to eliminate those ROS, thus inducing permanent damage to the foraminifera. This ROS production could also eventually damage the kleptoplasts resulting in higher kleptoplast degradation rates."

Comment: Ln. 21-23: A. tepida has no capacity to retain chloroplast according to the results, as fluoresce only persists for a couple of days, and even though some fluorescence is detected, the

functionality was not analysed. Therefore, chloroplasts might be present for a couple of days, but not functional. The O2 consumption is not a proxy of functionally of kleptoplasts, and just because respiration rates were lower at 300 uE does not mean that chloroplasts were functioning. Be careful not to mix up correlation with causation.
Reply: The functionality was measured using *Fv/Fm*. Although high *Fv/Fm* values are not an absolute guarantee that all photosynthetic processes are functional (e.g. the Calvin cycle) we can be sure that low or zero *Fv/Fm* are a result of impaired or absence of photosynthesis.

Ln. 28-32: This is very interesting. I wonder what caused this significant reduction in tolerance in these chloroplasts. Maybe the lack of a cellular protection? Would be great to see a sentence or two with thoughts from the authors of why such dramatic decrease. It would be possible that in situ the chloroplast are not functional at all.
Reply: Agreed it is very interesting however we can only make suppositions and some of them are discussed in this article mainly from P14 line 1 to 10
Please also note that irradiance levels are very quickly attenuated in muddy sediments. For example, at an ambient light of 1500 µmol photons m^{-2} s^{-1}, light levels at 500 µm deep will be reduced to 75 µmol photons m^{-2} s^{-1} in muddy sediments with a light attenuation coefficient of 8 mm^{-1}.

Effect of light on photosynthetic efficiency of sequestered chloroplasts in intertidal benthic foraminifera (*Haynesina germanica* and *Ammonia tepida*)

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Abstract

Some benthic foraminifera have the ability to incorporate functional chloroplasts from diatoms (kleptoplasty). Our objective was to investigate chloroplast functionality of two benthic foraminifera (*Haynesina germanica* and *Ammonia tepida*) exposed to different irradiance levels (0, 25, 70 µmol photon m^{-2} s^{-1}) using spectral reflectance, epifluorescence observations, oxygen evolution and pulse amplitude modulated (PAM) fluorometry (maximum photosystem II quantum efficiency (*Fv/Fm*) and rapid light curves (RLC)). Our results clearly showed that *H. germanica* was capable of using its kleptoplasts for more than one week while *A. tepida* showed very limited kleptoplastic ability with maximum photosystem II quantum efficiency (*Fv/Fm* = 0.4), much lower than *H. germanica* and decreasing to zero in only one day. Only *H. germanica* showed net oxygen production with a compensation point at 24 µmol photon m^{-2} s^{-1} and a production up to 1000 pmol O_2 $cell^{-1}$ day^{-1} at 300 µmol photon m^{-2} s^{-1}. *Haynesina germanica Fv/Fm* slowly decreased from 0.65 to 0.55

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1 in 7 days when kept in darkness; however, it quickly decreased to 0.2 under high light.
2 Kleptoplast functional time was thus estimated between 11 and 21 days in darkness and
3 between 7 and 8 days at high light. These results emphasize that studies about foraminifera
4 kleptoplasty must take into account light history. Additionally, this study showed that the
5 kleptoplasts are unlikely to be completely functional, thus requiring continuous chloroplast
6 resupply from foraminifera food source. The advantages of keeping functional chloroplasts
7 are discussed but more information is needed to better understand foraminifera feeding
8 strategies.

9 **1 Introduction**

10 Benthic foraminifera colonize a wide variety of sediments from brackish waters to deep-sea
11 environments and can be the dominant meiofauna in these ecosystems (Gooday 1986; Pascal
12 et al. 2009). They may play a relevant role in the carbon cycle in sediments from deep sea
13 (Moodley et al. 2002) to brackish environments (Thibault de Chanvalon et al. 2015). Their
14 minor role in organic carbon cycling in aerobic sediments, compared to bacteria, contrasts
15 with their strong contribution to anaerobic organic matter mineralisation (Geslin et al. 2011)
16 and they can be responsible for up to 80% of benthic denitrification (Pina-Ochoa et al. 2010;
17 Risgaard-Petersen et al. 2006).

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18 Some benthic foraminiferal species are known to sequester chloroplasts from their food
19 source and store them in their cytoplasm (Lopez 1979; Bernhard and Bowser, 1999) in a
20 process known as kleptoplasty (Clark et al. 1990). A kleptoplast is thus a chloroplast,
21 functional or not, that was "stolen" and integrated by an organism. Kleptoplastic foraminifera
22 are found in intertidal sediments (e.g. *Haynesina*, *Elphidium* and *Xiphophaga*) (Lopez 1979;
23 Correia and Lee 2000, 2002a, b; Goldstein et al. 2010; Pillet et al. 2011), low oxygenated
24 aphotic environments (*Nonionella, Nonionellina, Stainforthia*) (Bernhard and Bowser 1999;
25 Grzymski et al. 2002) and shallow-water sediments (*Bulimina elegantissima*) (Bernhard and
26 Bowser, 1999). The role of chloroplasts sequestered by benthic foraminifera is poorly known
27 and photosynthetic functions have only been studied in a few mudflat species (*Elphidium*
28 *williamsoni*, *Elphidium excavatum* and *Haynesina germanica*) (Lopez 1979; Correia and Lee
29 2000, 2002a, b; Cesbron et al. submitted). Amongst the deep-sea benthic foraminifer living in
30 the aphotic zone, only *Nonionella stella* has been studied (Grzymski et al. 2002). The authors
31 suggest that the sequestered chloroplasts in this species may play a role in the assimilation of
32 inorganic nitrogen, even when light is absent. It has also been hypothesised that chloroplast

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retention may play a major role in foraminiferal survival when facing starvation periods or in anoxic environments (Cesbron et al. submitted). Under these conditions, kleptoplasts could potentially be used as a carbohydrate source, and participate in inorganic nitrogen assimilation (Falkowski and Raven 2007) or, when exposed to light, to produce oxygen needed in foraminiferal aerobic respiration (Lopez 1979).

Foraminifera pigment and plastid ultrastructure studies have shown that the chloroplasts are sequestered from their food source, i.e. mainly from diatoms (Lopez 1979; Knight and Mantoura 1985; Grzymski et al. 2002; Goldstein 2004). This was confirmed by experimental feeding studies (Correia and Lee 2002a; Austin et al. 2005) and by molecular analysis of kleptoplastic foraminifera from different environments (Pillet et al. 2011, Tsuchiya et al. 2015). Foraminifera from intertidal mudflat environments (e.g. *H. germanica*, *A. tepida*) feed mostly on pennate diatoms (Pillet et al. 2011) which are the dominant microalgae in intertidal mudflat sediments (MacIntyre et al. 1996; Jesus et al. 2009). Furthermore, in this transitional coastal environments (e.g. estuaries, bays, lagoons) *A. tepida* and *H. germanica* are usually the dominant meiofauna species in West Atlantic French coast mudflats (Debenay et al. 2000, 2006; Morvan et al. 2006; Bouchet et al. 2009; Pascal et al. 2009; Thibault de Chanvalon et al. 2015). Their vertical distribution in the sediment is characterised by a clear maximum density at the surface (Alve and Murray 2001; Bouchet et al. 2009; Thibault de Chanvalon et al. 2015) with access to light, followed by a sharp decrease in the next two centimetres (Thibault de Chanvalon et al., 2015).

Foraminiferal kleptoplast retention times can vary from days to months (Lopez 1979; Lee et al. 1988; Correia and Lee 2002b; Grzymski et al. 2002). The source of this variation is poorly known but longer kleptoplast retention times were found in dark treatments (Lopez 1979; Correia and Lee 2002b), thus suggesting an effect of light exposure, similar to what is observed in kleptoplastic sacoglossans (Trench et al. 1972; Clark et al. 1990; Evertsen et al. 2007; Vieira et al. 2009), possibly related to the absence of some components of the kleptoplast photosynthetic protein complexes in the host (Eberhard et al. 2008).

Most recent studies on kleptoplastic foraminifera focused on feeding, genetics and microscopic observation related to chloroplast acquisition (e.g., Austin et al. 2005, Pillet et al. 2011, Pillet and Pawlowski 2013). To our knowledge little is known about the effects of abiotic factors on photosynthetic efficiency of sequestered chloroplasts in benthic foraminifera, particularly on the effect of light intensity on kleptoplast functionality. Non-

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1 invasive techniques are ideal to follow photosynthesis and some have already been used to
2 study foraminifera respiration and photosynthesis, e.g. oxygen evolution by microelectrodes
3 (Rink et al. 1998; Geslin et al. 2011) or ^{14}C radiotracer (Lopez, 1979). Recently, pulse
4 amplitude modulated (PAM) fluorometry has been used extensively in the study of
5 kleptoplastic sacoglossans (Vieira et al. 2009; Costa et al. 2012; Jesus et al. 2010; Serodio et
6 al. 2010; Curtis et al. 2013; Ventura et al. 2013). This non-invasive technique has the
7 advantage of estimating relative electron transport rates (rETR) using rapid light curves
8 (RLC) and photosystem II (PSII) maximum quantum efficiencies (*Fv/Fm*) very quickly and
9 without incubation periods. The latter parameter has been shown to be a good parameter to
10 estimate PSII functionality (e.g. Vieira et al. 2009; Jesus et al. 2010; Serodio et al. 2010;
11 Costa et al. 2012; Curtis et al. 2013; Ventura et al. 2013).

12 The objective of the current work was to investigate the effect of irradiance levels on
13 photosynthetic efficiency and chloroplast functional times of two benthic foraminifera feeding
14 in the same brackish areas, *H. germanica*, which is known to sequester chloroplasts and *A.*
15 *tepida*, not known to sequester chloroplasts. These two species were exposed to different
16 irradiance levels during one week and chloroplast efficiency was measured using
17 epifluorescence, oxygen microsensors and PAM fluorometry.

18

19 ## 2 Materials and methods

20 ### 2.1 Sampling

21 *Haynesina germanica* and *A. tepida* were sampled in January 2015 in Bourgneuf Bay
22 (47.013°N, 2.019°W), a coastal bay with a large mudflat situated south of the Loire estuary on
23 the French west coast. In this area, all specimens of *A. tepida* belong to genotype T6 of
24 Hayward et al. (2004) (Schweizer pers. comm.). In the field, a large amount (~20 kg) of the
25 upper sediment layer (roughly first 5 mm) was sampled and sieved over 300 and 150 µm
26 meshes using *in situ* sea-water. The 150 µm fraction was collected in dark flasks and
27 maintained overnight in the dark at 18°C in the laboratory. No additional food was added. In
28 the following day, sediment with foraminifera was diluted with filtered (GF/C, 1.2 µm,
29 Whatman) autoclaved sea-water (temperature: 18°C and salinity: 32) and *H. germanica* and
30 *A. tepida* in healthy conditions (i.e. with cytoplasm inside the test) were collected with a brush
31 using a stereomicroscope (Leica MZ 12.5). The selected specimens were rinsed several times

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using Bourgneuf bay filtered-autoclaved seawater to minimize bacterial and microalgal contamination.

2.2 Size and biovolume determination

Foraminifera test mean maximal elongation (µm, the length of the axes going from the last chamber to the other side of the test and passing by the umbilicus) was measured using a micrometer mounted on a Leica stereomicroscope (MZ 12.5). Mean foraminiferal volume was approximated with the equation of a half sphere, which is the best resembling geometric shape for *H. germanica* and *A. tepida* (Geslin et al. 2011). The cytoplasmic volume (or biovolume) was then estimated by assuming that the internal test volume corresponds to 75% of the total foraminiferal test volume (Hannah et al. 1994).

2.3 Spectral reflectance

Pigment spectral reflectance was measured non-invasively to determine and compare the relative pigment composition on 50 fresh specimens of *H. germanica*, on 50 fresh specimens of *A. tepida* and on a benthic diatom as explained in Jesus et al. (2008). Concisely, a USB2000 (Ocean Optics, Dunedin, FL, USA) spectroradiometer with a VIS-NIR optical configuration controlled by OObase32 software (Ocean Optics B.V., Duiven, the Netherlands) was used. The spectroradiometer sensor was positioned so that the surface was always viewed from the nadir position. Foraminiferal reflectance spectra were calculated by dividing the upwelling spectral radiance from the foraminifera (Lu) by the reflectance of a clean polystyrene plate (Ld) for both of which the machine dark noise (Dn) was subtracted (eq. 1).

$$\rho = \frac{(Lu - Dn)}{(Ld - Dn)} \qquad \text{(eq.1)}$$

2.4 Image analysis

Foraminifera kleptoplast fluorescence was measured using epifluorescence microscopy (×200, Olympus Ax70 with Olympus U-RFL-T, excitation wave length 485 nm). Two Tif images (1232 × 964 px) of each foraminifer were taken (one bright field photography and one epifluorescence photography) using LUCIA G^{TM} software. The bright field photography was used to trace the contours of the foraminifer and an ImageJ macro was used to extract the

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mean pixel values of the corresponding epifluorescence photography. Higher mean pixel values corresponded to foraminifera emitting more fluorescence and thus, as a proxy, contain more chlorophyll. In a RGB image each channel contains pixels between 0 and 255 values. The majority of the information regarding chlorophyll fluorescence is encoded in the red channel, therefore the green and blue channel were discarded and only the red channel was kept. The images from the different treatments were directly comparable as all images were taken using the same acquisition settings. Thus, the mean red pixel values were used as a proxy for chlorophyll fluorescence.

2.5 Oxygen measurements

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Oxygen was measured using advanced Clark type oxygen microelectrodes of 50 µm in diameter (Revsbech, 1989) (OXI50 - Unisense, Denmark). Electrodes were calibrated with a solution of sodium ascorbate at 0.1 M (0%) and with seawater saturated with oxygen by bubbling air (100%). Foraminiferal photosynthesis and oxygen respiration rates were measured following Høgslund et al. (2008) and Geslin et al. (2011). Measurements were carried out in a micro-tube made from glass Pasteur pipette tips with an inner diameter of 1 mm. The micro-tube was fixed to a small vial, filled with filtered autoclaved seawater from Bourgneuf Bay. The vial was placed in an aquarium with water kept at room temperature (18°C). A small brush was used to position a pool of 7 to 10 foraminifera in the glass micro-tube after removing air bubbles. Oxygen micro-profiles started at a distance of 200 µm above the foraminifers to avoid oxygen turbulences often observed around the foraminifers. Measurements were registered when the oxygen micro-profiles were stable; they were then repeated five time in the centre of the micro-tube, using 50 µm steps until 1000 µm away from the foraminifers (Geslin et al. 2011). The oxygen flux (J) was calculated using the first law of Fick:

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$$J = -D \times \frac{dC}{dx} \qquad \text{(eq. 2)}$$

Where D is the oxygen diffusion coefficient (cm² s^{-1}) at experimental temperature (18°C) and salinity (32) (Li and Gregory, 1974), and dC/dx is the oxygen concentration gradient (pmol O_2 cm^{-1}). The O_2 concentration gradients were calculated with the oxygen profiles and using the R^2 of the regression line to determine the best gradient. Total O_2 consumption and production rates were calculated as the product of O_2 fluxes by the surface area of the micro-

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tube and subsequently divided by the foraminifera number to finally obtain the cell specific rate (pmol O_2 cell^{-1} d^{-1}) (Geslin et al. 2011).

2.6 Fluorescence

All pulse amplitude modulated fluorescence measurements were carried out with a Water-PAM fluorometer (Walz, Germany) using a blue measuring light. Chloroplast functionality was estimated by monitoring PSII maximum quantum efficiency (*Fv/Fm*) and by using P-I rapid light curves (RLC, e.g., Perkins et al. (2006)) parameters (α, initial slope of the RLC at limiting irradiance; rETRmax, maximum relative electron transport rate; Ek, light saturation coefficient; and Eopt, optimum light) (Platt et al. 1980). Rapid light curves were constructed using eight incremental light steps (0, 4, 15, 20, 36, 48, 64, 90 and 128 μmol photons m^{-2} s^{-1}), each lasting 30 seconds. The PAM probe was set up on a stand holder at a 2 mm distance from a group of 10 foraminifera.

2.7 Experimental design

Haynesina germanica, a species known to sequester chloroplasts, were placed in plastic Petri dishes and starved during 7 days under three different light conditions: dark (D and Dark-RLC), low light (LL, 25 μmol photons m^{-2} s^{-1}) and high light (HL, 70 μmol photons m^{-2} s^{-1}); whereas for comparison, *A. tepida*, a foraminifer not known to sequester chloroplasts was starved but only exposed to the dark condition. A short term experiment was thus carried out (7 days) to study the effect of light on healthy specimens rather than the effect of starvation. For each condition, ten specimens were used per replicate and three replicates per light treatment; furthermore all plastic Petri dishes were filled with Bourgneuf bay filtered-autoclaved seawater. This experiment was carried out in a thermo-regulated culture room at 18° C, equipped with cool light fluorescent lamp (Lumix day light, L30W/865, Osram) and using a 14:10 h (Light:Dark) photoperiod. The distances between the light and the experimental conditions were assessed using a light-meter and a quantum sensor (ULM-500 and MQS-B of Walz) to obtain the desirable light intensities. Concerning the dark condition, the Petri dishes were place in a box covered with aluminium foil.

Haynesina germanica kleptoplast fluorescence was measured using epifluorescence microscopy, as explain above, before and after the different light treatments. At the beginning of the experiment it was done on 30 independent specimens to assess the natural and initial

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variation of *Haynesina germanica* kleptoplast fluorescence. At the end of the experiment, the measurement were done on all foraminifera exposed to the different light condition (a total of 30 specimens per condition). This was also measured on *A. tepida*, but results are not presented because no chlorophyll fluorescence was observed at the end of the experiment.

Haynesina germanica and *A. tepida* oxygen production and consumption were measured at the beginning of the experiment on three independent replicates with 7 specimens in each replicate. Six different light steps were used to measure O_2 production (0, 25, 50, 100, 200 and 300 µmol photons m^{-2} s^{-1}) for *H. germanica* and only two light steps (0 and 300 µmol photons m^{-2} s^{-1}) for *A. tepida*. Photosynthetic activity (P) data of *H. germanica* were fitted with a Haldane model, as modified by Papacek et al. (2010) and Marchetti et al. (2013) but without photoinhibition (eq. 3).

$$P(I) = \frac{Pm \times I}{I + Ek} - Rd \qquad \text{(eq. 3)}$$

Where Pm is the maximum photosynthetic capacity (pmol O_2 $cell^{-1}$ d^{-1}), I the photon flux density (µmol photons m^{-2} s^{-1}), Ek the half-saturation constant (µmol photons m^{-2} s^{-1}) and Rd the dark respiration, expressed as an oxygen consumption (pmol O_2 $cell^{-1}$ d^{-1}). The initial slope of the P–I (Photosynthesis –Irradiance) curve at limiting irradiance α (pmol O_2 $cell^{-1}$ day^{-1} (µmol photons m^{-2} $s^{-1})^{-1}$)) and the compensation irradiance Ic were calculated according to equations 4 and 5.

$$Ic = \frac{Ek \times Rd}{Pm - Rd} \qquad \text{(eq. 4)}$$

$$\alpha = \frac{Rd}{Ic} \qquad \text{(eq. 5)}$$

Oxygen measurements were repeated at 300 µmol photons m^{-2} s^{-1} and in the dark at the end of the experiment (7 days of incubation) for all different light treatments (D, LL, HL) using 10 specimens, to assess their production or consumption of oxygen at these two light levels (300 µmol photons m^{-2} s^{-1} and in the dark) in all treatments.

For All conditions (D, LL, HL and Dark-RLC) *Fv/Fm* were measured daily at early afternoon, after a one-hour dark adaptation period and were done in triplicate for each Petri Dish.

Rapid light curves were also carried out in all light treatments at the beginning and end of the experiment, after one-hour dark adaptation for the 2 tested species. Additionally, RLC were

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Haynesina germanica, a species known to sequester chloroplasts, were placed in plastic Petri dishes and starved during 7 days under three different light conditions: dark (D and Dark-RLC, 3×10 foraminifera), low light (LL, 25 µmol photons m^{-2} s^{-1}, 3×10 foraminifera) and high light (HL, 70 µmol photons m^{-2} s^{-1}, 3×10 foraminifera) on a 10:14 h (Light:Dark) cycle; whereas for comparison, *A. tepida* (3×10 foraminifera), a foraminifer not known to sequester chloroplasts were placed in plastic Petri dishes and only starved under dark conditions.

carried out daily in an extra triplicate kept in the dark (Dark-RLC) throughout the duration of the experiment.

2.8 Statistical analysis

Data are expressed as mean ± standard deviation (SD) when n = 3 or standard error (SE) when n = 30. Statistical analyses consisted of a t-test to compare the foraminifera test mean maximal elongation, a non parametric test (Kruskal Wallis) to compare the mean chlorophyll fluorescence of the foraminifera exposed to the different experimental conditions and a multifactor (experimental conditions (D, LL, HL), irradiance (0-300 µmol photons m^{-2} s^{-1})) analysis of variance (ANOVA) with a Fisher's LSD test to compare the respiration rates at the end of the experiment. Differences were considered significant at $p<0.05$. Statistical analyses were carried out using the Statgraphics Centurion XV.I (StatPoint Technologies, Inc.) software.

3 Results

3.1 Size and biovolume

Ammonia tepida specimens were larger than *H. germanica* with a mean maximal elongation of 390 ± 42 µm (SD, n = 34) and 366 ± 45 µm (SD, n = 122), respectively ($p < 0.01$, $F_{121,33} = 1.15$). This resulted in cytoplasmic biovolumes equal to $1.20 \times 10^7 \pm 3.9 \times 10^6$ µm^3 (SD) and $1.01 \times 10^7 \pm 3.65 \times 10^6$ µm^3 (SD), respectively.

3.2 Chloroplast functionality

Fresh *Haynesina germanica* and *A. tepida* showed very different spectral reflectance signatures (Figure 1). *Haynesina germanica* showed a typical diatom spectral signature with high reflectance in the infrared region (>740 nm) and clear absorption features around 585, 630 and 675 nm; the absorption feature around 675 nm correspond to the presence of chlorophyll *a*; the 585 nm feature is the result of fucoxanthin and the 630 nm absorption feature is the result of chlorophyll *c* (arrows, Figure 1). *Ammonia tepida* showed no obvious pigment absorption features apart from 430 nm (Figure 1).

Epifluorescence images showed a clear effect of the different light treatments (Dark, Low Light, Hight Light) on *H. germanica* chlorophyll fluorescence (Figure 2). Visual observations showed a clear decrease in chlorophyll fluorescence for the LL and HL treatments from the

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Supprimé: <#>Oxygen measurements¶
Oxygen was measured at the beginning and end of the experiment using advanced Clark type oxygen microelectrodes of 50 µm in diameter (Revsbech, 1989) (OXI50 - Unisense, Denmark). Electrodes were calibrated with a solution of sodium ascorbate at 0.1 M (0%) and with seawater saturated with oxygen by bubbling air (100%). Foraminiferal photosynthesis and oxygen respiration rates were measured following Høgslund et al. (2008) and Geslin et al. (2011). Measurements were carried out in a micro-tube made from glass Pasteur pipette tips with an inner diameter of 1 mm. The micro-tube was fixed to a small vial, filled with filtered autoclaved seawater from Bourgneuf Bay. The vial was placed in an aquarium with water kept at room temperature (18°C). A small brush was used to position 7 to 10 foraminifera in the glass micro-tube after removing air bubbles. Oxygen micro-profiles started at a distance of 200 µm above the foraminifers in the centre of the micro-tube and measurements were carried out in 50 µm steps until 1000 µm away from the foraminifers (Geslin et al. 2011). For each condition, three replicates were performed with different specimens. The oxygen flux (J) was calculated using the first law of Fick: ¶

$$J = -D \times \frac{dC}{dx} \quad \text{(eq. 2)}$$

Where D is the oxygen diffusion coefficient (cm^2 s^{-1}) at experimental temperature (18°C) and salinity (32) (Li and Gregory, 1974), and dC/dx is the oxygen concentration gradient (pmol O_2 cm^{-1}). The O_2 concentration gradients were calculated using the oxygen profiles. Total O_2 consumption and production rates were calculated as the product of O_2 fluxes by the surface area of the micro-tube and subsequently divided by the foraminifera number to finally obtain the cell specific rate (pmol O_2 $cell^{-1}$ d^{-1}) (Geslin et al. 2011). ¶
Haynesina germanica and *A. tepida* oxygen production and consumption were measured at the beginning of the experiment using 3 replicates of 7 foraminifera each. Six different light steps were used to measure O_2 production (0, 25, 50, 100, 200 and 300 µmol photons m^{-2} s^{-1}) for *H. germanica* and two light steps (0 and 300 µmol photons m^{-2} s^{-1}) for *A. tepida*. Photosynthetic activity (P) data of *H. germanica* were fitted with a Haldane model, as modified b ...

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beginning of the experiment (Figure 2A) to the end of a 7 day period of light exposure (Figure 2C and 2D, respectively). Samples kept in the dark did not show an obvious decrease but showed a more patchy distribution compared to the beginning of the experiment (Figure 2B). This was confirmed by a non-parametric test (Kruskal Wallis) showing that the differences in chlorophyll *a* fluorescence were significant ($p < 0.01$, Df = 3, Figure 3). It is also noteworthy to mention that there was a large individual variability within each treatment leading to large standard errors in spite of the number of replicates ($n = 30$).

Oxygen measurements carried out at the beginning of the experiment (T0) differed considerably between the two species. *Ammonia tepida* did not show any net oxygen production although respiration rates measured at 300 µmol photons m^{-2} s^{-1} were lower (2485 ± 245 pmol O_2 $cell^{-1}$ d^{-1}) than the ones measured in the dark (3531 ± 128 pmol O_2 $cell^{-1}$ d^{-1}) ($F_{2,2} = 3.7$, $p = 0.02$). *Haynesina germanica* showed lower dark respiration rates (1654 ± 785 pmol O_2 $cell^{-1}$ d^{-1}) and oxygen production quickly increased with irradiance, showing no evidence of photoinhibition within the light range used (Figure 4). Compensation irradiance (Ic) was reached very quickly, as low as 24 µmol photons m^{-2} s^{-1} (95% coefficient bound: 17-30 µmol photons m^{-2} s^{-1}, values calculated from the fitted model eq.4) and the half-saturation constant (Ek) was also reached at very low light levels, i.e. at 17 µmol photons m^{-2} s^{-1}. No photoinhibition was observed under the experimental light conditions (0 to 300 µmol photons m^{-2} s^{-1}), which resulted in an estimation of ~2800 pmol O_2 $cell^{-1}$ d^{-1} for maximum photosynthetic capacity. The P-I curve initial slope at limiting irradiance (α) was estimated at 70 pmol O_2 $cell^{-1}$ d^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$ (95% coefficient bound: 58-88).

Oxygen measurements carried out at the end of the experiment (T7) showed significant different dark and light respiration rates, with light respiration being lower than dark respiration but not reaching net oxygen production rates (D, LL, HL) (Table 1). Moreover, respiration rates were different between conditions ($p < 0.001$), with significantly lower respiration rates of specimens incubated under High Light conditions than those under Dark and Low Light conditions ($p < 0.05$, Fisher's LSD test).

PAM fluorescence rapid light curve (RLC) parameters (α, rETRmax, Ek and Eopt) showed significant differences between foraminiferal species and over the duration of the experiment (Figures 5 and 6). Highest rETRmax, α and Eopt were always observed in *H. germanica.* After only one starvation day *A. tepida* RLC parameters dropped to zero or close to zero. Contrastively, *H. germanica* RLC parameters showed a slow decrease throughout the

experiment (Figures 5 and 6) with rETRmax and α decreasing from 6 to 4 and 0.22 to 0.15, respectively (Figures 6A and B). The parameters Ek and Eopt stayed constant over the 7 days of the experiment, with values oscillating around 30 and 90, respectively (Figures 6C and D).

PSII maximum quantum yields (*Fv/Fm*) were clearly affected by light and time (Figure 7). Both species showed high initial *Fv/Fm* values, i.e. > 0.6 and 0.4 for *H. germanica* and *A. tepida,* respectively (Figure 7). However, while *A. tepida Fv/Fm* values quickly decreased to zero after only one starvation day, *H. germanica* exhibited a large variability between light conditions (D, LL, HL) throughout the duration of the experiment (Figure 7); decreasing from 0.65 to 0.55 in darkness (D), from 0.65 to 0.35 under low light (LL) conditions and from 0.65 to 0.20 under high light (HL). Using these *Fv/Fm* decreases, *H. germanica* kleptoplast functional times were estimated between 11-21 days in the dark (D), 9-12 days in low light (LL) and 7-8 days in high light (HL); depending if an exponential or linear model was applied. *Ammonia tepida* chloroplast functional times were estimated between 1-2 days (exponential and linear model, respectively) and light exposure reduced the functional time to less than one day (data not shown).

4 Discussion

4.1 Chloroplast functionality

Our results clearly show that only *H. germanica* was capable of carrying out net photosynthesis. *Haynesina germanica* had typical diatom reflectance spectra (Figure 1), showing the three major diatom pigment absorption features: chlorophyll *a*, chlorophyll *c*, and fucoxanthin (Meleder et al. 2003; Jesus et al. 2008; Kazemipour et al. 2012; Meleder et al. 2013). Conversely, in *A. tepida* these absorption features were not detected, suggesting that diatom pigments ingested by this species were quickly digested and degraded to a degree where they were no longer detected by spectral reflectance measurements. These non-destructive reflectance measurements are thus in accordance with other studies on benthic foraminifera pigments by HPLC showing that *H. germanica* feed on benthic diatoms (Knight and Mantoura, 1985). Similarly, Knight and Mantoura (1985) also detected higher concentrations and less degraded diatom pigments in *H. germanica* than in *A. tepida.*

Furthermore, *H. germanica* has the ability to produce oxygen from low to relatively high irradiance, as shown by the low compensation point (Ic) of 24 µmol photons m^{-2} s^{-1} and the

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1 high onset of light saturation (>300 µmol photons m^{-2} s^{-1}) (Figure 4). Thus, *H. germanica*
2 seems to be well adapted to cope with the high light variability observed in intertidal
3 sediments that can range from very high irradiance levels (>1000 µmol photons m^{-2} s^{-1}) at the
4 surface of the sediment during low tide to very low levels within the sediment matrix or
5 during high tide in turbid mudflat waters. *Ammonia tepida* was found to carry out aerobic
6 respiration, but respiration rates measured at 300 µmol photons m^{-2} s^{-1} were lower than those
7 measured in the dark. We thus suppose that in *A. tepida* oxygen production by ingested
8 diatom or chloroplasts might be possible, provided that this species is constantly supplied
9 with fresh diatoms. However, another possibility to explain this reduction in oxygen
10 consumption could be a decrease of its metabolism or activity under light exposure. The light
11 and dark oxygen production or consumption values measured for both species are in
12 accordance with previous studies (Geslin et al. 2011).

13 According to Lopez (1979), measured oxygen data can be used to estimate *H. germanica*
14 carbon fixation rates. Thus, using 1000 pmol O_2 $cell^{-1}$ d^{-1} at 300 µmol photons m^{-2} s^{-1}, ~200 to
15 4000 cells per 50 cm^3 in the top 0.5 cm (Morvan et al. 2006; Bouchet et al. 2007) and
16 assuming that photosynthesis produced one mol O_2 per mol of C fixed, *H. germanica* primary
17 production would be between 1.8×10^{-5} and 4.0×10^{-4} mol C m^{-2} d^{-1}. This is a very low value
18 compared to microphytobenthos primary production in Atlantic mudflat ecosystems, which
19 usually range from 1.5 to 5.9 mol C m^{-2} d^{-1} (e.g. Brotas and Catarino 1995, reviewed in
20 MacIntyre et al. 1996). The estimated values represent thus less than 0.1% of
21 microphytobenthos fixated carbon and are in the same range of values than what has been
22 described by Lopez (1979) using ^{14}C radioactive tracers. These results should be interpreted
23 with caution because a wide variety of factors probably affect *H. germanica in situ* primary
24 production, e.g. diatom availability, kleptoplast densities, nutrient supply, light exposure, sea
25 water turbidity and migration capability are all factors that can potentially affect *H.*
26 *germanica* kleptoplast functionality. Nevertheless, although carbon fixation seems not to be
27 relevant at a global scale, the oxygen production could be important at a microscale and
28 relevant in local mineralization processes in/on mudflat sediments (e.g. iron, ammonium,
29 manganese).

30 At sampling time (T0) *H. germanica* rETR and *Fv/Fm* values were similar to
31 microphytobenthic species (i.e. *Fv/Fm* > 0.65) (Perkins et al. 2001), suggesting that the
32 kleptoplast PSII and electron transport chain were not much affected after incorporation in the

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foraminifers' cytoplasm. In contrast, *A. tepida Fv/Fm* and RLC parameters were already much lower on the sampling day and quickly decreased to almost zero within 24 hours, suggesting that plastids were not stable inside the *A. tepida* cytoplasm. Complete diatoms inside *A. tepida* were already observed in feeding studies (Le Kieffre, pers. com), this low *Fv/Fm* value might thus come from recently ingested diatoms by *A. tepida*. *Fv/Fm* has previously been used to determine kleptoplast functional times and to follow decrease in kleptoplast efficiency in other kleptoplastic organisms, e.g. the sea slug *Elysia viridi*s (Vieira et al. 2009). *Fv/Fm* measurements carried out on *H. germanica* at different light conditions showed that light had a significant effect on the estimation of kleptoplast functional time, with the longest functional time estimated at 21 days for dark condition. This time frame would qualify *H. germanica* as a long term kleptoplast retention species (Clark et al. 1990); however, our seven days estimation for the high light treatment would place *H. germanica* in the medium-term retention group. This clearly shows that light exposure has an important effect on this species kleptoplast functionality. Concerning *A. tepida,* the short dark diatom or chloroplast functional time (<2 days) places this species directly in the short or medium-term retention group.

Additionally, *H. germanica* kept in darkness showed a slow decrease of the RLC parameters, α and rETRmax, throughout the seven experimental days; this decrease is likely related to overall degradation of the light-harvesting complexes and of other components of the photosynthetic apparatus, which gradually induced a reduction of light harvesting efficiency and of carbon metabolism. This decrease was much amplified in low and high irradiance and it should be pointed out that the actual light level of the HL treatment (i.e. 70 µmol photons m^{-2} s^{-1}) is very low as compared to irradiances in their natural environment, which are easily going above 1000 µmol photons m^{-2} s^{-1}, showing that the foraminifera kleptoplasts lack the high photoregulation capacity exhibited by the benthic diatoms that they feed upon (Cartaxana et al. 2013). This is consistent with the observation at the end of the experiment that no net oxygen production was occurring under the different light conditions. Nevertheless, a small difference was still found between dark and light respiration (Table 1), suggesting that some oxygen production was still occurring but it was not sufficient to compensate for the respiration oxygen consumption. We also noticed that the respiration was higher in the foraminifera maintained in low light and dark conditions in comparison to the high light foraminifera. In the line of the lower *Fv/Fm* values observed, this suggests that kleptoplasts and possibly other metabolic pathways might have been damaged by the excess

of light. Clearly, in *H. germanica* light exposure had a strong effect on PSII maximum quantum efficiency and on the retention of functional kleptoplasts (Figure 7), which can explain the absence of net oxygen production after the 7 days of the experiments. Comparable results for *H. germanica* were also obtained by counting the number of chloroplasts over time with cells exposed or not to light (Lopez 1979). One of the most probable explanations for the observed *Fv/Fm* decrease is the gradual inactivation of the protein D1 in PSII reaction centres. This protein is an essential component in the electron transport chain and its turnover rate is frequently the limiting factor in PSII repair rates (reviewed in Campbell and Tyystjärvi 2012). Normally, protein D1 is encoded in the chloroplast and is rapidly degraded and resynthesized under light exposure with a turnover correlated to irradiance (Tyystjärvi and Aro 1996). However, although D1 is encoded by the chloroplast genome, its synthesis and concomitant PSII recovery require further proteins that are encoded by the algal nuclear genome (Yamaguchi et al. 2005). Thus, when D1 turnover is impaired it will induce an *Fv/Fm* decrease correlated to irradiance (Tyystjärvi and Aro 1996) consistent to what was observed in the present study. In another deep sea benthic species (*Nonionella stella*) the D1 and other plastid proteins (RuBisCO and FCP complex) were still present in the foraminifer one year after sampling (Grzymski et al. 2002). This shows that some foraminifera can retain both nuclear (FCP) and chloroplast (D1 and RuBisCO) encoded proteins. However, contrary to *H. germanica*, *N. stella* lives in deeper environments never exposed to light and thus is unlikely to carry out oxygenic photosynthesis (Grzymski et al. 2002). This fundamental difference could explain why kleptoplast functional times are much longer in *N. stella*, reaching up to one year in specimens kept in darkness (Grzymski et al. 2002). On the other hand, it has been shown that isolated chloroplasts are able to function for several months in Sacoglossan sea slugs provided with air and light in aquaria (Green et al. 2001; Rumpho et al. 2001), which demonstrates the existence of interactions between the kleptoplast and the host genomes, and/or of mechanisms facilitating and supporting such long-lasting associations. In *H. germanica* exposed to HL it is also possible that reactive oxygen species (ROS) production rates of the sequestered chloroplasts might exceed the foraminifera capacity to eliminate those ROS, thus inducing permanent damage to the foraminifera. This ROS production could also eventually damage the kleptoplasts resulting in higher kleptoplast degradation rates.

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4.2 Possible advantages of kleptoplasty for intertidal benthic foraminifera

Much is still unknown about the relationship between kleptoplastic benthic foraminifera and their sequestered chloroplasts. The relevance of the photosynthetic metabolism compared to predation or organic matter assimilation is unknown; however, it would be of great interest to understand the kleptoplast role in the foraminiferal total energy budget. Oxygenic photosynthesis comprises multiple reactions leading to the transformation of inorganic carbon to carbohydrates. However, to produce these carbohydrates all the light driven reactions have to be carried out, as well as the Calvin cycle reactions. With fresh kleptoplasts this hypothesis seems possible (e.g. Lopez 1979), especially if the plastid proteins are still present and functional. However, we showed that the maximum quantum efficiency of the PSII decreased quickly under light exposure, suggesting that substantial direct carbohydrate production is unlikely without constant chloroplast replacement. Conversely, the production of intermediate photosynthetate products such as adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) could be possible and would be of metabolic value for the foraminifera. It is also possible that *in situ* the foraminifera have better photoregulation capacities. Not only they will have easy access to fresh diatom chloroplasts, as *H. germanica* is mainly living in the first few mm of the superficial sediment (Alve and Murray 2001, Thibault de Chanvalon et al. 2015), but they will also have the possibility of migrating within the sediment (Gross 2000) using this behavioural feature to enhance their photoregulation capacity, similarly to what is observed in benthic diatoms from microphytobenthic biofilms (e.g. Jesus et al. 2006; Mouget et al. 2008; Perkins et al. 2010). However, below the photic limit (max 2 to 3 mm in estuarine sediments (reviewed in MacIntyre et al. 1996, Cartaxana et al. 2011)) it is unlikely that oxygenic photosynthesis will occur, even if live *H. germanica* are also found below this limit (Thibault de Chanvalon et al. 2015, Cesbron et al. in press).

5 Conclusion

Comparing *H. germanica* with *A. tepida* showed that the former species potentially has the capacity of retaining functional kleptoplasts up to 21 days, much longer than *A. tepida* that showed almost no PSII activity after 24 hours. Nevertheless, the capacity of *H. germanica* to keep functional kleptoplasts was significantly decreased by exposing it even to low irradiance levels, which resulted in low *Fv/Fm* values and decreased oxygen production. This shows clearly that in our experimental conditions, *H. germanica* had reduced photoregulation capacities. These results emphasize that studies on kleptoplast photophysiology of benthic

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Supprimé: Using kleptoplasts, *H. germanica,* like other kleptoplastic organisms (e.g. *Elysia viridis* (Teugels et al. 2008)), is also theoretically capable of assimilating inorganic nitrogen via the glutamine synthetase and glutamate 2-oxo-glutarate aminotransferase (GS-GOGAT) pathways to produce glutamate and glutamine after the successive reduction of nitrate to nitrite and nitrite to ammonia or directly through ammonium uptake (Zehr and Falkowski 1988). However, the first reduction occurs in the diatom cytoplasm via the enzyme nitrate reductase (NR) and not inside the chloroplast. It is not known if *H. germanica* has this enzyme but it is present in *N. stella* (Grzymski et al. 2002). Interestingly, nitrogen (i.e. nitrite and ammonium) assimilation by sacoglossans (e.g. *Elysia viridis*) was observed under light and dark conditions with significantly higher nitrogen assimilation observed under light condition (Teugels et al. 2008). The uptake of ammonium and nitrite are light dependent as their relevant enzymes require kleptoplast electron donors (NiR and GOGAT); i.e. reduced ferredoxin formed in the photosynthetic electron transport chain are used as electron donors in the reaction involving the nitrite reductase [NiR]. Furthermore, the GS metabolic reaction is ATP-dependent, and gene expression of some key enzymes (NiR, GS and GOGAT) is light regulated (Grossman and Takahashi 2001). This suggests that kleptoplasts might also have an added value in providing extra nitrogen source to metabolic pathways in foraminifera under light exposure and also possibly over short periods under dark conditions. It is also noteworthy that ammonium incorporation might take place through the glutamine dehydrogenase (GDH) pathway in the mitochondria that converts glutamate to α-ketoglutarate, which can subsequently be assimilated in the kleptoplast via the GOGAT pathway (Teugels et al. 2008).¶
Diatoms are also known to assimilate organic nitrogen (Antia et al. 1991), to use their ornithine-urea cycle for anaplerotic carbon fixation into nitrogenous compounds (Allen et al. 2012) and some of the benthic species present on mudflats are also able to assimilate organic carbon (Admiraal and Peletier 1979). Apparently some benthic diatoms can alternate between an auto- or heterotrophic metabolism in function of the environment. Analysing the kleptoplast DNA would provide interesting data to determine if foraminifera are capable of selecting facultative heterotrophic diatoms to improve their ability to assimilate dissolved organic compounds. Finally, another possible added value of incorporating kleptoplasts is the possibility of using them as an energy stock to be digested during food-impoverished periods particularly when foraminifera are transported below the photic zone of the sediment by macrofaunal bioturbation.¶

foraminifera must be interpreted with care, as results are strongly influenced by the foraminiferal light history before incubation. Additionally, this study shows that the cellular machinery necessary for chloroplast maintenance is unlikely to be completely functional, suggesting that *H. germanica* has to continuously renew its chloroplasts to keep them functional. We hypothesize that kleptoplasts might have an added value by providing extra carbon and fueling nitrogen metabolic pathways to foraminifera, mainly under light exposure, but also as energy stock to be digested during food impoverished periods, in dark or light conditions.

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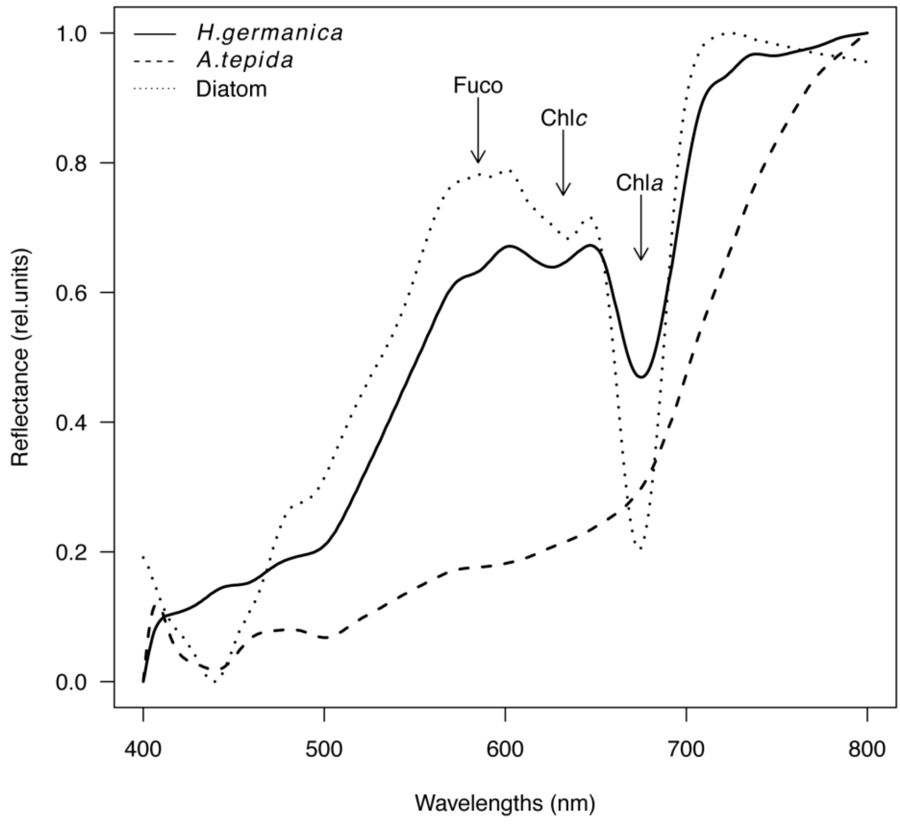
Table 1. Light and dark respiration rates (pmol O_2 cell^{-1} d^{-1}) ± SD of *Haynesina germanica* in the three experimental conditions (Dark, Low Light and High Light) at the end of the experiment (Df, degree of freedom, PFD Photon Flux Density).

Condition	PFD	Respiration Rate (pmol O_2 cell^{-1} d^{-1})
D	300	2452 ± 537
	0	3542 ± 765
LL	300	3468 ± 305
	0	4015 ± 110
HL	300	1179 ± 261
	0	1905 ± 235

Anova		Df	F-test	p
Condition	p (α=0.05)	2	13.1	<0.001
PFD	p (α=0.05)	1	5.4	0.026
Interaction	p (α=0.05)	2	0.3	0.78



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2 Figure 1. Spectral reflectance signatures of *Haynesina germanica, Ammonia tepida* and of a

3 benthic diatom in relative units (X-axis legend: Wavelength (nm)).

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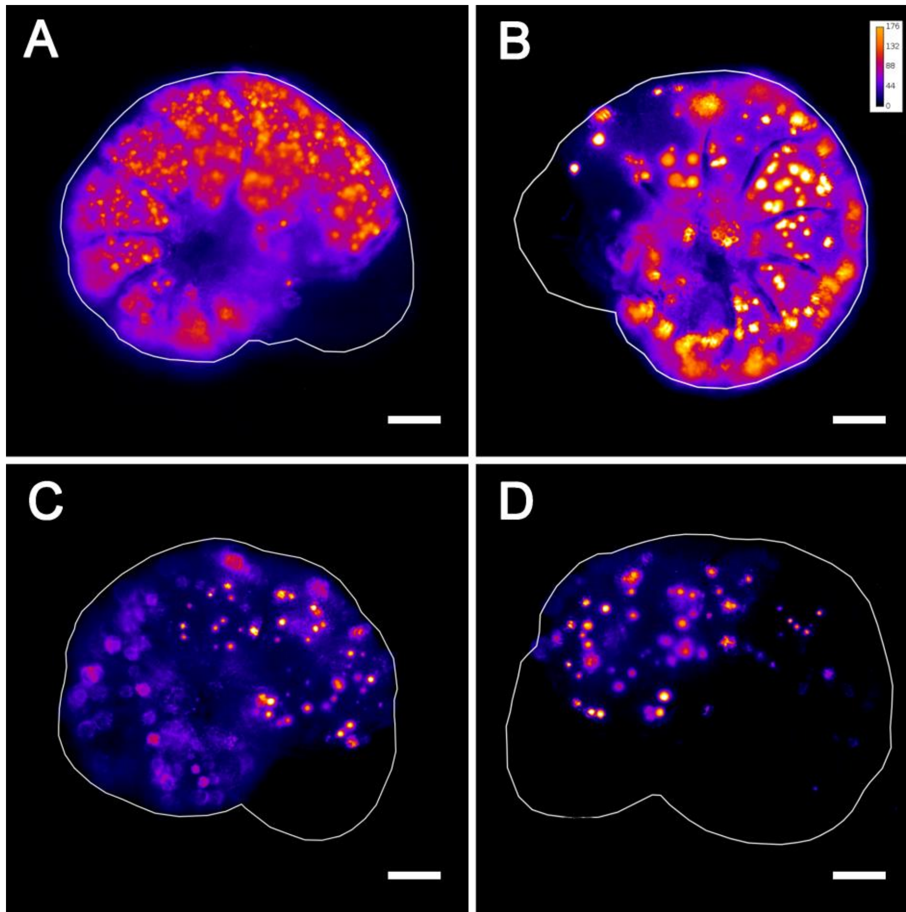
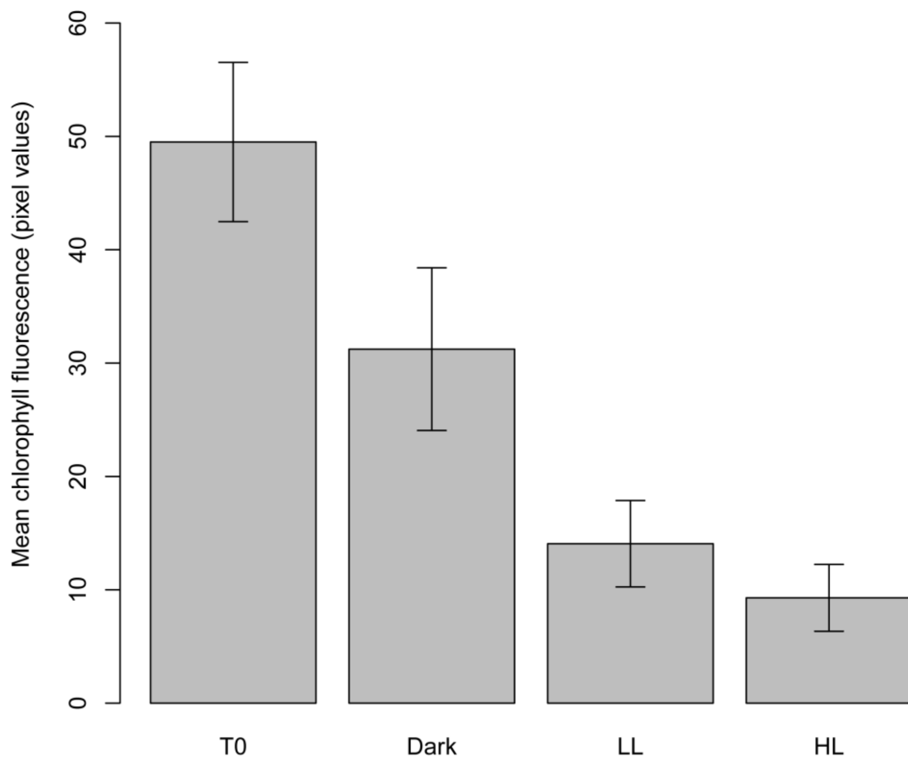


Figure 2. Illustration of *Haynesina germanica* chloroplast content at the beginning (A) and at the end of the experiment for the three experimental conditions, Dark (B), Low Light (C) and High Light (D). Higher colour scale values correspond to foraminifera emitting more fluorescence and likely containing more chlorophyll *a*; fluorescence in pixel values between 0 and 255, (scale bar = 50 µm).



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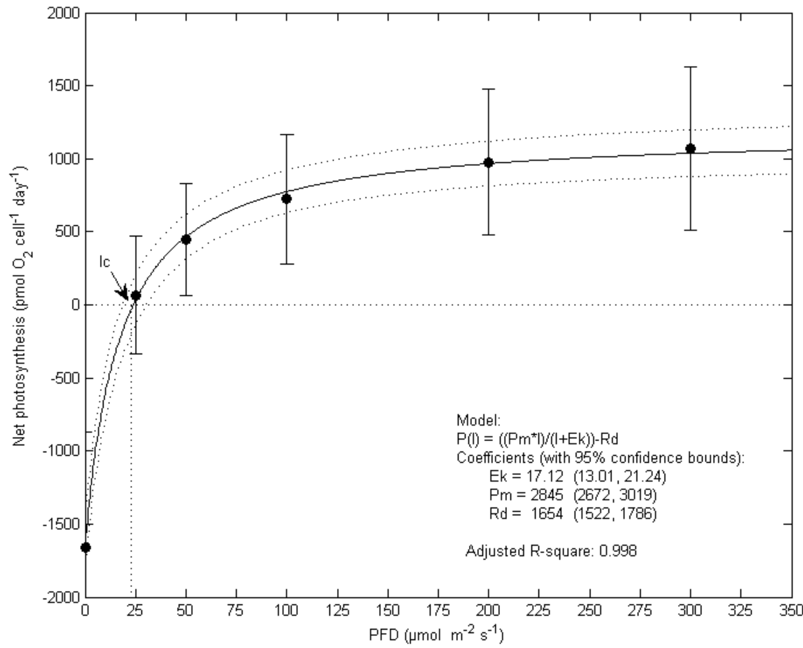
2 Figure 3. Mean chlorophyll *a* fluorescence (± SE, n = 30) at the end for the three experimental
3 conditions (Dark, Low Light and High Light) and the beginning (T0) of the experiment using
4 *Haynesina germanica*. Higher mean values likely corresponded to foraminifera containing
5 more chlorophyll.
6



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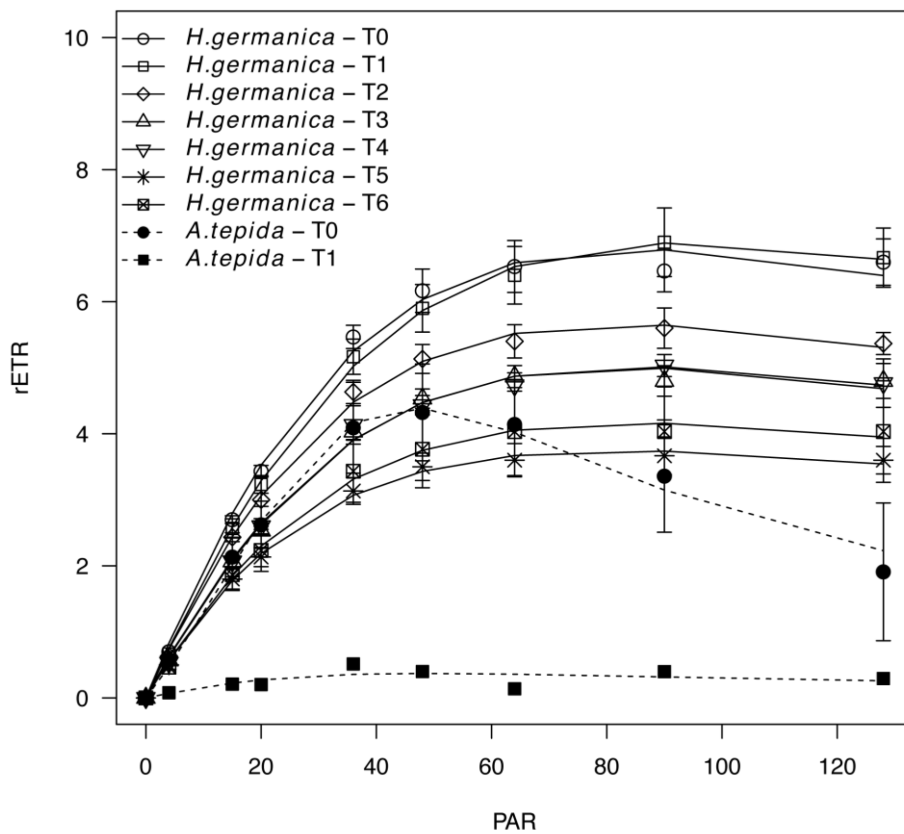
2 Figure 4. Net photosynthesis of *Haynesina germanica* (pmol O_2 cell^{-1} d^{-1}) as a function of the
3 photon flux density (PFD, µmol photons m^{-2} s^{-1}). The half-saturation constant, Ek, was found
4 at 17 (13-21), the dark respiration, Rd, at 1654 (1522-1786) pmol O_2 cell^{-1} d^{-1} and the
5 maximum photosynthetic capacity, Pm, at 2845 (2672-3019) pmol O_2 cell^{-1} d^{-1}.The Ic,
6 calculated compensation irradiance (24 (17-30) µmol photons m^{-2} s^{-1}).The adjusted R^2 of the
7 model was equal to 0.998, n = 3.

8



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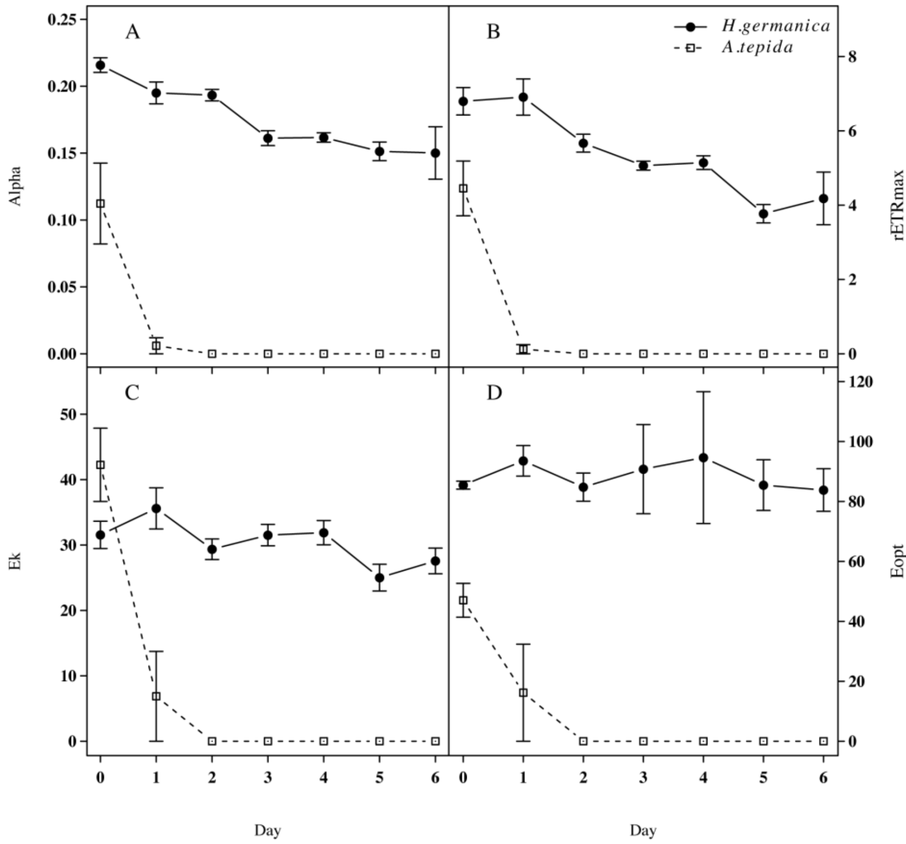
2 Figure 5. Rapid light curves (RLC, n = 3) expressed as the relative electron transport rate
3 (rETR) as a function of the photosynthetic active radiation (PAR in µmol photons m^{-2} s^{-1}) of
4 *Haynesina germanica* (black lines) and *Ammonia tepida* (black dashed lines) during the seven
5 days of the experiment.

6



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2 Figure 6. Rapid light curve (RLC, n = 3) parameters for *Haynesina germanica* (Dark-RLC)
3 and *Ammonia tepida* maintained in the dark during the experiment, Alpha is the initial slope
4 of the RLC at limiting irradiance, rETRmax is the maximum relative electron transport rate,
5 Ek is the light saturation coefficient and Eopt is the optimum light, all of them were estimated
6 by adjusting the experimental data to fit the model of Platt et al. (1980).

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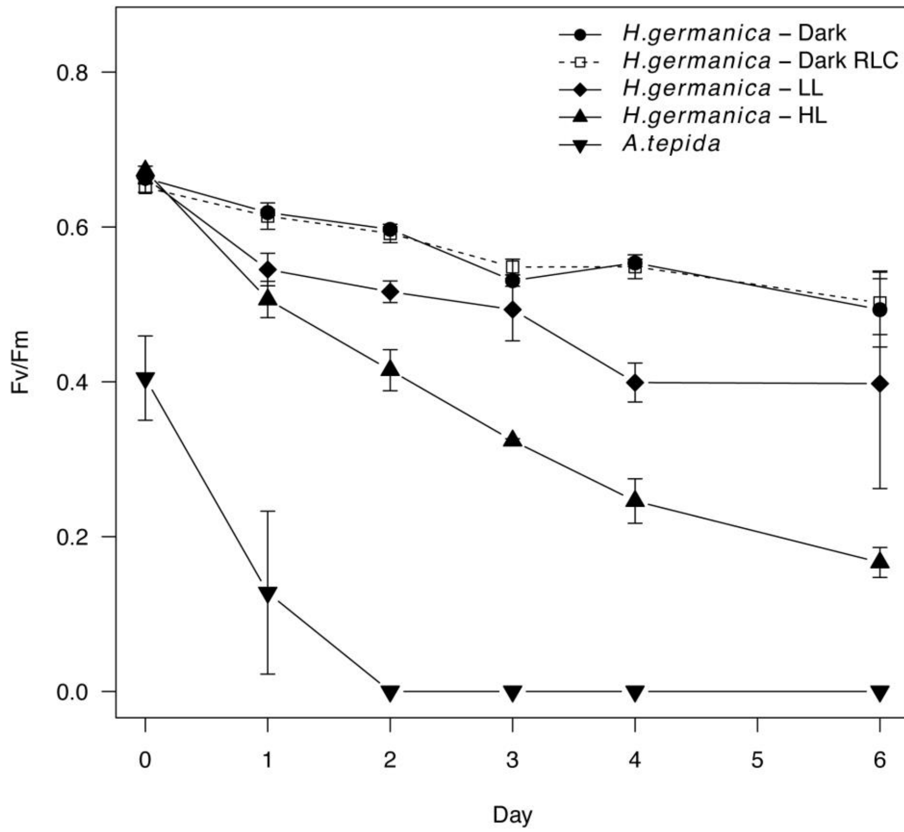

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2 Figure 7. Maximum quantum efficiency of the photosystem II (*Fv/Fm*, n = 3) during the
3 experiment for the different applied conditions (Dark, Low Light and High Light) and species
4 (*Haynesina germanica* and *Ammonia tepida*).