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1 **Low levels of ultra-violet radiation mitigate the deleterious effects of nitrate**  
2 **and thermal stress on coral photosynthesis**

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15 **Abstract**

16

17 Reef ecosystems are under increasing pressure from global and local stressors. Rising  
18 seawater temperature and high ultraviolet radiation (UVR) levels are the main drivers of the  
19 disruption of the coral-dinoflagellate symbiosis (bleaching). Bleaching can also be  
20 exacerbated by nitrate contamination in coastal reefs. However, the underlying physiological  
21 mechanisms are still poorly understood. Here, we assessed the physiological and oxidative  
22 state of the scleractinian coral *Pocillopora damicornis*, maintained eight weeks in a crossed-  
23 factorial design including two temperatures (26 °C or 30 °C), and two nitrate (0.5 and 3 µM-  
24 enriched), and UVR (no UVR and 25/1.5 Wm<sup>-2</sup> UVA/B) levels. Nitrate enrichment, and high  
25 temperature, significantly impaired coral photosynthesis. However, UVR alleviated the  
26 nitrate and temperature-induced decrease in photosynthesis, by increasing the coral's  
27 antioxidant capacity. The present study contributes to our understanding of the combined  
28 effects of abiotic stressors on coral bleaching susceptibility. Such information is urgently  
29 needed to refine reef management strategies.

30 **Keywords**

31 Eutrophication, nitrate, coral, photosynthesis, symbiosis, ultra-violet

## 32 **Introduction**

33 Coral reefs are among the most diverse and productive marine ecosystems on the  
34 planet despite thriving in oligotrophic waters (i.e. low levels of nitrogen and phosphorus;  
35 Crossland et al., 1991). They provide a range of important economic services to local  
36 population, such as tourism and recreation, fisheries and coastal protection (Moberg and  
37 Folke, 1999). Reefs rely on the nutritional and optimized symbiosis between corals and  
38 autotrophic dinoflagellates of the Symbiodiniaceae family (LaJeunesse et al., 2018).  
39 Symbionts efficiently take up and assimilate dissolved inorganic nutrients from the  
40 oligotrophic seawater (Godinot et al., 2009; Muscatine et al., 1984; Rådecker et al., 2015)  
41 and are able to recycle the host's metabolic wastes (Rahav et al., 1989). They also transfer  
42 most of their photosynthates to the coral host, which relies on this major source of nutrients  
43 to sustain its respiratory demand, growth and reproduction (Muscatine et al., 1984; Tremblay  
44 et al., 2014).

45 Despite their ecological and economical value, reef ecosystems are under increasing  
46 pressure from multiple global stressors, which disrupt the stability of the coral-dinoflagellate  
47 symbiosis (Ellis et al., 2019). The loss of symbionts (i.e. bleaching; Weis 2008) significantly  
48 impairs the coral nutritional state and may ultimately lead to significant mortality rates. Coral  
49 bleaching is thus one of the greatest threats to reef ecosystems nowadays (Eakin et al., 2019;  
50 Suggett and Smith, 2020). Large-scale bleaching events have increased in frequency over the  
51 last decades mainly due to heatwaves, and elevated sea surface temperatures (SST)  
52 maintained over several weeks (Couch et al., 2017; Hughes et al., 2017). Coral bleaching  
53 susceptibility and severity, however, depends on other environmental parameters such as the  
54 irradiance levels, both photosynthetically active radiation (PAR) and ultra-violet radiation  
55 (UVR), either UVA (400-320 nm) or UVB (320-280 nm). UVR can penetrate the water  
56 column down to 150 m depth in very oligotrophic waters (Barron et al., 2009; Kahng et al.,  
57 2019; Tedetti and Sempéré, 2006). While the thermotolerance threshold of corals is generally  
58 decreased under exposure to high PAR or UVR levels (Downs et al., 2013; Lesser and  
59 Farrell, 2004; Torregiani and Lesser, 2007), the contrary has also been observed (Halac et al.,  
60 2010; McCauley et al., 2018; Rosic et al., 2020). For example, Rosic et al. (2020) showed a  
61 synergetic effect of low PAR (no UVR exposure) and thermal stress that caused higher  
62 bleaching levels of the scleractinian coral *Acropora millepora* compared to thermal stress and  
63 high PAR. Antagonistic interactions of temperature and UVR were also observed on  
64 Caribbean octocorals (McCauley et al., 2018). Finally, two experiments showed an

65 enhancement of the photosynthetic performances of coral larvae under UVR exposure (Zhou  
66 et al., 2017, 2016). This discrepancy in the effects of UVR on coral physiology is most likely  
67 due to the different doses of UVR to which organisms are exposed (Barron et al., 2009;  
68 Overmans and Agustí, 2020). Therefore, UVR levels may be a tipping point towards  
69 postponing or speeding up DNA damage and coral bleaching (Ben-Zvi et al., 2019).

70 In addition to global change stressors, coral reefs also face local anthropogenic  
71 impacts. These include upwelling events (Richardson et al., 2020), nutrient enrichment of  
72 coastal seawater through land run off, agriculture and urban wastes (Fabricius, 2011, 2005).  
73 Under these conditions, inorganic nitrogen (N) and phosphorus (P) concentrations can be  
74 much higher (up to 4  $\mu\text{M}$  N and 0.5  $\mu\text{M}$  P, (Brodie et al., 2011; Govers et al., 2014; Naumann  
75 et al., 2015; Rouzé et al., 2015)) than the usual levels measured on reefs (ca. 0.5  $\mu\text{M}$  N and  
76 0.1  $\mu\text{M}$  P (Charpy, 2001; Kinsey and Davies, 1979; Lomas and Lipschultz, 2006; Meeder et  
77 al., 2012)). Recent findings have shown that concentrations of 2 to 5  $\mu\text{M}$  of nitrate, which is  
78 the main nitrogen compound resulting from human activities, can enhance coral bleaching  
79 during thermal stress, (Burkepile et al., 2019; Ezzat et al., 2016; Marangoni et al., 2020;  
80 Rosset et al., 2017; Wiedenmann et al., 2013). However, as for UVR exposure, the effect of  
81 nitrate on thermal stress-induced bleaching largely varies, depending for example on coral  
82 species, symbiont densities, nitrate and other nutrient concentrations (Fabricius et al., 2013;  
83 Schlöder and D’Croz, 2004; Serrano et al., 2018).

84 Variations in coral bleaching susceptibility under either UVR exposure or nitrate  
85 enrichment, may depend on changes in UVR or nitrate levels, and more specifically on  
86 corals’ antioxidant capacity (Ezzat et al., 2015; Krueger et al., 2015; Marangoni et al., 2020;  
87 Muller-Parker et al., 2015). Under thermal stress, the concentrations of reactive oxygen  
88 species (ROS) and nitrogen species (RNS) within the host and symbiont cells drastically  
89 increase (Weis, 2008). These compounds cause cellular damage through DNA degeneration,  
90 protein oxidation and lipid peroxidation (Freeman and Crapo, 1982; Suggett and Smith,  
91 2020). In order to cope with high levels of ROS and RNS, corals produce various protecting  
92 molecules, such as ascorbate, catalase (CAT), glutathione peroxidases and super-oxide  
93 dismutases (Krueger et al., 2014; Liñán- Cabello et al., 2010). Damages to coral tissue and  
94 bleaching occur when ROS and RNS production exceed the antioxidant capacity of the coral  
95 holobiont. While UVR and nitrate can respectively be sources of ROS and RNS (Lesser,  
96 2006; Moniczewski et al., 2015), the oxidative status of corals under the combination of the  
97 two stressors, has never been investigated and thus needs further attention.

98           The present study aims to investigate the effects of nitrate enrichment on coral  
99 bleaching susceptibility, when combined with different temperatures and/or UVR conditions.  
100 For this purpose, we used the widespread scleractinian coral species *Pocillopora damicornis*.  
101 We measured the changes in the physiological and oxidative status of the corals following  
102 exposure to the individual and combined stressors. We used a UVR level that can be received  
103 by tropical corals living at 10-15 m depth (daily dose of 125 Wd<sup>-1</sup> UVA and 7.5 Wd<sup>-1</sup> UVB),  
104 instead of the three times higher doses measured in shallow reefs. We hypothesized that  
105 thermally-stressed or UVR-stressed corals exposed to nitrate enrichment would experience  
106 increased levels of oxidative stress compared to corals maintained with no nitrate enrichment.  
107 Given the increasing exposure of reefs to human pollution and recurrent heat waves events,  
108 this study aims to identify the worse combination of factors for the survival of reef building  
109 corals such as *Pocillopora damicornis*. A deeper understanding of the impacts of multiple  
110 stressors on reef building corals will help identify adaptive strategies to better protect corals  
111 and associated coral reefs.

## 112 **Material and Methods**

113

### 114 ***1. Experimental design***

115 Ten colonies of the scleractinian coral *Pocillopora damicornis* were used to generate  
116 160 nubbins (16 nubbins from each colony), which were then equally divided in 16 aquaria  
117 of 25 L (one nubbin per colony, 10 per aquaria). Nubbins were left for three weeks in similar  
118 conditions (see below) for healing and were fed once a week with *Artemia salina* nauplii.  
119 During this period, aquaria were continuously supplied (at a rate of 8 L.h<sup>-1</sup>) with oligotrophic  
120 seawater (0.5 μM nitrate, 0.2 μM phosphorus) and corals were maintained at 25 °C under a  
121 photosynthetically active radiation (PAR) of 200 ± 10 μmol photons.m<sup>-2</sup>.s<sup>-1</sup> (12:12h  
122 photoperiod). PAR was delivered by 400 W metal halide lamps (HPITS, Philips) above the  
123 aquaria and PAR intensity was measured using a LI-COR data logger (LI-100) connected to a  
124 quantum sensor (LI-I93). Temperature was controlled by heaters connected to an ELLIwell PC  
125 902/T controller. Nutrient levels were measured once a week using an AA3 Seal autoanalyzer  
126 according to Aminot et al. (2009).

127 The 16 aquaria were then divided into 4 sets of 4 aquaria, each set being maintained  
128 under a different nitrate and UVR level (Fig. 1). The first set was a control condition (C26),  
129 which was not enriched with nitrate (0.5 μM nitrate) and not exposed to UVR. A second set  
130 of 4 aquaria (UV26) was not enriched with nitrate, but received 25 Wm<sup>-2</sup> of UVA and 1.5  
131 Wm<sup>-2</sup> of UVB during 5 hours per day (between 10:00 and 15:00) provided by two Q-panel  
132 UVA 340 lamps. The spectral distribution of these lamps is shown in Shick et al (1999). The  
133 two other sets of aquaria were enriched with 3 μM nitrate and maintained with or without the  
134 levels of UVR described above (N26 or UVN26 respectively). Nitrate enrichment was  
135 performed using stock solutions of 300 μM NaNO<sub>3</sub>. The solutions were delivered to the  
136 aquaria with a peristaltic pump and were renewed every week. The enrichment condition  
137 chosen was similar to the nitrate concentration observed in reefs experiencing nutrient  
138 enrichment (Brodie et al., 2011; Costa et al., 2000; Govers et al., 2014; Naumann et al., 2015;  
139 Rouzé et al., 2015). The intensity of UVR provided to the two sets of 4 aquaria was recorded  
140 using an International Light ILT1400 portable radiometer equipped with two detectors:  
141 SEL033/UVA and SEL240/UVB and the coefficient of attenuation of UVR irradiance in  
142 water was taken into account. UVA and UVB values, applied during only 5 h, corresponded  
143 to a daily dose of 125 Wd<sup>-1</sup> UVA and 7.5 Wd<sup>-1</sup> UVB). Such doses can be considered low  
144 compared to those received by corals on shallow reefs, which can reach more than 350 Wd<sup>-1</sup>

145 UVA and  $19 \text{ Wd}^{-1}$  UVB (Barron et al., 2009; Overmans and Agustí, 2020). In these reefs, in  
146 summer, peaks of  $60$  to  $70 \text{ Wm}^{-2}$  UVA and  $3 \text{ Wm}^{-2}$  UVB can occur at midday (Kaneohe Bay,  
147 Hawaii and Heron Island, Australia) (Rosic et al., 2020; Torregiani and Lesser, 2007). Our  
148 conditions, therefore, correspond to tropical corals living at 15 m depth, where UVR levels  
149 are significantly reduced.

150 The factorial design with UVR and nutrient levels was maintained for six weeks,  
151 before a thermal stress was applied to half of the aquaria (Fig. 1). Temperature was increased  
152 by  $0.5 \text{ }^{\circ}\text{C}$  every two days until reaching  $30 \text{ }^{\circ}\text{C}$ . This temperature was maintained for one  
153 week until bleaching was visually observed, and the measurements described below were  
154 performed.

155

## 156 **2. Physiological measurements**

### 157 Photosynthesis parameters

158 Rates of net photosynthesis (Pn) and respiration (R) were estimated on 6 nubbins per  
159 condition (from different colonies, and 3 nubbins per aquarium). For this purpose, nubbins  
160 were placed in 60 mL Plexiglass chambers filled with  $0.45 \text{ }\mu\text{m}$ -filtered seawater, maintained  
161 at  $26 \text{ }^{\circ}\text{C}$  or  $30 \text{ }^{\circ}\text{C}$ , and stirred. Each chamber was equipped with an oxygen sensor (Polymere  
162 Optical Fiber, PreSens, Regensburg, Germany) connected to an Oxy-4 (Channel fiber-optic  
163 oxygen meter, PreSens, Regensburg, Germany). Oxygen concentration was recorded for 30  
164 min with the Oxy4v2-30fb software, in the dark for R and at  $200 \text{ }\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$  for Pn.  
165 Two calibrations were done at 0%  $\text{O}_2$  with nitrogen saturated seawater and at 100%  $\text{O}_2$  with  
166 air saturated seawater. The gross photosynthesis rate was obtained by adding the absolute  
167 value of the respiration rate to the corresponding net photosynthesis rate. This obtained gross  
168 photosynthesis rate is likely to be an underestimation of the actual gross photosynthesis rate  
169 (Schrameyer et al., 2014). At the end of the measurements, nubbins were frozen for the later  
170 determination of the symbiont density, proteins and chlorophyll a and c<sub>2</sub> (Chl) content. Pn,  
171 Pg, and R rates were expressed as  $\mu\text{mol O}_2.\text{h}^{-1}.\text{cm}^{-2}$ . The surface area was obtained with the  
172 single dip wax technique (Stimson and Kinzie III, 1991).

173

### 174 Symbiont density, chlorophyll and proteins content

175 Samples used in the previous measurements were thawed and the tissues were separated from  
176 the skeleton using a Water-Pick and 10-15 mL of filtered seawater. A  $100 \text{ }\mu\text{L}$  sub-sample  
177 was used for the determination of the symbiont density with a Z1 Coulter Particle Counter  
178 (Beckman Coulter, US). For each sample, five technical replicates were performed. Another



179 5 ml sub-sample was used for the chlorophyll content analysis. For this purpose, the sub-  
180 sample was centrifuged at 5530 g for 15 min at 4 °C to separate the animal tissue  
181 (supernatant) from the dinoflagellates (pellet). Then the supernatant was discarded, and the  
182 pellet resuspended in 5 ml of acetone 100%, to extract chlorophyll in the dark at 4 °C for 24  
183 hours. Finally, the extract was centrifuged at 5530 g for 15 minutes at 15 °C. The absorbance  
184 of the supernatant was recorded at 630 nm, 663 nm and 750 nm using a UVmc<sup>2</sup>  
185 Spectrophotometer (Safas, Monaco). The concentration of chlorophyll a and c<sub>2</sub> was then  
186 calculated using the equations by Jeffrey and Humphrey (1975). Finally, 500 µL were used  
187 for the proteins content of the total holobiont and incubated for 5 hours at 60 °C in sodium  
188 hydroxide. Measurements were done with the Bicinchoninic acid (BCA) assay kit according  
189 to Smith (1985). All measurements were normalized to the skeletal surface area of the  
190 nubbins.

191

### 192 **3. Oxidative stress analysis**

193 48 nubbins – 6 per conditions (from different colonies and 3 per aquaria) – were snap-frozen  
194 in liquid nitrogen and kept at -80 °C for oxidative stress analysis. For each analysis, a small  
195 coral fragment of 5-10 mm was collected from the main fragment.

#### 196 Reactive oxygen species (ROS) levels

197 The ROS level in the tissues was quantified using the fluorescent probe 5-(and-6)-carboxy-  
198 2',7'-dichlorofluorescein diacetate (H<sub>2</sub>DCFDA, Molecular Probes) as described by Ruiz-Leal  
199 and George (2004), with some modifications. In presence of ROS, H<sub>2</sub>DCFDA emits  
200 fluorescence quantified by spectrofluorometry. Coral fragments were fresh collected,  
201 sonicated on ice (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific, France) in a  
202 homogenizing buffer (pH 7.75) containing Tris-HCl 100 mM, EDTA 2 mM and MgCl<sub>2</sub>.6H<sub>2</sub>O  
203 5 mM in MilliQ Water. After sonication, the holobiont homogenates were centrifuged at  
204 10000 g for 10 min at 4 °C, and the protein content was quantified following the Bradford  
205 Protein Assay (Bradford, 1976). Samples were standardized to the protein concentration of  
206 0.5 µg.µL<sup>-1</sup> and 10 µL were then added in triplicates on a black 96-wells microplate with  
207 reaction buffer (pH 7.2) containing HEPES 30 mM, KCl 200 mM and MgCl<sub>2</sub>.6H<sub>2</sub>O 1 mM in  
208 MilliQ water. Finally, 10 µL of H<sub>2</sub>-DCFDA (16 µM in ethanol) was added in each well. The  
209 fluorescence (excitation: 488 nm; emission: 525 nm) was read every 5 minutes for up to 50  
210 minutes using a spectrofluorometer (Xenius®, SAFAS, Monaco). The area beneath the kinetic  
211 curve was considered the level of ROS in the sample. The results were expressed as  
212 fluorescence units per minute (F.U. x min).

213

214 Lipid peroxidation (LPO)

215 LPO was assessed following the Thiobarbituric Acid Reactive Substance protocol (TBARS  
216 method), in line with Oakes and Van Der Kraak (2003). This method quantifies damage to  
217 lipids through the reaction between malondialdehyde (MDA), a byproduct of lipid  
218 peroxidation, and thiobarbituric acid (TBA). Coral fragments were sonicated (Frequency 70  
219 kHz, Vibra-Cell™, Bioblock Scientific, France) in a homogenizing buffer (KCl (1,15%)  
220 solution containing 35 µM butylatedhydroxytoluene (BHT)) on ice, centrifuged at 10000 g  
221 for 10 min at 4 °C, and the protein content was quantified following the Bradford Protein  
222 Assay (Bradford, 1976). Samples were standardized to the protein concentration of 0.35  
223 µg.µL<sup>-1</sup> and incubated at 95 °C for 30 min in flat-bottomed Eppendorf with acetic acid  
224 solution (20%), thiobarbituric acid (TBA) solution (0.8%), SDS solution (8.1%). After  
225 cooling, 100 µl of MilliQ water and 500 µl of n-butanol were added with through vortexing.  
226 The tubes were then centrifuged at 10000 g for 10 minutes at 15 °C. Finally, 150 µl of the  
227 organic phase (supernatant) was placed, in duplicate, in 96-well black plates and the  
228 fluorescence was read at 553 nm and 515 nm each minute for up to three minutes using a  
229 spectrofluorometer (Xenius®, SAFAS, Monaco). Results were normalized considering the  
230 total protein content in the sample homogenates in each well and expressed as nmol  
231 MDA.mg protein<sup>-1</sup>, which were calculated from a standard curve built using hydrolysed  
232 tetramethoxypropane (TMP).

233

234 Protein tyrosine nitration (PTN)

235 The level of 3-nitrotyrosine modified proteins in corals, a product of PTN resulting from  
236 oxidative damage to proteins by peroxynitrite, was assessed using the Nitrotyrosine ELISA  
237 Kit (ab113848, Abcam, Cambridge, UK) following manufacturer's instructions. The coral  
238 fragments were sonicated (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific) in  
239 phosphate buffered saline (PBS) (pH 7.4) on ice, centrifuged at 10,000 g for 10 minutes at 4  
240 °C. The protein content was quantified following the Bradford Protein Assay (Bradford,  
241 1976). Protein content of each sample was standardized to 0.6 µg.µL<sup>-1</sup> and incubated on ice  
242 for 20 min in extraction buffer kit solution (1:1). Standards were made with 3-Nitrotyrosine-  
243 Bovin Serum Albumin (3-NT-BSA) (reconstituted in 1 mL of 1x Incubation Buffer kit  
244 solution). Samples and standards were added on a 96-well plate coated with an antibody  
245 specific for 3-nitrotyrosine. The plate was wrapped in foil and left to incubate for two hours

246 at room temperature, before each well was washed twice with Wash Buffer. Liquid excess  
247 was completely removed. Then a biotin labelled anti-3-nitrotyrosine detector antibody was  
248 added to each well and left to incubate for one hour before washing twice with Wash Buffer.  
249 Each well was then incubated for one hour with HorseRadish Peroxidase (HRP)-labeled kit  
250 solution and thoroughly washed three times with Wash Buffer. Finally, the HRP  
251 Development kit solution was added in each well and the absorbance was immediately read  
252 at 600 nm every minute up to 15 minutes. Results were normalized considering the total  
253 protein content in the sample homogenates in each well and expressed as ng 3NT-BSA.mg  
254 protein<sup>-1</sup>.

255

#### 256 Non-enzymatic total antioxidant capacity (TAC)

257 The non-enzymatic total antioxidant capacity was assessed using the “OxiSelect Total  
258 Antioxydant Capacity (TAC) Assay Kit” (STA-360, Cell Biolabs Inc., San Diego USA)  
259 according to manufacturer’s instructions. This assay measures antioxidant capacity of  
260 biomolecules via single electron transfer (SET) mechanism. The coral fragments were  
261 sonicated (Frequency 70 kHz, Vibra-Cell™, Bioblock Scientific) in PBS (pH 7.4) on ice,  
262 centrifuged at 10000 g for 10 min at 4 °C, and the protein content was quantified following  
263 the Bradford protein assay (Bradford, 1976). Samples protein content were standardized to  
264 0.9 µg.µL<sup>-1</sup>. Standards were made with a serial dilution of uric acid from 1mmol.L<sup>-1</sup> to  
265 0.00390 mmol.L<sup>-1</sup>. Samples and standard were placed on a 96-well plate with reaction buffer  
266 (Kit solution buffer and PBS). The absorbance was recorded at 490 nm using a  
267 spectrofluorometer (Xenius®, SAFAS, Monaco). Samples were compared with a known uric  
268 acid standard curve, with absorbance values being proportional to the sample’s total reductive  
269 capacity. Results were normalized considering the total protein content in the sample  
270 homogenates in each well and expressed as µM Copper Reducing Equivalents (CRE).mg  
271 protein<sup>-1</sup>.

272

#### 273 **4. Statistical analysis**

274 Statistical analyses were processed using the program RStudio Version 3.5.1. Data were  
275 checked for normality using the Shapiro-Wilk test and for homoskedasticity using Levene’s  
276 test on the residuals from the linear model (Edmunds and Gates, 2002). When the  
277 assumptions were not met, data were transformed with a log transformation or if necessary, a  
278 Box Cox transformation. Mixed effects models were used to test the effect of the three fixed  
279 factors: “Nutrient”, “UV” and “Temperature”, with the random factor “Aquarium” on the

280 different physiological and oxidative stress parameters measured: holobiont protein biomass,  
281 dinoflagellates density, total chlorophyll a and c<sub>2</sub> content, net and gross photosynthesis rate  
282 per skeletal surface area and per symbiont cell, respiration rate per skeletal surface area and  
283 per symbiont cell, ROS level, lipid peroxidation and protein nitration. The Mixed Effect  
284 model showed that the effect of the random factor “Aquarium” was negligible for each  
285 parameter tested; this random factor was, therefore, removed from the models and three-way  
286 ANOVA were used with the factors: “Nutrient”, “UV” and “Temperature”. When the  
287 homoskedasticity assumption was not met, a White-adjusted ANOVA was run, using a  
288 heteroskedasticity-consistent covariance matrix estimator (White, 1980). Analyses were  
289 followed by a pairwise comparison test when factors effect was significant. Differences were  
290 assumed significant when  $p < 0.05$ .

291

292 **Results**

293 ***1. Effect of UVR exposure and nitrate enrichment at normal temperature (26 °C)***

294 The result of the statistical tests are reported in the supplementary appendix in Table S1, for  
295 physiological parameters, Table S2, S3 for photosynthesis parameters normalized to skeletal  
296 surface area and to symbiont cell and Table S4, for oxidative stress parameters.

297 At 26 °C, no significant effect of UVR exposure and/or nitrate enrichment was observed on  
298 the tissue parameters (Fig. 2a, b, c) compared to the control (C26). No significant change was  
299 observed on the respiration rates per skeletal surface area (ANOVA,  $p > 0.91$ , Fig. 3c) or per  
300 symbiont cell (ANOVA,  $p > 0.27$ , Fig. S1c). However, there was a 80% decrease in net  
301 photosynthesis, in the N26 condition compared to C26 condition, when photosynthesis was  
302 expressed both per skeletal surface area (pairwise comparison,  $p < 0.004$ , Fig. 3a) or  
303 symbiont cell (pairwise comparison,  $p < 0.0095$ , Fig. S1a). However, net photosynthesis  
304 increased by 60% in the combined nitrate + UVR (UVN26), compared to the N26 condition  
305 (pairwise comparison - per surface:  $p < 0.003$ , Fig. 3a - per symbiont:  $p < 0.0004$ , Fig. S1a).  
306 Gross photosynthesis rates per skeletal surface area, also, increased under UVR exposure  
307 (ANOVA,  $p < 0.006$ , Fig. 3b). Finally, the non-enzymatic total antioxidant capacity (TAC)  
308 significantly increased in all UVR condition (ANOVA,  $p < 0.0001$ , Fig. 4a) as well as the  
309 protein nitration in the UVN26 compared to N26 condition (pairwise comparison,  $p < 0.004$ ,  
310 Fig. 4b).

311

312 ***2. Effect of UVR exposure and nitrate enrichment under thermal stress (30 °C)***

313 The results of the statistical tests are reported in the supplementary appendix in Table S1, for  
314 physiological parameters, Table S2, S3 for photosynthesis parameters normalized per skeletal  
315 surface area and per symbiont cell and Table S4, for oxidative stress parameters. The linear  
316 models (ANOVA) indicate that thermal stress had both independent and combined effects  
317 (with UVR exposure and nutrient enrichment) on coral physiology.

318 Compared to the same conditions at 26°C, thermal stress alone drove a significant decrease in  
319 symbiont density in all conditions (ANOVA,  $p < 0.0001$ , Fig. 2b). It also induced, an  
320 increase in Pg (ANOVA,  $p < 0.0001$ , Fig. S1b), in respiration rates per symbiont (ANOVA,  $p$   
321  $< 0.0001$ , Fig. S1c), and an increase in ROS levels (ANOVA,  $p < 0.03$ , Fig. 4c), and in lipid  
322 peroxidation damages (ANOVA,  $p < 0.00011$ , Fig. 4d). In addition, temperature interacted  
323 with nitrate enrichment, since the Pn rates per symbiont (White-adjust ANOVA,  $p < 0.04$ )

324 were lower in the N30 condition compared to the N26 condition (pairwise comparison,  $p <$   
325  $0.0004$ , Fig. S1a).

326 Temperature also interacted with UVR exposure for the protein nitration level, which is  
327 higher in the UV30 than in UV26 conditions (pairwise comparison,  $p < 0.02$ , Fig. 4b, T.  
328 S4b).

329 UVR exposure increased the antioxidant capacity of corals in all conditions (ANOVA,  $p <$   
330  $0.0001$ , Fig. 4a). In addition, at high temperature ( $30^{\circ}\text{C}$ ), UVR exposure led to an increased  
331 the protein biomass in coral tissue (pairwise comparison, C30/UV30 - N30/UVN30,  $p <$   
332  $0.047$ , Fig. 2a) and in total chlorophyll a and  $c_2$  content (ANOVA,  $p < 0.045$ , Fig. 2c)  
333 compared to corals non exposed to UVR. It also increased the Pg normalized to skeletal  
334 surface area (ANOVA,  $p < 0.006$ , Fig. 3b) and symbiont cell (pairwise comparison,  
335 C30/UV30 - N30/UVN30,  $p < 0.024$ , Fig. S1b). Finally, the level of protein nitration in the  
336 UV30 was significantly higher than in the C30 condition (pairwise comparison,  $p < 0.035$ ,  
337 Fig. 4b). The interaction between nitrate and UVR exposure impacted ROS levels in coral  
338 tissue (ANOVA,  $p < 0.012$ ), which significantly increased in the UVN30 compared to N30  
339 (pairwise comparison,  $p < 0.0013$ , Fig. 4c).

## 340 **Discussion**

341 Reef ecosystems are under increasing pressure from interacting multiple stressors (e.g. rising  
342 temperatures, and excessive irradiance and nutrient levels). Stressors can have synergistic,  
343 antagonistic or additive effects on coral physiology, but these interactions are, however, not  
344 well understood (Chumun et al., 2013; Higuchi et al., 2015). By quantifying the combined  
345 effects of UVR exposure, nitrate enrichment and thermal stress on the physiology and the  
346 oxidative stress response of the widespread scleractinian coral *Pocillopora damicornis*, this  
347 study brings a deeper understanding of the response of the coral symbioses to multiple  
348 environmental changes. Specifically, our results demonstrate that low UVR intensities (and  
349 low doses) can mitigate the negative effects of thermal stress by enhancing the antioxidant  
350 capacity of coral colonies. In addition, low UVR intensities can prevent the negative effects  
351 of nitrate on symbiont photosynthesis, most likely, by regulating the complex links between  
352 carbon and nitrogen metabolism.

353

### 354 ***1. Nitrate enrichment without UVR exposure impaired symbiont photosynthesis***

355 Nitrate enrichment in absence of UVR exposure induced a significant decrease in net  
356 photosynthesis (normalized to surface area or symbiont cell) of *P. damicornis*. Nitrate-  
357 induced decrease in carbon fixation or photosynthetic efficiency has already been observed in  
358 several coral species (Chumun et al., 2013; Courtial et al., 2017; Ezzat et al., 2015; Nordemar  
359 et al., 2003). This was coupled with a decline in the amount of photosynthates transferred  
360 from the symbionts to the host tissue (Ezzat et al., 2015). A bleaching with nitrate enrichment  
361 has sometimes been observed (Burkepile et al., 2019) and could explain the lower rates of  
362 photosynthesis observed here with the nitrate-enriched corals. However, this is unlikely as no  
363 significant reduction in symbiont density and/or chlorophyll a and  $c_2$  content occurred. The  
364 deleterious effects of nitrate on coral physiology may also be due to an imbalanced nitrogen  
365 to phosphorus ratio (N:P ratio). In this case, symbiont growth is promoted by an increase in  
366 nitrogen availability, but the lack of phosphorus weakens the lipid membranes of the  
367 symbionts, especially during thermal stress (Ezzat et al., 2016; Rosset et al., 2017;  
368 Wiedenmann et al., 2013). Although this might happen under an important phosphorus  
369 deficiency, here, phosphorus concentrations were around 0.2  $\mu\text{M}$  and symbiont growth was  
370 not enhanced by nitrogen supplementation. In addition, corals under UVR exposure and  
371 nitrate enrichment (UVN26) did not decrease their photosynthetic rates. All together, these

372 observations suggest that, the N:P ratio was not imbalanced in our study and has thus not  
373 driven the decrease in photosynthesis observed in both the N26 and N30 conditions.

374 The photosynthesis impairment under nitrate enrichment is likely due to a competition,  
375 between carbon and nitrogen assimilation for ATP and reductants generated by  
376 photosynthetic electron transport (Nunes-Nesi et al., 2010). Conversion of one nitrate  
377 molecule into ammonium indeed requires ATP and consumes one NADPH and six reduced  
378 ferredoxins. Therefore, in plants, 25% of the ATP and NADPH produced from light energy  
379 by the electron transfer complex is used for nitrate assimilation, and only 75% remains  
380 available for carbon fixation (Bloom, 2015). In plants, when light is limited, or when nitrogen  
381 metabolism is not paired with carbon metabolism, nitrate assimilation and carbon dioxide  
382 fixation will directly compete for both ATP and reductants generated by the photosynthetic  
383 electron transport (Nunes-Nesi et al., 2010). In this case, nitrate can cause a reduction in  
384 carbon assimilation rates. It can also disrupt the C:N balance and alter essential metabolites  
385 (Saiz-Fernández et al., 2017). In turn, shortage of carbon skeletons may delay the  
386 assimilation of nitrate into amino acids (Bloom, 2015). This can cause an oxidative stress  
387 condition, by an accumulation of nitrite inside the coral tissue. Although in this experiment  
388 there was no significant increase in ROS under nitrate enrichment, there was a slight increase  
389 in LPO, a proxy for cellular damage (Gutteridge, 1995). This suggests that corals might have  
390 experienced a slight damage from nitrate exposure, possibly through the generation of nitric  
391 oxide (Lundberg et al., 2008). In addition, TAC values decreased in the N30 treatment  
392 compared to control (C26), further indicating an oxidative imbalance. Overall, competition  
393 between carbon and nitrogen for both ATP and reductants is the most plausible explanations  
394 for the decrease in photosynthesis observed in the coral nubbins (Fig. 5). Any increase in  
395 carbon fixation might therefore reduce the nitrate effect on photosynthesis, as observed with  
396 corals exposed to low doses of UVR (see below).

## 397 ***2. Low doses of UVR are beneficial to corals, both under thermal stress or nitrate*** 398 ***enrichment***

399 In shallow reefs, corals receive high levels of UVR. However, they synthesize UV-absorbing  
400 compounds such as mycosporine-like amino acids (MAAs) which partially protect them  
401 from UVR (Shick et al. 1999). Some of these MAAs can also act as antioxidant compounds  
402 (Rosic and Dove, 2011; Yakovleva et al., 2004). In this experiment, exposure of *P.*  
403 *damicornis* to UVR increased TAC in coral tissue at 30 °C, which likely helped avoid an  
404 accumulation of ROS and, thus, a potential damage to biomolecules (as shown by LPO). On



405 the contrary, corals which were not exposed to UVR, experienced decreased TAC level at  
406 30°C and significant bleaching. Alternatively, UVR might have also protected corals from  
407 photoinhibition during exposure to high temperature and light. It has indeed been shown that  
408 UVR trigger photoreceptors that control extraction-contraction of coral polyps (Ben-Zvi et  
409 al., 2019). Contraction of the polyps under UVR may have prevented photodamage and  
410 bleaching in the thermal stressed corals.

411 In this experiment, UVR exposure also significantly enhanced photosynthesis at elevated  
412 temperature notably through an increased chlorophyll level in symbiont cells. A similar effect  
413 had previously been observed, with UVA, on the photosynthetic performances of either free  
414 algae (Gao et al., 2007; Gao and Xu, 2008; Xu and Gao, 2009) or of Symbiodiniacea in  
415 symbiosis with the larvae of *Pocillopora damicornis* and *Seriatopora calendrum* (Zhou et al.,  
416 2017, 2016). Such positive effect of UVA on photosynthesis might be due to the fact that the  
417 activity of the carbonic anhydrase (which transports inorganic carbon used for  
418 photosynthesis) is enhanced by UVA radiation. Enhanced photosynthesis was even recorded  
419 in free algae when exposed to UVB (Chen et al., 2020), which were shown to protect them  
420 against photoinhibition (Hanelt et al., 2006). The positive effect of UVR on coral  
421 photosynthesis contradicts the usual observation of increased bleaching under UVR exposure  
422 and thermal stress (Lesser and Farrell, 2004). However, bleaching is mainly observed under  
423 high UVR doses. On the contrary, the daily dose of UVR used in this study is lower, and  
424 corresponds to that received by coral colonies around 15 m depth (Barron et al., 2009;  
425 Overmans and Agustí, 2020; Rosic et al., 2020). Taken all together, these observations  
426 suggest that the effect of UVR on coral physiology is dose-dependent and that low levels of  
427 UVR may enhance coral photosynthesis by increasing cellular chlorophyll content or  
428 enhancing the carbonic anhydrase activity.

429 In addition to its photoprotective role at a high temperature, low doses of UVR protects  
430 symbionts against the negative effect nitrate has on photosynthesis. Indeed, the impairment of  
431 coral photosynthesis under nitrate enrichment was not observed when corals received UVR.  
432 Many studies have shown the effect of nutrient enrichment or depletion on the UV-induced  
433 decrease in photosynthesis (Rojo et al., 2019). However, this is the first time the contrary is  
434 observed - the alleviation of nitrate-induced decrease in photosynthesis by UVR. In  
435 macroalgae, activities of both nitrate reductase (involved in nitrate assimilation) and carbonic  
436 anhydrases (involved in carbon fixation) are stimulated by exposure to UVR (Kumar et al.,  
437 1996; Viñepla et al., 2006). They show a peak in the evening, whereas the peak is delayed

438 without UVR. In addition, Figueroa & Vinegla (2001) observed in two marine algae that  
439 UVR acts as an environmental signal involved in the control of carbon and nitrogen cycles,  
440 and regulates feedback processes that control N assimilation as a function of carbon content.  
441 Therefore, in corals, UVR may have prevented the nitrate-induced inhibition of  
442 photosynthesis by regulating the complex links between carbon and nitrogen metabolism  
443 (Fig. 5). In addition, since UVR promotes the synthesis of MAAs, rich in nitrogen (Korbee et  
444 al., 2005; Peinado et al., 2004; Shick et al., 2005; Zheng and Gao, 2009), it might have also  
445 stimulated the reduction of nitrate into ammonium and its subsequent incorporation into  
446 MAAs.

447 In conclusion, this multifactorial study shows that light conditions, and specifically UVR  
448 levels may influence the sensitivity of coral species to thermal stress and nutrient pollution.  
449 We, indeed, demonstrated that UVR plays an important role in mitigating the effects of  
450 thermal stress and nitrate enrichment on coral photosynthesis. However, since UVR can also  
451 be detrimental to coral's photosynthesis under high doses, future studies should aim at  
452 investigating the dose-effect response of corals to UVR exposure. Overall, our results  
453 indicate that UVR may be a critical factor, which not only affects the distribution of corals,  
454 but also their response to environmental stress.

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- 745



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750

751 **Authors' contributions**

752 Alice Blanckaert: Conceptualization, Methodology, Writing.

753 Laura F. B. Marangoni: Methodology, Writing.

754 Cécile Rottier: Methodology.

755 Renaud Grover and Christine Ferrier-Pagès: Conceptualization, Funding acquisition, Writing

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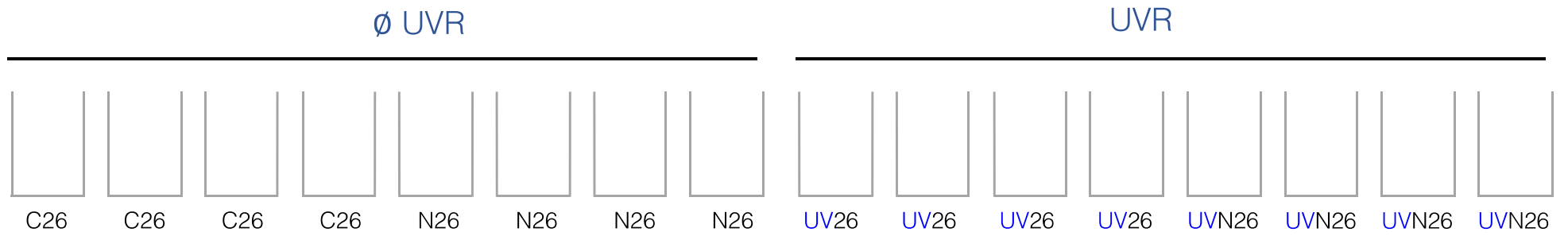
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760 **Conflict of interest**

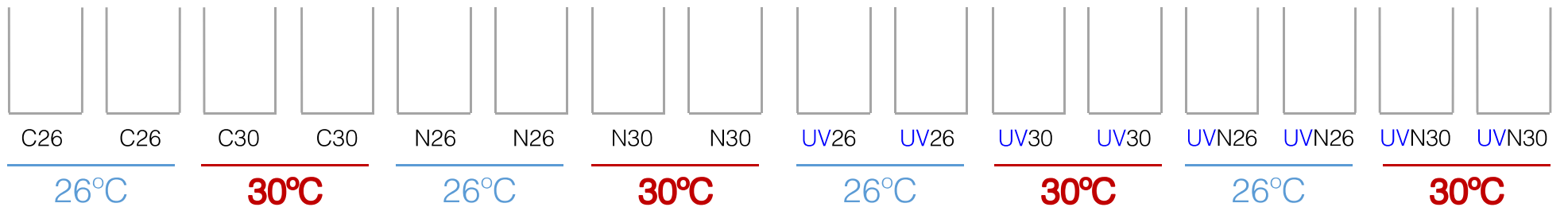
761 The authors declare that they have no conflict of interest.

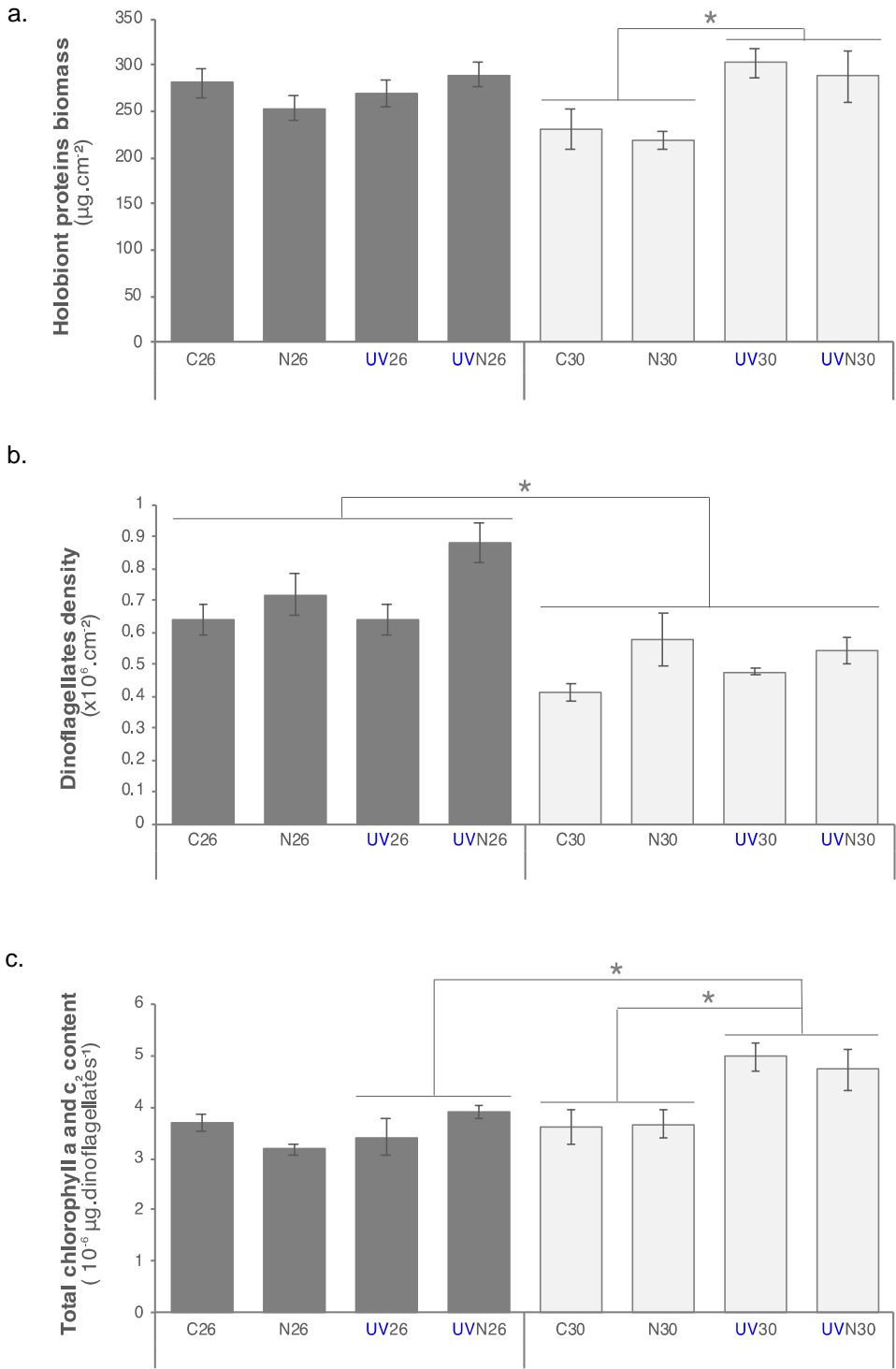
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First step : 6 weeks



Second step : 2 weeks





**Figure 3**

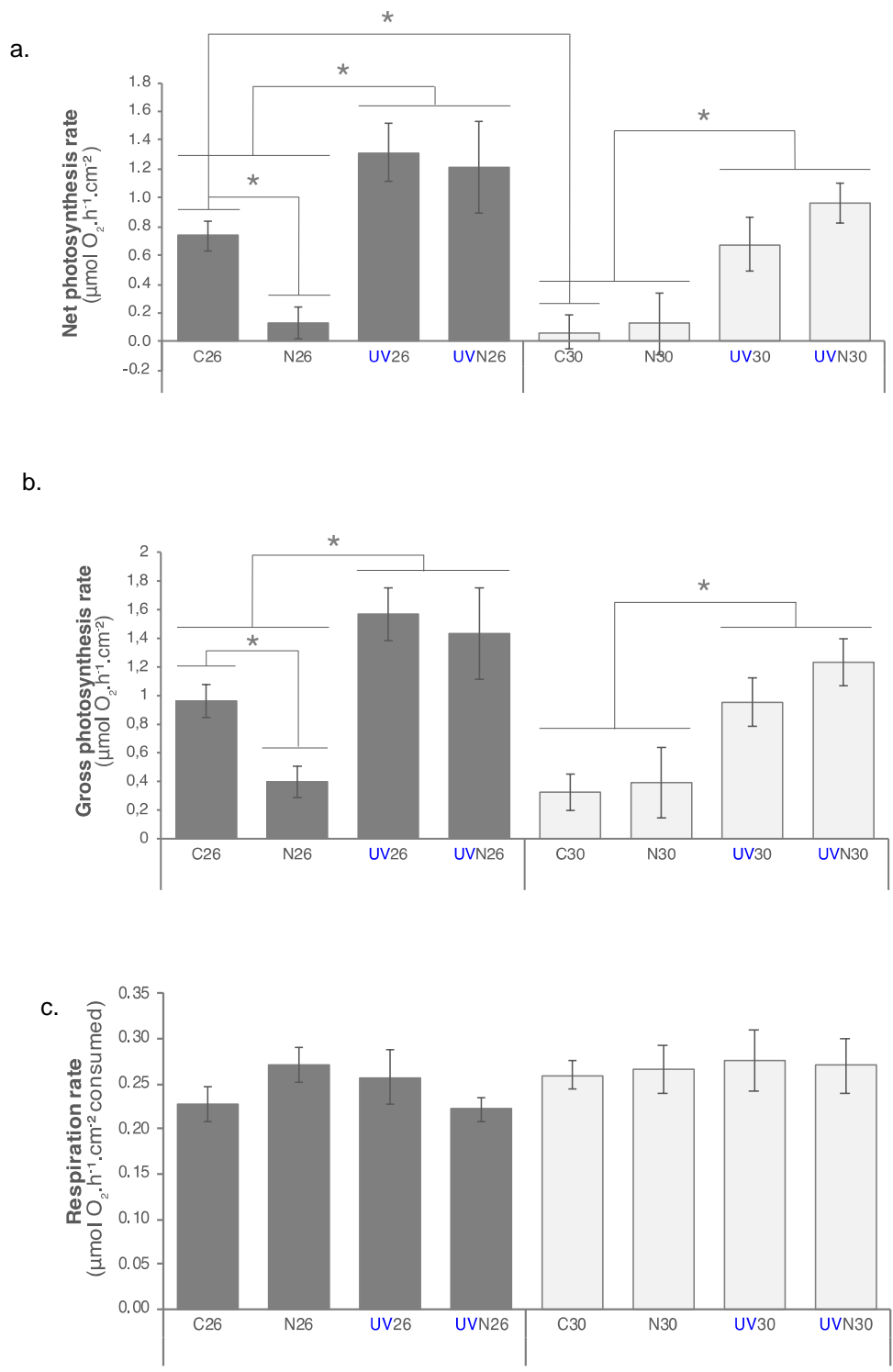


Figure 4

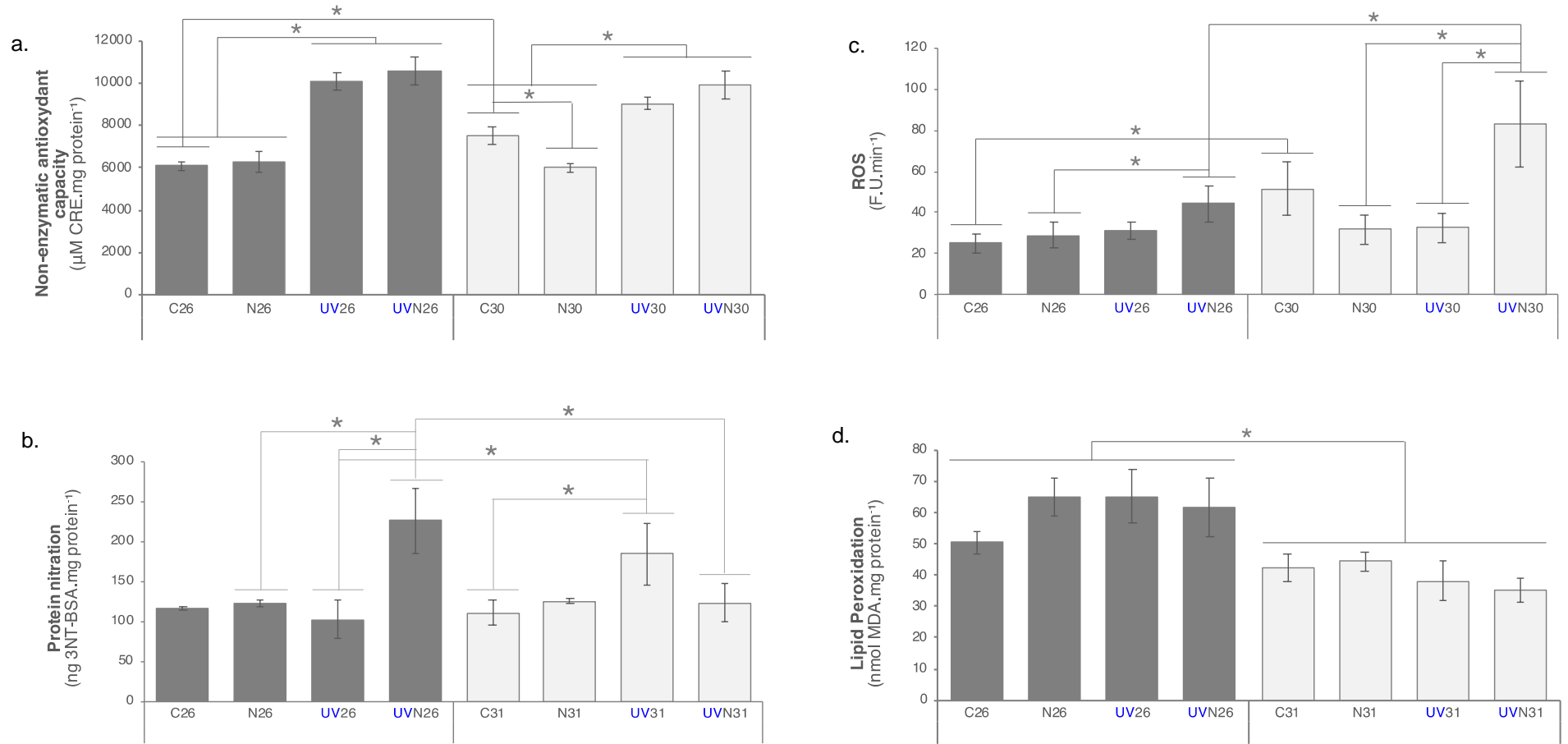
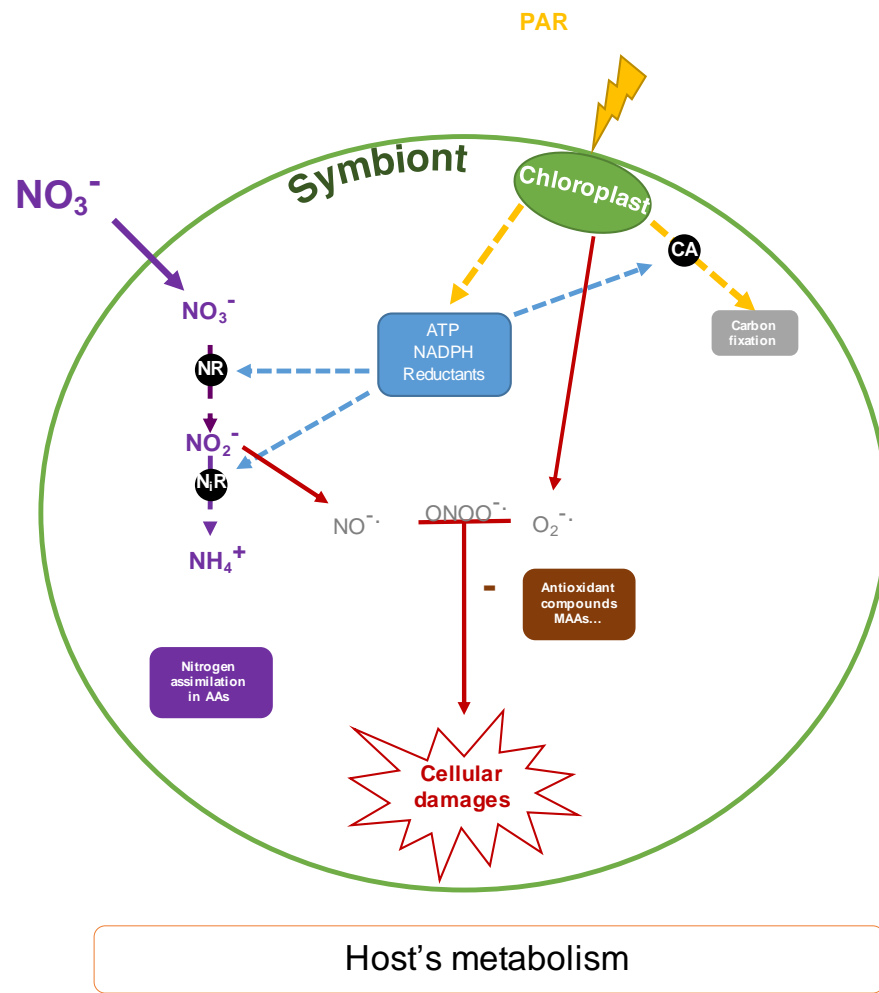
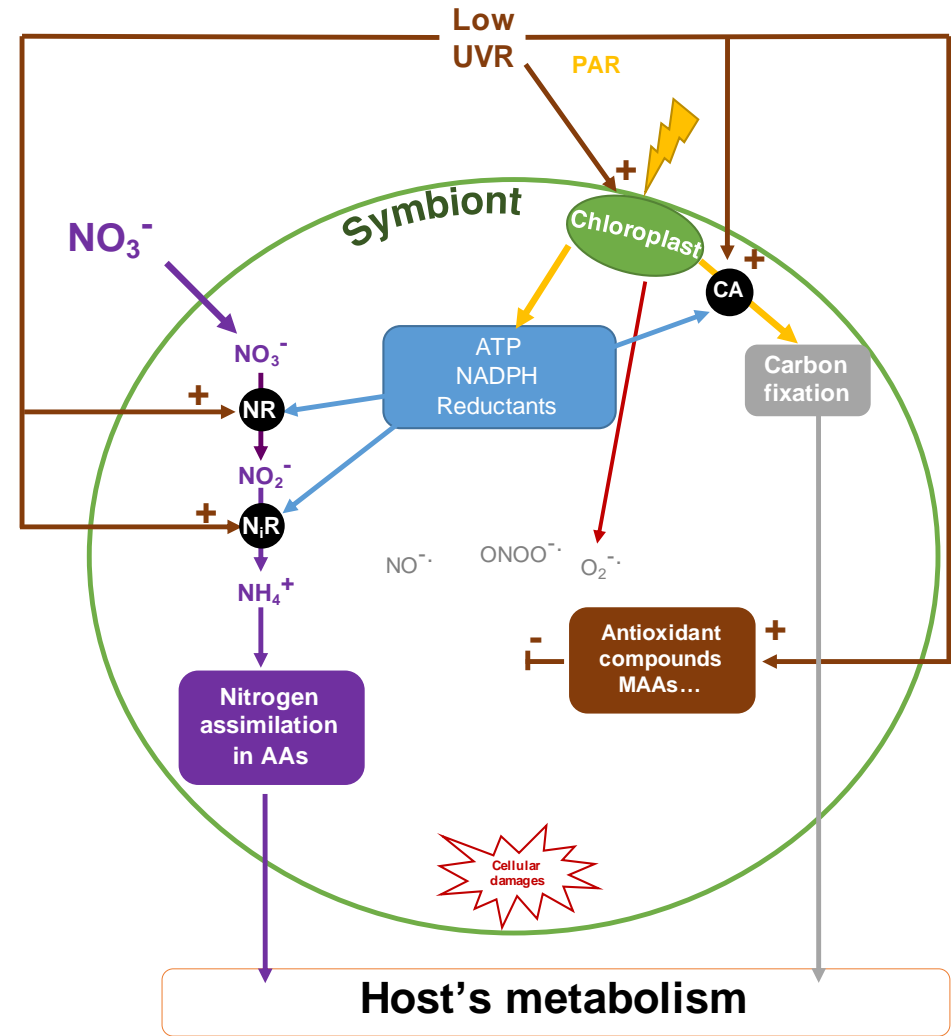


Figure 5



Nitrate enrichment  
No UVR exposure



Nitrate enrichment  
UVR exposure

766 **Figure legends**

767

768 **Figure 1**

769 Cross-factorial experimental design.

770 16 aquaria were used with 10 nubbins per aquaria. All tanks were supplied with continuous fresh  
771 seawater (0.5  $\mu\text{M}$  nitrate) and kept under PAR irradiance of  $200 \pm 10 \mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ . Corals  
772 were not fed.

773 During six weeks, 8 aquaria were kept under UVR exposure ( $25 \text{ Wm}^{-2}$  UVA and  $1.5 \text{ Wm}^{-2}$  UVB) and  
774 8 aquaria without UVR exposure. Out of the 8 aquaria, 4 were kept in control seawater and 4 were  
775 enriched with nitrate (3  $\mu\text{M}$  nitrate). Then, for 2 weeks, for each nitrate (N)-UVR condition 2 aquaria  
776 were kept at 26 °C and 2 aquaria were raised to 30 °C.

777 The experimental conditions are the following: **C26-C30**: control corals maintained at 26°C or 30°C  
778 without nitrate enrichment and UVR exposure, **N26-N30**: corals maintained under nitrate enrichment  
779 (3  $\mu\text{M}$  nitrate) at 26 °C or 30°C, **UV26-UV30**: corals exposed to UVR ( $25 \text{ Wm}^{-2}$  UVA and  $1.5 \text{ Wm}^{-2}$   
780 UVB) at 26 °C or 30°C, **UVN26-UVN30**: corals maintained under UVR exposure and nitrate  
781 enrichment (3  $\mu\text{M}$  nitrate) at 26 °C or 30°C.

782

783 **Figure 2**

784 Effects of temperature, UVR and nitrate levels on the physiological parameters of the coral

785 *Pocillopora damicornis*.

786 The barplots represents proteins biomass normalized by surface area ( $\mu\text{g.cm}^{-2}$ ) (a.), dinoflagellates  
787 density normalized by surface area ( $10^6.\text{cm}^{-2}$ ) (b.) and total chlorophyll a and c<sub>2</sub> content normalized  
788 by dinoflagellate cell ( $10^{-6} \mu\text{g.dinoflagellates}^{-1}$ ) (c.), under different nitrate (N)-UVR conditions. Dark  
789 grey bars represent corals maintained at 26°C (26) while light grey bars are corals maintained at 30°C  
790 (30) - **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR  
791 exposure, **N26-N30**: corals maintained under nitrate enrichment (3  $\mu\text{M}$  nitrate) at 26 °C or 30°C,  
792 **UV26-UV30**: corals exposed to UVR ( $25 \text{ Wm}^{-2}$  UVA and  $1.5 \text{ Wm}^{-2}$  UVB) at 26 °C or 30°C, **UVN26-**  
793 **UVN30**: corals maintained under UVR exposure and nitrate enrichment (3  $\mu\text{M}$  nitrate) at 26 °C or  
794 30°C.

795 Data represent mean and standard error of 6 replicates. Stars represent significantly different values (p  
796 value < 0.05).

797

798 **Figure 3**

799 Effects of temperature, UVR and nitrate levels on the oxygen fluxes (per skeletal surface area)  
800 measured for the coral *Pocillopora damicornis* maintained under different environmental conditions.

801 The barplots represent net (a.) and gross (b.) photosynthesis rates and respiration (c.) rates ( $\mu\text{mol}$   
802  $\text{O}_2.\text{h}^{-1}.\text{cm}^{-2}$ ) normalized per surface area under different nitrate (N)-UVR conditions. Dark grey bars  
803 represent corals maintained at 26°C (26) while light grey bars are corals maintained at 30°C (30) -  
804 **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR exposure,  
805 **N26-N30**: corals maintained under nitrate enrichment (3  $\mu\text{M}$  nitrate) at 26 °C or 30°C, **UV26-UV30**:  
806 corals exposed to UVR ( $25 \text{ Wm}^{-2}$  UVA and  $1.5 \text{ Wm}^{-2}$  UVB) at 26 °C or 30°C, **UVN26-UVN30**:  
807 corals maintained under UVR exposure and nitrate enrichment (3  $\mu\text{M}$  nitrate) at 26 °C or 30°C. Data  
808 represent mean and standard error of 6 replicates. Stars represent significantly different values (p  
809 value < 0.05).

810

811 **Figure 4**

812 Effects of temperature, UVR and nitrate levels on the oxidative stress paramaters of the coral  
813 holobiont *Pocillopora damicornis*

814 The barplots represent the non-enzymatic antioxidant capacity ( $\mu\text{M CRE.mg protein}^{-1}$ ) (a.), protein  
815 nitration level ( $\text{ng3NT-BSA.mg protein}^{-1}$ ) (b.), reactive oxygen species levels ( $\text{F.U.min}^{-1}$ ) (c.) and  
816 lipid peroxidation level ( $\text{nmol MDA.mg protein}^{-1}$ ) (d.) under different nitrate (N)-UVR conditions.

817 Dark grey bars represent corals maintained at 26°C (26) while light grey bars are corals maintained at  
818 30°C (30) - **C26-C30**: control corals maintained at 26°C or 30°C without nitrate enrichment and UVR  
819 exposure, **N26-N30**: corals maintained under nitrate enrichment (3 μM nitrate) at 26 °C or 30°C,  
820 **UV26-UV30**: corals exposed to UVR (25 Wm<sup>-2</sup> UVA and 1.5 Wm<sup>-2</sup> UVB) at 26 °C or 30°C, **UVN26-**  
821 **UVN30**: corals maintained under UVR exposure and nitrate enrichment (3 μM nitrate) at 26 °C or  
822 30°C. Data represent mean and standard error of six replicates. Stars represent significantly different  
823 values (p value < 0.05).

824

### 825 **Figure 5**

826 Summary of the main results obtained with nitrate-enriched corals with or without exposure to  
827 ultraviolet radiation.

828 The figure briefly summarizes the results obtained on the effect of nitrate enrichment on the  
829 dinoflagellate symbionts of the scleractinian coral *Pocillopora damicornis* in absence  
830 of ultraviolet radiation (**UVR**) (left panel) and with exposure to low UVR levels (right panel). Dashed  
831 arrows represents reduced processes while plain arrows (and increased text-box size) represent UVR-  
832 enhanced processes.

833 **PAR**: photosynthetically active radiation; **NR** and **N<sub>i</sub>R**: nitrate and nitrite reductase  
834 respectively; **NO<sub>3</sub><sup>-</sup>**, **NO<sub>2</sub><sup>-</sup>** and **NH<sub>4</sub><sup>+</sup>**: nitrate, nitrite and ammonium respectively, **NO<sup>•</sup>** and **ONOO<sup>-</sup>**

835 <sup>•</sup> are the reactive species of nitrogen: nitric oxide and peroxynitrite; **O<sub>2</sub><sup>-</sup>** is a reactive species of  
836 oxygen: superoxide anion; **CA**: carbonic anhydrase. **MAAs** and **AAs**: Mycosporine-like amino acids  
837 and amino acids respectively; **ATP** and **NADPH**: Adenosine triphosphate and Nicotinamide adenine  
838 dinucleotide phosphate

839 Carbon fixation and the reduction of nitrate into ammonium require ATP and other energetic  
840 molecules produced by photosynthesis. Low UVR exposure enhances photosynthesis and the  
841 production of energetic molecules (especially at 30°C), by increasing chlorophyll content. It is also  
842 known to stimulate the signaling pathway of the NR, N<sub>i</sub>R and CA, enhancing their activity. Therefore,  
843 nitrate reduction and carbon assimilation are enhanced in presence of low UVR levels. Finally, UVR  
844 stimulates the synthesis of antioxidant compounds such as MAAs, which offer a protection against  
845 oxidative stress.