1 **Warming effect on nitrogen fixation in Mediterranean macrophyte sediments**

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17 **Running head:** Nitrogen fixation in Mediterranean macrophytes

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20 Warming, Thermal dependence, Activation Energy, Q_{10}, Mediterranean Sea.

21 **Abstract**

22   The Mediterranean Sea is warming faster than the global Ocean, with important

23 consequences for organisms and biogeochemical cycles. Warming is a major stressor for key

24 marine benthic macrophytes. However, the effect of warming on marine N_2 fixation remains

25 unknown, despite the high productivity of macrophytes in oligotrophic waters is partially

26 sustained by the input of new nitrogen (N) into the system by N_2 fixation. Here, we assess the

27 impact of warming on N_2 fixation rates on three key marine macrophytes: *Posidonia oceanica*,

28 *Cymodocea nodosa*, and *Caulerpa prolifera*. We experimentally measured N_2 fixation rates in

29 vegetated and bare sediments at temperatures encompassing current summer mean, projected

30 summer mean and projected summer maximum seawater surface temperatures (SST) by the end

31 of the century under a scenario of moderate greenhouse-gas emissions. We found that N_2 fixation

32 rates in vegetated sediments were 2.8-fold higher than in bare sediments at current summer mean

33 SST, with no differences among macrophytes. Currently, the contribution of N_2 fixation to

34 macrophytes productivity could account for up to 7 %, 13.8 % and 1.8 % of N requirements for

35 *P. oceanica, C. nodosa,* and *C. prolifera,* respectively. We show the temperature dependence of

36 sediment N_2 fixation rates. However, the thermal response differed for vegetated sediments,

37 where rates showed an optimum at 31 °C followed by a sharp decrease at 33 °C, and bare

38 sediments, where rates increased along the range of experimental temperatures tested here. The

39 activation energy and Q_{10} were lower in vegetated sediments than in bare sediments. The

40 projected warming is expected to increase the contribution of N_2 fixation to Mediterranean

41 macrophytes' productivity. Therefore, the thermal dependence of N_2 fixation might have

42 important consequences for primary production in coastal ecosystems in the context of warming.

1. Introduction

43

44   Global mean surface temperatures have increased 0.85 ℃ from 1880 to 2012 and are

45   projected to increase between 1 and 3.5 ℃ by the end of the 21st century, relative to pre-

46   industrial times (IPCC, 2013). Similarly, heatwaves are more frequent since the second half of

47   the 20th century in Europe, Asia and Australia (IPCC, 2013;Perkins et al., 2012). Oceans store

48   most of the accumulated heat in the biosphere, warming at an average rate of 0.11 ℃ per decade

49   at surface (up to 75 m depth) since 1970 (IPCC, 2013), with longer and more frequent marine

50   heatwaves over the last century (Oliver et al., 2018). Warming is larger in small and enclosed

51   basins such as the Mediterranean Sea (Vargas-Yáñez et al., 2008), which is warming at 0.61 ℃

52   per decade (Belkin, 2009), with rapid migration of marine isotherms (Burrows et al., 2011) and

53   increased thermal extremes (Diffenbaugh et al., 2007). Specifically, the maximum surface

54   seawater temperature (SST_{max}) in the Balearic Islands, in the western Mediterranean Sea, is

55   projected to increase by 3.4 ± 1.3 ℃ by 2100 under a scenario of moderate greenhouse-gas

56   (GHG) emissions (A1B scenario from the IPCC Special Report on Emissions Scenarios,

57   equivalent to the RCP6.0 scenario of the IPCC Fifth Assessment Report) (Jordà et al., 2012),

58   with important consequences for marine organisms and ecosystems.

59   The effect of warming on Mediterranean marine organisms is reflected in changes in

60   abundance, survival, fertility, phenology, and migration (Marbà et al., 2015). In particular,

61   warming increases the mortality rates of the Mediterranean endemic and key marine angiosperm,

62   *Posidonia oceanica* (Marba and Duarte, 2010), which is predicted to be functional extinct by

63   2049 due to warming (Chefaoui et al., 2018;Jordà et al., 2012). In coastal benthic ecosystems,

64   warming also affects metabolic processes driving biogeochemical cycles. Warming enhances

65   sediment sulfate reduction rates (Robador et al., 2016), leading to an increase in sulfide

66   accumulation in coastal bare sediments (Sanz-Lázaro et al., 2011) and seagrass colonized

67   sediments (Koch et al., 2007). In the Mediterranean Sea, heatwaves and warming trigger sulfide

68   intrusion in *P. oceanica* shoots (García et al., 2013), which has toxic effects on plant meristems

69   (Garcias-Bonet et al., 2008) and increases shoot mortality (Calleja et al., 2007). Sanz-Lázaro et

70   al. (2011) found that warming enhances sediment oxygen uptake and CO_2 emissions in coastal

71   sediments, boosted by the addition of labile organic matter, in a mesocosm experiment.

72   Similarly, warming together with eutrophication have been identified as main drivers of hypoxia

73   in a Mediterranean macroalgae *Caulerpa prolifera* meadow (Vaquer-Sunyer et al., 2012).

74   However, studies on the effect of warming on atmospheric nitrogen fixation in coastal marine

75   ecosystems are lacking.

76   Nitrogen (N_2) fixation plays a fundamental role in balancing nutrient budgets at the basin

77   scale in the Mediterranean Sea, with most of this N_2 fixation associated with *P. oceanica*

78   seagrass meadows (Béthoux and Copin-Montégut, 1986). Indeed, endophytic nitrogen-fixing

79   bacteria have been detected in roots of *P. oceanica* (Garcias-Bonet et al., 2012;Garcias-Bonet et

80   al., 2016) and N_2 fixation has been reported in leaves and roots of *P. oceanica* (Agawin et al.,

81   2016;Lehnen et al., 2016) and *in situ* incubations (Agawin et al., 2017). Therefore, the high

82   productivity of *P. oceanica* in the oligotrophic Mediterranean waters is partially supported by N_2

83   fixation. However, the magnitude of N_2 fixation rates in the rhizosphere of *P. oceanica* is still

84   unknown, as well as N_2 fixation associated to other key macrophytes commonly found in the

85   Mediterranean Sea, such as *Cymodocea nodosa* and *Caulerpa prolifera*. In addition, whether

86   warming will affect N_2 fixation rates is still unknown.

87   Here, we experimentally assess the response N_2 fixation rates in coastal Mediterranean

88   ecosystems to warming. We focus specifically on three key Mediterranean macrophytes: two

89   seagrass species (*P. oceanica*, *C. nodosa*) and one green macroalgae species (*C. prolifera*). We

90   experimentally measured N_2 fixation rates in vegetated and bare sediments at temperatures

91   encompassing current summer mean SST (25 and 27 ºC), projected summer mean SST (29 and

92   31 ºC), and projected summer SST_{max} (33 ºC) by the end of the century under a scenario of

93   moderate GHG emissions to assess: i) differences between vegetated and bare sediments, ii)

94   differences among macrophyte species and iii) the thermal dependence of sediment N_2 fixation

95   rates.

96

97   **2. Materials and methods**

98

99   **2.1. Study site**

100   The study was conducted with communities sampled in Pollença Bay (Mallorca, Spain), a

101   bay located in the western Mediterranean Sea (39º 53.792' N; 3º 5.523' E). The study site was

102   selected based on the coexistence of the three most commonly found macrophyte species in the

103   region, including two seagrasses (*Posidonia oceanica* and *Cymodocea nodosa*) and one green

104   macroalgae (*Caulerpa prolifera*). The three macrophytes grow close to each other in

105   monospecific patches at 5-m depth. The study was conducted in mid-June 2017 when *in situ*

106   daily mean (±SE) SST was 26.4 ± 0.08 ºC.

107   We sampled sediment colonized by these three macrophytes and the adjacent bare

108   sediment using sediment cores (50 cm length and 4.5 cm in diameter). We collected the sediment

109   samples by pushing the cores down into the sediment with the help of a rubber hammer and

110   carefully extracting at least 15 cm of undisturbed top sediment. The cores were transported

111   immediately to the laboratory. We measured seawater salinity using a calibrated conductivity

112   meter (ProfiLine Cond 3310, WTW®, USA) and summer SST was monitored and recorded *in*

113   *situ* every 2 hours from 2012 to 2017 using a Hobo logger (Onset Computer Corporation®, MA,

114   USA). Full summer SST records are available for 2012, 2013, 2106, and 2017. No data is

115   available for 2015, and only partial temporal coverage is available for 2014 (Fig. 1). The summer

116   mean (±SE) SST varied from 26.29 ± 0.05 °C in 2013 to 27.03 ± 0.04 °C in 2017, with an

117   average summer mean SST of 26.54 ± 0.17 °C for the last years. Average summer minimum and

118   maximum SST were 22.92 and 29.08 °C, respectively. The highest maximum SST was 29.67 °C

119   and was registered in August 2017 (Table 1).

120

121   **2.2. Nitrogen fixation rates**

122   We measured sediment N_2 fixation rates by the Acetylene Reduction Assay (Capone and

123   Taylor, 1980) in *P. oceanica*, *C. nodosa* and *C. prolifera* vegetated sediments and the adjacent

124   bare sediment at five incubation temperatures: 25, 27, 29, 31, and 33 °C. The 25 and 27 °C

125   temperature treatments represent the current summer mean SST, covering the *in situ* recorded

126   average summer mean SST of 26.54 ± 0.2 °C (25 % percentile = 25.81 °C and 75 % percentile =

127   27.61 °C) from 2012 to 2017. The 29 and 31 °C temperature treatments represent the range of the

128   projected summer mean SST by the end of the century under a scenario of moderate GHG

129   emissions equivalent to RCP6.0, applying the projected mean SST increase of 2.8 ± 1.1 °C in the

130   region (Jordà et al., 2012) over the current summer mean SST. The 33 °C temperature treatment

131   represent the projected summer SST_{max} by the end of the century under a scenario of moderate

132   GHG emissions equivalent to RCP6.0, by applying the projected SST_{max} increase of 3.4 ± 1.3 °C

133   (Jordà et al., 2012) over the summer SST_{max} of 29.67 °C already recorded in 2017.

134   Once in the laboratory, the sediment from the cores was extruded carefully using a

135   plunger, and the first 10 cm of sediment below surface were collected, the rest of the sediment in

136   the core was discarded. For each replicate, 80 ml of sediment was placed in a 500 ml glass bottle.

137   Then, we added 200 ml of autoclaved seawater and the bottles were closed with a lid fitted with

138   a gas-tight valve. Finally, we added 20 ml of acetylene-saturated seawater through the gas-tight

139   valve of each bottle in order to achieve a final acetylene concentration of 4 mM. The acetylene-

140   saturated seawater was prepared according to Wilson et al. (Wilson et al., 2012). We run the

141   sediment incubations in triplicate for each type of sediment and each temperature treatment. The

142   sediment incubations were run under dark conditions in five water baths (i.e. one per temperature

143   treatment) equipped with thermometers and heaters located in a stable temperature room. The

144   target temperature for each water bath was maintained using an IKS-AQUASTAR system which

145   controlled and recorded the temperature every 10 minutes. During the 24 h incubations, the

146   temperature oscillation around the target temperatures ranged from 0.3 to 0.7 ºC, and the

147   temperature accuracy was ± 0.05 ºC on average (Table 2). The incubations lasted 24h, starting

148   after the addition of acetylene-saturated seawater. We sampled the headspace five times: at the

149   start of the experiment and at 12, 17, 20, and 24 h since the onset of the experiment. Specifically,

150   we withdrew 3 ml of air from the headspace with a gas-tight syringe. The headspace air sample

151   was immediately injected into a 3 ml vacuum vial for further analysis of ethylene concentration

152   on a gas chromatographer equipped with a flame ionization detector (FID-GC, Agilent 5890)

153   using a PoraPLOT U GC column (25 m × 0.53 mm × 20 μm, Agilent Technologies, USA). We

154   built a calibration curve using three ethylene standards of known concentration (1.02, 10.13 and

155   99.7 ppm) and Helium as a balance gas, supplied by Carburos Metálicos S.A. (Palma de

156   Mallorca, Spain). We estimated the concentration of dissolved ethylene, from the ethylene

157   concentration in the equilibrated air as described previously (Wilson et al., 2012) and applying

158   the solubility coefficient of ethylene according to Breitbarth et al. (Breitbarth et al., 2004) as a

159   function of temperature and salinity.

160   We run negative controls consisting in sediment without addition of acetylene-saturated

161   seawater in order to confirm that ethylene was not naturally produced by our samples, and

162   autoclaved seawater used in the preparation of the incubations with addition of acetylene-

163   saturated seawater in order to confirm that ethylene was not produced in the seawater. The

164   ethylene production rates were converted into N_2 fixation rates by applying the common ratio of

165   3 mol of acetylene:1 mol of N_2 (Welsh, 2000).

166   At the end of the incubation, we dried the sediment samples at 60 °C and recorded the dry

167   weight for further calculations. Moreover, we calculated the sediment organic matter (OM)

168   content of each replicate sediment sample by loss on ignition (Dean Jr, 1974). The sediment N_2

169   fixation rates were standardized to surface area integrated over 10 cm sediment depth by taking

170   into account the sediment bulk density.

171

172   **2.3. Statistical analysis**

173   Differences in sediment OM content and bulk density among *P. oceanica*, *C. nodosa,* and

174   *C. prolifera* vegetated sediments and bare sediment were tested with the non-parametric Kruskal

175   Wallis test. Differences in sediment N_2 fixation rates among the four types of sediment were

176   tested by Friedman test matching by temperature treatment. Differences in sediment N_2 fixation

177   rates between vegetated and bare sediments were tested by non-parametric Mann-Whitney *U*

178   test. Moreover, we tested the thermal dependence of sediment N_2 fixation rates in vegetated and

179   bare sediments by fitting Arrhenius function to estimate the activation energy, derived from the

180   relationship between the natural logarithm of N_2 fixation rates and the inverse of the temperature

181   multiplied by the Boltzmann's constant (Dell et al., 2011), and Q_{10} (Raven and Geider, 1988).

182   The activation energy and Q_{10} of N_2 fixation in vegetated sediments were calculated using the

183   increasing rates measured at four temperature treatments (25, 27, 29, and 31 °C), while the

184   declining rates measured at 33 °C were not included. The full range of temperature treatments

185   was used for bare sediments since no decline was detected. All statistical analyses were

186   performed using JMP (SAS Institute Inc., USA) and PRISM (GraphPad Software Inc., USA)

187   statistical software.

188

189   **3. Results**

190   Sediment OM content was significantly different in the sediments colonized by different

191   macrophyte species ($\chi^2_{3,56}$ = 50.33, p < 0.0001). *Posidonia oceanica* sediments had the highest

192   OM content (13.34 ± 0.56 %), whereas bare sediments had the lowest OM content (0.44 ± 0.50

193   %, Table 2). Sediment bulk density differed among sediment types ($\chi^2_{3,56}$ = 46.02, p < 0.0001,

194   Table 3).

195   Average N_2 fixation rates in bare sediments was 0.06 ± 0.01 (range from 0.01 to 0.09)

196   nmol N gDW^{-1} h^{-1}; while, average N_2 fixation rates in vegetated sediments was three-fold greater

197   at 0.19 ± 0.03 (range from 0.05 to 0.9) nmol N gDW^{-1} h^{-1}. Within the vegetated sediments, the

198   maximum mean N_2 fixation rate was detected in *C. prolifera* (0.22 ± 0.05 nmol N gDW^{-1} h^{-1}),

199   whereas the minimum mean N_2 fixation rate was measured in *C. nodosa* (0.15 ± 0.04 nmol N

200   gDW^{-1} h^{-1}). Nitrogen fixation rates differed among the four different sediment types (i.e. bare, *P.*

201   *oceanica*, *C. nodosa* and *C. prolifera* sediments) ($\chi^2_{3,56}$ = 10.68, p = 0.005) when expressed by

202   sediment dry weight. However, once the rates were converted into aerial basis, these differences

203   were no longer significant ($\chi^2_{3,56}$ = 6.12, p > 0.05), due to high variability in sediment bulk

204   densities. Sediment N_2 fixation rates were independent of OM content (linear regression,

205   p>0.05).

206   Nitrogen fixation rates in aerial basis were significantly higher in vegetated sediments

207   compared to bare ones (U = 154, p < 0.002) when pooling all temperature treatments together,

208   with sediments colonized by macrophytes supporting, on average, twice the nitrogen fixation rate

209   than bare sediments (mean ± SE = 3.86 ± 0.53 and 1.77 ± 0.20 mg N m^{-2} d^{-1}, respectively). The

210   differences between vegetated and bare sediment remained significant at current summer SST

211   (25-27 ºC) and projected summer mean SST (29-31 ºC) (U= 13, p < 0.01 and U = 23, p < 0.05,

212   respectively), with N_2 fixation rates of 3.15 ± 0.48 and 1.14 ± 0.3 mg N m^{-2} d^{-1} in vegetated and

213   bare sediments, respectively, at current summer SST (25-27 ºC) and N_2 fixation rates of 5.25 ±

214   1.17 and 2.18 ± 0.2 mg N m^{-2} d^{-1} in vegetated and bare sediments, respectively at projected

215   summer mean SST (29-31 ºC). However, vegetated and bare sediments did not differ in N_2

216   fixation rates between vegetated and bare sediments at projected summer SST$_{max}$ (33 ºC), with

217   N_2 fixation rates of 2.49 ± 0.248 and 2.21 ± 0.15 mg N m^{-2} d^{-1}, respectively (Fig. 2).

218   In vegetated sediments, N_2 fixation rates increased linearly with temperature up to 31ºC

219   (N_2 fixation (nmol N g DW^{-1} h^{-1}) = -0.63 + 0.03*Temperature, R^2 = 0.11, p < 0.05), with a

220   marked decrease from 0.32 ± 0.09 nmol N g DW^{-1} h^{-1} at 31 ºC to 0.11 ± 0.01 nmol N g DW^{-1} h^{-1}

221   at 33 ºC (Fig. 3). Nitrogen fixation rates in bare sediments increased linearly with temperature up

222   to 33 ºC (N_2 fixation (nmol N g DW^{-1} h^{-1}) = -0.11 + 0.01*Temperature, R^2 = 0.51, p < 0.005, Fig.

223   3). The associated activation energies were 0.91 ± 0.39 and 1.25 ± 0.39 eV for vegetated and

224   bare sediments, respectively (Fig. 4). Associated Q_{10} values were 3.84 ± 2.22 and 6.41 ± 2.97 for

225   vegetated and bare sediments, respectively.

4. Discussion

The overall average N_2 fixation rate found in Mediterranean vegetated sediments at current summer mean SST (3.15 ± 0.48 mg N m^{-2} d^{-1}) is within the range of those rates reported for sediments colonized by temperate seagrass species (from 1.2 to 6.5 mg N m^{-2} d^{-1} in *Zostera marina* sediments in the North Sea (McGlathery et al., 1998) and the Northwest Atlantic coast (Capone, 1982), and from 0.1 to 7.3 mg N m^{-2} d^{-1} in *Zostera noltii* in the Northeast Atlantic coast (Welsh et al., 1996)), but lower than the rates reported for tropical and subtropical seagrass species (see references in (Welsh, 2000)). The overall N_2 fixation rates in vegetated sediments are higher than in bare sediments, consistent with the long-recognized role of marine plants in enhancing N_2 fixation rates (Capone, 1988). Specifically, the vegetated sediments supported 3 to 4-fold higher N_2 fixation rates than bare sediments did.

The N_2 fixation rates we measured in *P. oceanica* sediments at current summer mean SST (2.86 ± 1.26 mg N m^{-2} d^{-1}) are higher than the rates reported by Agawin et al. (ranging from 0.06 to 1.51 mg N m^{-2} d^{-1}) in summer, at similar seawater temperature, using benthic bell-jar chambers containing *P. oceanica* shoots and the underlying sediment (Agawin et al., 2017). However, the different methodological approaches make comparisons difficult: while sediment slurries might slightly overestimate rates due to sediment structure disturbance and increase in organic matter availability, incubation chambers might underestimate rates due to poor diffusion of acetylene into the sediment (Welsh, 2000). Nevertheless, the N_2 fixation rates in bare sediments at current summer mean SST (1.14 ± 0.31 mg N m^{-2} d^{-1}) are very similar to those measured by benthic bell-jar in bare sediment adjacent to a *P. oceanica* meadow (from 0.01 to 1.99 mg N m^{-2} d^{-1}) (Agawin et al., 2017), suggesting that these differences in N_2 fixation rates in *P. oceanica* sediment might also be due to variability among sites. The N_2 fixation rates in *C.*

249   *nodosa* and *C. prolifera* sediments reported here are the first reports, to the best of our

250   knowledge, for these two important Mediterranean macrophyte species. Indeed, the analysis of

251   sediment N stocks in a *C. nodosa* meadow in the Mediterranean Sea suggested that N_2 fixation

252   might be contributing to enhance the N stocks compared to bare sediments (Pedersen et al.,

253   1997). The similar stable N isotope composition of *C. nodosa* tissues and those of *P. oceanica* in

254   the Mediterranean (Fourqurean et al., 2007) also suggests that they use similar sources of

255   nitrogen. The N_2 fixation rates at current summer mean SST in *C. prolifera* sediments found here

256   (0.17 ± 0.04 nmol N gDW^{-1} h^{-1}) are similar to the sediment N_2 fixation rates associated to the

257   invasive *C. taxifolia* in Monaco (0.12 ± 0.09 nmol N gDW^{-1} h^{-1}) but 20-fold lower than the N_2

258   fixation rates reported for *C. taxifolia* in France (3.96 ± 1.99 nmol N gDW^{-1} h^{-1}) (Chisholm and

259   Moulin, 2003).

260   Although the sediments colonized by these three macrophyte species hold similar rates,

261   the contribution of sediment N_2 fixation to the productivity of each plant is different. Taking into

262   account the average net production (2.63 and 1.47 g DW m^{-2} d^{-1} for *P. oceanica* and *C. nodosa*,

263   respectively (Duarte and Chiscano, 1999) and 5.16 g DW m^{-2} d^{-1} for *C. prolifera* (Marbà

264   unpublished)) and the tissue nitrogen content (from 1.55 to 1.63 % for *P. oceanica*, from 1.91 to

265   2.28 % for *C. nodosa* (Duarte, 1990;Fourqurean et al., 2007) and from 3 to 4.9 % for *C. prolifera*

266   (Morris et al., 2009)), the mean measured sediment N_2 fixation rates detected at current summer

267   mean SST (25 and 27 °C) could account for 6.7 to 7 %, 11.5 to 13.8 % and 1.1 to 1.8 % of the

268   nitrogen requirements for *P. oceanica*, *C. nodosa,* and *C. prolifera*, respectively. The calculated

269   contribution of N_2 fixation to seagrass growth requirements fall within the range of the N_2

270   fixation contributions reported for temperate seagrasses, ranging from 5 to 12 % for *Z. marina*

271   and *Z. noltii*, respectively (Welsh, 2000). The calculated contribution of N_2 fixation to fulfill the

272   macrophytes' growth requirements points N_2 fixation as partially supporting the high

273   productivity of these primary producers in the Mediterranean oligotrophic waters.

274   We experimentally demonstrate that N_2 fixation rates in coastal sediments are thermal

275   dependent, both in vegetated and bare sediments, in agreement with higher rates typically

276   reported in tropical and subtropical meadows compared to temperate and cold seagrass systems

277   (Herbert, 1999;McGlathery, 2008;Welsh, 2000); although an experimental demonstration was

278   lacking. The thermal dependence, as reflected by the activation energy and Q_{10}, for N_2 fixation

279   rates was, however, higher in bare sediments than in vegetated sediments, possibly due to

280   different bacterial communities. Westrich and Berner (1988) also found that sulfate reduction

281   exhibited a more pronounced thermal dependence in sediments supporting lower rates. The

282   activation energies for N_2 fixation in vegetated sediments (0.91 ± 0.4 eV or 87.8 ± 37.6 KJ mol^{-1})

283   and in bare sediments (1.25 ± 0.4 eV or 120.6 ± 37.6 KJ mol^{-1}) are within the range of the

284   activation energy reported for sediment sulfate reduction (range from 36 to 132 KJ mol^{-1}

285   (Robador et al., 2016;Westrich and Berner, 1988)) and for sediment organic matter degradation

286   (range from 54 to 125 KJ mol^{-1} (Middelburg et al., 1996)). The Q_{10} values associated to sediment

287   N_2 fixation (3.84 ± 2.22 and 6.41 ± 2.97 for vegetated and bare sediments, respectively) are

288   higher than those reported for sediment sulfate reduction (from 1.6 to 3.4 (Robador et al., 2016)),

289   but still similar to those values associated to organic matter degradation (from 2.2 to 6.3

290   (Middelburg et al., 1996)). Moreover, the thermal response differed for vegetated sediments,

291   where N_2 fixation rates showed an optimum at 31 ^{o}C followed by a sharp decrease at 33 ^{o}C, and

292   bare sediments, where N_2 fixation rates increased along the range of experimental temperatures

293   tested here. The thermal response of N_2 fixation in vegetated sediments found here is similar to

294   the thermal response reported for N_2 fixation in soil crusts (Zhou et al., 2016) and seagrass

295   rhizosphere (Garcias-Bonet et al., 2018), with an increase in rates up to 30 and 29 ºC,

296   respectively, and a marked decrease in rates at temperatures above the optimum. The forecasted

297   warming by the end of the century could potentially increase N_2 fixation rates by 36.7% in

298   vegetated sediments and 46.8% in bare sediments. However, the decrease in N_2 fixation rates in

299   vegetated sediments at 33 ºC would imply a reduction of a third in the contribution of N_2 fixation

300   to the macrophytes' productivity during heatwaves. Therefore, the thermal dependence of N_2

301   fixation in vegetated sediments might have important consequences for primary production in

302   coastal ecosystems in the context of warming. However, this may not be the case for *P.*

303   *oceanica*, as this species is projected to be critically compromised, to the extent that functional

304   extinction is possible, with projected Mediterranean warming rates by 2050 (Chefaoui et al.,

305   2018;Jordà et al., 2012).

306

307   **Conclusion**

308   Mediterranean macrophyte meadows are sites of intense N_2 fixation rates, twice as high

309   as those in adjacent bare sediments. As these rates increased greatly with warming, realized

310   warming of the Mediterranean Sea is expected to have led to enhanced N_2 fixation rates, both for

311   bare and vegetated sediments, with further warming leading to further increase in N_2 fixation

312   rates.

313

314   **Author contribution**

315   NG-B, RVS, CMD and NM designed the study. NG-B and RVS performed the field

316   work, and sample and data analysis. NG-B, RVS, CMD and NM interpreted the results. NG-B

317   wrote the first draft of the manuscript. All authors contributed substantially to the final

318   manuscript.

319

320   **Conflict of interests**

321   Authors declare that they have no conflict of interest

322

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329   **Figures**

330   Fig. 1. Summer *in situ* SST (from June 21st to September 22nd) from 2012 to 2017 at the study

331   site. Solid line indicates average summer median SST and dashed lines indicate the 25 and 75%

332   percentiles. Average summer median SST and percentiles do not include data from 2014 due to

333   partial temporal coverage in temperature records. No data available for 2015.

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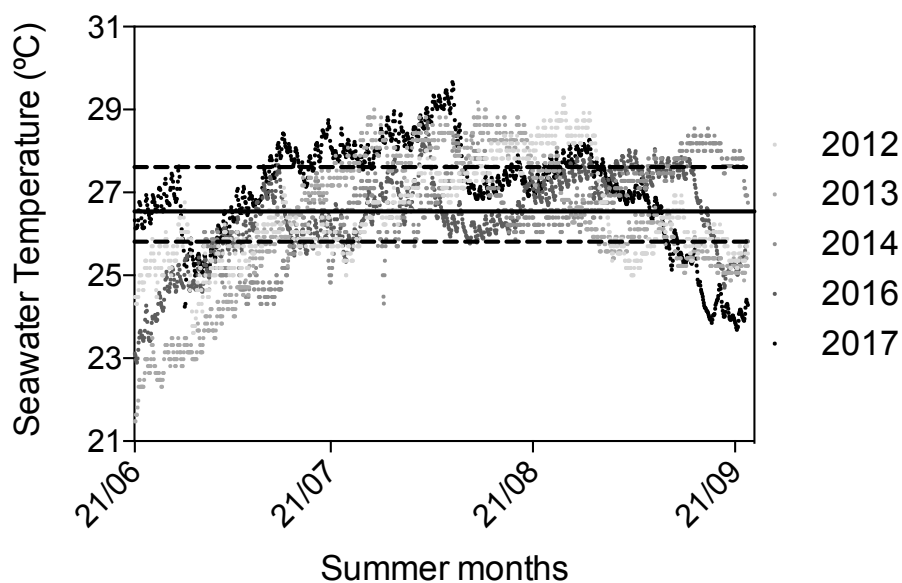
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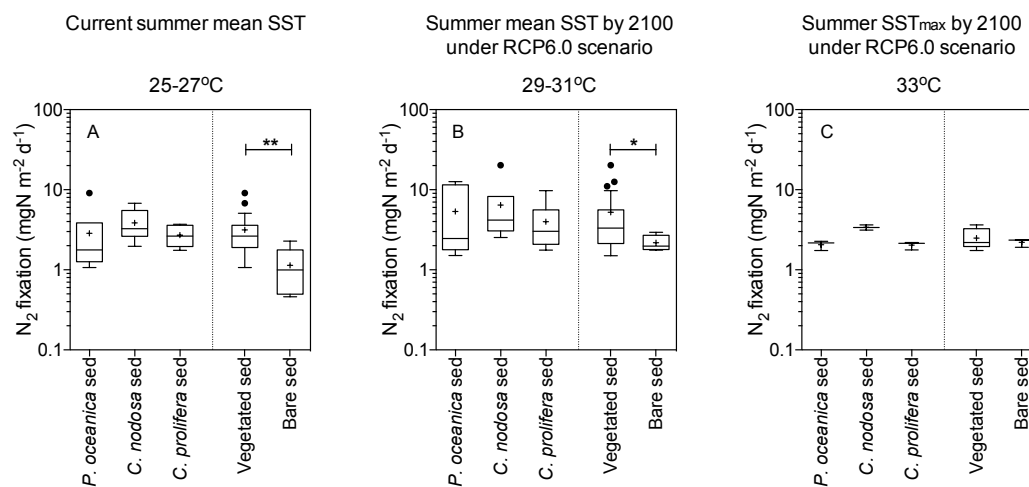
336

337   Fig. 2. Box plot of the N$_2$ fixation rates of sediments colonized by different macrophytes (as well

338   as grouping all vegetated sediments together) and bare sediment measured at current summer

339   SST (25-27 $^{\circ}$C, (A)), projected summer mean SST by 2100 under RCP6.0 scenario (29-31 $^{\circ}$C,

340   (B)), and projected summer SST$_{max}$ by 2100 under RCP6.0 (33 $^{\circ}$C, (C)).

341

342

343

344   Fig. 3. Relationship of experimental incubation temperature and sediment N$_2$ fixation rates in

345   vegetated (black dots) and bare sediments (white dots). Dots indicate mean values and errors

346   bars indicate standard error of the mean.

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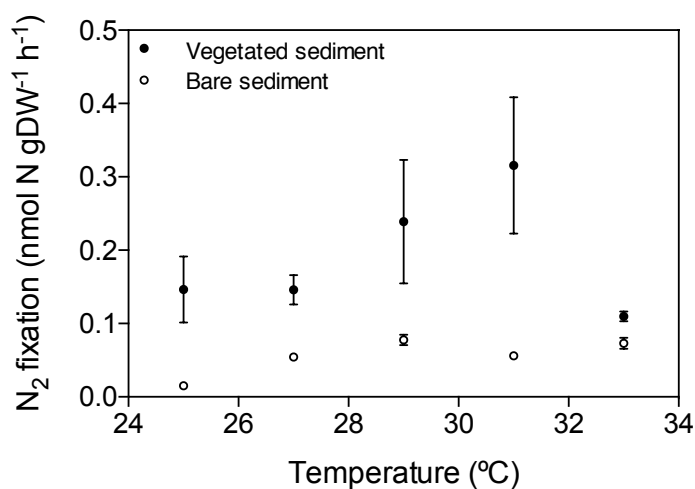
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351   Fig. 4. Arrhenius plot for N_2 fixation rates in vegetated (black dots and line) and bare sediments

352   (grey dots and line).

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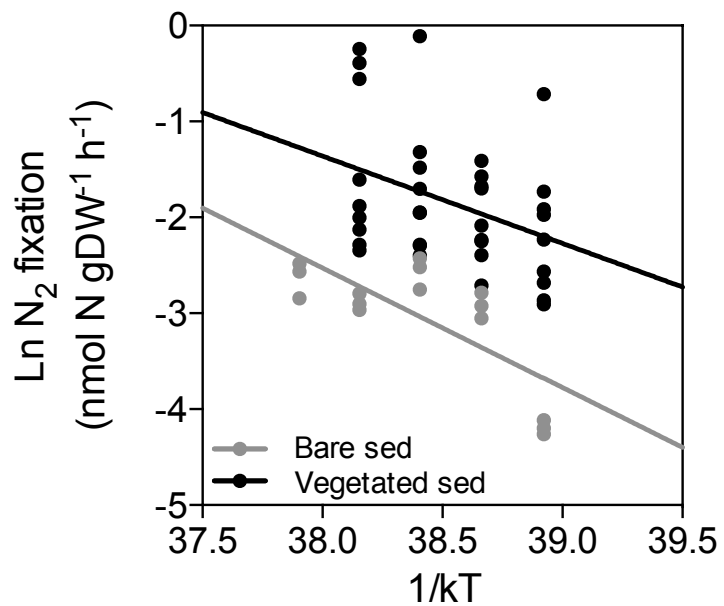
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357 **Tables**

358

359 Table 1. Summary of *in situ* summer SST at the study site (Pollença Bay, Mallorca) from June

360 21st to September 22nd from 2012 to 2017, showing the mean ± SEM, minimum (Min) and

361 maximum (max), median, and 25 and 75% percentiles. nd: no data, na: not available due to

362 partial temporal coverage.

363

Year	Summer Surface Seawater Temperature (SST)				
	Mean (±SEM)	Min - Max	Median	25% percentile	75% percentile
2012	26.44 ± 0.03	23.61 – 29.29	26.23	25.70	27.28
2013	26.29 ± 0.05	21.48 – 29.18	26.28	25.23	27.98
2014	na	na – 28.55	na	na	na
2015	nd	nd	nd	nd	nd
2016	26.39 ± 0.03	22.90 – 28.69	26.43	25.82	27.24
2017	27.03 ± 0.04	23.69 – 29.67	27.21	26.50	27.92
Average 2012 – 2017*	**26.54 ± 0.17**	**22.92 – 29.08**	**26.54**	**25.81**	**27.61**

364

365 *including data from 2012, 2013, 2016, and 2017 for which full summer records are available.

366

367

368   Table 2. Experimental temperature set up used in the sediment incubations, indicating the five

369   target temperatures and the actual mean (±SEM) temperature recorded in each incubation water

370   bath during the 24h incubations. The ranges (minimum – maximum values) are shown in

371   parenthesis.

372

Target temperature (ºC)	Incubation temperature (ºC)
25 ºC	25.07 ± 0.01 (24.9 – 25.2)
27 ºC	27.02 ± 0.01 (26.6 – 27.2)
29 ºC	29.05 ± 0.01 (28.8 – 29.3)
31 ºC	31.08 ± 0.01 (30.7 – 31.4)
33 ºC	33.05 ± 0.01 (32.7 – 33.2)

373

374

375

376   Table 3. Sediment organic matter content and bulk density in sediments colonized by different

377   macrophyte species and bare sediment in Pollença bay in June 2017. Mean values (±SEM) and

378   the ranges (minimum – maximum values) are shown.

379

	P. oceanica sediment	*C. nodosa* sediment	*C. prolifera* sediment	Bare sediment
Sediment organic matter content (%)	12.58 ± 0.95 (8.17 – 20.63)	6.14 ± 0.39 (4.49 – 10.22)	0.74 ± 0.12 (0.04 – 1.31)	0.44 ± 0.08 (0.02 – 1.41)
Sediment bulk density (g DW sed cm^{-3})	0.54 ± 0.03 (0.29 – 0.69)	1.01 ± 0.03 (0.74 – 1.24)	0.46 ± 0.02 (0.32 – 0.56)	0.96 ± 0.02 (0.84 – 1.09)

380

381

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