Warming effect on nitrogen fixation in Mediterranean macrophyte sediments

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Warming, Thermal dependence, Activation Energy, Q10, Mediterranean Sea.
Abstract

The Mediterranean Sea is warming faster than the global Ocean, with important consequences for organisms and biogeochemical cycles. Warming is a major stressor for key marine benthic macrophytes. However, the effect of warming on marine N$_2$ fixation remains unknown, despite the high productivity of macrophytes in oligotrophic waters is partially sustained by the input of new nitrogen (N) into the system by N$_2$ fixation. Here, we assess the impact of warming on N$_2$ fixation rates on three key marine macrophytes: *Posidonia oceanica*, *Cymodocea nodosa*, and *Caulerpa prolifera*. We experimentally measured N$_2$ fixation rates in vegetated and bare sediments at temperatures encompassing current summer mean, projected summer mean and projected summer maximum seawater surface temperatures (SST) by the end of the century under a scenario of moderate greenhouse-gas emissions. We found that N$_2$ fixation rates in vegetated sediments were 2.8-fold higher than in bare sediments at current summer mean SST, with no differences among macrophytes. Currently, the contribution of N$_2$ fixation to macrophytes productivity could account for up to 7%, 13.8% and 1.8% of N requirements for *P. oceanica*, *C. nodosa*, and *C. prolifera*, respectively. We show the temperature dependence of sediment N$_2$ fixation rates. However, the thermal response differed for vegetated sediments, where rates showed an optimum at 31 °C followed by a sharp decrease at 33 °C, and bare sediments, where rates increased along the range of experimental temperatures tested here. The activation energy and Q$_{10}$ were lower in vegetated sediments than in bare sediments. The projected warming is expected to increase the contribution of N$_2$ fixation to Mediterranean macrophytes’ productivity. Therefore, the thermal dependence of N$_2$ fixation might have important consequences for primary production in coastal ecosystems in the context of warming.
1. Introduction

Global mean surface temperatures have increased 0.85 °C from 1880 to 2012 and are projected to increase between 1 and 3.5 °C by the end of the 21st century, relative to pre-industrial times (IPCC, 2013). Similarly, heatwaves are more frequent since the second half of the 20th century in Europe, Asia and Australia (IPCC, 2013; Perkins et al., 2012). Oceans store most of the accumulated heat in the biosphere, warming at an average rate of 0.11 °C per decade at surface (up to 75 m depth) since 1970 (IPCC, 2013), with longer and more frequent marine heatwaves over the last century (Oliver et al., 2018). Warming is larger in small and enclosed basins such as the Mediterranean Sea (Vargas-Yáñez et al., 2008), which is warming at 0.61 °C per decade (Belkin, 2009), with rapid migration of marine isotherms (Burrows et al., 2011) and increased thermal extremes (Diffenbaugh et al., 2007). Specifically, the maximum surface seawater temperature ($SST_{\text{max}}$) in the Balearic Islands, in the western Mediterranean Sea, is projected to increase by $3.4 \pm 1.3$ °C by 2100 under a scenario of moderate greenhouse-gas (GHG) emissions (A1B scenario from the IPCC Special Report on Emissions Scenarios, equivalent to the RCP6.0 scenario of the IPCC Fifth Assessment Report) (Jordà et al., 2012), with important consequences for marine organisms and ecosystems.

The effect of warming on Mediterranean marine organisms is reflected in changes in abundance, survival, fertility, phenology, and migration (Marbà et al., 2015). In particular, warming increases the mortality rates of the Mediterranean endemic and key marine angiosperm, Posidonia oceanica (Marba and Duarte, 2010), which is predicted to be functional extinct by 2049 due to warming (Chefaoui et al., 2018; Jordà et al., 2012). In coastal benthic ecosystems, warming also affects metabolic processes driving biogeochemical cycles. Warming enhances sediment sulfate reduction rates (Robador et al., 2016), leading to an increase in sulfide.
accumulation in coastal bare sediments (Sanz-Lázaro et al., 2011) and seagrass colonized sediments (Koch et al., 2007). In the Mediterranean Sea, heatwaves and warming trigger sulfide intrusion in P. oceanica shoots (García et al., 2013), which has toxic effects on plant meristems (Garcias-Bonet et al., 2008) and increases shoot mortality (Calleja et al., 2007). Sanz-Lázaro et al. (2011) found that warming enhances sediment oxygen uptake and CO₂ emissions in coastal sediments, boosted by the addition of labile organic matter, in a mesocosm experiment. Similarly, warming together with eutrophication have been identified as main drivers of hypoxia in a Mediterranean macroalgae Caulerpa prolifera meadow (Vaquer-Sunyer et al., 2012).

However, studies on the effect of warming on atmospheric nitrogen fixation in coastal marine ecosystems are lacking.

Nitrogen (N₂) fixation plays a fundamental role in balancing nutrient budgets at the basin scale in the Mediterranean Sea, with most of this N₂ fixation associated with P. oceanica seagrass meadows (Béthoux and Copin-Montégut, 1986). Indeed, endophytic nitrogen-fixing bacteria have been detected in roots of P. oceanica (Garcias-Bonet et al., 2012; Garcias-Bonet et al., 2016) and N₂ fixation has been reported in leaves and roots of P. oceanica (Agawin et al., 2016; Lehn et al., 2016) and in situ incubations (Agawin et al., 2017). Therefore, the high productivity of P. oceanica in the oligotrophic Mediterranean waters is partially supported by N₂ fixation. However, the magnitude of N₂ fixation rates in the rhizosphere of P. oceanica is still unknown, as well as N₂ fixation associated to other key macrophytes commonly found in the Mediterranean Sea, such as Cymodocea nodosa and Caulerpa prolifera. In addition, whether warming will affect N₂ fixation rates is still unknown.

Here, we experimentally assess the response N₂ fixation rates in coastal Mediterranean ecosystems to warming. We focus specifically on three key Mediterranean macrophytes: two
seagrass species (P. oceanica, C. nodosa) and one green macroalgae species (C. prolifera). We experimentally measured N₂ fixation rates in vegetated and bare sediments at temperatures encompassing current summer mean SST (25 and 27 °C), projected summer mean SST (29 and 31 °C), and projected summer SST_max (33 °C) by the end of the century under a scenario of moderate GHG emissions to assess: i) differences between vegetated and bare sediments, ii) differences among macrophyte species and iii) the thermal dependence of sediment N₂ fixation rates.

2. Materials and methods

2.1. Study site

The study was conducted with communities sampled in Pollença Bay (Mallorca, Spain), a bay located in the western Mediterranean Sea (39° 53.792’ N; 3° 5.523’ E). The study site was selected based on the coexistence of the three most commonly found macrophyte species in the region, including two seagrasses (Posidonia oceanica and Cymodocea nodosa) and one green macroalgae (Caulerpa prolifera). The three macrophytes grow close to each other in monospecific patches at 5-m depth. The study was conducted in mid-June 2017 when in situ daily mean (±SE) SST was 26.4 ± 0.08 °C.

We sampled sediment colonized by these three macrophytes and the adjacent bare sediment using sediment cores (50 cm length and 4.5 cm in diameter). We collected the sediment samples by pushing the cores down into the sediment with the help of a rubber hammer and carefully extracting at least 15 cm of undisturbed top sediment. The cores were transported immediately to the laboratory. We measured seawater salinity using a calibrated conductivity
meter (ProfiLine Cond 3310, WTW®, USA) and summer SST was monitored and recorded in situ every 2 hours from 2012 to 2017 using a Hobo logger (Onset Computer Corporation®, MA, USA). Full summer SST records are available for 2012, 2013, 2016, and 2017. No data is available for 2015, and only partial temporal coverage is available for 2014 (Fig. 1). The summer mean (±SE) SST varied from 26.29 ± 0.05 °C in 2013 to 27.03 ± 0.04 °C in 2017, with an average summer mean SST of 26.54 ± 0.17 °C for the last years. Average summer minimum and maximum SST were 22.92 and 29.08 °C, respectively. The highest maximum SST was 29.67 °C and was registered in August 2017 (Table 1).

2.2. Nitrogen fixation rates

We measured sediment N\textsubscript{2} fixation rates by the Acetylene Reduction Assay (Capone and Taylor, 1980) in P. oceanica, C. nodosa and C. prolifera vegetated sediments and the adjacent bare sediment at five incubation temperatures: 25, 27, 29, 31, and 33 °C. The 25 and 27 °C temperature treatments represent the current summer mean SST, covering the in situ recorded average summer mean SST of 26.54 ± 0.2 °C (25 % percentile = 25.81 °C and 75 % percentile = 27.61 °C) from 2012 to 2017. The 29 and 31 °C temperature treatments represent the range of the projected summer mean SST by the end of the century under a scenario of moderate GHG emissions equivalent to RCP6.0, applying the projected mean SST increase of 2.8 ± 1.1 °C in the region (Jordà et al., 2012) over the current summer mean SST. The 33 °C temperature treatment represent the projected summer SST\textsubscript{max} by the end of the century under a scenario of moderate GHG emissions equivalent to RCP6.0, by applying the projected SST\textsubscript{max} increase of 3.4 ± 1.3 °C (Jordà et al., 2012) over the summer SST\textsubscript{max} of 29.67 °C already recorded in 2017.
Once in the laboratory, the sediment from the cores was extruded carefully using a plunger, and the first 10 cm of sediment below surface were collected, the rest of the sediment in the core was discarded. For each replicate, 80 ml of sediment was placed in a 500 ml glass bottle. Then, we added 200 ml of autoclaved seawater and the bottles were closed with a lid fitted with a gas-tight valve. Finally, we added 20 ml of acetylene-saturated seawater through the gas-tight valve of each bottle in order to achieve a final acetylene concentration of 4 mM. The acetylene-saturated seawater was prepared according to Wilson et al. (Wilson et al., 2012). We run the sediment incubations in triplicate for each type of sediment and each temperature treatment. The sediment incubations were run under dark conditions in five water baths (i.e. one per temperature treatment) equipped with thermometers and heaters located in a stable temperature room. The target temperature for each water bath was maintained using an IKS-AQUASTAR system which controlled and recorded the temperature every 10 minutes. During the 24 h incubations, the temperature oscillation around the target temperatures ranged from 0.3 to 0.7 °C, and the temperature accuracy was ± 0.05 °C on average (Table 2). The incubations lasted 24h, starting after the addition of acetylene-saturated seawater. We sampled the headspace five times: at the start of the experiment and at 12, 17, 20, and 24 h since the onset of the experiment. Specifically, we withdrew 3 ml of air from the headspace with a gas-tight syringe. The headspace air sample was immediately injected into a 3 ml vacuum vial for further analysis of ethylene concentration on a gas chromatograph equipped with a flame ionization detector (FID-GC, Agilent 5890) using a PoraPLOT U GC column (25 m × 0.53 mm × 20 µm, Agilent Technologies, USA). We built a calibration curve using three ethylene standards of known concentration (1.02, 10.13 and 99.7 ppm) and Helium as a balance gas, supplied by Carburos Metálicos S.A. (Palma de Mallorca, Spain). We estimated the concentration of dissolved ethylene, from the ethylene
concentration in the equilibrated air as described previously (Wilson et al., 2012) and applying the solubility coefficient of ethylene according to Breitbarth et al. (Breitbarth et al., 2004) as a function of temperature and salinity.

We run negative controls consisting in sediment without addition of acetylene-saturated seawater in order to confirm that ethylene was not naturally produced by our samples, and autoclaved seawater used in the preparation of the incubations with addition of acetylene-saturated seawater in order to confirm that ethylene was not produced in the seawater. The ethylene production rates were converted into N₂ fixation rates by applying the common ratio of 3 mol of acetylene:1 mol of N₂ (Welsh, 2000).

At the end of the incubation, we dried the sediment samples at 60 °C and recorded the dry weight for further calculations. Moreover, we calculated the sediment organic matter (OM) content of each replicate sediment sample by loss on ignition (Dean Jr, 1974). The sediment N₂ fixation rates were standardized to surface area integrated over 10 cm sediment depth by taking into account the sediment bulk density.

2.3. Statistical analysis

Differences in sediment OM content and bulk density among P. oceanica, C. nodosa, and C. prolifera vegetated sediments and bare sediment were tested with the non-parametric Kruskal Wallis test. Differences in sediment N₂ fixation rates among the four types of sediment were tested by Friedman test matching by temperature treatment. Differences in sediment N₂ fixation rates between vegetated and bare sediments were tested by non-parametric Mann-Whitney U test. Moreover, we tested the thermal dependence of sediment N₂ fixation rates in vegetated and bare sediments by fitting Arrhenius function to estimate the activation energy, derived from the
relationship between the natural logarithm of N\textsubscript{2} fixation rates and the inverse of the temperature multiplied by the Boltzmann’s constant (Dell et al., 2011), and Q\textsubscript{10} (Raven and Geider, 1988). The activation energy and Q\textsubscript{10} of N\textsubscript{2} fixation in vegetated sediments were calculated using the increasing rates measured at four temperature treatments (25, 27, 29, and 31 °C), while the declining rates measured at 33 °C were not included. The full range of temperature treatments was used for bare sediments since no decline was detected. All statistical analyses were performed using JMP (SAS Institute Inc., USA) and PRISM (GraphPad Software Inc., USA) statistical software.

3. Results

Sediment OM content was significantly different in the sediments colonized by different macrophyte species ($\chi^2_{3,56} = 50.33, p < 0.0001$). *Posidonia oceanica* sediments had the highest OM content (13.34 ± 0.56 %), whereas bare sediments had the lowest OM content (0.44 ± 0.50 %, Table 2). Sediment bulk density differed among sediment types ($\chi^2_{3,56} = 46.02, p < 0.0001$, Table 3).

Average N\textsubscript{2} fixation rates in bare sediments was 0.06 ± 0.01 (range from 0.01 to 0.09) nmol N gDW\textsuperscript{-1} h\textsuperscript{-1}; while, average N\textsubscript{2} fixation rates in vegetated sediments was three-fold greater at 0.19 ± 0.03 (range from 0.05 to 0.9) nmol N gDW\textsuperscript{-1} h\textsuperscript{-1}. Within the vegetated sediments, the maximum mean N\textsubscript{2} fixation rate was detected in *C. prolifera* (0.22 ± 0.05 nmol N gDW\textsuperscript{-1} h\textsuperscript{-1}), whereas the minimum mean N\textsubscript{2} fixation rate was measured in *C. nodosa* (0.15 ± 0.04 nmol N gDW\textsuperscript{-1} h\textsuperscript{-1}). Nitrogen fixation rates differed among the four different sediment types (i.e. bare, *P. oceanica, C. nodosa* and *C. prolifera* sediments) ($\chi^2_{3,56} = 10.68, p = 0.005$) when expressed by sediment dry weight. However, once the rates were converted into aerial basis, these differences
were no longer significant ($\chi^2_{3,56} = 6.12, p > 0.05$), due to high variability in sediment bulk densities. Sediment N$_2$ fixation rates were independent of OM content (linear regression, p>0.05).

Nitrogen fixation rates in aerial basis were significantly higher in vegetated sediments compared to bare ones ($U = 154, p < 0.002$) when pooling all temperature treatments together, with sediments colonized by macrophytes supporting, on average, twice the nitrogen fixation rate than bare sediments (mean ± SE = 3.86 ± 0.53 and 1.77 ± 0.20 mg N m$^{-2}$ d$^{-1}$, respectively). The differences between vegetated and bare sediment remained significant at current summer SST (25-27 ºC) and projected summer mean SST (29-31 ºC) ($U= 13, p < 0.01$ and $U = 23, p < 0.05$, 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 bare sediments, respectively, at current summer SST (25-27 ºC) and N$_2$ fixation rates of 5.25 ± 1.17 and 2.18 ± 0.2 mg N m$^{-2}$ d$^{-1}$ in vegetated and bare sediments, respectively at projected summer mean SST (29-31 ºC). However, vegetated and bare sediments did not differ in N$_2$ fixation rates between vegetated and bare sediments at projected summer SST$_{\text{max}}$ (33 ºC), with N$_2$ fixation rates of 2.49 ± 0.248 and 2.21 ± 0.15 mg N m$^{-2}$ d$^{-1}$, respectively (Fig. 2).

In vegetated sediments, N$_2$ fixation rates increased linearly with temperature up to 31ºC (N$_2$ fixation (nmol N g DW$^{-1}$ h$^{-1}$) = -0.63 + 0.03*Temperature, $R^2 = 0.11, p < 0.05$), with a 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$^{-1}$ at 33 ºC (Fig. 3). Nitrogen fixation rates in bare sediments increased linearly with temperature up 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. 3). The associated activation energies were 0.91 ± 0.39 and 1.25 ± 0.39 eV for vegetated and bare sediments, respectively (Fig. 4). Associated Q$_{10}$ values were 3.84 ± 2.22 and 6.41 ± 2.97 for vegetated and bare sediments, respectively.
4. Discussion

The overall average N\textsubscript{2} fixation rate found in Mediterranean vegetated sediments at current summer mean SST (3.15 ± 0.48 mg N m\textsuperscript{-2} d\textsuperscript{-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\textsuperscript{-2} d\textsuperscript{-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\textsuperscript{-2} d\textsuperscript{-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\textsubscript{2} fixation rates in vegetated sediments are higher than in bare sediments, consistent with the long-recognized role of marine plants in enhancing N\textsubscript{2} fixation rates (Capone, 1988). Specifically, the vegetated sediments supported 3 to 4-fold higher N\textsubscript{2} fixation rates than bare sediments did.

The N\textsubscript{2} fixation rates we measured in *P. oceanica* sediments at current summer mean SST (2.86 ± 1.26 mg N m\textsuperscript{-2} d\textsuperscript{-1}) are higher than the rates reported by Agawin et al. (ranging from 0.06 to 1.51 mg N m\textsuperscript{-2} d\textsuperscript{-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\textsubscript{2} fixation rates in bare sediments at current summer mean SST (1.14 ± 0.31 mg N m\textsuperscript{-2} d\textsuperscript{-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\textsuperscript{-2} d\textsuperscript{-1}) (Agawin et al., 2017), suggesting that these differences in N\textsubscript{2} fixation rates in *P. oceanica* sediment might also be due to variability among sites. The N\textsubscript{2} fixation rates in *C.*
nodosa and *C. prolifera* sediments reported here are the first reports, to the best of our knowledge, for these two important Mediterranean macrophyte species. Indeed, the analysis of sediment N stocks in a *C. nodosa* meadow in the Mediterranean Sea suggested that N$_2$ fixation might be contributing to enhance the N stocks compared to bare sediments (Pedersen et al., 1997). The similar stable N isotope composition of *C. nodosa* tissues and those of *P. oceanica* in the Mediterranean (Fourquean et al., 2007) also suggests that they use similar sources of nitrogen. The N$_2$ fixation rates at current summer mean SST in *C. prolifera* sediments found here (0.17 ± 0.04 nmol N gDW$^{-1}$ h$^{-1}$) are similar to the sediment N$_2$ fixation rates associated to the invasive *C. taxifolia* in Monaco (0.12 ± 0.09 nmol N gDW$^{-1}$ h$^{-1}$) but 20-fold lower than the N$_2$ fixation rates reported for *C. taxifolia* in France (3.96 ± 1.99 nmol N gDW$^{-1}$ h$^{-1}$) (Chisholm and Moulin, 2003).

Although the sediments colonized by these three macrophyte species hold similar rates, the contribution of sediment N$_2$ fixation to the productivity of each plant is different. Taking into account the average net production (2.63 and 1.47 g DW m$^{-2}$ d$^{-1}$ for *P. oceanica* and *C. nodosa*, respectively (Duarte and Chiscano, 1999) and 5.16 g DW m$^{-2}$ d$^{-1}$ for *C. prolifera* (Marbà unpublished)) and the tissue nitrogen content (from 1.55 to 1.63 % for *P. oceanica*, from 1.91 to 2.28 % for *C. nodosa* (Duarte, 1990;Fourquean et al., 2007) and from 3 to 4.9 % for *C. prolifera* (Morris et al., 2009)), the mean measured sediment N$_2$ fixation rates detected at current summer 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 nitrogen requirements for *P. oceanica*, *C. nodosa*, and *C. prolifera*, respectively. The calculated contribution of N$_2$ fixation to seagrass growth requirements fall within the range of the N$_2$ fixation contributions reported for temperate seagrasses, ranging from 5 to 12 % for *Z. marina* and *Z. noltii*, respectively (Welsh, 2000). The calculated contribution of N$_2$ fixation to fulfill the
macrophytes’ growth requirements points N₂ fixation as partially supporting the high 
productivity of these primary producers in the Mediterranean oligotrophic waters. 

We experimentally demonstrate that N₂ fixation rates in coastal sediments are thermal 
dependent, both in vegetated and bare sediments, in agreement with higher rates typically 
reported in tropical and subtropical meadows compared to temperate and cold seagrass systems 
(Herbert, 1999; McGlathery, 2008; Welsh, 2000); although an experimental demonstration was 
lacking. The thermal dependence, as reflected by the activation energy and Q₁₀, for N₂ fixation 
rates was, however, higher in bare sediments than in vegetated sediments, possibly due to 
different bacterial communities. Westrich and Berner (1988) also found that sulfate reduction 
exhibited a more pronounced thermal dependence in sediments supporting lower rates. The 
activation energies for N₂ fixation in vegetated sediments (0.91 ± 0.4 eV or 87.8 ± 37.6 KJ mol⁻¹) 
and in bare sediments (1.25 ± 0.4 eV or 120.6 ± 37.6 KJ mol⁻¹) are within the range of the 
activation energy reported for sediment sulfate reduction (range from 36 to 132 KJ mol⁻¹ 
(Robador et al., 2016; Westrich and Berner, 1988)) and for sediment organic matter degradation 
(range from 54 to 125 KJ mol⁻¹ (Middelburg et al., 1996)). The Q₁₀ values associated to sediment 
N₂ fixation (3.84 ± 2.22 and 6.41 ± 2.97 for vegetated and bare sediments, respectively) are 
higher than those reported for sediment sulfate reduction (from 1.6 to 3.4 (Robador et al., 2016)), 
but still similar to those values associated to organic matter degradation (from 2.2 to 6.3 
(Middelburg et al., 1996)). Moreover, the thermal response differed for vegetated sediments, 
where N₂ fixation rates showed an optimum at 31 °C followed by a sharp decrease at 33 °C, and 
bare sediments, where N₂ fixation rates increased along the range of experimental temperatures 
tested here. The thermal response of N₂ fixation in vegetated sediments found here is similar to 
the thermal response reported for N₂ fixation in soil crusts (Zhou et al., 2016) and seagrass
rhizosphere (Garcias-Bonet et al., 2018), with an increase in rates up to 30 and 29 °C, respectively, and a marked decrease in rates at temperatures above the optimum. The forecasted warming by the end of the century could potentially increase N₂ fixation rates by 36.7% in vegetated sediments and 46.8% in bare sediments. However, the decrease in N₂ fixation rates in vegetated sediments at 33 °C would imply a reduction of a third in the contribution of N₂ fixation to the macrophytes’ productivity during heatwaves. Therefore, the thermal dependence of N₂ fixation in vegetated sediments might have important consequences for primary production in coastal ecosystems in the context of warming. However, this may not be the case for *P. oceanica*, as this species is projected to be critically compromised, to the extent that functional extinction is possible, with projected Mediterranean warming rates by 2050 (Chefaoui et al., 2018; Jordà et al., 2012).

**Conclusion**

Mediterranean macrophyte meadows are sites of intense N₂ fixation rates, twice as high as those in adjacent bare sediments. As these rates increased greatly with warming, realized warming of the Mediterranean Sea is expected to have led to enhanced N₂ fixation rates, both for bare and vegetated sediments, with further warming leading to further increase in N₂ fixation rates.

**Author contribution**

NG-B, RVS, CMD and NM designed the study. NG-B and RVS performed the field work, and sample and data analysis. NG-B, RVS, CMD and NM interpreted the results. NG-B
wrote the first draft of the manuscript. All authors contributed substantially to the final manuscript.

Conflict of interests

Authors declare that they have no conflict of interest

Acknowledgements

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Fig. 1. Summer *in situ* SST (from June 21st to September 22nd) from 2012 to 2017 at the study site. Solid line indicates average summer median SST and dashed lines indicate the 25 and 75% percentiles. Average summer median SST and percentiles do not include data from 2014 due to partial temporal coverage in temperature records. No data available for 2015.
Fig. 2. Box plot of the N\textsubscript{2} fixation rates of sediments colonized by different macrophytes (as well as grouping all vegetated sediments together) and bare sediment measured at current summer SST (25-27 °C, (A)), projected summer mean SST by 2100 under RCP6.0 scenario (29-31 °C, (B)), and projected summer SST\textsubscript{max} by 2100 under RCP6.0 (33 °C, (C)).
Fig. 3. Relationship of experimental incubation temperature and sediment N$_2$ fixation rates in vegetated (black dots) and bare sediments (white dots). Dots indicate mean values and error bars indicate standard error of the mean.
Fig. 4. Arrhenius plot for $N_2$ fixation rates in vegetated (black dots and line) and bare sediments (grey dots and line).
Tables

Table 1. Summary of *in situ* summer SST at the study site (Pollença Bay, Mallorca) from June 21st to September 22nd from 2012 to 2017, showing the mean ± SEM, minimum (Min) and maximum (max), median, and 25 and 75% percentiles. nd: no data, na: not available due to partial temporal coverage.

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean (±SEM)</th>
<th>Min - Max</th>
<th>Median</th>
<th>25% percentile</th>
<th>75% percentile</th>
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</thead>
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<tr>
<td>2012</td>
<td>26.44 ± 0.03</td>
<td>23.61 – 29.29</td>
<td>26.23</td>
<td>25.70</td>
<td>27.28</td>
</tr>
<tr>
<td>2013</td>
<td>26.29 ± 0.05</td>
<td>21.48 – 29.18</td>
<td>26.28</td>
<td>25.23</td>
<td>27.98</td>
</tr>
<tr>
<td>2014</td>
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<td>na</td>
<td>na</td>
<td>na</td>
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<tr>
<td>2015</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>2016</td>
<td>26.39 ± 0.03</td>
<td>22.90 – 28.69</td>
<td>26.43</td>
<td>25.82</td>
<td>27.24</td>
</tr>
<tr>
<td>2017</td>
<td>27.03 ± 0.04</td>
<td>23.69 – 29.67</td>
<td>27.21</td>
<td>26.50</td>
<td>27.92</td>
</tr>
</tbody>
</table>

**Average 2012 – 2017**

**26.54 ± 0.17** | **22.92 – 29.08** | **26.54** | **25.81** | **27.61**

*including data from 2012, 2013, 2016, and 2017 for which full summer records are available.*
Table 2. Experimental temperature set up used in the sediment incubations, indicating the five target temperatures and the actual mean (±SEM) temperature recorded in each incubation water bath during the 24h incubations. The ranges (minimum – maximum values) are shown in parenthesis.

<table>
<thead>
<tr>
<th>Target temperature (ºC)</th>
<th>Incubation temperature (ºC)</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>25 ºC</td>
<td>25.07 ± 0.01 (24.9 – 25.2)</td>
<td></td>
</tr>
<tr>
<td>27 ºC</td>
<td>27.02 ± 0.01 (26.6 – 27.2)</td>
<td></td>
</tr>
<tr>
<td>29 ºC</td>
<td>29.05 ± 0.01 (28.8 – 29.3)</td>
<td></td>
</tr>
<tr>
<td>31 ºC</td>
<td>31.08 ± 0.01 (30.7 – 31.4)</td>
<td></td>
</tr>
<tr>
<td>33 ºC</td>
<td>33.05 ± 0.01 (32.7 – 33.2)</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Sediment organic matter content and bulk density in sediments colonized by different macrophyte species and bare sediment in Pollença bay in June 2017. Mean values (±SEM) and the ranges (minimum – maximum values) are shown.

<table>
<thead>
<tr>
<th></th>
<th>P. oceanica sediment</th>
<th>C. nodosa sediment</th>
<th>C. prolifera sediment</th>
<th>Bare sediment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment organic matter content (%)</td>
<td>12.58 ± 0.95 (8.17 – 20.63)</td>
<td>6.14 ± 0.39 (4.49 – 10.22)</td>
<td>0.74 ± 0.12 (0.04 – 1.31)</td>
<td>0.44 ± 0.08 (0.02 – 1.41)</td>
</tr>
<tr>
<td>Sediment bulk density (g DW sed cm⁻³)</td>
<td>0.54 ± 0.03 (0.29 – 0.69)</td>
<td>1.01 ± 0.03 (0.74 – 1.24)</td>
<td>0.46 ± 0.02 (0.32 – 0.56)</td>
<td>0.96 ± 0.02 (0.84 – 1.09)</td>
</tr>
</tbody>
</table>
References


IPCC: Climate change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report (AR5) of the Intergovernmental Panel on Climate Change Cambridge Univ Press, New York, USA, 2013.


