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Warming enhances sulphide stress of Mediterranean seagrass (*Posidonia oceanica*)

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ABSTRACT

We experimentally investigated the effect of Mediterranean seawater summer warming projected for the 21st century under Intergovernmental Panel on Climate Change (IPCC) scenarios of greenhouse gas emissions on *Posidonia oceanica* sulphide stress. The results reveal that projected warming would enhance sulphide toxicity in *P. oceanica* and, hence, the risk of seagrass loss. We assessed sulphide stress of seagrasses by estimating the sulphide intrusion ($F_{sulphide}$) into leaf tissue from the sulphur isotopic signature in sediments ($\delta^{34}S_{sulphide}$), in the water column ($\delta^{34}S_{sulphate}$) and in *P. oceanica* leaves ($\delta^{34}S_{tissue}$). Seagrasses were grown at 6 seawater temperature treatments, ranging from 26 °C to 32 °C, in 3 replicated experimental mesocosms for 50 days. At the end of the experiment, the seagrass shoots in all of the treatments exhibited sulphide intrusion ($F_{sulphide} > 0\%$), which had been negligible when the experiment started. Similarly, the leaf $\delta^{34}S_{tissue}$ was lighter at the end of the experiment than at the beginning, when seagrasses in the field were experiencing a seawater temperature of 23 °C. $F_{sulphide}$ linearly increased with experimental warming, while leaf $\delta^{34}S_{tissue}$ declined with increasing temperature at a rate of 0.3‰ for each centigrade degree of warming. The daily production of new leaves per shoot decreased as the sediment became more reduced. Leaf $\delta^{34}S_{tissue}$ below 20.5‰, indicative of 1% sulphide intrusion, is expected to compromise *P. oceanica* performance.

1. Introduction

Mediterranean surface seawater temperature has been increasing since the early 20th century as a consequence of global warming, and under IPCC greenhouse emission scenarios, it is projected to further increase by, on average, 4 °C during the 21st century (IPCC, 2007). As temperature is a key parameter regulating chemical and biological reactions (e.g. Brown et al., 2004), Mediterranean warming is expected to affect the biological and biogeochemical components of Mediterranean ecosystems and modify their ecological structure and functioning. Despite the scarcity of information on climate change impacts on marine system biodiversity (Richardson and Poloczanska, 2008), there is evidence that heat waves are stressing Mediterranean marine biota (e.g. sponges, corals and seagrasses) by, for instance, triggering mortality, particularly of sessile organisms (e.g. Coma et al., 2009; Marbà and Duarte, 2010), and changing sexual reproduction rates (e.g. Diaz-Almela et al., 2007). Warming impacts on marine biota result not only from the direct impact of increasing temperature on organism physiology but also from the effect of warming on other biological (e.g. microbial activity and metabolic rates) and abiotic (e.g. oxygen solubility) components of ecosystem function (e.g. Vaquer-Sunyer and Duarte, 2010).

Seagrasses are clonal marine angiosperms that play an important role in coastal ecosystems through their capacity for ecosystem engineering (Gambi et al., 1990), the supply of carbon to food webs (Perkins-Visser et al., 1996) and their effect on biogeochemical cycles (Marbà et al., 2006; Mateo et al., 2006; Duarte et al., 2011; Fourqurean et al., 2012). Seagrass meadows rank among the most vulnerable ecosystems, declining globally at rates of 2–5% per year (Duarte et al., 2008; Waycott et al., 2009), leading to the loss of the functions, goods and services these ecosystems provide. Coastal eutrophication together with warming resulting from climate change is recognised as the prevalent causes for this global decline (Duarte, 2002). *Posidonia oceanica*, the dominant and endemic seagrass species in the Mediterranean, has already shown increased shoot mortality and enhanced net shoot losses in pristine meadows with increased warming over the 2002–2007 period, with recruitment unable to counterbalance the losses (Marbà and Duarte, 2010). The increased mortality with warming observed for *P. oceanica* is steeper than expected from physiological responses alone (Marbà and Duarte, 2010) and may also result from indirect effects, such as effects of warming on oxygen dynamics and benthic biogeochemical processes.

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Seagrasses are sensitive to elevated sulphide levels in sediment porewaters, which have been shown to severely suppress photosynthesis, growth and meristematic activity and ultimately reduce seagrass survival (Carlson et al., 1994; Goodman et al., 1995; Calleja et al., 2007; Koch et al., 2007; Garcias-Bonet et al., 2008). Sulphides are produced by sulphate reducing bacteria in anoxic marine sediments and are highly toxic to eukaryotic cells at concentrations as low as 1–10 μM (Fenchel and Finlay, 1995). The intrusion of sulphides into seagrass tissues seems to be responsible for sudden die-off events, which have been observed during the past few decades for both temperate and tropical seagrasses (Robblee et al., 1991; Zieman et al., 1999; Holmer and Bondgaard, 2001; Greve et al., 2003), as well as long-term reductions in seagrass abundance (Calleja et al., 2007; Marbà et al., 2007). Sulphide intrusion into seagrass tissues appears to be driven by a combination of high sulphide concentrations in sediments and low oxygen concentrations in the water column (Holmer and Nielsen, 2007; Mascaró et al., 2009). Under these circumstances, and despite the lacunae system in seagrasses that allows the diffusion of oxygen from the leaves to the rhizomes and roots (Borum et al., 2006), the oxygen partial pressure in roots and rhizomes is reduced, and sediment sulphides can intrude into tissues, where they move rapidly between the plant compartments by gas phase diffusion through the lacunae (Pedersen et al., 2004; Borum et al., 2005), eventually crossing shoot meristems and reaching the leaves. Sulphide intrusion in seagrass tissues is readily reflected in their sulphur isotopic signatures ($\delta^{34}S$, Frederiksen et al., 2006; Marbà et al., 2007). The proportion of total sulphur in the plant coming from sulphide, or sulphide intrusion (F_{sulphide}), can be estimated from the $\delta^{34}S$ signatures in the plant tissue, water column and sediments (Frederiksen et al., 2006). Oxygen consumption rates and sulphate reduction rates increase with increasing temperatures (Knoblauch and Jørgensen, 1999); thus, increasing warming has the potential to intensify sulphide intrusion into seagrasses by enhancing the build up of sulphides in sediments and reducing the partial pressure of oxygen. However, the expectation that increased warming should lead to enhanced suphide intrusion in seagrass tissues has not yet been tested.

Here, we test whether the warming projected for the 21st century under IPCC scenarios of greenhouse gas emissions (IPCC, 2007) triggers *Posidonia oceanica* sulphide stress by increasing sulphide intrusion in seagrass leaves. In addition, we test if the sulphide intrusion constrains *P. oceanica* performance. We do so using experimental mesocosms including both *P. oceanica* and its associated sediment community for 50 days. We examine the changes in sulphur isotopic signature ($\delta^{34}S$) in sediments, water column and *P. oceanica* leaves in response to experimental warming as well as the response of *P. oceanica* growth to changes in sediment isotopic signature.

2. Material and methods

2.1. Experimental design

Fragments of *Posidonia oceanica* horizontal rhizomes with roots and one apical and approximately 7 vertical shoots attached were collected in June 2009 from sandy sediments at 6–7 m depth at Cala Millor (Mallorca, Spain, 39.60° N, 3.39° E). Seagrass fragments were gently washed free of sediment and, within 3 h after collection, the cut end of horizontal rhizomes was sealed with a non-toxic polyvinylsilicoxane silicone elastomer to maintain gas pressure inside the rhizome. Eight to ten rhizome fragments were planted on 5 cm thickness of thoroughly homogenised pre-washed beach sand with a low organic matter content (1.78%), in 30 l circular mesocosms (i.d. 35 cm; approximate density 500–600 shoots m^{-2}) filled with seawater. The leaves were trimmed to 20 cm to prevent exceeding the water height in the mesocosms, which could produce desiccation and damage of the leaves as well as self-shading and, thus, growth limitation by light availability (e.g. Cebrian et al., 1998). A total of 18 mesocosms were set up and equally distributed between six water-baths consisting of 500 l fibreglass tanks filled with freshwater and heated with 3 Hagen heaters per tank (two 300 W and one 150 W), adjusted to achieve ambient temperature. A pump inside each tank re-circulated the water for a homogenous water bath. We therefore obtained a total of 6 different treatment tanks with 3 replicate mesocosms in each. Planted mesocosms were left to acclimate for 3 days at ambient temperature (23 °C). Temperatures were then increased at 0.4 °C per day to achieve target treatment temperatures of 26, 27, 28.5, 30, 31.2 and 31.8 °C, which were maintained for 50 days before terminating the experiment.

Temperature was monitored continuously and recorded every 30 min over the length of the experiment with a Fourier microlite temperature logger located inside one mesocosm of each treatment. The temperature was also checked in each mesocosm at regular intervals with a hand-held mercury thermometer to ensure the target temperature was correct. The mean temperature of each replicated mesocosm was within <0.5 °C of the mean temperature recorded by the temperature logger. Hence, the temperature for each treatment was calculated by averaging the measurements from the data loggers during the experimental period. Throughout the experiment, the salinity was kept between 35 and 40 (average ± standard error, 37.8 ± 0.04), and approximately one quarter of the mesocosm water volume was changed every week. The PAR light intensity was 433 μmol photons m^{-2} s^{-1} at the canopy level (two T5 lamps with two 53 W fluorescent bulbs each) in a 12:12 h diurnal cycle.

2.2. Sediment analysis

During harvesting of seagrass material in Cala Millor, three sediment cores were collected for the determination of $\delta^{34}S$ in 16 cm^3 of homogenised sediment from the sediment depth interval of 5–10 cm. Approximately 8 cm^3 of sediment was also collected with 60 ml cut-end syringes from two random mesocosms of three random treatment tanks after the acclimation period at ambient temperature and homogenised into one sample per tank; thus three replicates were obtained to determine the initial conditions. At the end of the experiment, two 8 cm^3 sediment samples were collected per mesocosm and homogenised into one sample; thus three replicates per treatment were obtained for the determination of final conditions. Homogenised sediments were transferred into 50 ml tubes with 15 ml of 1 M ZnAc and kept frozen until analysis. After thawing, the sediment was then distilled following to the two-step method described by Fossing and Jørgensen (1989), where acid volatile sulphide (AVS), consisting of FeS and porewater sulphide, is extracted first, followed by chromium reducible sulphide (CRS), consisting of FeS_2 and S_o, extracted in a second step. The extracted sulphide was precipitated as Ag_2S and analysed for $\delta^{34}S$ by weighing the precipitate (ca. 450 μg) in tin capsules together with vanadium pentoxide (ca. 3 mg) and analysed by element analyser combustion continuous flow isotope ratio mass spectroscopy (EA-C-CF-IRMS) at Iso-analytical (United Kingdom). The stable isotopic signatures were reported in standard delta notation (‰) as follows:

$$\delta^{34}S = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}}\right) - 1\right] \times 100$$