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Interactive effect of temperature, acidification and ammonium enrichment on the seagrass *Cymodocea nodosa*

L.G. Egea*, R. Jiménez-Ramos, J.J. Vergara, I. Hernández, F.G. Brun

Department of Biology, Faculty of Marine and Environmental Sciences, University of Cadiz, 11510, Puerto Real, Cadiz, Spain

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ABSTRACT

Global (e.g. climate change) and local factors (e.g. nutrient enrichment) act together in nature strongly hammering coastal ecosystems, where seagrasses play a critical ecological role. This experiment explores the combined effects of warming, acidification and ammonium enrichment on the seagrass *Cymodocea nodosa* under a full factorial mesocosm design. Warming increased plant production but at the expense of reducing carbon reserves. Meanwhile, acidification had not effects on plant production but increased slightly carbon reserves, while a slight stimulation of net production and a slight decrease on carbon reserves under ammonium supply were recorded. When all the factors were combined together improved the production and carbon reserves of *Cymodocea nodosa*, indicating that acidification improved ammonium assimilation and buffered the enhanced respiration promoted by temperature. Therefore, it could indicate that this temperate species may benefit under the simulated future scenarios, but indirect effects (e.g. herbivory, mechanical stress, etc.) may counteract this balance.

1. Introduction

In the last century, human activities have triggered changes at a global scale that are affecting ecosystems worldwide, with coastal vegetated ecosystems being one of the most threatened (Large, 2009). These ecosystems are expected to come under increased pressure from climate change and direct anthropogenic factors in the next decades, (Nicholls et al., 2007). In coastal vegetated habitats worldwide, seagrasses (i.e. marine flowering plants) form extensive meadows in intertidal and subtidal environments. These habitats are increasingly recognised for their ecological function and provisioning of human services, including nutrient regeneration (Costanza et al., 1997), water quality improvement (Waycott et al., 2005), reduction in human and wildlife pathogens (Lamb et al., 2017; Sullivan et al., 2017), shoreline protection (Bos et al., 2007; Christianen et al., 2013), suitable breeding habitats (including those for economically relevant species; Cullen-Unsworth et al., 2014), biodiversity hotspots (Duffy, 2006; González-Ortiz et al., 2014a) and carbon sequestration (Fourqurean et al., 2012). These keystone habitats thus are considered one of the richest and most relevant ecosystems worldwide (Ruiz-Frau et al., 2017; Short et al., 2011), with high economic value for humans (e.g. Campagne et al., 2014). This importance is recognised worldwide by different legislations and international conventions like the Convention on Biological Diversity (1992) or the European Habitats Directive (92/43/EEC). Favoured by this legislative framework, seagrass habitats have been specifically targeted for conservation and restoration (Green and Short, 2004). Regrettably, the proximity of seagrasses to anthropogenic littoral impacts and their shallow distribution in estuarine and coastal areas have led to widespread seagrass losses, with a global decline of 7% yr^{-1} (Waycott et al., 2009) and almost 14% of all seagrass species currently endangered (Short et al., 2011). Therefore, it is crucial to understand the responses of these ecosystems to multiple co-stressors in order to provide sound advice on managing for possible future trajectories (Brierley and Kingsford, 2009; Hoegh-Guldberg and Bruno, 2010; Unsworth et al., 2014).

Climatic change effects (e.g. increase in temperature, seawater acidification, frequency of storms, sea level rise, etc.) in combination with coastal anthropogenic and natural stressors (e.g. nutrient load, changes in salinity and littoral current, diseases, etc.) act together in coastal areas, and their effects are expected to increase in the near future (Halpern, 2014; Nicholls et al., 2007). Increased CO_2 concentration in the air and subsequent solubility in seawater reduces pH and modifies the balance of the different dissolved carbonate species (Zeebe and Wolf-Gladrow, 2001; Koch et al., 2013). Partial pressure of carbon dioxide in water is raised under such conditions, which can benefit seagrass primary production as seagrass photosynthesis is generally considered to be carbon limited (Beer et al., 1980; Beardall et al., 1998; Beer and Koch, 1996; Invers et al., 2001). Thus, higher CO_2 is predicted

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* Corresponding author at: Department of Biology, University of Cadiz, Campus of International Excellence (CEIMAR), 11510, Puerto Real, Cádiz, Spain.
E-mail address: gonzalo.egea@uca.es (L.G. Egea).

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to lead to higher photosynthesis, growth rates, biomass (Hall-Spencer et al., 2008; Jiang et al., 2010; Palacios and Zimmerman, 2007; Short and Neckles, 1999; Takahashi et al., 2016; Zimmerman et al., 1997) and internal non-structural carbohydrates (NSC) concentrations (Campbell and Fourqurean, 2013; Egea et al., 2018; Garrard and Beaumont, 2014; Zimmerman et al., 1997), in the absence of other factors limiting the growth (e.g. nutrients, light). However, it is important to note that extrapolating laboratory results to predict long-term responses in seagrasses is not always easy, since some long-term experiments have shown no significant changes in biomass, shoot density and/or growth rates under CO_2 enrichment (Alexandre et al., 2012; Campbell and Fourqurean, 2013; Cox et al., 2016; Palacios and Zimmerman, 2007).

Several studies have highlighted the importance of temperature in the seagrass metabolism and in the maintenance of a positive carbon balance, since warmer temperatures favour photosynthesis and respiration through their effects on kinetic reactions and metabolism (Evans et al., 1986; Pérez and Romero, 1992; Zimmerman et al., 1989). Some previous experiments have demonstrated that warmer temperature may benefit the flowering (Ruiz et al., 2017), growth and biomass of seagrass species (under high saturating light conditions; Bulthuis, 1987), while reducing the reserves of non-structural carbohydrates through enhancing respiration (Hernán et al., 2017). However, other studies have shown negative effects on plants (Collier and Waycott, 2014; Jordà et al., 2012; Moreno-Marin et al., 2018; Repolho et al., 2017). The final effect will depend on the thermal tolerance of a species and its optimal temperature for photosynthesis, respiration, and growth (Bulthuis, 1987; Collier et al., 2011; Masini and Manning, 1997; Short and Neckles, 1999).

In addition to these variables affected by climate change, the current increase in nutrient load in coastal waters has been identified as a key factor that has the potential to negatively impact seagrass meadows (Antón et al., 2011; Burkholder et al., 2007; Cabaço et al., 2008; Hughes et al., 2004). Several reports have indicated that moderate increases in nutrient load may stimulate seagrass production and biomass (Alcoverro et al., 1997; Jiménez-Ramos et al., 2017a; Pérez et al., 1991; Short, 1987; Udy et al., 1999). However, under conditions of high nitrogen availability, direct ammonium toxicity can curtail plant growth, biomass and survival (Brun et al., 2002; van Katwijk et al., 1997). As with temperature, the net outcome will depend on the effects of nutrient load on the photosynthesis rates and non-structural carbohydrate reserves, which are needed for a rapid ammonium assimilation (Brun et al., 2008; Villazán et al., 2013a).

These three factors directly affect photosynthetic rate, plant production, biomass and non-structural carbohydrate reserves. However, while CO_2 enrichment may have either a positive effect or no effect on seagrasses, temperature and nutrient enrichment may cause positive or negative effects. The net response may depend on the species, the physiological status of the plants and, notably, the interaction between these factors. For instance, higher CO_2 may benefit plants subject to higher temperatures because both the higher photosynthetic and respiration rates expected under higher temperature can benefit from elevated CO_2 levels (e.g. reducing the carbon limitation; Ow et al., 2016; Zimmerman et al., 1997), higher levels of non-structural carbohydrates (e.g. needed for respiration processes; Campbell and Fourqurean, 2013) and higher biomass (e.g. more photosynthetic tissues; Jiang et al., 2010; Palacios and Zimmerman, 2007; Russell et al., 2013). In contrast, warmer temperature may have a detrimental effect on plants subjected to ammonium enrichment because of the decrease in non-structural carbohydrate reserves due to enhanced respiration rates, as demonstrated by van Katwijk et al. (1997) and Moreno-Marin et al. (2018). However, CO_2 enrichment may counterbalance this negative interaction to some extent, because of its associated enhanced rates in photosynthetic and higher non-structural carbohydrate reserves, which are known to reduce ammonium toxicity symptoms (Brun et al., 2002, 2008). In addition, higher nutrient levels (mainly nitrogen) may be beneficial under elevated CO_2 levels, since the resulting higher photosynthesis and growth rates increase the demand for nutrients (Coskun et al., 2016; Stitt and Krapp, 1999).

Therefore, while the plant response to a single factor can be well described and predicted, the combination of multiple factors acting together under natural conditions can induce a complex response difficult to predict, as plants may exhibit non-additive responses when exposed to multiple stressors (Gunderson et al., 2016; Moreno-Marin et al., 2018). Non-additive effects may be antagonistic (i.e. the combined effect is less than the expected additive effect) or synergistic (i.e. greater than the expected additive effect). Some previous works have found mainly non-additive responses when using a multifactorial design with some of the aforementioned stressors (warmer temperature, enhanced CO_2, ammonium enrichment) (Brun et al., 2008; Burnell et al., 2013; Collier et al., 2011; De los Santos et al., 2010; Egea et al., 2018; Jiménez-Ramos et al., 2017b; Koch et al., 2013; La Nafie et al., 2012; Lee et al., 2007; Moreno-Marin et al., 2016, 2018; Repolho et al., 2017; Salo and Pedersen, 2014; Villazán et al., 2013a). Therefore, if plants have a non-additive response, predicting the effects of environmental change from single factor experiments may under- or overestimate the combined effect of multiple stressors.

This work aims to study the response of a temperate seagrass (*Cymodocea nodosa*) to the forecasted global change factors (high temperature, CO_2 increase and ammonium enrichment) using a multifactorial mesocosm experiment, testing whether the combined effects of these stressors are additive or non-additive. Based on previous studies, we hypothesize that the combination of the three factors will have a positive effect on plant production and biomass, while non-structural carbohydrates will be reduced because of their depletion by ammonium assimilation and the enhanced respiratory processes promoted by higher temperature. In addition, we predict that most of the factor combinations will produce non-additive responses.

2. Material and methods

2.1. Field plant collection

Individual shoots of *Cymodocea nodosa* (Ucria) Ascherson were randomly collected from a depth of 1–2 m in submerged seagrass meadows at Cadiz Bay (southern Spain, 36°29′19.79”N; 6°15′53.05″E). Healthy looking vertical shoots with intact rhizomes were transported to the laboratory within 2 h of collection in an ice chest. Once in the laboratory, a large pool of experimental shoots were selected bearing similar lengths, numbers of leaves and roots, and they were cleaned of visible epiphytes. They were acclimated for 5 days in aerated water collected from the sampling site under sub-saturating light (ca. 150 μmol photons $m^{-2} s^{-1}$) with a 16:8 h light:dark cycle at 20 °C before they were used in the experiment.

2.2. Mesocosm experiment

The study was conducted in an open-water indoor mesocosm system at the Faculty of Marine and Environmental Sciences of the University of Cadiz during four weeks in November 2013. The plants were allocated to 1.5 L incubation chambers ($n = 24$) (Fig. 1). In each chamber, about 18–21 individual *C. nodosa* shoots were planted individually by hand while maintaining similar fresh biomass values (FW) in each chamber, which resulted in a total of ca. 500 shoots planted among all chambers. The total fresh weight (FW) of plants (including leaves, rhizome and root biomasses) in each chamber (B_0, FW) was annotated at the beginning of the experiment. Each chamber had been previously filled with 0.5 L of pre-washed sandy sediment that had been sieved (1 mm) to remove fauna and large particles. We ran a full-factorial indoor mesocosm experiment in a temperature-controlled climate room set at 22 °C to test the effects of three factors: warming, acidification and ammonium enrichment in the seagrass *C. nodosa*. We used two temperature levels, control temperature (CT) ca. 22 °C and high