TESIS DOCTORAL

Carbon metabolism and dissolved organic carbon (DOC) fluxes on seagrass communities: insights from plant colonization states, eutrophication and global change related factors

Metabolismo de carbono y flujos de carbono orgánico disuelto (COD) en comunidades de angiospermas marinas: dependencia del estadío de colonización, eutrofización y factores relacionados con el cambio global

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Los directores: Dr. D. Ignacio Hernández Carrero, Catedrático de Ecología y Dr. D. Fernando G. Brun Murillo, Profesor Titular de Ecología de la Universidad de Cádiz.

CERTIFICAN:

Que la presente memoria titulada, "Metabolismo de carbono y flujos de carbono orgánico disuelto (COD) en comunidades de angiospermas marinas: dependencia del estadío de colonización, eutrofización y factores relacionados con el cambio global", presentada por Luis Gonzalo Egea Tinoco, ha sido realizada bajo su dirección y autorizan su presentación y defensa, para optar al grado de Doctor por la Universidad de Cádiz.

Y para que así conste y surta los efectos oportunos, firman los presentes en Puerto Real, a 12 de septiembre de 2017.

Prof. Dr. Ignacio Hernández Carrero

Prof. Dr. Fernando G. Brun Murillo

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Felicidad es estar con la naturaleza, mirarla y conversar con ella

Leo Tolstoy

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TABLE OF CONTENTS

ABSTRACT

Coastal vegetated communities are among the most productive ecosystems on Earth. Their role in the global carbon cycle and how they cope with global change may be more relevant than previously believed. They export large quantities of matter, both in particulate and dissolved forms to adjacent communities. Dissolved organic carbon (DOC) flux play a central role in the marine carbon cycle as an important driver of primary production for other compartments of the food web. While there has been extensive research on DOC dynamics in the open ocean, the role of coastal ecosystems in the global DOC cycle is still inadequately understood, even though these habitats tend to accumulate large amounts of DOC. Few studies have examined the DOC fluxes by marine macrophytic communities (macroalgae and seagrasses) under *in situ* approaches to determine their overall contribution in the whole system and their subsequent exchange with adjacent communities. Moreover, coastal vegetated communities, especially those dominated by seagrasses, are currently considered one of the most threatened ecosystems on Earth because of anthropogenic pressures, including nutrient increase and climate change. Thus, the overall objective of this Thesis was to evaluate the carbon metabolism and DOC fluxes in communities dominated by seagrasses and elucidate the effects of human–induced disturbances on the carbon dynamics of the community.

The results of this Thesis showed that macrophytic communities are highly autotrophic, with large and variable contributions of their different components of the community, and a DOC source for the plankton community. Increase in nutrients concentration triggered that the communites dominated by the macroalge *Caulerpa prolifera* and the seagrass *Cymodocea nodosa* moved from autotrophy to heterotrophy in certain seasons of the year and could increase or decrease the DOC release. The response of seagrass communities when subjected to a pH decrease was complex and showed to be species-specific. The pH decrease triggered a significant increase in gross primary production (GPP) and community respiration (R) in seagrasses, which was translated into sucrose increase in aboveground tissues rather than a higher DOC release. Water temperature was the stressor that had a higher positive effect on carbon metabolism, yielding higher seagrass productivity, growth and DOC release. A direct relationship between productivity increase and larger DOC release was found in communities dominated by seagrasses. In addition, a high correlation between DOC release and both water temperature and current velocity was found. This Thesis demonstrated that climatic change and to some extent nutrient enrichment in coastal areas may not be so detrimental than previously believed at least for temperate seagrasses, and even may benefit the productivity and resistance of some temperate species (e.g. *Cymodocea nodosa*) in the future.

The results of this Thesis underline the high productivity of vegetated coastal ecosystems at a local level, which support new insights in the role of the marine primary production in the ocean C sink and the role of the carbon coastal cycle in the global carbon cycle. Finally, this Thesis underscores that the role of seagrass meadows in the carbon coastal cycle will be more relevant in the near future, as higher C uptake and DOC release may occur under forecasted global change conditions.

RESUMEN

Las comunidades de macrófitos costeros son consideradas como uno de los ecosistemas más productivos del planeta. Su papel en el ciclo global del carbono y como ellos luchan contra el cambio global podría ser más relevante de lo que hasta ahora se creía. Exportan grandes cantidades de materia en forma particulada y disuelta hacia comunidades adyacentes. Los flujos de carbono orgánico disuelto (COD) juegan un papel esencial en el ciclo del carbono marino como un importante mecanismo de transferencia de la producción primaria a otros compartimentos de la cadena trófica. A pesar de que la investigación de la dinámica de COD en el océano abierto ha sido extensamente estudiada, el papel de los ecosistemas costeros en el ciclo global del COD es aún poco conocido, pese a que éstos hábitats tienden a acumular grandes cantidades de COD. Pocos estudios han examinado los flujos de COD en comunidades de macrófitos marinos (macroalgas y angiospermas marinas) bajo un enfoque *in situ* para determinar la contribución del sistema completo y el consecuente intercambio con comunidades adyacentes. Además, las comunidades de macrófitos costeros, especialmente las angiospermas marinas, son actualmente consideradas como uno de los ecosistemas más amenazados de la Tierra debido a las presiones antropogénicas, incluyendo el incremento de nutrientes y el cambio climático. Por ello, el objetivo principal de esta Tesis fue evaluar el metabolismo de carbono y flujos de COD en comunidades de angiospermas marinas y elucidar los efectos producidos por las presiones antropogénicas en la dinámica del carbono de la comunidad.

Los resultados de esta Tesis mostraron que las comunidades de macrófitos costeros son altamente autotróficas, con una gran y variable contribución de los diferentes componentes de la comunidad, siendo una fuente de COD que refuerza la comunidad planctónica. El aumento en la concentración de nutrientes dio lugar a que las comunidades dominadas por la macroalga *Caulerpa prolifera* y la angiosperma marina *Cymodocea nodosa* pasaran de autotrofia a heterotrofia en determinadas temporadas del año además de aumentar o disminuir la liberación de COD. La respuesta de las comunidades de angiospermas marinas sometidas a una disminución de pH fue compleja y mostró ser especifica de la especie. La disminución del pH provocó un aumento significativo de la producción primaria bruta (PPB) y la respiración de la comunidad (R) en angiospermas marinas lo que se tradujo en un aumento de sacarosa en la biomasa subterránea, en lugar de aumentar la liberación de COD. La temperatura fue el factor de estrés que tuvo el mayor efecto positivo en el metabolismo del carbono, dando lugar a una mayor productividad de las angiospermas marinas, crecimiento y liberación de COD. Una relación directa entre el aumento de la productividad y el incremento de la liberación de

COD fue encontrada en las comunidades de angiospermas marinas. Además, se demostró una alta correlación entre la liberación de COD y la temperatura del agua y las condiciones hidrodinámicas (velocidad de corriente). Por lo tanto, esta Tesis demuestra que el cambio climático y en cierta medida el enriquecimiento de nutrientes en las zonas costeras puede no ser tan perjudicial como hasta ahora se creía, al menos para angiospermas marinas de latitudes templadas, e incluso podría beneficiar a la productividad y resistencia de la especie de angiosperma marina *C. nodosa* en el futuro.

Los resultados de esta Tesis subrayan la alta productividad de los ecosistemas de macrófitos costeros a nivel local, lo que apoya nuevas ideas en el papel de la producción primaria marina como sumidero oceánico de carbono, así como el ciclo costero de carbono en el ciclo global del carbono. Finalmente, esta Tesis subraya que el papel de las praderas de angiospermas marinas en el ciclo costero del carbono será mayor en el futuro, debido a la mayor captación de carbono y liberación de COD que puede ocurrir bajo las condiciones previstas de cambio global.

RESUMO

As comunidades de macrófitas costeiras são consideradas como um dos ecossistemas mais produtivos do planeta. O seu papel no ciclo global do carbono e a forma como eles lutam contra a alteraçõe global podem ser mais relevantes do que se pensava anteriormente. Eles exportam grandes quantidades de matéria em formas particuladas e dissolvidas para comunidades adjacentes. Os fluxos de carbono orgânico dissolvido (COD) desempenham um papel essencial no ciclo do carbono marinho como um mecanismo importante para transferir a produção primária para outros compartimentos na cadeia alimentar. Embora a pesquisa sobre a dinamização da COD no oceano aberto foi amplamente estudada, o papel dos ecossistemas costeiros no ciclo global de COD ainda é pouco compreendido, mesmo que esses habitats tendam a acumular grandes quantidades de COD. Poucos estudos examinaram os fluxos de COD em comunidades de macrófitas marinhas (macroalgas e angiospermas marinhas) sob uma abordagem *in situ* para determinar a contribuição do sistema completo e a conseqüente troca com comunidades adjacentes. Além disso, as comunidades de macrófitas costeiras, especialmente as angiospermas marinhas, são atualmente consideradas como um dos ecossistemas mais ameaçados da Terra devido a pressões antropogênicas, incluindo aumento de nutrientes e alterações climáticas. Portanto, o objetivo principal desta tese foi avaliar o metabolismo do carbono e os fluxos de COD em comunidades de angiospermas marinhas e elucidar os efeitos produzidos pelas pressões antropogênicas sobre a dinâmica de carbono da comunidade.

Os resultados desta tese mostraram que as comunidades de macrófitas costeiras são altamente autotróficas, com uma contribuição grande e variável dos diferentes componentes da comunidade, sendo uma fonte de COD que reforça a comunidade planctônica. O aumento na concentração de nutrientes resultou nas comunidades formadas pela macroalga *Caulerpa prolifera* e o angiosperma marinha *Cymodocea nodosa* para passar da autotrofia para heterotrofia em certas estações do ano além de aumentar ou diminuir a liberação de COD. A resposta das comunidades angiosperma marinha quando submetidas a uma diminuição do pH foi complexa e mostrou-se específica para cada espécie. A diminuição do pH causou um aumento significativo na produção primária bruta (PPB) e na respiração comunitária (R) em angiospermas marinhas, o que resultou em um aumento da sacarose na biomassa subterrânea, em em vez de aumentar a liberação de COD. A temperatura foi o fator de estresse que teve o maior efeito positivo no metabolismo do carbono, resultando em aumento da produtividade de angiospermas marinhas, crescimento e liberação de COD. Foi encontrada uma relação direta entre o aumento da produtividade e o aumento da liberação de COD em comunidades de angiospermas marinhas. Além disso, foi demonstrada uma alta correlação entre a liberação de COD e a temperatura da água e as condições hidrodinâmicas (velocidade atual). Portanto, esta tese demonstra que as alterações climáticas e até certo ponto o enriquecimento de nutrientes nas áreas costeiras podem não ser tão prejudiciais como se acreditava anteriormente, pelo menos para angiospermas marinhas de latitudes temperadas, e poderiam mesmo beneficiar a produtividade e resistência da espécie de angiosperma marinha *C. nodosa* no futuro.

Os resultados desta tese sublinham a alta produtividade dos ecossistemas de macrófitas costeiras a nível local, apoiando novas ideias no papel da produção primária marinha como dissipador oceânico de carbono, assim como o ciclo do carbono costeiro no mercado global carbono. Finalmente, esta tese enfatiza que o papel dos prados marinhos no ciclo do carbono litoral será maior no futuro, devido à maior absorção de carbono e liberação de COD que pode ocorrer nas condições de alterações global esperadas.

DEFINITIONS

The following terms are defined according to their meaning in this work.

Carbon metabolism: Process that includes the pathway by which photosynthetic organisms use the energy captured from light in the form of NADPH and ATP to transform inorganic carbon forms (e.g. CO_2) into organic carbon molecules such as carbohydrates. The organic molecules produced by photosynthesis form the autotrophic basis of the food web. In addition, includes the process by which the organic carbon is respired or remineralised by the autotrophic and heterotrophic components of the system. Therefore, carbon metabolism comprises both the gross primary production (GPP) and community respiration (R) processes.

Gross primary production (GPP) (mmol C m^{-2} d^{-2}): Autochthonous production of organic carbon by the autotrophic components of the system using inorganic carbon forms (e.g. CO_2) and energy captured from light.

Community respiration (R) (mmol C m^{-2} d^{-2}): Remineralization of organic carbon (in the form of CO_2) by the autotrophic and heterotrophic components of the system. Then, it is the consumption of organic carbon by all the components of the community.

Net community production (NCP) (mmol C m^{-2} d^{-2}): Difference between GPP of the autotrophic components of the community and R. Then, it represents the excess of carbon community production and can be positive, negative or equal to zero.

Autotrophic system: A system where the production of organic carbon is higher than the consumption of this organic carbon (i.e. GPP > R). Then, a net autotrophic system is a potential C sink (i.e. CO_2 sink).

Heterotrophic system: A system where the consumption of organic carbon exceeds its production (i.e. R > GPP). Then, a net heterotrophic system is a potential C source (i.e. CO_2 source).

Carbon captured: Carbon fixed by photosynthetic organisms during the photosynthetic process that is not remineralised directly through community respiration. It represents the total inorganic carbon removed from the system to be transformed into organic carbon. The carbon capture is quantified as the NCP. Part of this organic carbon will be exported to other ecosystems and/or remineralised by herbivores or bacteria through respiration, but part of this carbon will not return to inorganic forms after centuries or millennia (i.e. carbon sequestered).

Carbon sequestered (carbon buried): Carbon captured that is not remineralised during a long period. It remains sequestered in the sediment or in deep waters, and then, does not return as inorganic carbon (i.e. CO_2) to the atmosphere after centuries or millennia. It represents the long-term storage of carbon (i.e. C sink), which partially offset the increase in atmospheric CO_2 derived of human activities.

Black carbon: Particles derived from incomplete combustion of fossil fuels, biofuel and biomass as a consequence of both anthropogenic and naturally occurring activity. Black carbon has a greater effect on radiation transmission and climate change.

Green carbon: Carbon sequestered by the biosphere through the photosynthesis process.

Blue carbon: Fraction of green carbon that is sequestered in vegetated coastal habitats, in particular salt marshes, mangroves, macroalgal beds and seagrass meadows.

Global change: planetary–scale changes in the Earth system as a consequence of human activity including ocean acidification, temperature increase, eutrophication, species loss, etc.

Climate change: Part of global change, which includes the planetary–scale changes in the Earth system at climate level (i.e. ocean acidification and temperature increase).

Stressor: Environmental factor derived from human activities or natural events, which causes any pressure in one or more species or to the whole ecosystem. As a consequence of this pressure, the species or ecosystem may be damaged or otherwise benefited depending on the magnitude, duration and addition with other stressors.

GENERAL INTRODUCTION

Dime y lo olvido, enséñame y lo recuerdo, involúcrame y lo aprendo.

Benjamin Franklin

The role of coastal ecosystems in the marine carbon cycle

The coastal zone is defined as the part of land most affected by its proximity to the sea, and that part of the ocean most affected by its proximity to the land (Hinrichsen 1998). It covers approximately the area from inshore waters up to 200 m in depth to about an altitude of 200 m above sea level (Pernetta & Milliman 1995). On a global scale, the coastal zone represents a limited area of the planet (circa 18%). However, it has a great importance because of their considerable role in the biogeochemical cycles (e.g. carbon cycle), the large variety of ecosystems it contains, and the high biodiversity it supports. As the consequence, a quarter of the global primary production is generated in this area (Pernetta & Milliman 1995, Gattuso et al. 1998).

Coastal zone plays an essential role in the biogeochemical cycles since it receives massive inputs of terrestrial organic matter and nutrients, exchanges large amounts of matter and energy with the open ocean and is among the most geochemically and biologically active areas of the biosphere (Gattuso et al. 1998). The traditional models analysing the global carbon cycle do not incorporate the coastal zone, but directly link oceans and continents (Fig. 1). The role of the coastal zone in these global models is still a matter of debate because of the limited knowledge in the magnitude of the interchange of carbon between the coastal zone and the open sea. The complexity of the carbon dynamics in coastal areas has led to uncertainty regarding the role of the coastal ocean as a sink or source of CO_2 to the atmosphere. Traditionally, coastal areas usually have been considered as a CO_2 source to the atmosphere (e.g. Smith 1993) and only recently it has been established that, overall, continental shelves absorb CO_2 (Chen & Borges 2009). Hence, more studies of carbon metabolism in coastal areas are needed to evaluate the real contribution of this zone to the global carbon cycle.

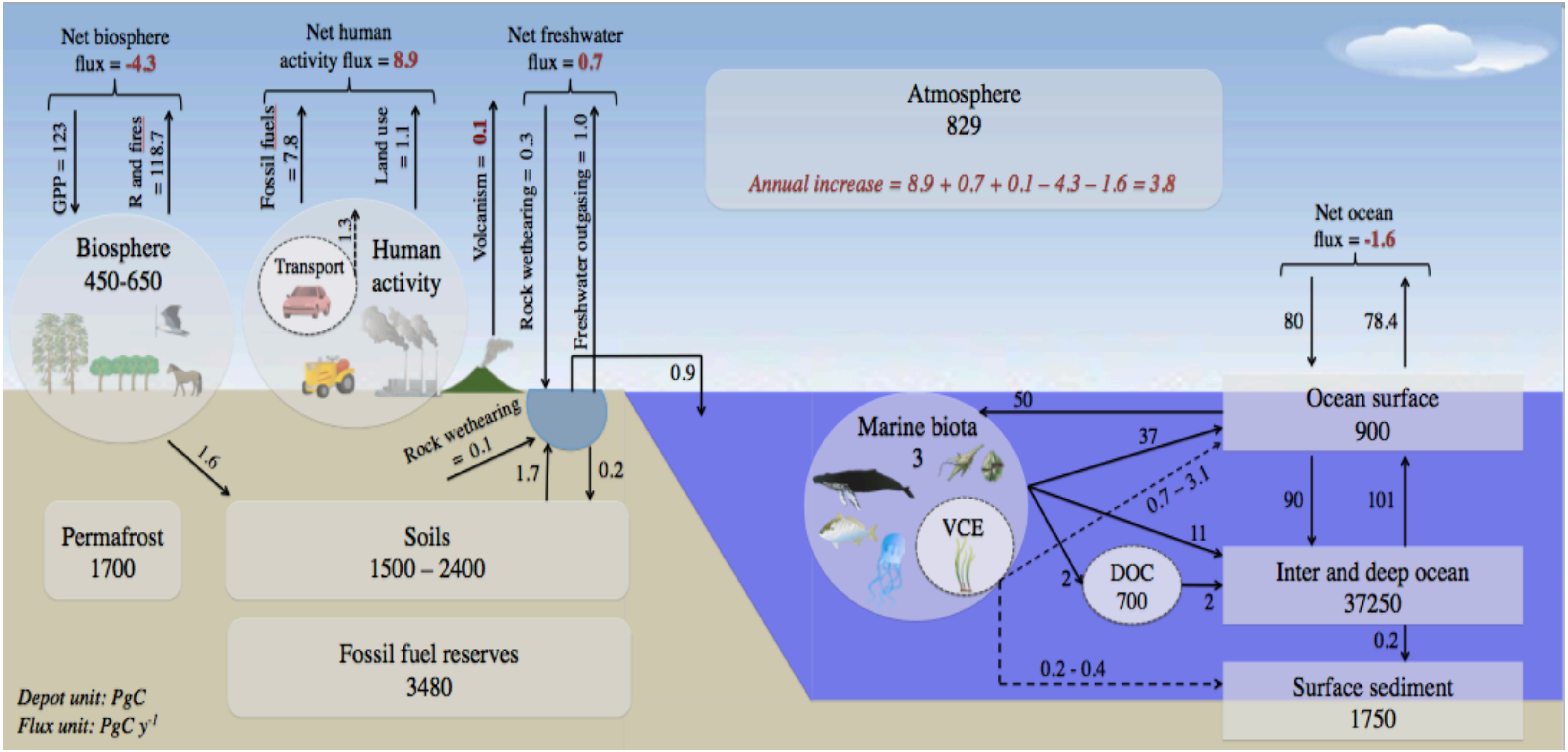


Figure 1. Simplified drawing of the global carbon cycle. Numbers represent current estimated of carbon reservoirs in PgC (1 PgC = 10^{15} gC) and annual carbon exchanged fluxes in PgC y^{-1}. VCE: vegetated coastal ecosystems. Negative values indicate atmospheric CO_2 removal and positive values indicate atmospheric CO_2 emission. Adapted from Carbon and other biogeochemical cycles (IPCC 2013).

The coastal zone bears a large variety of habitats in both terrestrial and marine parts (Martínez et al. 2007). Within the marine part, vegetated coastal ecosystems (including seagrass meadows, macroalgal beds, mangroves and salt marshes) occupy only a limited surface on a global scale (<2% of the ocean surface) but have some of the highest rates of primary carbon production (Duarte & Cebrián 1996, Nellemann et al. 2009). As a consequence of their high productivity, vegetated coastal ecosystems provide a large number of ecosystem goods and services (Costanza et al. 1997) and play an important but overlooked role in the marine carbon cycle (Duarte 2017). Marine carbon fluxes so far have mainly focused on microorganism activity, since bacteria and microalgae are the dominant sources of primary production and respiration in the ocean (e.g. Duarte & Cebrián 1996, del Giorgio & Duarte 2002). However, recent analyses of the carbon cycling in vegetated coastal areas suggest that these fluxes may be more important than previously considered as a consequence of their high productivity (Duarte et al. 2005) and their role as carbon sequestration filters (Watanabe & Kuwae 2015). The excess organic carbon produced by vegetated coastal ecosystems has two possible fates: to be exported to adjacent ecosystems in the way of particulate or dissolved form, or to be stored in sediments contributing to C burial (Fig. 1). The analysis of the cycle of carbon in vegetated coastal ecosystems suggests they globally export to the open ocean between 769 – 3,177 Tg C y^{-1} (Duarte et al. 2005), which is ready to be used (i.e. respired) and probably largely leading to the open ocean to a net heterotrophic status (Duarte & Agustí 1998, del Giorgio & Duarte 2002) or buried in the open ocean, for example through the transportation of organic carbon at depth areas by the biological pump (e.g. Agusti et al. 2015). On the other hand, the C buried by vegetated coastal ecosystem from the called earth's blue carbon sink accounts for between 235 – 450 Tg C y^{-1}, which represent ca. 50 – 70% of all carbon stored in ocean sediment (Duarte et al. 2005, Laffoley & Grimsditch 2009, Nellemann et al. 2009, Mcleod et al. 2011), and is equivalent to up to 17 – 33% of the emissions from the entire global road transport sector, which was estimated in ca. 1,376 Tg C y^{-1} in 2010 (Sims et al. 2014).

Regrettably, vegetated coastal habitats are among the most threatened ones worldwide, with a rate of loss much higher than any other ecosystem on the planet (in some instances up to four times that of rainforests). Currently, on average, between 2 – 7% of these ecosystems are lost annually (Nellemann et al. 2009) with current increasing rates of loss in many habitats, such as seagrass meadows (Waycott et al. 2009). This growing loss in vegetated coastal habitats is draining not only their sequestering potential, but also may result in the release of the carbon already buried in their sediments, which may turn back to the atmosphere (Mcleod et al. 2011). Therefore, studying the carbon metabolism and the interactions among all components shaping the food web in the community will lead to a greater understanding of the productivity and carbon cycle in these ecosystems, which is necessary for their effective management (Polis et al. 1997, Barbier et al. 2011).

Dissolved organic carbon (DOC) fluxes in the coastal carbon cycle

The knowledge of the marine dissolved carbon cycle has focused mainly on the dissolved inorganic carbon (DIC), which is estimated in about 38,150 PgC (37,250 from inter and deep ocean and 900 PgC from surface ocean; Fig. 1). However, dissolved organic carbon (DOC), with about 700 PgC, is the other important C pool in the ocean (Ciais et al. 2013). This is mainly composed of many different classes of organic compounds such as sugars or amino acids, as well as fractions that are more coarsely classified, such as humic acids (Hansell & Carlson 2001, Dafner & Wangersky 2002). The DOC has a wide range of turnover times, from minutes to millennia, and is typically referred to as biologically labile, semi-labile, or refractory (Williams & Druffel 1987, Carlson 2002). The role of DOC in the global carbon cycle is still a matter of discussion because several studies suggested that most of DOC is largely old and refractory and thus unavailable for biological consumption (Hansell & Carlson 2001). On the contrary, other studies have highlighted the central role played by DOC in the global carbon cycle (Hedges et al. 1997, Hansell & Carlson 2002), since a significant fraction of this DOC (between 30–67% according to Dachs et al. 2005, Ruiz-Halpern et al. 2014) is comprised by a wide variety of largely unresolved volatile and semi-volatile organic compounds (VOC and SOC), and thus, it is available to marine organisms. This DOC acts as a quick transfer of C and energy across the food web, as it is easily assimilated by marine organisms, and fully involved in the C exchange between communities (Hansell & Carlson 2001, Navarro et al. 2004). Therefore, DOC is an important driver of primary production for other compartments of the food web, as was already suggested decades ago (Jørgensen 1976). In addition, some studies suggest that DOC is important to the functioning of the oceanic biological pump (Sugimura & Suzuki 1988, Suzuki et al. 1985). Hence, the dynamic of DOC cycling affects the transportation of organic carbon to deep zones, which ultimately affects the oceanic-atmospheric CO_2 balance, and hence, the atmospheric CO_2 concentration (Hedges 1992).

Oceanic DOC originates from river inputs (estimated about 0.17 Gt C y^{-1}), although with important lack of data from some regions (Mayorga et al. 2010), and from marine biological production, which remains poorly understood. On the other hand, while there has been extensive research on DOC dynamics in the open ocean (reviewed by Hansell &

Carlson 2001), the role of coastal ecosystems in the global DOC cycle is still inadequately understood, even though these habitats tend to accumulate huge amounts of DOC (Dafner & Wangersky 2002). Ultimately, DOC dynamics in shallow coastal areas are controlled by the interaction of primary producers and heterotrophic bacteria. Primary producers act as source of new organic matter either particulate or as dissolved form. Heterotrophic bacteria can act either as a sink (Boto et al. 1989) or a source of DOC through direct excretion, virus–mediated cell lysis, by exoenzymes released by bacteria as well as zooplankton–mediated sloppy feeding (Stoderegger & Herndl 1998, Glud & Mathias 2004). In addition, heterotrophic bacteria provide the major pathway for the transformation of particulate organic material (POM) to DOC (Fig 2).

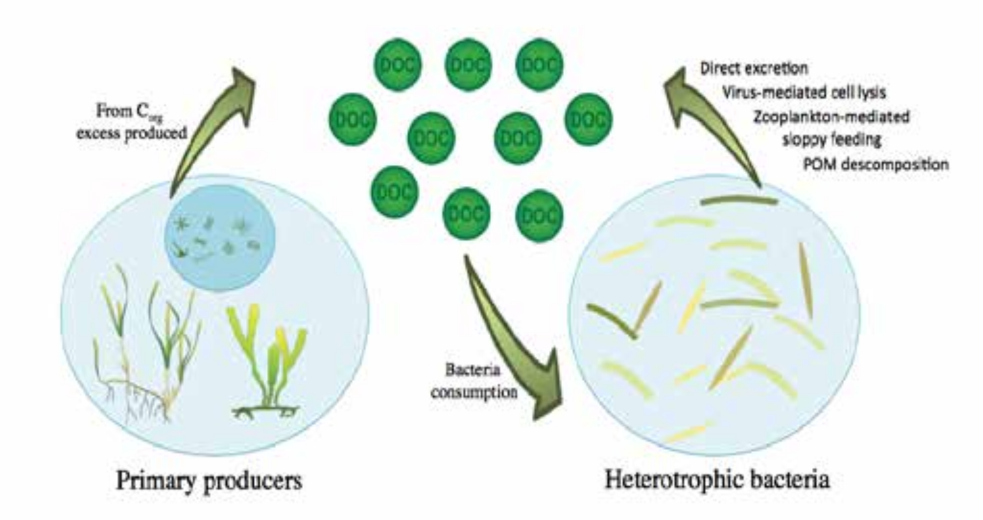


Figure 2. Simplified conceptual model showing the DOC dynamic in shallow coastal areas.

The DOC flux in vegetated coastal ecosystems remains poorly studied, especially under *in situ* approaches, to determine the overall contribution of the whole system (e.g. Ziegler & Benner 1999, Santos et al. 2004, Barrón & Duarte 2009, Maher & Eyre 2011). Moreover, the analysis of the contribution of each component of the community in the DOC dynamic along with their inter–annual variation, as well as the dependence of DOC fluxes with other environmental factors, is still poorly comprehended. This is essential to estimate the energy and organic matter exchanged between coastal communities and to evaluate the present and future role of DOC fluxes, nor only in the coastal zone but in the global carbon cycle.

Seagrass ecosystems

Seagrasses are marine flowering plants that form one of the richest and most relevant coastal ecosystems (Short et al. 2011) as a consequence of the large number of ecological services they provide (Costanza et al. 1997), including long-term carbon catchment (Nellemann et al. 2009, Tokoro et al. 2014). Seagrasses are present in shallow coastal areas around all continents, except Antarctica (Hemminga & Duarte 2000). All seagrass species are monocotyledons and they exhibit low taxonomic diversity (ca. 60 species worldwide, compared with ca. 8,000 species of macroalgae) classified in 4 families (*Posidoniaceae*, *Zosteraceae*, *Hydrocharitaceae* and *Cymodoceae*).

Despite being the most geographically extended coastal ecosystem, they cover less than 0.2% of the ocean surface. To date, there is no global inventory of seagrass area covered. The documented seagrass area is circa 177,000 km^2 (Green & Short 2004), but this is an acknowledged underestimate of the total area covered, since for many areas, seagrasses have not been well documented (especially insular Southeast Asia, the east coast of South America and the west coast of Africa). Hence, the estimates most commonly used in the literature suggest a minimum surface of 300,000 km^2 and a high of 600,000 km^2 (Duarte et al. 2005a, Nellemann et al. 2009, Mcleod et al. 2011), with the global area potentially suitable to support seagrass growth estimated at 4,320,000 km^2 (Gattuso et al. 2006). Particularly, Spain counts on almost 1620 km^2 (equivalent to the surface of Gran Canaria) of seagrass meadows (Ruiz et al. 2015).

Although seagrass meadows account for only a relatively small area of the coastal zone, they are well recognised as highly important due to the large number of ecological services they provide as a consequence of their high productivity (Orth et al. 2006, Short et al. 2011, Campagne et al. 2014), such as nutrient regeneration, water quality improvement, shoreline protection, creation of suitable breeding habitats (including those for species of economic relevance) and biodiversity (Costanza et al. 1997, Green & Short 2004, Duffy 2006, González-Ortiz et al. 2014).

The role of seagrasses in the coastal carbon cycle

Within vegetated coastal ecosystems, the role of seagrasses in the coastal carbon cycle has been widely studied in the last years, probably since, as it was stated above, it is one of the most productive ecosystems on the planet, leading to a significant carbon capture. In addition, the total carbon buried in coastal sediments does not seem as negligible as hitherto believed. Thus, as a consequence of their high productivity, seagrass meadows tends to be largely autotrophic in an annual basis (sometimes more than the Amazonian forest, Grace et al. 1995), and therefore yield a net community production (averaging ca. 120 g C m^{-2} y^{-1}) (Duarte et al. 2010). On the other hand, seagrass meadows bury C at a rate that is 35 times faster than tropical rainforests, and their sediments never become saturated (Mcleod et al. 2011). Furthermore, while terrestrial forests bind C for decades or centuries, seagrass meadows can bind C for millennia (Mateo et al. 1997, Macreadie et al. 2012, Serrano et al. 2012). In a comprehensive survey of seagrass C stocks collected from almost 1,000 meadows, Fourqurean et al. (2012) estimated that seagrasses can store circa 4.2 – 8.4 Pg C, 26 times higher than previous estimations (Duarte & Chiscano 1999).

The total primary production of the seagrass community is contributed almost equally by the angiosperms themselves and the epiphytes and macroalgae they support (Hemminga & Duarte 2000). Thus, the number of studies regarding the productivity of the whole community dominated by seagrasses has increased in the last years (Duarte et al. 2010), and tends to estimate the C fluxes by measuring *in situ* dissolved oxygen (DO) changes (Silva et al. 2009) to estimate the net contribution of the whole community (e.g. Barrón & Duarte 2006). The mean net community production of seagrasses (ca. 120 g C m^{-2} y^{-1}) (Duarte et al. 2010), represents globally between ca. 36 – 72 Tg C y^{-1} considering the current estimated range of seagrass cover area, and plays an remarkable role in the carbon cycle of the coastal zone (Duarte & Chiscano 1999, Hemminga & Duarte 2000). The fate of this large amount of C has being widely studied. According to Duarte & Cebrián (1996), circa 18% is consumed by herbivores, circa 44% is exported either as particulate or dissolved form and finally circa 16% may be sequestered in the sediment, and thus contributing to the marine C burial (i.e. blue carbon). However, these mean values at a global level were obtained by extrapolating results from few studies, that were focused primarily on tropical or subtropical species and therefore, these percentages may vary considerably. For example, in a more recent study (Kennedy et al. 2010), it is suggested that between 30 – 50% of NCP of seagrass meadows is potentially buried. On the other hand, besides the entry of organic carbon through the primary production of the community (i.e. NCP), there are a sedimentation of allochthonous organic carbon particles as a consequence of the reduction of water flow when crossing the seagrass canopy (Peralta et al. 2008, González-Ortiz et al. 2014, Macreadie et al. 2014). Indeed, about half of the organic C buried in seagrass sediments derives from the seagrass meadow itself and the other 50% derives from allochthonous organic carbon (Kennedy et al. 2010).

On the other hand, seagrass communities export significant amounts of organic carbon to adjacent ecosystems as particulate or dissolved forms (Duarte & Cebrián 1996, Barrón & Duarte 2009). While the origin and fate of particulate organic matter has been widely studied (Hemminga et al. 1994, Kennedy et al. 2004, Williams et al. 2009, Magen et al. 2010), the role of the DOC has only been recently highlighted (Mateo et al. 2006, Barrón & Duarte 2009, Van Engeland et al. 2013, Barrón et al. 2014). To date, the most common approach about DOC fluxes in seagrass ecosystems has been based on isolated plant incubations (Brylinsky 1977, Wetzel & Penhale 1979, Moriarty et al. 1986, Haas et al. 2010, Kaldy 2012). However, this procedure underestimates the magnitude of the community net release of DOC because it neglects the amount processed by other community compartments, such as epiphytes, the leaching of DOC from decomposing material in the sediments and the consumption by bacteria. Unfortunately, few studies have examined *in situ* DOC release by marine macrophyte communities and subsequent exchange with adjacent communities. The total number of net DOC flux estimations from seagrass meadows is mainly limited to the temperate zone and further efforts are needed to extend and increase these estimates to other climatic areas (Barrón et al. 2014).

Effect of anthropogenic pressures on the seagrass carbon metabolism and DOC fluxes

The coastal zone has been the centre of human activity during millennia. The world population (7.5 billion already reached in 2017), is steadily increasing, especially since the Industrial Revolution (Bongaarts 2009). Human population is expected to rise by 50% during the 21th century (Steck 2014). Today, 40% of the world's population lives in coastal areas (Burke et al. 2001, IOC/UNESCO, IMO, FAO, UNDP, 2011) and is expected to reach 75% by 2025 (Connelly 2008). Hence, the magnitude of human pressure is becoming larger on the coasts worldwide (Mora et al. 2011).

In the last century, human activities have triggered changes at a global scale that are affecting ecosystems worldwide, being coastal vegetated ecosystems one of the most threatened (Large 2009). In the next decades, these ecosystems are expected to increase their exposition to adverse consequences related to climate change, exacerbated by increasing direct human induced pressures (Nicholls et al. 2007). The shallow distribution of seagrasses in estuarine and coastal areas and its vulnerability against anthropogenic impacts has 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). The value of seagrass ecosystems is worldwide recognised by different legislations and international Conventions like the Convention on Biological Diversity (1992) or the

European Habitats Directive (92/43/EEC), which not only protect the habitats where seagrasses thrive but include the habitat of *Posidonia oceanica* as priority one in the European Union. In addition, the OSPAR Convention considers seagrass habitats at risk (www.ospar.org). Favoured by this legislation framework, seagrass habitats are nowadays specifically targeted for conservation and restoration (Green & Short 2004). To succeed in this challenge, it is necessary to understand the response of the ecosystems when subjected to multiple co-stressors derived from human pressures in order to provide management advices, including the modelling of future trajectories (Brierley & Kingsford 2009, Hoegh-Guldberg & Bruno 2010, Brodie et al. 2014). Marine organisms are typically exposed to more than one environmental stressor at any time and organisms are generally expected to be more sensitive to a given stressor when simultaneously affected by another (e.g. Myers 1995, Paine et al. 1998). The combined effect of two or more stressors may be additive or non–additive, while non–additive effects may be synergistic or antagonistic. Some studies also indicate that the effects of multiple stressors on seagrasses can be synergistic (e.g. De Los Santos et al. 2009, La Nafie et al. 2012, Salo et al. 2014, Villazán et al. 2015). The possible presence of non–additive effects of multiple stressors makes it difficult to predict consequences of the ongoing effects of anthropogenic pressures on seagrasses, since these changes are expected to alter several environmental and potentially stressful factors at the same time (e.g. temperature, acidification, light conditions, salinity, nutrient availability, etc.).

Eutrophication

Increase of nutrient levels in the water column, especially nitrogen and phosphorus, along onshore areas (particularly in urbanized metropolitan ones), may lead to eutrophication, which has been identified as one of the most important threats to coastal marine ecosystems (Nixon 1995, Smith & Schindler 2009) and seagrass meadows (Hughes et al. 2004, Burkholder et al. 2007, Cabaço et al. 2008, Antón et al. 2011). Increasing nutrient availability affects seagrass stands in two ways: alterations of the ecosystem and alteration of the individual plants. Thus, nutrient excess promotes the proliferation of fast–growing species, including phytoplankton, epiphytes and opportunistic macroalgae (Sand-Jensen & Borum 1991, Duarte 1995), species that compete with seagrasses for light and can cause them mortality by shading (Short et al. 1995, Hauxwell et al. 2001, McGlathery 2001, Brun et al. 2003, Moreno-Marín et al. 2016). Nutrient enrichment may further intensify the flux of organic matter to the sediment, thereby enhancing the risk of anoxia and sulphide toxicity (Borum et al. 2005, Pérez et al. 2007, Olivé et al. 2009). Moreover, increased nutrient availability stimulates seagrass consumption by herbivores, probably through an increase in the nutritional quality of the food resource (McGlathery 1995, Ruiz et al. 2001, Cebrian et al. 2009, Jimenez-Ramos et al. 2017). This can lead to significant reductions in photosynthetic biomass and consequently to seagrass mortality. Nutrient enrichment may not only alter seagrasses and associated algae, but also affect the composition and abundance of associated faunal assemblages (community associated). However, experimental evidence of this is rather sparse and mostly reduced to influences over key herbivores (Valentine & Heck 2001, Castejón-Silvo et al. 2012). The latter could decrease seagrass DOC release if the main DOC consumer abundance increases as a consequence of changes in the community associated.

In addition to causing alterations in the ecosystem, eutrophication has also an effect on individual plants. Several studies have pointed out that moderate increases (<10 µM) in the availability of dissolved inorganic nitrogen (DIN) (e.g. NO_3^-, NH_4^+ and NH_3^-) may stimulate growth of seagrasses in pristine, oligotrophic environments (Short 1987, Pérez et al. 1991, Alcoverro et al. 1997, Udy et al. 1999). However, under conditions of reduced availability of dissolved inorganic nitrogen (nitrate and ammonium), especially when ammonium is the most abundant form, plant growth and survival can be curtailed by direct ammonium toxicity (Burkholder et al. 1992, 1994, Van Katwijk et al. 1997, Brun et al. 2002, 2008, Villazán et al. 2013). High availability of NH_4^+ may cause the accumulation of this compound within the plant, which can affect intracellular pH, enzyme kinetics, photosynthetic ATP production and uptake of other ions (Marschner 1995, Britto & Kronzucker 2002, Villazán et al. 2013). In addition, intracellular accumulation of NH_4^+ may yield an imbalance in the carbon economy as a consequence of the internal demand of energy and carbon skeletons needed for rapid ammonium assimilation (i.e. the synthesis of amino acids and proteins). This carbon demand is met by resources diverted from growth and other metabolic processes (Marschner 1995, Brun et al. 2002, 2008, Villazán et al. 2013).

In summary, this study presents the hypothesis that under nutrient enrichment, there will be a decrease in the seagrass production and DOC release derived from macroalgal competition, changes in the associated community and ammonium toxicity.

Global change effect in coastal ecosystems

In the last decades, global change (i.e. global scale changes in the Earth system including ocean acidification, temperature increase, eutrophication, species loss, etc.) has been acknowledged as one of the main threats for the biosphere and the ecosystem services it provides, especially for marine and coastal ecosystems (Nicholls et al. 2007, Chapin et al. 2008, Halpern 2014).

Ocean acidification

Over the last century, human activities have produced large amounts of CO_2 through fossil fuel burning, intensive agriculture and deforestation. The concentration of CO_2 in the atmosphere has risen from 280 ppm in preindustrial times to 409 ppm in 2016, as measured by the Mauna Loa Observatory. It is projected that atmospheric CO_2 will increase at a rate of 0.5% per year throughout the XXI century, a rate of change that is approximately 100–times faster than has occurred in the past 650,000 years (Meehl et al. 2007). Thus, by the middle of this century, atmospheric CO_2 levels could reach more than 500 ppm, and surpass 800 ppm by the end of it (Friedlingstein et al. 2006); levels not reached since the Cretaceous Period (Retallack 2001).

The ocean has absorbed about one–quarter of this anthropogenic CO_2 (Sabine & Feely 2004, Canadell et al. 2007) acting as a carbon sink and thus contributing to the key ecosystem service of climate regulation. A portion of this CO_2 absorbed by the oceans is stored in living biomass and sequestered in sediments, but a large fraction remains in its inorganic form. In the ocean, carbon is available predominantly as dissolved inorganic molecules (DIC, ~38,150 PgC) (see Fig. 1); that is carbonic acid (dissolved CO_2 in water), bicarbonate and carbonate ions, which are tightly coupled via ocean chemistry (Fig. 3). The increase of inorganic carbon stored in the oceans has driven a reduction in seawater pH and has promoted changes in the seawater chemistry in a process commonly referred to as "ocean acidification" (OA) (Fig. 3) (Caldeira & Wickett 2003, 2005, Orr et al. 2005, Doney et al. 2009, Koch et al. 2013). The global ocean pH is expected to fall to between 8.05 and 7.6 by the end of this century (Prinn et al. 2011, IPCC 2013).

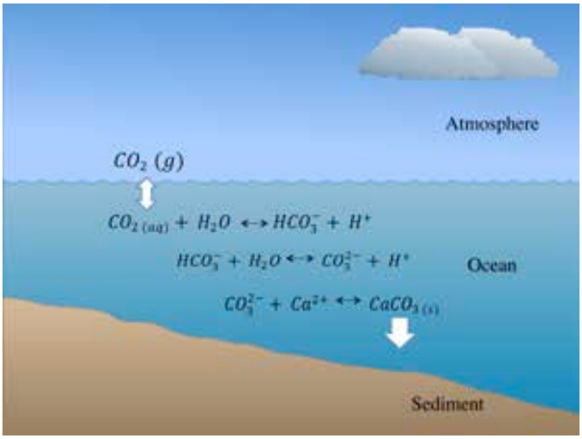


Figure 3. Simplified diagram of inorganic carbon speciation in the ocean.

This raises concerns about the possible impacts of these changes on marine organisms. The OA is an ubiquitous stressor that is likely to lead to negative consequences in the future for marine organisms (Kroeker et al. 2010), ecosystems (Fabry 2008) and ecosystem services provision (Cooley et al. 2009). In seagrass ecosystems, the OA could either ameliorate or aggravate the current decline of seagrasses (Koch et al. 2013). To date, it is known that seawater pH decrease as a result of increasing CO_2 concentration can benefit the seagrass primary productivity, as these plants are generally considered to be photosynthetically CO_2 limited (Beer & Koch 1996, Beardall et al. 1998). Then, an increase in growth rates and biomass in the absence of other growth limiting factors (e.g., nutrients, light) is expected (Short & Neckles 1999, Palacios & Zimmerman 2007, Hall-Spencer et al. 2008, Harley et al. 2012, Garrard & Beaumont 2014). In addition, if CO_2 increase may enhance primary production, a fraction of this new production can be released as DOC and thus affecting the picture of the pathway of C fluxes in coastal communities.

However, the effects of increased CO_2 concentrations on seagrasses may depend on the degree of carbon limitation in natural systems. Carbon limitation has been partly attributed to the thickness of the diffusive boundary layer (DBL) surrounding leaf surfaces at low current velocities (Larkum 1989, Madsen & Sand-Jensen 1991, Koch 1994), or to a relatively inefficient HCO_3^- uptake system (Beer et al. 1980). Hence, the specific outcome of a CO_2 increase is still unclear because there is an array of environmental variables, which could ameliorate or aggravate the effects of the CO_2 increase.

Therefore, this study presents the hypothesis that under pH decrease condition, there will be an increase in seagrass production and DOC release, as these plants are generally considered to be photosynthetically CO_2 limited.

Temperature increase

Global warming is emerging as a major threat to ecosystems worldwide (IPCC, 2007). Mean global sea–surface temperatures has increased by circa 0.8 °C over the last century (Levitus et al. 2001, Hansen et al. 2006) and, by the end of this century, is projected to increase by circa 3–4 °C (Meehl et al. 2007).

The effect of temperature increase on seagrasses has been widely studied, since temperature is a key factor for seagrass health, growth and community metabolic rates (Koch et al. 2013). Seagrasses are affected by thermal stress in a number of ways. The direct effects of increased temperature in seagrasses will depend on the species thermal tolerance and its optimum temperature for photosynthesis, respiration and growth (Short & Neckles 1999). The temperature optima for photosynthesis in tropical seagrasses range from 27 to 33 °C and for temperate species from 21 to 32 °C, while their growing temperatures average approximately 3 and 8 °C lower, respectively (reviewed in Lee & Kim 2007). Should there are no limitations of nutrients or light, a slight increase in water temperature can increase photosynthetic rates in seagrasses not growing near their upper limits of thermal tolerance (Campbell et al. 2006), which could lead to an increase in plant grow. In addition, several studies have highlighted the effect of temperature in the seagrass metabolism and in the maintenance of a positive carbon balance (Evans et al. 1986, Bulthuis 1987, Zimmerman et al. 1989). However, temperature increase affects not only the plant, but all the other components of the community. Thus, although temperature can increase photosynthetic rates in seagrasses, the heterotrophic community inhabiting the meadow will also increase their respiration rate. The metabolic theory of Ecology (MTE) predicts respiration rates to increase faster with warming than does primary production (Brown et al. 2004, Harris et al. 2006, Sarmento et al. 2010), which results in a reduction of the P:R ratio and a greater likelihood of shifting the system to heterotrophy. On the other hand, recent studies have shown how the net DOC flux in seagrass communities was significantly correlated with seasonal changes in water temperature (Barrón & Duarte 2009, Barrón et al. 2014).

Therefore, this study presents the hypothesis that under temperature increase, there will be a decrease in the seagrass community production as a consequence of a faster increase in respiration rates. It is also expected an increase in DOC release, probably as a consequence of an increase in metabolic processes.

Extreme climatological events

The frequency and intensity of extreme climatological events (e.g. heat waves, flooding, cyclones and hurricanes) are expected to increase as a consequence of climate change (Easterling 2000, Duarte et al. 2006, IPCC 2007) and then, their effects on seagrass beds may be higher, causing great impacts and mortality. Understanding how ecosystems respond to extreme climatic events is necessary to predict how ecosystems and biodiversity will respond to global change (Jentsch & Beierkuhnlein 2008). In particular, understanding the response of communities dominated by foundational plant species (i.e. seagrasses) to extreme climatic events is essential, as this will largely shape the ecological response at an ecosystem scale (Royer et al. 2011).

Heat waves

Temperature change in climate change research is generally concerned with the variation in ecosystems structure and functions associated with gradually increasing mean temperatures (Parmesan & Yohe 2003). However, extreme climatic events such as heat waves will also govern the response of ecosystems to climate change (Hegerl et al. 2011). There is a lack of a rigorous definition of a heat wave, which usually is defined as a period of abnormal increase of temperature and humidity (IPCC 2012). Typically, a heat wave lasts few days, and the increase in air temperature usually translates into an increase in 2–4 °C sea surface temperature (e.g. see Marbà & Duarte 2010). In spite of this sudden temperature increase, its effects on seagrass stands may drive more impacts than a gradual warming (Marbà & Duarte 2010). To date, most studies about heat waves have focused on terrestrial ecosystems. However, marine ecosystems also exhibit extreme ecological responses to these events. For instance, studies on coral reefs have reported widespread mortality following heat waves (Hoegh-Guldberg 1999, Baker et al. 2008). In seagrasses, most of the knowledge is based on monitoring programs, which correlated seagrass shoot mortality with previous heat waves events in summer (Marbà & Duarte 2010, Fraser et al. 2014, Thomson et al. 2015, Bergmann et al. 2010). However, no heat wave experiments *in situ* have been carried out to study the effect of a sudden and temporary temperature increase in the seagrass community, especially its effects in the C metabolism and DOC fluxes.

Therefore, this study presents the hypothesis that heat waves will decrease seagrass production and DOC release as a consequence of seagrass damage.

Increase of storm events

Global change processes are expected to increase the frequency and intensity of storms and wave stress (Bernstein et al. 2007, Young et al. 2011). As a consequence, an increase in the intensity and frequency of high current velocities derived from storms, tropical cyclones and hurricanes may be expected. In addition, hydrodynamic conditions in coastal areas worldwide may change in consequence of anthropogenic engineering activities that change tidal flows and may yield high current velocity in particular systems (Kennish 2013). The effects of an increase in the water current velocity along with interaction with other environmental factors can be decisive for seagrasses. For example, as it is noted previously, the effects of higher CO_2 concentrations on seagrasses depend on the degree of carbon limitation in natural systems, which has been partly attributed to the thickness of the DBL surrounding leaf surfaces at low current velocities (Larkum 1989, Madsen & Sand-Jensen 1991, Koch 1994). The faster the water moves, the thinner the DBL becomes (Massel 1999) and, consequently, the faster the transfer of CO_2 molecules from the water column to the seagrass cells. Thus, a higher current velocity leads to a reduction of the DBL, and this favours the CO_2 uptake and photosynthesis (Koch et al. 2006), as has been already demonstrated for nutrient uptake (e.g. ammonium; Thomas & Cornelisen 2000, Morris et al. 2008). However, increasing flow velocity may reduce photosynthetic rates due to enhanced sediment resuspension (i.e. decreasing light levels; Koch 2001) and by leaves self-shading, as leaves collapse onto each other when currents are strong (Koch et al. 2006). Hence, current velocity conditions can produce a positive or a negative effect on productivity, depending on what effect will have more relevance: either a possible increase in CO_2 uptake as a result of the DBL reduction, or alternatively, a decrease in photosynthesis as a consequence of leaf self-shading or sediment resuspension. On the other hand, if hydrodynamic affects photosynthesis, and thus productivity (Koch et al. 2006), changes in current velocities may also produce an effect on DOC fluxes released by seagrass populations, which will affect the C fluxes in coastal communities.

Therefore, this study will test the hypothesis that under high current velocity conditions, seagrass production and DOC release can increase or decrease, a question still open and unresolved.

What happens next? The role of natural recovery

The slow natural recovery of seagrass beds (Reynolds et al. 2013) after extreme climatological events has been very little studied (e.g. Lee et al. 2007, Kim et al. 2015), despite the fact that most of the seagrass beds found in tropical areas are regularly exposed to flooding cyclones and tsunamis. These events impact negatively on seagrasses, as for example, via burial, scouring and direct removal of plants and seed banks (Preen & Marsh 1995, Bach et al. 1998, Campbell & McKenzie 2004). Recovery of seagrass meadows from large–scale disturbance has been shown to take from 2 to 4 years (Preen et al. 1995), or even more than 5 years (Birch & Birch 1984, Onuf 2000, Black & Ball 2001). Studies on seagrass colonization (e.g. Barrón et al. 2004, van Katwijk et al. 2016) and seagrass recovery show that relatively young seagrass meadows have different structural characteristics, faunal composition and sediment stabilization pattern than that of mature ones, which may affect the productivity and DOC fluxes in the community. Little attention has been given to the differences in carbon metabolism and DOC fluxes between different ages of seagrass stands after extreme climatological events, even when their implications may be critical for the overall ecosystem recovery.

Summary of the effect of anthropogenic pressures on the seagrass carbon metabolism and DOC fluxes and the hypothesis tested in this work

	Effect	**Hypothesis for seagrass C metabolism**	**Hypothesis for seagrass DOC release**
Nutrient enrichment	Macroalgae competition Change in community associated Ammonium toxicity	Decrease in seagrass production due to seagrass damage	Decrease due to seagrass damage
Ocean acidification	Can benefit the seagrass primary productivity if they are CO_2 limited.	Increase in seagrass production	Increase due to higher carbon capture
Temperature	Can increase photosynthetic rates in seagrasses Community respiration increase	Decrease in seagrass production because of faster respiration rates	Increase due to increase in metabolic processes
Heat waves	Shoot mortality and dieback	Decrease in seagrass production as a consequence of seagrass damage	Decrease as a consequence of seagrass damage
Higher current velocity	Remain poorly understood	Possible increase of CO_2 uptake derived from thinner DBL. Possible decrease in photosynthesis due to leaf self-shading	Possible increase or decrease depend on effect in C metabolism

Objectives and outline of the Thesis

Natural ecosystems are being increasingly threatened by the growth of human population, which is increasingly concentrated on the coast. Among them, coastal ecosystems, where a high proportion of planet's biodiversity converges, play a key role in the global cycles and will be one of the most vulnerable ecosystems in the next decades. However, the role of coastal ecosystems in the carbon cycle is still an issue that needs to be studied in depth, especially in those highly productive and with high capability for carbon captured ability as seagrass meadows. Therefore, the evaluation of the carbon captured and its fate in seagrass dominated ecosystems is necessary to understand the productivity and carbon cycle in these areas, which is essential not only for an effective management but also to forecast the effects of the main global threats at the Earth scale. On the other hand, the effects of environmental factors and stressors derived from human–induced activities, and their effects on the C metabolism and DOC released by seagrasses is still poorly understood, in spite that seagrass meadows are one of the most vulnerable ecosystems in the face of anthropogenic impacts.

The main hypothesis of this Thesis is that seagrass carbon metabolism and DOC fluxes can be substantially altered by global human–induced disturbances, and that the trajectory of such changes are not unique but depends on the complex interactions among stressor factors. The overall objective of this Thesis was then to evaluate the carbon metabolism and DOC fluxes in seagrass communities and elucidate the effects of human–induced disturbances on the carbon dynamics of coastal communities with special emphasis in seagrass dominated ones. In addition, this study assessed the contribution of the seagrass production to offset the CO_2 increase under future scenarios of climate change. Thus, three specific objectives were outlined in this work:

- To evaluate the carbon metabolism and DOC fluxes in benthic coastal communities dominated by seagrass species.
- To assess the effects of main global stressors in the carbon dynamics of these communities.
- To estimate the contribution of the seagrass production to partially offset the CO_2 increase under future scenarios of climate change.

To address these goals, this Thesis combines different experimental approaches, starting from an *in situ* approach where the carbon metabolism and DOC fluxes of the whole community were measured, and then shifting to *in situ* manipulative and mesocosm experiments, where the influence of human–induced disturbances in the C metabolism and DOC fluxes were evaluated.

The first part of the Thesis comprises the assessment of the carbon metabolism and DOC fluxes in benthic coastal communities. Thus, carbon metabolism and DOC fluxes in benthic communities (i.e. *Cymodocea nodosa*, the rhizophytic green algae *Caulerpa prolifera* and bare, unvegetated sediments) and in the pelagic plankton community were studied *in situ* in the inner Cádiz Bay (Spain) during an annual cycle (**Chapter 1**). The carbon metabolism and DOC fluxes in the tropical seagrass *Halodule wrightii* and unvegetated sediments were also evaluated *in situ* in Balandra Bay (Mexico) (**Chapter 2**). In addition, differences in the C metabolism and DOC fluxes among seagrass patches with different colonization status after a natural recovery process following an extreme climatological event were studied.

The second part of the Thesis involves four experiments to address the influence of human–induced disturbances and stressors on seagrass communities. Thus, the effects of nutrient enrichment during three months on the C metabolism and DOC fluxes were evaluated *in situ* in a community dominated by the seagrass *C. nodosa* in two contrasting seasons (winter and summer) (**Chapter 3**). The effect of heat waves on the C metabolism and DOC fluxes was assayed *in situ* in a community dominated by the seagrass *C. nodosa* in two contrasting seasons (winter and summer) (**Chapter 4**). The effect of ocean acidification under different hydrodynamic conditions on the carbon metabolism and DOC fluxes in the seagrass *Zostera noltei* was assessed using an open–water outdoor mesocosm system during four weeks (**Chapter 5**). Finally, the Thesis attempted to elucidate the influence of three key factors of global change (warming, CO_2 increase and ammonium enrichment) on the productivity of the temperate seagrass *C. nodosa* under a full factorial mesocosm experiment (**Chapter 6**).

The Thesis presents as main novelties the study of carbon metabolism and DOC fluxes at the same time (Chapters 1 and 2) and how they change with the changes in environmental factors studies (Chapters 3 – 6). In addition, these environmental factors or stressors were studied *in situ* (Chapters 3 and 4), that allows a more realistic approximation of how human-induced disturbances can alter the carbon dynamics of the community. This Thesis also underlines the importance of studying environmental factors that interact in nature using a multifactorial approach. As seagrass meadows are influenced by several environmental factors simultaneously, this approach yields a more realistic approximation of the possible effects of global change and anthropogenic impacts on seagrass ecosystems (Chapters 5 and 6).

The third part of this Thesis focuses on the role of vegetated coastal ecosystems in the carbon coastal cycle, and therefore how seagrass productivity may partially offset the expected CO_2 increase as a consequence of climate change. In addition, the current and future role of seagrasses in the ocean C sink capacity is also discussed.

SPECIES STUDIED

Cymodocea nodosa (Ucria) Ascherson

This is a common seagrass species throughout the Mediterranean Sea, and extends into the Atlantic Ocean north to mid-Portugal, and south to Madeira, the Canary Islands and Cape Verde, as well as to Mauritania and Senegal on the coasts of Africa.

Commonly occurs in shallow waters (from a few cm to a depth of 2.5 m) but can reach a depth of 30-40 m, usually growing in sandy substrate and sheltered areas. Occasionally it is accompanied by the macroalgae *Caulerpa prolifera* in muddy sediments. *C. nodosa* forms unispecific meadows in the Mediterranean Bioregion (Short et al. 2007) but also thrives in mixed stands with *Posidonia oceanica* (it is out-competed by this species) or *Zostera noltei*. The meadows of *C. nodosa* are currently threatened by the coastal development. Locally can be strongly affected by mechanical damage from trawling and anchoring from boats. Eutrophication is also a serious threat. The population is thought to be currently stable and listed as Least Concern according to the IUCN Red List.

Cymodocea nodosa from Cádiz bay (Spain)

Zostera noltei Hornemann

Commonly named dwarf eelgrass, occurs in the eastern Atlantic as well as the Baltic, Mediterranean, Black, Caspian and Aral Seas. It also occurs in western Africa in Mauritania, the Canary Islands and Cape Verde.

Z. noltei is a fast growing species. It is a small seagrass that thrive in intertidal and subtidal areas (den Hartog 1970). This species can occur in areas of low salinity and co-occurs with *Ruppia* spp. at the inner edges (0.5-1.5 m depth) of *Z. marina* beds (Green & Short 2003). There have been local declines in some regions due to water turbidity and coastal development. It is sensitive to eutrophication (Short & Burdick 1995) and is highly affected by shading (Van Lent et al. 1991). However the declines are not significant enough to include the species in any of the threatened categories of the IUCN Red List, which listed *Z. noltei* as Least Concern. However, this species is considered as especially protected according to the Andalucian legislation (Decree 23/2012).

Zostera noltei from Cádiz bay (Spain)

Halodule wrightii **Ascherson**

Commonly known as shoalgrass, is found in the eastern tropical Pacific from the Gulf of California to the Gulf of Panama. In the Indian Ocean it is found from the northern extent of the Bay of Bengal to along the Coromandel Coast as well as Oman. It is also found from southern Somalia to the north part of South Africa, including the Mozambique Channel, Mauritius and Madagascar.

H. wrightii is typically found on sandy to muddy bottoms and can be found in mixed beds. It is highly tolerant to a range of environmental conditions, including wide ranging salinity, high temperatures, turbidity, and eutrophication (Green & Short 2003, Larkum et al. 2006). The overall population trend for this species is stable, and possibly increasing in some parts of its range. Although is highly tolerant to a range of environmental conditions, it is affected locally by coastal development and destructive anthropogenic activities. This species is listed as Least Concern according to the IUCN Red List.

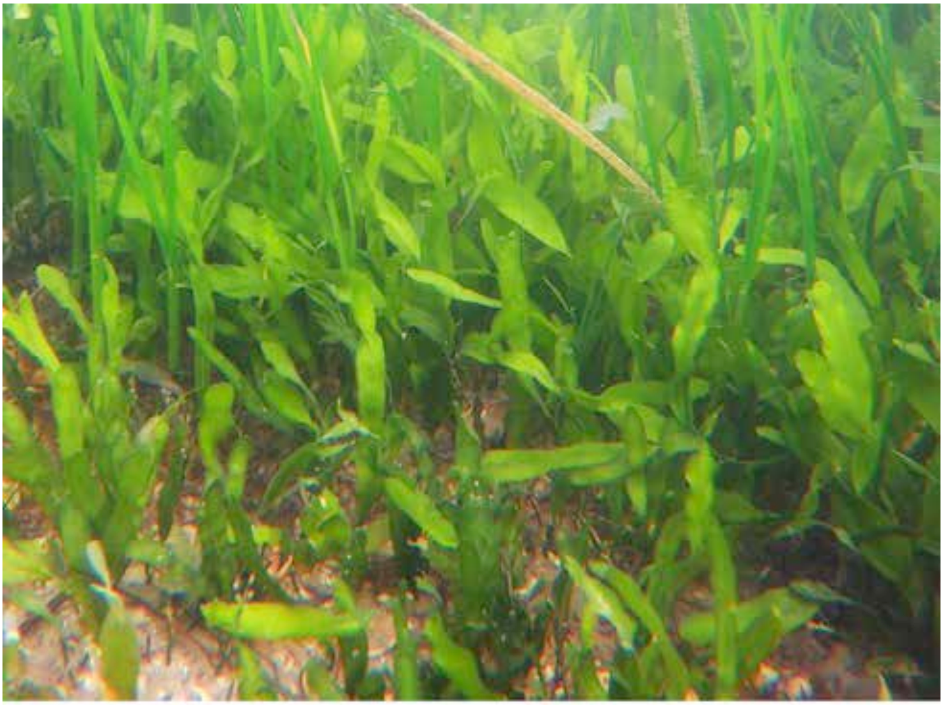
Halodule wrightii from Caleta Balandra (BCS, México)

Caulerpa prolifera (Forsskål) J.V. Lamouroux

This macroalgal species is a chlorophyta that can be found in shallow European waters, the Mediterranean Sea and the warm eastern Atlantic Ocean and also in the eastern seaboard of the United States, Mexico and Brazil, as well as certain other scattered locations.

Commonly, it helps to consolidate the seabed and enable seagrasses to colonize the area. However, under conditions of stress by temperature, an unusual sexual reproduction by holocarpy can be experienced (van Tussenbroek et al. 2005). It is highly tolerant to a range of environmental conditions. This species is not evaluated in the IUCN Red List.

General information from: Short FT, Carruthers TJR, van Tussenbroek B & Zieman J (2010) The IUCN Red List of Threatened Species and AlgaeBase Retrieved August 18, 2011.



Caulerpa prolifera from Cádiz bay (Spain)

CHAPTER 1

Coupling carbon metabolism and dissolved organic carbon fluxes in benthic and pelagic coastal communities

Submitted to Ecosystems

Recorres el mundo en busca de una felicidad que está siempre al alcance de la mano.

Horacio

Coupling carbon metabolism and dissolved organic carbon fluxes in benthic and pelagic coastal communities

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ABSTRACT

The carbon metabolism and net dissolved organic carbon (DOC) fluxes were estimated for benthic communities dominated by the seagrass *Cymodocea nodosa*, the rhizophytic green algae *Caulerpa prolifera* and unvegetated sediments, and the pelagic plankton community of inner Cádiz Bay, southern Spain, at bimonthly intervals during an annual cycle from summer 2012 to autumn 2013. Vegetated benthic communities were highly autotrophic at the annual scale with a mean net community production (NCP) of 29.6 mol C m^{-2} y^{-1} for *C. nodosa* and 13.9 mol C m^{-2} y^{-1} for *C. prolifera*. However, the carbon metabolism of *C. prolifera* shifted seasonally between autotrophic and heterotrophic. Unvegetated benthic communities were slightly heterotrophic throughout the year (-8.8 mol C m^{-2} y^{-1}), while the plankton community was always net autotrophic (6.2 mol C m^{-2} y^{-1}). Regarding DOC, benthic communities were net DOC producers undergoing a marked seasonality with maximum net DOC production during the summer. *Cymodocea nodosa* was the only community with a positive correlation between NCP and DOC. The plankton community was the only net DOC consumer indicating a strong coupling between the benthic and pelagic compartments in the bay. The NCP estimated for the whole benthic community of Cádiz Bay was 8,300 Tons C y^{-1}, and the net output of net DOC flux was 2,355 Tons C y^{-1}. Finally, this study indicates how changes in the cover of adjacent benthic communities belonging to the same shallow coastal area can prompt great changes to the C metabolism and net DOC fluxes of the whole system.

INTRODUCTION

Coastal vegetated habitats, including seagrass meadows, macroalgae, mangroves and salt marshes are among the most productive ecosystems on Earth and play an important role in the global carbon cycle in spite of the limited surface area they occupy on a global scale (<2% of the ocean surface) (Duarte et al. 2005, Laffoley & Grimsditch 2009, Nellemann et al. 2009, Mcleod et al. 2011). They are well recognised due to the large number of ecological services they provide as a consequence of their high productivity (Costanza et al. 1997), such as nutrient regeneration, water quality improvement, shoreline protection, creation of suitable breeding habitats (including those for species of economic relevance), biodiversity and substantial C burial (46.9 % of the total carbon buried by marine ecosystems, i.e. *blue carbon*) (Duffy 2006, Nellemann et al. 2009, González-Ortiz et al. 2014). Seagrasses are marine founder species that form one of the richest and most important coastal vegetated habitats (Short et al. 2011), which is being considered in recent years as a key ecosystem to cope with global change (Nellemann et al. 2009, Kennedy et al. 2010) because of their high productivity and the proportion of C captured that is then sequestered for millennia (Mateo et al. 1997, Macreadie et al. 2012, Serrano et al. 2012). The global estimate of seagrass net community production (NCP) is between 21–101 TgC y^{-1} (Duarte et al. 2010), which represents up to 1% of the total annual anthropogenic CO_2 emission estimated in ca. 10,000 Tg C y^{-1} (IPCC 2014). Vegetated coastal habitats are among the most threatened worldwide (Nellemann et al. 2009) with currently increasing rates of loss in many habitats, such as seagrass meadows (Waycott et al. 2009). This growing loss in vegetated coastal habitats is draining not only their sequestering potential, but also may result in the release of carbon already buried in their sediments back to the atmosphere (Mcleod et al. 2011). Therefore, studying the C capture and the transfer of energy among all components shaping the food web in the community will lead to a better understanding of the productivity and carbon cycle in these ecosystems, which is necessary for an effective management (Polis et al. 1997, Barbier et al. 2011).

The net community production (NCP) of vegetated coastal areas can be used as a proxy of the trophic state of the system (i.e. autotrophic *vs* heterotrophic). Primary productivity and carbon cycle in seagrass communities have usually been measured using the “punching method” (Zieman 1974, Peralta et al. 2000). This technique generally estimates the aboveground production, neglecting or underestimating the C fixed in belowground tissues, which could be even greater than that of the aboveground component (Brun et al. 2003). In addition, productivity in macroalgal communities is usually approached by calculating changes in thallus surface or weight (Lobban & Harrison 1985).

These methods, however, do not accurately yield the total community production because they do not consider other components (both autotrophic and heterotrophic), whose contributions can be occasionally even greater than the macrophyte production itself (Kaldy et al. 2002). Thus, recent studies on C fluxes in macrophyte-dominated communities use a more realistic approach by measuring *in situ* changes in dissolved oxygen (DO) at the whole community level, which includes all the biological components using Winkler method (e.g. Ziegler & Benner 1998, Barrón & Duarte 2006) or methods based on continuous O_2 measurements (e.g. Champenois & Borges 2012).

To predict how the ocean will respond to global change, and whether coastal ecosystems will be able to absorb the forecasted additional carbon dioxide released to the atmosphere, it is necessary to understand the spatiotemporal patterns and the fate of organic material with high carbon content in the ocean (Dafner & Wangersky 2002, Samper-Villarreal et al. 2016). Marine macrophyte communities often produce an excess of organic matter that can be either stored in sediments or exported to adjacent ecosystems as particulate or dissolved forms (Duarte & Cebrián 1996, Barrón & Duarte 2009). While the origin and fate of particulate organic matter have been widely studied (Hemminga et al. 1994, Kennedy et al. 2004, Williams et al. 2009, Magen et al. 2010), the role of the dissolved organic carbon (DOC) has only been recently highlighted (Mateo et al. 2006, Barrón et al. 2009, Van Engeland et al. 2013, Barrón et al. 2014). Although the DOC pool represents only 2% of oceanic carbon (about 700 PgC), it is the most abundant reservoir of exchangeable organic carbon in the marine carbon pool, which is a cornerstone in the global carbon cycle (Hedges et al. 1997, Hansell & Carlson 2001). Most of the oceanic DOC originates from biological production by marine organisms and a large fraction of this DOC is later remineralized by microorganisms on time scales ranging from hours to days. Therefore, DOC is an important driver of primary production in other compartments of the food web, as it was suggested decades ago (Jørgensen 1976). While there has been extensive research on DOC dynamics in the open ocean (reviewed by Hansell & Carlson 2001), the role of coastal ecosystems in the global DOC cycle is still inadequately understood, even though these habitats tend to accumulate huge amounts of DOC (Dafner & Wangersky 2002). To date, few studies have examined *in situ* DOC release by marine macrophyte communities and subsequent exchange with adjacent communities. The most common approach has been based on isolated plants incubations (Brylinsky 1977, Wetzel & Penhale 1979, Moriarty et al. 1986, Haas et al. 2010, Kaldy 2012). However, this procedure underestimates the magnitude of the community net release of DOC because it neglects the amount processed by other community compartments, such as epiphytes, the leaching of DOC from decomposing material in the sediments and the consumption by bacteria.

The current understanding of changes in C metabolism and net DOC fluxes in coastal ecosystems is mostly constrained by the limited attention given to *in situ* measurements; therefore, this study was based on *in situ* changes in dissolved oxygen and DOC using benthic and pelagic chambers. We evaluated the annual C metabolism and net DOC fluxes in three subtidal benthic communities inhabiting a shallow bay in southern Spain: communities dominated by the macrophytes *Cymodocea nodosa* (seagrass) and *Caulerpa prolifera* (rhizophyte), and unvegetated sediments. In addition, the annual C metabolism and net DOC flux of the plankton community were evaluated. Throughout bimonthly incubations we were able to assess the contribution of the main compartments of shallow coastal areas to the C balance of the system and net DOC export to the open ocean, which are crucial to understanding the effects of global change on this ecosystem.

MATERIAL AND METHODS

Sampling site. The study was carried out in Cádiz Bay, southern Spain, which is a shallow macrotidal and sheltered embayment of approximately 12,000 ha. The bay is subdivided into a deeper outer bay directly connected to the open sea and a shallow inner bay (3,545 ha), which receives water from the outer bay, being both basins connected through a strait (Fig. 1). Climatically it fits into a semi-warm subtropical thermal regime whose normal temperature range oscillates between 11 to 28 ºC and 593.4 mm as average annual precipitation. There is very little fresh water input into the system so the average salinity ranges between 34.1 to 35.6 and the average solids in suspension varies from 10 to 30 mg/l (more information in Muñoz & Sánchez 1994). The sea bottom of the inner bay is dominated by three benthic marine macrophytes: the seagrasses *Zostera noltei* Hornem. and *Cymodocea nodosa* Ucria (Ascherson), and the rhizophytic green algae *Caulerpa prolifera* (Forsskål) J. V. Lamouroux. Within the macrophyte meadows there are some scattered unvegetated areas. The study was conducted in the inner bay, near the site called Santibañez (36° 28’ 12.79” N, 6° 15’ 7.07” W), in *C. nodosa* and *C. prolifera*-dominated communities growing at a mean depth of 1.5 m as well as in unvegetated sediments (Brun et al. 2015).

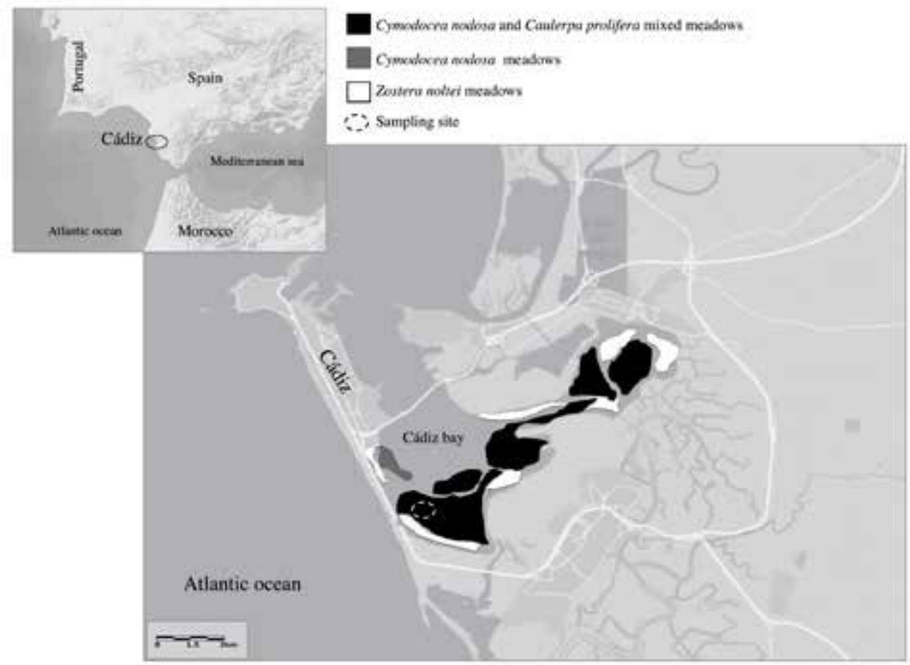

Figure 1. Study site at Cádiz Bay, Spain.

Field incubations. Community carbon metabolism and net DOC flux were assessed seasonally in subtidal benthic communities (from July 2012 to October 2013). Bimonthly samplings were carried out using *in situ* benthic chambers or "incubations", which were placed by scuba diving. It should be noted that although each community is dominated either by macrophytes (*Cymodocea nodosa* or *Caulerpa prolifera*) or unvegetated sediment, it is actually an assemblage of several biological components, such as plankton, epiphytes, macroalgae, infauna and sediment biofilms (i.e. microphytobentos, bacteria, etc). Therefore, the results in this study integrate the entire community and its changes throughout the whole year. Replicated incubations were conducted randomly in patches dominated by *C. nodosa* and *C. prolifera* (three replicates per community), as well as in adjacent unvegetated areas (n=3). Incubations consisted of a rigid cylinder made of a polyvinyl chloride (20 cm diameter) with a sharpened lower end firmly inserted 7–10 cm into the sediment, and an air-tight polyethylene plastic bag fitted over the upper end of the cylinder (Hansen et al. 2000). Each bag was provided with a sampling port to withdraw water samples. The walls of the bags were flexible allowing its movement with the hydrodynamics and preventing water stagnation. Incubations were placed in the evening just a few hours before nightfall. To avoid collecting resuspended material resulting from physical disturbance during installation of the chambers, the first sample was taken 2h after setting up them. To measure community carbon metabolism (through DO concentration) and net DOC flux, water enclosed within each incubation was taken through the sampling port using a 50 ml acid-washed syringe (standard plastic previously subjected to blank control) at three times during the day: i) just before sunset (S1), ii) right after sunrise (S2) and iii) 6 h after sunrise (S3). In this way, community carbon metabolism and net DOC flux in dark and light periods can be distinguished (Barrón and Duarte 2009). To calculate the real water volume of each incubation, 20 ml of a 0.1 M uranine solution (sodium fluorescein, $C_{20}H_{10}Na_2O_5$) was injected into each incubation bag at the end of the experiment, allowing 5 min for mixing. Thereafter, water samples were collected and kept frozen until spectrophotometric determination according to Morris et al. (2013). The mean volume of water enclosed in the bags was 9.13 ± 0.17 l (n=72). Once the bag was removed, macrophyte biomass inside the chamber was harvested, rinsed and dried at 60 ºC to estimate aboveground and belowground biomass. Alongside benthic incubations, plankton community carbon metabolism and net DOC flux were also studied *in situ* by incubating 12 Winkler bottles (0.5 l each): 6 wrapped with black bags for dark incubations and 6 without wrapping for light incubations. Winkler bottles were placed during sunrise and left for 24 hours tied to a buoy near the surface, so that they dipped one meter into the water almost to the depth of the canopy of the meadows. Incubations were conducted every two months from summer 2012 to autumn 2013. Temperature and light were continuously monitored with three SQ-212 Quantum Sensors set on bare sediment close to the experimental plots that registered PAR (photosynthetic active radiation) values during the incubation periods. To better compare results and reduce the environmental variability, sampling days were chosen to be representative of the analysed season and with a similar tidal range as well as other environmental conditions (e.g. cloud cover, rain, wind, etc.).

Laboratory analysis. Water samples (15 ml) for DO concentration were fixed *in situ* immediately after collection in the supporting vessel, kept in darkness, refrigerated, and determined using a spectrophotometric modification of the Winkler titration method (Pai et al. 1993, Roland et al. 1999). In benthic chamber incubations, hourly rates of community respiration (CR^h) were estimated as the difference in DO concentrations between samplings S2 and S1 ($R^h = DO^{S2} - DO^{S1}$). Hourly rates of net community production (NCP^h) were estimated from the difference in DO concentrations between samplings S3 and S2 ($NCP^h = DO^{S3} - DO^{S2}$). Hourly rates of gross primary production (GPP^h) were computed as the sum of the hourly rates of R and NCP ($GPP^h = CR^h + NCP^h$). Finally, daily rates of gross primary production (GPP^d), community respiration (CR^d) and net community production (NCP^d) were estimated following the calculations (where photoperiod correspond to photoperiod in each sampling day):

$$GPP^d = GPP^h * \text{Photoperiod} \; ; \; CR^d = CR^h * 24h \; ; \; NCP^d = GPP^d - CR^d$$

Regarding the plankton community, NCP was calculated directly from Winkler incubation bottles without any wrapping, CR was calculated from Winkler bottles with black wrapping, and GPP was estimated as the sum of CR and NCP rates. In order to make comparable planktonic measurements (i.e. measured in a volume) with benthic ones (i.e. measured per surface), plankton metabolism was transformed to square meters by taking into account the mean depth in the area (1.5 meters), and therefore, the volume of water column equivalent to one square meter of sediment surface would be 1,500 L (1.5 m depth per 1 m^2). Metabolic rates in DO units were converted to carbon units assuming photosynthetic and respiratory quotients of 1, a value used widely for seagrasses (e.g. Barrón et al. 2004). Although these assumptions could introduce uncertainties into the estimates of CR, GPP and NCP, this should be a minor concern when the global metabolism of each community is assessed. Dissolved oxygen concentrations in benthic chambers were, on average, 11.8 mg O_2 l^{-1} (S1), 6.4 mg O_2 l^{-1} (S2) and 10.6 mg O_2 l^{-1} (S3). Thus, these changes in DO during the incubations seem relatively moderate, minimizing the propagation of errors when computing metabolic rates (e.g. converting DO to carbon units or assuming respiration at night-time is the same than at daytime) and artefacts due to physiological stress on the communities.

DOC fluxes were estimated by changes in DOC concentration during light and dark periods. Water samples (20 ml) from benthic chambers were filtered through pre-combusted (450 °C for 4 h) Whatman GF/F filters (0.7 µm) and were kept with 0.08 ml of H_3PO_4 (diluted 30%) at 4 ºC in acid-washed material (glass vials encapsulated with silicone-PTFE caps) until analyses. Concentrations of DOC were derived by catalytic oxidation at high temperature (720 °C) and chemiluminescence by using a Shimadzu TOC-VCPH analyzer. DOC-certified reference material (Low and Deep), provided by D. A. Hansell and W. Chen (University of Miami), of 41 to 45 of µmol DOC and 1 µmol DOC were used to assess the accuracy of the estimations (http://yyy.rsmas.miami.edu/groups/biogeochem/CRM.html). The instrument blank ranged between 0 and 17 µmol DOC l^{-1} across the different analytical batches. Net DOC flux were calculated by the sum of the DOC flux during the dark period (i.e. the differences between S2 and S1 DOC concentrations) and DOC flux during the light period (i.e. after extrapolating the differences between S3 and S2 DOC concentrations for all hours of light). Thus, when net DOC flux was positive, the community was considered to act as a net DOC producer (i.e. source). When net DOC flux was negative, however, the community was considered to act as a net DOC consumer (i.e. sink). DOC concentrations in benthic chambers were, on average, 1.9 mg l^{-1} (S1), 2.2 mg l^{-1} (S2) and 2.7 mg l^{-1} (S3). Thus, these changes in the DOC during the incubations seem relatively moderate, minimizing the propagation of errors on the computation of metabolic rates and artefacts due to physiological stress on the communities.

Data and statistical analysis. Annually NCP and net DOC production for the whole bay can be estimated integrating all daily rates of the eight samplings along the study period using the trapezoidal method, then scaling up the results and combining it with the global coverage of each community in the bay: 1,700 ha for *C. nodosa*; 1,553 ha for *C. prolifera* and 292 ha for unvegetated sediments (Muñoz & Sánchez 1994, Brun et al. 2015).

Prior to any statistical analysis, data were checked for normality (Shapiro-Wilk normality test) and homoscedasticity (Bartlett test of homogeneity of variance test). When necessary, data were transformed to comply with these assumptions. Differences in GPP among communities were analysed using 2-way ANOVAs. When significant differences were found, a Tukey post-hoc test was applied. Even after trying several transformations, the CR values did not meet the normality assumption and, therefore, CR differences among communities were analysed using the Kruskal-Wallis test with the Wilcoxon signed-rank test. The relationships between GPP and CR with temperature, light and biomass were analysed using Pearson correlations, whereas the relationship between GPP and CR and the relationship between DOC with NCP, GPP and CR were analysed using the Spearman correlation. Mean NCP and net DOC fluxes were calculated through a trapezoidal method. Even after several transformations, NCP and net DOC flux values did not meet the normality assumption; therefore, significant differences in NCP and net DOC fluxes among communities were analysed using the Kruskal-Wallis test with the Wilcoxon signed-rank test. Differences in specific periods for NCP between vegetated and plankton communities were analysed using a Wilcoxon test or Welch test when data were not homoscedastic. Data are presented as mean ± SE. The significance level (α) set in all tests performed was 0.05. Statistical analyses were computed with R statistical software 3.0.2 (R Development Core Team 2013).

RESULTS

Abiotic variables. Both water temperature and photon flux followed a seasonal pattern, with values similar to those recorded for this location (Pérez-Lloréns et al. 2004, Olivé et al. 2013). Average daily water temperature measured during the days of the incubations showed an unimodal response and ranged from 11.5 ± 0.15 ºC (winter) to 27.4 ± 0.05 ºC (summer). Average underwater photon flux during sunny hours ranged from 152.0 ± 11.9 µmol photons m^{-2} s^{-1} in winter to 529.9 ± 53.6 µmol photons m^{-2} s^{-1} in early summer (Table 1). The above- and belowground biomass of *C. nodosa* increased during the spring and peaked in summer, whereas that of *C. prolifera* reached its highest in spring. Both species showed the lowest biomass in winter (Table 1).

Table 1. Water temperature, photoperiod, mean photon flux density, and total (above- + belowground) biomass of *Cymodocea nodosa* and *Caulerpa prolifera*. Data represent mean ± SE (*n*=3).

Date		**Temperature (ºC)**	**Photoperiod (h)**	**Light (µmol photon m^{-2} s^{-1})**	***C. nodosa* biomass (kg FW m^{-2})**	***C. prolifera* biomass (kg FW m^{-2})**
2012	Aug	26.82 ± 0.03	14.17	212.8 ± 16.1	1.06 ± 0.28	0.51 ± 0.02
	Oct	25.01 ± 0.07	11.67	375.3 ± 33.4	1.03 ± 0.07	0.9 ± 0.08
	Dic	11.48 ± 0.15	9.82	152.0 ± 12.0	0.96 ± 0.35	0.85 ± 0.02
2013	Feb	15.31 ± 0.02	10.38	133.4 ± 18.9	0.49 ± 0.05	1.01 ± 0.19
	Apr	19.39 ± 0.02	13.73	224.1 ± 22.7	2.6 ± 0.3	3.92 ± 0.41
	Jun	25.61 ± 0.09	14.63	529.9 ± 53.6	3.05 ± 0.3	3.15 ± 0.09
	Aug	27.39 ± 0.05	14.17	316.9 ± 18.6	2.74 ± 0.44	1.7 ± 0.04
	Oct	19.40 ± 0.04	11.57	329.5 ± 19.4	1.55 ± 0.24	1.31 ± 0.19

Community metabolism. Overall, the GPP^h in vegetated communities (dominated either by *C. nodosa* or *C. prolifera*) was significantly higher (7- and 6-fold for *C. nodosa* and *C. prolifera*, respectively) than unvegetated ones ($p < 0.001$). In addition, respiration in vegetated bottoms was higher than in unvegetated ones ($p < 0.001$), although there were no significant differences between the two vegetated communities ($p = 0.19$) (Fig. 2). The GPP^h in the vegetated communities showed different temporal trends (Fig. 2A, 2B). The *C. nodosa* stands showed maximum GPP^h in late summer (i.e. summer-autumn, 22.20 ± 4.33 mmol C m^{-2} h^{-1}) and minimum in winter (i.e. February, 4.03 ± 1.44 mmol C m^{-2} h^{-1}). In contrast, *C. prolifera* stands had maximum GPP^h values in spring (i.e. April, 23.82 ± 2.11

mmol C m^{-2} h^{-1}) and minimum ones in autumn (i.e. October, 5.54 ± 1.12 mmol C m^{-2} h^{-1}). No correlation was found between GPP^h values with water temperature or photon flux for all the communities. Hourly rate of community respiration (CR^h) was neither correlated with photon flux nor biomass but was correlated with water temperature in all the communities (significantly correlated in vegetated communities). No correlation between GPP^h and biomass was found for the *C. nodosa*-dominated community, whereas a significant correlation was observed in the *C. prolifera*-dominated community (r=0.79) (Table 2).

Table 2. Pearson correlations between gross production rate (GPR: mmol C m^{-2} h^{-1}) and respiration (R: mmol C m^{-2} h^{-1}) with temperature, light and biomass for communities dominated by *Cymodocea nodosa*, *Caulerpa prolifera* and unvegetated sediments. *r* is the correlation coefficient. Asterisks show significance level $p < 0.05$.

	C. nodosa		***C. prolifera***		**Unvegetated**	
			GPP			
	r	*p*-value	*r*	*p*-value	*r*	*p*-value
Biomass	0.24	0.57	0.79	0.02*	NA	NA
Light	0.50	0.21	-0.10	0.82	0.33	0.43
Temperature	0.61	0.11	-0.32	0.44	0.01	0.98
			R			
	r	*p*-value	*r*	*p*-value	*r*	*p*-value
Biomass	0.29	0.48	-0.03	0.94	NA	NA
Light	0.52	0.18	0.51	0.20	0.46	0.26
Temperature	0.76	0.03*	0.89	0.01*	0.66	0.08

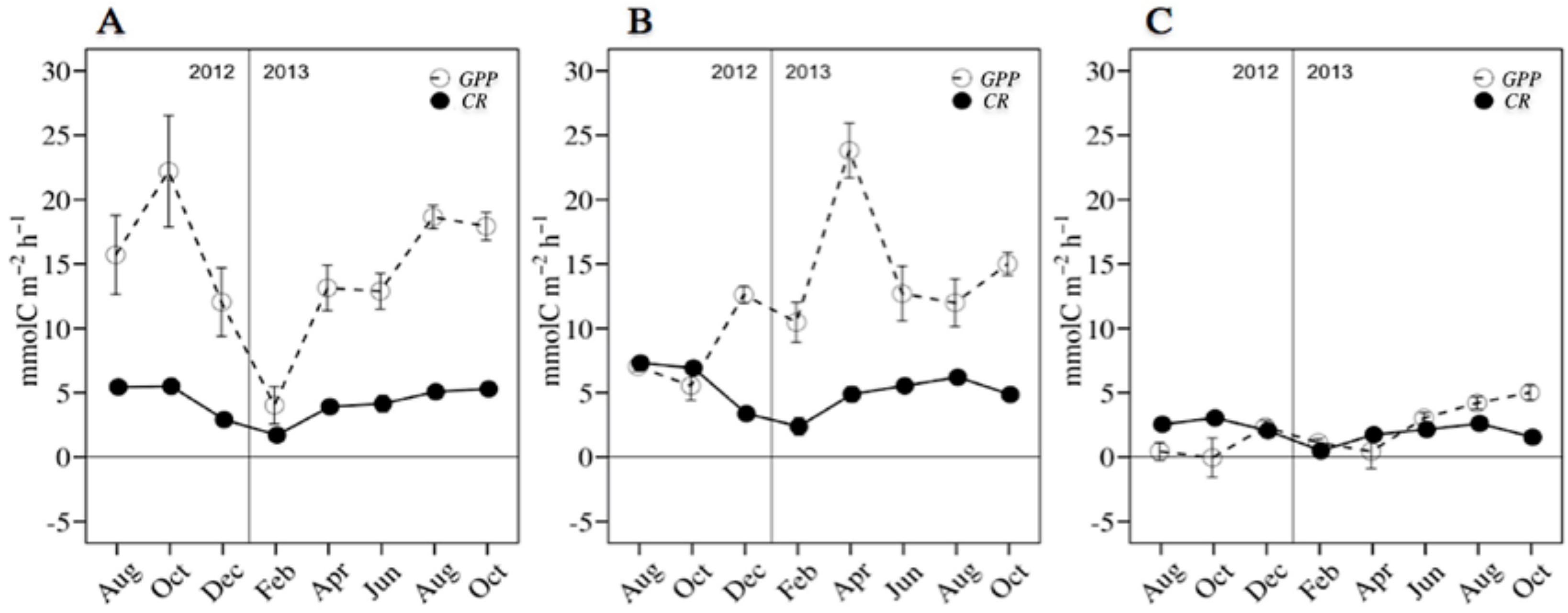

Figure 2. Seasonal variation in gross primary production (GPP) and community respiration (CR) in the three most representative benthic communities studied: A) dominated by *Cymodocea nodosa*, B) dominated by *Caulerpa prolifera* and C) unvegetated sediments.

NCP^d showed an unimodal pattern, peaking in late summer for *C. nodosa* (i.e. August, 148 ± 11 mmol C m^{-2} d^{-1}) and in spring for *C. prolifera* (i.e. April, 210 ± 16 mmol C m^{-2} d^{-1}), and always significantly exceeded that of the unvegetated one ($p < 0.05$) (Fig. 3). Moreover, the intensity and amplitude of the seasonal variations in NCP^d were much higher in the *C. prolifera*-dominated community than in the *C. nodosa* one, reaching negative values in some periods of the year. Seasonality was not observed either for the unvegetated community or the planktonic one. Observed over the whole year, vegetated bottoms tended to be net autotrophic (except *C. prolifera* in summer 2012), in contrast to the unvegetated bottoms, which were mostly net heterotrophic (Fig. 3). The planktonic community was autotrophic throughout the year, although its contribution compared with the NCP^d of each vegetated community was generally low (5% up to 23% of NCP^d of *C. nodosa* and 6% up to 11% of NCP^d of *C. prolifera*). However, in a few cases, planktonic NCP^d was significantly higher than that recorded in vegetated communities, such as in winter 2013 (*C. nodosa*) and summer 2012 and 2013 (*C. prolifera*).

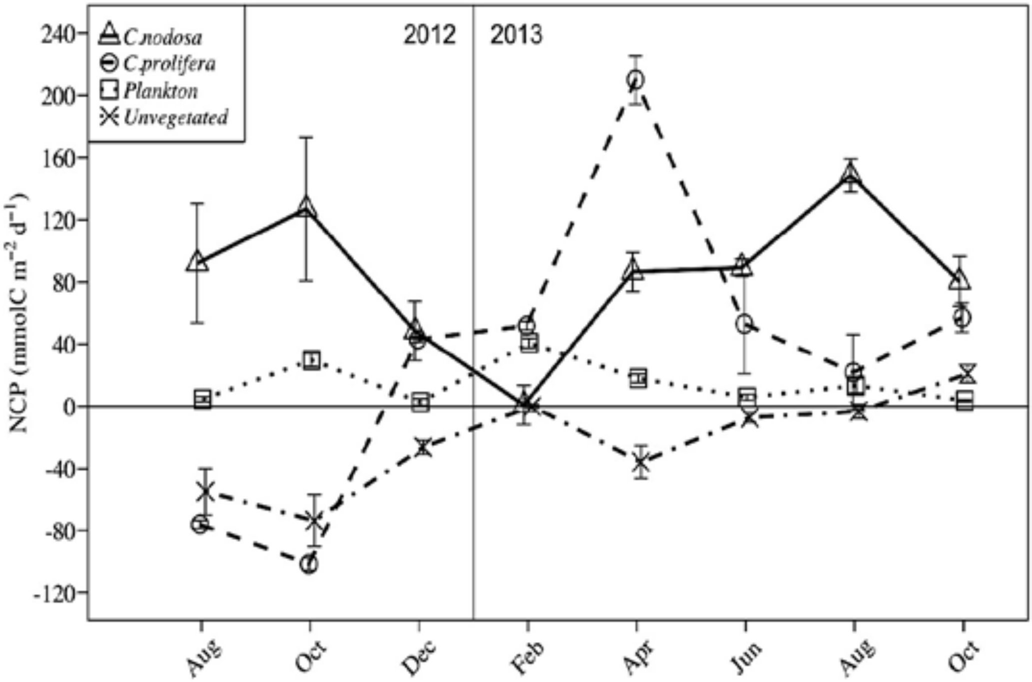

Figure 3. Seasonal variation in net community production (NCP) of the benthic communities dominated by *Cymodocea nodosa*, *Caulerpa prolifera* and unvegetated sediments, and the pelagic community dominated by plankton.

The gross production to respiration ratio (P:R) in vegetated communities was typically higher than 1, with the exception of *C. prolifera* in summer (Table 3). In the *C. nodosa* community, it ranged from 1.7 to 1.9 all year around, except in winter when it dropped to 1.0. In the *C. prolifer*a community, P:R ranged from 0.9 to 2.8, revealing the broadest range in C metabolism. The P:R ratio in the unvegetated community was generally lower than 1 throughout the year.

Table 3. Productivity:Respiration (P:R) ratio for communities dominated by *Cymodocea nodosa*, *Caulerpa prolifera* and unvegetated sediments (Mean ± SE). The data for summer and autumn months show the mean P:R ratio over the two consecutive years 2012 and 2013.

Date	***C. nodosa***	***C. prolifera***	**Unvegetated**
Aug	1.9 ± 0.16	0.9 ± 0.14	0.5 ± 0.20
Oct	1.8 ± 0.17	0.9 ± 0.25	0.8 ± 0.38
Dic	1.7 ± 0.20	1.5 ± 0.03	0.5 ± 0.07
Feb	1.0 ± 0.32	2.2 ± 0.47	1.1 ± 0.15
Apr	1.9 ± 0.09	2.8 ± 0.10	0 ± 0.57
Jun	1.9 ± 0.07	1.4 ± 0.26	0.9 ± 0.07
Mean	1.7 ± 0.09	1.4 ± 0.17	0.6 ± 0.14

DOC flux. Vegetated bottoms, especially those covered by *C. prolifera*, were largely net DOC producers compared with unvegetated ones. In contrast, the plankton community was generally a strong net DOC consumer, acting as a large sink for DOC (Fig. 4). Furthermore, net DOC flux followed a seasonal pattern in benthic communities, with higher values during summer and lower ones during winter. No significant differences were detected between the macrophyte communities (Fig. 4). No seasonality was recorded for the plankton community.

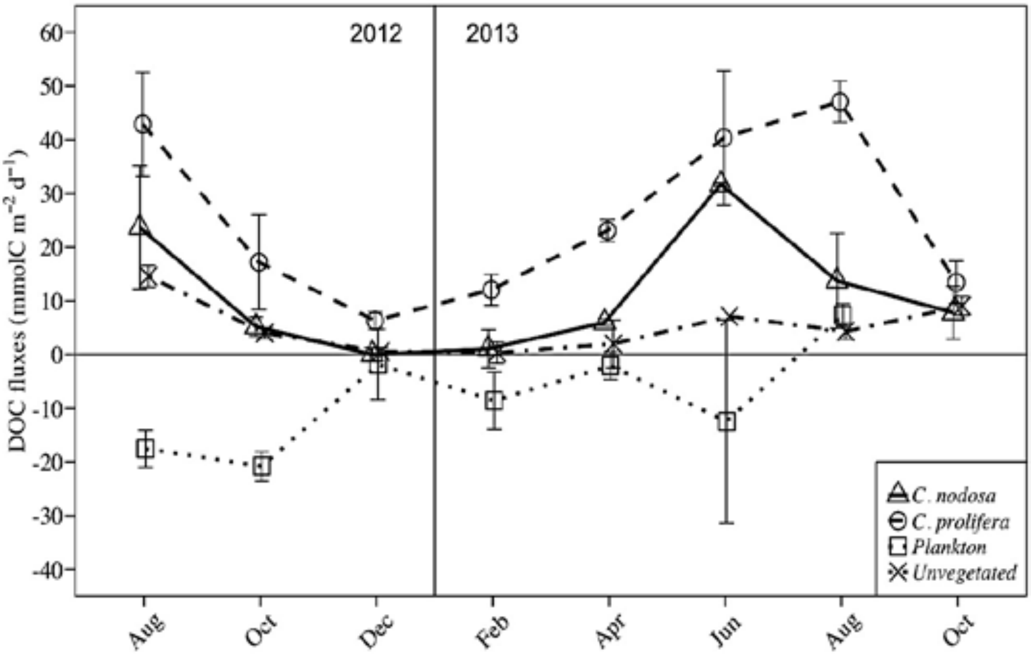

Figure 4. Seasonal variation in net dissolved organic carbon (DOC) flux of communities dominated by *Cymodocea nodosa*, *Caulerpa prolifera*, unvegetated sediments and plankton.

Plotting NCP^d *vs* DOC allows us to distinguish between the different metabolic trends in the benthic communities of Cádiz Bay (Fig. 5). Therefore, at an annual scale, the *C. nodosa*-dominated community was net autotrophic (29.6 ± 7.3 mol C m^{-2} y^{-1}) and a net DOC producer (3.3 ± 1.3 mol C m^{-2} y^{-1}), whereas the unvegetated community was heterotrophic (-8.8 ± 2.7 mol C m^{-2} y^{-1}) and a net DOC producer (1.5 ± 0.6 mol C m^{-2} y^{-1}). In contrast, the planktonic community was autotrophic (6.2 ± 1.0 mol C m^{-2} y^{-1}) and the only net DOC consumer (-3.3 ± 1.1 mol C m^{-2} y^{-1}). The *C. prolifera* community, however, shifted its metabolism throughout the year from net autotrophic or heterotrophic, although it was net autotrophic (13.9 ± 4.0 mol C m^{-2} y^{-1}) and a net DOC producer (8.8 ± 1.9 mol C m^{-2} y^{-1}) when averaged over an annual basis.

The relationship between net DOC flux and community productivity (NCP^d, GPP^d and R^d) changed depending on the community (Table 4). The *C. nodosa* community showed a significant positive correlation between DOC and the three parameters of community productivity (NCP^d, GPP^d and R^d), whereas the *C. prolifera* community only showed a significant positive correlation between DOC and R^d.

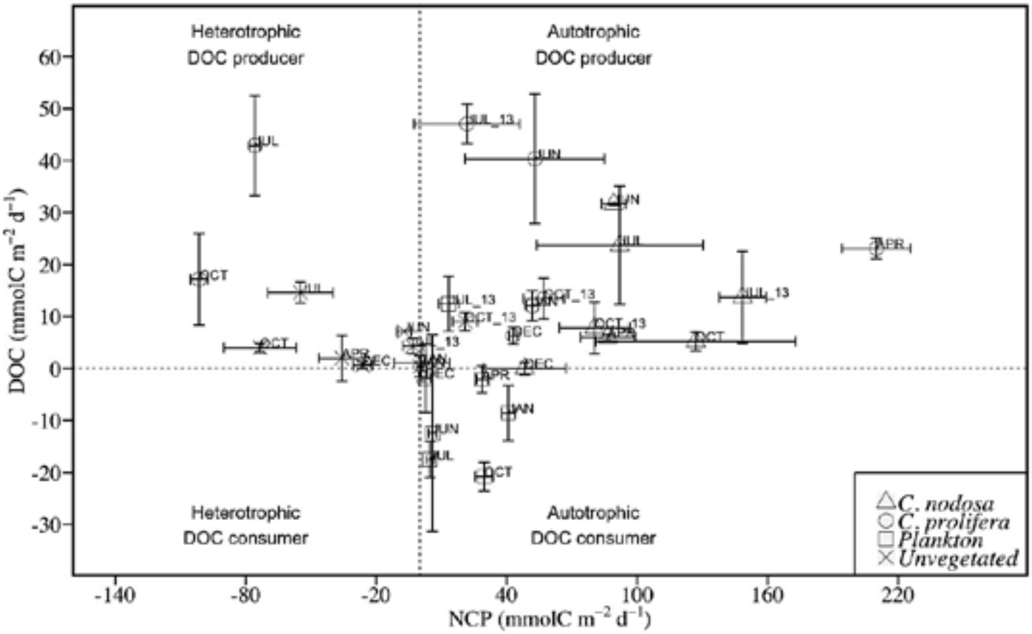

Figure 5. Relationship between dissolved organic carbon (DOC) and net community production (NCP) in communities dominated by *Cymodocea nodosa*, *Caulerpa prolifera*, unvegetated sediments and plankton. Data represent mean ±SE for each sampling event.

Table 4. Spearman correlations between dissolved organic carbon (DOC, mmolC m^{-2} d^{-1}) flux with net community production (NCP, mmolC m^{-2} d^{-1}), gross primary production (GPP, mmolC m^{-2} d^{-1}) and respiration (R, mmolC m^{-2} d^{-1}) for communities dominated by *Cymodocea nodosa, Caulerpa prolifera* and plankton, and unvegetated communities, annually. *r* is the correlation coefficient. Asterisks show significance level $p < 0.05$.

		NCP		GPP		R	
		r	*p*	*r*	*p*	*r*	*p*
DOC	*C. nodosa*	0.52	0.01*	0.48	0.02*	0.45	0.03*
	C. prolifera	-0.19	0.41	0.1	0.65	0.67	0.0007*
	Unvegetated	-0.2	0.37	-0.05	0.83	0.26	0.25
	Plankton	-0.36	0.11	0.14	0.55	-0.1	0.68

Scaling up to entire Cádiz Bay. On a yearly basis, the benthic communities inhabiting the bay reached a net NCP value of ca. 8,300 Tons C y^{-1} (6,000 Tons C y^{-1} for *C. nodosa*; 2,600 Tons C y^{-1} for *C. prolifera* and -300 Tons C y^{-1} for unvegetated sediments) and a net DOC production of ca. 2,355 Tons C y^{-1} (700 Tons C y^{-1} for *C. nodosa*; 1,600 Tons C y^{-1} for *C. prolifera* and 55 Tons C y^{-1} for bare sediments).

DISCUSSION

Autotrophic at the annual scale, the studied vegetated communities (*Cymodocea nodosa and Caulerpa prolifera*) contribute potentially to the net storage of C, in agreement with previous reports (e.g. Barrón & Duarte 2006, Duarte et al. 2010). In contrast, bare sediments were heterotrophic on a yearly basis, serving as a net source of C (Fig. 2 and 3). On an annual basis, the GPP values in the community dominated by *C. nodosa* (777 g C m^{-2} y^{-1}) were similar to those reported by Duarte et al. (2010) for the same species (986 g C m^{-2} y^{-1}). The net contribution of the two vegetated communities to the overall metabolism of Cádiz Bay is highly significant, as both of them showed P:R ratios higher than 1 (Table 3) and covered around 43% (*C. nodosa*) and 48% (*C. prolifera*) of the seabed in the bay (Muñoz & Sánchez 1994, Brun et al. 2015). The methodology used in this experiment has been widely utilized (e.g. Barrón & Duarte 2006) because it allows an effective approach of the *in situ* metabolic responses of the whole community. There are other less invasive methodologies available, such as eddy correlation method. However, the technical complexity as well as, the high sensor costs (Chipman et al. 2012), make difficult their use to evaluate several benthic communities at the same time with their replicates, as it was done in this work.

The vegetated communities showed high NCP^d values with both intra and inter-annual variability (especially in *C. prolifera* community), although a longer data-set should be necessary to determine the range of variability at both scales. Vegetated communities showed certain seasonality, but peaking at different seasons: summer (*C. nodosa*) and spring (*C. prolifera*). The marked seasonality was also accompanied by periods of low productivity in both vegetated communities. The summer minimum NCP^d values in the *C. prolifera* community, supported by the pattern found by Ruiz-Halpern et al. (2014) for this species in a Mediterranean bay, could be attributed to low GPP^d because there were no significant differences in R^d at the annual scale between the two macrophyte communities. In addition, the low summer biomass of *C. prolifera* found in this experiment matched those reported by Vergara et al. (2012) and could be linked to low GPP^d (Table 2) as a consequence of a decline in the effective photosynthetic quantum yield due to excess of underwater photon flux (Häder et al. 1997) as this specie seems to have preference for low or moderate irradiances (Malta et al. 2005). In addition the high temperature in summer can promoted the respiration in the full OM sediment of *C. prolifera*. The *C. nodosa* community had low productivity periods as well, having a nearly balanced C metabolism in winter, probably because its production is dependent on photoperiod (Peralta et al. 2002, Olivé et al. 2013). The amplitude in seasonal variation was much higher in the *C. prolifera*-dominated community, which showed shifts in its net metabolism throughout the year, ranging from autotrophic to heterotrophic (Fig. 3). However, the *C. nodosa*-dominated community may be able to dampen the consequences of this metabolic variability of the *C. prolifera* community, allowing for a more stable metabolic balance in the whole bay.

This study reveals how the different components of the ecosystem (i.e. macrophyte, sediment or plankton community) can have relevant and variable contributions to the NCP of shallow coastal areas, especially in periods when production of the dominant primary producers is low, as noted in earlier works (e.g. Pomeroy 1959, Moncreiff et al. 1992, Ziegler & Benner 1999). The planktonic annual NCP in benthic system of Cádiz Bay was 48 g C m^{-3} y^{-1}, in contrast to oceanic planktonic communities which metabolism seems to be nearly balanced on annual basis (Williams 1998) or can even be net heterotrophic in some coastal systems (e.g. Navarro et al. 2004). Nevertheless, the planktonic NCP^d was relatively less important than that from vegetated communities throughout the year. Thus, the planktonic annual NCP represented between 5% and 23% of the value estimated for the *C. nodosa*-dominated community, except in winter when plankton was the main primary producer (Fig. 3). The extrapolation of the plankton estimations to the whole year should be considered with caution because of the experimental time frame. Probably the bimonthly period was long enough to include the seasonal metabolic changes that occur in the benthic communities, but it may not be sufficient to cover those occurring in the plankton community because of the rapid variability that this community can register in short time periods (e.g. blooms in few days, Garcia 2002). On the other hand, when extrapolating the NCP of the plankton to the whole water column, an over-estimation of the GPP of deeper plankton community can occur, as a consequence of the exponential light reduction through the water column. However, Winkler bottles were dipped one meter into the water column, so that they were deployed at half-depth of the water column and close to the top of the canopy. Therefore, an underestimation of the GPP of the surface plankton community can occur too. Therefore, although it can balance the previous over-estimation, the use of this methodology to make our extrapolations, brings some uncertainties that have to be considered. Epiphyte production in *C. nodosa* meadows in Cádiz Bay was about 7.7 mg C m^{-2} d^{-1} in spring and 4.3 mg C m^{-2} d^{-1} in winter (Shönemann 2015), representing about 0.8% and 40%, respectively, of the NCP^d calculated for each season in this community. On the other hand, the annual NCP of the bare sediment community ranged between -2% and -60% of that estimated for the *C. nodosa*-dominated community. The extrapolation from the bare sediment community to the *C. nodosa* sediment community should be taken cautiously because microbiomes will probably not be exactly the same even though the small patches of bare sediment were completely surrounded by *C. nodosa* meadows. However, this allowed us to make a rough estimate of the contribution of the sediment community in *C. nodosa* meadows to the annual NCP. Seagrass productivity measured in the zone through punching method (Zieman 1974, Peralta et al. 2000) ranged from 25 to 80 mmol C m^{-2} d^{-1} (Brun et al. 2003, 2015). Note that through this method, the estimated contribution of the seagrass to annual NCP of the *C. nodosa*-dominant community was between 40-90% of the annual NCP. This lower value compared with that implied by our study results from the fact that the punching method only calculates the GPP in the aboveground tissues, which may account for less than 60 % of the total plant GPP (Brun et al. 2003). These results show how the sediment, epiphyte and plankton communities can account for a large and variable fraction of the net primary production of the communities dominated by seagrasses.

Vegetated coastal areas are not only highly productive but also represent areas with large DOC production; they are even more important as net DOC producers than previously recorded (Barrón & Duarte 2009, Barrón et al. 2014). By comparing previous studies, it can be estimated that the global net DOC export from seagrass meadows (calculated by Barrón et al. 2014) represents 46% of the global NCP of seagrass meadows (calculated by Duarte et al. 2010). However, this global average value has been obtained by extrapolating results from few studies, mostly in tropical or subtropical species. Our results will allow adjusting better the global average as we used temperate seagrasses, where studies are scarcer. In our work, annual net DOC flux in *C. nodosa* and *C. prolifera* communities represented 11% and 62% of the annual NCP, respectively. For the plankton community, annual net DOC flux consumed by plankton was equivalent to half of its annual NCP. Our study reveals the importance of DOC in the metabolism of shallow coastal areas since benthic communities could contribute largely as DOC sources for the planktonic one. This is supported by Navarro et al. (2004) study, although in this case plankton acted as a net DOC consumer due to their own heterotrophic behaviour. In our study, the planktonic community was autotrophic but the only net DOC consumer among the communities studied (Fig. 4), suggesting that it uses the organic carbon released as DOC by adjacent benthic communities. Ultimately, DOC dynamics in shallow coastal areas are controlled by the interaction of primary producers and heterotrophic bacteria. Primary producers act as a source of new organic matter either in particulate or in dissolved form. Heterotrophic bacteria can act either as a sink (Boto et al. 1989) or as a source of DOC through direct excretion, virus-mediated cell lysis, by exoenzymes released by bacteria as well as zooplankton-mediated sloppy feeding (Stoderegger & Herndl 1998, Glud & Mathias 2004). In addition, heterotrophic bacteria provide the major pathway for the transformation of particulate organic material (POM) to DOC (Fig. 6). Probably in the planktonic compartment of Cádiz Bay there is a high heterotrophic bacteria activity, which led plankton to act as DOC consumer in spite of being net autotrophic. Therefore, DOC from benthic production may be an important source for planktonic metabolism in shallow coastal areas, such as Cádiz Bay. It is supported by previous reports suggesting the key of DOC in boosting the epiphytic microheterotroph community on seagrass leaves (i.e. sessile bacteria and invertebrates) (Kirchman et al. 1984) and the plankton community (Valiela et al. 1997, Soetaert et al. 2000), with bacterioplankton being the component that primarily uses DOC (Van Engeland et al. 2013). This source of C not only helps to fuel plankton productivity but fuels the NCP of the whole ecosystem when environmental conditions are less favourable for benthic macrophytes. Therefore, it can be drawn that macrophyte diversity in coastal areas and their strong metabolic interactions may help to buffer shifts in productivity when environmental conditions are less favourable for vegetated communities.

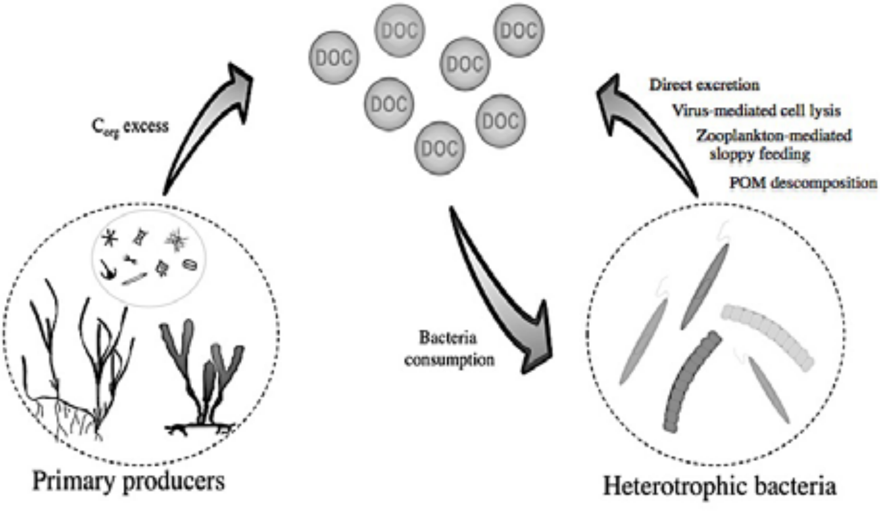

Figure 6. Conceptual model showing the DOC dynamic in shallow coastal areas.

Former studies have linked the DOC released by macrophytes with their standing biomass. For example, Ruiz-Halpern et al. (2014) found a strong dependence of net DOC flux on *C. prolifera* biomass. However, we did not find such correlation either in *C. nodosa* (Pearson r= 0.56, p= 0.15) or in *C. prolifera* (Pearson r= 0.24, p= 0.57). The relationship between net DOC flux and community productivity (NCP^d, GPP^d and CR^d) depended on the community type (Table 4), with the *C. nodosa* one showing the highest correlation at an annual scale. Therefore, high productivity in seagrass communities seems linked to larger net DOC flux. Probably the considerable organic matter content of sediments overgrown by *C. prolifera* (Vergara et al. 2012) drove the net heterotrophic community metabolism, thus masking the positive DOC-productivity relationship.

This study also revealed how changes in neighbouring benthic communities sharing the same shallow coastal landscape might result in large changes in the C metabolic of the system. Therefore, the actual extent of the area covered by benthic communities in Cádiz Bay resulted in a large autotrophic benthic system with a high NCP and net DOC production (ca. 8,300 Tons C y^{-1} and 2,355 Tons C y^{-1}, respectively) (Fig. 7). Vegetated coastal habitats are acknowledged as the most endangered marine habitats (Large 2009). Around 29% of the global area covered by seagrasses at the beginning of the twentieth century has already been lost, and generally replaced with unvegetated, unconsolidated, muddy and sandy soils (Fourqurean et al. 2012). Under such situation, it is likely that a large fraction of the organic C stored in their sediments will be released back to the ocean-atmosphere CO_2 pool (Pendleton et al. 2012). In the case of Cádiz Bay, the degradation of vegetated areas into bare sediments would constitute a radical change in the C metabolism of the whole bay, shifting it from the currently highly autotrophic and net DOC producer to a heterotrophic and lower net DOC producer (NCP $\approx$ -3,700 Tons C y^{-1}; net DOC flux $\approx$ 655 Tons DOC y^{-1}). However, if management policies would favour the spread of *C. nodosa* meadows, NCP and net DOC production could increase up to ca. 12,600 Tons C y^{-1} and ca. 1,440 Tons DOC y^{-1}, respectively (Fig. 7). This scaling up at annual scale should be considered with caution because of possible artefacts associated with the experimental design. On one side, small areas (i.e. those enclosed in the incubations) were used in scaling-up the coverage of each community at the whole Cádiz Bay. In doing it, we assume that the benthic communities share the same conditions in all bay (e.g. depth, biomass, density, etc.). On the other hand, there are uncertainties associated to the annual extrapolation of measurements, recorded only for a certain days. Although climatic conditions during such days were within the normal seasonal range and could be considered as representative of the sampling season, it should be noted that not every day (in the same season) has the same climatic conditions than the day in which measurements were taken (e.g. storms, wind force, etc.). Hence, probably the data variability associated will be, at least, a couple of magnitude orders lower than the average obtained. However, as we are mostly interested in underline the high productivity of this kind of ecosystem at a local level, the variability associated with this extrapolation does not mask this objective.

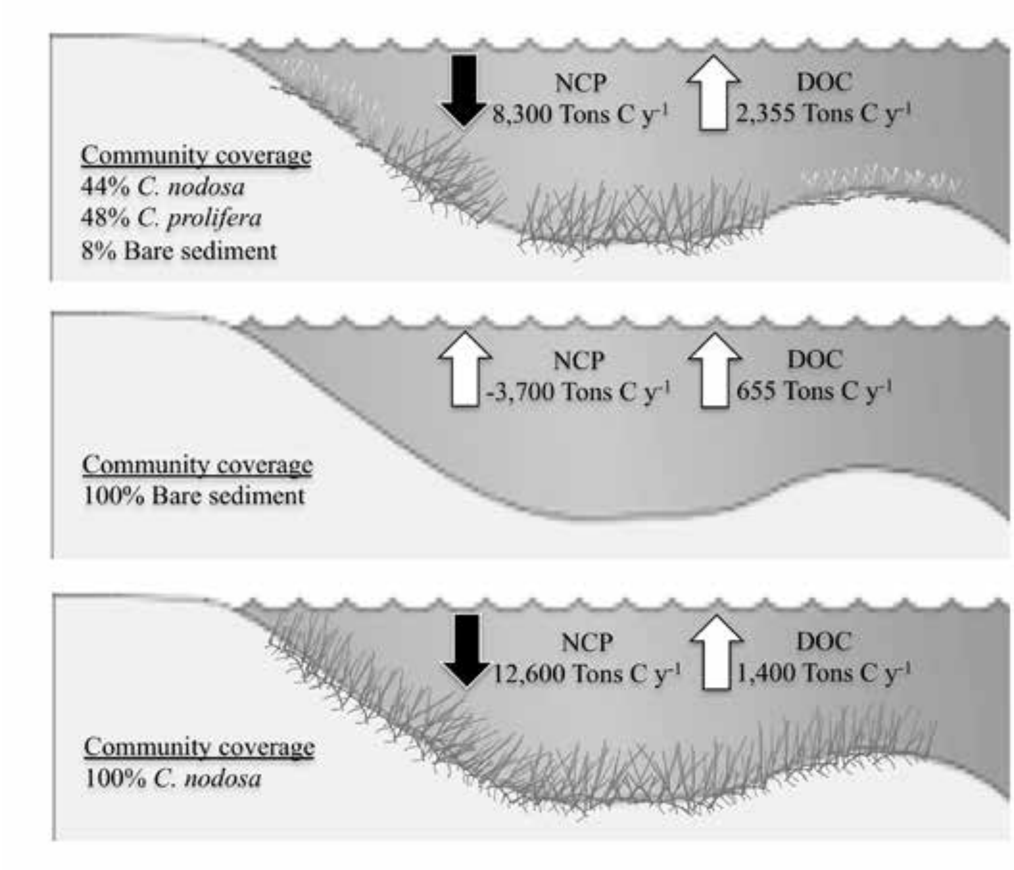

Figure 7. Simplified models of Cádiz Bay with different compositions of benthic communities (first for actual community coverage according to Muñoz and Sanchez 1994; Brun et al. 2015). Net community production (NCP) and net dissolved organic carbon (DOC) production resulting from the whole benthic ecosystem were estimated, as well as the DOC flux input or output taking into account the net DOC consumption by the plankton community and water renewal in the bay.

In conclusion, this research evidences that shallow coastal areas inhabited by different communities (e.g. seagrass, macroalgae and bare sediment) are highly dynamic, displaying large seasonal and community–specific variability in C metabolism. It can be drawn that macrophyte diversity in coastal areas and their strong metabolic interactions may help to buffer shifts in productivity when environmental conditions are less favourable, being seagrasses the main contributor to a more stable metabolic balance in the whole ecosystem. Seagrass communities are highly autotrophic but different components of the community (i.e. seagrasses, biotic components of the sediment, epiphytes or plankton) can have large and variable contributions along the year to the total NCP of the whole community. On the other hand, vegetated coastal communities contribute largely as net DOC source for the plankton community, boosting this community. Moreover, this study has shown a highly significant correlation between the net DOC released and the NCP in this seagrass community. Finally, the results indicated that vegetated coastal communities are highly autotrophic, suggesting that these ecosystems contribute to the net storage of C, which may help to offset the rise of carbon dioxide levels and, thus, acting as natural hot spots in counterbalancing climate change (Nellemann et al. 2009, Duarte et al. 2013). However, the C sink capacity of these ecosystems depends ultimately on the amount of carbon accumulated at long-term in the sediment as organic matter (Larkum et al. 2006). The remaining material is either consumed and/or exported elsewhere, so the actual burial capacity of Cádiz Bay will probably be lower (Kennedy et al. 2010 suggested that 30% to 50% of the seagrass NCP is buried *in situ*). Future research should delve into the C burial in shallow coastal areas such as Cádiz Bay to explore their potential in fighting against global change. This could promote the need for greater protection of these valuable ecosystems under the framework of the IPCC.

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CHAPTER 2

Changes in carbon metabolism and dissolved organic carbon fluxes between patches of different colonization states of the seagrass *Halodule wrightii* during the natural recovery after an extreme climatological event

In preparation

Nadie es como otro. Ni mejor ni peor. Es otro. Y si dos están de acuerdo, es por un malentendido.

Jean Paul Sartre

Changes in carbon metabolism and dissolved organic carbon flux between patches of different colonization states of the seagrass *Halodule wrightii* during the natural recovery after an extreme climatological event

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ABSTRACT

Seagrasses are marine flowering plants that form one of the most productive ecosystems worldwide, exporting large quantities of organic matter, both in particulate and dissolved forms to adjacent communities. The majority of the seagrass meadows found in tropical areas are regularly exposed to the impacts of extreme climatological events (e.g. tropical storms, hurricanes or cyclones), which are expected to increase as a consequence of climate change. The slow natural recovery of seagrass meadows after these events has been scarcely studied, especially in relation to the productivity and dissolved organic carbon (DOC) dynamics. This work presents an *in situ* experiment evaluating the carbon metabolism and DOC fluxes in three patches of *Halodule wrightii* with different colonization states after a tropical storm. The results showed that, as the colonization state increased, carbon metabolic rates (i.e. net community production) decreased as a consequence of the higher complexity of the associated community. In addition, the export of DOC was significantly higher in younger meadows, probably as a consequence of changes in the characteristic of the community together with the increase in the hydrodynamic conditions within the meadow due to a lower seagrass density and biomass. This study also suggests that the CO_2 uptake rates and organic C export rates in seagrass ecosystems are likely underestimated if the frequency and intensity of extreme climatological events increase as a consequence of climate change.

INTRODUCTION

Seagrasses are marine flowering plants present in shallow coastal areas around all continents except Antarctica (Hemminga & Duarte 2000) forming one of the richest and most relevant coastal ecosystems (Short et al. 2011). They are well recognised for providing ecosystem goods and services that have been estimated to be of high value compared with other marine and terrestrial habitats (Costanza et al. 1997, Orth et al. 2006). Seagrasses rank among the most productive ecosystems on Earth (Duarte & Chiscano 1999). It has been estimated that they contribute circa 12% of the net ecosystem primary production in the ocean (Duarte & Cebrián 1996). This high productivity comes equally from the epiphytes and macroalgae they support and by the angiosperms themselves (Hemminga & Duarte 2000), contributing to the carbon cycle in coastal waters and to the carbon sequestration from the atmosphere (Tokoro et al. 2014). Accounting for this growing interest, the number of studies where the productivity of the whole community dominated by seagrasses is included, have increased in the last decade (Barrón & Duarte 2006, Duarte et al. 2010).

The excess of organic matter derived from the high productivity of seagrass communities can be exported to adjacent ecosystems as particulate or dissolved form (Duarte & Cebrián 1996, Barrón & Duarte 2009). Seagrasses are known to release important amounts of dissolved organic carbon (DOC; Penhale & Smith 1977, Moriarty et al. 1986, Barrón et al. 2014). This compartment is the most abundant reservoir of exchangeable organic carbon in the marine pool and thus is a cornerstone in the global carbon cycle (Hedges et al. 1997, Hansell & Carlson 2001). The DOC is an important driver of primary production in other compartments of the food web, as it is easily assimilated by marine organisms (Jørgensen 1976) and is fully involved in the C exchange between communities (Hansell & Carlson 2001, Navarro et al. 2004, Egea et al. 2017a). The global net DOC export from seagrass meadows calculated by Barrón et al. (2014) represents 46% of the global net community production (NCP) of seagrass meadows calculated by Duarte et al. (2010) and this DOC released may be critical to keep the annual high productivity of communities dominated by seagrass meadows (Egea et al. 2017a).

The shallow distribution of seagrasses in estuarine and coastal areas and its proximity to anthropogenic littoral impacts has led to widespread seagrass losses, with a global decline of 7% yr^{-1} (Waycott et al. 2009). Besides coastal ecosystem disturbances from human activities, the majority of seagrass meadows found in tropical areas (Green & Short 2004) are regularly exposed to flooding, cyclones or hurricanes, which negatively impact seagrasses either physically (via burial, scouring and direct removal of plants and seed banks; Preen & Marsh 1995, Bach et al. 1998, Campbell & McKenzie 2004) or physiologically (via light limitation, nutrient excess and low salinity; Bjork et al. 1999, Ralph et al. 2007). The frequency and intensity of these extreme climatological events are expected to increase as a consequence of climate change (e.g. Duarte et al. 2006) and then, its effects in seagrass beds may be higher, causing great impacts and mortality. The slow natural recovery of seagrass beds (Reynolds et al. 2013) after extreme climatological events has been scarcely studied (e.g., Lee et al. 2007, Kim et al. 2015). Recovery of seagrass meadows from large-scale disturbance has been shown to take 2 – 4 years (Preen et al. 1995) and even more than 5 years (Birch & Birch 1984, Onuf 2000, Blake & Ball 2001). Studies on seagrass colonization (e.g. Barrón et al. 2004) and seagrass recovery (van Katwijk et al. 2016) showed that relatively young seagrass meadows have different structure, fauna and sediment stabilization comparing with mature meadows. Through seagrass recovery, aboveground and belowground biomass change (Duarte & Sand-Jensen 1990), which trigger changes in the hydrodynamics and particle sedimentation (Morris et al. 2008), dissolved nutrient exchanges (Adhitya et al. 2016), carbon stocks (Pérez et al. 2001) and fauna biodiversity and abundance (González-Ortiz et al. 2014, 2016). Then, seagrass recovery after an extreme climatological event, in turn, may affect productivity and DOC fluxes of seagrass communities.

The current understanding of carbon metabolism and DOC fluxes in coastal ecosystems is limited by the little attention that has been given to these responses *in situ*. Although there are many studies with estimates of seagrass community metabolism (reviewed in Duarte et al. 2010), they are unevenly distributed, with a substantial gap of information on community metabolic rates for seagrass meadows along the coasts of the Southern Hemisphere and North and West Pacific regions (Duarte et al. 2010). Similarly, the total number of net DOC flux estimates from seagrass meadows is mainly limited in the temperate zone. Therefore further efforts are needed in the tropical zone (Barrón et al. 2014). In addition, little attention has been given to assess the differences in carbon metabolism and DOC fluxes among seagrass meadows of different recovery states (i.e. patches with different moment of appearance since an extreme climatological event), in spite of the fact that carbon metabolism and DOC fluxes may be critical for the overall ecosystem recovery. Therefore, this study aims to explore the consequences in productivity and DOC fluxes in three different colonization state of the tropical seagrass *Halodule wrightii* (meadows of 1, 2 and 5 years old) since an extreme climatological event, which buried and/or removed the seagrass meadow. The study was based on changes in dissolved oxygen (DO) and DOC using *in situ* benthic chambers.

MATERIAL AND METHODS

Sample site. The study was carried out in Balandra Bay, a shallow coastal wetland covering approximately 450 ha in the Sonoran Desert located in Bahía de La Paz (southern Gulf of California, Mexico) (Fig. 1). It is a unique place for their biological and social values (Calderón et al. 2008) stemming from a coastal lagoon, mangroves, rocky reefs and patches of the seagrass *Halodule wrightii*. The genus Halodule is widely distributed along the coasts of tropical seas of the Atlantic and Indo-Pacific regions (Den Hartog 1970, Rosas & Ruelas 1985, Phillips & Menez 1988) and is considered a relatively rapid coloniser with fast clonal growth rates (Rasheed 2004). Balandra Bay belongs to the Ramsar international wetland convention since 2008, and it is considered a Protection Area since 2012 by the Mexican Government and a Natural Heritage by the UNESCO since 2013. Due to its geographical location, Balandra Bay is prone to tropical cyclones that produce strong tidal currents and sediment fluxes impacting markedly in the dynamic of patches of *H. wrightii* (Pérez-Estrada, in prep.). In fact, Pérez-Estrada (in prep.) found a large variety of patches with different recovery states since an extreme climatological event (tropical storm), which buried and/or removed completely the seagrass meadow, in a monitoring work starting in 2010.

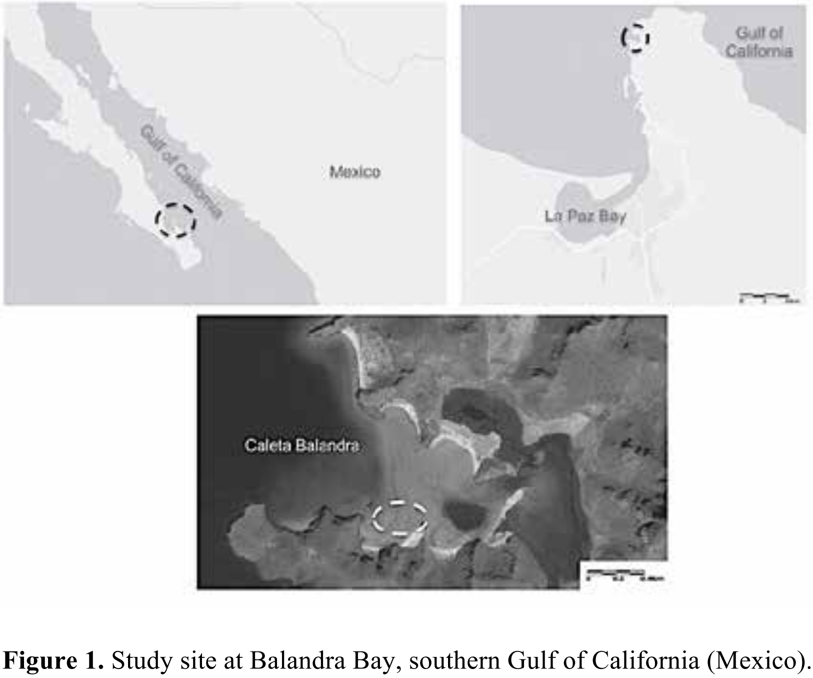

Figure 1. Study site at Balandra Bay, southern Gulf of California (Mexico).

The study was conducted in a 4 m deep (high tide) *H. wrightii* patches (24°19'N 110°20'W) with 3 different recovery states (i.e. three different colonization states meadow since patch was re-established): i) from 2010 (old patches), ii) from 2012 (medium patches) iii) from 2014 (young patches) plus bare sediments. The different patches were spaced at least 10 meters far away each other.

Field experiment. The carbon community metabolism and DOC fluxes were assessed in April 2016 using *in situ* benthic chambers (called incubations from here on), which were settled by scuba diving. It is important to point out that although each community was dominated by the macrophyte (i.e. *H. wrightii*) or by unvegetated sediments, they were finally composed by a set of other biological components such as plankton, epiphytes, macroalgae, infauna and sediment microbial community, that vary among incubations. Therefore, the results in this study included the entire community. Haphazardly replicated incubations were performed in the three patches of *H. wrightii* communities (three replicates per colonization state), and three additional incubations were carried out in adjacent bare sediments. The incubations consisted of a polyvinyl chloride rigid cylinder (PRC) of 19.5 cm diameter firmly inserted, with a sharpened side, about 15 cm into the sediment and a gas-tight polyethylene plastic bag fitted to the cylinder (Hansen et al. 2000). Due to strong currents inside the bay we installed an additional structure surrounding the PRC consisting in a metal ring (20.7 cm diameter) attached by pressure to the PRC with four legs (25x1.3x0.3 cm) inserted into the sediment. Each incubation had a sampling port to withdraw water samples. To avoid water stagnation inside of the incubation, the walls of the chambers were made flexible, so that they moved gently with hydrodynamics. The chambers were deployed in the experimental plots at low tide in the evening, just few hours before nightfall. To avoid the collection of resuspended material coming from experimental setup, the first sampling started 2h after the laying of the chambers. The enclosed water for community carbon metabolism (through DO concentration) and DOC fluxes was sampled though the sampling port using a 50 ml (polyethylene) acid-washed syringe at three times: i) just before sunset (S1); then leaving overnight the chambers and ii) taking samples right after sunrise (S2); and iii) 6 h after sunrise (S3). In this way, community carbon metabolism and DOC fluxes in dark and light periods can be discriminated (Barrón & Duarte 2009). To calculate the volume of each incubation chamber at the end of the experiment, 20 ml of a 0.1 M uranine (sodium fluorescein, $C_{20}H_{10}Na_2O_5$) solution was injected into each incubation bag, allowing 5 min for mixing. Then, water samples were collected and kept frozen until spectrophotometric determination in order to calculate the volume according to Morris et al. (2013). The volume of water enclosed in the benthic incubations was in average 9.8 ± 0.03 L (*n*=12).

Once removed the plastic bag, the entirely community in each incubation was harvested (including belowground biomass). Then, the fauna and flora (*H. wrightii*, epiphytes, and macroalgae) were separated, weighed and classified. Finally *H. wrightii* and macroalgae were rinsed and dried at 60 ºC to estimate aboveground and belowground biomass in the case of the seagrass and total biomasss for macroalgae. Organic matter content of the sediment was measured by a standard combustion procedure in a 2.5 cm diameter x 5 cm depth core. Temperature (ºC) and light (lumens·m^{-2}) were continuously monitored during the sampling period with HOBO data loggers (UA-002-64) set on each seagrass patches and in bare sediments close to the experimental plots.

Laboratory analysis. The DO was measured in water samples (15 ml) that were fixed immediately after collection, kept in darkness and refrigerated. The oxygen concentration was determined using a spectrophotometric modification of the Winkler titration method (Pai et al. 1993, Roland et al. 1999). Hourly rates of community respiration (R) were estimated as the difference in DO concentrations between samplings S2 and S1. Hourly rates of net community production (NCP) were estimated from the difference in DO concentrations between samplings S3 and S2. Hourly rates of gross primary production (GPP) were computed as the sum of the hourly rates of R and NCP. Finally, daily rates of GPP were calculated by multiplying the hourly GPP by the photoperiod. Daily rates of R were calculated by multiplying the hourly respiration by 24 (hours). Daily rates of NCP were estimated as the difference between daily rates of GPP and R. Metabolic rates in DO units were converted to carbon units assuming photosynthetic and respiratory quotients of 1, a value used widely in seagrasses (e.g. Barrón & Duarte 2006). Although these assumptions could set uncertainties in the estimates of R, GPP and NCP, this must be a minor concern when the global metabolism of each community is assessed.

The flux of DOC was estimated by changes in its concentration during the light and night periods. Water samples from the incubations were filtered through pre-combusted (450 °C for 4 h) Whatman GF/F filters and were kept adding 0.08 ml H_3PO_4 (diluted 30%) and 4 ºC in acid-washed material (glass vials encapsulated with silicone-teflon caps) until analyses. Concentrations of DOC were derived by catalytic oxidation at high temperature (720 °C) and chemiluminescence by using a Shimadzu TOC-VCPH analyzer. The DOC certified reference material (Low and Deep), provided by D. A. Hansell and W. Chen (University of Miami) (http://yyy.rsmas.miami.edu/groups/biogeochem/CRM.html) were used to assess the accuracy of the estimations. Net DOC fluxes were calculated by the sum of the DOC fluxes during the night period (i.e. the differences between S2 and S1 DOC concentration) and DOC fluxes during the light period (i.e. after extrapolate the differences between S3 and S2 DOC concentration for all hours of light). Thus, when net DOC fluxes were positive, the community acted as a net DOC producer. On the contrary, when net DOC fluxes were negative, the community acted as net DOC consumer.

Data and statistical analysis. To transform light in lumens m^{-2} to µmols photons m^{-2} s^{-1}, we used the most commonly conversion factor used in the literature (Carruthers et al. 2001) (1 lumens m^{-2} = 51.2 µmols photons m^{-2} s^{-1}). Number of species, abundance and diversity index of Shannon–Wiener (H’) (Shannon & Weaver 1963) and equitativity were calculated. H’ and equitativity were calculated according to the following formulae:

$$H' = -\sum (\rho_i) \ln (\rho_i)$$

$$Equitativity = \frac{\ln (s)}{H'}$$

where ρ_i is the relative abundance and s is the number of species.

Prior to any statistical analysis, data were checked for normality (Shapiro-Wilk normality test) and homocedasticity (Bartlett test of homogeneity of variances). Statistical differences between the three patches of different colonization states and unvegetated sediments were analysed using a 1-way ANOVA. When significant differences were found a Tukey post-hoc test was applied. Data are presented as mean ± SE. The significance level (α) set in all tests performed was 0.05. Statistical analyses were computed with R-scientific software 3.0.2 (R Development Core Team 2013).

RESULTS

Abiotic variables. Average water temperature measured during the study period was 23.1 ± 0.05 ºC during night hours and 23.4 ± 0.18 ºC at midday. The mean irradiance from 10:00 to 11:00 was ca. 590 µmol photons m^{-2} s^{-1} in bare sediment and 160 µmol photons m^{-2} s^{-1} inside the meadows.

Meadow characteristics. The average community biomass (i.e. the sum of seagrasses, epiphytes and macroalgae) was significantly lower ($p < 0.05$) in the young patches than in the other patches (x3 and x2 for medium and old patches respectively). *Halodule wrightii* was clearly the dominant macrophyte in the meadow, since the biomass of macroalgae (mainly *Polysiphonia sp., Ceramium sp., Acanthophora spicifera, Caulerpa sertularioides* and *Spyridia filamentosa*) and cyanobacteria (mainly *Lyngbia majuscule*) in the three patches of different colonization states were negligible, even in bare sediments. The particulate organic matter (POM) in the sediments was almost negligible (< 0.4% in all patches), recording no significant differences between patches of different colonization states (Table 1).

Table 1. Characteristic of patches of different colonization states and unvegetated sediment. Data are mean ± SE (*n*=3).

	Community biomass (gFW)	**Macroalgae and cyanobacteria biomass (%)**	**Shoot density ($shoot \cdot m^{-2}$)**	**POM (g)**	**POM (%)**
Unvegetated	NA	NA	NA	0.160	0.32
Young	3.8 ± 0.4	0.01 ± 0.004	78 ± 16	0.197	0.37
Medium	10.9 ± 1.3	0.07 ± 0.058	136 ± 40	0.157	0.31
Old	7.7 ± 0.8	0.07 ± 0.061	195 ± 50	0.209	0.39

Community metabolism. Overall, daily community metabolism in the communities dominated by seagrasses were significantly higher (5, 3 and 9-fold for GPP, R and NCP respectively) than unvegetated ones ($p < 0.01$) (Table 2). In addition, there were significant differences among patches of different colonization states. Thus, The GPP, R and NCP in the young patches were ca. 1.5-fold higher than the average of the other two older patches (Table 2). This difference turned significant when data was normalized by biomass, being GPP, R and NCP in the young patches more than 3-fold higher than the average of the other two patches (Figure 2). No significant differences were found between medium and old patches.

Table 2. Mean of carbon community metabolism in unvegetated sediment and patches of different colonization states. Data are mean ± SE (*n*=3) and are expressed in mmolC m^{-2} d^{-1}. GPP: Gross community primary production; R: Community Respiration; NCP: Net community production. P:R: Productivity:Respiration ratio.

	GPP	**R**	**NCP**	**P:R**
Unvegetated	25.5 ± 7.0	-17.4 ± 3.9	8.1 ± 10.9	1.9 ± 1.0
Young	165.1 ± 40.2	-62.6 ± 9.6	102.4 ± 31.5	2.6 ± 0.3
Medium	110.9 ± 28.1	-38.4 ± 4.6	72.5 ± 27.9	3.0 ± 0.7
Old	112.1 ± 4.2	-51.5 ± 6.1	60.7 ± 4.7	2.2 ± 0.2

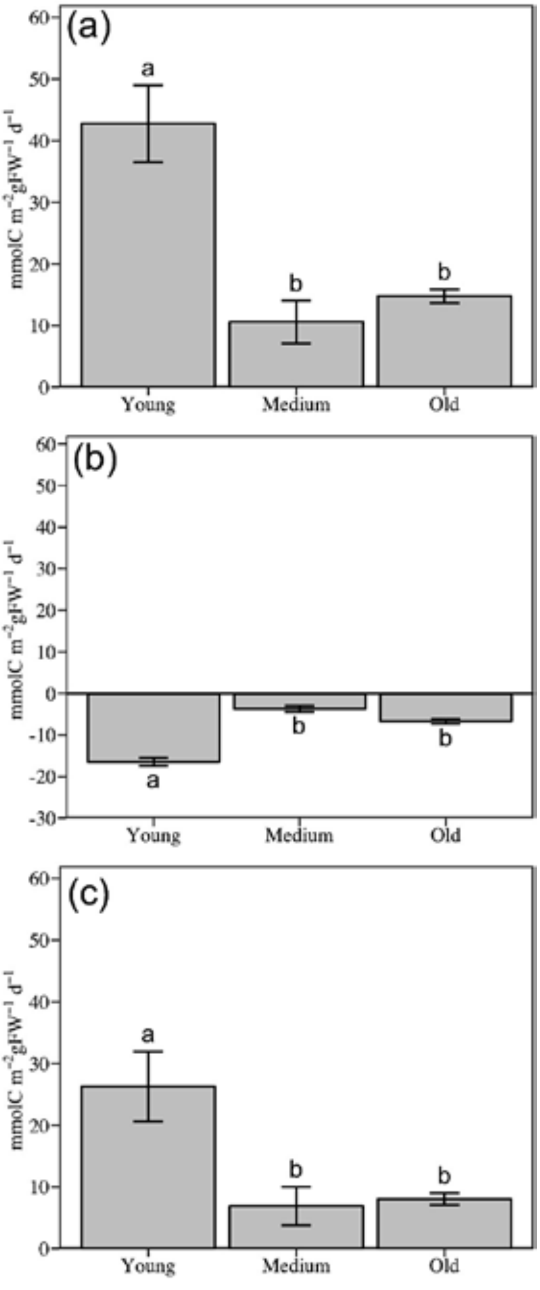

Figure 2. Carbon community metabolism in patches of different colonization states from colonization. (a) Gross community primary production (GPP), (b) Community Respiration (R) and (c) Net community production (NCP). Different letters indicate significant differences between patches. Data are presented and mean ± ES (*n*=3).

DOC fluxes. Young patches were the greatest net DOC producers (44.2 ± 3 mmol C m^{-2} d^{-1}) with rates significantly higher than the average of the other communities (medium, old patches and unvegetated sediments; Fig. 3). Unvegetated sediments and medium patches were also net DOC producers, but at a very low rate (3.2 ± 6.7 mmol C m^{-2} d^{-1} and 1.6 ± 1.1 mmol C m^{-2} d^{-1}, respectively). On the contrary, old patches were net DOC consumers (-5.6 ± 5.3 mmol C m^{-2} d^{-1}), although differences were not significant.

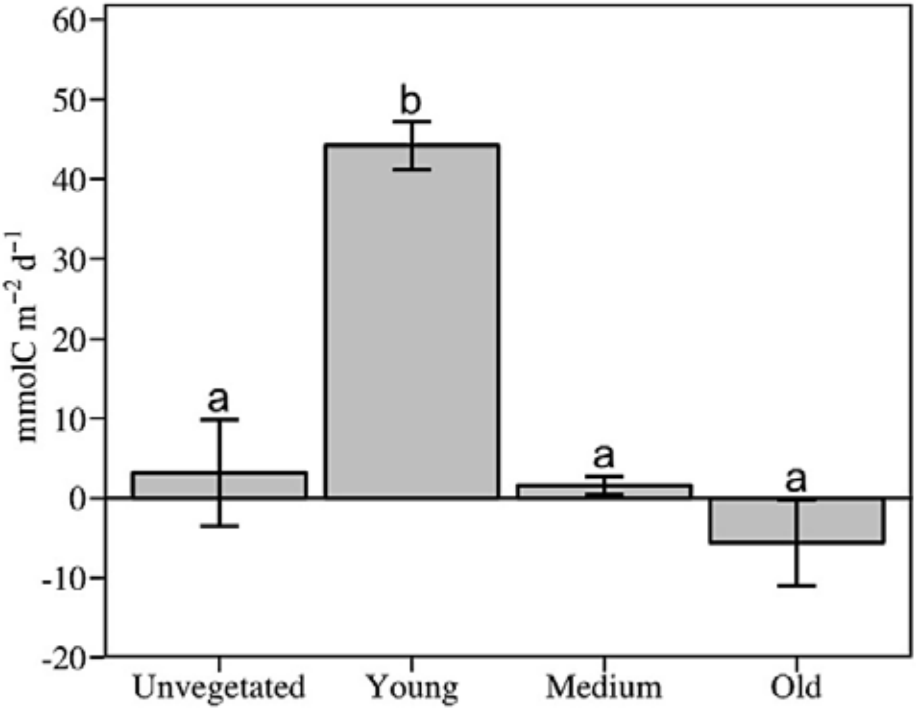

Figure 3. Net DOC fluxes in unvegetated sediments and patches with different colonization states. Different letters indicate significant differences between rates in the different communities. Data are showed as mean ± SE (*n*=3).

Fauna. Younger patches showed the lowest number of species and density of individuals, while medium and old patches showed similar number of species and density of individuals (Table 3). The number of species was circa 1.5 to 1.8 higher, while the density of individuals was from 3 to 4 times higher in medium and old patches when compared to young ones. Old patches showed the highest Shannon diversity index and equitativity (Table 3). The most abundant taxa in all the patches was *Cnidaria*, followed by *Annelida*, but this taxa decreased with the state since colonization. *Molusca* and *Crustacea* were relatively abundant as well. *Equinoderma* and *Cordada* were found in the young patches, whereas *Nematoda* were found in the other two colonization states. However, the abundance of the latter taxa was relatively low (Table 4).

Table 3. Number of species, abundance, Shannon index of diversity and equitativity in patches of different colonization states. Data are presented as mean ± SE (*n*= 3).

	Young	**Medium**	**Old**
Number of species	10 ± 1	16 ± 2	19 ± 1
Abundance (ind m^{-2})	51 ± 21	197 ± 17	167 ± 49
Shannon-Wiener Index (H’)	1.07 ± 0.13	0.82 ± 0.07	1.42 ± 0.12
Equitativity	0.46 ± 0.03	0.30 ± 0.02	0.49 ± 0.04

Table 4. Relative abundance of taxa (%) in patches of different colonization states.

	Young	**Medium**	**Old**
Cnidaria	66	85	74
Annelida	14	6	7
Molusca	9	4	10
Crustacea	7	4	7
Equinoderma	2	-	-
Cordada	2	-	-
Nematoda	-	1	2

DISCUSSION

This study has demonstrated that carbon community metabolism and dissolved organic carbon (DOC) fluxes are significantly affected by the time after the estabishment of a seagrass patch. The community dominated by *Halodule wrightii*, independently of the colonization state, were highly autotrophic as all meadows showed P:R ratios clearly higher than 1 and also significantly higher than those recorded in unvegetated sediments (Table 2). Regarding net DOC fluxes, the community of *Halodule wrightii* acted as a net DOC source or net DOC consumer depending on the time since colonization. The reported values are within the same range of values given by Duarte et al. (2010) for the carbon community metabolism and Barrón et al. (2014) for DOC exportation (21.87 ± 32.1 mmol C m^{-2} d^{-1}) in this seagrass specie.

Previous studies about colonization by seagrasses showed how vegetated biomass increase with time after patch establishment (Duarte & Sand-Jensen 1990) triggered an increase in fauna diversity and abundance (González-Ortiz et al. 2014, 2016) and the organic matter of the sediment (Pérez et al. 2001). Here, seagrass communities showed a decrease in net community production (NCP) with time after patch establishment (Table 2). The difference between the youngest patches and the other two patches became significant when they were normalised by macrophytic biomass (Fig 2). The lower net ecosystem production may be accounted for by shifts in the community associated (i.e. diversity and organism abundance) with time after patch establishment. Thus, Shannon diversity index and organism abundance increase since *H. wrightii* colonization (Table 3), in agreement to the patterns found in previous studies where fauna and seagrass structure enhanced with colonization time (e.g. Gartner et al. 2015, Mcskimming et al. 2016). As the communities hold more macrofauna associated, the net community production decrease. This hypothesis has been supported for other macrophyte-based systems where the net community production was lower than expected (e.g. Santos et al. 2004).

Our results showed a high variability in net DOC fluxes in meadows of *Halodule wrightii* depending on the time after patch establishment (Fig. 3). Thus, the net DOC flux ranged from highly DOC producer in the young patch (44.2 ± 3 mmol C m^{-2} d^{-1}, which represent the 43% of their NCP) to DOC consumer in the old patch (-5.6 ± 5.3 mmol C m^{-2} d^{-1}). Meanwhile the medium patche had a nearly balanced net DOC flux but being lightly net DOC producer (1.6 ± 1.1 mmol C m^{-2} d^{-1}, which represent the 2% of their NCP) (Fig 3). Barrón et al. (2014) highlighted the fact that other marine macrophytes with high rates of colonisation also show high variability in net DOC fluxes. This variability can be attributed to the effect of three different processes. Firstly, as a consequence of the characteristic of the associated community. Thus, in the youngest meadow co-habited more *Annelida*, which could release DOC when feeding on the organic matter of the sediment, and *Crustacea*, which could release DOC when they ripped the seagrass leaves, than in the two older patches (Table 4). Secondly, as a consequence of the hydrodynamic regimen in the meadow (Egea et al. 2017b). Although all *H. wrightii* patches of different colonization states grew in the same areas under similar hydrodynamic conditions, the youngest meadow evidenced significantly lower biomass and density (Table 1), which can facilitate the penetration of water within the meadow (González-Ortiz et al. 2014) and the sediment erosion (Peralta et al. 2008). Higher current velocity is expected to enhance the DOC transference from the sediment to the water column, as demonstrated for others compounds (van Duren & Middelburg 2000, Koch et al. 2006, Chipman et al. 2010, Corbett 2010). Therefore, it is concluded that the decrease in net DOC fluxes in the plots dominated by *Halodule* is partly explained by changes in the hydrodinamic regime within the meadow and changes in the characteristics of the associated community as the patches become older.

Previous studies have linked the DOC fluxes with NCP (Ziegler & Benner 1999, Egea et al. 2017a), which is in agreement with the remarkable direct relationship between DOC and NCP found in the three communities of different colonization states dominated by *H. wrightii* ($r^2 = 0.97$; $p = 0.09$) (Fig. 4).

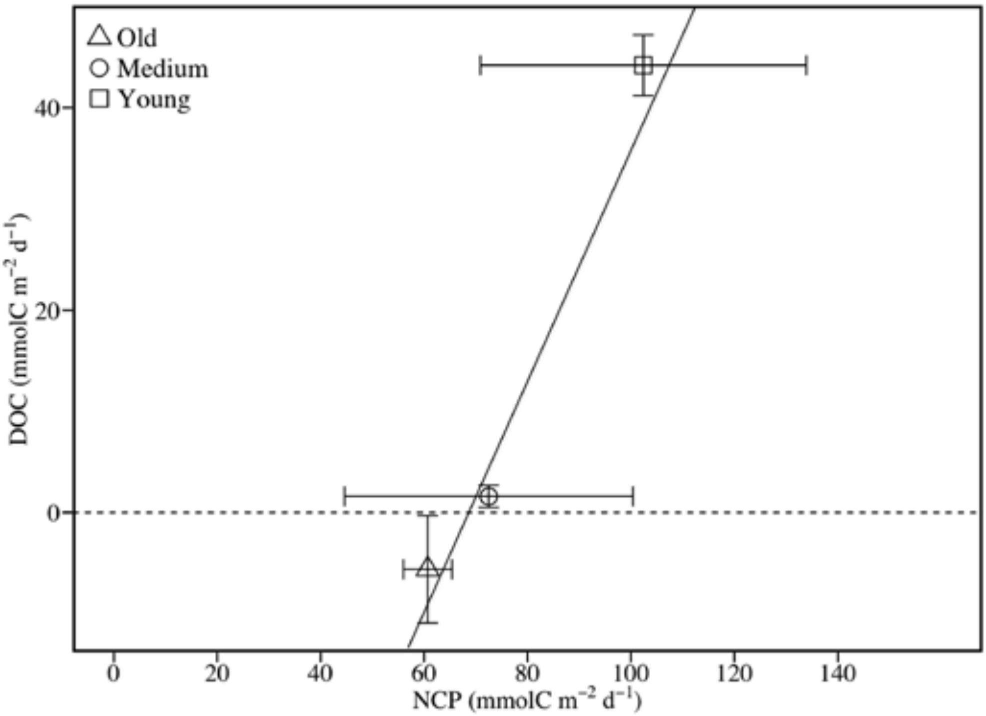

Figure 4. Relationship between dissolved organic carbon (DOC) and net community production (NCP) in patches of *H. wrightii* with different colonization states. Data are shown as mean ±SE (*n*= 3).

The old and medium patches showed similar carbon community metabolism, DOC fluxes as well as similar fauna diversity. The carbon metabolism and DOC fluxes were significantly lower than young patches. This demonstrates that patches dominated by *H. wrightii* evidence highest metabolic rates and DOC production in the early years after an extreme climatological event. Hence in areas where the degradation and recovery of seagrass meadow are often such as areas with highly mobile submerged dunes (Marbà & Duarte 1995) or in areas where seagrass are usually facing extreme climatological event, the CO_2 uptake rate could be higher and, as a consequence, the organic C export (POM and DOC) to adjacent ecosystems could be higher, as it has been demonstrated here for DOC. This can have noteworthy consequences in the estimates of CO_2 uptake and organic C export by seagrass communities. These two rates (CO_2 uptake and organic C export) are likely to be underestimated if the frequency and intensity of extreme climatological events increase as is expected by the consequences of climate change (Duarte et al. 2006). However our research did not study the continuing effect of degradation and recovery of the seagrass meadow as a consequence of an increase in the frequency of extreme climatological events, which could affect or modify the patterns observed here. Future research should delve into the shifts after extreme climatological events in seagrass meadows to predict and mitigate the effects of climate change in coastal marine ecosystems.

CONCLUSIONS

The present study showed that carbon metabolism and net DOC fluxes in patches dominated by *Halodule wrightii* have a high variability even when they are measured in the same species, area and date. The NCP decreased with the colonization state as a consequence of an increase in the associated community (i.e. macrophyte biomass and fauna). This can decrease the carbon exportation to adjacent ecosystems, as it was estimated for the reduction in net DOC fluxes. Thus, DOC released from the seagrass community decreased with the colonization state, which was probably due to a lower hydrodynamic regime within the patch as a consequence of the higher seagrass density and biomass and the characteristic of the associated community. Finally, this study highlights the importance of extreme climatological events that can produce a regression of seagrass communities to early colonization state, shifting the net community production from autotrophic to heterotrophic and hence affecting the rates of CO_2 uptake and organic C export.

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CHAPTER 3

Effect of nutrient enrichment on carbon metabolism and dissolved organic carbon (DOC) fluxes in vegetated coastal communities

In preparation

La cura es siempre agua salada: el sudor, las lágrimas y el mar.

Karen Biden

Effect of nutrient enrichment on carbon metabolism and dissolved organic carbon (DOC) fluxes in vegetated coastal communities.

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ABSTRACT

Coastal vegetated communities are among the most productive ecosystems on Earth. Their role in the global carbon cycle and in coping with global change may be more relevant than previously believed. They export large quantities of matter, both in particulate and dissolved forms to adjacent communities. Unfortunately, they are currently considered one of the most threated ecosystems on Earth because of anthropogenic pressures, including water nutrient enrichment. This work presents an *in situ* experiment evaluating the effects of nutrient enrichment in carbon metabolism and dissolved organic carbon (DOC) fluxes in shallow coastal communities dominated by the seagrass *Cymodocea nodosa* and by the macroalga *Caulerpa prolifera* during two contrasting seasons (winter and summer). Nutrient enrichment produced alterations at the ecosystem level, affecting to the primary producers and fauna abundance and diversity, which triggered the communities to moving from autotrophy to heterotrophy during part of the year. A decrease in the net community production (NCP) and a reduction in DOC fluxes in both macrophyte communities and seasons were found as a consequence of the increase in faunal abundance and biodiversity promoted by nutrient enrichment. However, a strong increase in DOC release was found in the community dominated by *C. nodosa* in the summer as a consequence of the increase in plant biomass and the proliferation of fast–growing species, including epiphytes and opportunistic macroalgae.

INTRODUCTION

Coastal vegetated habitats, including seagrass meadows, macroalgae, mangroves and salt marshes, are among the most productive ecosystems on Earth and play an important role in the global carbon cycle in spite of the limited surface they occupy on a global scale (< 2% of the ocean surface) (Duarte et al. 2005, Laffoley & Grimsditch 2009, Nellemann et al. 2009, Mcleod et al. 2011). They are well recognised due to the large number of ecological services they provide as a consequence of their high productivity (Costanza et al. 1997, Short et al. 2011), such as nutrient regeneration, water quality improvement, shoreline protection, creation of suitable breeding habitats (including those for species of economic relevance) and biodiversity (Duffy 2006, González-Ortiz et al. 2014). As a part of these ecosystems, seagrasses are marine foundation species that form one of the richest and most important coastal ecosystems (Short et al. 2011) currently considered a key element to cope with global change (Nellemann et al. 2009, Kennedy et al. 2010) as a consequence of their high productivity and the proportion of C captured that can be sequestered for millennia (Mateo et al. 1997, Macreadie et al. 2012, Serrano et al. 2012). The high productivity of seagrasses and vegetated coastal ecosystems contributes to the carbon uptake in the coast; carbon that can be stored, consumed, buried or exported to adjacent ecosystems as particulate or dissolved molecules (Duarte & Cebrián 1996, Barrón & Duarte 2009). Dissolved organic carbon (DOC) is the most abundant reservoir of exchangeable organic carbon in the marine C pool; a central factor in the global carbon cycle (Hedges et al. 1997, Hansell & Carlson 2001). The DOC usually acts as a quick transfer of C in the food web because it is easily assimilated by marine organisms and fully involved in the C exchange between communities (Hansell & Carlson 2001, Navarro et al. 2004, Egea et al. 2017).

The proximity of vegetated coastal habitats to anthropogenic littoral impacts entails these habitats are among the most threatened worldwide (Nellemann et al. 2009), especially seagrasses as they have experienced widespread losses with a global decline of 7% yr^{-1} (Waycott et al. 2009). This currently regression may be exacerbated by the increase of dissolved nutrient levels in the water column, especially nitrogen and phosphorus, along coastal areas (particularly in urbanized metropolitan ones). This increase can lead to eutrophication processes, which have been identified as one of the most important threats to the marine ecosystems (Nixon 1995, Smith & Schindler 2009), including seagrass meadows (Hughes et al. 2004, Burkholder et al. 2007, Cabaço et al. 2008, Antón et al. 2011).

Increased nutrient availability affects seagrass stands in two ways: alterations of the whole ecosystem and alteration of the individual plants. Thus, surplus of nutrients promotes the proliferation of fast–growing species, including phytoplankton, epiphytes and opportunistic macroalgae (Sand-Jensen & Borum 1991, Duarte 1995), species that compete with seagrasses for light and that can cause mortality by shading (Short et al. 1995, Hauxwell et al. 2001, McGlathery 2001, Brun et al. 2003, Moreno-Marín et al. 2016). Nutrient enrichment may further intensify the flux of organic matter to the sediment, thereby enhancing the risk of anoxia and sulphide toxicity (Borum et al. 2005, Pérez et al. 2007, Olivé et al. 2009). Moreover, increased nutrient availability stimulates seagrass consumption by herbivores, probably through an increase in the nutritional quality of their tissues (McGlathery 1995, Ruiz et al. 2001, Cebrian et al. 2009, Jimenez-Ramos et al. 2017a). This can lead to significant reductions in photosynthetic biomass and consequently to seagrass mortality. Nutrient enrichment may not only alter seagrasses and associated algae, but also affect the composition and abundance of associated faunal assemblages (Jimenez-Ramos et al. 2017b). However, experimental evidence of this is rather sparse and mostly reduced to influences over key herbivores (Valentine & Heck 2001, Castejón-Silvo et al. 2012).

In addition to causing alterations in the ecosystem, high nutrients availability also affect individual plants. Several studies have pointed out that moderate increases (<10 µM) in the availability of dissolved inorganic nitrogen (DIN) may stimulate the growth of seagrasses in pristine, oligotrophic environments (Short 1987, Pérez et al. 1991, Alcoverro et al. 1997, Udy et al. 1999). However, high availability of nitrogen in their reduced forms (i.e. ammonia and ammonium), can curtail plant growth and survival by direct ammonium toxicity (Burkholder et al. 1992, 1994, Van Katwijk et al. 1997, Brun et al. 2002, 2008, Villazán et al. 2013). Apparently, seagrasses are unable to down-regulate ammonium uptake, probably because of a lack of inhibitory feedback mechanisms (Rabe 1990, Touchette & Burkholder 2000). Therefore, high availability of NH_4^+ may cause the accumulation of this compound within the plant, which can affect intracellular pH, enzyme kinetics, photosynthetic ATP production and uptake of other ions (Marschner 1995, Britto & Kronzucker 2002, Villazán et al. 2013). In addition, intracellular accumulation of NH_4^+ may yield an imbalance in the carbon economy in seagrasses as a consequence of the enhanced internal demands of energy and carbon skeletons needed for rapid ammonium assimilation (i.e. the synthesis of amino acids and proteins). This carbon demand is met by resources diverted from growth and other metabolic processes (Marschner 1995, Brun et al. 2002, 2008, Villazán et al. 2013). Although the individual effects of nutrient enrichment on seagrasses are generally well studied (for review, see Burkholder et al.

2007, Koch et al. 2013), the *in situ* effects at the community level and including the community carbon dynamics, remain poorly understood.

The present study aims to gain insights into how two vegetated coastal communities, a temperate one dominated by the seagrass *Cymodocea nodosa* and other dominated by the macroalga *Caulerpa prolifera*, are affected by nutrient enrichment at a long term by analysing changes at the community level in the C metabolism, DOC fluxes and meadow characteristic (community biomass, macroalgae biomass, epiphyte biomass and fauna abundance and diversity) . The *in situ* experiment was replicated in two seasons (winter and summer) to determine if both communities have a differential response when acclimated to such contrasting environmental conditions.

MATERIAL AND METHODS

Study area. The study was conducted in a subtidal community dominated by the seagrass *Cymodocea nodosa* Ucria (Ascherson) growing at a depth of 3.5 m (low tide) in Santibañez, in the inner part of Cádiz bay, southern Spain (36.47°; 6.25º W) (Fig. 1). Climatically it fits into a semi-warm subtropical thermal regime whose normal temperature range varies between 11 to 28 ºC, with annual precipitation averaging 593 mm. There is very little fresh water input into the system so the average salinity ranges between 34.1 and 35.6 PSU and the average suspension solids vary from 10 to 30 mg/l. The benthic vegetation is dominated by three marine communities: two dominated by the seagrasses *Zostera noltei* Hornem. and *C. nodosa*, and another dominated by the rhizophytic green algae *Caulerpa prolifera* (Forsskål) J. V. Lamouroux. In between the macrophyte communities, there are also some unvegetated, bare areas. In the water column, nutrient peaks usually occur in winter, with values up to 1.4 µM NO_2^-, 12 µM NO_3^-, 25 µM NH_4^+ and 1.5 µM PO_4^{3-} (Tovar et al. 2000). For detailed information of the study area, see previous descriptions in Muñoz & Sánchez 1994, Morris et al. (2009) and Egea et al. (2017).

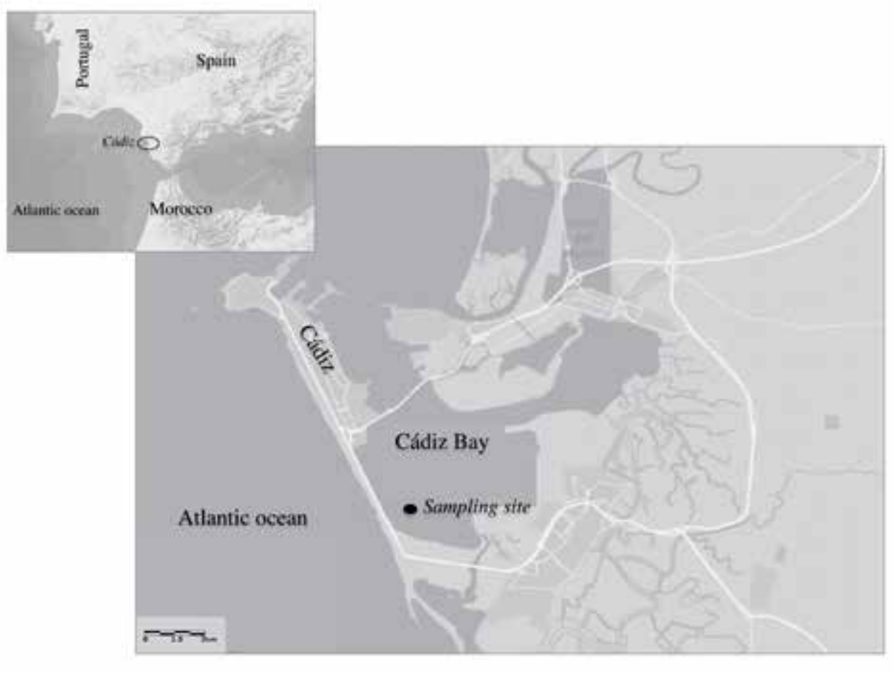

Figure 1. Study site at Cádiz Bay, Spain.

Experimental setup. The experiment was conducted in spring-summer 2016 (from May to July) and winter 2017 (from January to March), from now on called winter and summer trials respectively since they correspond with the period of maximum and minimum growth and biomass of these macrophytes in the area (Peralta et al. 2008, Egea et al. 2017). An *in-situ* nutrient enrichment was conducted in two vegetated coastal communities: one dominated by *Cymodocea nodosa* and one dominated by *Caulerpa prolifera* over three months in each trial. Although the community was dominated by these macrophytes, is actually an assemblage of several biological components, such as plankton, epiphytes, other macroalgae, infauna and sediment microbes, that will likely have a differential response to nutrient enrichment. Therefore, the results of this study integrate the entire community as a way to undertake a more realistic approach. Twenty–four experimental plots were selected in each trial (twelve for *C. nodosa* and twelve for *C. prolifera*) for the two treatments (control and enriched plots). Then each community and treatment consisted of six replicates distributed haphazardly (three for setting incubations and three for the collection of plant biomass and fauna samples). Experimental plots (50x50 cm) were separated by at least ten meters each and delimited by eight sticks placed within the canopy of the seagrass or macroalga beds. At the top of each stick (15 cm above the seafloor in *C. nodosa* and 7 cm above the seafloor in *C. prolifera*), a small mesh bag filled with slow-release fertilizer (Osmocote^{TM}; N:P:K; 18:9:3) was employed to alter nutrient levels in the water column. An empty mesh bag was attached to the sticks of the control replicates representing ambient conditions. In each enriched plot 80 g of Osmocote® was applied (0.5 kg m^{-2}) distributed in each mesh bag equally. Water samples were collected every two weeks in the central part of the square at 15 cm and 7 cm of above the seafloor in *C. nodosa* and *C. prolifera* meadows respectively using a silicone tube joined to sterilized plastic syringes and filtered through Whatman GF/F filters (0.45µm) to measure nutrients availability. At this moment, mesh bags containing Osmocote® were checked and replaced, in the case that some of them were lost. Temperature (ºC) and light (lumens m^{-2}) were monitored with three HOBO data loggers (UA-002-64) set on each community during two days at the beginning, half and at the end of the experimental period, next to the experimental plots.

Sample procedure. After three months, three replicates for each treatment (control and enriched plots) and community (*C. nodosa* and *C. prolifera*) were randomly selected from the experimental plots (i.e. those delimited by sticks), and benthic chambers (from now called incubations) were placed by scuba diving to estimate the C metabolism and DOC fluxes. The incubations consisted of a rigid cylinder made of a polyvinyl chloride (20 cm diameter) with a sharpened lower end firmly inserted 7–10 cm into the sediment, and an air-tight polyethylene plastic bag fitted over the upper end of the cylinder (Hansen et al. 2000). Each bag was provided with a sampling port to withdraw water samples. To prevent water stagnation in the incubations, the walls were made flexible so that they gently moved with the hydrodynamics. Incubations were placed in the evening just few hours before nightfall. To avoid the collection of resuspended material resulting from disturbance during installation of the experiments, the first sample was taken 2h after setting up the incubations.

To measure community carbon metabolism (through dissolved oxygen –DO– concentration) and DOC fluxes, water enclosed within each incubation was taken through the sampling port using a 50 ml acid-washed syringe at three times during the day: i) just before sunset (S1), ii) right after sunrise (S2) and iii) 6 h after sunrise (S3). In this way, community carbon metabolism and DOC fluxes in dark and light periods can be distinguished (Barrón & Duarte 2009). To calculate the volume of each incubation at the end of the experiment, 20 ml of a 0.1 M uranine solution (sodium fluorescein, $C_{20}H_{10}Na_2O_5$) was injected into each bag, allowing 5 min for mixing. Then, water samples were collected and kept frozen until spectrophotometric determination according to Morris et al. (2013). The volume of water enclosed in the chamber was 11 ± 0.1 L (*n*=24). Once the polyethylene plastic bag was removed, macrophyte biomass in each chamber was harvested, rinsed and dried at 60 ºC to estimate aboveground and belowground biomass. To better compare the two periods of study (summer and winter), sampling days in each season were chosen with similar tidal range as well as weather forecast (e.g. presence of clouds, no rain, wind, etc.) in order to reduce the environmental variability.

The remaining three replicates of each treatment and community were collected manually to examine the above (AG)- and belowground (BG) biomass, including the epi- and infaunal diversity, using a 400 cm^2 quadrat placed in the center of the experimental plots (controls and enriched). The complete community including all macrophytes, fauna and sediment (till 15 cm depth) were removed and placed into a plastic bag. The samples were then transported to the laboratory within 45 min after collection in an ice chest.

Laboratory analysis. Water samples (15 ml) for DO concentration were fixed *in situ* immediately after collection in the supporting boat, and were kept in darkness, refrigerated and finally measured using a spectrophotometric modification of the Winkler titration method (Pai et al. 1993, Roland et al. 1999). Hourly rates of community respiration (R^h) were estimated as the difference in DO concentrations between samplings S2 and S1 ($R^h = DO^{S2} - DO^{S1}$). Hourly rates of net community production (NCP^h) were estimated from the difference in DO concentrations between samplings S3 and S2 ($NCP^h = DO^{S3} - DO^{S2}$). Hourly rates of gross primary production (GPP^h) were computed as the sum of the hourly rates of R and NCP ($GPP^h = R^h + NCP^h$). Finally, daily rates of gross primary production (GPP^d), respiration (R^d) and net community production (NCP^d) were calculated following the calculations (where photoperiod corresponded to photoperiod in each sampling day):

$$GPP^d = GPP^h * \text{Photoperiod} \; ; \; R^d = R^h * 24h \; ; \; NCP^d = GPP^d - R^d$$

Metabolic rates in DO units were converted to carbon units assuming photosynthetic and respiratory quotients of 1, a value used widely for seagrasses (e.g. Barrón et al. 2004). Although these assumptions could introduce uncertainties into the estimates of R, GPP and NCP, this should be a minor concern when the global metabolism of each community is assessed. Dissolved oxygen concentrations in benthic chambers were, on average, 8.1 mg O_2 l^{-1} (S1), 5.5 mg O_2 l^{-1} (S2) and 8.1 mg O_2 l^{-1} (S3) in winter trial and 11.9 mg O_2 l^{-1} (S1), 9.0 mg O_2 l^{-1} (S2) and 10.8 mg O_2 l^{-1} (S3) in summer trial. Thus, these changes in DO during the incubations seem relatively moderate, minimizing the propagation of errors when computing metabolic rates (e.g. converting DO to carbon units or assuming respiration at night-time is the same than at daytime) and artefacts due to physiological stress on the communities.

DOC fluxes were estimated by changes in DOC concentration during light and dark periods. Water samples (20 ml) from the benthic chambers were filtered through pre-combusted (450 °C for 4 h) Whatman GF/F filters (0.7 µm) and were kept with 0.08 ml of H_3PO_4 (diluted 30%) at 4 ºC in acid-washed material (glass vials encapsulated with silicone-PTFE caps) until analyses. Concentrations of DOC were derived by catalytic oxidation at high temperature (720 °C) and chemiluminescence by using a Shimadzu TOC-VCPH analyzer. DOC-certified reference material (Low and Deep), provided by D. A. Hansell and W. Chen (University of Miami), were used to assess the accuracy of the estimations (http://yyy.rsmas.miami.edu/groups/biogeochem/CRM.html). The instrument blank ranged between 0 to 13 µmol DOC l^{-1} across the different analytical batches. Net DOC flux was calculated by the sum of the DOC flux during the dark period (i.e. the differences between S2 and S1 DOC concentrations) and DOC flux during the light period (i.e. after extrapolating the differences between S3 and S2 DOC concentrations for all hours of light). Thus, when net DOC flux was positive, the community was considered to act as a net DOC producer (i.e. source). However, when net DOC flux was negative, the community was considered to act as a net DOC consumer (i.e. sink). DOC concentrations were, on average, 2.49 mg l^{-1} (S1), 2.35 mg l^{-1} (S2) and 2.46 mg l^{-1} (S3) in winter trial and 3.15 mg l^{-1} (S1), 3.29 mg l^{-1} (S2) and 3.92 mg l^{-1} (S3) in summer trial. Thus, these changes in the DOC during the incubations seem relatively moderate, minimizing the propagation of errors on the computation of metabolic rates and artefacts due to physiological stress of the communities.

Macrophyte biomass was separated into leaves (AG), and roots and rhizomes (BG), counted, weighed and dried for 1 week at 60°C to obtain dry weight (DW). Biomass was expressed as g DW m^{-2}. Shoot density in *C. nodosa* was determined from the total number of shoots collected in each experimental plot. Epiphytes in *C. nodosa* were gently scraped with a razor blade from five haphazardly separated plants, weighed and DW attained by drying samples at 60°C for 2 days. Epiphyte biomass corresponding to each replicate was calculated using the average epiphyte DW values and the shoot density. Data were expressed as g DW m^{-2}. Epi- and endofauna specimens were separated from macrophytic biomass samples and immediately placed in Rose Bengal. All specimens were identified to the lowest possible taxonomic level under stereomicroscope, measured (length, width and thickness) and counted to assess any change in the community structure. Samples were subsequently dried at 60°C for 1 week and weighted (DW). Biomass values were expressed by area of the corresponding experimental plots (g DW m^{-2}).

Data and statistical analysis. To transform light values in lumens m^{-2} to µmols photons m^{-2} s^{-1}, the most commonly conversion factor given in the literature under sunlight was used (1 lumens m^{-2} = 51.2 µmols photons m^{-2} s^{-1}; Carruthers et al. 2001). Differences in seawater nutrients concentration among treatments were analysed using a paired *t*-test.

Ecological indices such as number of species, species abundance, diversity index of Shannon–Wiener (H') (Shannon & Weaver 1963) and equitativity were calculated. H' and equitativity were calculated according to the following formulae:

$$H' = -\sum (\rho_i) \ln (\rho_i)$$

$$Equitativity = \frac{\ln(\mathrm{s})}{H'}$$

where ρ_i is the relative abundance and s is the number of species.

Statistical differences between treatment for each community and season trial were made through a statistical meta-analysis of the effect size to avoid the possible misleading influence of sample size. While null hypothesis significance testing only informs about the probability of an observation, the presentation of the effect size along with its confidence interval (CI) provides the two most important pieces of statistical information for biologists: the magnitude estimate of an effect of interest and the precision of that estimate (Nakagawa & Cuthill 2007). To estimate the effect size of the parameters under study, the Hedges' d metric was chosen (Hedges & Olkin 1985), as it is an unbiased estimator that provides a better estimate for small sample sizes. The effect size was presented as Hedges' d according to Nakagawa & Cuthill (2007). Hedges' d metric values along with its confidence interval (CI) above 0 indicate a pronounced positive effect, bellow 0 indicate a pronounced negative effect, and equal to 0 indicates no effect on the parameter under investigation. The bigger the number either on the positive or negative direction tells about the magnitude of the effect.

Data are presented as mean ± SE. Statistical analyses were computed with R statistical software 3.0.2 (R Development Core Team 2013).

RESULTS

Abiotic variables. Average water temperature measured during the study period varied between 15.6 ± 0.2 ºC in winter to 25.8 ± 0.3 ºC in summer. Underwater daily irradiance at the canopy level of *C. nodosa* bed was 165 ± 11 µmol photons m^{-2} s^{-1} at midday during the winter trial and 339 ± 33 µmol photons m^{-2} s^{-1} during the summer trial. In *C. prolifera* bed, the underwater daily irradiance was 194 ± 13 µmol photons m^{-2} s^{-1} during the winter trial and 375 ± 17 µmol photons m^{-2} s^{-1} during the summer trial. Nutrient concentrations differed significantly between enriched and control treatments along the experimental time, revealing large differences in nutrients availability for the community depending on the experimental treatment for both communities and seasons (Table 1).

Table 1. Seawater phosphate and ammonium concentrations in plots dominated by *C. nodosa* and *C. prolifera.* Different letters indicate significant differences between treatments in each trial. Data are expressed as mean ± SE (*n*=6).

	Cymodocea nodosa			
	Summer		**Winter**	
	Control	**Nutrient enriched**	**Control**	**Nutrient enriched**
Phosphate (µM)	0.37 ± 0.04^{a}	0.83± 0.31^{b}	0.22 ± 0.02^{a}	0.75± 0.27^{b}
Ammonium (µM)	5.69 ± 3.43^{a}	23.32 ± 6.68^{b}	3.71 ± 1.26^{a}	18.14 ± 4.72^{b}
	Caulerpa prolifera			
	Summer		**Winter**	
	Control	**Nutrient enriched**	**Control**	**Nutrient enriched**
Phosphate (µM)	0.41 ± 0.03^{a}	0.93± 0.45^{b}	0.11 ± 0.04^{a}	0.66± 0.10^{b}
Ammonium (µM)	7.27 ± 2.53^{a}	19.15 ± 5.61^{b}	2.76 ± 0.74^{a}	12.76 ± 3.69^{b}

Meadow characteristics. The average biomass in the community dominated by *Cymodocea nodosa* (i.e. seagrass, epiphytes and macroalgae) was slightly higher (x1.4) in the summer trial than in the winter one. Whereas no effect in community biomass was found in winter under nutrient enrichment conditions, during the summer the average biomass was significantly higher (x3) in nutrient enriched plots with respect to the control. *C. nodosa* was the dominant macrophyte in the meadow, but the importance of macroalgae and epiphytic biomasses varied along the season and treatment (Table 2). Whereas no significant differences were found in macroalga biomass between control treatments in both trials, the biomass of epiphytes in summer doubled the winter values in the controls. Nutrient enrichment triggered a significant increase in macroalga and epiphyte biomasses in both trials (x2 and x3 for macroalgae and x1.5 and x2 for epiphytes in winter and summer trials respectively). Regarding the community dominated by *Caulerpa prolifera*, the average community biomass was significantly higher in the summer trial than in the winter one. Nutrient enrichment did not produce significant differences in the average community biomass. *C. prolifera* clearly was the dominant macrophyte in the meadow in both trials since the presence of other macroalga species was negligible.

Table 2. Meadow characteristics in the experimental plots. NA, when the presence was negligible. *Cn*, *Cymodocea nodosa*. *Cp*, *Caulerpa prolifera*

Specie	Season	Treatment	Community biomass (g DW)	Macroalgal biomass (g FW)	Macroalgal biomass (%)	Epiphytes biomass (g DW*shoot^1)
Cn	**Winter**	**Control**	11.9 ± 1.9	12.4 ± 4.2	22	3 ± 0.1
Cn	**Winter**	**Nutrient enriched**	13.1 ± 1.7	27 ± 9.1	45	4.5 ± 0.3
Cn	**Summer**	**Control**	16.9 ± 1.5	13.9 ± 0.8	12	6.9 ± 0.1
Cn	**Summer**	**Nutrient enriched**	53.7 ± 3.9	43.5 ± 1.1	23	13.5 ± 0.5
Cp	**Winter**	**Control**	2.2 ± 0.5	NA	NA	NA
Cp	**Winter**	**Nutrient enriched**	3.8 ± 1.1	NA	NA	NA
Cp	**Summer**	**Control**	2.4 ± 0.1	NA	NA	NA
Cp	**Summer**	**Nutrient enriched**	5 ± 2.4	NA	NA	NA

Effects on the community metabolism. Nutrient enrichment generally produced a decrease in net community production (NCP) in the stands dominated by *C. nodosa* and *C. prolifera* in both seasons (Fig. 2c). The exception was the community dominated by *C. nodosa* in the summer trial, when a slight increase in NPC was found (x1.2). In winter, *C. nodosa* was slightly autotrophic in the control treatment, but decreased significantly its NCP (x1.5) and became heterotrophic under nutrient enrichment. In *C. prolifera* the nutrient enrichment caused a decrease in the NCP, especially in the summer trial (x1.6) when became heterotrophic. Nutrient enrichment generally produced a decrease in the community respiration (R) and the gross primary production (GPP) (Figs. 2a and 2b). This effect was especially dramatic in the community dominated by *C. prolifera* during the summer trial. The only exception was the slight increase in GPP in the meadow dominated by *C. nodosa* during the summer trial.

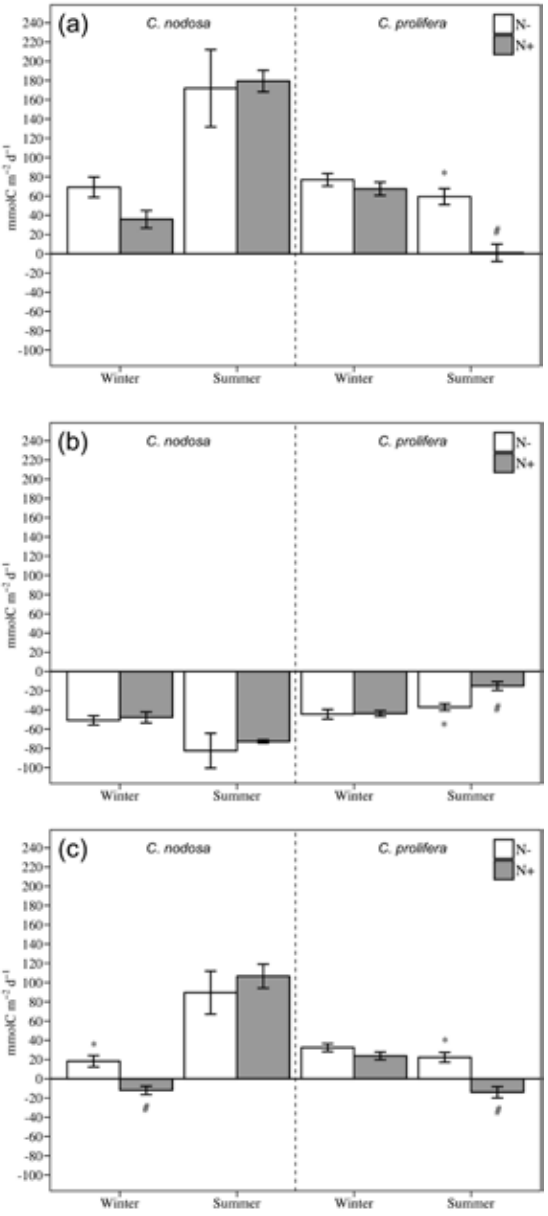

Figure 2. Effect of nutrient enrichment on (a) Gross Primary Production (GPP), (b) Respiration (R) and (c) Net Community Production (NCP) in winter and summer in two communities dominated by *Cymodocea nodosa* (left) and *Caulerpa prolifera* (right). N-: control treatment; N+: nutrient enrichment treatment. Different symbols indicate significant differences between treatments in each season. Data are expressed as mean ± SE (*n*=3).

DOC fluxes. Nutrient enrichment produced a decrease in DOC release in both seasons except in the meadow dominated by *C. nodosa* during the summer trial (Fig. 3), when a strong increase was found (x7). This community was marginally a DOC consumer in the control treatment during the winter trial, while under nutrient enrichment the consumption of DOC increased significantly (x3). On the contrary, the decrease in DOC fluxes observed in the community dominated by *C. prolifera* was not significant in any of the trials.

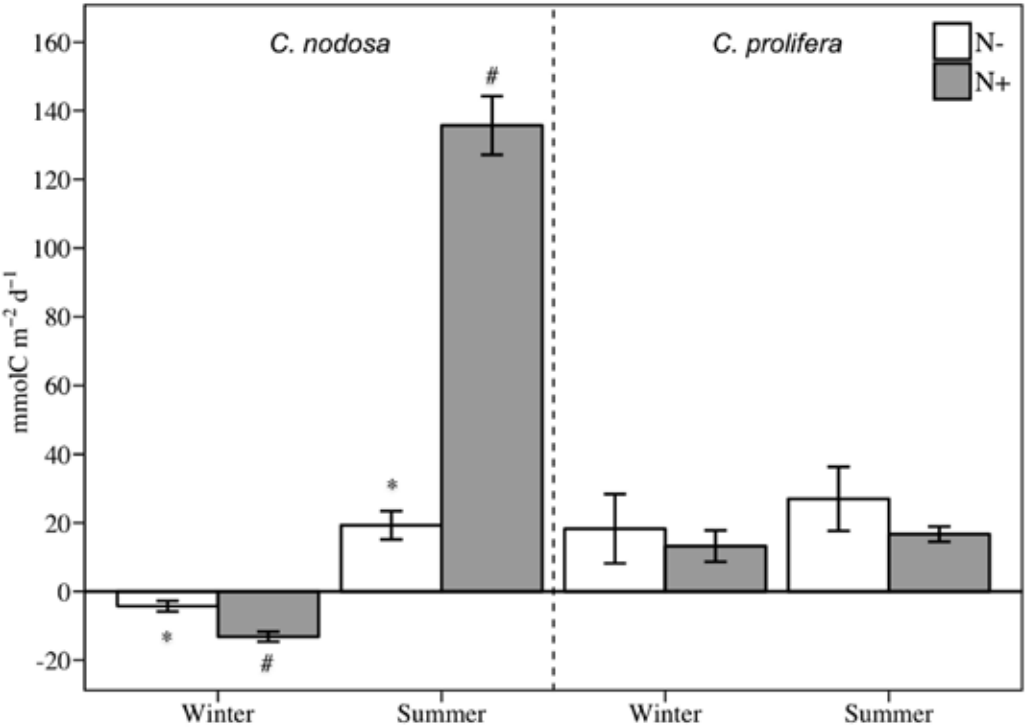

Figure 3. Effect of nutrient enrichment on the net DOC fluxes in winter and summer in two communities dominated by *Cymodocea nodosa* (left) and *Caulerpa prolifera* (right). N-: control treatment; N+: nutrient enrichment treatment. Different letters indicate significant differences between treatments. Data are expressed as mean ± SE (*n*=3).

Fauna. In the community dominated by *Cymodocea nodosa*, nutrient enrichment produced a pronounced increase in the number of species, abundance (x2 in the winter trial and x5.8 in the summer trial) and in the Shannon–Wiener diversity index (especially in summer) under nutrient enrichment (Table 3). Regarding the meadow dominated by *Caulerpa prolifera*, the differences between nutrient conditions was less accused, with an increase in the abundance and diversity index (x1.5 and x1.2 respectively) in the winter trial, but a decrease (x0.7 and x0.9 respectively) in the summer trial (Table 3).

Nutrient enrichment affected the relative abundance of the main taxa (Table 4). The most abundant taxa in the meadow of *C. nodosa* belonged to *Gasteropoda* and *Bivalvia* whereas the most abundant taxa in the meadow of *C. prolifera* was *Bivalvia* under both nutrient conditions studied. In meadows of *C. nodosa*, the taxa more abundant under nutrient enrichment were *Annelida* and *Bivalvia*, as it was also observed for *Bivalvia* in stands of *C. prolifera* during the summer trial. The relative abundance of *Gasteropoda* was not affected in the beds of *C. nodosa* but decreased in meadows of *C. prolifera* in both season trials. *Amphipoda* decreased in *C. nodosa* beds under nutrient enrichment during the summer trial.

Table 3. Number of species, abundance, Shannon index of diversity and equitativity in the experimental plots (*n*= 3)

Species	***Cymodocea nodosa***				***Caulerpa prolifera***			
Trial	**Winter**		**Summer**		**Winter**		**Summer**	
Treatment	**Control**	**Nutrient enriched**	**Control**	**Nutrient enriched**	**Control**	**Nutrient enriched**	**Control**	**Nutrient enriched**
Number of species	6 ± 0.3	13 ± 0.6	3 ± 0.6	7 ± 0.7	6 ± 0.3	6 ± 0.3	2 ± 0.9	3 ± 0.7
Abundance (ind m^{-2})	23 ± 1.9	50 ± 6.3	20 ± 4.7	118 ± 10.1	2 ± 0.7	4 ± 1.5	14 ± 9.9	11 ± 7.5
Shannon-Wiener Index (H')	1.7 ± 0.1	2.4 ± 0.1	1 ± 0.2	1.8 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	0.7 ± 0.4	0.8 ± 0.2
Equitativity	0.9 ± 0	0.9 ± 0	1 ± 0	1 ± 0	0.2 ± 0.1	0.2 ± 0.1	0.6 ± 0.3	0.9 ± 0

Table 4. Taxa relative abundance in experimental plots (*n*= 3)

Species	*Cymodocea nodosa*				*Caulerpa prolifera*			
Trial	**Winter**		**Summer**		**Winter**		**Summer**	
Treatment	**Control**	**Nutrient enriched**	**Control**	**Nutrient enriched**	**Control**	**Nutrient enriched**	**Control**	**Nutrient enriched**
Bivalvia	0.29 ± 0.08	0.39 ± 0.05	0.04 ± 0.04	0.36 ± 0.17	0.44 ± 0.29	0.69 ± 0.23	0.29 ± 0.15	1 ± 0
Gasteropoda	0.34 ± 0.1	0.3 ± 0.05	0.57 ± 0.3	0.51 ± 0.11	0.22 ± 0.22	0.06 ± 0.06	0.71 ± 0.15	0 ± 0
Amphipoda	0.2 ± 0.11	0.23 ± 0.01	0.24 ± 0.24	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Annelida	0 ± 0	0.04 ± 0.02	0 ± 0	0.13 ± 0.07	0.33 ± 0.33	0.25 ± 0.25	0 ± 0	0 ± 0
Crustacea	0 ± 0	0 ± 0	0,22 ± 0,05	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Effect size. The community dominated by *C. prolifera* revealed that during the summer, the GPP of this community was the most affected (Fig.4), suggesting a marked GPP decrease as a consequence of the nutrient enrichment. This community also evidenced a pronounced positive effect in respiration. Regarding NCP, the former community and that dominated by *C. nodosa* in the winter trial revealed a strong negative effect of nutrient enrichment. Also, the release of DOC in the community dominated by the seagrass was markedly affected by nutrient enrichment with two contrasting results: a pronounced DOC decrease during the winter trial and a pronounced DOC increase during the summer trial. At this moment was when this community showed a noteworthy increase in the total biomass. This increase due to the nutrients effect was especially marked for macroalgae and epiphytic biomasses, although the latter was also observed during the winter trial (Fig. 4).

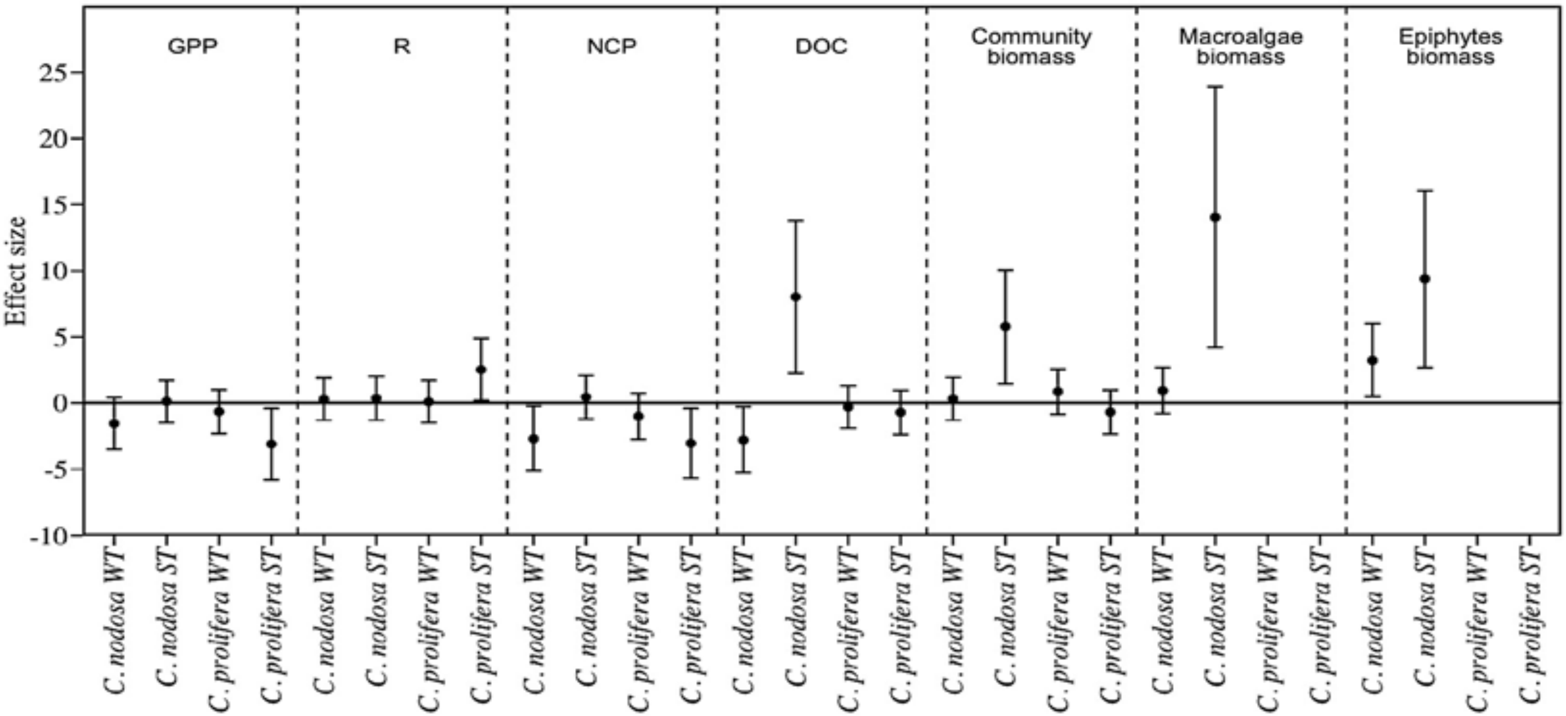

Figure 4. Effect sizes (*n*=3) between the nutrient enrichment treatment and the control for gross primary production (GPP, mmol C m^{-2} d^{-1}), community respiration (R, mmol C m^{-2} d^{-1}), net community production (NCP, mmol C m^{-2} d^{-1}), dissolved organic carbon flux (DOC, mmol C m^{-2} d^{-1}), community biomass (gDW), macroalgae biomass (gFW) and epiphytes biomass ($gDW*shoot^{-1}$) in communities dominated by *Cymodocea nodosa* or by *Caulerpa prolifera* (winter trial *WT*; summer trial *ST*). Error bars indicate the 95% confidence intervals.

DISCUSSION

This study has shown that, a long term (three months), the increase in nutrients *in situ* yields changes at different levels of the vegetated coastal communities, affecting to the different components of the community (i.e. macrophytes, fauna, macroalgae and epiphytes). These changes moving the whole community from autotrophy to heterotrophy in certain seasons of the year. Moreover, the increase in nutrients can affect the net DOC flux, reducing or promoting the DOC release by the community depending on the season of the year. Thus, the most notable effect in DOC flux was found in *C. nodosa*-dominant community where nutrient enrichment can produce a higher DOC release in summer.

This study showed that the increase in nutrients at long term modified the seagrass community, since the proportion of primary producers and consumers in the community was affected. Thus, an increase in the proliferation of fast–growing species, including epiphytes and opportunistic macroalgae, was found especially in summer trials (Table 2) in accordance with previous studies (Sand-Jensen & Borum 1991, Duarte 1995). This has important consequences as these primary producers compete with seagrasses for light and can cause mortality by shading (Short et al. 1995, Hauxwell et al. 2001, McGlathery 2001, Brun et al. 2003). In this case, the increase in fast–growing species has probably not been so detrimental since an increase in seagrass biomass in summer (when environmental conditions are optimal for seagrass growth) was found (Fig. 4). Then, the moderate increase in the availability of nitrogen stimulated the growth of *C. nodosa* as a consequence of an increase in productivity, as noted in Short (1987), Pérez et al. (1991), Alcoverro et al. (1997) or Udy et al. (1999). In addition, fast–growing species can reduce the effect of ammonium toxicity in seagrasses and therefore improve their growing conditions (Moreno-Marín et al. 2016). On the other hand, nutrient enrichment yielded a significant increase in faunal biomass and biodiversity in the summer trial (Table 3 and 4), especially *Bivalvia* (which may indicate that the community acted as a high DOC producer as they are strong DOC consumers) and *Annelida* (which may indicate changes in DOC flux as they are bioturbators). These changes in community structure triggered changes in carbon metabolism and dissolved organic carbon (DOC) fluxes.

Both vegetated communities were autotrophic in the control treatment, either in winter and summer trials (Fig. 2). Particularly, the community dominated by *C. nodosa* was highly autotrophic and acted as DOC source in the summer trial (Fig. 3), since this is the season with the most favourable environmental conditions for seagrass productivity and growth in temperate zones (Touchette & Burkholder 2000, Champenois & Borges

2012, Olivé et al. 2013, Egea et al. 2017). In contrast, this community acted as a DOC consumer during winter (Fig. 3), probably as a consequence of the nearly balanced carbon metabolism in this season (Egea et al. 2017). On the other hand, the community dominated by the macroalgae was autotrophic and acted as a DOC source both in the winter and the summer trial. The reported values in the control treatments were within the same range of values described for both macrophytic communities in the same study area (Egea et al. 2017) and by Duarte et al. (2010) for carbon community metabolism and Barrón et al. (2014) for DOC exportation.

When the two vegetated communities were subjected to nutrient enrichment *in situ*, significant differences were found in the C metabolism and DOC fluxes. Nutrient enrichment produced a decrease in NCP (Fig. 2, 4) and a reduction in DOC released (Fig 3, 4) except in the community dominated by *C. nodosa* during the summer. The decrease in NCP can be attributed, among other reasons, to ammonium toxicity, which affects enzyme kinetics and photosynthetic ATP production (Marshner 1995, Britto & Kronzucker 2002). In addition, an increase in the flux of organic matter to the sediment (which consume O_2, Borum et al. 2005, Pérez et al. 2007, Olivé et al. 2009) can drive an increase in the heterotrophic activity, bearing an increase in respiration in the community. The decrease in DOC release can be the result of the lower productivity and the higher complexity of the macrofaunal community (increase in biodiversity and abundance), which led to an increase in the heterotrophic activity and DOC consumption. The community dominated by *C. nodosa* did not increase significantly the NCP (x1.2) during the summer trial but evidenced a significant strong increase in the DOC released respect to the control (x7). This marked increase in DOC release in this season corroborates previous findings by Egea et al. (2017), and can attributed to an increase in the community biomass (x3) in the most DOC release season by seagrass according to (Mather & Eyre 2000, Barrón et al. 2014, Egea et al. 2017). Moreover, the proliferation of fast–growing species, including epiphytes (x2) and opportunistic macroalgae (x3), tends to release large amounts of DOC (Mateo et al. 2006, Apostolaki et al. 2011, Wada & Hama 2013), explaining the increase of the DOC released by the community. In addition, the intensification of the flux of organic matter from the sediments due to higher temperatures and nutrient availability can also explain the increase de DOC release.

In conclusion, this study showed that the high productivity and organic C exportation in vegetated coastal ecosystems could be overrated under future conditions of high nutrients availability as a consequence of the increase in human activity in the coastal zone. A significant decrease in C capture capacity, and therefore, a significant decrease in

C sequestration is expected, which may hinder their service as natural hot spots in counterbalancing climate change (Nellemann et al. 2009, Duarte et al. 2013). On the other hand, the DOC release by seagrass–dominant communities can be promoted or reduced depending on the season, which has important ecological implications, as DOC released from seagrass populations means an increase in the turnover and a more efficient transference of carbon and energy from primary producers to higher trophic levels (e.g. plankton community) (Navarro et al. 2004, Barrón & Duarte 2009, Egea et al. 2017), which may boost secondary production in the area.

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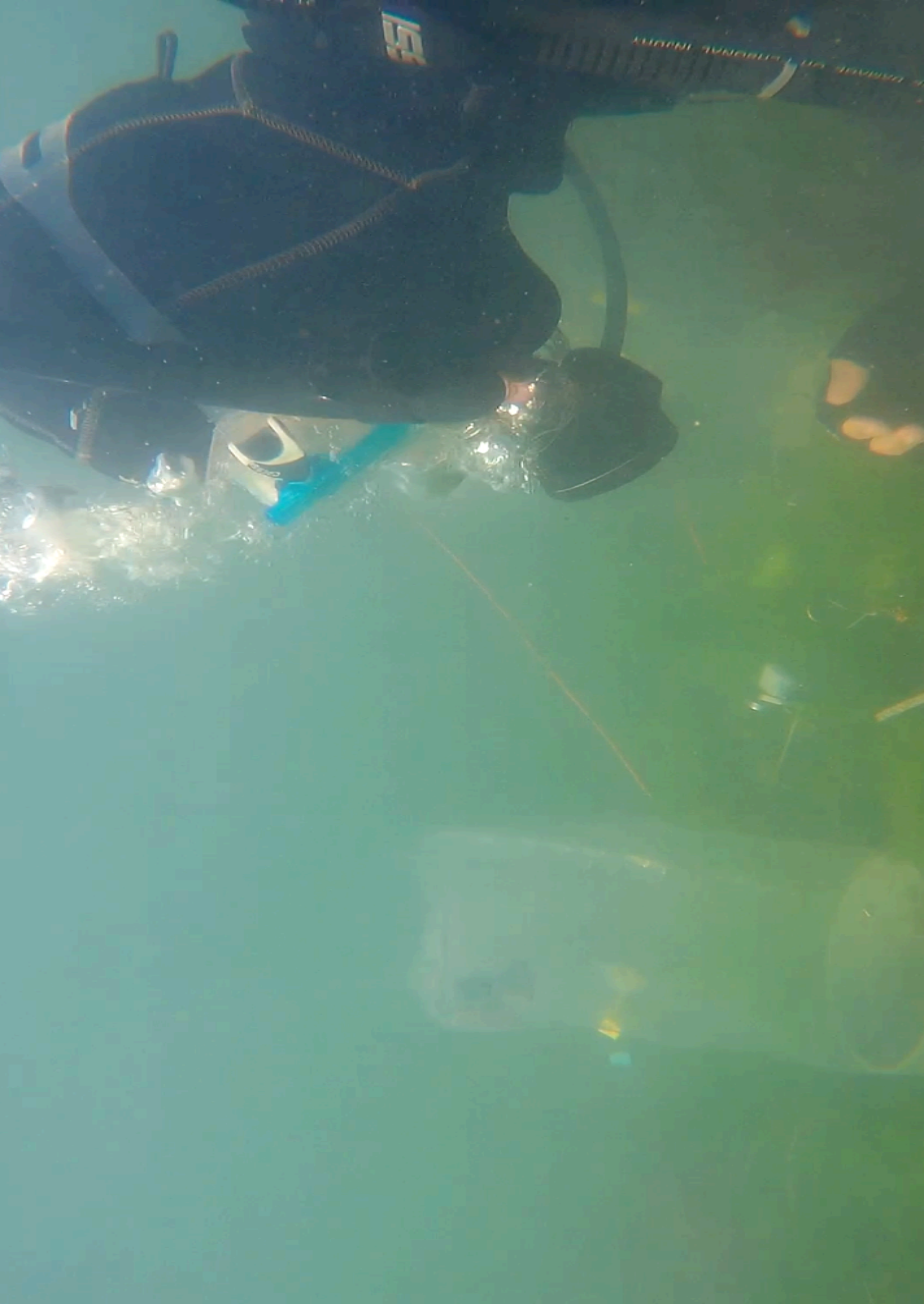

CHAPTER 4

Effect of heat waves on carbon metabolism and dissolved organic carbon (DOC) fluxes in temperate seagrass communities

Submitted to Scientific Reports

Lo que sabemos es una gota de agua; lo que ignoramos es el océano.

Isaac Newton

Effect of heat waves on carbon metabolism and dissolved organic carbon (DOC) fluxes in temperate seagrass communities

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ABSTRACT

Seagrasses are marine foundation plants that form one of the most productive ecosystems worldwide, exporting large quantities of matter, both in particulate and dissolved forms to adjacent communities. Unfortunately, they are currently considered one of the most threated ecosystems on Earth because of anthropogenic pressures, including local and global factors. Regarding global related factors, climate change research is generally focused on the effect of a gradual increase in mean temperatures. However, the frequency of extreme climatic events, such as heat waves, are also expected to increase and may even drive more adverse effects than gradual warming. This work presents an *in situ* experiment evaluating for the first time the effects of heat waves in carbon metabolism and dissolved organic carbon (DOC) fluxes in a community dominated by the seagrass *Cymodocea nodosa* during two contrasting seasons (winter and summer). The results showed that a sudden temporary increase in water temperature did not produce significant differences in carbon community metabolism and DOC fluxes in winter. In contrast, high temperature conditions in summer enhanced significantly the carbon community metabolism and affected positively to DOC fluxes, which increased at a rate of ca. 3 mmol C m^{-2} d^{-1} per each degree increased. This study demonstrates that in this temperate seagrass community, heat waves had a beneficial effect on the community carbon metabolism and DOC fluxes, in contrast to previous researches suggesting negative effects on seagrasses.

INTRODUCTION

Global warming is emerging as a major threat to ecosystems worldwide (Sala et al. 2000, Walther et al. 2002, IPCC 2007). Mean global sea-surface temperatures have increased by circa 0.8 °C over the last century (Levitus et al. 2001, Hansen et al. 2006) and, by the end of this century, is projected to increase by circa 3–4 °C (Meehl et al. 2007). Besides mean sea-surface temperature alteration as a consequence of global change, the frequency and magnitude of extreme climatic events such as sudden heat waves across the globe are expected (Easterling 2000, Meehl & Tebaldi 2004, IPCC 2007). Climate change research is generally concerned with the variation in ecosystems structure and functions associated with gradually increasing mean temperatures (Parmesan & Yohe 2003). However, extreme climatic events such as heat waves will also dictate the response of ecosystems to climate change (Hegerl et al. 2011). There is a lack of a rigorous definition of a heat wave, which usually is defined as a period of abnormal increase of temperature and humidity (IPCC 2012). Typically a heat wave lasts few days, and the increase in air temperature usually translates into an increase in 2–4 °C sea surface temperature (e.g. see Marbà & Duarte 2010). Although heat waves are usually associated with summer periods in the northern hemisphere, temporary and abnormal temperature rises can occur during all year, even in winter according to data from National Oceanic and Atmospheric Administration (NOAA). Understanding how ecosystems respond to extreme climatic events is necessary to predict how ecosystems and biodiversity will respond to climate change (Jentsch & Beierkuhnlein 2008). In particular, understanding the response of communities dominated by foundational plant species (i.e. seagrasses) to extreme climatic events is essential as this will largely shape the ecological response at an ecosystem scale (Royer et al. 2011).

Seagrasses are marine foundation species that form one of the richest and most important coastal habitats (Short et al. 2011). They are globally distributed and well recognised by the ecosystem services they provide, such as high rates of productivity, coastal nutrient cycling, and support to other ecosystems as a habitat and food source (Orth et al. 2006). Most of seagrass species have relatively high light requirements compared to algae and phytoplankton, and this usually constrains them to shallow waters in estuarine and coastal areas (Dennison et al. 1993), where vulnerability to thermal extremes is highest (Anthony & Kerswell 2007, Jiménez et al. 2012). The shallow distribution of seagrasses and its proximity to anthropogenic littoral impacts has led to widespread seagrass losses with a global decline of 7% yr^{-1} (Waycott et al. 2009). This currently regression may be exacerbated by global change (Short & Neckles 1999), including extreme temperature events (Seddon et al. 2000, Moore et al. 2014), which may drive more impacts than gradual warming (Marbà & Duarte 2010).

Seagrass meadows rank among the most productive ecosystems on Earth (Duarte & Chiscano 1999) due to the seagrasses themselves, the epiphytes they support and the accompanying macroalgae (Hemminga & Duarte 2001). The high productivity of seagrass communities contributes to carbon uptake in coastal ecosystem, carbon that can be stored, consumed, buried or exported to adjacent ecosystems in the way of particulate or dissolved form (Duarte & Cebrián 1996, Barrón & Duarte 2009). Dissolved organic carbon (DOC) is the most abundant reservoir of exchangeable organic carbon in the marine C pool; a central factor in the global carbon cycle (Hedges et al. 1997, Hansell & Carlson 2001). The DOC usually acts as a quick transfer of C in the food web because it is easily assimilated by marine organisms and fully involved in the C exchange between communities (Hansell & Carlson 2001, Navarro et al. 2004, Egea et al. 2017). The global net DOC export from seagrass meadows calculated by Barrón et al. (2014) represents 46% of the global net community production (NCP) of seagrass meadows calculated by Duarte et al. (2010). This DOC released may be critical to keep the annual high productivity of communities dominated by seagrass meadows (Egea et al. 2017). Previous studies have shown that the net DOC fluxes in seagrass communities is significantly correlated with water temperature (Barrón & Duarte 2009, Barrón et al. 2014), although these studies are based on seasonal monitoring programs. However, the effect of a sudden increase in water temperature (e.g. heat waves) on DOC fluxes in coastal vegetated habitats is largely unknown.

The effect of warming on seagrasses has been widely studied, since temperature is a key factor for seagrass health, growth and community metabolic rates (Koch et al. 2013). Respiratory rates generally increase faster than photosynthetic ones with temperature, which can lead to a negative carbon balance in the community (Pérez & Romero 1992, Harris & Nixon 2006, Moore & Short 2006). However, little attention has been given to the effect of sudden heat waves on seagrass carbon metabolism in situ, but taking into account the whole community. To date, most studies related to heat waves have focused on the response of terrestrial ecosystems (e.g. Bragazza 2008, De Boeck et al. 2010) and some of them highlight a reduction of ecosystems productivity and respiration (Reichstein et al. 2007). Marine ecosystems also exhibit extreme ecological responses to these events. For instance, studies on coral reefs (Hoegh-Guldberg 1999, Baker et al. 2008), rocky benthic communities (Garrabou et al. 2009) and seaweeds (Wernberg et al. 2013) have reported widespread mortality or reduction in the abundance following heat waves. In seagrasses, most of the knowledge is based on monitoring programs, which correlated seagrass shoot mortality with previous heat waves events in summer (Marbà & Duarte 2010, Fraser et al. 2014, Thomson et al. 2015, Bergmann et al. 2010). However, no heat waves experiments in situ have been carried out to date, in order to study the effects of extreme climate events in seagrass communities at different times of the year. Bearing this in mind, the present study aims to gain insights into how a temperate seagrass community is affected by sudden heat waves by analysing changes at the community level in C metabolism and DOC fluxes. The in situ experiment was replicated in two seasons (winter and summer) to determine if *Cymodocea nodosa* community has a differential response when acclimated to such contrasting environmental conditions.

MATERIAL AND METHODS

Study area. The study was conducted in a subtidal community dominated by the seagrass *Cymodocea nodosa* growing at a depth of 3.5 m (low tide) in Santibañez, in the inner part of Cádiz bay, southern Spain (36.47°; 6.25º W) (Fig. 1). Climatically it fits into a semi-warm subtropical thermal regime whose normal temperature range oscillates between 11 to 28 ºC and 593 mm as average annual precipitation. There is very little fresh water input into the system so the average salinity ranges between 34.1 to 35.6 PSU and the average suspension solids varies from 10 to 30 mg/l. This area is dominated by three benthic marine communities: the two formed by the seagrasses *Zostera noltei* Hornem. and *Cymodocea nodosa* Ucria (Ascherson), and the community dominated by the rhizophytic green algae *Caulerpa prolifera* (Forsskål) J. V. Lamouroux. In between the macrophyte communities, there are also some unvegetated areas. For detailed information of the study area, see previous descriptions in Muñoz & Sánchez de Lamadrid (1994), Morris et al. (2009) and Egea et al. (2017).

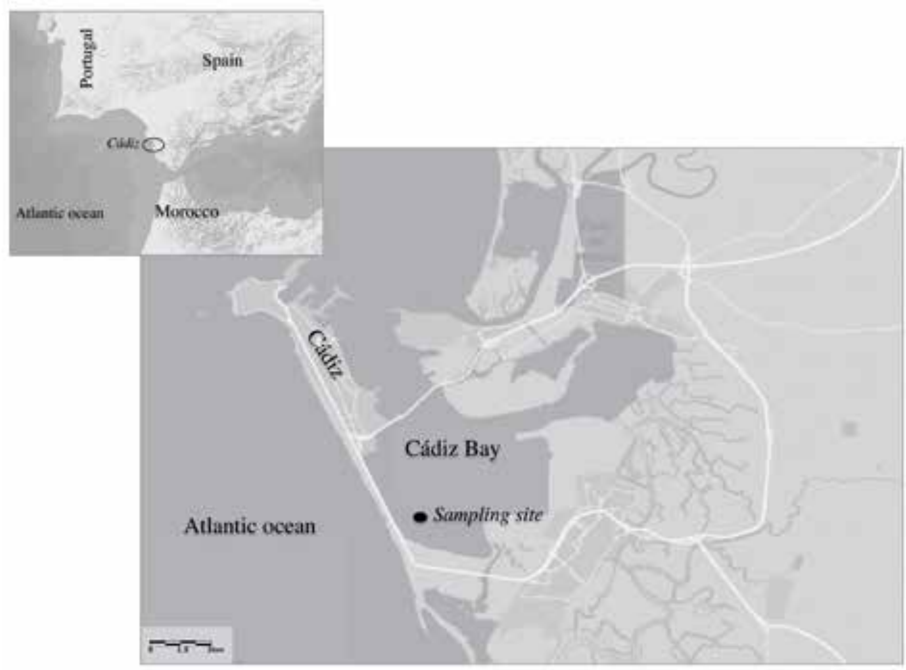

Figure 1. Study site at Cádiz Bay, Spain.

Installation of incubations. The experiment was conducted in late winter (March 2016) and late summer (September 2016), from now called winter and summer trials respectively. Six areas, three replicates for control temperature (CT) and three replicates for high temperature (HT) were randomly selected within a large *C. nodosa* meadow, and benthic chambers (from now called incubations) were placed by scuba diving. Although the community was dominated by *C. nodosa*, is actually an assemblage of several biological components, such as plankton, epiphytes, macroalgae, infauna and sediment microbes. Therefore, the results in this study integrate the entire community as a way to undertake a more realistic approach. Incubations consisted of a rigid cylinder made of a polyvinyl chloride (20 cm diameter) with a sharpened lower end firmly inserted 7–10 cm into the sediment, and an air-tight polyethylene plastic bag fitted over the upper end of the cylinder (Hansen et al. 2000). Each bag was provided with a sampling port to withdraw water samples. To prevent water stagnation in the incubations, the walls of the incubations were made flexible so that they moved with the hydrodynamics. In addition, the three HT incubations had underwater heaters to warm up the water between 1.5 – 2.5 °C in comparison to the surrounding water during the sampling period (circa 24 hours). Incubations were placed in the evening just few hours before nightfall. To avoid the collection of resuspended material resulting from disturbance during installation of the experiment, the first sample was taken 2h after setting up the incubations.

Sampling procedure. To measure community carbon metabolism (through dissolved oxygen –DO– concentration) and DOC fluxes, water enclosed within each incubation was taken through the sampling port using a 50 ml acid-washed syringe at three times during the day: i) just before sunset (S1), ii) right after sunrise (S2) and iii) 6 h after sunrise (S3). In this way, community carbon metabolism and DOC fluxes in dark and light periods can be distinguished (Barrón & Duarte 2009). To calculate the volume of each incubation at the end of the experiment, 20 ml of a 0.1 M uranine solution (sodium fluorescein, $C_{20}H_{10}Na_2O_5$) was injected into each incubation bag, allowing 5 min for mixing. Then, water samples were collected and kept frozen until spectrophotometric determination according to Morris et al. (2013). The volume of water enclosed in the chamber was 10.1 ± 0.5 L (*n*=24). Once the polyethylene plastic bag was removed, macrophyte biomass in each chamber was harvested, rinsed and dried at 60 ºC to estimate aboveground and belowground biomass. Temperature (ºC) and light (lumens m^{-2}) were continuously monitored with HOBO data loggers (UA-002-64) set on each incubation, and in bare sediment close to the experimental plots, which were measured during the sampling period. To better compare the two periods of study (winter and summer), sampling days in each season were chosen with similar tidal range as well as other environmental conditions (e.g. presence of clouds, no rain, wind, etc.) in order to reduce the environmental variability.

Laboratory analysis. Water samples (15 ml) for DO concentration were fixed immediately after collection, kept in darkness, refrigerated, and determined using a spectrophotometric modification of the Winkler titration method (Pai et al. 1993, Roland et al. 1999). Hourly rates of community respiration (R^h) were estimated as the difference in DO concentrations between samplings S2 and S1 ($R^h = DO^{S2} - DO^{S1}$). Hourly rates of net community production (NCP^h) were estimated from the difference in DO concentrations between samplings S3 and S2 ($NCP^h = DO^{S3} - DO^{S2}$). Hourly rates of gross primary production (GPP^h) were computed as the sum of the hourly rates of R and NCP ($GPP^h = R^h + NCP^h$). Finally, daily rates of gross primary production (GPP^d), respiration (R^d) and net community production (NCP^d) were calculated following the calculations (where photoperiod corresponded to photoperiod in each sampling day):

$$GPP^d = GPP^h * \text{Photoperiod} \; ; \; R^d = R^h * 24h \; ; \; NCP^d = GPP^d - R^d$$

Metabolic rates in DO units were converted to carbon units assuming photosynthetic and respiratory quotients of 1, a value used widely for seagrasses (e.g. Barrón et al., 2004). Although these assumptions could introduce uncertainties into the estimates of R, GPP and NCP, this should be a minor concern when the global metabolism of each community is assessed. Dissolved oxygen concentrations in benthic chambers were, on average, 12.0 mg O_2 l^{-1} (S1), 7.7 mg O_2 l^{-1} (S2) and 10.5 mg O_2 l^{-1} (S3). Thus, these changes in DO during the incubations seem relatively moderate, minimizing the propagation of errors when computing metabolic rates (e.g. converting DO to carbon units or assuming respiration at night-time is the same than at daytime) and artefacts due to physiological stress on the communities.

DOC fluxes were estimated by changes in DOC concentration during light and dark periods. Water samples (20 ml) from benthic chambers were filtered through pre-combusted (450 °C for 4 h) Whatman GF/F filters (0.7 μm) and were kept with 0.08 ml of H_3PO_4 (diluted 30%) at 4 ºC in acid-washed material (glass vials encapsulated with silicone-PTFE caps) until analyses. Concentrations of DOC were derived by catalytic oxidation at high temperature (720 °C) and chemiluminescence by using a Shimadzu TOC-VCPH analyzer. DOC-certified reference material (Low and Deep), provided by D. A. Hansell and W. Chen (University of Miami), were used to assess the accuracy of the estimations (http://yyy.rsmas.miami.edu/groups/biogeochem/CRM.html). The instrument blank ranged between 0 to 12 μmol DOC l^{-1} across the different analytical batches. Net DOC flux was calculated by the sum of the DOC flux during the dark period (i.e. the differences between S2 and S1 DOC concentrations) and DOC flux during the light period (i.e. after extrapolating the differences between S3 and S2 DOC concentrations for all hours of light). Thus, when net DOC flux was positive, the community was considered to act as a net DOC producer (i.e. source). However, when net DOC flux was negative, the community was considered to act as a net DOC consumer (i.e. sink). DOC concentrations were, on average, 2.09 mg l^{-1} (S1), 2.37 mg l^{-1} (S2) and 2.54 mg l^{-1} (S3). Thus, these changes in the DOC during the incubations seem relatively moderate, minimizing the propagation of errors on the computation of metabolic rates and artefacts due to physiological stress on the communities.

Data and statistical analysis. To transform light values in lumens m^{-2} to µmols photons m^{-2} s^{-1}, the most commonly conversion factor given in the literature under sunlight was used (1 lumens m^{-2} = 51.2 µmols photons m^{-2} s^{-1}; Carruthers et al. 2001). Light daily rate was calculated using the average daily hours of light (photoperiod) in each station (14,33 and 10,65 h in summer and winter respectively). Prior to any statistical analysis, data were checked for normality (Shapiro-Wilk normality test) and homoscedasticity (Bartlett test of homogeneity of variance test). When necessary, data were transformed to comply with these assumptions through neperian logarithm. Even after several transformations, water temperature values did not meet the normality assumption; therefore, significant differences in water temperature among factors in each trial were analysed using the Kruskal–Wallis test with the Wilcoxon signed–rank test. Statistical differences between factors (temperature and season) in productivity (GPP, R and NCP) and DOC fluxes were analysed by a 2–way ANOVA. When significant differences were found, a Tukey post–hoc test was applied.

When statistical differences were not found using 2–way ANOVA, but differences between treatments were large, it may indicate the existence of a power issue because of the limited sample size (n=3), and then a statistical meta-analysis of the effect size was used to avoid the possibly misleading influence of sample size. While null hypothesis significance testing only informs about the probability of an observation, the presentation of the effect size along with its standard error (SE) provides the two most important pieces of statistical information for biologists: the magnitude estimate of an effect of interest and the precision of that estimate (Nakagawa & Cuthill 2007). Thus, if there are non-significant differences but large effects, it may suggest further research with greater power (Fritz & Morris 2012). To estimate the effect size of the parameters under study, the Hedges' d metric was chosen (Hedges & Olkin 1985), as it is an unbiased estimator that provides a better estimate for small sample sizes. The effect size was presented as Hedges' d ± asymptotic standard error for the effect size according to Nakagawa & Cuthill (2007). Hedges' d metric values above 0 indicate a positive effect, bellow 0 indicate a negative effect, and equal to 0 indicates no effect on the parameter under investigation. The bigger the number either on the positive or negative direction tells about the magnitude of the effect. Cohen (1988) has proposed ‘conventional’ values as benchmarks for what are considered to be ‘small’, ‘medium’, and ‘large’ magnitude of the effects (d = 0.2, 0.5, 0.8, respectively) (Nakagawa & Cuthill 2007).

Data are presented as mean ± SE. The significance level (α) set in all tests performed was 0.05. Statistical analyses were computed with R statistical software .0.2 (R Development Core Team 2013).

RESULTS

Abiotic variables. Mean water temperatures in CT treatment varied between 16.5 ± 0.02 ºC in winter to 24.6 ± 0.03 ºC in summer. Water in HT treatment was statistically higher in both sampling events ($p < 0.001$) when compared to CT treatment, averaging 18.4 ± 0.02 ºC and 26.7 ± 0.03 ºC in winter and summer respectively (Table 1). Underwater daily irradiance at the canopy level of *C. nodosa* meadow at midday was 147 ± 13 µmol photons m^{-2} s^{-1} during the winter trial and 260 ± 18 µmol photons m^{-2} s^{-1} during the summer trial.

Table 1. Water temperature in the different treatments and seasons. All data are in ºC and expressed as mean ± SE. CT: Control temperature; HT: High temperature. Superscript letters indicate significant differences between treatments in each season at α = 0.05.

Season	Treatment	Water temperature	Temperature range	Temperature increase
Winter	CT	16.5 ± 0.02^{a}	16 – 17.4	-
	HT	18.4 ± 0.03^{b}	17.9 – 18.9	1.9 ± 0.01
Summer	CT	24.6 ± 0.02^{a}	23.9 – 25.3	-
	HT	26.7 ± 0.03^{b}	26 – 27.3	2.1 ± 0.01

Effects on community metabolism. High temperature treatments produced an increase in Productivity:Respiration (P:R) ratio of 15% during the winter trial (from 1.3 ± 0.04 to 1.5 ± 0.05) and 6% in the summer trial (from 1.8 ± 0.09 to 1.9 ± 0.12). The GPP and NCP in CT were significantly higher in summer than in the winter trial. Temperature increase only affected significantly to GPP, R and NCP during the summer but not in the winter trial. Hence, GPP, R and NCP were ca. 1.6, 1.5, 1.8 times higher under HT than under CT in the summer trial (Fig. 2, Table 2).

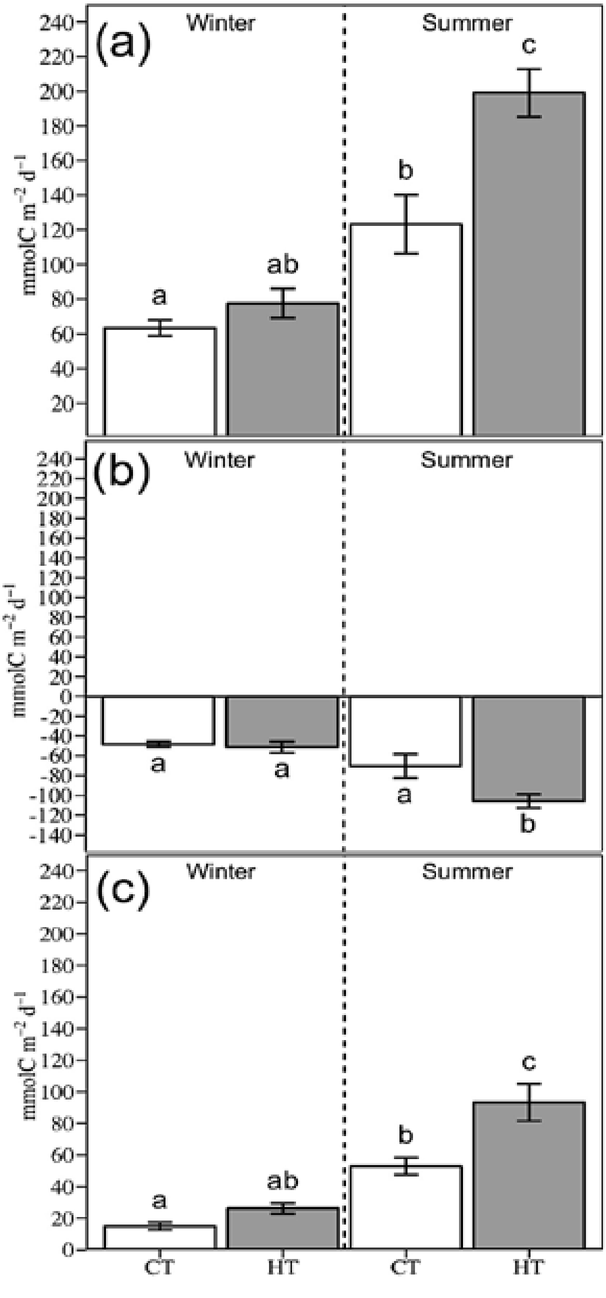

Figure 2. Effect of heat waves on (a) Gross Primary Production (GPP), (b) Respiration (R) and (c) Net Community Production (NCP) in winter and summer. CT: Control temperature; HT: High temperature. Different letters indicate significant differences between treatments and seasons. Data are expressed as mean ± SE (*n*=3).

Table 2. Results of the 2–way ANOVA analysis of the factors temperature and season in the carbon community metabolism. GPP: gross primary production; R: respiration; NCP: net community production.

		GPP		
	df	**MS**	**F**	***p***
Season	1	24703	58	**<0.001**
Temperature	1	6072	14	**0.005**
Season & Temperature	1	2839	6.687	**0.032**
Residuals	8	425		
		R		
	df	**MS**	**F**	***p***
Season	1	4380	25	**0.001**
Temperature	1	1099	6	**0.037**
Season & Temperature	1	785	4.485	0.067
Residuals	8	175		
		NCP		
	df	**MS**	**F**	***p***
Season	1	8279	61	**<0.001**
Temperature	1	2004	15	**0.005**
Season & Temperature	1	638	4.669	0.063
Residuals	8	137		

DOC fluxes. The dissolved organic carbon flux was similar in CT and HT treatments in the winter trial. In contrast, during the summer trial, the DOC flux in HT doubled that of the CT (Fig. 3). Although, differences were not significant (Table 3), effect sizes revealed that temperature largely affected positively to DOC fluxes in the summer trial (Hedges' $d = 0.79 \pm 0.85$). Overall, DOC fluxes ranged from ca. 25 – 30% of NCP in the summer trial to ca. 100% of NCP in the winter trial and even exceeding the NCP under CT (126% of NCP).

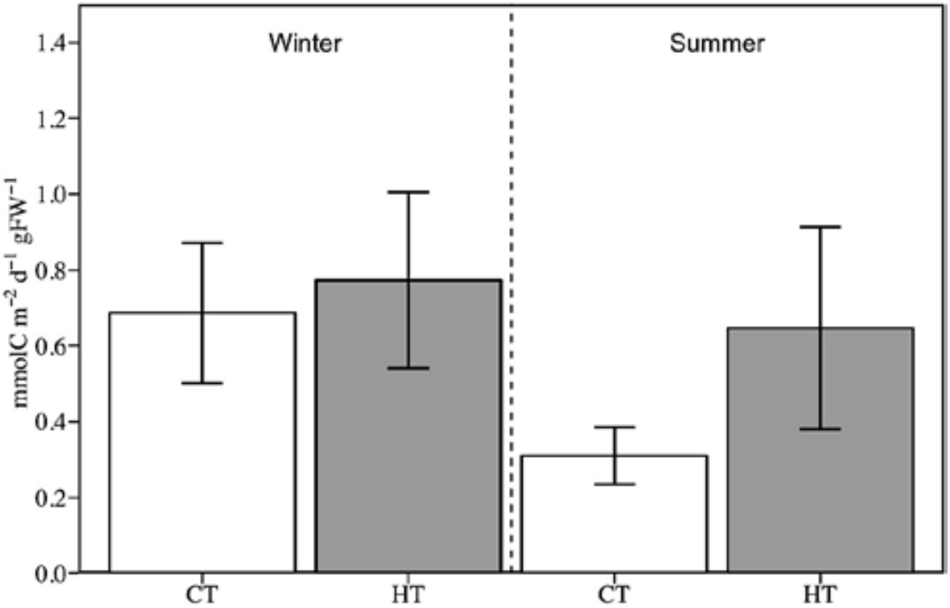

Figure 3. Effect of warming on net DOC fluxes in winter and summer. CT: Control temperature; HT: High temperature. Data are expressed as mean ± SE (*n*=3).

Table 3. Results of the 2–way ANOVA analysis of the factors temperature and season in the dissolved organic carbon (DOC) fluxes.

	df	**MS**	**F**	***p***
Season	1	0.19027	1.538	0.250
Temperature	1	0.13548	1.095	0.326
Season & Temperature	1	0.04746	0.384	0.553
Residuals	8	0.12370		

DISCUSSION

This study demonstrates that a sudden and temporal increase in water temperature *in situ*, which characterizes heat waves, may have significant consequences in seagrass communities, especially during the summer, a fact that has not been previously reported in any seagrass study. Thus, this sudden and temporal increase in water temperature enhances carbon community metabolism (i.e increased P:R ratio, GPP and NCP) and, in addition, affected positively the DOC fluxes.

Cymodocea nodosa community was highly autotrophic and acted as a DOC source in both treatments (high and control temperature) and seasons (winter and summer), which support previous findings in the same study area (Egea et al. 2017). Moreover, the reported values are within the same range of values described by Duarte et al. (2010) for carbon community metabolism and Barrón et al. (2014) for DOC exportation. In the winter trial, there were not significant differences in the carbon community metabolism and DOC fluxes. In contrast, during the summer trial, high temperature conditions significantly increased the carbon community metabolism (Fig. 2 and table 2), and affected positively to DOC fluxes, which was twice of the control (Fig. 3). Hence, this study demonstrated that a sudden and temporal increase in water temperature can produce a positive effect in the carbon community metabolism and in the DOC fluxes in a seagrass dominated community, but depending on the season of the year. The *Cymodocea nodosa* population may be far from its optimum temperature for growth during the winter but very close during the summer (Pérez & Romero 1992), and therefore opposite trends can be expected as it was found (i.e. an improvement of carbon metabolism and fluxes in winter). One of the major differences between both seasons is the light received at the top of the canopy, being ca. 240% higher in summer than in winter (ca. 13,300 ± 900 mmol photons m^{-2} d^{-1} in summer and ca. 5,600 ± 480 mmol photons m^{-2} d^{-1} in winter). Thus, the limited response found in the community to temperature increase in winter may be due to the significantly lower light incidence, which prevents photosynthetic organisms in the community from increasing their metabolism and DOC release with high temperature, as light limitation is one of the most important factors for seagrass metabolism (Touchette & Burkholder 2000, Peralta et al. 2002, Brun et al. 2003, Mateo et al. 2006), and also has been recently suggested as a key factor in the DOC release by coastal marine meadows (Barrón et al. 2014).

Our results showed that the positive effect in carbon metabolism derived from a sudden increase in temperature does not only affect to the seagrass *Cymodocea nodosa* but also to the whole community (e.g. plankton, epiphytes, macroalgae, infauna and sediment microorganisms). The net community production (NCP) significantly increased under high temperature when compared to CT treatment in summer (76% from 52.9 to 93.3 mmol C m^{-2} d^{-1}) (Fig. 2c, table 2). The higher increase in GPP (61% from 123 to 199 mmol C m^{-2} d^{-1}) when compared to respiration (50% from -70 to -105 mmol C m^{-2} d^{-1}) (Fig. 2a, b) was the main responsible of such significant increase in NCP. Thus, the P:R ratio was typically higher than 1 in all treatments and, in fact, the HT treatments produced an increase in P:R ratio (15% in winter and 6% in summer). This is in contrast with the Metabolic Theory of Ecology (MTE) which predicts respiration rates to increase faster with warming than primary production rates do (Brown et al. 2004, Harris et al. 2006, Sarmento et al. 2010), because activation energies for autotrophs are half of that for heterotrophs, which results in a reduction of P:R ratio and a greater chance of shifting the system to heterotrophy (Harris et al. 2006). However MTE is based on the gradual warming due to climate change and does not take into account the effect of a sudden increase in temperature due to heat waves, as has been analysed in this work.

Most of the previous research in heat waves in seagrass ecosystems showed negative consequences on seagrasses, including shoot mortality and dieback (Bergmann et al. 2010, Collier & Waycott 2014, Fraser et al. 2014, Thomson et al. 2015). These studies contrast with the results found in the present work. Previous studies were usually based on data collected after heat wave events or heat waves stress induced experimentally under mesocosm conditions. In the first case, the results reported are probably a consequence of the interaction between elevated temperatures and other factors such as light limitation, as noted by different authors (Fraser et al. 2014, Thomson et al. 2015), or because the experiment were developed with species living at the extreme of their thermal tolerance region (Thomson et al. 2015). In the second case, they usually focused on the ecophysiological response of isolated seagrass plants, without considering the whole community and their interactions and feedbacks, and the results may be subjected to uncertainties derived from seagrass manipulation and possible artefacts associated with the experimental chambers or design. In contrast, the present work makes an *in situ* approximation at the community level, with a minimum disturbance of natural seagrass communities, and hence can be considered as a more integrative response and closer to the natural conditions.

The significant increase in NCP recorded here can trigger noteworthy consequences to the whole seagrass community, as this turn the community into more autotrophic and then amplify their role as a major CO_2 sink. This C uptake surplus may help seagrasses to synthesize more carbon skeletons that can be directly used for growth or stored, supporting seagrass growth during unfavorable growing conditions (Alcoverro et al. 2000, Brun et al. 2002, 2008). The NCP of vegetated coastal areas can be used as a proxy of the trophic state of the system (i.e. autotrophic vs heterotrophic), and then determine whether it acts as a sink or source of atmospheric CO_2 (Maher & Eyre 2012, Tokoro et al. 2014). Thus, this study showed that in a future scenario where the frequency of extreme climate events will be more common, probably the metabolism of temperate seagrass ecosystems dominated by seagrasses increases, moving towards autotrophy, and hence tending to act as a greater CO_2 sink, which may help to partially offset the rise of carbon dioxide levels.

Besides their high productivity, seagrass communities represent areas with large DOC production; they are even more important as net DOC producers than previously recorded (Barrón & Duarte 2009, Barrón et al. 2014). It can be estimated that the global net DOC export from seagrass meadows (calculated by Barrón et al. 2014) represents 46% of the global NCP of seagrass meadows (calculated by Duarte et al. 2010). In the present study, winter and summer DOC fluxes in control temperature treatment were ca. 310 and 680 µmol C gFW^{-1} m^{-2} d^{-1}, which is similar to the net DOC fluxes recorded in both seasons in an annual study for this specie in the same location (Egea et al. 2017), and within the range of values reported by Barrón et al. (2014). These results represented ca. 30% and 126% of the NCP respectively. Previous studies have shown that the net DOC flux in seagrass communities was significantly correlated with water temperature (Barrón & Duarte 2009, Barrón et al. 2014), but it is important to note that are based on seasonal monitoring programs. Barrón et al. (2014) indicated that each degree of temperature increase led to an increase of about 1.5 mmol C m^{-2} d^{-1} in net DOC flux in *Posidonia oceanica* stands. The results of the present study confirmed this relationship between temperature and the DOC released in seagrass communities, which was independent of the season. Thus, an increase of ca. 3 mmol C m^{-2} d^{-1} by each degree of temperature raised was recorded, both in winter and in summer. However, it is known that seagrass biomass may vary greatly in this area throughout the year (Duarte 1989, Brun et al. 2003), and hence the results can vary when normalized by biomass. In this case, we still recorded a weak increase (13%) in DOC release in winter (from ca. 680 to 770 µmol C gFW^{-1} m^{-2} d^{-1}) in contrast to the summer when the net DOC flux doubled (from ca. 310 to ca. 650 µmol C gFW^{-1} m^{-2} d^{-1}). Hence, this research evidences that an increase in the frequency of heat waves in the next decades will yield an increase in seagrass DOC fluxes and especially in summer, when the net DOC fluxes in coastal vegetated ecosystems are highest (Barrón & Duarte 2009, Egea et al. 2017). This has important ecological implications since an increased DOC release from seagrass stands means a quicker and more efficient transference of carbon and energy from primary producers to higher trophic levels (e.g. plankton community) (Navarro et al. 2004, Barrón & Duarte 2009, Egea et al. 2017), which may boost secondary production in the area.

In summary, if the frequency of sudden extreme climate events such as heat waves is expected to increase in the next decades, it will trigger a significant increase in the metabolism of the community dominated by *C. nodosa*, leading to higher CO_2 uptake in the system that can be captured or exported in particulate or dissolved form to other ecosystems. The latter supports the increase in DOC fluxes recorded when water temperature increases, especially in summer. These results demonstrate that heat waves had a positive effect on seagrass communities, in contrast to that recorded in previous studies. However, this finding has to be restricted to this temperate seagrass community, and therefore further research following this integrative *in situ* approach should be done in communities bearing different species and from different bioregions.

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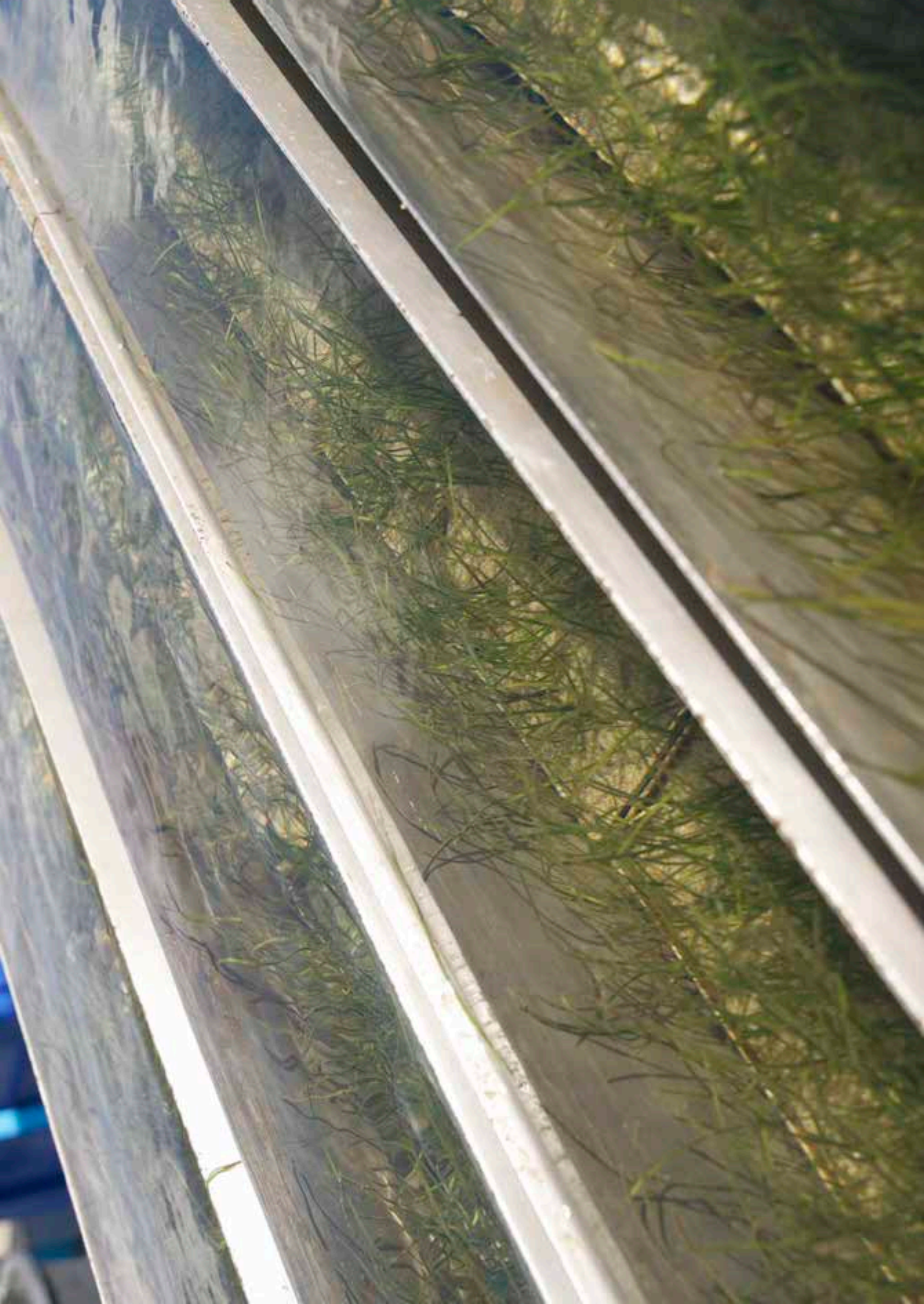

CHAPTER 5

Effects of ocean acidification and hydrodynamic conditions on carbon metabolism and dissolved organic carbon (DOC) fluxes in seagrass populations

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Nadie excepto nosotros mismos puede liberar nuestra mente.

Bob Marley

Effects of ocean acidification and hydrodynamic conditions on carbon metabolism and dissolved organic carbon (DOC) fluxes in seagrass populations

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ABSTRACT

Global change has been acknowledged as one of the main threats for biosphere and ecosystem services they provide, especially for marine ecosystem. Although seagrasses play a critical ecological role in coastal ecosystem, their response to ocean acidification (OA) and climate change is not well understood. Previous studies focused on the effect of OA separately, but the combined effect with other co-factors that will act together during climate change should be addressed. Thus, the impact of different hydrodynamic regimes and CO_2 increase on seagrass performance remains unknown. We here attempted to elucidate the effects of OA under different current velocities on the productivity of the seagrass *Zostera noltei* (using changes in dissolved oxygen as a proxy for the seagrass carbon metabolism) and dissolved organic carbon (DOC) fluxes, using an open-water outdoor mesocosm system during four weeks. Under current pH condition, current velocity had a positive effect in productivity, but it depended on shoot density. However, the differences between current velocity levels disappear under OA conditions, when a significant increase in gross production rate and respiration was found, suggesting that *Z. noltei* is carbon limited at the current inorganic carbon concentration of seawater (pCO_2). In addition, an increase in non-structural carbohydrates under OA conditions was found, which may drive better growing conditions and a higher resilience for seagrasses subjected to environmental stressors. Regarding DOC fluxes, a direct and positive relationship was found between current velocity and DOC released, both at current pCO_2 and OA conditions. Therefore, it is concluded that OA under high current velocity may produce a favourable growth scenario for *Z. noltei* populations, increasing its productivity, non-structural carbohydrate concentrations, and enhancing DOC release. Our results help to better understand how seagrass ecosystems may face up climate change with the important implications regarding the resilience and conservation of these threatened ecosystems.

INTRODUCTION

Over the last century, human activities have produced large amounts of CO_2 through fossil fuel burning, intensive agriculture and deforestation (IPCC 2014). The concentration of CO_2 in the atmosphere has risen from 280 ppm in preindustrial times to 400 ppm in 2015 measured at Mauna Loa Observatory, one of the longest records station of direct measurements of atmospheric carbon dioxide (Espaciais-inpe & Jos 1996). By the middle of this century, atmospheric CO_2 levels could reach more than 500 ppm, and surpass 800 ppm by the end of the century (Friedlingstein et al. 2006). During the industrial era, the ocean has absorbed about one-quarter of this anthropogenic CO_2 (Sabine & Feely 2004, Canadell et al. 2007) acting as a carbon sink and thus contributing to the key ecosystem service of climate regulation. A portion of CO_2 absorbed by oceans is stored in living biomass and sequestered in sediments, but a large amount remains in its inorganic form. The increase of inorganic carbon stored in the oceans has driven a reduction in seawater pH and has promoted changes in the seawater chemistry in a process commonly referred to as "ocean acidification" (OA) (Caldeira & Wickett 2003, 2005, Orr et al. 2005, Doney et al. 2009, Koch et al. 2013). The global ocean pH is expected to fall to between 8.05 and 7.6 by the end of this century (IPCC 2013, Prinn et al. 2011). This raises concern about the possible impacts of these changes on marine organisms. The OA is a ubiquitous stressor which is likely to lead to negative consequences in the future for marine organisms (Kroeker et al. 2010), ecosystems (Fabry 2008) and ecosystem services provision (Cooley et al. 2009). In recent years studies underscore the crucial role of shallow coastal ecosystems function not only as transition zones between land and ocean but also as carbon sequestration filter (Nellemann et al. 2009, Watanabe & Kuwae 2015)

Seagrasses are marine flowering plants that form one of the richest and most relevant coastal ecosystems (Short et al. 2011) as a consequence of the large number of ecological services they provide (Nelleman et al. 2009, Short et al. 2011, Tokoro et al. 2014) including long-term carbon catchment (Nellemann et al. 2009, Tokoro et al. 2014). They cover less than 0.2% of the ocean surface, but still they contribute to a disproportionate amount of marine net primary productivity (NPP) (1%) and are responsible for approximately 15% of carbon storage in the oceans (Laffoley & Grimsditch 2009). Seagrass-dominated ecosystems are highly productive habitats (Duarte & Chiscano 1999) playing an important role in the carbon cycle of coastal areas (Duarte & Chiscano 1999, Hemminga & Duarte 2000). The excess of organic matter they produce can be exported to adjacent ecosystems in the way of particulate or dissolved form (Duarte & Cebrián 1996, Barrón & Duarte 2009). Dissolved organic carbon (DOC), with about

700 PgC, represents only 2% of the carbon pool in the ocean (Ciais et al. 2013). However, it is a central factor in the global carbon cycle (Hedges et al. 1997, Hansell & Carlson 2001) acting as a quick transfer of C and energy in the food web, as it is easily assimilated by marine organisms and fully involved in the C exchange between communities (Hansell & Carlson 2001, Navarro et al. 2004). The global net DOC export from seagrass meadows, as calculated by (Barrón et al. 2014), represents as much as the 46% of the global NCP of seagrass meadows, as reported by (Duarte et al. 2010). Hence, this DOC released may be critical to keep the annual high productivity of communities dominated by seagrasses (Ziegler & Benner 1999). However, few studies have studied the relation between productivity and DOC fluxes and how much DOC is released regarding the productivity of the community *in situ* (for review, see Barrón et al. 2014). While there has been extensive research in the open ocean DOC dynamics (for review, see (Hansell & Carlson 2001, Carlson 2014) the role of coastal ecosystems in the global DOC cycle is still inadequately understood despite being the area where a higher proportion of ocean DOC tends to accumulate (Dafner & Wangersky 2002).

In spite of the ecological importance and the services that seagrass meadows provide, increased anthropogenic pressure has led to widespread seagrass losses in shallow estuarine and coastal zones (Orth et al. 2006). With a global surface meadow decline of 7% yr^{-1} (Waycott et al. 2009), the loss of seagrasses is currently the highest loss-rate of any ecosystem on the planet. The OA could either ameliorate or aggravate the current decline of seagrasses (Koch et al. 2013). Thus, the responses of seagrasses to OA must be considered for an effective management of coastal regions in the future. The effects of increased CO_2 concentrations on seagrasses may depend on the degree of carbon limitation in natural systems. Carbon limitation has been partly attributed to the thickness of the diffusive boundary layer (DBL) surrounding leaf surfaces at low current velocities (Larkum 1989, Madsen & Sand-Jensen 1991, Koch 1994) or to a relatively inefficient HCO_3^- uptake system (Beer et al. 1980). The faster the water moves, the thinner the DBL becomes (Massel 1999) and, consequently, the faster the transfer of CO_2 molecules from the water column to the seagrass cells. Thus, higher current velocity leads to a reduction of the DBL thickness and this favours CO_2 uptake and photosynthesis (Koch et al. 2006). However, increasing flow velocity may reduce photosynthetic rates due to enhanced sediment resuspension (i.e. decreasing light levels; Koch 2001) and by leaves self-shading because of leaves collapse onto each other when currents are strong affecting productivity (Koch et al. 2006). Hence, current velocity conditions can produce a positive or a negative effect on seagrass productivity, depending on what effect will have more relevance: either a possible increase in CO_2 uptake as a result of the DBL decrease, or alternatively, a reduction in photosynthesis as a consequence of leaf self-shading or sediment resuspension. It is important to highlight that hydrodynamic conditions in coastal areas worldwide may change as a consequence of anthropogenic engineering activities that change tidal flows (Kennish 2013) and nowadays as a result of climate change, which is expected to increase the frequency and intensity of storms and waves stress (Young et al. 2011).

The effects of OA on seagrasses so far have mainly focused on how elevated CO_2 concentrations will affect seagrass productivity, light requirements and nutrients content (Beer & Koch 1996, Zimmerman et al. 1997, Palacios & Zimmerman 2007, Jiang et al. 2010). However, the specific outcome of a CO_2 increase is still unclear because there is an array of ambient variables, which could ameliorate or aggravate the effects of the CO_2 increase. The interaction between CO_2 increase and hydrodynamic conditions have not been addressed in deep, in spite of the importance of the latter at different seagrass ecosystem levels (Morris et al. 2008, De Los Santos et al. 2009, Bal et al. 2011, González-Ortiz et al. 2014). Likewise, little attention has been given to the effect of hydrodynamic on DOC fluxes in seagrass meadows. Recent studies have highlighted the direct relationship between productivity and DOC fluxes (Barrón et al. 2014). Therefore, if hydrodynamic affects photosynthesis, and thus productivity (Koch et al. 2006), different current velocities may also produce a significant effect on DOC fluxes released by seagrass populations, which could affect C fluxes in coastal communities. Therefore, this study aims to explore the consequences in productivity and DOC fluxes in the temperate seagrass *Zostera noltei* subjected simultaneously to ocean acidification and different current velocities.

MATERIAL AND METHODS

Experimental design. The study was conducted on the temperate seagrass *Zostera noltei* Hornemann in an open-water outdoor mesocosm system at the Royal Netherlands Institute for Sea Research (NIOZ) during four weeks in summer 2014. This time span is enough to detect any treatment-driven changes in physiological and morphological traits in this fast-growing species (e.g. Peralta et al. 2002). Twelve small flume tanks were used to expose plants to three contrasting current velocities (i.e., four flume tanks per current velocity), which were connected to two big seawater reservoirs (ca. 1,500 l and ca. 5,000 l). The twelve flume tanks were constructed as independent rectangular stainless-steel containers (13 × 26 × 130 cm). Each flume tank used individual water pumps (further details are given in (Peralta et al. 2006). The reservoirs fed the flume tanks with seawater subjected to two pH levels (i.e six flume tanks per pH level). The factors were manipulated in a fully crossed design, making a total of six-crossed combination of factors (i.e. treatments), each with two replicates (12 units). Each reservoir received daily ca. 300 l pre-filtered (2 µm) water from Oosterschelde estuary with the aid of a pump. The reservoirs were placed higher than the fume tanks to use the gravity to fill them with their treated seawater at a turnover time of 1 d. Excess seawater outflowed slowly through the edge of the flume tanks (Fig. 1). This high rate of seawater renewal ensured homogenous temperature maintenance among the flume tanks. In each small flume, 4 stainless-steel pots (12 × 12 × 25 cm) were allocated. Pots were completely filled (3.6 l) with a homogeneous mix of clay, sand and gravel. In each pot, about 150-170 individual *Z. noltei* shoots were planted one by one by hand (ca. 22.5 ± 0.5 g FW pot^{-1}) resulting in a total of ca. 8,000 shoots planted among all units (the twelve flume tanks where the factors velocity and CO_2 were combined). Light and temperature conditions were natural over the duration of the experiment (ca. 1,250 mol photons m^{-2} d^{-1} and 23.5 °C measured daily at 10:00 am). To reduce differences in nutrient availability in the water column due to current velocity effects on sediment fluxes, all sediment was thoroughly washed and the water column was daily renewed in each flume tank. Total fresh plant biomass of each pot was measured at the start and at the end of the experiment. Algae, epiphytes and dead leaves were removed daily and leaves were fresh weighed.

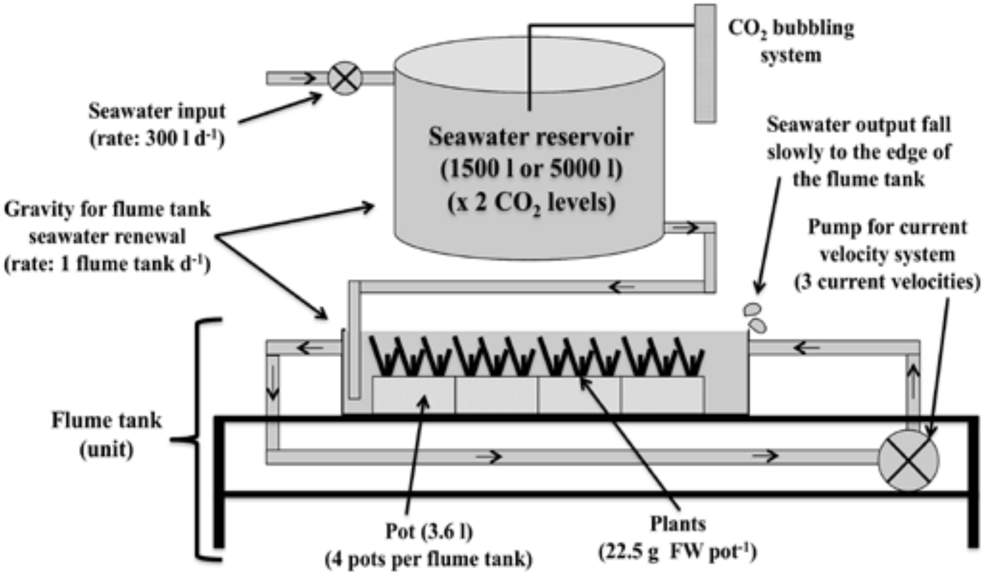

Figure 1. Simplified diagram of the open-water mesocosm system.

Field plant collection. Individual shoots of *Z. noltei* were randomly collected from an intertidal bed in the mudflats of Viane (51^{0}39N, 4^{0}01E; Oosterschelde estuary), in the southwestern part of The Netherlands. The permits for collect seagrass were issued by the province of Zeeland (local government) and have the numbers 09002713/NB.08.068 and 12076427. Healthy looking shoots with intact rhizomes were transported to the laboratory within 60 min of collection in an ice chest where they were cleaned of visible epiphytes, and were held in aerated natural water over 5 days under sub-saturating light (ca. 150 µmol photons m^{-2} s^{-1}), at 20 ºC in a 16:8 h light:dark cycle.

Table 1. Summary of seawater chemistry in the different treatments. Total alkalinity (TA), pH total (pH_T), dissolved inorganic carbon (DIC) and pCO_2. Salinity were ca. 30 and temperature were ca. 20.9 ^{0}C in all cases. Data are means ± SE, *n*=8. LV: Low velocity; MV: Medium velocity; HV: High velocity; CpH: Current pH; FpH: Forecasted pH.

Factors		**TA (μmol kg^{-1})**	**pH_T**	**DIC (μmol kg^{-1})**	**pCO_2 (ppm)**
pH	**Velocity**				
CpH	LV	2,530 ± 18	8.1 ± 0.02	2,232 ± 14	372 ± 15
FpH	LV	2,457 ± 6	7.8 ± 0.02	2,314 ± 11	820 ± 34
CpH	MV	2,642 ± 71	8.1 ± 0.02	2,363 ± 57	448 ± 13
FpH	MV	2,484 ± 11	7.8 ± 0.02	2,340 ± 13	835 ± 55
CpH	HV	2,471 ± 8	8.1 ± 0.02	2,214 ± 9	436 ± 27
FpH	HV	2,473 ± 11	7.8 ± 0.01	2,328 ± 8	810 ± 26

Acidification and current velocities. Acidification was manipulated in the two large reservoirs with one (1,500 l) for current pH (CpH) and another bigger (5,000 l) for forecasted pH (FpH). The CpH reservoir was kept at control conditions (mean target pH in total scale pH = 8.10) and the FpH reservoir was manipulated according to the scenario forecasted by the Intergovernmental Panel on Climate Change (IPCC 2013), and was acidified via bubbling with a CO_2 enriched gas mixture (Westfalen Gassen Netherland BV 250 bar) (mean target pH in total sale pH = 7.65). The FpH reservoir was spiked only once per day, just before the filling of the reservoir during high tide. Changes in CO_2 concentration were controlled through daily measurements of water pH, salinity and temperature (at 9:30, 13:00 and 19:00 local hour) at the top of the seagrass canopy in each flume tanks. Electrodes were calibrated weekly using standardized pH buffers. As a consequence of plant productivity, pH in each flume tank varied slightly throughout the day (ranged from 7.8 to 7.9 in FpH and from 8.1 to 8.3 in CpH) but the high water renewal allow to kept pH almost constant during the whole day. Seawater samples were collected weekly and immediately filtered (Whatman GF/F filters, 0.7 µm) and stored before adding $HgCl_2$ at 4°C. Total alkalinity (TA) was measured by gran titration using a 888 pH electrode Metrohm meter combined with glass electrode (Metrohm 6.0259.100), and was finally calculated by the nonlinear least square method. Carbon chemistry parameters were derived using pH (on the total scale), total alkalinity (TA), temperature and the CO2SYS package (Lewis & Wallace 1998) with the K1 and K2 constants from (Mehrbach et al. 1973), as modified by (Dickson & Millero 1987), and the $KHSO_4$ constant from (Dickson 1990). The pH, TA, temperature, salinity and carbon speciation within each flume tank are shown in Table 1. Current velocities were manipulated in the small flume tanks. The low (LV), medium (MV) and high velocities (HV) were adjusted to approximately 0.01, 0.10

and 0.35 $m \cdot s^{-1}$, respectively, using individual water pumps for each flume. The selected velocities were a good approximation of the lowest and highest velocities that might be found at vegetated intertidal areas in the Oosterschelde estuary (Bouma et al. 2005, Peralta et al. 2006).

Sample collection. The DOC fluxes were determined in each small flume tank weekly. Three water samples per flume tank were taken from the surface using a 50 ml (polyethylene) acid-washed syringe in 3 different experimental times: i) just before sunset; ii) then leaving the chambers overnight and sampling right after sunrise; and iii) 6 h after sunrise. Thus, night and light period for DOC fluxes were discriminated (Barrón & Duarte 2009). To transform the flume tanks in a closed system during the sampling period for DOC, water renovation was stopped. To measure the DOC exchanges between the flume tank and the atmosphere, samples were collected at the beginning of the experiment (i.e. when flume tanks were filled and before plants were planted) to subtract the atmosphere-water DOC exchange effect. The seagrass biomass planted in the units ranged between 1 to 2.5 g l^{-1} which was within the biomass-volume ratio used in previous incubation chambers (e.g. Barrón & Duarte 2009).

Carbon metabolism of *Z. noltei* population was assessed using four benthic chambers (ca. 0.7 l) (named incubations from here onwards) haphazardly installed at the end of the experiment in each small flume tanks (one per pot). Therefore, four replicates per unit were deployed. The incubations consisted of a polyvinyl chloride rigid cylinder of 8 cm in diameter firmly inserted, with a sharpened side, about 5 cm into the sediments and a gas-tight polyethylene plastic bag fitted to the cylinder (Hansen et al. 2000) enclosing *Z. noltei* plants. Each incubation had a sampling port to withdraw water samples. To avoid water stagnation inside the incubations, their walls were made flexible so that they could move to some extent by the wind effect. The incubations were deployed in the experimental pots in the evening, two hours before nightfall to avoid the collection of resuspended material from the incubations. The first sampling event started 1h after setting them up. The enclosed water was sampled through the sampling port using a 50 ml (polyethylene) acid-washed syringe in three different experimental periods following the three DOC sampling periods aforementioned. This is the usual time lapse used for this methodology (e.g. Barrón & Duarte 2009), because is enough large to determine DO changes while avoiding oxygen oversaturation in the incubations. To calculate the volume, 20 ml of a 0.1 M uranine solution (sodium fluorescein, $C_{20}H_{10}Na_2O_5$) was injected into each incubation at the end of the incubation period, allowing 5 min for mixing, and determining the resulting dilution according to (Morris et al. 2013). Water volume enclosed in the incubations averaged 0.68 ± 0.02 L. Once the polyethylene plastic bag was removed, *Z. noltei* biomass was collected (including belowground biomass), rinsed, dried at 60 ºC and weighed.

Biomass samples for carbohydrates (in both aboveground and belowground tissues) were collected at the end of the experiment. Plant production was estimated through biomass differences in each pot measured at the beginning and end of the experiment.

Laboratory analysis. Fluxes of DOC were estimated by changes in DOC during the night and light period. Water samples from the incubations were filtered through pre-combusted (450°C for 4 h) GF/F filters, and were kept frozen in acid-washed material (glass vials encapsulated with silicone-teflon caps) until analyses. Concentrations of DOC were derived by catalytic oxidation at high temperature (720 °C) and NDIR by using a Shimadzu TOC-VCPH analyzer. The DOC certified reference material (Low and Deep), provided by D. A. Hansell and W. Chen (University of Miami; http://yyy.rsmas.miami.edu/groups/biogeochem/CRM.html) were used to assess the accuracy of the estimations. Net DOC fluxes were calculated as the differences between the final and the initial DOC concentrations in the water samples, and thus when net DOC fluxes were positive, system behaved as DOC producer. On the contrary, when net DOC fluxes were negative, system behaved as DOC consumer.

Samples of water for dissolved oxygen (DO) concentration were fixed immediately after collection, kept in darkness and refrigerated (4ºC) and determined using a spectrophotometric modification of the Winkler titration method (Pai et al. 1993, Roland et al. 1999). Changes in DO concentrations between the three different collection periods (S1, S2 and S3) were used later to estimate the net production (NPP), gross production (GPP) and respiration (R). The R during the night hours was estimated as the difference in O_2 concentrations between sampling S2 and S1. The NPP during light hours was estimated as the difference in O_2 concentrations between sampling S3 and S2. Then, GPP during light hours was calculated as the sum of hourly rates of R and NPP, assuming similar night and light respiration. Metabolic rates were converted into carbon units assuming photosynthetic and respiratory quotients of 1, a value used widely in seagrasses (e.g. Barrón & Duarte 2006). Daily rates of GPP were calculated by multiplying the hourly GPP by the photoperiod. Daily rates of R were calculated by multiplying the hourly R by 24 h because it was assumed the same R in night and light hours. Finally, daily rates of NPP were estimated as the difference between daily rates of GPP and R.

The concentration of non-structural carbohydrates (sucrose and starch) was measured in duplicate leaf and rhizome samples from each incubation. Samples were freeze-dried and ground prior to the analyses. Total non-structural carbohydrates were measured following (Brun et al. 2002). Sugars (sucrose and hexoses) were first solubilized by 4 sequential extractions in 96% (v/v) ethanol at 80 °C for 15 min. The ethanol extracts were evaporated under a stream of air at 40 °C and the residues were then dissolved in 10 ml of deionized water for analysis. Starch was extracted from the ethanol-insoluble residue by keeping it for 24 h in 1 N NaOH. The sucrose and starch content of the extracts was determined spectrophotometrically using resorcinol and anthrone assays with an absorbance of 486 and 640 nm respectively, and sucrose as a standard.

Data and statistical analysis. Prior to any statistical analysis, data were checked for normality (Shapiro-Wilk normality test) and homocedasticity (Bartlett test of homogeneity of variances test). Statistical differences between factors (pH and current velocity) were analysed using a 2-way ANOVA. When significant differences were found, a Tukey post-hoc test was applied. After several transformations, Respiration values did not meet the normality assumption, therefore Respiration differences among communities were analysed using the Kruskal-Wallis test with the Wilcoxon signed-rank test. Data were presented as mean ± SE. The significance level (α) in all tests performed was set at 0.05 of probability. Statistical analyses were computed with *R* 3.0.2 (*R* Development Core Team 2013).

RESULTS

Abiotic variables. Average daily water temperature showed an unimodal response, averaging 23.4 ± 0.8 ºC and ranging from ca. 21 ºC (midnight) to ca. 25 ºC (midday). This low daily temperature variation was due to the high water renewal (see M&M section). Regarding light doses, mean daily light level at the water surface at 10:00 am was ca. 1,250 µmol photons m^{-2} s^{-1}.

Effects on seagrass metabolism. Reduced pH produced a significant response in GPP and R, with FpH ca. 1.5 times higher than CpH in both GPP and R. However, no significant responses in NPP were found under both pH levels. Current velocity produced a non significant response in GPP, R and NPP. However, medium velocity was ca. 1.6, 1.7 and 1.8 times higher for GPP, R and NPP respectively than the average of the other two levels of water velocity under current pH (CpH) conditions. Under forecasted pH (FpH) conditions, the differences between current velocity levels practically disappeared. The combined effect of reduced pH and current velocity resulted in significant differences in R, showing the units FpH + LV higher R than the combined effect of CpH + HV (Fig. 2 and Table 2).

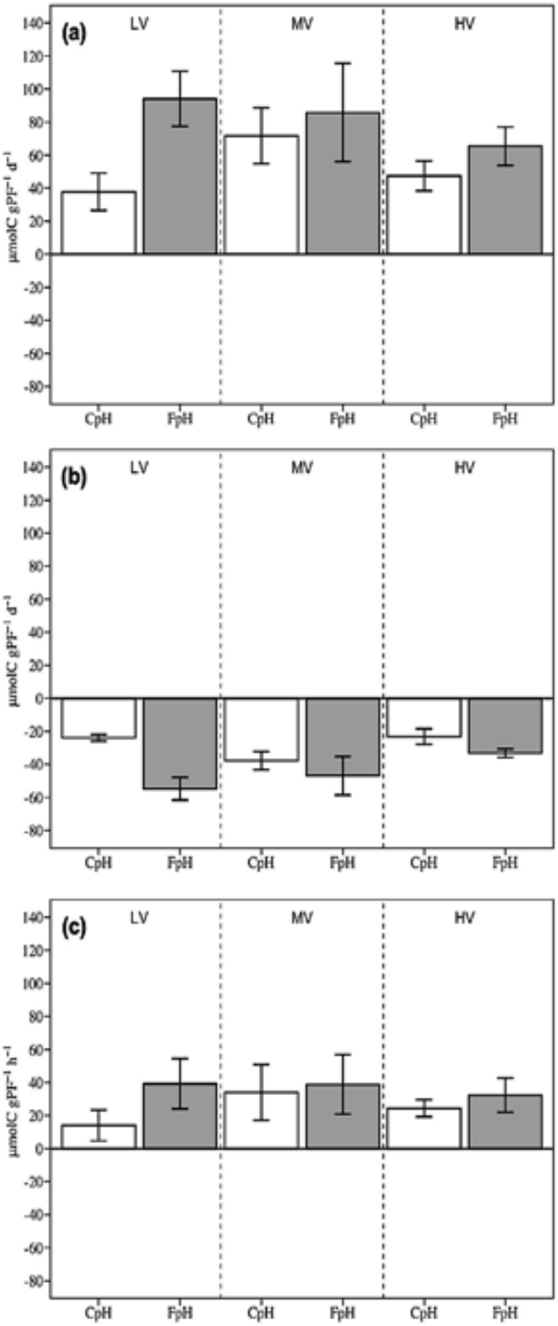

Figure 2. Effects of seawater acidification and current velocity on (a) Gross Primary Production (GPP), (b) Respiration (R) and (c) Net Primary Production (NPP). LV: Low velocity; MV: Medium velocity; HV: High velocity; CpH: Current pH; FpH: Forecasted pH.

Table 2. Two-way ANOVA results for the effect of pH and current velocity (CV) on gross primary production (GPP), respiration (R), net primary production (NPP), dissolved organic carbon flux (DOC) and biomass loss (BL). Bold letters indicate significant differences at $p < 0.05$.

	GPP	**R**	**NPP**	**DOC**	**BL**
pH	**0.046**	<**0.01**	0.255	0.159	0.252
CV	0.343	0.212	0.669	<**0.001**	**0.005**
pH:CV	0.374	**0.014**	0.705	0.664	0.199

Effects on non-structural carbohydrates content. Sucrose levels of aboveground tissues were affected significantly by reduced pH, with FpH units averaging ca. 1.6 times higher contents than those under CpH (Fig. 3a and Table 3). On the contrary, no significant response for sucrose content in belowground tissues, and both above and belowground tissues for starch among pH levels was found (Fig. 3b and Table 3). On the other hand, current velocity produced non-significant responses either in sucrose or starch. Similarly, the combined effect of reduced pH and current velocity produced non significant responses in carbohydrates content nevertheless, the sucrose content of the belowground tissues was ca. 0.4 times lower in the combination of low velocity (LV) + CpH than the mean of all factor combination (Figs. 3c, d and Table 3).

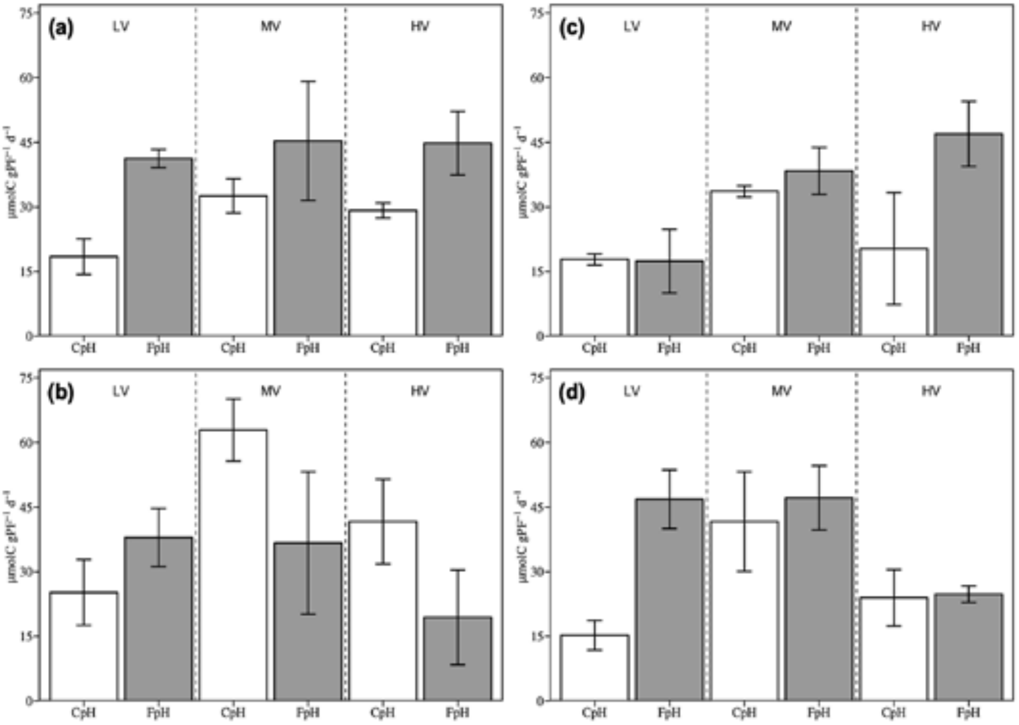

Figure 3. Effects of seawater acidification and current velocity on (a) aboveground sucrose, (b) belowground sucrose, (c) aboveground starch and (d) belowground starch. LV: Low velocity; MV: Medium velocity; HV: High Velocity; CpH: Current pH; FpH: Forecasted pH.

Table 3. Two-way ANOVA results for the effect of pH and current velocity (CV) on aboveground sucrose, belowground sucrose, aboveground starch and belowground starch (mg sucrose or starch g DW^{-1}). Bold letters indicate significant differences at $p < 0.05$.

	Above sucrose	**Below sucrose**	**Above starch**	**Below starch**
pH	**0.0232**	0.210	0.131	0.060
CV	0.433	0.195	0.085	0.071
pH:CV	0.766	0.200	0.220	0.139

DOC fluxes. Reduced pH produced a non-significant response in dissolved organic carbon (DOC) fluxes. Meanwhile, current velocity affected DOC fluxes significantly. Fluxes of DOC under high velocity (HV) were ca. 6 times higher than fluxes under LV.

Also, DOC fluxed under medium velocity (MV) were ca. 2.4 times higher than fluxes under low current velocity. Overall, DOC fluxes ranged from circa of 16% of NCP in LV to 64% of NCP in MV and even exceeding the NCP under HV (120% of NCP). The combined effect of reduced pH and current velocity produced non significant responses in DOC fluxes (Fig. 4 and Table 2).

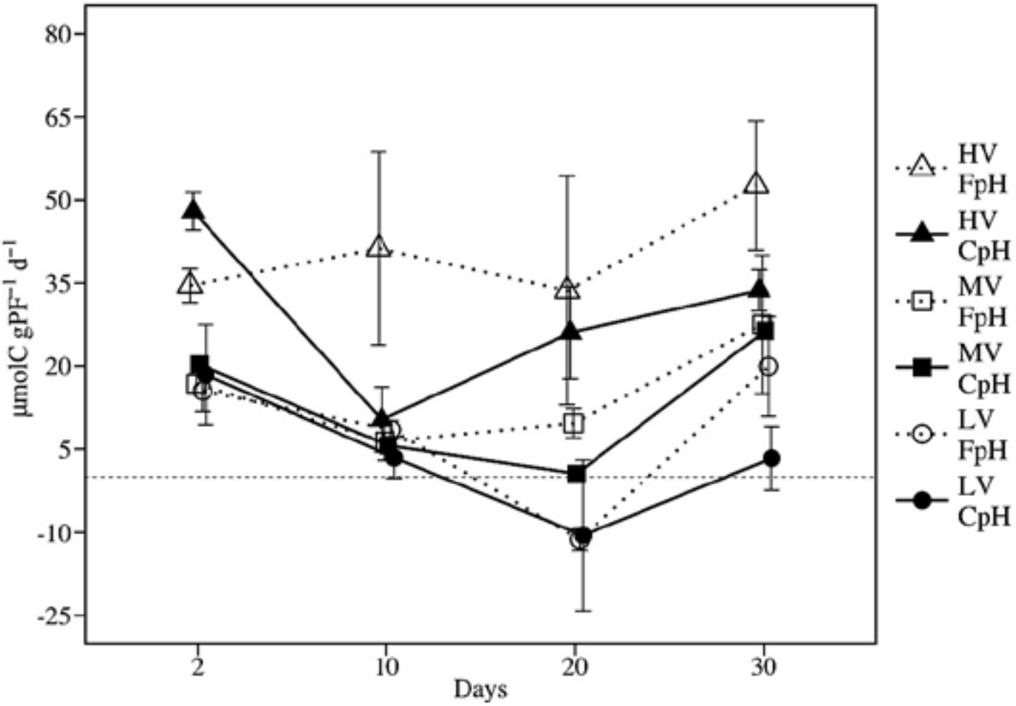

Figure 4. Effects of seawater acidification and current velocity on net DOC fluxes. LV: Low velocity; MV: Medium velocity; HV: High velocity; CpH: Current pH; FpH: Forecasted pH.

Biomass loss. Reduced pH produced non significant response in biomass loss. On the contrary, current velocity produced significant differences in this variable, being higher under LV current (29 ± 3.1 %) than MV (21 ± 2.6 %) and HV (16 ± 2.5 %) currents. The combined effect of reduced pH and current velocity produced non significant effect (Table 2) however, the HV and FpH combination was the treatment with lowest biomass loss, being 1.7 times lowest than the average of all other combinations (13.7% vs. 23.5% biomass loss).

DISCUSSION

The two factors assayed (pH and current velocity) produced significant changes in the carbon metabolism and dissolved organic carbon (DOC) fluxes in *Zostera noltei* population. Reduced pH enhanced mainly seagrass productivity, whereas current velocity enhanced mainly DOC fluxes. *Z. noltei* was net autotrophic independently of the combination of factors, and in all the cases kept a high productivity level. In average, GPP was ca. 65 ± 7 µmol C gPF^{-1} d^{-1}, within the range of values described by (Duarte et al. 2010) for a large number of seagrass species. A significant increase in GPP and R under forecasted pH (FpH) conditions was found (Fig. 2 and Table 2), being both, on average, 1.5 times higher than under current pH (CpH). This may indicate that photosynthesis of *Z. noltei* is carbon limited at the current inorganic carbon concentration of seawater (i.e. CO_2), as noted in previous studies done in this species (e.g. Silva et al. 2005, Alexandre et al. 2012), and also in other seagrass species (Beer & Koch 1996, Zimmerman et al. 1997, Invers et al. 2001). Hence, *Z. noltei* may benefit from future CO_2 enrichment by enhancing the photosynthetic rates at higher CO_2 concentrations (Alexandre et al. 2012), which lead to a significant increase in C uptake that can be stored in their tissues (e.g. enhancing non structural carbohydrates reserves, which is in agreement with the patterns observed here) or exported in particulate or dissolved form, as also recorded in this experiment, contributing to increase the productivity of adjacent communities.

On the contrary, current velocity had not significant effects in plant productivity, although under CpH condition, plants subjected to MV had higher GPP (1.7 times higher) than the average of the other two velocity levels. Probably, under low current velocities the thickness of the DBL surrounding leaf surface increased, which reduces carbon uptake, as previously demonstrated for inorganic carbon (Beer et al. 1980, Larkum 1989, Madsen & Sand-Jensen 1991, Koch 1994) and also for others nutrients (Cornelisen & Thomas 2004, Morris et al. 2008, Villazán et al. 2016). In contrast, under high current velocities the self-shading in the population increased since leaves allocated almost horizontal, collapsing each other and affecting light absorption and hence productivity (Koch et al. 2006). Likely, the intermediate current velocity allows a more favourable balance between light absorption and DBL reduction, allowing an increase in plant productivity. Some previous studies undertaken in the same small flumes showed opposite trends (mainly at high current velocity) as those found in this experiment (Peralta et al. 2006, De Los Santos et al. 2009, Villazán et al. 2016). That is, in these previous studies higher current velocities favoured the growth and development of *Z. noltei* plants, mainly because of the more horizontal position of the leaves, which enhanced light capture. Meanwhile, the lower plant growth under MV was explained by the insufficient oxygenation of the rhizosphere, which entail a drain of energy to offset the effects of the anoxic sediment (Smith et al. 1984, Zimmerman et al. 1995, Brun et al. 2003). However, the main difference between our experimental design and these previous ones is shoots density. Whilst in our design we used a high shoots density (c.a. 5,650 shoots m^{-2}), in previous studies shoots density were clearly lower (c.a. 150 shoots m^{-2}), and under such experimental conditions, the interaction between density and current velocity can produce opposite effects. Under MV conditions the higher complexity of the belowground network of *Z. noltei* bed may led to a greater oxygenation of the rhizosphere, and under HV conditions light capture may decrease because of self-shading of the leaves in the canopy. This conditional outcome depending on shoots density deserves further research. On the other hand, under FpH, the differences in GPP between current velocity levels disappear, which suggests that *Z. noltei* plants were CO_2 saturated under such conditions. CO_2 enrichment may limit the impact of light limitation on seagrasses (Zimmerman et al. 1997), offsetting the lower light absorption under HV conditions. Likewise, the higher CO_2 concentration probably offsets the increase in the thickness of DBL surrounding leaf surface. Thus, the interaction between pH and current velocity in the productivity of *Z. noltei* population is not so straightforward as expected, since under CpH conditions current velocity may have a positive effect depending on shoots density, but under FpH, this positive effect of current velocity may disappear (Fig. 5).

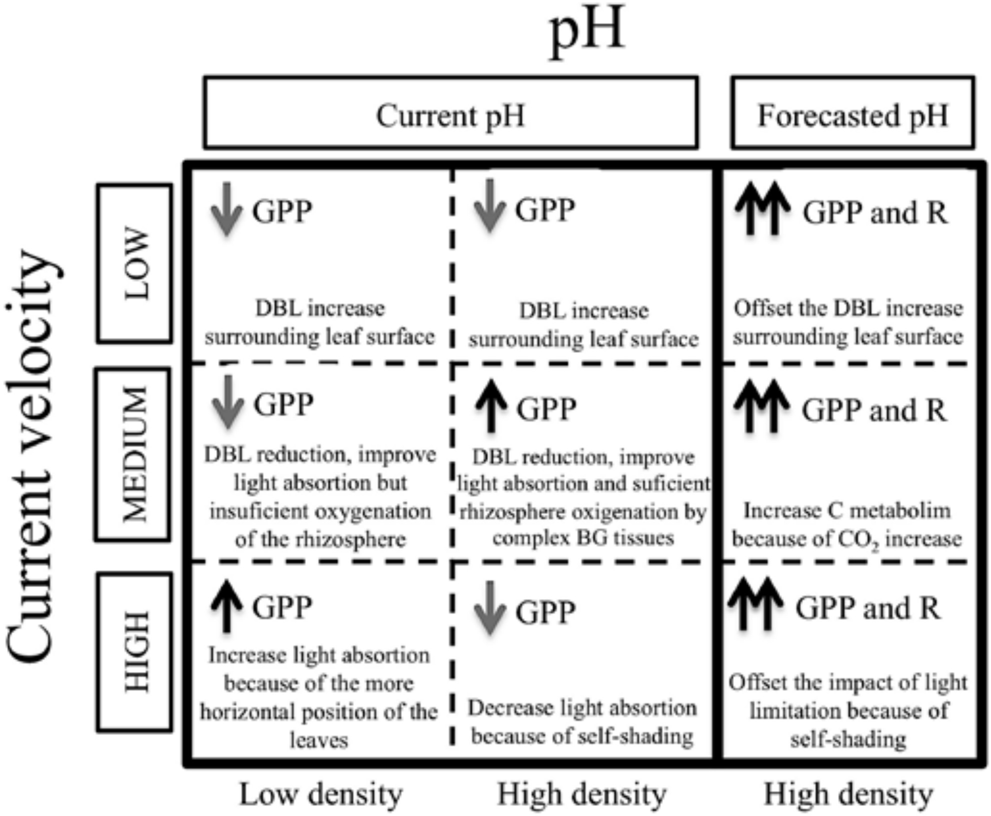

Figure 5. Conceptual model showing the likely effects of CO_2 concentration, current velocity and shoots density on Gross Production (GPP) and Respiration (R). (🡅) Increase; (🡇) Decrease; (🡅🡅) Significant increase.

Sucrose increased in aboveground tissues under FpH condition while a non-significant response was found in belowground sucrose and both above and belowground starch. It was noticed, however, that belowground starch increased in all factors combination between 30-260% respect LV + CpH plants (Fig. 3 and Table 3), which is consistent with short-term experiments of OA in seagrasses, where the concentration of belowground non-structural carbohydrates increased (Jiang et al. 2010, Campbell & Fourqurean 2013). Carbon reserves are essential for seagrass survival under stressful environmental conditions, because environmental stressors such as periods of light limitation (Zimmerman et al. 1995, Burke et al. 1996, Ralph et al. 2007, Govers et al. 2015), ammonium toxicity and/or sediment anoxia (Brun et al. 2002, 2008) increase plant carbon demands and force plants to use stored carbohydrates to provide a critical source of carbon skeletons and energy to alleviate these periods of low photosynthetic C fixation.

One of the most noteworthy results of this study is the recorded increase in DOC fluxes with current velocity independently of pH levels (Figs. 4, 6 and Table 2). This increase in DOC fluxes can be attributed to the sum of two different processes. Firstly, as demonstrated in this work, as the higher the current velocity, the higher the GPP of *Z. noltei* population, and since a fraction of the GPP is released as DOC (Barrón et al. 2014), a higher DOC release is expected as velocity increase. Secondly, these ecosystems stored large amounts of particulate organic carbon in the sediment (Kennedy et al. 2010), which can be potentially transformed to DOC because of heterotrophic bacteria activity (Boto et al. 1989, Maher & Eyre 2010). Although the relative contribution of each compartment (e.g. DOC released from sediment) to the net DOC fluxes in seagrass communities remains poorly understood (Barrón et al. 2014), higher current velocity is expected to enhance also the DOC transference from the sediment to the water column, as demonstrated for others compounds (van Duren & Middelburg 2000, Koch et al. 2006, Chipman et al. 2010, Corbett 2010). This finding has interesting ecological implications since an increased DOC release from seagrass populations means a quicker and more efficient transference of carbon and energy from primary producers to higher trophic levels, and may indicate that populations thriving in areas with higher hydrodynamics may produce higher DOC fluxes.

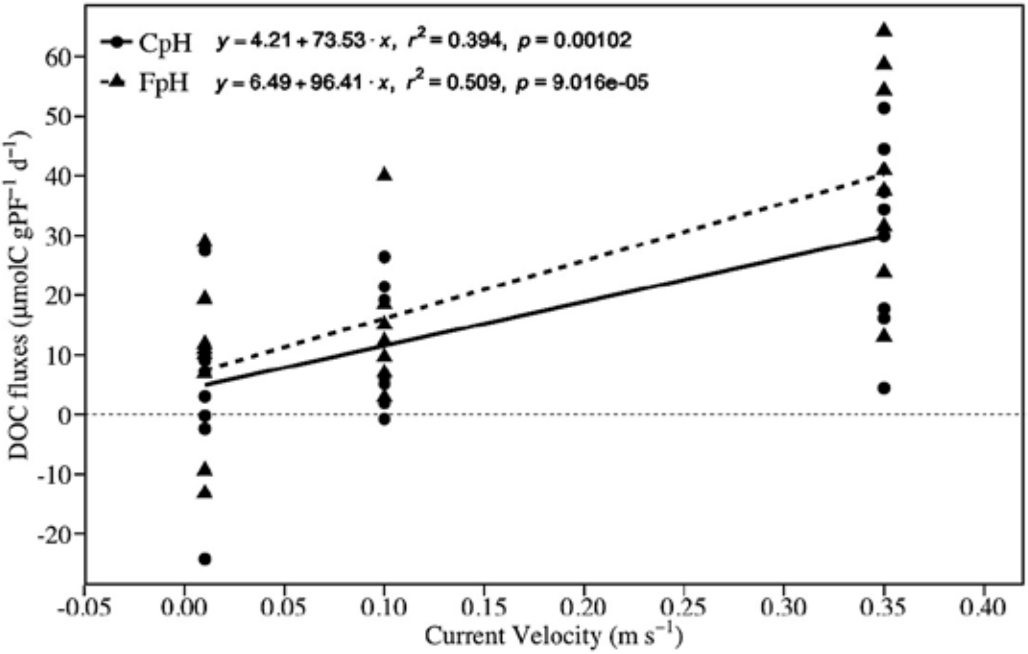

Figure 6. Relationship between DOC fluxes and current velocity. CpH: Current pH; FpH: Forecasted pH.

Besides seawater acidification and current velocity, other factors related with climate change, such as warming, may severely impact the productivity and even the survival of marine organisms (Harley et al. 2006). By the end of this century, mean global surface temperatures is projected to increase by ~3-4 °C (Meehl et al. 2007). Temperature is a key factor for seagrass health, growth and community metabolic rates (Koch et al. 2013). Thus, temperature increase may exacerbate or moderate the results found here. For example, the Metabolic Theory of Ecology (MTE) predicts respiration rates increase faster with warming than primary production rates do (Brown et al. 2004, Harris et al. 2006, Sarmento et al. 2010), which results in a reduction of P:R ratio and a greater chance of shifting the system to heterotrophy. On the other hand, recent studies have shown that the net DOC flux in seagrass communities was significantly correlated with water temperature (Barrón & Duarte 2009, Barrón et al. 2014). Hence, future researches should delve into the interaction of ocean acidification and current velocity with other environmental or anthropogenic stressors related with climate change such as warming, in order to understand and mitigate the effects derived of this global threat in seagrass ecosystems.

In summary, the best scenario in this study for *Z. noltei* was OA under high current velocity, which led to a significant increase in productivity and non-structural carbohydrate concentrations (especially aboveground sucrose and starch) and produced the lowest biomass loss. In addition, a direct relationship between current velocity and DOC fluxes was recorded at the end of the experiment both under control carbon and OA conditions. Since both factors (i.e. OA and hydrodynamics) are expected to increase under climate change, and leaving other indirect interactions such as warming, sea level change, herbivory, etc, seawater acidification and current velocity may produce a favourable scenario for *Z. noltei* populations increasing its productivity, their non-structural carbohydrate levels and DOC release, which means a quicker and more efficient transference of carbon and energy to higher trophic levels and a higher resistance and resilience to external stressors.

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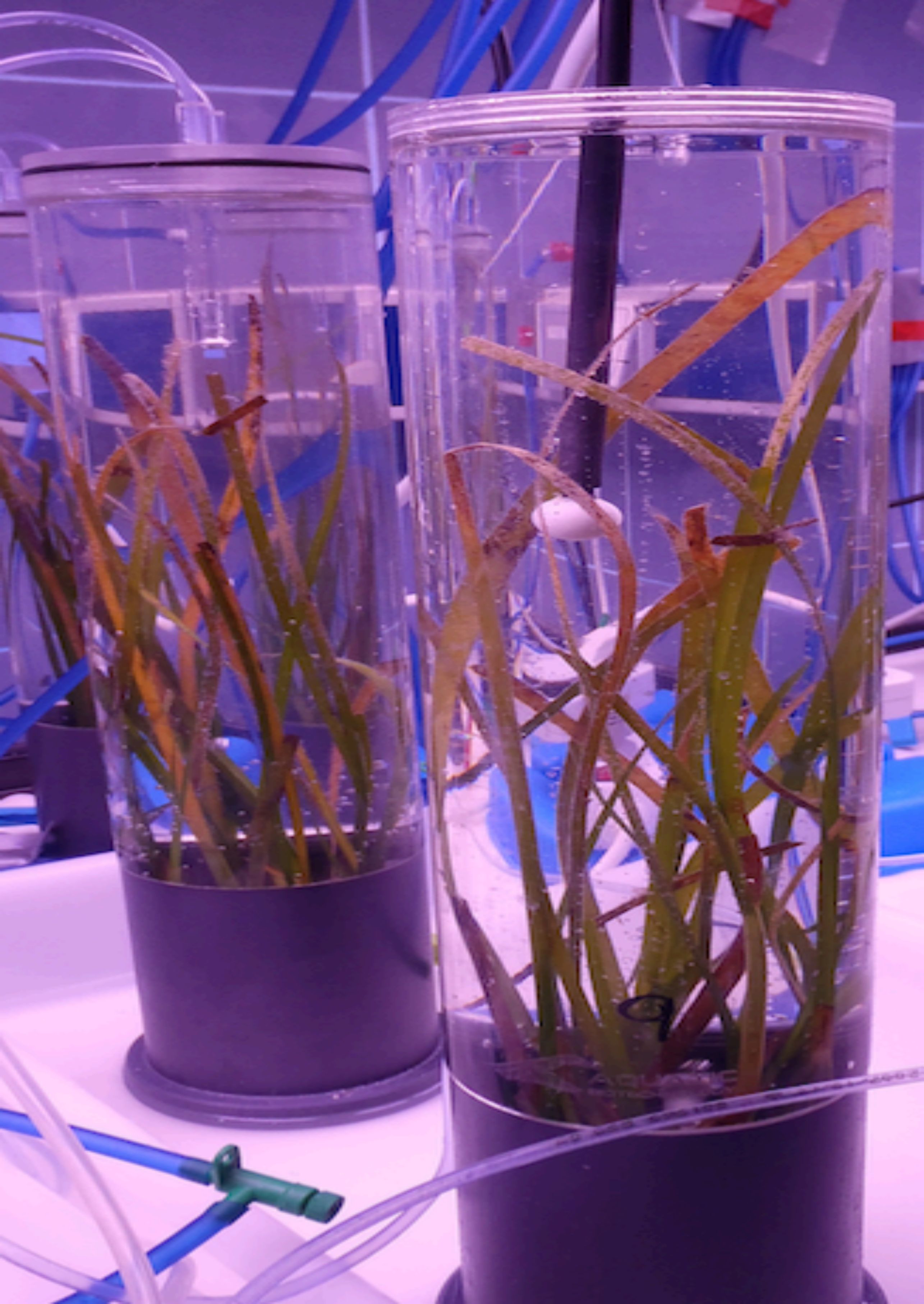
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CHAPTER 6

Interactive effect of temperature, pH and ammonium enrichment in the seagrass *Cymodocea nodosa* (Ucria) Aschers.

Submitted to Marine Pollution Bulletin

El cerebro no es un vaso por llenar, sino una lámpara por encender.

Plutarco

Interactive effect of temperature, pH and ammonium enrichment in the seagrass *Cymodocea nodosa* (Ucria) Aschers.

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ABSTRACT

Global change, eutrophication, coastal over-development are strongly hammering coastal ecosystems since most of the human population lives currently there. One of the major challenges for scientists is to forecast future trajectories when multiple factors are acting together, since the response can be non-additive (i.e. synergist or antagonist). Therefore, this study attempts to elucidate the effects of three main factors (warming, CO_2 increase and ammonium enrichment) on the seagrass *Cymodocea nodosa* under a full factorial mesocosm experiment. Overall, warming demonstrated to have a positive effect in plant production but at the expense of reducing carbon reserves. Meanwhile, loss leaf rates increased with ammonium supply and CO_2 increase did not produce significant effects. However, the combination of these factors improved plant growth and preserved carbon reserves, which is essential to face stressful environmental conditions. Thus, it suggests that future scenarios may benefit to this temperate seagrass improving their growth and carbon reserves.

INTRODUCTION

In the last century, human activities have triggered changes at a global scale that are affecting ecosystems worldwide, being coastal vegetated ecosystems one of the most threatened (Large 2009). In the next decades, these ecosystems are expected to increase their exposition to adverse consequences related to climate change, exacerbated by increasing direct human induced pressures (Nicholls et al. 2007). Within coastal vegetated habitats, seagrasses (i.e. marine flowering plants) form one of the richest and most relevant coastal ecosystems (Short et al. 2011). They are globally distributed and well recognised as highly important in coastal areas due to the large number of ecological services they provide (Orth et al. 2006, Short et al. 2011, Campagne et al. 2014). However, the shallow distribution of seagrasses in estuarine and coastal areas and its proximity to anthropogenic littoral impacts has 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). The value of seagrass ecosystems is worldwide recognised by different legislations and international Conventions like the Convention on Biological Diversity (1992) or the European Habitats Directive (92/43/EEC). Favoured by this legislation framework, seagrass habitats are specifically targeted for conservation and restoration (Green & Short 2004), which increases the necessity of understanding the responses of these ecosystems to multiple co-stressors in order to provide management advices, including the modelling of its future trajectories (Brierley & Kingsford 2009, Hoegh-Guldberg & Bruno 2010). This challenge requires a multiscalar approach from the organism response to populations, communities and the entire ecosystem (Russell et al. 2012), considering the emergence of likely synergistic and/or antagonist effects and non-linear responses when multiple stressors are acting together (Woodward et al. 2010, Villazán et al. 2016).

Climatic change effects (e.g. seawater acidification, increase in temperature, frequency of storms, sea level rise, etc) joined with coastal anthropogenic and natural stressors (e.g. nutrient load, salinity and littoral current changes, diseases, etc…) are acting together in coastal areas and their effects are expected to increase in the near future ((Nicholls et al. 2007, Halpern 2014). Thus, in a future scenario, pH is expected to fall below 7.9 and global sea-surface temperature increase in 3–4 °C (Meehl et al. 2007). In addition, ocean acidification and high temperature can locally interact with excessive nutrient loads and can aggravate changes in ecosystem structure and functioning (Russell et al. 2009). Previous reports have studied the response of seagrasses to some of these factors separately (for review, see Short & Neckles 1999, Burkholder et al. 2007, Koch et al. 2013) or in a lower extent in multifactorial designs (De los Santos et al. 2009, La Nafie et al. 2013, Villazán et al. 2013, 2015, 2016, Khan et al. 2016). For instance, it is known that seawater pH decrease as a result of increasing CO_2 concentration can benefit the seagrass primary productivity, as these plants are generally considered to be photosynthetically CO_2 limited (Beer & Koch 1996, Beardall et al. 1998). Then, an increase in growth rates and biomass in the absence of other growth limiting factors (e.g., nutrients, light) is expected (Short & Neckles 1999, Palacios & Zimmerman 2007, Hall-Spencer et al. 2008, Harley et al. 2012, Garrard & Beaumont 2014). On the other hand, the direct effects of increased temperature in seagrasses will depend on the species thermal tolerance and its optimum temperature for photosynthesis, respiration, and growth (Short & Neckles 1999). Several studies have highlighted the effect of temperature in seagrass metabolism and the maintenance of a positive carbon balance (Evans et al. 1986, Bulthuis 1987, Zimmerman et al. 1989). Thus, temperate species such as *Cymodocea nodosa* (Ucria) Aschers. may increase photosynthesis and respiration rates over a wide range of temperatures (Pérez & Romero 1992, Terrados & Ros 1995). In addition to those variables affected by climate change, nutrients increase in coastal waters (e.g. ammonium), has been identified as a key process negatively impacting seagrass meadows (Hughes et al. 2004, Burkholder et al. 2007, Cabaço et al. 2008, Antón et al. 2011). Several reports have pointed out that moderate increases in nutrient loads may stimulate seagrass growth and production (Short 1987, Pérez et al. 1991, Alcoverro et al. 1997, Udy et al. 1999). However, under conditions of high N availability, especially when ammonium is the most abundant form, plant growth and survival can be curtailed by direct ammonium toxicity (Van Katwijk et al. 1997), which may depend on the internal demand of energy and C skeletons needed for rapid ammonium assimilation (Burkholder et al. 1992, Brun et al. 2002, 2008).

Regrettably, the interactive effect of the main climate change factors (i.e. temperature and pH) and high nutrient load has not been assayed so far, in spite of the fact that synergistic and antagonistic responses are expected. Therefore, this research is aimed to study the response of the seagrass *Cymodocea nodosa* to the forecasted global change using a multifactorial mesocosm experiment with the 3 aforementioned factors (pH decrease, high temperature and ammonium enrichment). The results of this study will allow a better understanding of the responses of this species facing the challenges posed by global change, which is crucial for modelling future trajectories of coastal ecosystems (Brierley & Kingsford 2009, Hoegh-Guldberg & Bruno 2010, Unsworth et al. 2014).

MATERIAL AND METHODS

Field plant collection. Individual shoots of *Cymodocea nodosa* were randomly collected from 1-2 m depth submerged meadows of Cádiz Bay (southern Spain, 36°29'19.79"N; 6°15'53.05"E). Healthy looking shoots with intact rhizomes were transported to the laboratory within 2 hours of collection in an ice chest where they were cleaned of visible epiphytes and held in aerated water from the sampling site over 5 days under sub-saturating light (ca. 150 µmol photons m^{-2} s^{-1}) in a 16:8 hours light:dark cycle at 20°C until used in the experiment for laboratory acclimation.

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 Cádiz during four weeks in November 2013. The plants were allocated in 1.5 L incubation chambers (*n*=24) (Fig. 1).

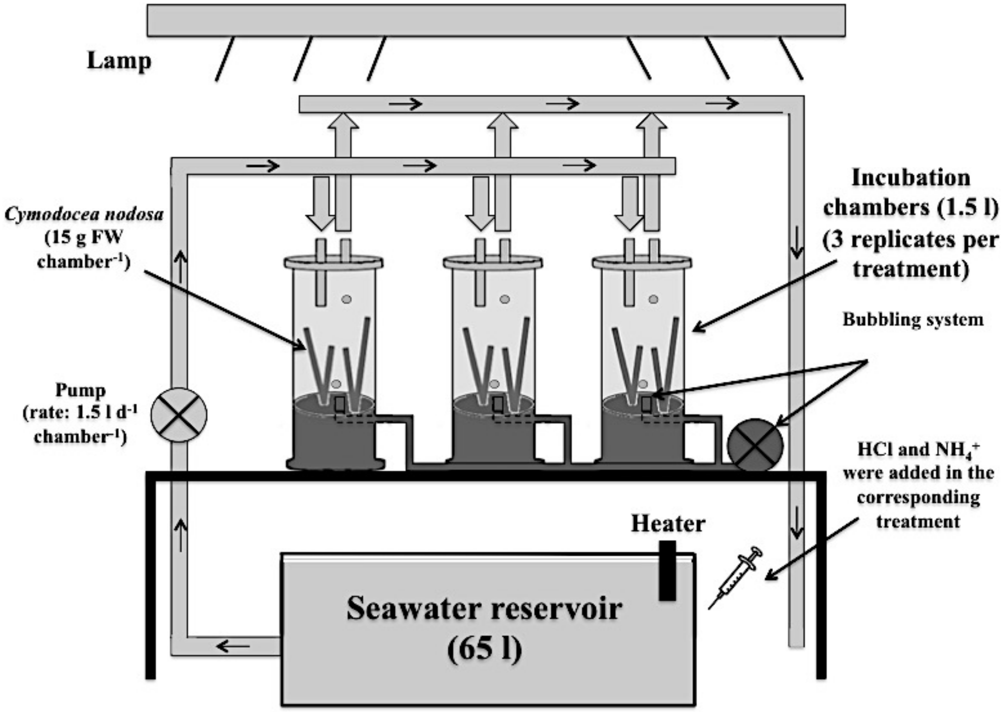

Figure. 1. Simplified diagram of one of the experimental treatments. (see description in the text).

In each chamber, about 18-21 individual *C. nodosa* shoots were planted one by one by hand (15.1 ± 0.5 g FW chamber^{-1}) resulting in a total of ca. 500 shoots planted among all chambers. Each chamber was previously filled with 0.5 l pre-washed and sieved (1 mm) sandy sediment 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: (a) control temperature (CT) with temperature ca. 22 ºC and (b) high temperature (HT) with seawater heated 4 °C; two pH levels: (a) current pH (CpH) with pH ca. 8.12, equivalent to ca. 415 ppm CO_2 and (b) forecasted pH (FpH) with pH ca. 7.69, equivalent future conditions to ca. 720 ppm CO_2; and two ammonium levels: (a) control NH_4^+ (CNH_4^+) without NH_4^+ addition and (b) enrichment NH_4^+ (ENH_4^+). The nutrient in ENH_4^+ treatment was added to kept a constant concentration ca. 31 µM NH_4^+, with no negative effects on plants shown by previous studies from our group (Villazán et al. 2016). The factors were manipulated in a fully crossed design, making a total of eight treatments (Table 1).

Table 1. Water chemical characteristics in each treatment (salinity was 30 psu and light was ca. 325 µmol photons m^{-2} s^{-1}). Data are means ± SE (*n*=90). CT: control temperature; HT: High temperature; CpH: Current pH; FpH: Forecasted pH; CNH_4^+: control NH_4^+; ENH_4^+: Enrichment NH_4^+.

Treatments			**NH_4^+ (µM) ***	**pH**	**pCO_2 (ppm)**	**Temp. (ºC)**
Temp.	**pH**	**NH_4^+**				
HT	FpH	ENH_4^+	32.1 ± 1.4	7.67 ± 0.01	729 ± 12	26.14 ± 0.03
HT	CpH	ENH_4^+	31.4 ± 1.5	8.10 ± 0.02	402 ± 19	26.07 ± 0.03
HT	CpH	CNH_4^+	0	8.10 ± 0.01	412 ± 18	26.08 ± 0.02
HT	FpH	CNH_4^+	0	7.66 ± 0.01	736 ± 12	26.10 ± 0.03
CT	FpH	ENH_4^+	31.8 ± 1.7	7.68 ± 0.01	750 ± 18	21.95 ± 0.08
CT	CpH	ENH_4^+	30.4 ± 1.3	8.13 ± 0.02	424 ± 22	21.94 ± 0.08
CT	CpH	CNH_4^+	0	8.14 ± 0.01	447 ± 21	21.93 ± 0.08
CT	FpH	CNH_4^+	0	7.66 ± 0.01	744 ± 17	21.85 ± 0.05

Notes: All measurements were conducted in the incubation chambers, except for NH_4^+ (µM) added (*) which was conducted in the corresponding reservoirs.

These variables were manipulated in 65 L seawater reservoirs. Each reservoir, which received sand-filtered seawater from the bay at a rate of 4.5 l d^{-1}, fed three replicated incubation chambers at a rate of 1.5 l d^{-1} (Fig. 1). The natural seawater used in the reservoirs contained low levels of ammonium (ca. 0.7 µM), nitrate and phosphate (1−2 µM). The incubation chambers were illuminated by lamps with cool fluorescent tubes (T5 High Output Blau Aquaristic aquarium color extreme fluorescents) in a 16:8 hours light:dark cycle. This light source created a homogenous field of irradiance in each chamber (325 ± 20 µmol photons m^{-2} s^{-1}). Water temperature and pH in the incubation chambers fluctuated temporally between light and night periods just as it happens in natural seagrass beds. Aeration was individually supplied in each incubation chamber (20 cm^3/h) in order to homogenise the water and reduce the diffusive boundary layer. Once a week, epiphytes growing in the chamber walls were removed and incubation chambers were hazardously reallocated in order to minimize spatial differences. In addition, all dead leaves were continuously removed throughout the experimental period. At the end of the experiment all surviving plants of each incubation chamber were harvested and weighed (fresh weight, FW).

Temperature, inorganic carbon and ammonium treatments. Levels of temperature and pH were manipulated according to the scenario forecasted by the Intergovernmental Panel on Climate Change (IPCC 2013, Prinn et al. 2011). Temperatures were maintained by recirculating water through a heater (Tetra HT 100W). The pH in the forecasted pH reservoirs were obtained by adding small amounts of HCl in the seawater until reaching the pH value necessary for the required CO_2 concentration (ca. 720 ppm total scale) (e.g. Netten et al. 2013). Changes in CO_2 concentration were controlled through daily measures of water pH, salinity and temperature in the incubation chambers. Weekly carbon chemistry parameters were derived using pH (on the total scale), alkalinity, temperature and salinity. At each incubation chamber, alkalinity samples just before sunrise were collected in 250 mL borosilicate bottles and a saturated solution of $HgCl_2$ was added following the methods outlined in the DOE handbook (DOE 1994). Alkalinity was determined with the Gran titration technique with HCl 0.1 N using a Methrom Ion Analysis (tiamo, version 1.2 light with titrando 808, stirrer 801, pH meter 780 and sonda metrohm 6.0262.100 (Metrohm AG CH-9101, Herisau, Switzerland) according to Perez et al. (2000). Total inorganic carbon (Ci) and partial pressure of CO_2 (pCO_2) were estimated from pH, alkalinity, temperature and salinity data using the CO2SYS package (Lewis & Wallace 1998), with the K1 and K2 constants from Mehrbach et al. (1973), as modified by Dickson & Millero (1987), and the $KHSO_4$ constant from Dickson (1990). The pH, total alkalinity (TA), temperature, salinity and carbon speciation within the incubation chambers are shown in Table 2. Regarding ammonium concentrations, control NH_4^+ reservoirs resembled field conditions. The enriched NH_4^+ reservoirs were obtained by adding ammonium to the reservoir from a NH_4^+ stock solution every day to keep the concentrations as close as possible to the target concentration (ca. 31 μM NH_4^+). The NH_4^+ addition corresponded to ca. 700 μmol g FW^{-1} d^{-1} in the enriched NH_4^+ treatments. The concentration of ammonium was monitored according to Invers et al. (2004) every two-three days in all chambers and every day in all reservoirs. Water samples were collected right after addition of ammonium, although the first ammonium measures in the incubation chambers started two days after the start of the experiment.

Table 2. Summary of seawater chemistry: temperature, total alkalinity, Ω calcium ion and aragonite and inorganic carbon content in the different treatments (salinity were ca. 30 and temperature were ca. 21ºC in all treatments). The values for pCO_2, CO_3^{2-}, HCO_3^-, Ω Calcium and Ω Aragonite were calculated by the computer programme CO2SYS package (version 2.1) (Lewis and Wallace, 1998). Aragonite is abbreviated as Ar and calcium as Ca. Data are means ± SE, *n*=9. CT: control temperature; HT: High temperature; CpH: Current pH; FpH: Forecasted pH; CNH_4^+: control NH_4^+; ENH_4^+: Enrichment NH_4^+.

Incubation treatments			**A_T (μmol kg^{-1})**	**pH_T**	**DIC (μmol kg^{-1})**	**pCO_2 (ppm)**
Temp.	**pH**	**NH_4^+**				
HT	FpH	ENH_4^+	1286 ± 15	7.6 ± 0.01	1238 ± 15	739 ± 14
HT	CpH	ENH_4^+	2485 ± 11	8.1 ± 0.01	2214 ± 12	419 ± 10
HT	CpH	CNH_4^+	2560 ± 22	8.1 ± 0.01	2278 ± 23	423 ± 16
HT	FpH	CNH_4^+	1259 ± 50	7.6 ± 0.01	1210 ± 49	709 ± 19
CT	FpH	ENH_4^+	1345 ± 33	7.6 ± 0.01	1287 ± 30	697 ± 11
CT	CpH	ENH_4^+	2460 ± 20	8.1 ± 0.01	2179 ± 17	396 ± 13
CT	CpH	CNH_4^+	2653 ± 31	8.1 ± 0.01	2358 ± 30	432 ± 11
CT	FpH	CNH_4^+	1329 ± 27	7.6 ± 0.03	1275 ± 22	735 ± 36

Laboratory analysis. After 30 days of culture, plants were collected to measure production (net production, leaf loss and gross production rates), non-structural carbohydrates (i.e. sucrose and starch in aboveground and belowground tissues), internal ammonium (aboveground tissues) and C:N atomic ratio (aboveground tissues). For production measurements, the net production rate (NPR) was obtained by the difference between initial fresh biomass and the fresh biomass at the end of the experiment (after removal of dead leaves) divided between the elapsed days (i.e. 30 days). The leaf loss rate (LLR) was obtained by dividing the fresh biomass of dead leaves by the experimental time. Finally, gross production rates (GPR) were obtained by the difference between initial fresh biomass and the fresh biomass at the end of the experiment, but including dead leaves, divided by the experimental time. The concentration of non-structural carbohydrates (i.e. sucrose and starch) was measured in duplicated leaf and rhizome samples from each incubation chamber. Samples were freeze-dried and ground prior to analysis. Total non-structural carbohydrates were measured following Brun et al. (2002). Sugars (sucrose and hexoses) were first solubilized by 4 sequential extractions in 96% (v/v) ethanol at 80°C for 15 min. The ethanol extracts were evaporated under a stream of air at 40 °C and the residues were then dissolved in 10 ml of deionized water for analysis. Starch was extracted from the ethanol-insoluble residue by keeping it for 24 h in 1 N NaOH. The sucrose and starch content was determined spectrophotometrically using a resorcinol and anthrone assay with an absorbance of 486 and 640 nm, respectively, with sucrose as standard. For internal ammonium, the intracellular concentrations of NH_4^+ were measured in duplicated leaf samples from each incubation chamber. Samples were rinsed in deionized water and ca. 0.5 g (FW) was ground in 20 ml of boiling deionized water (Dortch et al. 1984). Samples were sonicated for 10 min and then centrifuged for 20 min at 5000 g. The concentration of NH_4^+ was finally measured in the supernatant according to Bower & Holm-Hansen (1980) and Grasshoff et al. (1983). For C:N atomic ratio, total C and N content was determined in duplicated freeze-dried, ground samples of leaves and roots/rhizomes from each aquarium using a Carlo-Erba NA-1500 CHNS analyzer.

Data and statistical analysis. Prior to any statistical analysis data were checked for normality (Shapiro-Wilk normality test) and homocedasticity (Bartlett test of homogeneity of variance test). The main effects of treatment factors (temperature, acidification and ammonium addition) on gross production rate (GPR), loss leaves rates (LLR), net production rates (NPR), non-structural carbohydrates, C:N and internal NH_4^+ concentrations were tested using a 3-way ANOVA. When significant differences were found, the Tukey post-hoc test was applied to compare both the levels and interaction factors. Data were presented as mean ± SE. The significance level (α) set in all tests performed was 0.05. Statistical analyses were computed with R 3.0.2 (R Development Core Team 2013).

RESULTS

Ammonium concentration in seawater. Ammonium added was removed effectively by plants along the experiment at control temperature (CT) treatments under NH_4^+ enrichment (Fig. 2A). Meanwhile, some ammonium accumulated in the seawater in the treatment CpH + HT during the first two days of the experiment. The maximum accumulation was recorded in the treatment FpH + HT (4-6 µM NH_4^+ during the first two weeks of the experiment; Fig. 2B).

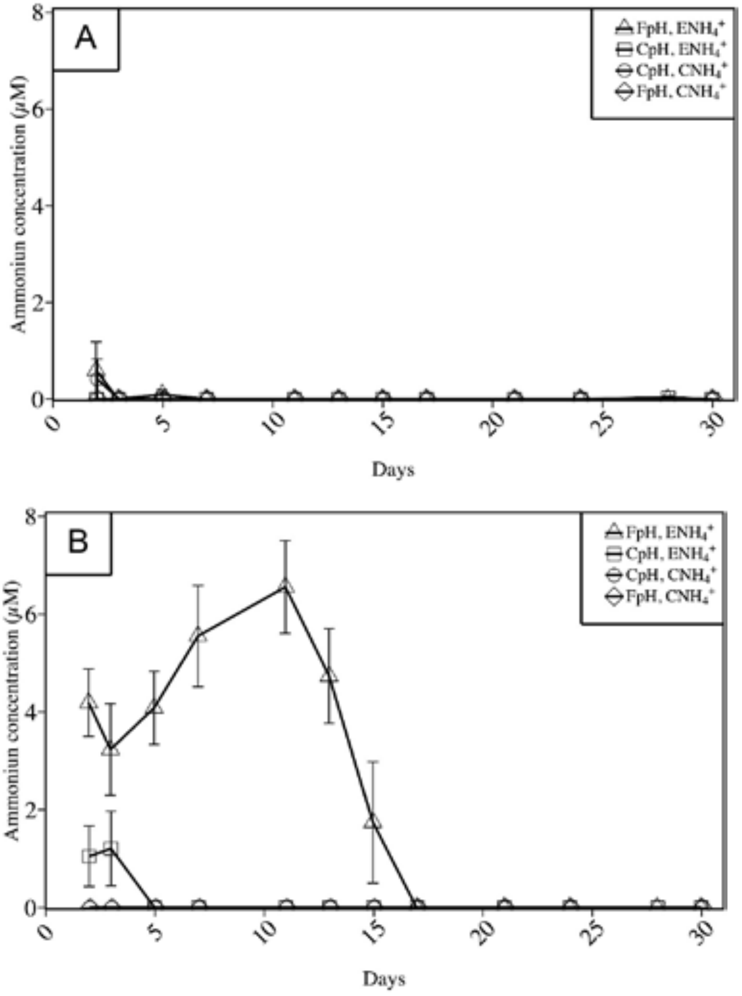

Figure 2. Ammonium concentrations in seawater on [A] control temperature (CT) and [B] high temperature (HT). CpH: Current pH; FpH: Forecasted pH; CNH_4^+: control NH_4^+; ENH_4^+: Enrichment NH_4^+

Effects on plant productivity. Temperature significantly affected GPR. The production in the treatment CpH + CNH_4^+ + HT was, on average, 1.8 times higher than that in all other treatments (Fig. 3A, Table 3). The combined effect of ENH_4^+ + HT and the combined effect of FpH + ENH_4^+ + HT produced significant differences in GPR, mainly due to the effect of temperature. The LLR was significantly affected by the NH_4^+ enrichment, being, on average, 1.5 times lower than CNH_4^+ treatments (Fig. 3B and Table 3). Overall, the NPR increased significantly under HT treatments and also under the combination of the three factors assayed (Fig. 3C and Table 3). Thus, the treatments with higher NPR were CpH + CNH_4^+ + HT (being 2.4 times higher than the average of the other treatments) and FpH + ENH_4^+ + HT (being 2 times higher than the average of the other treatments). No significant response in any of the three variables was found at forecasted pH as a single factor.

Table 3. Results of the 3-way ANOVA test on treatments (temperature, acidification and ammonium addition) and relevant interactions for Gross Production Rate (GPR: g FW d^{-1}) Loss Leaves Rates (LLR: g FW d^{-1}) and Net Production Rates (NPR: g FW d^{-1}). All data were normally distributed. * Significance level $p < 0.05$. ** Significance level $p < 0.01$.

	GPR	**LLR**	**NPR**
Temperature	0.001 **	0.025 *	0.003 **
pH	0.102	0.102	0.161
NH_4^+	0.330	<0.001 **	0.097
Temperature : pH	0.3	0.937	0.455
Temperature : NH_4^+	0.025 *	0.005 **	0.149
pH : NH_4^+	0.114	0.075	0.206
Temperature : pH : NH_4^+	0.042 *	0.019 *	0.004 **

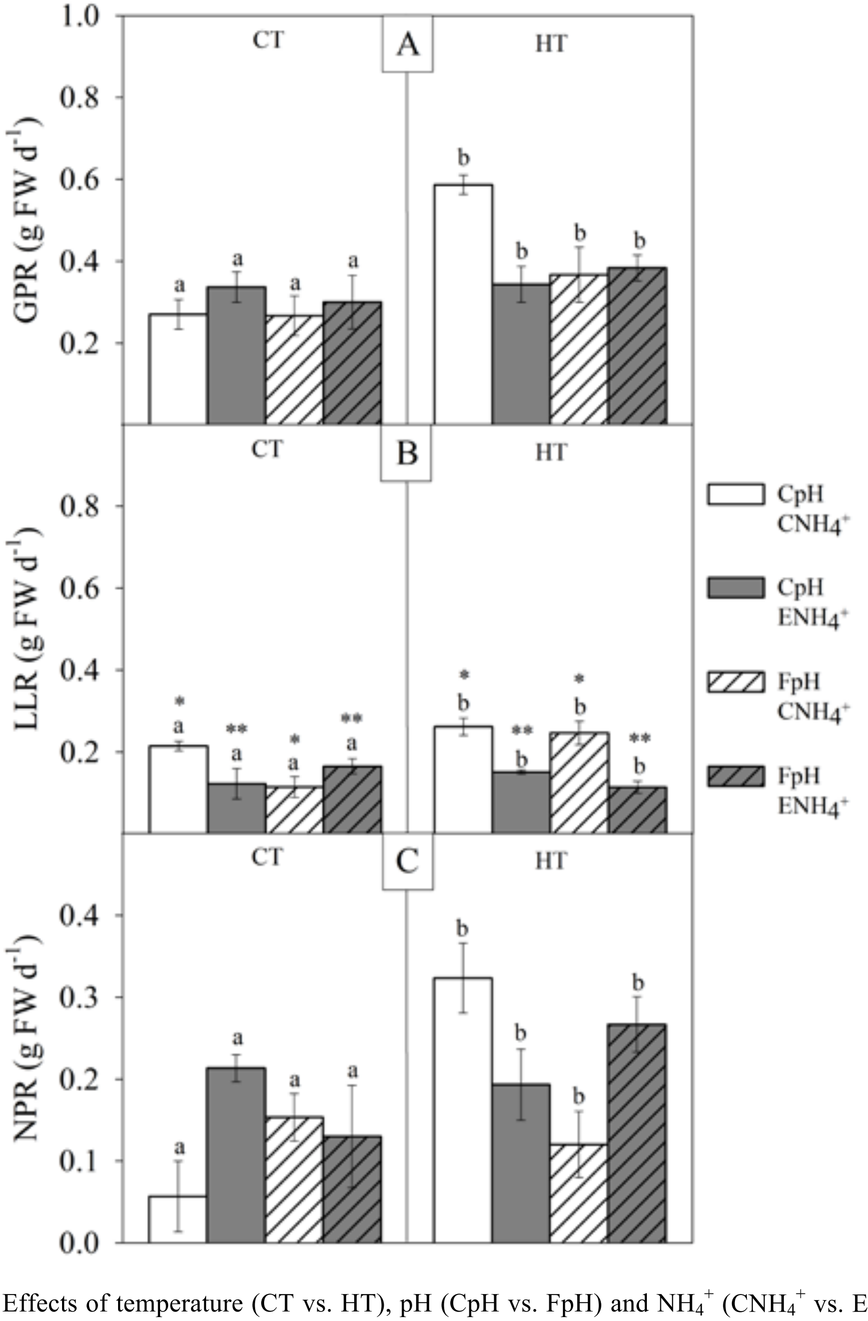

Figure 3. Effects of temperature (CT vs. HT), pH (CpH vs. FpH) and NH_4^+ (CNH_4^+ vs. ENH_4^+) on [A] Gross Production Rate (GPR), [B] Leaves Loss Rate (LLR) and [C] Net Production Rate. (NPR). Letters above the bars represent significant differences in temperature levels; asterisks above the bars represent significant differences in NH_4^+ levels.

Effects on non-structural carbohydrates content. Non-structural carbohydrates content in both aboveground (leaves) and belowground (rhizomes and roots) tissues were affected by temperature. Thus, while there were no significant responses in sucrose content in aboveground tissues among treatments, belowground sucrose content was affected significantly at HT treatments being, on average, 1.5 times lower than treatments under control temperature (Fig. 4A, 4B and Table 4). Regarding starch, aboveground tissues were significantly affected by HT treatments being, on average, 1.5 times lower than CT treatments (Fig. 4C and Table 4). Although there were no significant changes in belowground starch content when the three factors were tested separately, the combined effect of the three factors caused a significant decrease in starch content (Fig. 4D and Table 4). In addition, although forecasted pH did not yield significant effects on sucrose and starch contents, there was a weak effect. Thus, forecasted pH yielded, on average, 1.25 higher contents in aboveground tissues and 1.3 lower contents in belowground ones than under control pH (Fig. 4C, 4D and Table 4).

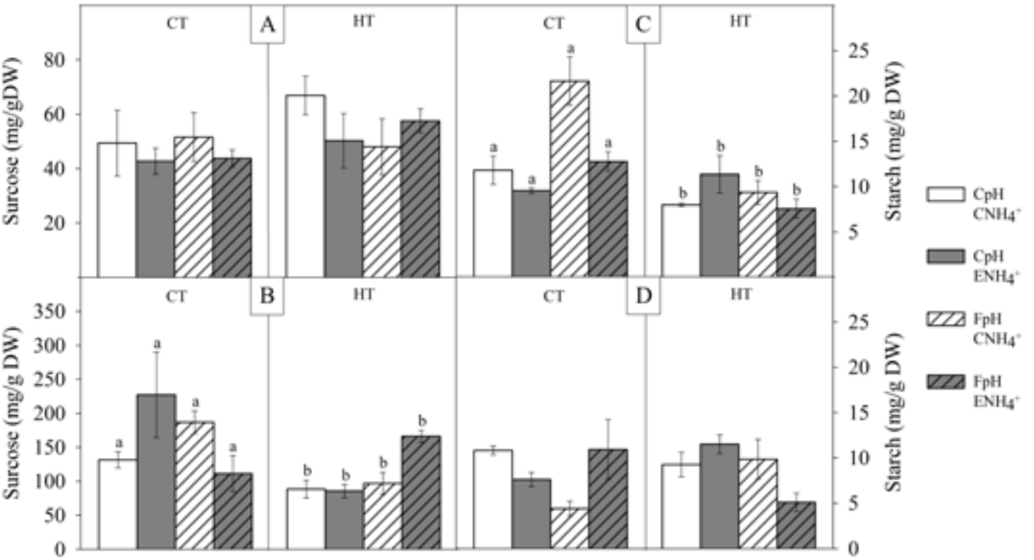

Figure 4. Effects of temperature (CT vs. HT), pH (CpH vs. FpH) and NH_4^+ (CNH_4^+ vs. ENH_4^+) on [A] aboveground sucrose, [B] belowground sucrose, [C] aboveground starch and [D] belowground starch concentrations. Letters above the bars represent significant differences in temperature levels.

Table 4. Results of the 3-way ANOVA test on treatments (temperature, acidification and ammonium addition) and relevant interactions for aboveground sucrose, belowground sucrose, aboveground starch and belowground starch concentrations (mg sucrose or starch g DW^{-1}). All data were normally distributed, except for below sucrose and above starch to which a natural logarithmic transformation was applied. * Significance level $p < 0.05$. ** Significance level $p < 0.01$.

	Above sucrose	**Below sucrose**	**Above starch**	**Below starch**
Temperature	0.147	0.004 **	<0.001**	0.679
pH	0.722	0.371	0.094	0.069
NH_4^+	0.366	0.349	0.108	0.861
Temperature : pH	0.531	0.031 *	0.006 **	0.557
Temperature : NH_4^+	0.754	0.190	0.04 *	0.223
pH : NH_4^+	0.296	0.343	0.032 *	0.560
Temperature : pH : NH_4^+	0.256	0.003 **	0.582	0.002 **

Effects on tissue C:N and ammonium tissue content. Temperature and pH did not affect C:N ratio and internal ammonium concentration in aboveground tissues. On the contrary, ammonium addition had a significant effect on plants, averaging lower C:N ratios (ENH_4^+ treatments showed a reduction of ca. 0.26 times than those with no addition) and higher internal ammonium content (ENH_4^+ treatments increased this content ca. 1.5 times compared with those with no NH_4^+ addition) (Fig. 5A, 5B and Table 5). The combination of factors did no produce significant effects on both variables excepting for the combination ENH_4^+ + HT on internal ammonium, which was 1.7 times higher than the average of the other treatments (Fig. 5B; Table 5).

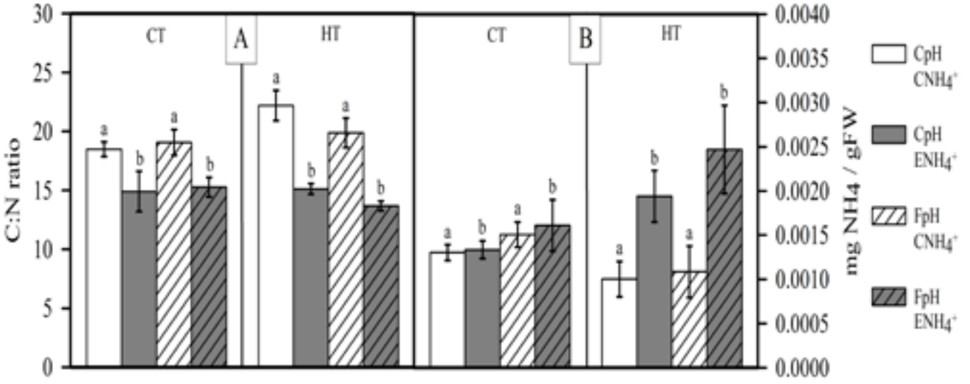

Figure 5. Effects of temperature (CT vs. HT), pH (CpH vs. FpH) and NH_4^+ (CNH_4^+ vs. ENH_4^+) on [A] C:N and [B] NH_4^+ internal concentration (mg NH_4^+ gFW^{-1}). Letters above the bars represent significant differences in NH_4^+ levels.

Table 5. Results of the 3-way ANOVA test on treatments (temperature, acidification and ammonium addition) and relevant interactions for C:N and NH_4^+ internal concentration (mg NH_4^+ gFW^{-1}). All data were normally distributed. * Significance level $p < 0.05$. ** Significance level $p < 0.01$.

	C:N	**Internal NH_4^+**
Temperature	0.302	0.342
pH	0.355	0.171
NH_4^+	<0.001 **	0.005 **
Temperature : pH	0.132	0.863
Temperature : NH_4^+	0.063	0.011 *
CO_2 : NH_4^+	0.818	0.505
Temperature : pH : NH_4^+	0.72	0.631

DISCUSSION

This work showed that warming, acidification and ammonium enrichment produced both, alone and in combination significant effects in plant productivity, non-structural carbohydrates (NSC) content and internal nitrogen. Temperature was the factor that had a higher effect on plant productivity, causing a significant increase in GPR and NPR (Fig. 3A, 3C; Table 3) probably due to the positive effect of temperature on the enzymatic machinery of photosynthesis as previously demonstrated for *Cymodocea nodosa* (Pérez & Romero 1992, Terrados & Ros 1995). Plants also showed changes in NSC under high temperature condition, with lower belowground contents in those plants subjected to high temperature (Figs. 4B, 4C; Table 4). As temperature enhances metabolic activity and respiration, it may cause plants to use the stored carbohydrates (mainly sucrose) as response for the increase in energy requirements and carbon demand as noted in previous studies (Van Katwijk et al. 1997, Brun et al. 2002, 2008). In contrast, no significant response was found on internal measured variables (C:N ratio and NH_4^+ tissue content) under high temperature (Fig. 5; Table 5). Therefore, it suggests that temperature increases production and growth but decreases the internal carbon reserves. Internal carbon reserves are essential for plants when environmental conditions become more stressful such as light limitation, high ammonium concentration or anoxia conditions (Van Katwijk et al. 1997, Terrados et al. 1999, Brun et al. 2002, 2003) and then, under these conditions plants could become more susceptible due to the limited reserves of carbon reserves and energy.

Acidification increased CO_2 availability but had little effect on seagrass growth (Fig. 3; Table 3), agreeing with previous studies (e.g. Schwarz et al. 2000, Martínez-Crego et al. 2014). However, acidification (FpH) produced an increase in above starch at the expense of below starch concentrations (Fig. 4C, 4D; Table 4), but no significant responses were found in internal characteristics (C:N ratio and internal NH_4^+ concentration) (Fig. 5; Table 5). This weak response to pH decrease may be explained if plants were already carbon saturated (i.e. in the proximity of the RuBisCO), because of the use of carbon concentration mechanisms (CCM) or because they are already using bicarbonate directly (Beer et al. 1980, Schwarz et al. 2000, Invers et al. 2001).

Ammonium enrichment had no significant effect on GPR and NPR but caused a significant decreased in LLR (Fig. 3; Table 3). Ammonium can be toxic for seagrasses at high concentrations and in order to avoid such toxicity, ammonium must be rapidly assimilated into amino acids at the expense of a consumption of energy and carbon skeletons (Brun et al. 2002). In our experiment, ammonium toxicity was unlikely because there was no negative response in growth and in the concentration of NSC (Fig. 4; Table 4), which is generally considered as an early warning symptom of ammonium toxicity (Brun et al. 2002, Villazán et al. 2015, Moreno-Marín et al. 2016). This can be explained both by the high levels of irradiance on the incubation chambers which provide ATP, reducing power and carbon skeletons to undertake ammonium assimilation (Brun et al. 2002, 2008, Villazán et al. 2015, Moreno-Marín et al. 2016), but also by the lower sensitivity to ammonium toxicity (i.e. a higher concentration is necessary to produce negative effects) that seems to display *Cymodocea nodosa* when compared to other seagrass species (e.g. *Zostera noltei* or *Z. marina*; Moreno-Marín et al. 2016).

The most noteworthy results of this study derived from the combination of the three factors assessed simultaneously. Thus, an accumulation of ammonium in seawater was found under ENH_4^+ + HT treatment especially under the forecasted pH value, which had a lower capacity to assimilate the extra ammonia injected in the water in the early days of the incubation (Fig. 2). It is known that temperature enhance the toxicity process in seagrasses if ammonium is used as the N source rather than of nitrate (Van Katwijk et al. 1997, Brun et al. 2002), which may then prone to those plants subjected to such combination of factors to a higher vulnerability against ammonium. However, ammonium accumulation in the water disappeared after two weeks in such treatments, which may be due to the increase in the size of the plants (i.e. lowering the ratio ammonium/biomass) or, what is most likely, and increase of the microbial benthic community in the sediment, which is able to considerably reduce ammonium concentrations in the water column (Moreno-Marín et al. 2016).

As a result of the interaction of the three factors, a remarkable finding of this experiment was the increase in *Cymodocea nodosa* production, being the treatment FpH + ENH_4^+ + HT one of the treatments with higher increase in net production (2 times) (Fig. 3). In addition, this treatment led to a significant increase in below sucrose (1.25 times) (Fig. 4; Table 4). Thus, these results demonstrated that the combined effect of the three factors triggered a positive response of *Cymodocea nodosa* improving the productivity in this species and enhancing NSC concentrations, which may in addition improve plants resistance to other stressors. Hence, based on these results it seems that climatic change and to some extent nutrient enrichment in coastal areas may not be so detrimental than previously believed (Orth et al. 2006), and even may benefit *C. nodosa* productivity and resistance in the future. In this regard, seagrass meadows are natural hot spots to fight against climate change as they benefit from these changes, and have a large potential to uptake the excess of anthropogenic CO_2 (Duarte and Cebrián 1996, Kennedy et al. 2010, Russell et al. 2013). Therefore, conservation management to protect and increase seagrass meadow is a potential solution to the global problems we face.

CONCLUSIONS

The present study showed that although the environmental factors studied in this experiment may produce a limited response in this seagrass species (as in the case of pH decrease), a significant synergistic response can occur when all factors interact (Gunderson et al. 2016). In this case, we highlights a positive response of *Cymodocea nodosa* under forecasted future conditions of temperature increase, pH decrease and ammonium enrichment as their productivity enhances without decreasing non-structural carbohydrates reserves, which are essential when environmental conditions become more stressful. However as it was pointed out, although there may be a positive synergy with the direct effect of environmental factors, other indirect effect may hamper them (such as algal blooms or larger epiphytic biomass under ammonium enrichment). Overall, our results will be useful to understand how these threatened ecosystems will deal with the challenges that pose global change. This research also highlights the importance of studying environmental factors that interact in nature using a multifactorial approach, which yield a more realistic approximation of the possible effects of global change and anthropogenic impacts on seagrass ecosystems.

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GENERAL DISCUSSION

En la vida ni se gana ni se pierde, ni se fracasa ni se triunfa. En la vida se aprende, se crece, se descubre; se escribe, borra y reescribe; se hila, se deshila y se vuelve a hilar.

Ana Celia Blum

Evaluation of the carbon metabolism and DOC fluxes in seagrass communities

Throughout this Thesis it has been verified that seagrass communities are highly autotrophic, suggesting that ecosystems dominated by these communities have a large contribution to the net storage of C. In addition, they acted as a net DOC source in coastal areas boosting the transfer of energy and matter in these areas. However a great variability in their contribution to both processes was found depending on the seagrass species (Chapter 1, 2 and 5), season of the year (Chapter 1, 3 and 4) and intrinsic characteristics of the meadow (e.g. different colonization states) (Chapter 2) (Table 1).

Table 1. Net community production (NCP, mmolC m^{-2} d^{-1}), dissolved organic carbon (DOC) fluxes (mmol C m^{-2} d^{-1}) and the percentage of NCP released as DOC (%) in the communities dominated by three seagrass species sampled in this Thesis. Results for *C. nodosa* were obtained *in situ* from the annual average (bold letters) and from each season of the year (plain letters) of communities dominated by this species. Results for *H. wrgihtii* were obtained *in situ,* in spring (bold letters) and from each colonization state (plain letters) from communities dominated by this species. Results for *Z. noltei* were obtained from the mesocosm experiment done in summer under medium current velocity conditions.

NCP	DOC	% NCP as DOC	Species	Site
81 ± 20	**9 ± 4**	**11**	***Cymodocea nodosa***	**Cádiz (Spain)**
25 ± 15	1 ± 2	4	Winter	
88 ± 6	16 ± 6	18	Spring	
117 ± 21	18 ± 6	15	Summer	
104 ± 24	6 ± 2	6	Autumn	
79 ± 14	**13 ± 8**	**16**	***Halodule wrightii***	**La Paz (Mexico)**
102 ± 31	44 ± 3	43	Young patch	
73 ± 28	2 ± 1	3	Medium patch	
61 ± 5	-6 ± 5	10	Old patch	
17 ± 3	**13 ± 4**	**76**	***Zostera noltei***	**Yerseke (The Netherlands)**

The methodology used in this Thesis has been widely utilized elsewhere (e.g. Barrón & Duarte 2006) because it allows an effective approach of the *in situ* metabolic responses of the whole community. However, this methodology presents limitations such as an increase in pH and O_2 as a result of photosynthetic activity, which do not occur to the same degree in the natural meadow where the high turbulent mixing avoids the oversaturation (Champenois & Borges 2012). This can causes a decrease of the photosynthetic rates (Invers et al. 1997) and, consequently, an underestimation of NCP. On the other hand, community respiration can be underestimated under low O_2 conditions when incubation is in darkness during long time (Zimmerman et al. 1989, Buapet et al. 2013). Nonetheless, a recent study underscored that this uncertainties usually are produced in experiment where NCP was estimated at solar noon or during several hours (more than 6 h.) (Olivé et al. 2015). Here, the NCP was estimated during 5–6 hours after sunrise, and therefore it was assumed that the underestimation is of little relevance in this work.

This Thesis has showed how the different components of the community (i.e. seagrass, biotic components in the sediment, macroalgae, epiphytes and plankton community) can have large and variable contributions to the NCP of the whole community (Fig. 1) (Chapter 1). However, this variability in the contribution of NCP was highly dependent on the season of the year. For example the plankton and epiphytes became the dominant primary producers in the community dominated by *C. nodosa* during the winter whereas the bare sediments showed a higher net heterotrophic activity in spring and summer. As was argued in Chapter 1, the extrapolations of results from the sediment and the plankton community to the *C. nodosa* community should be taken with caution. The structure of the community thriving in bare sediment will probably not be the same than that in sediments dominated by *C. nodosa* (González-Ortiz et al. 2016). Moreover, the bimonthly period used in Chapter 1 may not be sufficient to cover the metabolic changes occurring in the plankton community. Despite these bias introduced due to the methodology, it was considered that does not hinder the objective of estimating approximately the contribution of each biological component in the NCP of the whole community dominated by *C. nodosa*.

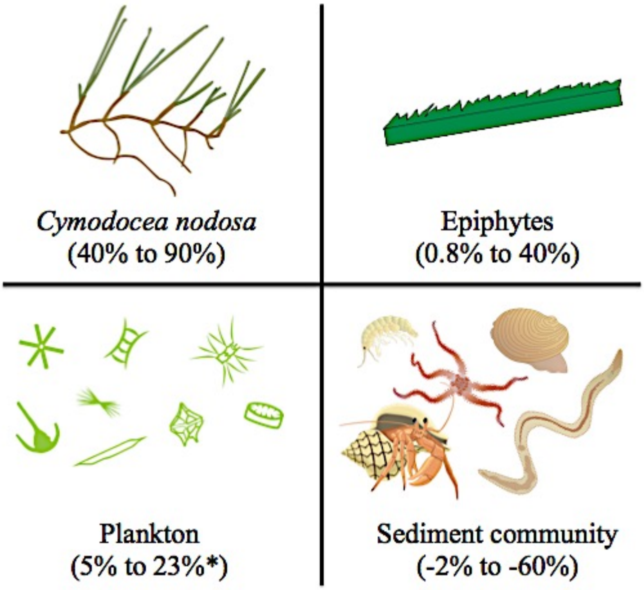

Figure 1. Percentage of NCP of each biological component in the total NCP of the community dominated by *C. nodosa*. Negative values indicate a net heterotrophic activity and tend to decrease the NCP of the whole community. Productivity data for *C. nodosa* were obtained from unpublished data through the punching method, ephiphytes from Shönemann (2015) and the plankton and sediment community from those studied in Chapter 1 respectively. * Percentage of NCP from plankton except in winter, when plankton became the main primary producer.

In addition, this study has demonstrated that shifts in the associated community (i.e. diversity and abundance of organisms) within the seagrass meadow can drive strong variations in the carbon community metabolism and DOC fluxes, even within the same seagrass species, season and place (Chapter 2). This difference in the community associated was attributed to a different meadow states after the colonization of bare sediments. Thus, vegetated communities showed a decrease in the net community production (NCP) with the colonization state (Table 1).

On the other hand, in this Thesis it was studied an annual cycle of the carbon community metabolism and DOC fluxes in vegetated coastal communities (*C. prolifera*, *C. nodosa*) and the plankton one simultaneously, which allowed to study the carbon dynamics between different compartments of the ecosystem (Chapter 1). Seasonality in C metabolism and DOC fluxes was found in both communities, the seagrass and macroalgae. Temperature and light are probably the environmental variables that drove these inter–annual variations (Peralta et al. 2002, Olivé et al. 2013). However, the amplitude in seasonal variation was much higher in the community dominated by *C. prolifera*, which shifted its net metabolism throughout the year, ranging from autotrophic to heterotrophic. As *C. nodosa* remained autotrophic throughout the year we can conclude that this community may be able to dampen the consequences of metabolic variability of *C. prolifera*, allowing for a more stable metabolic balance in the whole bay. Moreover, it was found that the main primary producer in the whole bay varied along the year. Vegetated communities were autotrophic at annual scale and macrophytes were the main primary producers in the system during the whole year, but plankton became the main primary producer in the winter. Thus, the main primary producers in the system were *C. prolifera* in spring, *C. nodosa* in summer and the plankton community in winter.

The excess of production from the seagrass meadows can be either stored at medium or long-term in the sediment (thus contributing to partially offset the climate change), or exported to adjacent ecosystems as particulate or dissolved forms (Duarte & Cebrián 1996, Barrón & Duarte 2009). Seagrass meadows represent areas with great DOC production (Barrón & Duarte 2009, Barrón et al. 2014). By comparing previous studies, it can be estimated that the global net DOC exported from seagrass meadows (Barrón et al. 2014) represents 46% of the global NCP of seagrass meadows (Duarte et al. 2010). Here, DOC flux represented 11% of their annual NCP in *C. nodosa* and 16% of their NCP in *H. wrightii* (Table 1). The high global average value from Barrón et al. (2014) is a consequence of extrapolating results from a few studies, most in tropical zones and during periods of high productivity. This Thesis allowed a better adjustment of this global average, since we studied a temperate seagrass species during a whole year (Chapter 1) and a tropical seagrass in a region where seagrasses have received little attention (Chapter 2). The low percentage of DOC in relation to NCP found here is probably a consequence of including whole communities with potential DOC consumers (i.e. infauna, bacteria from sediments, plankton, etc). This hypothesis is reinforced when the results for *Z. noltei* (76 %, see Table 1) and the other two species (*C. nodosa* and *H. wrightii*) were compared, since in the case of *Z. noltei* experiment, plants were collected without the associated infauna (Chapter 5), while in the latter studies the whole community was considered. In addition, the study of *H. wrightii* evidenced that DOC represented 44% of the NCP in the youngest meadow, which showed a lower abundance and diversity of fauna (Chapter 2).

A great variability in DOC fluxes were found in seagrass meadows depending on the species (Chapter 1 and 2) and season (Chapter 1), which agrees with a previous review (Barrón et al. 2014), and reinforced the great variability found in seagrass productivity. However, this Thesis has demonstrated that the variability in DOC fluxes can be explained, to a great extent, as a consequence of the meadow characteristics (i.e. hydrodynamic regime and characteristic of the community associated), which varied with the colonization state, as found in Chapter 2. Thus, the highest DOC release was found in the early states of the *H. wrightii* meadow, as a result of the increase in the hydrodynamic regime within the meadow due to the lower seagrass density and biomass, which was demonstrated later in Chapter 5, and the lower abundance and diversity of potential DOC consumers.

The results of this study have shown a high and significant correlation between the DOC flux and community production, especially for the NCP (Chapters 1 and 2). Therefore, a high productivity in seagrass communities seems to be linked to larger DOC fluxes, as supported with previous findings (Ziegler & Benner 1999). Hence, any factor that contributes to increase the primary production of the community (e.g. increase in water temperature -Chapters 4 and 6-, increase in CO_2 -Chapters 5 and 6-) may trigger higher fluxes of DOC, which may boost the flux of matter and energy in these coastal areas.

The DOC released by seagrass communities can be very relevant for the food web in the community and for adjacent ecosystems, because it boosts the turnover of C and energy, as it is easily assimilated by marine organisms and fully involved in the C exchange between componets of the ecosystem (Hansell & Carlson 2001, Navarro et al. 2004). For example, metazoans such as sponges can consume DOC (which may provide up to 80% of their organic carbon demand; Mueller et al. 2014). This Thesis supports the importance of DOC in the metabolism of shallow coastal areas, showing that benthic communities could contribute largely as DOC source for the planktonic one (Chapter 1), as previously suggested by Navarro et al. (2004). Hence, benthic DOC helps to fuel the plankton productivity, and then indirectly, fuels the NCP of the whole ecosystem when environmental conditions are less favourable for the primary production of benthic macrophytes. Therefore, it can be drawn that macrophyte diversity in coastal areas and their strong metabolic interactions may help to buffer shifts in productivity when environmental conditions are less favourable.

The next step regarding DOC should be to study the contribution of each component of the community. As has been argued, the role of the potential DOC consumer such as plankton or infauna is relevant (Chapters 1 and 2). However, multiple components of the seagrass community might be responsible for DOC uptake or release and this is still poorly understood. For example, as far as we are aware, there are no estimates in the literature of the contribution of the degradation of organic compounds from the sediment (benthic DOC flux) in the net DOC flux in seagrass meadows. However, taking into account the large amount of particulate organic matter (POM) stored in these sediments (Kennedy et al. 2010), that may be potentially transformed into DOC through heterotrophic bacteria activity (Boto et al. 1989, Maher & Eyre 2010), the contribution of benthic DOC flux into the total DOC flux may be also significant. Furthermore, some authors using stable and radioactive isotopes (Brun et al. 2003, Vonk et al. 2008), reported that seagrass leaves take up dissolved organic nitrogen and also DOC (Van Engeland et al. 2011). However, little attention has been given to this DOC uptake. Hence, the contribution of each component of the seagrass community in the total DOC release is an open question that requires further attention.

Effects of human–induced disturbances on the carbon dynamics of the community

The effects of nutrient enrichment on vegetated coastal ecosystems under ecosystem approach have mainly focus so far on the shading caused by the proliferation of epiphytes and opportunistic macroalgae (Short et al. 1995, Brun et al. 2003), the intensification of the flux of organic matter to the sediment (Borum et al. 2005, Pérez et al. 2007) or the stimulation of seagrass consumption by herbivores (McGlathery 1995, Ruiz et al. 2001). This Thesis underscores a new approach that shows how increase in nutrients at long term yields alterations at the ecosystem level, which can drive the community to heterotrophy in some seasons of the year (e.g. winter for *C. nodosa* and summer for *C. prolifera*; Chapter 3) (Table 2). Probably, the increase in biodiversity and benthic fauna (Jimenez-Ramos et al. submitted) and the increase in the flux of organic matter to the sediment (which consume O_2, Borum et al. 2005, Pérez et al. 2007, Olivé et al. 2009)) trigger a higher heterotrophic activity at the community level. As a consequence of the increase in heterotrophic activity, the C uptake ability decreases, and therefore, a significant decrease in C sequestration is expected, which may hamper future efforts to mitigate climate change. On the other hand, the increase in nutrients produced an increase in seagrass biomass in summer (when environmental conditions are more favourable for seagrass growth). Then, a moderate raise in the availability of nutrients stimulates the seagrass growth as a consequence of an increase in productivity (Short 1987, Pérez et al. 1991, Alcoverro et al. 1997, Udy et al. 1999). However, high ammonium conditions evidenced no effect in seagrass productivity (Chapter 6). This weak response can be attributed to the seagrass manipulation in the mesocosm facilities or, what is more likely, as a consequence of use isolated plants instead of the whole community. This suggests that future efforts in the research of human–induced disturbances should be focused on the whole community in order to better understand the response of these ecosystems to face up climate change.

Water acidification as a consequence of CO_2 increase was evaluated by two methods in two temperate seagrass species: via bubbling with CO_2 in *Z. noltei* (Chapter 5) and via water acidification in *C. nodosa* (Chapter 6). Both approaches increased dissolved inorganic carbon (DIC) availability and mainly CO_2. In an open–atmosphere system (Chapter 5), the bubbling method was the easiest way to maintain constant conditions over long periods of time. Logistic problems did not allow the use of this methodology in Chapter 6, because of that water was acidified. As incubation chambers in Chapter 6 were atmospheric–isolated and a strong water turnover was established, the bias derived from the possible total alkalinity (TA) decrease was reduced. This Thesis has found a significant increase in GPP and R (Chapter 5) but a weak response in seagrass growth (Chapter 6)

(Table 2). The weak response to pH decrease in *C. nodosa* in Chapter 6 may be explained by the fact that plants were already carbon saturated, since they can uptake bicarbonate directly (Beer et al. 1980, Invers et al. 2001, Schwarz et al. 2000). On the other hand, *Z. noltei* showed a significant increase in GPP and R (Chapter 5). This C surplus was translated into sucrose increase in aboveground tissues, which is consistent with the gene expression under high CO_2 conditions in seagrasses (Ruocco at al. 2015), instead of growth and DOC release. The results of this Thesis confirmed that the responses of seagrass ecosystems to CO_2 increase will be complex and depends on the seagrass species and the carbon saturation stage of the plants, which still presents many uncertainties (Silva et al. 2009). Probably the CO_2 increase will have more effect in the associated community (e.g. epiphyte composition and abundance; Brodie et al. 2014, Martinez-Crego et al. 2014, Nogueira et al. 2017), which will affect plants indirectly. Hence, a more integrative ecosystem-based approach is necessary in future researchs about the CO_2 increase effect in seagrasses.

Among the stressors considered in this Thesis: nutrient enrichment, pH decrease, changes in the hydrodynamic regime and water temperature increase, the latter was the stressor that had a stronger positive effect on carbon metabolism (i.e increased P:R ratio, GPP and NCP) (Chapter 4), yielding higher seagrass productivity and growth (Chapter 6) (Table 2). The increase in temperature affects positively the enzymatic machinery of photosynthesis (within a range), as previously demonstrated in *C. nodosa* (Pérez & Romero 1992, Terrados & Ros 1995). This C surplus led to a higher DOC release, especially in summer (Chapter 4). In addition, temperature increase may have positive consequences for temperate seagrass meadows, as the C surplus may help seagrasses to synthesize more carbon skeletons that can be directly used for growth or stored, supporting plant growth during unfavourable growing conditions. However, an increase in water temperature for longer time triggered a decrease in the carbon reserves (mainly sucrose), as shown in chapter 6, probably as a consequence of the higher metabolic activity and respiration, as reported in previous studies (Van Katwijk et al. 1997, Brun et al. 2002, 2008). Nonetheless, in a future scenario, high temperature will interact with other environmental factors (e.g. pH decrease, ammonium enrichment, etc.) and the final outcome is not so straightforward, since this Thesis demonstrated that the interaction of several environmental factors may produce non-additive effects (i.e. synergist or antagonist). Thus, high temperature under pH decrease and ammonium enrichment conditions increased the *Cymodocea nodosa* production, growth and sucrose, as was demonstrated in Chapter 6.

Table 2. Summary of the effects of human-induced disturbances on the carbon dynamics of the seagrass community. GPP: gross primary production (mmol C m^{-2} d^{-1}); R: respiration (mmol C m^{-2} d^{-1}); NCP: net community production (mmol C m^{-2} d^{-1}); P:R: production:respiration rates; NPR: net production rates (g FW d^{-1}); DOC: dissolved organic carbon (mmol C m^{-2} d^{-1}) fluxes; % NCP as DOC: the percentage of NCP released as DOC. Numbers in parentheses indicate how many times the variable increases or decreases

	GPP	**R**	**NCP**	**P:R**	**NPR**	**Carbon reserve**	**DOC**	**% NCP to DOC**	**Chapter**
Nutrient enrichment	Decrease	No significant effect	Decrease in winter (1.7) and shif to heterotrophy	Decrease in winter (0.5), increase in summer (1.2)	No effect	No effect	Consumer in winter, high producer in summer (7)	Increase (6) in summer	3 and 6
pH decrease	Significant increase (1.5)	Significant increase (1.5)	No significant response	-	No effect	Decrease in aboveground (1.25) and belowground (1.3)	No effect	-	5 and 6
Hydrodynamic regimen change	No significant response but medium velocity increase (1.6)	No significant response but medium velocity increase (1.7)	No significant response but medium velocity increase (1.8)	-	Significant decrease in biomass lost in high (1.4) and medium (1.3) velocity respect low velocity	No significant response	Increase in high (6) and medium (2.4) velocity respect low velocity	Increase in low (0.2), medium (0.6) and high (1.2) velocity	5
Temperature Increase	Increase in summer (1.6)	Increase in summer (1.5)	Increase in summer (1.8)	Increase in winter (0.15) and summer (0.06)	Increase (1.5)	Decrease sucrose belowground and starch aboveground (1.5)	Increase in summer (2)	-	4 and 6

Regarding DOC fluxes in coastal vegetated communities, the increase in nutrients triggered significant changes in these fluxes, either increasing or decreasing the DOC release depending on season of the year (Table 2; Chapter 3). The decrease in DOC release can be the result of a lower productivity or the result of a higher carbon demand after a rapid ammonium assimilation, which is met by resources diverted from other metabolic processes. Although there are no studies on this, probably part of the DOC released from the seagrass comes from metabolic processes that under these conditions are more limited. The high DOC release during the summer in the community dominated by *C. nodosa* can be a consequence of the proliferation of fast–growing primary producers, either phytoplankton, epiphytes or opportunistic macroalgae, which tend to release large DOC amounts, especially in summer. It can be also attributed to an intensification of the flux of organic matter from the sediments due to higher temperatures and nutrient availability. Moreover, a high correlation between DOC release in communities dominated by seagrasses and water temperature was found. Therefore, this study suggests that an increase in temperature because of ocean warming and heat waves may yield an increase in seagrass DOC release, especially in summer (Chapter 4). One of the environmental factors studied in this Thesis that most affected the DOC release was the current velocity. This was attributed to a higher GPP and the enhancement of DOC transference from the sediment to the water column (Chapter 5). Thus, it was demonstrated the hypothesis established in chapter 2 that attributed the larger DOC release in the youngest seagrass patch to the higher current velocity. In summary, this Thesis demonstrated that human–induced disturbance can yield important consequences in the DOC fluxes in seagrass communities. Under forecasted global change conditions in water temperature and nutrient enrichment, the seagrass DOC released will be significantly higher in summer (x2 because of high temperature and x7 because of nutrient enrichment; Table 2) when the net DOC fluxes in coastal vegetated ecosystems are highest (Chapter 1). This increase can be even greater under higher current velocities in relation to a greater number of storms or because the meadows thrive in areas of high hydrodynamic conditions (Chapter 5). This has noteworthy ecological implications since an increase in DOC released from seagrass populations means a faster and more efficient transference of carbon and energy from primary producers to higher trophic levels.

Finally, this Thesis has studied the changes in C metabolism and DOC fluxes through the slow natural recovery of seagrass beds after extreme climatological events (e.g. cyclones, hurricanes or monsoons) which are expected to increase as a consequence of climate change, especially in tropical areas where larger areas inhabited by seagrass meadows can be found. Thus, this Thesis showed that during the early colonization states in patches dominated by *H. wrightii* the NCP and DOC release was highest, and a decrease in NCP and DOC release as the colonization state advances was found. This pattern was attributed to an increase in the community associated (Chapter 2). The DOC release from the seagrass community decreased as the colonization state advances probably due to the existence of more potential DOC consumers in the community and because of the lower hydrodynamic regime as a consequence of the higher seagrass density and biomass, as was demonstrated later in Chapter 5. If NCP and DOC release are highest, the transference of carbon and energy from primary producers to higher trophic levels is higher. Hence, it can be questioned to what extent is adverse for the seagrass community the existence of periods of extreme climatological events that renew the system as, for example, forest fires in land (Long 2006). The response does not depend exclusively on the C metabolism and DOC release. For example, it is know that biodiversity is essential for the ecosystem performance (Najem et al. 1994, Duarte 2000) and productivity (Duffy et al. 2017) and this work found an increase in biodiversity and fauna abundance with the colonization state. Anyway, if NCP and DOC release are highest during the early states of recovery, the rates of C uptake and organic C export in seagrass ecosystems are likely to be underestimated if the frequency and intensity of extreme climatological events increase as a consequence of climate change. However, this work did not study the continuing effect of degradation and recovery of the seagrass meadow as a consequence of an increase in the frequency of extreme climatological events, which could affect or modify the patterns observed here. Future research should delve into the shifts after extreme climatological events in seagrass meadows to predict and mitigate the effects of climate change in coastal marine ecosystems.

In summary, this Thesis has tried to untangle the complex interrelations between the different components of coastal communities, which can question the foreseen consequences of the human–induced changes, in the sense that they cannot be as straightforward as expected. Thus, communities can change their net carbon metabolism from autotrophic to heterotrophic or from net DOC producers to net DOC consumers depending on which one is the dominant vegetated species, season of the year, colonization state and the interaction between environmental variables including nutrient enrichment, temperature, pH decrease and hydrodynamic conditions. Therefore, future studies on human–induced disturbance should be addressed *in situ*, including the whole community in order to better understanding the response of these ecosystems to face up climate change. On the other hand, this Thesis underlines the importance of studying environmental factors that interact in nature using a multifactorial approach, since the interactions of several environmental factors can produce non-additive (i.e. synergist or antagonist) effects. Based on the results of this study, it seems that global change and, to some extent, nutrient enrichment in coastal areas may not be so detrimental than previously believed (Orth et al. 2006) at least for temperate seagrass, and even may benefit *C. nodosa* productivity and resilience in the future. Moreover, forecasted global change conditions of water temperature and nutrient enrichment may increase the seagrass DOC release, especially in summer, when the net DOC fluxes in coastal vegetated ecosystems are highest. Therefore, this Thesis underscored that the role of seagrass meadows in the carbon coastal cycle will be higher in the future as larger C uptake and DOC release may occur under forecasted environmental conditions.

The contribution of the seagrass production to offset the CO_2 increase under future scenarios of climate change.

The results of this study underline the high productivity of vegetated coastal ecosystems at a local scale (Chapter 1). For example, in Chapter 1 vegetated communities were net autotrophic at annual basis (6,000 Tons C y^{-1} for *C. nodosa*; 2,600 Tons C y^{-1} for *C. prolifera*) and then, acting as a potential CO_2 sink, while bare sediments were net heterotrophic (-300 Tons C y^{-1}), and therefore potentially acting as a net source of CO_2. Therefore, the carbon capture (i.e. NCP) in Cádiz bay is about 8,300 Tons C y^{-1} (Chapter 1, Fig. 7). This benthic NCP in Cádiz Bay is equivalent to 45% of the CO_2 emitted by all the cars in the nearby city of Cádiz (120,000 residents approximately) if these cars were driving about 10,000 km y^{-1} and assuming that the carbon released by a standard car is 150 g CO_2 km^{-1}. However, the C sink capacity of these ecosystems depends ultimately on how much organic matter is stabilized in seagrass sediments at long-term (Larkum et al. 2006) which is affected by biotic and abiotic factors (Serrano et al. 2016). Our knowledge about organic carbon dynamics in seagrass ecosystem will requires further attention to this organic matter stabilized in seagrass sediments (Belshe et al. 2017). The remaining material is either consumed and/or exported elsewhere, so the actual burial capacity of Cádiz Bay will probably be lower (30 – 50% of the seagrass NCP according to Kennedy et al. (2010)). Recent literature shows that coastal vegetated ecosystems may act as natural hot spots for carbon sequestration (e.g. Nellemann et al. 2009, Kennedy et al. 2010, Fourqurean et al. 2012, Lavery et al. 2013, Pergent et al. 2014), which has led to suggest the key role played by seagrasses, salt-marshes, estuaries and mangroves, in order to support strategies to mitigate climate change (Laffoley & Grimsditch 2009, Mcleod et al. 2011, Duarte et al. 2013). These works usually denote the limited size of the existing database and encourage to rise the efforts to extend and increase these estimates. This Thesis tries to contribute to extend this database, which will improve our capacity to make predictions at a global scale. Future research should delve into the C burial, in particular shallow coastal areas such as Cádiz Bay, to explore their potential to cope with global change. This could promote the need for greater protection of these valuable ecosystems under the framework of the IPCC.

However, in spite of the relevance of the capacity to capture C in seagrass ecosystems, it can exist some controversy to what extent the C flux in the biosphere is comparable to the C efflux derived from human activity. Our current understanding of the carbon cycle tends to separate the natural flux from the anthropogenic CO_2 emission (i.e. perturbation flux), especially in the ocean where organic carbon flux seems to be in equilibrium (50 PgC y^{-1} in and out) despite the annual increase in dissolved inorganic carbon (ca. 1.6 PgC y^{-1}) (see Fig. 1 in general introduction section). Marine primary production has been considered similar for centuries, a background cycle of CO_2 that has contributed to the balance that established the pre-industrial CO_2 concentration (280 ppm). Thus, current marine primary production is considered negligible to cope with the CO_2 increase derived of human activities, and even, the direct contribution of blue carbon to the uptake of increase in CO_2 is still unclear. The capacity of the ocean as a C sink is mainly related to the downward transport of the dissolved carbon due to high CO_2 solubility in cold waters in areas of deep-water formation. Thus, this C saturated water sink to the bottom of the oceans and are stored for centuries due to the thermohaline circulation (Jutterström & Anderson 2010, MacGilchrist et al. 2014), the also called solubility pump (Volk & Hoffert 1985, Heinze et al. 2015). However, the biological component should have more relevance than previously noted. To date, the biological component has been focused on the so-called biological pump (Volk & Hoffert 1985), which consists in the uptake of DIC by biota in the surface ocean to produce organic particulate matter (POC) and their transportation to deep zones. However, less than 1% of POC reaches the open-ocean seafloor by sedimentation (Lee et al. 2004), and this probably may explain why the biological component has not been sufficiently considered for the sequestration of anthropogenic carbon. However, recent studies indicate that the amount of organic material that reaches the deep ocean is greater than previously believed (Agusti et al. 2015). The results of this Thesis encourage to the reflection of the role of the marine primary production (which include oceanic plankton and vegetated coastal ecosystems) in the ocean C sink and the role of the coastal vegetated zones in the global carbon cycle. The magnitude of C capture and DOC export between vegetated coastal communities at local level, which have been showed in this Thesis, support this reflection. Furthermore, at a global scale, blue carbon sink accounts for between 235 – 450 Tg C y^{-1}, which represents half or more than the annual carbon stored in the deep ocean sediment (Fig. 1) (Duarte et al. 2005, Laffoley & Grimsditch 2009, Nellemann et al. 2009, Mcleod et al. 2011). On the other hand, vegetated coastal habitats play an indirect role in the ocean C sink by boosting the plankton compartment (through DOC released from benthic communities, Chapter 1), which is involved in the biological pump. Further studies about the carbon cycle in vegetated coastal ecosystems are necessary for a better understanding of the global carbon cycle to cope with climate change.

This study also revealed how changes in neighbouring benthic communities sharing the same shallow coastal landscape might result in large changes in the C metabolism of the whole system, and consequently in the potential to act as C source or sink (Chapter 1). For example, in Chapter 1, if large areas of the bay were covered by seagrasses, the NCP in Cádiz bay can reach 12,600 Tons C y^{-1} (Chapter 1, Fig. 7), equivalent to 70% of the CO_2 emitted by all cars in the city of Cádiz (under the same conditions explained previously). This would imply that up to 35% (0.5 x 70% of the CO_2 emitted, according to Kennedy et al. 2010) of this CO_2 could be sequestered by the communities dominated by seagrasses. Unfortunately, the declining of vegetated coastal ecosystems is becoming generalized. Thus, circa 29% of the area covered by seagrasses at the beginning of the twentieth century has already disappeared, and generally replaced with bare, unconsolidated mud and sand soils (Fourqurean et al. 2012). When seagrass and macroalgae meadows decline, not only blue carbon is lost, but also it is likely that a large fraction of the organic C stored in the sediment is released back to the ocean-atmosphere CO_2 pool (Pendleton et al. 2012, Fourqurean et al. 2012). Hence, the lost of vegetated areas and their replacement by unvegetated communities would be a radical shift in the metabolism of the whole bay, moving from a highly autotrophic system and DOC producer, to a heterotrophic community (releasing 3,700 Tons C y^{-1}) that also consume DOC (Chapter 1, Fig. 7). Nevertheless, these possible changes in benthic communities of Cádiz bay should be considered with caution. It is unlikely that seagrasses will be able to cover the mudflats of the whole Cádiz bay, even with a reduction of the human pressure, because of the large array of environmental conditions. Similarly, considering an increase of the human pressure in the bay, the likelihood of a total decline of the vegetated coastal ecosystems in the bay is low, as different environmental mitigation measures have been set up to increase the environmental quality of this shallow ecosystem. In summary, acknowledging the uncertainties and variabilities associated with this extrapolation, it does not mask the objective of underline the high productivity of vegetated coastal areas at a local level, which is essential to improve our forecast capacity and may trigger new conservation strategies for this threaten ecosystem.

Finally, based on the results of this study, human-induced disturbance can produce important consequences in C metabolism and DOC fluxes in seagrass meadows, which will affect the ability to future C sequestration from this key ecosystem in the blue carbon. Thus, increase in nutrient enrichment conditions can decrease the production in some seasons of the year (Chapter 3). In contrast, variables related to climate change such as temperature increase, pH decrease join with the increase in current velocity may enhance the production and, therefore, the C captured (Chapters 4-6) (Table 2). Then, the net community production in seagrasses (averaging ca. 120 g C m^{-2} y^{-1} according Duarte et al. 2010) could increase in the future. This increase in seagrass productivity will affect the large number of ecological services they provide, including the long term C sequestration. If C captured increase, probably the proportion of C that is buried in the sediment will be higher since it can produce an increase in belowground tissues or a possible reduction of the herbivory pressure by increase the C / N ratio making them less palatable (Heck & Valentine 2006, Cebrian et al. 2009). Hence, this Thesis underscores that the role of seagrass meadows in the blue carbon will be higher in the future scenario of climate change as larger C uptake may occur, which may help to partially offset the rise of carbon dioxide levels. However, this finding has to be restricted to temperate seagrass community studied in this Thesis. Future researches should delve into climate change effect on C metabolism and C sequestration capacity following *in situ* approach bearing different seagrass species and from different bioregions to better understand the future role of this key ecosystem to cope with global change.

CONCLUSIONS

CONCLUSIONS

1. Vegetated coastal communities are highly autotrophic and act as a dissolved organic carbon (DOC) source, indicating that these areas may contribute greatly to the net storage of C.

2. Different components of the seagrass community (i.e. seagrasses, biotic components of the sediment, epiphytes or plankton) showed a large and variable contribution along the year to the net community production (NCP).

3. The increase in nutrient concentration triggered that seagrass and macroalgae–dominated communities moved from autotrophy to heterotrophy in certain seasons of the year. This may be a consequence of the promoted increase in species richness and fauna abundance.

4. The response of seagrass communities when subjected to a pH decrease was complex and showed to be species-specific. The pH decrease triggered a significant increase in GPP and R, which translated into sucrose increase in aboveground tissues.

5. Water temperature caused a high positive effect on carbon metabolism (i.e increased P:R ratio, GPP and NCP) and increased seagrass productivity, growth and DOC fluxes.

6. There was a high significant correlation between the DOC released and the seagrass community production.

7. The DOC released from benthic communities contributed to fuel plankton productivity. However, there was a great variability in community production and DOC fluxes depending on the species, season and colonization states of the meadow.

8. DOC released in early states of seagrass colonization was higher and may be the consequence of an increase in the hydrodynamic regime within the meadow due to a lower seagrass density and biomass, and also due to the lower diversity of potential DOC consumers.

9. Nutrient enrichment can decrease DOC fluxes as a consequence of the reduction in seagrass productivity. However, nutrient enrichment can produce a higher DOC

release in summer as well, as a consequence of the proliferation of fast–growing primary producers, either epiphytes or opportunistic macroalgae.

10. A high correlation between DOC release and both water temperature and current velocity was found. Temperature increased productivity and C metabolism of seagrasses, while high current velocity increased GPP and enhanced DOC transference from the sediment to the water column.

11. Forecasted global change conditions in water temperature and nutrient enrichment may increase the DOC released by seagrass community, especially in summer when the net DOC fluxes in coastal vegetated ecosystems are highest.

12. Climatic change and to some extent moderate nutrient enrichment in coastal areas may not be so detrimental than previously believed, at least for temperate seagrasses, and even may benefit the productivity and resistance of some species (e.g. *Cymodocea nodosa*) in the near future.

13. This Thesis underscores that the role of seagrass meadows in the carbon coastal cycle will be more relevant in the future, as larger C uptake and DOC release may occur under forecasted global change conditions.

CONCLUSIONES

1. Las comunidades vegetadas costeras son altamente autotróficas y actúan como fuente de carbono orgánico disuelto (COD), lo que indica que estas áreas pueden contribuir en gran medida al almacenamiento neto de carbono.

2. Diferentes componentes de la comunidad de fanerógamas marinas (es decir, fanerógamas marinas, componentes bióticos del sedimento, epífitos o plancton) mostraron una gran y variable contribución a la producción neta de la comunidad (PNC) a lo largo del año.

3. El aumento de la concentración de nutrientes provocó que las comunidades dominadas por fanerógamas marinas y macroalgas pasaran de autotrofia a heterotrofia en ciertas estaciones del año. Esto puede ser una consecuencia del aumento en la riqueza de especies y abundancia de fauna.

4. La respuesta de las comunidades de fanerógamas marinas cuando se sometieron a una disminución del pH fue compleja y mostró ser específica para cada especie. La disminución del pH desencadenó un aumento significativo de producción bruta (PB) y respiración (R), que se tradujo en un aumento de la sacarosa en los tejidos aéreos.

5. La temperatura del agua causó un elevado efecto positivo en el metabolismo del carbono (aumento de la relación P:R, PB y PNC) aumentando la productividad, crecimiento y flujos de COD.

6. Hubo una alta correlación significativa entre el COD liberado y la producción de la comunidad de fanerógamas marinas.

7. El COD liberado de las comunidades bentónicas contribuyó a aumentar la productividad del plancton. Sin embargo, hubo una gran variabilidad en la producción comunitaria y los flujos de COD dependiendo de la especie, la estación y los estadíos de colonización de la pradera.

8. El COD liberado en las primeras etapas de colonización de la pradera fue mayor y puede ser la consecuencia de un aumento en el régimen hidrodinámico dentro de la pradera debido a una menor densidad de haces y biomasa, y también debido a la menor diversidad de consumidores potenciales de COD.

9. El enriquecimiento de nutrientes puede disminuir los flujos de COD como consecuencia de la reducción en la productividad de las fanerógamas marinas. Sin embargo, el enriquecimiento de nutrientes también puede producir una mayor liberación de COD en verano como consecuencia de la proliferación de productores primarios de rápido crecimiento, ya sean epífitos o macroalgas oportunistas.

10. Se encontró una alta correlación entre la liberación de COD con la temperatura del agua y la velocidad de la corriente. La temperatura aumentó la productividad y el metabolismo de carbono de las fanerógamas marinas, mientras que la alta velocidad de la corriente aumentó la PB y la transferencia de COD del sedimento a la columna de agua.

11. Las condiciones previstas para el cambio global en la temperatura del agua y en el enriquecimiento de nutrientes pueden aumentar el COD liberado por la comunidad de fanerógamas marinas, especialmente en verano, cuando los flujos netos de COD en los ecosistemas vegetados costeros son más altos.

12. El cambio climático y, en cierta medida, el enriquecimiento moderado de nutrientes en las zonas costeras puede no ser tan perjudicial como se creía anteriormente, al menos para las praderas marinas de regiones templadas, e incluso podría beneficiar la productividad y resistencia de algunas especies (por ejemplo, *Cymodocea nodosa*) en un futuro próximo.

13. Esta Tesis Doctoral subraya que el papel de las praderas de fanerógamas marinas en el ciclo costero del carbono será más relevante en el futuro, ya que mayor captación de carbono y liberación de COD puede ocurrir bajo las condiciones de cambio global previstas.

CONCLUSÕES

1. As comunidades de vegetação costeira são altamente autotróficas e atuam como fonte de carbono orgânico dissolvido (COD), o que indica que essas áreas podem contribuir muito para o armazenamento líquido de carbono.

2. Diferentes componentes da comunidade de angiosperma marinha (p. ex., angiosperma marinha, componentes bióticos dos sedimentos, epífitas ou plancton) mostraram uma contribuição ampla e variável para a produção comunitária líquida (PNL) ao longo do ano.

3. Aumento da concentração de nutrientes causou comunidades dominadas por angiosperma marinha e macroalgas para passar da autotrofia para heterotrofia em certas estações do ano. Isso pode ser uma consequência do aumento da riqueza de espécies e da abundância de fauna.

4. A resposta das comunidades angiosperma marinha quando submetidas a uma diminuição do pH foi complexa e mostrou-se específica para cada espécie. A diminuição do pH desencadeou um aumento significativo na produção bruta (PB) e na respiração (R), o que resultou em um aumento da sacarose nos tecidos aéreos.

5. A temperatura da água causou um alto efeito positivo no metabolismo do carbono (aumento da relação P: R, PB e PNC), aumentando a produtividade, o crescimento e os fluxos de COD.

6. Houve uma alta correlação significativa entre a DOC liberada e a produção da comunidade angiosperma marinha.

7. COD liberado das comunidades bentônicas contribuiu para aumentar a produtividade do plâncton. No entanto, houve grande variabilidade na produção comunitária e nos fluxos de DQO, dependendo da espécie, estação e estágios da colonização da pradaria.

8. COD liberado nos estágios iniciais da colonização da pradaria foi maior e pode ser conseqüência de um aumento no regime hidrodinâmico dentro da pradaria devido a uma menor densidade de vigas e biomassa e também devido à menor diversidade de consumidores potenciais de COD.

9. Enriquecimento de nutrientes pode diminuir os fluxos de COD como conseqüência da redução da produtividade de angiospermas marinhas. No entanto, o enriquecimento de nutrientes também pode levar a uma maior liberação de COD no verão como resultado da proliferação de produtores primários de rápido crescimento, sejam epífitas ou macroalgas oportunistas.

10. Uma correlação alta foi encontrada entre a liberação de COD com a temperatura da água e a velocidade da corrente. A temperatura aumentou a produtividade e o metabolismo do carbono dos angiospermas marinhos, enquanto a alta velocidade da corrente aumentou a transferência de PB e COD do sedimento para a coluna de água.

11. As condições previstas para a alterações global na temperatura da água e no enriquecimento de nutrientes podem aumentar a COD liberado pela comunidade angiosperma marinha, especialmente no verão, quando os fluxos líquidos de COD nos ecossistemas da vegetação costeira são maiores.

12. As alterações climáticas e, até certo ponto, o enriquecimento moderado de nutrientes nas zonas costeiras podem não ser tão prejudiciais como se acreditava anteriormente, pelo menos para angiosperma marinhas temperadas, e podem até beneficiar a produtividade e a resiliência de algumas espécies (por exemplo, *Cymodocea nodosa*) no futuro próximo.

13. Esta Tese de Doutorado sublinha que o papel dos leitos de angiospermas marinhas no ciclo do carbono costeiro será mais relevante no futuro, uma vez que uma maior absorção de carbono e liberação de COD podem ocorrer sob condições de alterações global previstas.

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