DOCTORAL THESIS

CARBON SINKS IN SEAGRASS MEADOWS: RATES, CONTROL, VULNERABILITY AND THE ROLE OF CARBONATE.

Inés Mazarrasa Elósegui
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DOCTORAL THESIS

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A mi familia, que me inspira y motiva, siempre.

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Abbreviations and Acronyms

List of most common abbreviations and acronyms used through the Thesis.

C: carbon
C_{org}: organic carbon.
C_{inorg}: inorganic carbon.
$CaCO_3$: calcium carbonate
Ca^{2+}: calcium ion.
$[Ca^{2+}_{sw}]$: calcium ion concentration in seawater.
$[Ca^{2+}_{sat}]$: calcium ion concentration in a seawater solution saturated with calcium carbonate.
CF: CS: Constant Flux: Constant Sedimentation sediment dating model.
CH_4: methane.
Chl a: chlorophyll a.
CIC: Constant Initial Concentration sediment dating model.
C: N: ratio between organic carbon and nitrogen.
CO_2: carbon dioxide.
CO_3^{2-}: carbonate ion.
$[CO_3^{2-}{}_{sw}]$: carbonate ion concentration in seawater.
$[CO_3^{2-}{}_{sat}]$: carbonate ion concentration in a seawater solution saturated with $CaCO_3$.
CRS: Constant Rate of Supply sediment dating model.
DBD: dry bulk density.
$\delta^{13}C$: carbon isotopic signature.
DIC: dissolved inorganic carbon.
DOC: dissolved organic carbon.
DW (%): percentage of dry weight.
EA: elemental analyzer.
GHG: green house gas.
HCO^-_3: bicarbonate ion.
HP: human pressure.
IPCC: Intergovernmental Panel on Climate Change.
K^*_{sp}: stoichiometric solubility product.
λ: decay rate.
^{210}Pb: lead radioisotope 210.
$^{210}Pb_{ex}$: ^{210}Pb in excess.
LOI: Loss of Ignition technique.
NCP: net community production.
N: nitrogen.
OM: organic matter.
Ω: carbonate saturation state.
Ω_{Ca}: calcite saturation state.
Ω_{Ar}: aragonite saturation state.
Ø: sediment grain size.
pCO_2: partial pressure of carbon dioxide.
PIC: particulate inorganic carbon.
P: phosphate.
POC: particulate organic carbon.
ψ: molar ratio CO_2 flux : $CaCO_3$ precipitation.

REDD: reduced emissions from deforestation and forest degradation.
$T_{1/2}$: radionuclide half-life.
T^a: temperature.
TA: total alkalinity.
UNFCCC: United Nations Framework Convention on Climate Change.

Summary

Coastal vegetated ecosystems (i.e. mangroves, saltmarshes and seagrasses) have been recognized as being among the most significant natural carbon sinks on Earth due to a high capacity of absorbing and trapping carbon that gets buried and stored in the sediment compartment for a long-term. Yet "Blue Carbon" ecosystems, as they are usually referred to, rank among the most threatened globally and their decline implies the loss of all the ecosystem services they provide including that of carbon sink. Hence, the protection of these ecosystems has been proposed as a suitable way to mitigate climate change to be considered within the current, or similar, strategies addressed to enhance the protection of terrestrial carbon sinks (i.e. forests) through economic incentives (i.e. REDD+ programs). However, the knowledge on the carbon sink capacity of these ecosystems, especially seagrass meadows, was very weak at the time this thesis was planned and certain gaps of knowledge needed to be urgently addressed.

This thesis was planned with the aim to contribute to the understanding of the processes that govern the carbon sink capacity in seagrass meadows and its vulnerability. Different key questions were examined including the spatial and temporal (~last century) variability in the sediment organic carbon (C_{org}) sinks (stocks and burial rates), the effect of environmental factors and human pressure, the vulnerability of the C_{org} deposits to be remineralized when seagrass meadows are lost, and the role seagrass meadows play as carbonate (i.e. $CaCO_3$) sedimentary reservoirs as well as the implications of carbonate in seagrass meadows efficiency as carbon sinks.

Based on *Posidonia oceanica* meadows around the Balearic Islands, this thesis identifies significant differences in the sediment C_{org} stocks and burial rates among meadows that could be better explained by the level of human pressure than by the environmental factors considered (water depth and wave exposure). High human pressure surprisingly led to higher C_{org} burial rates both spatially and temporally, as the rate of C_{org} burial increased towards the present, coinciding with the increase in anthropogenic pressure in the region of study that took place around the 60's (following tourism industry development), and led to a higher contribution of seston derived organic carbon to the sediment deposits. The enhanced eutrophication of coastal waters derived from an increasing human pressure is suggested as the most parsimonious explanation to the trends observed, indicating that anthropogenic pressure is a relevant factor conditioning the carbon sink capacity in seagrass meadows.

The vulnerability of seagrass C_{org} deposits to remineralization and to be lost as CO_2 is proved, for the first time, in this thesis. The risk of remineralization is however variable and depends on sediment biogeochemical characteristics, being enhanced in sediments rich in nitrogen and sestonic organic matter and prevented as sediment carbonate content increases. Therefore, an enrichment of seagrass sediments with sestonic inputs enhanced by the increase in anthropogenic pressure, as the observed in the Balearic Islands, leads to a "weakening" of the sedimentary carbon sinks since they become more vulnerable to remineralization.

This thesis also demonstrates that seagrass sediments are a globally significant $CaCO_3$ reservoir and that, despite the precipitation of carbonate leading to CO_2 emissions, sedimentary carbonate prevents organic matter remineralization and enhances C_{org} burial efficiency in seagrass sediments.

Hence, the research presented in this dissertation significantly contributes to the knowledge on the role seagrass meadows play as carbon sinks by examining different aspects that, until now, had been largely unexplored or not considered.

Resumen

Los ecosistemas costeros vegetados (manglares, marismas y praderas de fanerógamas marinas) se encuentran entre los sumideros de carbono más importantes del planeta gracias a una elevada capacidad de absorber y atrapar carbono y enterrarlo en el sedimento a largo plazo. Sin embargo, los ecosistemas de "Carbono Azul", como se les conoce, se encuentran entre los más amenazados a nivel global y su declive implica la pérdida de todos los servicios ecosistémicos que proveen, incluido el de sumidero de carbono. La protección de estos ecosistemas podría ser considerada como una medida de mitigación del cambio climático dentro de las estrategias ya existentes, dirigidas a promover, mediante incentivos económicos, la conservación de los sumideros naturales de carbono terrestres, como los bosques (ej.: programa REDD+). Sin embargo, el conocimiento sobre la capacidad como sumidero de carbono de los ecosistemas costeros, en especial de las praderas submarinas, era todavía muy limitado cuando esta tesis se planteó.

El objetivo de esta tesis es contribuir al conocimiento de los procesos que determinan la capacidad como sumideros de carbono de las praderas submarinas así como la vulnerabilidad de esta función. Se han evaluado distintos aspectos claves, incluyendo la variabilidad espacial y temporal (~ último siglo), el efecto de factores ambientales y de la presión antrópica, la vulnerabilidad de los depósitos a ser remineralizados cuando las praderas se degradan y el papel de las praderas como depósitos de carbonato ($CaCO_3$) así como sus implicaciones en la función de sumidero de carbono.

En base al estudio del sedimento de praderas de *Posidonia oceanica* de las Islas Baleares, esta tesis identifica importantes diferencias en los depósitos y tasas de enterramiento de carbono orgánico (C_{org}) en el sedimento que se explican mejor por el distinto nivel de presión antrópica que por los factores ambientales considerados (profundidad de las praderas y exposición al oleaje). Una alta presión antrópica ha dado lugar a mayores tasas de enterramiento tanto espacial como temporalmente, como muestra su incremento hacia el presente coincidiendo con el aumento de la presión antrópica en la región en los 60's asociado con el desarrollo del turismo; y también a una mayor contribución de carbono derivado de seston. Estos resultados apuntan al incremento en la eutrofización del agua, derivado de la incidencia humana, como la explicación más parsimoniosa a las tendencias que se observan, indicando que la presión antrópica es un factor determinante en los sumidero de carbono de las praderas submarinas.

La vulnerabilidad de los depósitos de C_{org} a ser remineralizados y emitidos en forma de CO_2 queda demostrada, por primera vez, en esta tesis. El riesgo a ser remineralizados depende de características biogeoquímicas, siendo mayor en sedimentos ricos en nitrógeno y en materia orgánica de origen sestónico y menor cuanto mayor es la concentración de carbonato en el sedimento. Por tanto, un enriquecimiento del sedimento de las praderas con aportes sestónicos, posiblemente favorecido por un incremento en la presión antrópica, como el observado en las Islas Baleares, da lugar a un debilitamiento de los sumideros de carbono que se vuelven más vulnerables a ser remineralizados.

Esta tesis también demuestra que los sedimentos de las praderas submarinas son un importante reservorio de carbonato a escala global y que, aunque su precipitación da lugar a la emisión de CO_2, el $CaCO_3$ inhibe la remineralización de la materia orgánica e incrementa la eficiencia en el enterramiento de C_{org} en el sedimento de las praderas.

Por tanto, la investigación presentada en esta tesis contribuye significativamente al conocimiento sobre el papel de las praderas submarinas como sumideros de carbono, evaluando diferentes aspectos que, hasta la fecha, habían sido poco o nada explorados.

Resum

Els ecosistemes costaners vegetats (manglars, maresmes i praderies de fanerògames marines) es troben entre els embornals de carboni més importants del planeta gràcies a una elevada capacitat d'absorbir i atrapar carboni, i d'enterrar-lo en el sediment a llarg termini. No obstant, els ecosistemes de "Carboni Blau", com se'ls coneix, es troben entre els més amenaçats a nivell global i el seu declivi implica la pèrdua de tots els serveis ecosistèmics que proveeixen, inclòs el d'embornal de carboni. La protecció d'aquests ecosistemes podria ser considerada com una mesura de mitigació del canvi climàtic dins de les estratègies ja existents, dirigides a promoure, mitjançant incentius econòmics, la conservació dels embornals naturals de carboni terrestres (per exemple, boscos) (programa REDD+). No obstant, el coneixement sobre la capacitat com a embornal de carboni dels ecosistemes costaners, especialment de les praderies submarines, era encara molt limitat quan aquesta tesi es va plantejar.

L'objectiu d'aquesta tesi és contribuir al coneixement dels processos que determinen la capacitat de les praderies submarines com a embornals de carboni, així com la vulnerabilitat d'aquesta funció. S'han avaluat diferents aspectes claus, incloent la variabilitat espacial i temporal (~ últim segle), l'efecte de factors ambientals i de la pressió antròpica, la vulnerabilitat dels dipòsits a ser remineralitzats quan les praderies es degraden i el paper de les praderies com dipòsits de carbonat ($CaCO_3$), així com les seves implicacions en la funció d'embornal de carboni.

En base a l'estudi del sediment de praderies de *Posidonia oceanica* de les Illes Balears, aquesta tesi identifica importants diferències en els dipòsits i taxes d'enterrament de carboni orgànic (C_{org}) en el sediment, les quals s'expliquen millor pel diferent nivell de pressió antròpica que per els factors ambientals considerats (profunditat de les praderies i exposició a l'onatge). Una alta pressió antròpica ha donat lloc a majors taxes d'enterrament tant espacial com temporalment; com a mostra, el seu increment cap al present coincidint amb l'augment de la pressió antròpica a la regió en els anys 60 associat amb el desenvolupament del turisme, i també a una major contribució de carboni derivat de sèston. Aquests resultats apunten a l'increment en l'eutrofització de l'aigua, derivada de la pressió antròpica, com l'explicació més parsimoniosa a les tendències que s'observen, indicant que la pressió antròpica és un factor determinant en els embornal de carboni de les praderies submarines.

La vulnerabilitat dels dipòsits de C_{org} a ser remineralitzats i emesos en forma de CO_2 queda demostrada, per primera vegada, en aquesta tesi. El risc a ser remineralitzats depèn de característiques biogeoquímiques, sent major en sediments rics en nitrogen i en matèria orgànica d'origen sestònic i menor com més gran és la concentració de carbonat en el sediment. Per tant, un enriquiment del sediment de les praderies amb aportacions sestòniques, possiblement afavorit per un increment en la pressió antròpica, com l'observat a les Illes Balears, dóna lloc a un debilitament dels embornals de carboni que es tornen més vulnerables a ser remineralitzats.

Aquesta tesi també demostra que els sediments de les praderies submarines són un important reservori de carbonat a escala global i que, encara que la seva precipitació dóna lloc a l'emissió de CO_2, el $CaCO_3$ inhibeix la remineralització de la matèria orgànica i incrementa l'eficiència en l'enterrament de C_{org} en el sediment de les praderies.

Per tant, la investigació presentada en aquesta tesi contribueix significativament al coneixement sobre el paper de les praderies submarines com a embornals de carboni, avaluant diferents aspectes que, fins ara, havien estat poc o gens explorats.

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Tres medusas transparentes…
flotando en el mar las
tres juntas se…
divierten!
(Cuento, autor desconocido)

I

General Introduction
and
Goals

> *“…. our grandchildren will surely blame us if they find that*
> *we understood the reality of anthropogenic climate change*
> *and failed to do anything about it” (Oreskes 2004)*

I.1. The threat of climate change and the search for solutions in the natural environment

Climate change is one of the most important challenges that humanity is facing. The steady increase in temperature and its derived consequences, such as the increase in frequency and strength of extreme weather events, are causing important impacts to ecosystems and societies, as the natural resources upon which they depend are also under threat (IPCC 2014). Today there is overwhelming evidence supporting that the observed increase in temperature since the mid 20th century is the consequence of an increase in the concentration of green house gases (GHGs) in the atmosphere, derived from the intensification of fossil fuel burning and land-use change since industrial revolution (IPCC 2014). Among GHGs, CH_4 and CO_2 are the most relevant (Trumper et al. 2009), with CO_2 contributing the most to the atmospheric composition (Ciais et al. 2013). The awareness of the human responsibility in this phenomenon has lead to the generally used of the term “anthropogenic climate change” among the scientific community (Parry et al. 2004; Rosenzweig et al. 2008).

The recognition of the existence of this threat lead to the creation of an international agreement for climate, the United Nations Framework Convention on Climate Change (UNFCCC) in 1992, with the ultimate goal of reaching “*the stabilization of greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system”*(UNFCCC 1992). Since the UNFCC was established, several strategies have been developed in order to promote the reduction of GHGs emissions to the atmosphere from industrial activities and land use conversion (e.g. Kyoto Protocol from 1997). Yet, reducing emissions is not enough to successfully achieve the stabilization and reduction of CO_2 atmospheric concentration and it needs to be combined with strategies that enhance its sequestration and storage from the atmosphere (Batjes 1998; IPCC 2005).

The capacity of certain ecosystems to absorb and store carbon has promoted the development of climate change mitigation strategies based on protection and restoration of these important ecosystems (Trumper et al. 2009). Moreover, the conservation of ecosystems that act as natural carbon sinks additionally benefits societies through the other multiple ecosystem services they provide (Canadell and Raupach 2008).

I.1.1. Natural carbon sinks

According to the (UNFCCC 1992), a carbon sink is any process, activity or mechanism that removes carbon from the atmosphere and a carbon reservoir is any component of the climate system where carbon is stored. In the Earth System, carbon is cycled across different reservoirs, mainly the atmosphere, the ocean, fresh water systems, vegetation, soils and sediments (Ciais et al. 2013). When the amount of carbon in any reservoir other than the atmosphere increases it is commonly referred to as carbon sequestration (IPCC 2007). An ecosystem may be considered a natural carbon sink when carbon is sequestered and accumulated at a faster rate than is being released; or as a source when carbon is released at a higher rate than it is absorbed (Trumper et al. 2009).

Photosynthetic organisms (ranging from phytoplankton to terrestrial plants) play a significant role as carbon sinks by absorbing CO_2 from the atmosphere and transforming it to essential compounds for life (Trumper et al. 2009). Whereas a fraction of this carbon might be released back to the atmosphere through respiration, there is a significant amount that remains stored in the living organisms as biomass. Once organisms die, biomass may be preserved in soils, the sea floor or sedimentary rocks (Trumper et al. 2009).

Terrestrial ecosystems store 3 times more carbon than the atmosphere (828 Pg in 2011; Ciais et al. 2013), both in vegetation living biomass (450-650 Pg C; Prentice et al. 2001) and, a major fraction, as dead biomass and litter in soils (1500-2500 Pg C; Batjes et al. 1996). Yet, the ocean is the major carbon reservoir on the biosphere (Ciais et al. 2013), with most carbon being in the form of dissolved inorganic carbon (DIC, 38,000 Pg) and, a minor fraction, as dissolved organic carbon (DOC, 700 Pg) or living biomass, predominantly phytoplankton (2 Pg) (Ciais et al. 2013). The capacity of the oceans to absorb CO_2 depends on two different mechanisms, the solubility pump and the biological pump (Falkowski et al. 2000). The former relies on the solubility of CO_2 into water and its transformation into DIC; the efficiency of which is temperature dependent, being CO_2 solubility favored in cold waters (Falkowski et al. 2000). The biological pump is the transfer of CO_2 taken up by phytoplankton in the ocean surface via photosynthesis leading to a decrease in the CO_2 concentration in surface waters that favors CO_2 flux from the atmosphere into the ocean. Phytoplankton later sinks to deeper depths in the form of organic matter and debris, where it may either be stored or remineralized. Approximately a 25% of the biomass produced in the ocean surface that sinks to the deeper ocean is remineralized through heterotrophic respiration (Falkowski et al. 1998). Yet, once the organic carbon sinks beneath the ocean thermocline it is effectively sequestered from the atmosphere for centuries or even millennia (Falkowski et al. 1998). As a result of their high carbon sequestration capacity, the open ocean and terrestrial ecosystems are estimated to have absorbed more than half of the CO_2 emitted since industrial revolution (Ciais et al. 2013).

However, not only in the open ocean but also along the coast, marine photosynthetic organisms play a highly significant role as carbon sinks (Smith 1981; Duarte et al. 2005). This is mainly driven by vegetated coastal ecosystems, especially mangroves, seagrass meadows, and saltmarshes, that, similar to phytoplankton in the ocean, have shown a great capacity to trap and store carbon (Romero et al. 1994; Mateo et al. 1997; Chmura et al. 2003; Duarte et al. 2005; Bouillon et al. 2008). However, these ecosystems have been traditionally overlooked in the estimates of the global carbon budget and on the climate change scientific and political debate. It has not been until relatively recent that their valuable role as carbon sinks and the urgent need to take them into account for climate change mitigation strategies has been highlighted (Nelleman et al. 2009).

I.1.2. "Blue Carbon" ecosystems

The carbon sequestered by marine organisms has been termed "Blue Carbon" and it accounts for 55% of the carbon sequestered photosynthethically in the Biosphere (Nellemann et al. 2009). In coastal areas, Blue Carbon sequestration is mainly conducted by vegetated ecosystems: seagrass meadows, mangroves and saltmarshes. Despite being responsible of only 10% of the primary production of the ocean, the generally low turn over rate of these organisms allows them to hold a significant fraction (~2/3) of the marine autotrophic biomass (Smith 1981; Duarte and Cebrián 96).

Most of the carbon stored in these ecosystems is located in the sediment compartment as living (roots and rhizomes) or dead biomass (e.g. detritus) along with organic particles trapped from the water column (Alongi et al. 2001; Bouillon et al. 2003; Kennedy et al. 2010; Mcleod et al. 2011; Fourqurean et al. 2012).

Whereas the carbon bound in biomass and soils of terrestrial ecosystems can be preserved for a maximum of decades to centuries (Chambers et al. 2001; Schlesinger and Lichter 2001) the carbon stored in below-ground biomass in coastal ecosystems has been reported to remain for even millennia (Mateo et al. 1997). In addition, vegetated coastal ecosystems enhance sediment vertical accretion thanks to the growth of their below-ground biomass (i.e. roots and rhizomes) and their capacity to favor particle sedimentation (Duarte et al. 2013a). This property likely enhance their capacity to act as long-term carbon sinks as, unlike in terrestrial soils, their sediment would not get carbon saturated (Stewart et al. 2007; Mcleod et al. 2011). In addition, as they are submerged or partially submerged, Blue Carbon ecosystems are not vulnerable to fires, that are, in the case of terrestrial carbon sinks, a major cause of the release of stored carbon as CO_2 (Canadell and Raupach 2008). Thereby coastal ecosystems are especially suitable for carbon burial and storage, with a capacity at least comparable to that of terrestrial forests (Mcleod et al. 2011).

Yet, ecosystems can shift from being carbon sinks to carbon sources as a result of changes in climate conditions, atmospheric composition and land-use (eg. deforestation, afforestation, agricultural practices) (Oechel et al. 1993; Andersen 2003; Houghton et al. 2012). For instance, the conversion and/or degradation of terrestrial ecosystems has been responsible for the release of 1.1-1.5 Pg C y^{-1} during the decades 1980-2010, representing 12% of the anthropogenic CO_2 emissions to the atmosphere (Houghton et al. 2012). In order to protect these valuable sinks, different strategies that promote their conservation have been developed, such as the Reduced Emission from Deforestation and Degradation program (REDD+), under which countries willing or able to reduce emissions from deforestation can be financially compensated (Scholz and Schmidt 2008). Yet, these strategies have been restricted to terrestrial ecosystems for which carbon inventories, sequestration rates and the consequences of their conversion and degradation have been explored for decades and are relatively well described (FAO 2010; Ciais et al. 2013). Blue Carbon ecosystems are suffering an intense degradation as well (Duarte et al. 2013a), but their inclusion in such conservation strategies depends on the development of a broader knowledge on the functioning of these ecosystems as carbons sinks as well as the impact of their degradation in the carbon cycle, than that available at present (Mcleod et al. 2011).

I.2. Seagrass meadows: ecological functions and main threats

Among Blue Carbon ecosystems, seagrass meadows are especially relevant on a global scale, due to their broad distribution along the coasts of all continents except Antarctica (Hemminga and Duarte 2000). Seagrasses are a group of 60 species of flowering plants adapted to live in the marine environment (Hemminga and Duarte 2000; Kuo and den Hartog 2001). As they are rhizomatous and modular plants, seagrasses expand mainly through clonal growth forming extensive meadows occupying the coastal fringe from the intertidal to up to 90 m depth, where they are limited by light availability (Duarte 1991a).

Contrasting to the little charisma that seagrass meadows have traditionally had compared to other coastal ecosystems (e.g. corals) (Duarte et al. 2008), seagrass meadows provide important services to humans and rank among the most valuable ecosystems in the biosphere (Costanza et al. 1997). Seagrass meadows are one of the most productive net autotrophic systems on the planet (Duarte and Chiscano 1999; Duarte et al. 2010) oxygenating both the water column and the sediment and acting as the base of complex food webs that can extend far away from the meadow it-self, influencing adjacent ecosystems (Suchanek et al. 1985; Duarte and Cebrián 1996; Heck et al. 2008). Their physical structure also provides refuge and nursery ground, thereby serving as the habitat and food for many different groups of organism, including charismatic species such as dugongs or manatees, currently under threat (Green and Short 2003; IUCN 2010), and many species of fish, molluscs and crustaceans of commercial interest (Watson et al. 1993; De la Torre-Castro and Rönnback 2004). In addition, the seagrass meadow canopy acts as an efficient filter that traps suspended particles and absorb nutrients from the water column, enhancing water clarity and nutrient cycling (Short and Short 1984; Gacia et al. 2002; Green and Short 2003). Seagrass meadows also play a relevant role as ecosystem engineers (Jones et al. 1994; Koch et al. 2006a) reducing water flow velocity (Fonseca et al. 1982; Gambi et al. 1990) and attenuating wave action (Fonseca and Cahalan 1992; Granata et al. 2001; Bouma et al. 2005), favoring sediment accumulation and stabilization and preventing shoreline erosion (Koch et al. 2006a).

Finally, seagrass meadows have been identified as hot spots for carbon burial in the ocean (Duarte et al. 2005). Despite occupying 0.1% of the ocean surface, they are responsible for 20 % of the global carbon buried in marine sediments (Duarte et al. 2005; Kennedy et al. 2010). A recent study showed that carbon burial in seagrass meadows is comparable to that of mangroves and saltmarshes (when considering global extents) and more than one order of magnitude higher than that of terrestrial forests when standardizing by surface area (Mcleod et al. 2011), thereby placing seagrass ecosystems among the most intense natural carbon sinks on earth.

Despite the relevant services seagrass ecosystems provide, they rank among the most threaten biomes on the planet (Duarte et al. 2008) due mainly to the high influence of humans in coastal areas (Short and Wyllie-Echeverria 1996). Thirty-seven percent of the human population and 70% of the world megacities are located within 100 km from the coast (LOIC 2002; Duarte 2009). Increasing urbanization, construction or usage of coastal areas for recreational or industrial purposes (e.g. aquaculture, fishing or recreation boating) usually favors eutrophication and reduces water clarity, enhances the input of pollutants, leads to physical damage of the seabed (e.g. through anchoring or trawling), and favors changes in the patterns of sedimentation and erosion (Short and Wyllie-Echeverria 1996; Duarte 2002). All these factors cause significant impacts in seagrass meadows (Cambridge et al. 1986; Duarte 1995; Short and Wyllie-Echeverria 1996; Pasqualini et al. 1999; Apostolaki et al. 2007). As a consequence, 29% of the global seagrass extent mapped has been lost since 1980, at a decay rate of 0.9% y^{-1} that has accelerated after the 1990's to up to 7% y^{-1} (Waycott et al. 2009).

The extent of human impacts in seagrass meadows is expected to increase as human population and usage of the coasts continue to grow rapidly (Duarte 2002). In addition, environmental factors such as rising temperatures and extreme weather events (i.e. hurricanes) that also trigger seagrass meadows decline (Short and Wyllie-Echeverria 1996; Marbà and Duarte 2010; Marbà et al. 2014) are expected to increase in the near future as a consequence of anthropogenic climate change. Hence, under this scenario and in the absence of proper management measures, seagrass meadows will be following a widespread decline leading to the loss of all ecosystem services they provide, including carbon sequestration (Duarte 2002; Marbà et al. 2014).

I.2.1. Carbon sinks in seagrass meadows: mechanisms and main gaps of knowledge

The carbon sink capacity of seagrass meadows is derived from both their high primary production rates (Duarte et al. 2010) and from their capacity to trap particles from the water column (i.e.seston) (Gacia et al. 2002; Kennedy et al. 2010) that may derive from different sources, including terrestrial organic matter debris and/or phytoplankton (Figure I.1). This pool of carbon is referred as allochthonous carbon to differentiate from the autochthonous carbon pool, that derives from the seagrass biomass produced through photosynthesis. Seagrass plant biomass is distributed in two different compartments: above-ground (i.e. leaves) and below ground tissue (roots and rhizomes), which is the major component of seagrass biomass (Duarte and Chiscano 1999).The rate of seagrass leaf consumption by herbivores tends to be low (<10% of their production) (Mateo et al. 2006) and seagrass leaf detritus usually show low decomposition rates due to a low N and P content (Enriquez et al. 1993). However, carbon stored as above-ground biomass is still vulnerable to grazers and bacterial decomposition and it is considered a short-term carbon sink (Macreadie et al. 2014). On the contrary, roots and rhizomes tend to be highly recalcitrant due to a low nutritional value and high lignin content (Kuo and Cambridge 1978; Harrison 1989; Enriquez et al. 1993; Klap et al. 2000) and are much more efficiently preserved compared to above-ground tissue (Enriquez et al. 1993; Fourqurean and Schrlau 2003).

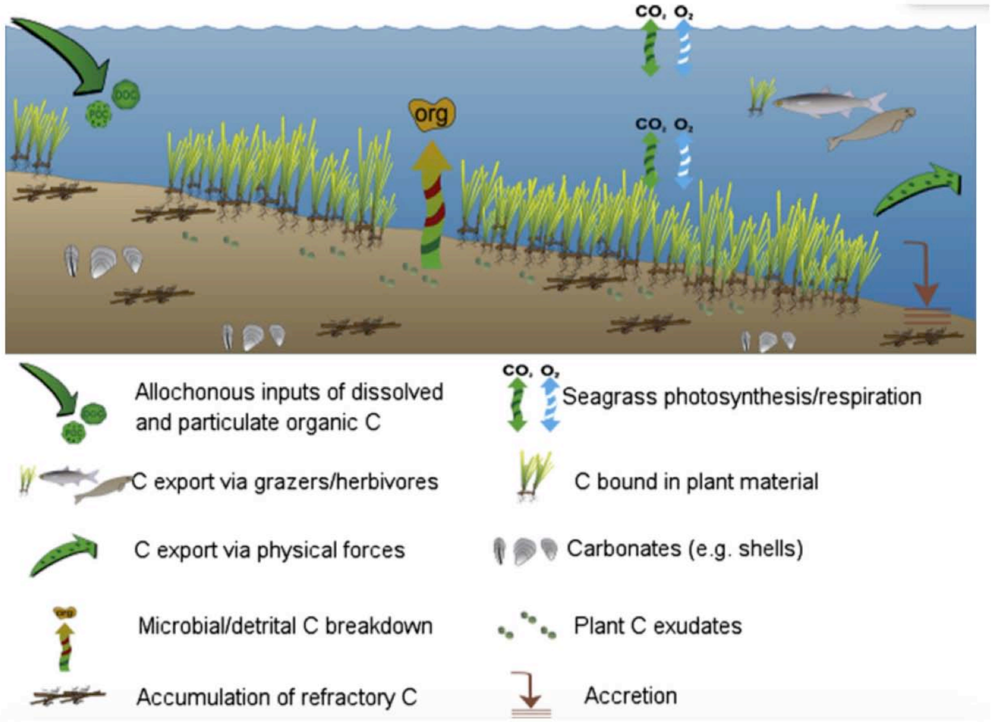


Figure I.1. Conceptual scheme of the main fluxes and reservoirs of carbon in seagrass meadows (extracted from Macreadie et al. 2014).

The high fraction of primary production that is allocated to below-ground biomass and the sedimentation of particles from the water column, lead to a flux of autochthonous and allochthonous carbon into the sediment, where preservation is enhanced (even of non refractory compounds) due to the characteristic anoxic conditions of seagrass sediments (Mateo et al. 2006). Hence, the largest carbon deposits and long-term sinks in seagrass meadows are located in the sediment compartment (Fourqurean et al. 2012).

Other factors enhance the carbon burial capacity in seagrass meadows compared to other natural carbon sinks such as the dampening effect that the canopy exerts against waves and currents, preventing erosion, resuspension (Gambi et al. 1990; Bouma et al. 2005; Koch et al. 2006a) and subsequent sediment aeration.

As done in most of the literature on natural carbon sinks, along this introduction the term “carbon” (i.e. C) has referred only to organic carbon which represents the CO_2 efficiently sequestered from the atmosphere and trapped as organic matter by photosynthesis (Burdige 2007). Yet, in seagrass meadows there is also a significant pool of particulate inorganic carbon in the form of calcium carbonate ($CaCO_3$) that accumulates in the sediment, derived from the shells of different organisms inhabiting the meadows (Hendler et al. 1995; Perry and Beavington-Penney 2005; Panieri 2006) or through the accumulation of particles from the water column (Gacia et al. 2003) (Figure I.1). $CaCO_3$ accumulation in seagrass meadows may have important implications in the role these ecosystems play as carbon sinks (Mateo and Serrano 2012; Macreadie et al. 2014) that will be further developed in section I.4.6. Yet, by the time this thesis started, the inorganic carbon fraction had never been considered in any of the available global assessments of the carbon sequestration capacity of seagrass meadows (Duarte et al. 2005; Mcleod et al. 2011; Fourqurean et al. 2012).

From now on in this thesis, the terms C_{org} (i.e. organic carbon) or POC (i.e. particulate organic carbon) may be also used, along with the term “C” (i.e. carbon), to distinguish the organic carbon from the inorganic carbon fraction, that is referred as C_{inorg}, PIC (i.e. particulate inorganic carbon) or as the mineral form, $CaCO_3$ (i.e. calcium carbonate).

Carbon sinks in Posidonia oceanica

Posidonia oceanica, endemic to the Mediterrenanean Sea, is a remarkable species in terms of carbon sequestration and burial capacity, being the seagrass species storing the largest C_{org} sediment stocks described so far (Fourqurean et al. 2012). It has been the species of study for most of the research presented in this thesis and therefore it deserves a separate section that describes its peculiarities, especially those relating to its function as a carbon sink. Its high capacity for carbon burial is explained on the basis of structural and physiological properties. It is a large, highly productive and long-lived species (Pergent et al. 1994; Hemminga et al. 1999; Gobert et al. 2006) that develops long and dense canopies, very efficient as particle traps (Dauby et al. 1995; Gacia and Duarte 2001), and a large bellow-ground biomass (Duarte and Chiscano 1999), highly recalcitrant (Piovetti et al. 1984; Klap et al. 2000) that expand both horizontally and vertically forming a typical structure called “matte” (Romero et al. 1994; Gobert et al. 2006). The anoxic conditions found in *P. oceanica* sediments enhances the preservation of the organic matter for even millennia (Mateo et al. 1997; Mateo et al. 2006). In addition, *P.oceanica* vertical shoots respond to sedimentation by growing towards the surface, leading to a progressive accretion of the matte (Boudouresque and Jeudy de Grissac 1983), that may reach several meters high (Lo Iacono et al. 2008), and to the burial of the organic matter accumulated (Romero et al. 1994).

Similar to global trends, *Posidonia oceanica* has lost approximately 12.9% of its areal extend during the last century, at a decay rate of 1.79 % y^{-1}, leading to the loss of the dominant Blue Carbon ecosystem of the Mediterranean Sea (Marbà et al. 2014).

I.3. Motivation of this thesis

The significant role of seagrass meadows as carbon sinks and the high rates of seagrass loss render these ecosystems as suitable candidates for the development of carbon mitigation strategies through their protection, conservation and restoration (i.e. Blue Carbon strategies) (Laffoley and Grimsditch 2009; Nellemann et al. 2009), similar to those that already exist for terrestrial ecosystems. Yet, this step requires to increase the knowledge on the functioning of these ecosystems as C sinks and how they respond to present and future threats derived from human pressures (Mcleod et al. 2011).

It was within this context that this thesis was planned; with the aim to contribute to the understanding of the process that govern the carbon sink capacity in seagrass meadows and its vulnerability. Specifically, there were certain gaps of knowledge highlighted by different authors that needed to be urgently addressed (Mcleod et al. 2011; Duarte et al. 2013a; Macreadie et al. 2014) and that were used as take off points to guide the specific goals of this thesis.

I.4. Gaps of knowledge on the carbon sinks in seagrass meadows

I.4.1. Scarce and biased data on sediment carbon stocks and burial rates

By the time this thesis was planned, data on sediment C_{org} stocks from different species were already available and have grown during the last years (Fourqurean et al. 2012; Lavery et al. 2013). On the contrary, data on C_{org} burial rates were remarkably scarce (Duarte et al. 2005, 2013a) and corresponded, in most cases, to indirect estimates based on the excess of the organic matter primary production remained once accounting for the loss through herbivory, export, DOC released and/or decomposition (Duarte et al. 2005; Mateo et al. 2006). Only few direct measurements of carbon burial along the sediment profile existed but they were restricted to few *Posidonia oceanica* meadows in the Mediterranean Sea (Romero et al. 1994; Mateo et al. 1997; Serrano et al. 2012) (Table I.1).

Table I.1. Direct measurements of C_{org} burial rates in seagrass sediments available by the time this thesis started (WM: Western Mediterranean).

Species	**Location**	**C_{org} burial ($g\ Cm^{-2}\ y^{-1}$)**	**Time scale (y)**	**Reference**
P.oceanica	Ischia, Italy	19-191	~100-1000	Romero et al. 1994
P. oceanica	Ischia, Italy	30	~3370	Mateo et al. 1997
	Culip, WM	9		
	Port Lligat, WM	75		
	Campello, WM	112.1		
	Tabarca, WM	61.7 - 104.2		
	Medas, WM	12.6		
P.oceanica	Port Lligat, WM	21	~4300	Serrano et al. 2012

Considering only the C_{org} stock in seagrass sediments is not enough to assess their role as carbon sinks as it does not indicate if the ecosystem is stable or if it is actually acting as a sink (i.e. the reservoir of C_{org} is growing) or as source (i.e. carbon is been released) (Macreadie et al. 2014). In addition, the variability in net community metabolism (Duarte et al. 2010) and structural features (Duarte 1991b) among seagrass species and the high variability in the C_{org} burial rates among meadows of the same species (Mateo et al. 1997; Table I.1) suggest that C sinks might be constrained by the meadow dominant species and by environmental conditions.

Hence, a proper assessment of the global Blue Carbon sinks in seagrass meadows needed to expand the quantification of seagrass sediment C_{org} stocks and, especially, C_{org} burial rates across different species and locations (Duarte et al. 2013a; Macreadie et al. 2014).

I.4.2. External control of the carbon sink capacity: the role of environmental factors

The effect of environmental factors on the C sink capacity of seagrass meadows remained still largely unexplored. Yet, the identification of these factors would be very useful for a proper management of seagrass ecosystems as C sinks, to prioritize the protection of certain meadows against others and to identify the most suitable areas for Blue Carbon restoration projects (Mcleod et al. 2011).

Seagrass meadows expand across a broad range of depositional environments, from sheltered estuaries to highly exposed coastal areas (Carruthers et al. 2007) and along a broad gradient of depths, from the intertidal to up to 90 m (Duarte 1991a). Both coastal exposure and depth are likely to affect the carbon sink capacity of these ecosystems.

In sheltered bays, where hydrodynamic forces are reduced, sedimentation is enhanced whereas erosion and export are prevented, when compared to meadows under a higher exposure to wave energy (Van Keulen and Borowitzka 2003; Bradley and Houser 2009; Hansen and Reidenbach 2012). As a result, sediment of seagrass meadows located in sheltered areas tend to have smaller grain sizes and higher organic carbon content than sediment of meadows located in highly exposed sites (Murphey and Fonseca 1995; Fonseca and Bell 1998). Hence, the carbon burial capacity of seagrass meadows is expected to be higher in sheltered than in exposed meadows, as sedimentation of allochthonous organic carbon from the water column is expected to be enhanced whereas sediment erosion and export of autochthonous organic carbon would be reduced.

On the other hand, seagrass biomass productivity tend to decrease with depth due to the decrease in light availability (Duarte 1991a; Collier et al. 2007). Therefore, a lower accumulation of autochthonous carbon might occur as the depth of the meadows increases. Yet, hydrodynamic forces are usually higher in shallower meadows compared to deeper ones (Koch et al. 2006b) favoring the export of organic matter (Mateo and Romero 1997; Katwijk and Hermus 2000), sediment suspension (Koch 1999) and aeration, which may enhance carbon remineralization (Harrison 1989), and thus a lower C_{org} burial efficiency than in deeper meadows. Yet, neither the effects of exposure to wave energy or water depth on seagrass carbon sink capacity had been assessed before.

I.4.3. Origin of the C_{org} being sequestered in seagrass sediment deposits

As explained before, C_{org} deposits in seagrass sediments form as a result of the accumulation of seagrass derived organic matter and allochthonous carbon both from the water column (i.e.seston) and/or from other benthic macrophytes present in the ecosystem (e.g. macroalgae) (Gacia et al. 2002; Kennedy et al. 2004; Dubois et al. 2012). Based on a global compilation of data, Kennedy et al. (2010) identified that the contribution of allochthonous carbon to the top 10 cm of seagrass sediments is on average around 50%, but it is highly variable among meadows and might constitute the dominant fraction (95%).

Sestonic mater (e.g. phytoplankton) and fast-growing macroalgae usually show much faster decomposition rates than seagrass detritus (Enriquez et al. 1993; Banta et al. 2004). As a result, seagrass sediment deposits enriched with any of those labile sources of organic matter are more prone to be remineralized (Holmer et al. 2004) and present lower carbon burial efficiencies, as much of the sedimentary C_{org} deposit is emitted as CO_2. Therefore, knowledge about the origin of the C_{org} accumulated in seagrass sediments is highly relevant to evaluate its prevalence as a long-term deposit.

The spatial variability in C_{org} sources among seagrass meadows might be explained by species features of seagrass, that determine their efficiency to trap allochthonous carbon (Kennedy et al. 2010), and/or environmental factors such as the degree of exposure of the meadow to wave energy (that affects the particle sedimentation rates), the proximity to other productive coastal ecosystems (e.g.mangroves) (Samper-Villarreal et al. 2016) or human activities in adjacent watersheds, such as deforestation or fish farming (Kennedy et al. 2004, 2010). Carbon sources contribution to the sediment deposits may also vary along time in relation to the maturity stage of a seagrass meadow (Marbà et al. 2015) or to a shift in the dominant primary producer community, such as what occurs during an eutrophication event (McGlathery 2001). Due to the important implications that the composition of the carbon deposits may have for long-term burial efficiency, the spatial and temporal variability of C_{org} sources contribution need to be assessed and the factors leading to this variability need to be further explored.

I.4.4. The fate of the C_{org} deposits when seagrass meadows are lost

The decline of seagrass meadows leads to the loss of all the ecosystem functions they support including that of C_{org} sequestration (Orth et al. 2006; Marbà et al. 2014). Furthermore, the loss of seagrass cover may also lead to the erosion of the sediment carbon deposits, as the buffering capacity of the seagrass canopy against waves and currents disappears (Ward et al. 1984; Duarte et al. 2013a).

Recent studies demonstrated that C_{org} deposits may be partially eroded following vegetation loss (Macreadie et al. 2015; Marbà et al. 2015) but the fate of this carbon could not be resolved. The stored C_{org} may be exported and buried in adjacent sediments (Macreadie et al. 2014), or, once exposed to aerobic conditions, it could be remineralized and released as CO_2 back to the water-atmosphere pool as reported for other coastal vegetated ecosystems (Huang et al. 2010; Lovelock et al. 2011). Therefore, the decline of seagrass meadows may not only imply the loss of their carbon sequestration capacity but also it may lead to their conversion from a carbon sink to a carbon source (Mcleod et al. 2011).

The potential emissions of CO_2 following seagrass degradation has been considered in previous assessments assuming that between 25-100% of the carbon deposits within the top first meter of the sediment would be remineralized once exposed to aerobic conditions (Pendleton et al. 2012; Fourqurean et al. 2012). Yet, the release of CO_2 from disturbed seagrass sediments has not been empirically proven and it is likely to depend on intrinsic properties of the deposits, such as the nature of the carbon stored and its refractory conditions (Pendleton et al. 2012) which, as explained in the previous section, might be very variable both spatially and temporally (Kennedy et al. 2010; Marbà et al. 2015).

If the vulnerability of the carbon stocks to remineralization after seagrass meadows loss is demonstrated and quantified, the development of conservation and protection strategies would become even more urgent (Duarte et al. 2013a) and the inclusion of seagrass meadows within the GHG's emissions reduction and offset schemes that already exist for terrestrial ecosystems would be promoted (Herr et al. 2012). Therefore, filling this gap of knowledge was one of the top priorities towards research efforts needed to be addressed (Duarte et al. 2013a; Macreadie et al. 2014).

I.4.5. Impact of coastal human pressure on seagrass carbon sinks

The more than 50 billion people that live within 50 km from the coastline and the increasing usage of coastal areas (Orth et al. 2006; Waycott et al. 2009) have placed seagrass meadows under a high level of human pressure. Among all human impacts, the increase in the input of nutrients is likely the most widespread on seagrass ecosystems (Short and Wyllie-Echeverria 1996; Ralph et al. 2006; Orth et al. 2006) with eutrophication even causing the complete loss of seagrass meadows (Kemp et al. 1983; Short and Burdick 1996).

Yet, nutrient enrichment may affect seagrass carbon sinks in different ways before causing seagrass meadows extinction. A moderate increase in the nutrient inputs to oligotrophic waters may favor seagrass primary productivity and biomass growth (Powell et al. 1989, 1991) which may lead to an increase in the C_{org} burial capacity. On the other hand, eutrophication increases allochthonous carbon production and abundance in the water column (i.e. microalgae and total suspended particles) that, despite affecting seagrass through the reduction of light availability (Short et al. 1995; McGlathery 2001), may potentially enhance C_{org} burial as it settles and accumulates in the sediments. Hence, the interaction between human influence in coastal areas and the adjacent seagrass meadows carbon sinks needs to be explored, especially as human population and usage of coastal areas are projected to increase in the future (Duarte 2009; Mcleod et al. 2011).

I.4.6. The inorganic carbon deposits. Implications in the carbon sink capacity of seagrass meadows

Seagrass meadows are also active ecosystems regarding $CaCO_3$ accumulation, precipitation and dissolution processes. The high metabolic rate of seagrass meadows increases pH within the canopy (Invers et al. 1997; Hendriks et al. 2014a) enhancing $CaCO_3$ precipitation (Yates and Halley 2006) and favoring the settlement of a broad range of calcifying organisms, ranging from epiphytes (Canals and Ballesteros 1997; Perry and Beavington-Penney 2005; James et al. 2009) to benthic invertebrates, such as echinoderms, mollusks and foraminifera (Hendler et al. 1995; Panieri 2006).The carbonate skeletons and shells of these organisms tend to accumulate in the sediments along with carbonate particles trapped from the water column by the seagrass canopy (Gacia et al. 2003). As a result, seagrass meadows enhance the formation of biogenic carbonate sediments (Land 1970; Canals and Ballesteros 1997; Perry and Beavington-Penney 2005) and are a significant source of sand for beach formation and maintenance (De Falco et al. 2003).

The accumulation of particulate inorganic carbon (PIC) in the form of $CaCO_3$ in seagrass meadows may constrain their role as CO_2 sinks. Although $CaCO_3$ consists of bound carbon that could otherwise end up as CO_2 in the water-atmosphere pool

(Macreadie et al. 2014), its precipitation leads to a net release of CO_2 to the atmosphere (Ware et al. 1992) as illustrated by the equation (1) (Stumm and Morgan 1981).

$$Ca^{2+} + 2HCO^{-}_3 <-> CaCO_3 + H_2O + CO_2 \qquad (I.1)$$

The amount of CO_2 released is defined by the molar ratio CO_2 flux : $CaCO_3$ precipitation (ψ), which varies according to different temperatures and CO_2 concentrations (Frankignoulle et al. 1994). Smith (2013) identified that for shallow environments, where seagrass meadows develop, ψ is approximately of 0.63 moles of CO_2 released per mole of $CaCO_3$ precipitated. This has lead to the idea of the need to consider not only the stocks of particulate organic carbon (POC) but also the stocks of inorganic carbon (PIC) stored in seagrass sediments to actually assess the role these ecosystems play as CO_2 sinks (Mateo and Serrano 2012; Serrano et al. 2012; Macreadie et al. 2014).

The accumulation of PIC in seagrass sediments is likely to vary across different species and latitudes. Larger species with larger surface areas, slower turn over rates and denser canopies, tend to accumulate more epiphyte load including calcifying organisms (Heijs 1985; Cebrian et al. 1994; Gacia et al. 2003) and are expected to be more efficient at reducing water flow and at favoring particle sedimentation (Koch et al. 2006a). On the other hand, $CaCO_3$ precipitation and the development of calcifying communities depend on the seawater carbonate saturation state (Ω). Ω is defined as the product of the ions Ca^{2+} and CO_3^{-2} concentrations in seawater at the *in situ* conditions of salinity and temperature, divided by the stoichiometric solubility product (K^*_{sp}) for the same conditions:

$$\Omega = ([Ca^{2+}_{sw}] * [CO_3^{2-}{}_{sw}]) / K^*_{sp}$$

K^*_{sp} is defined as:

$$K^*_{sp} = [Ca^{2+}_{sat}] * [CO_3^{2-}{}_{sat}]$$

where $[CO_3^{2-}{}_{sat}]$ is the total (free and complex) carbonate ion concentration in a seawater solution saturated with $CaCO_3$ (Zeebe and Wolf-Gladrow 2001). Hence, when $\Omega > 1$, seawater is supersaturated and $CaCO_3$ precipitation occurs whereas when $\Omega < 1$ dissolution of $CaCO_3$ is favored. In the ocean $[Ca^{2+}_{sat}]$ does not significantly vary and therefore Ω is mainly conditioned by the carbonate ion concentration (CO_3^{2-}). CO_3^{2-} concentration in seawater varies with environmental conditions such as temperature and salinity and any biogeochemical process that leads to a change in inorganic carbon speciation (Zeebe and Wolf-Gladrow 2001). In general, cold, low-pH and fresh water leads to low CO_3^{2-} concentrations (Mucci 1983; Zeebe and Wolf-Gladrow 2001) and therefore low Ω saturation states and low $CaCO_3$ precipitation rates or even dissolution. As a consequence, Ω tends to decrease with latitude from warm tropical and subtropical open ocean waters to cold subpolar and polar waters (Takahashi et al., 2014).

At the time this thesis began, there was a need to quantify the magnitude of the $CaCO_3$ deposits in seagrass meadows worldwide and to explore its variability among species or regions. Yet, whereas strong efforts had been conducted during the last decade to estimate the organic carbon deposits (POC) in seagrass meadows across different regions and worldwide (Fourqurean et al. 2012; Lavery et al. 2013; Campbell et al. 2014) a global compilation of PIC sediment deposits in seagrass meadows was missing. On the other hand, in biogenic rich sediments, organic matter tends to associate to $CaCO_3$ particles through surface sorption or through intracrystaline interactions, becoming less accessible to microorganisms and decomposers (Suess 1973; Ransom et al. 1998; Bock and Mayer 2000; Ingalls et al. 2004). In addition, the organic matter-mineral interactions likely embed the organic matter in a more anoxic environment

(Burdige 2007). Hence $CaCO_3$ accumulated in seagrass sediments may enhance C_{org} burial efficiency in seagrass meadows, a hypothesis that had not been formulated before and needed to be explored.

I.5. Aims, goals and specific objectives of the thesis

The general aim of this thesis is to increase the knowledge about the capacity of seagrass meadows to act as carbon sinks, which I approach by attempting to fill in the main gaps of knowledge outlined in this introduction; focusing on the assessment of the effect of external factors (environmental and anthropogenic), the relevance of the inorganic carbon deposits in seagrass sediments at a global scale, the vulnerability of the carbon sediment deposits to be lost as CO_2 and the implications that sedimentary carbonate may have in the carbon sink capacity of seagrass meadows.

The research presented responds to three main goals. To accomplish each goal different specific objectives are addressed.

Goal 1. To assess the effect of human pressure and two environmental factors, wave exposure and meadow depth using *P. oceanica* meadows around the Balearic Islands as a case study. Specific objectives:

1.1. Increase the data available on C_{org} stocks and especially on burial rates in seagrass meadows focusing on assessing the effect of two environmental factors, the exposure to wave energy and meadow depth, on the C_{org} burial rates of seagrass meadows.

1.2. To determine the contribution of different sources of C_{org} to the sediment carbon stock and explore its spatial (among meadows) and temporal (last century) variability.

1.3. To assess the effect of anthropogenic pressure on C_{org} burial rates and sources contribution during the last century.

Goal 2. To quantify seagrass inorganic carbon (i.e. PIC) deposits globally and to examine the latitudinal variability of one of the main sources of PIC to seagrass sediment: the carbonate epiphyte load on seagrass leaves. Specific objectives:

2.1. To quantify the magnitude of the inorganic carbon deposits (PIC) in seagrass sediments globally, exploring its variability among species and latitudinal regions.

2.2. To assess the effect that latitude, through changes in temperature and Ω, has in the accumulation of carbonate epiphyte load on seagrass leaves.

Goal 3. To characterize the vulnerability of C_{org} sediment deposits to be lost through remineralization when sediments are disturbed and exposed to oxic conditions, as well as to assess the role that $CaCO_3$ may have in C_{org} burial efficiency.
Specific objectives:

3.1. To assess the risk of remineralization of C_{org} deposits when sediments are suspended and exposed to aerobic conditions taking into account the different composition of the carbon deposits in terms of C_{org} sources and $CaCO_3$ content.

3.2. To assess the effect of the accumulation of $CaCO_3$ in the C_{org} burial efficiency in seagrass sediments.

I.6. Thesis structure

This thesis is structured in 5 different sections. The first section consists of this general introduction. A second section describes the main approaches and methods used in the research developed. The results are presented in section III, which is distributed in 3 different parts (section III.1, section III.2 and section III.3) according to the different goals addressed. Each part includes two different chapters, each with a specific introduction, methodological section and a particular discussion to the specific objectives.

> *Section III.1. Carbon sinks: the effect of environmental factors and anthropogenic pressure on coastal areas during the last century.*
> This first block of the results addresses Goal 1 and includes the following chapters.
>
> Chapter 1:
> “Effect of environmental factors (wave exposure and depth) and anthropogenic pressure in the C sink capacity of *Posidonia oceanica* meadows” (Objectives 1.1 & 1.3)
> Chapter 2:
> “Dynamics of carbon sources supporting burial in seagrass sediments during rapid coastal development” (Objectives 1.2 & 1.3)
>
> *Section III.2. Inorganic Carbon deposits (PIC): Global distribution and environmental controls.*
> This second block of results addresses Goal 2 and includes the following chapters:
>
> Chapter 3:
> “Seagrass meadows as a globally significant carbonate reservoir” (Objective 2.1).
> Chapter 4:
> “Latitudinal gradient in carbonate loads on seagrass leaves” (Objective 2.2).
>
> *Section III.3: Vulnerability of carbon deposits to be lost through remineralization and potential interactions with accumulated $CaCO_3$.*
> The last block of results responds to Goal 3 and includes the following chapters:
>
> Chapter 5:
> “Assessing the risk of remineralization of seagrass carbon deposits following sediment suspension” (Objective 3.1).
> Chapter 6:
> “Carbonate deposition increases carbon burial in seagrass sediments” (Objective 3.2).

After the results section, section IV presents a general discussion developed from the results obtained along the different chapters. Finally, section V summarizes the general conclusions that can be extracted from the research conducted in this thesis.

II

General Methods

The broad spectrum of goals addressed in this thesis needed the application of different approaches that ranged from field seagrass meadows surveys to an extensive data review and laboratory experiments. This section aims to provide a general overview of the main approaches and methods applied in the research presented along this dissertation. Particularities addressed to respond to specific goals are described in more detail in the methods section of each chapter included in section III.

II.1. Approaches

II.1.1. Seagrass meadows surveys

Sediment cores

The exploration of sediment C_{org} deposits, burial rates and sources in seagrass meadows and the examination of the effect of environmental factors and anthropogenic pressure (results presented in Chapter 1 and 2) have been conducted through an extensive survey of *Posidonia oceanica* sediment cores around the Balearic Island.

P.oceanica is an interesting species to focus on. Besides its high carbon sink capacity, already explained in the General Introduction, sediments accumulated by *Posidonia* species can serve as valuable paleoenvironmental records, providing information about changes in environmental conditions in coastal areas (Mateo et al. 2010; Macreadie et al. 2012; Serrano et al. 2013). In addition, endemic to the Mediterranean Sea, *P.oceanica* meadows develop under a wide range of levels of anthropogenic pressure that has, in general, grown during the last century mainly as a consequence of the growth in the population density and development of the tourism industry in the region (Boudouresque et al. 2009; Holon et al. 2015). In particular, the archipelago of the Balearic Islands is one of the top touristic destinations in Europe (Hof and Schmitt 2011). The development of the tourism industry led to a boom in urbanization and population density around the 60's that has caused significant impacts on the natural environment, including coastal ecosystems (Morey et al. 1992; Rullan 1998; Pons et al. 2014).

Thus, the meadows of *Posidonia oceanica* around the Balearic Islands constituted an ideal case study to assess the effect of human pressure on seagrass sediments carbon sinks, both spatially and temporally, focusing on the last century, the period in which the highest increase in human pressure has taken place.

Leaves carbonate epiphyte load

The effect of latitude on the accumulation of leaves carbonate epiphyte load (presented in Chapter 4) was assessed by conducting an extensive survey of shoots collected in eight meadows formed by *Zostera* genus distributed along a broad latitudinal gradient, from 27ºS up to 64ºN. Specifically, between 9-12 shoots were collected from each of six *Zostera marina* meadows, one *Heterozostera tasmanica* meadow and one *Zostera capricorni* meadow.

Zostera genus was selected for this study as it is distributed worldwide and it is present in most of the seagrass biogeographic regions (Green and Short 2003). The inclusion of other seagrass genera was not considered in order to avoid potential confounding effects derived from specific structural and physiological features, such as differences in leaf turnover rates (Stevenson 1988), and to focus on the effect of latitude.

II.1.2. Data review

The global assessment of the particulate inorganic carbon (PIC) stored in seagrass sediments (presented in Chapter 3) was assessed through a global compilation of published and unpublished studies reporting PIC or $CaCO_3$ content in sediment of seagrass meadows and adjacent bare sand patches. Published data were extracted from the Web of Knowledge (using the search terms “seagrass” AND “inorganic carbon” AND [“calcific* OR sediment* OR $CaCO_3$ OR dissolut* OR dia- genesis”]) and Google Scholar (using the search terms “seagrass carbonate”), whereas unpublished data were extracted from the database compiled by Fourqurean et al. (2012) and provided by the authors that contributed to the study.

II.1.3. Laboratory experiments

The vulnerability of seagrass sediments organic carbon deposits to remineralization and to be released as CO_2 (Chapter 5) and the effect of sediment $CaCO_3$ stocks in the organic carbon burial efficiency (Chapter 6) were assessed by conducting two long-term incubation experiments in laboratory controlled conditions.

Specifically for the goals addressed in Chapter 5, sediments from four different *Posidonia oceanica* meadows were incubated in suspension and maintained in a temperature control chamber (~20ºC), in the dark and under aerobic conditions during almost a year. To respond to the questions addressed in Chapter 6, fresh seagrass leaves from the same *Posidonia oceanica* meadow were incubated under four different treatments of $CaCO_3$ concentration for a maximum of 280 days, also at 20ºC, in the dark and under aerobic conditions.

In both experiments, the change in biogeochemical parameters along time was assessed by conducting 5 different periodical samplings distributed in geometrically distributed time intervals, following a logarithmic distribution, encompassing the total of the duration of each experiment.

II.2. Methods

II.2.1. Core sampling and processing

Sediment cores examined in Chapter 1 and 2 were extracted by manually hammering PVC tubes (9 cm ID* 60 cm L) (Figure II.1a). Sediment compression during hammering occurs inevitably (Glew et al. 2001; Serrano et al. 2014) and it may derive in errors when estimating carbon stocks and sediment age along the depth profile if it is not considered. Compression was measured as the difference between the outer and inner distance from the top of the core to the sediment surface divided by the difference between the core length and the outer distance from the top of the core to the sediment surface (i.e. the core length dug in the sediment) (Figure II.1b). Sediment depth was corrected assuming a linear sediment compression along the core depth during sampling and distributing the spatial discordances proportionally between the expected and the observed sediment column layer (Serrano et al. 2014).

Cores were transported to the laboratory and preserved in the freezer until processing. Processing consisted in slicing each core every 1 or 2 cm (depending on the core and the analysis to be conducted) (Figure II.1.c). After measuring the slice fresh volume, each slice was dried in an oven at 60º for approximately 48h until constant weight, and dry weight was measured (Fourqurean et al. 2014) (Figure II.1.d). A fraction of dry sediment of each slice from cores sliced every 1 cm was preserved for granulometric analysis. The reminder sediment samples were pulverized to fine powder and further processed for sediment aging and other biogeochemical parameters determination.

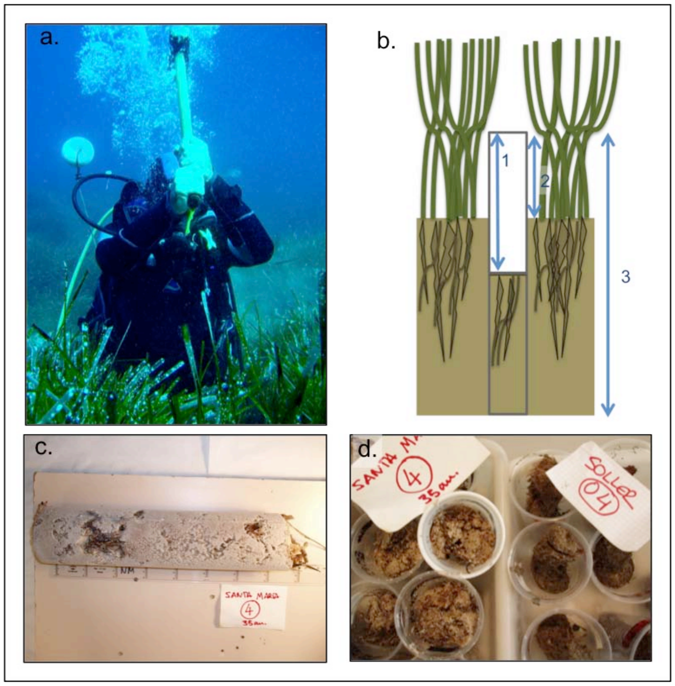


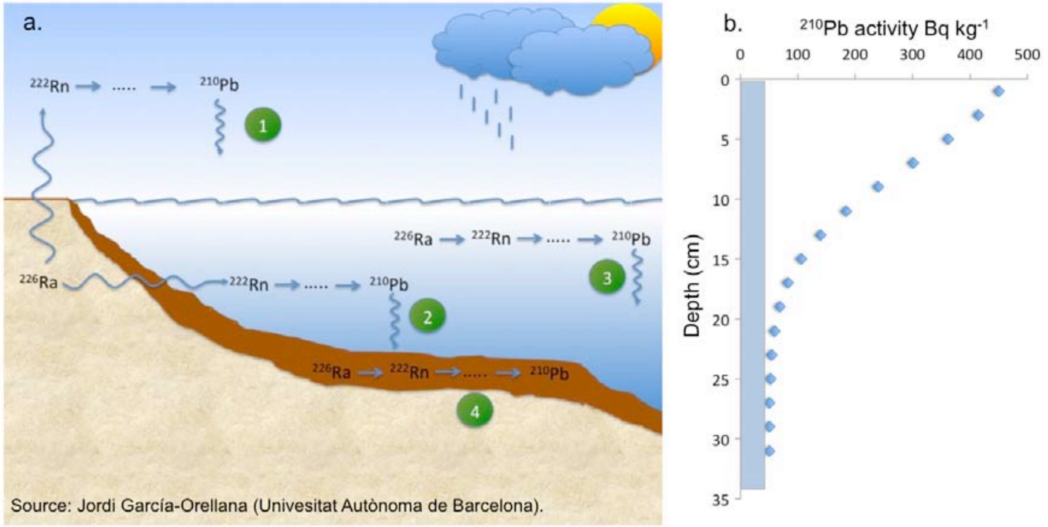
Figure II.1. a) Core hammering; b) Example of sediment compression during hammering and measures for sediment decompression: 1. inner distance; 2. outer distance and 3. core length; c) Example of a sediment core profile before processing and d) sediment slices after oven dried.

II.2.2. Sediment aging

In order to assess the carbon burial rate in seagrass (*P.oceanica*) meadows and its change during the last century (addressed in Chapter 1 and 2), the estimation of an approximate year of sediment deposition along the cores depth profiles was needed. For the purposes of this thesis, seagrass sediments have been dated using ^{210}Pb, especially suitable for recent time periods encompassing approximately the last 100-150 years (Appleby 2001).

Briefly, ^{210}Pb is a radionuclide that occurs naturally in the environment. It forms as a part of the decay chain of ^{238}U and it has a half-life of $T_{1/2}= 22.3 \pm 0.1$ y. In marine sediments, ^{210}Pb may derive from two sources (Figure II.2a). On one hand, ^{210}Pb is formed as a consequence of the natural decay of its parent, ^{226}Ra, which is part of the sediment minerals. This fraction of ^{210}Pb is called supported ^{210}Pb and is considered to be constant as it is in equilibrium within the ^{226}Ra, although changes in the lithology may modify its concentration. On the other hand, ^{210}Pb may also accumulate from the water column associated to sedimentation particles (Appleby 2001). In coastal waters, ^{210}Pb accumulates in the water mass, mainly from the atmosphere (Hancock and Hunter 1999), where it is present as a result of the disintegration of another of its precursors in the ^{238}U chain, ^{222}Rn, derived from the disintegration of ^{226}Ra, that escapes the soil matrix and incorporates into the atmosphere through diffusion (Turekian et al. 1977).

The atmospheric flux of ^{210}Pb into the sediment is considered to be constant at both a regional and annual scale, but it may vary geographically depending on atmospheric circulation, climate parameters (e.g. rainfall) or ^{222}Rn sources. The fraction of ^{210}Pb that accumulates in the sediment through sedimentation processes is named as excess ^{210}Pb (^{210}Pb$_{ex}$). Once in sediment, excess ^{210}Pb is subject to radioactive disintegration at a constant known rate (λ= 0.0311 y^{-1}).



Source: Jordi García-Orellana (Univesitat Autònoma de Barcelona).

Figure II.2. a) ^{210}Pb cycle in coastal ecosystems: 1) ^{222}Rn escapes from the soil matrix and incorporates to the atmosphere where it disintegrates to ^{210}Pb that deposits into the water mass mainly through rainfall 2) ^{222}Rn escapes from the sediment matrix straight to the water mass where it disintegrates to ^{210}Pb that deposits in the sediment associated to sedimentation particles 3) ^{226}Ra dissolved in the seawater disintegrates to ^{222}Rn and then to ^{210}Pb that accumulates in the sediment associated to particles and 4) sediment ^{226}Ra disintegrates to ^{210}Pb that constitutes the supported ^{210}Pb. b) ideal ^{210}Pb profile in sediments. Blue area represents the supported ^{210}Pb activity.

As older sediments get buried under more recently deposited layers, the examination of the ^{210}Pb$_{ex}$ profiles along the sediment depth may allow estimating the age model of the sediment. Excess ^{210}Pb along a sediment profile is determined as the difference between the total ^{210}Pb in each sediment section and the sediment supported ^{210}Pb. Supported ^{210}Pb might be estimated through the determination of ^{226}Ra or as the average ^{210}Pb concentration of the deepest layers once ^{210}Pb reached constant values, as done for sediment dating in this thesis.

In an ideal situation, ^{210}Pb along the depth profile declines following the radioactive decay law towards the supported ^{210}Pb value (Figure II.2b),

$$C_x=C_0\cdot e^{-\lambda\cdot t}$$

where C_x represents the measured excess ^{210}Pb activity, C_0 that (unknown) at the time of burial and λ the constant disintegration of ^{210}Pb (λ= 0.0311 y^{-1}).

For any dating method, it is assumed that the system has been closed during the study period and the decrease in ^{210}Pb activity is only due to its radioactive disintegration. Based on that main assumption, three different models have been developed: CIC (Constant Initial Concentration), CF:CS (Constant Flux: Constant Sedimentation) and CRS (Constant Rate of Supply). The seagrass meadows sediment records examined in the research presented in this thesis were mainly dated using the CRS method, which is, at present, the most widely used method (Appleby 2001), whereas only two sediment cores were dated using the CF:CS (Krishnaswamy et al. 1971). Briefly, CF: CS assumes a constant flux of excess ^{210}Pb to the sediment and a constant sedimentation rate from the water column, whereas CRS model is more suitable in areas where sedimentation rate has varied over time and only the flux of $^{210}Pb_{ex}$ is assumed to be constant.

II.2.3. Analysis of biogeochemical parameters

The main biogeochemical parameters measured and the techniques used along the research presented in this dissertation are described below. Table II.1 summarizes the techniques applied for the determination of different variables in each of the chapters included in the Results section.

Sediment dry bulk density (DBD)

Sediment dry bulk density (DBD) was measured as the ratio between sediment dry weight (oven dried at 60ºC until constant weight) and sediment fresh volume, before determination of other biogeochemical parameters.

Sediment grain size determination

Granulometric analysis were performed at the Universitat de Barcelona on a Beckman Coulter LS GB500. The sediment was classified according to the Udden-Wentworth grain size scale (Ø<4 µm, clay; Ø: 4 - 63 µm, silt; Ø: 63 - 2000 µm, sand and Ø: 2000-4000 µm, gravel).

Organic matter content (OM %DW)

Organic matter as percentage of dry weight (%DW) was estimated through the Loss of Ignition Technique (LOI). LOI is a measure of the fractional mass sample lost after combustion and is calculated as follows (Fourqurean et al. 2014):

%LOI= ((Dry mass before combustion)-(Dry mass after combustion))/ (Dry mass before combustion) *100

Sediment combustion temperature and timing applied may vary between 450-550ºC during approximately 4 to 8 hours (Heiri et al. 2001; Fourqurean et al. 2014). For the research presented in this thesis, temperature combustion ranked between 500ºC-550ºC during 4-5 hours, the most commonly used methods in seagrass sediments (Fourqurean et al. 2012). For the determination of OM content (%DW) in seagrass leaves, combustion was carried out at 450ºC during 2 hours, following Gacia et al. (2003).

Particulate organic carbon content (C_{org} or POC %DW)

Sediment organic carbon content has been measured at Iso-Analytical Laboratory (United Kingdom) using an elemental analyzer (EA-IRMS), in samples previously acidified with 1M hydrochloride acid during one night to eliminate the inorganic carbon fraction.

Due to budget limitation, this analysis had to be restricted to a certain fraction of the samples sets. For the remainder samples, organic carbon (%DW) was estimated using empirical relationships (expressed as a linear regression equations) obtained by comparing the organic carbon measured with the elemental analyzer and the organic matter determined by LOI in the same samples, as recommended by Fourqurean et al. (2014). Different empirical relationships were obtained for the different sets of samples and applied to estimate organic carbon (%DW) for those samples where only LOI (%DW) measures were available (Chapters 1 and 2) or, for the contrary, applied to estimate the C_{org} (%DW) in the whole set of samples (Chapter 5).

Dissolved organic carbon content (DOC)

Dissolved organic carbon (DOC) was measured from 50 ml water samples, centrifuged at 1500 rpm during 15 minutes and acidified with 20 µl of H_3PO_4. Samples were sent to the Organic Geochemistry Laboratory at the CSIC Instituto de Investigaciones Marinas (Vigo, Spain) where they were analyzed with a Total Organic Carbon analyzer (Shimadzu TOC-V).

Carbonate ($CaCO_3$ %DW) and inorganic carbon content (PIC %DW)

Particulate inorganic carbon in seagrass sediments is present in the form of carbonates (i.e. calcium carbonate, $CaCO_3$). The measurement of either PIC or $CaCO_3$ mass allows the estimation of the other variable assuming that inorganic carbon constitute a 12% of the molecular weight of calcium carbonate ($CaCO_3$) (Fourqurean et al. 2014).

In sediment samples, PIC has been estimated as the total C (%DW) measured with an elemental analyzer (EA-IRMS) in samples pre-burned at 500ºC during 4 hours; whereas $CaCO_3$ has been measured through the acidification method as the percentage of weight lost overnight of samples acidified with 1 M hydrochloric acid (analysis conducted in Iso-Analytical Laboratory, United Kingdom) (Fourqurean et al. 2014).

Calcium carbonate in leaves samples has been measured as the weight loss of the sample pre-combusted at 450ºC for OM %DW determination, after combustion at 1000ºC for 1 hour (Gacia et al. 2003).

Carbon sources contribution: isotopic analysis

For the research presented in this thesis, the identification of different organic carbon sources to seagrass sediments has been conducted using stable isotopes analysis, specifically $\delta^{13}C$. The use of stable isotopes to determine individual contributions of different sources to the total organic matter sedimentary stock is especially suitable when the potential sources are significantly different in their isotopic signatures (Fry et al. 1977; Gacia et al. 2002; Kennedy et al. 2004, 2010). This is the case for seagrass sediments as seagrass tissue is known to be isotopically heavier in their carbon isotopic signature (-10 and -11%, Hemminga and Mateo 1996) compared to other sources of organic matter in coastal areas, such as phytoplankton, benthic macroalgae or terrestrial organic matter (-22 ‰, -17 ‰ and -27 ‰ respectively, France 1995; Moncreiff and Sullivan 2001; Diefendorf et al. 2010).

For the region where seagrass sediments examined are located (i.e. The Balearic Islands), the main sources of carbon to the sediment stock were assumed to be seagrass (*P.oceanica*) derived organic matter and sestonic inputs, based on results found by (Papadimitriou et al. 2005). Hence to estimate the contribution of these sources to the total sediment carbon stock at an specific meadow, a two-component isotope-mixing model (Phillips and Gregg, 2003) was applied:

$$\delta^{13}C_{sediment} = \delta^{13}C_{seagrass}\, f + \delta^{13}C_{seston}\,(1 - f)$$

Sediment carbon isotopic signature ($\delta^{13}C_{sediment}$) was measured in Iso-Analytical Laboratory (United Kingdom) using an Elemental Analyser - Isotope Ratio Mass Spectrometry (EA-IRMS) after removing potential carbonate content by acidifying the samples with 1 M hydrochloric overnight. The $\delta^{13}C_{seagrass}$ (± SE) values corresponded to averages of the $\delta^{13}C$ measured in leaves and rhizomes of *P.oceanica* shoots collected between the years 2008 and 2009 in each particular meadow examined and the $\delta^{13}C_{seston}$ values were extracted from Papadimitriou et al. (2005).

The application of $\delta^{13}C$ in the estimation of different carbon sources contributions to seagrass sediment along time should be used with care as the ^{13}C signature of the sources may have not remained constant along the time period considered. Changes in environmental variables such as dissolved inorganic carbon (DIC) concentration or isotopic signature, irradiance or nutrient availability are known to cause shifts in the $\delta^{13}C$ of primary producers including seagrass and phytoplankton (Peterson and Fry 1987; Hemminga and Mateo 1996; Popp et al. 1999; Vizzini and Mazzola 2003; Hill et al. 2008; Serrano et al. 2011), which most likely affects the final $\delta^{13}C$ that can be measured in the sediment C_{org} deposits. Therefore, time variability in the sediment $\delta^{13}C$ could be wrongly attributed to a shift in the contribution of carbon sources with time when not considering the potential change in the sources $\delta^{13}C$ signature. This constraint was especially relevant for the goals addressed in Chapter 2, and was solved by taking into account the possible variability in the seston and *P.oceanica* $\delta^{13}C$ signature with time due to different trends in environmental factors when interpreting shifts in the sediment $\delta^{13}C$ signature. In addition, sediment C:N ratio was also measured as it helps to better discriminate the sources of organic matter and to the interpretation of temporal trends, thanks to the lower values usually observed in marine microalgae (C:N<10), a main component of seston loads, compared to those found in seagrass tissue (Atkinson and Smith 1970; Meyers 1994).

Nitrogen and C:N ratios

Nitrogen content (N %DW) was measured using a CN elemental analyzer (Truspec CN determinator, LECO).

C:N ratios were calculated only considering the organic fraction of sediment carbon (C_{org}) measured as explained above.

Table II.1. Summary of the analysis conducted for measuring the different biogeochemical variables examined in the different chapters included in the Results section, except for Chapter 3, that is based on a compilation of published and unpublished data. (*) Indicates analysis conducted by external laboratories.

Biogeochemical parameters	**Chapter 1**	**Chapter 2**	**Chapter 4**	**Chapter 5**	**Chapter 6**
Sediment grain size	*Beckman Coulter LS GB500				
Organic matter (OM %DW)	Sediments: LOI (550ºC, 4h)	Sediments: LOI (550ºC, 4h)	Leaves: LOI (450ºC, 2 h)	Sediments: LOI (500ºC, 4 h)	Leaves + sand treatments: LOI (500ºC, 4 h)
Particulate organic carbon (C_{org}, POC %DW)	Sediments: *EA in acidified samples	Sediments: *EA in acidified samples		Sediments: *EA in acidified samples	
Dissolved organic carbon (DOC)				Centrifuged water samples and *TOC	
Carbonate content ($CaCO_3$ %DW)			Leaves: combustion (1000ºC, 1h)	Sediment: *acidification	Leaves: combustion (1000ºC,1h) Leaves + $CaCO_3$: *EA in pre-combusted samples (500ºC, 4h)
Stable isotope analysis ($\delta^{13}C$)		Sediments: *EA-IRMS in acidified samples		Sediments: *EA-IRMS in acidified samples	
Nitrogen content (N %DW)		Sediment: EA		Sediment: EA	

III

Results

III.1

Carbon sinks: the effect of environmental factors and anthropogenic pressure on coastal areas during the last century

Chapter 1

Effect of environmental factors (wave exposure and depth) and anthropogenic pressure in the C sink capacity of Posidonia oceanica meadows.

Abstract

Seagrass are among the most important natural carbon sinks on Earth with *Posidonia oceanica* (Mediterranean Sea) considered as the most relevant species. Yet, the number of direct measurements of organic carbon burial rates in *P.oceanica* is still scarce and the effect of local environmental factors remains largely unexplored. In addition, *P.oceanica* meadows are declining due to the increase in anthropogenic pressure in coastal areas during the last century. The aim of this study is to assess the recent carbon sink capacity of *P.oceanica* and particularly the effect of human pressure and two environmental factors, water depth and exposure to wave energy (based on a fetch index), on the carbon burial rate since 1900. We conducted an extensive survey of sediment cores in meadows distributed across a gradient of depth, fetch and human pressure around The Balearic Islands. Sediment and carbon accumulation rates were obtained from ^{210}Pb concentrations profiles. Top-30 centimeters carbon stocks (6.1 ± 1.4 kg C m^{-2}) and burial rates (26 ± 6 g C m^{-2} yr^{-1}) varied up to 5-fold across meadows. No significant effect of water depth or fetch in carbon burial rates was observed, despite fetch was significantly correlated with sediment mean grain size, confirming the effect of wave exposure in the patterns of sedimentation. Human pressure was, on the contrary, determinant in the carbon burial rates, which tended to be higher in meadows under a high or very high human pressure and to increase since the onset of the rise in anthropogenic pressure.

1.1. Introduction

Seagrass meadows rank among the most intense natural carbon sinks of the biosphere (Nellemann et al. 2009; Fourqurean et al. 2012; Duarte et al. 2013b). Due to their high carbon sink capacity and their broad distribution along the coastal areas of all continents, except the Antarctica (Hemminga and Duarte 2000), seagrass meadows contribute about 50 - 64 % of the organic carbon (C_{org}) sequestered annually by coastal vegetated ecosystems (Duarte et al. 2013b) and bury 20% of the carbon buried in the global ocean (Duarte et al. 2005; Kennedy et al. 2010).

The endemic Mediterranean seagrass, *Posidonia oceanica*, stands out as the species supporting the highest carbon burial rates (Duarte et al. 2005; Lavery et al. 2013) and the largest sediment organic carbon stocks (Fourqurean et al. 2012; Lavery et al. 2013) among all seagrass studied thus far.

P.oceanica supports millenary clones (Arnaud-Haond et al. 2012), with shoots up to tens of decades in age (Marbà et al 2005) connected by thick and robust rhizomes that extend both horizontally and vertically forming reefs that can reach 6 m thick (Lo Iacono et al. 2008). The massive carbon stocks accumulated by *P.oceanica* have been explained on the basis of intrinsic properties of the species, including its long life span, high productivity, high belowground biomass and recalcitrant nature of its tissues (Mateo et al. 2006) along with dense canopies that buffer particle resuspension (Gambi et al. 1990; Gacia et al. 1999; Terrados and Duarte 2000) while promoting particle trapping (Hendriks et al. 2008; Kennedy et al. 2010), high sedimentation rates and hence, high burial capacity (Mateo et al. 2006). However, estimates of C_{org} burial rates in *P.oceanica* meadows are still scarce (Mateo et al. 1997; Serrano et al. 2012) and the high variability in C_{org} burial rates identified between different meadows (Mateo et al. 1997) suggests that the C_{org} sink capacity is also controlled by environmental factors, which are, as for other species, poorly understood (Macreadie et al. 2014).

A large number of environmental factors are likely to affect the carbon sink capacity of *P.oceanica* meadows. Among them, the exposure to waves, which determines the patterns of sedimentation and may lead to sediment resuspension and erosion during high-energy events (Van Keulen and Borowitzka 2003; Bradley and Houser 2009; Hansen and Reidenbach 2012). Indeed, indexes of wave exposure, such as effective fetch, have been successfully used as predictors of sediment grain size and percentage of organic carbon (% C_{org}) in seagrass meadows, those with smaller sediment grain size and higher C_{org} concentration usually being found in low-energy environments (Murphey and Fonseca 1995; Fonseca and Bell 1998). Conversely, because of the close coupling between sediment grain size and coastal hydrodynamics, grain size may be used as an indicator of hydrodynamic conditions (Cabaço et al. 2010). Hence, differences in wave exposure among locations may help explain differences in carbon burial rates among *P.oceanica* meadows. In addition, a recent study identified that water depth constrained the C sink capacity of seagrass meadows, observing higher stocks and burial rates in shallower meadows that tended to decrease towards deeper meadows (Serrano et al. 2014). This study attributed this finding to differences in light availability induced by the depth gradient (Serrano et al. 2014) as irradiance controls shoot density and net C balance (Alcoverro et al. 1995).

P.oceanica meadows are experiencing a widespread decline across the Mediterranean Sea, with over 1/3 of their cover lost over the past 50 years (Marbà et al. 2014), consistent with global declining trends for seagrass meadows (Waycott et al. 2009). *P.oceanica* loss is largely attributable to anthropogenic pressures, such as eutrophication (Duarte 2002; Marbà et al. 2014), which in the Mediterranean Sea mainly derive from the fast urbanization and the intensive development of tourism industry in coastal areas initiated around the 60's (Boudouresque et al. 2009).

The decline of *P.oceanica* meadows may imply the loss of its C_{org} sink capacity (Marbà et al. 2014) along with other ecosystem services. In addition, the increasing urbanization and usage of the coast may have affected the carbon burial capacity of *P.oceanica* meadows over time by altering the patterns of sedimentation and the inputs of autochthonous and allochthonous carbon into meadows sediments. High human activity usually enhances nutrient inputs to coastal areas (Bowen and Valiela 2001) that, when moderate, may favor seagrass productivity (Powell et al. 1989). On the contrary, an excess input of nutrients may lead to eutrophication, reducing water quality and seagrass production (Short and Burdick 1996) while increasing the load of allochthonous carbon such as phytoplankton and organic detritus (Duarte et al. 1995; Borum 1985) that may get trapped and buried in seagrass sediments.

The aim of this study is to assess the recent carbon sink capacity (stocks and burial rates) of *P.oceanica* meadows and particularly to assess the effect of human pressure and two environmental factors, water depth and exposure to wave energy (based on a fetch index), on the C_{org} burial rate since 1900. We do so by examining sediment cores across eleven *P. oceanica* meadows sampled along a gradient of water depth, wave exposure and level of human pressure in The Balearic Islands (Western Mediterranean) and by comparing the C_{org} burial between two periods: before and after human pressure increased at each site.

1.2. Methods

Description of study sites

The Balearic Islands rank among the top touristic destinations in Europe (Hof and Schmitt 2011). Tourism industry is the main economic sector in the region and it has leaded to an intensive urbanization, particularly in the coastal zone (Murray et al. 2008), during the last century (Pons et al. 2014). Tourism in The Balearic Islands started booming around the 1960's (Rullan 1998) leading to a high affluence of tourists during the summer period and to the increase in the population density in the touristic areas, mainly located in the coast, due to the arrivals of rural islanders and migrants (Pons et al. 2014). As a result, in only 50 years, the coastline has been rapidly transformed through an intensive construction of touristic resorts and development of urban areas (Pons et al. 2014). The Balearic Islands are also a globally significant spot for recreational boating (Balaguer et al. 2011), which delivers additional pressure to *P.oceanica* meadows through intensive anchoring (Ceccherelli et al. 2007; Montefalcone et al. 2008) and through the input of organic matter and nutrients through wastewater discharges (Matthew Leon and Warnken 2008) from boats and harbours.

Agriculture extensions are mainly located inland (Basterretxea et al. 2010) and the influence of rivers and fresh water inputs in coastal water is minimal as the karstic condition of the sediment prevents the formation of perennial rivers (Kent et al. 2002) with only ephemeral streams forming in the wettest seasons (Basterretxea et al. 2010).

Therefore, the degree of human influence in the coastal zone of the region of study is intimately related to tourism industry development and derived from a high population density, intensive urbanization and a high usage of coastal areas, including recreational boating. These factors are in fact among the most significant pressures to *P.oceanica* meadows identified in other regions of the Mediterranean Sea (Holon et al. 2015).

Eleven *P.oceanica* meadows were selected around the archipelago of The Balearic Islands, 6 in Mallorca, 2 in Formentera and 3 in the Cabrera National Park (Figure 1.1), distributed along a gradient of water depth, wave exposure and human pressure (Table 1.1).

Figure 1.1. Location of the sampling sites in the Balearic Islands.

Mallorca, is the largest island with 49% of the surface considered to be artificial or modified, built or transformed by human activities (Balaguer et al. 2008). Formentera is the less populated island from the Archipelago but supports intense pressure from summer tourism, including boats affluence (e.g. Ses Illetes) and rapid urbanization of certain areas, such as Es Pujols (Morey et al. 1992; Roig-Munar et al. 2013).

The Archipelago of Cabrera is a non-permanently inhabited group of small islands used as an army station from 1940 until it was declared as a national park in 1991. Santa Maria bay, closed to visitors since 1993, and Es Castell, where anchoring is not permitted, represent the two most pristine *P.oceanica* meadows included in this study. The meadow at Es Port bay is, on the contrary, under a relatively higher level of human pressure as it is where the Park's visitor center and the main services are located and where visitors arriving by touristic ferries or sailing boats are allowed to moor in one of the 50 buoys available. Es Port waters receive the sewage produced by visitors in moored boats, few of which are equipped with holding tanks (Marbà et al. 2002) and the outflow of the treated sewage produced by the visitors on land (PNMTAC 2009).

P.oceanica meadows currently cover an approximate area of 600 km^2 around the archipelago of The Balearic Islands (Álvarez et al. 2015). Yearly surveys of permanent plots conducted in the meadows during the 3 - 11 years before sampling demonstrate that most of the studied meadows had a similar shoot density (643 ± 75 - 435 ± 72 shoots m^{-2}) with the exception of Es Pujols in Formentera (969 ± 94 shoots m^{-2}), Cala d'Or (Mallorca, 389 ± 20 shoots m^{-2}) and Es Port (Cabrera National Park, 227 ± 12 shoots m^{-2}) (Table 1.1). In addition, the net population growth in all meadows studied for the last decade was negative, indicating that their shoot density tended to decline during this period (Table 1.1).

Table 1.1. Location and main features of sampled seagrass meadows. Wave exposure (fetch), water column depth, human pressure (standardized) index, level of human pressure assigned to each meadows and the estimated year of increase of human pressure (Year$_{HP}$) are the main factors assessed. Meadow shoot population parameters (avg ± SE), density (shoot m^{-2}), net population growth (y^{-1}) and surface area covered (%) are also provided. Data on shoot density and population growth are estimates from yearly surveys of permanent plots in each meadow during the last 3-11 years before the study was conducted (Marbà et al. unpublished) and data on % surface covered correspond to two field campaigns (years 2008 and 2009) and one field campaign in Es Port (Cabrera) in 2000 (Marbà et al. unpublished; Marbà et al. 2006).

		Meadow geophysical parameters			**Human pressure**			**Meadow shoot population**		
Station	**Island**	**Latitude / Longitude**	**Fetch (km)**	**Depth (m)**	**Index**	**Level**	**Year$_{HP}$**	**Density (Shoots m$^{-2)}$**	**Net population growth (Avg ± SE)**	**% Surface covered**
Pollença	Mallorca	39º54,16.97"/ 3º05'34.51"	16.63	4	9.8	Very High	1965	643 ± 75	-0.037 ± 0.06	91.5 ± 8.5
Sta.Ponça	Mallorca	39º30'48.37" / 2º 28'09.71"	90.66	5	6.6	Very High	1970	378 ± 36	NA	78.5 ± 9
Cala d'Or	Mallorca	39º22'08.85" / 3º13'52.23"	176.44	7	5.4	Very High	1970	389 ± 20	-0.026 ± 0.021	84.5 ± 8
Portocolom	Mallorca	39º 25'.02.56' / 3º 16'01.53"	139.35	9	4.9	High	1970	568 ± 41	-0.14 ± 0.09	94 ± 3.5
Sóller	Mallorca	39º 47' 40.05" / 2º 41' 06.48"	185.45	11	1.2	High	1960	435 ± 72	NA	56.8 ± 15.8
Magalluf	Mallorca	39 º30'13.46" / 2 º 32.38.35"	166.69	6	0.2	High	1970	479 ± 35	-0.12 ± 0.03	59.5 ± 37.5
Es Port	Cabrera	39º08'45.41" / 2º56'42.21"	6.73	17	-3.9	Moderate	1991	227 ± 12	-0.06 ± 0.026	36.9 ± 1.9
Es Pujols	Formentera	38º 43'42.16" / 1º 27'17.78"	187.06	4	-4	Moderate	1970	969 ± 94	-0.04 ± 0.04	92.5 ± 4
Ses Illetes	Formentera	38º45'20.77" / 1º25'49.06"	95.69	5	-6.5	Low	1970	556 ± 38	-0.05 ± 0.04	87.3 ± 0.3
Es Castell	Cabrera	39º09'09.93" / 2º55'49.93"	0.46	5	-6.8	Low	1991	515 ± 39	-0.1 ± 0.05	80.5 ± 19.5
Sta.María	Cabrera	39º09'05.40" / 2º56'46.50"	16.93	13	-6.8	Low	1991	470 ± 37	-0.1 ± 0.033	98 ± 2

Wave exposure

Wave exposure among sites was characterized based on a fetch index, “the effective fetch” (Hakanson 1981), which is based in 15 distances (χ_i in km) from the study site to the opposite shore measured for every deviation angle γi from a central radius (0º) where γi equals 0°, ±6°, ±12°, ±18°, ±24°, ±30°, ±36° and ±42°. The central radius is placed in the direction where the opposite shore is located at the highest distance (Figure SI 1.1). The effective fetch (L_f) is then calculated using the equation (1.1).

$$L_f = (\Sigma\, \chi_i \cos \gamma i)/(\Sigma \cos \gamma i) \tag{1.1}$$

Human pressure

Taking into account the specific context of the region of study we chose 8 indicators of human activity to characterize the level of human pressure in the selected meadows (Table SI 1.1): population density; number of hotel beds; the number of sewage pipes and uncontrolled punctual discharges to each bay within 1 km distance from each sampling site; number of ports, marinas and/or yatch clubs and the number of berths located in the same bay of each meadow; number of boats potentially sailing regularly in coastal waters of each site; and fish farming activity in the area.

In order to classify the meadows according to their level of human pressure, indicators of pressure were standardized following the Z-score scaling method (Milligan and Cooper 1988) where the values for each indicator were recalculated as (V - mean V)/ s, being V the value of the indicator, and mean V and s the average value and the standard deviation, respectively, of the indicator in the region. Standardized variables were added per site to obtain a human pressure index per meadow (Table 1.1, Table SI 1.1). According to the human pressure index obtained, meadows were classified across 4 different categories of human pressure: Low (sum < -5), Moderate (sum= -5 - 0), High (sum= 0 - 5) and Very high (> 5) (Table 1.1, Table SI 1.1). The spatial gradient of chlorophyll a concentration across the studied meadows agrees with the gradient of human pressure defined, as chlorophyll a concentration tends to increase from meadows under low to high and very high human pressure (Table SI 1.1).

We identified the onset of human pressure boom at each site as the year when the temporal trend of the cumulative number of hotel beds from 1931 to 2010 visually showed the first rapid increase (Figure SI 1.2, Table 1.1). We support this date with that of the construction of the first yacht club or marina in the area (Figure SI 1.2). For Ses Illetes, a non urbanized area in Formentera and yet one of the top touristic spots in the island for which visitors affluence records are not available, we assumed a similar date of human pressure increase as for Es Pujols (Figure SI 1.2). We considered that human pressure started increasing at Cabrera sites the year when it was declared National Park (1991), when visitors were first allowed in the island. Hence, the year when human pressure started to rise (year_{HP}) at the studied sites ranged between 1960 and 1991 (Table 1.1). The periods considered before and after the year of the onset of human pressure (year_{HP}) at each site, respectively, encompassed from 1900 to year_{HP} and from year_{HP} to sampling year.

Sediment sampling and analysis

Seagrass meadows were sampled in June 2012, except Santa Maria that was sampled in June 2013. Three cores were extracted from each meadow by hammering 60 cm long and 9 cm diameter PVC tubes into a maximum sediment depth ranging between 23 cm in Ses Illetes and 45 cm in Sta. Maria (Table 1.2). The compression of the sediment due to sampling was estimated in 9 of the meadows and varied between 5 ± 3 % in Es Pujols and 38 ± 4 % in Es Castell (Table 1.2). The compression factor was used to correct the thickness of sediment layers to estimate the carbon stocks (g C_{org} cm^{-2}) assuming a linear sediment compression along the core depth during sampling and distributing the spatial discordances proportionally between the expected and the observed sediment column layer, as done in previous studies (Serrano et al. 2014).

Table 1.2. Number of cores, maximum sediment depth sampled and estimated core compression during sampling, mean sediment grain size (± SE) (and the depth of the core used for granulometric analysis) and the observed depth and year (± SE) of the oldest sediment layer accumulated since 1900 (Oldest Age) estimated from ^{210}Pb dating techniques in one core per site.

Station	**Sampling**			**Granulometry**		**^{210}Pb dating**	
	N cores	**Max. core lenght (cm)**	**Compression % (Mean ± SE)**	**Grain size (µm, Mean ± SE)**	**Depth (cm)**	**Oldest age (Mean ± SE)**	**Depth (cm)**
Pollença	3	39	35 ± 4	203 ± 12	37	1912 ± 3	17
Sta.Ponça	3	34	NA	547 ± 23	32	1918 ± 7	19
Cala d'Or	3	32	17 ± 3	535 ± 18	32	1930 ± 1	21
Portocolom	3	35	21 ± 7	545 ± 24	27	1906 ± 7	29
Sóller	3	39	NA	1026 ± 141	37		
Magalluf	3	43	13 ± 2	648 ± 39	41	1912 ±4	15
Es Port	3	36	31 ± 3	338 ± 24	35	1918 ± 4	17
Es Pujols	3	27	5 ± 3	367 ± 15	15		
Ses Illetes	3	23	11 ± 4	547 ± 33	21		
Es Castell	3	35	38 ± 4	315 ± 10	33	1921 ± 9	17
Sta.Maria	4	45	20 ± 3	550 ± 16	27	1943 ± 2	13

One core per site was sliced at 1 cm intervals and was used for sediment grain size (Ø) and ^{210}Pb concentration analysis. Grain size analysis was performed at Universitat de Barcelona on a Beckman Coulter LS GB500. The sediment was classified according to the Udden-Wentworth grain size scale (Ø: < 4µm, clay; Ø: 4 - 63 µm, silt;Ø: 63 - 2000 µm, sand and Ø: 2000-4000 µm, gravel). Concentrations of ^{210}Pb were determined by alpha spectrometry through the measurement of its granddaughter ^{210}Po, assuming radioactive equilibrium between both radionuclides. About 150 - 300 mg of each sample were spiked with ^{209}Po and microwave digested with a mixture of concentrated HNO_3 and HF. A second digestion was carried out with boric acid to complex fluorides. The resulting solutions were evaporated and diluted to 100 mL 1M HCl and polonium isotopes were autoplated onto pure silver disks. Polonium emissions were measured by alpha spectrometry using PIPS detectors (CANBERRA, Mod. PD-450.18 A.M). Reagent blanks were comparable to the detector backgrounds (i.e. 1-2 x 10^{-5} c s^{-1}) and analysis of replicate samples and reference materials were carried out systematically to ensure the accuracy and the precision of the obtained results.

The supported ^{210}Pb was estimated as the average ^{210}Pb concentration of the deepest layers once ^{210}Pb reached constant values. Excess ^{210}Pb concentrations were obtained by subtracting the supported ^{210}Pb from the total ^{210}Pb. Age models of the sediment records were obtained by modeling the excess ^{210}Pb concentration profiles along the accumulated mass at each site. The age of the sediment layers along the core profiles was estimated using the Constant Rate of Supply model (CRS, Appleby and Oldfield 1978), except for Cala d'Or and Porto Colom where the Constant Flux : Constant Supply model was applied (CF:CS, Krishnaswamy et al. 1971).

The remaining portions of the dated cores together with the other additional cores per site, sliced at 2 cm intervals, were used to determine sediment density and sediment organic matter concentration (OM, in %DW). Sediment density was estimated as the ratio between the sediment sample dry weight (g) (dried at 60ºC during a minimum of 48h) and the sample fresh volume (cm^3). OM (%DW) was estimated as the fractional weight loss of dry sediment in the samples after combustion at 550 ºC during 4 hours. In addition, the sediment organic carbon concentration (C_{org}, in %DW) was analysed in alternate 2 cm slices from one core per site on an Elemental Analyser - Isotope Ratio Mass Spectrometry (EA-IRMS) at Iso-Analytical Laboratory (United Kingdom). The relationship between OM (%DW) and C_{org} (%DW) contents measured in the same sample (n= 101, Figure SI 1.3), described by the fitted regression equation (1.2), was used to estimate C_{org} in those samples analyzed for OM only:

$$C_{org} = -0.601\ (\pm\ 0.090) + 0.290\ (\pm\ 0.009)\ OM\ (F_{100=} 973.8, R^2=0.91, p < 0.001) \qquad (1.2)$$

The density of C_{org} (g C_{org} cm^{-3}) along the sediment profile was estimated as the product of % C_{org} by the sediment density of each sediment slice, divided by 100.

Carbon stocks and burial rates estimation

The C_{org} stock (g C cm^{-2}) along the sediment profile was estimated as the product of the C_{org} density (g C cm^{-3}) by the sediment slice decompressed thickness.

We estimated carbon stocks for the sediment top-30 centimeters by multiplying the average C_{org} stocks per slice (g C cm^{-2}) for a minimum of 3 replicated cores per station, within the top-30 cm of the sediment, by 30. The reported C_{org} stocks (kg C m^{-2}) correspond to decompressed stocks (as explained above), except for two meadows where sediment compression could not be measured during sampling (Sóller and Sta. Ponça). To compare with global estimates we extrapolate top-30 cm C_{org} stocks (observed and decompressed) to a one-meter depth and to a one-hectare scale (Table SI 1.3) taking into account data on meadow surface cover (%) per site (Table 1). Extrapolation to one-meter depth is supported by the fact that, as shown in the results, no general trend in C_{org} concentration (%DW) with sediment depth among the meadows examined was found.

Average C_{org} burial rates (g C m^{-2} yr^{-1}) at each station were calculated for different periods (i.e. since 1900, before and after the increase of human pressure) using the data on accumulated stocks of C_{org} (g C cm^{-2}) during each period, identified by the ^{210}Pb derived-chronology, divided by the number of years of each period. The temporal variability in the C_{org} burial was examined calculating the ratio of burial rates after and before the onset of the increase of human pressure, with values below or above 1 denoting a decrease or an increase in the C_{org} burial after the onset of anthropogenic pressure increase.

To estimate the C_{org} stocks accumulated between 1900 and present we multiplied the estimated average C_{org} burial rate since 1900 by the number of years until sampling was conducted (112 for all the meadows except for Sta. Maria which was 113).

An overestimation of the carbon buried in the most recent period compared to that stored in the period before may occur if a potential remineralisation of the sediment organic matter along time is not considered. In order to assess the magnitude of this error, we estimate recent C_{org} stocks and burial rates after decomposition during a number of years equivalent to the age difference between the two periods examined. We did so by considering the highest available decay rate of organic matter buried in *P. oceanica* sediments (-0.0005 y^{-1}, Serrano et al. 2012) and applying the most commonly used equation in the literature on seagrass tissue decomposition (Enriquez et al. 1993; Pergent et al. 1994; Serrano et al. 2012)

$$W_t = W_o \cdot e^{-\lambda t} \qquad (1.3)$$

where W_t is the predicted C_{org} stock, W_o is the observed C_{org} for the most recent period, λ is the decay rate (0.0005 yr^{-1}) and t is the difference between the average age of the sediment accumulated during the periods after and before the anthropogenic pressure increase.

Statistical analysis

One-way ANOVA test was used for determining significant differences in average mean grain size and C_{org} concentration along the whole core profiles and top meter stocks (decompressed) between stations. A post-hoc Tukey test was used to identify the sites with significantly different C_{org} stocks. Wilcoxon Signed rank test was used to assess the difference in the C_{org} burial rates between the periods before and after the anthropogenic pressure increased. Linear regression analysis was used to assess the change of C_{org} (%DW) with sediment depth, the effect of fetch on the mean sediment grain size and the effect of water depth, fetch and human pressure index on the C_{org} burial rates.

Propagated standard errors (SE) were calculated for the top-30 centimeters stocks and C_{org} burial rates taking into account the SE associated to sediment age and that derived from C_{org} stocks of three replicate cores per site (Supplementary Information).

1.3. Results

Sediments were dominated by the sand fraction (Ø: 63 -2000 μm), which represented an average (± SE) of 84 ± 1 % of the seagrass sediments examined. However, there were significant differences in the mean grain size (ANOVA, F_{229}=23.6, $p<0.0001$; Table 1.2) and in the contribution of different grain size fractions between sites (Fig. SI 4). The sediments from Sóller had the largest mean grain size, followed by those from Magalluf, whereas those from Pollença and Es Port were characterized by presenting the largest proportion of silt and clay fractions (Figure SI 1.4).

The concentration of sediment C_{org} (%DW) in the *P. oceanica* meadows studied averaged (± SE) 1.8 ± 0.5 C_{org} %DW and showed significant differences between meadows (ANOVA, F_{780} =112, $p < 0.0001$), ranging from a minimum average concentration of 0.7 ± 0.3 C_{org} (%DW) in Es Castell to a maximum of 5.8 ± 0.5 C_{org} (%DW) in Pollença. C_{org} (%DW) significantly changed along the sediment depth profile in six of the eleven meadows examined, decreasing with depth at average rates of -0.08

± 0.02 (%DW) cm^{-1}, -0.04 ± 0.005 (%DW) cm^{-1}, -0.05 ± 0.009 (%DW) cm^{-1} and -0.03 ± 0.01 (%DW) cm^{-1} in Es Pujols, Es Port, Porto Colom and Sta. Ponça, respectively, and increasing with sediment depth at average rates of 0.03 ± 0.005 C_{org} (%DW) cm^{-1} and 0.014 ± 0.004 C_{org} (%DW) cm^{-1} in Magalluf and Sóller respectively (Figure 1.2; Table SI 1.2).

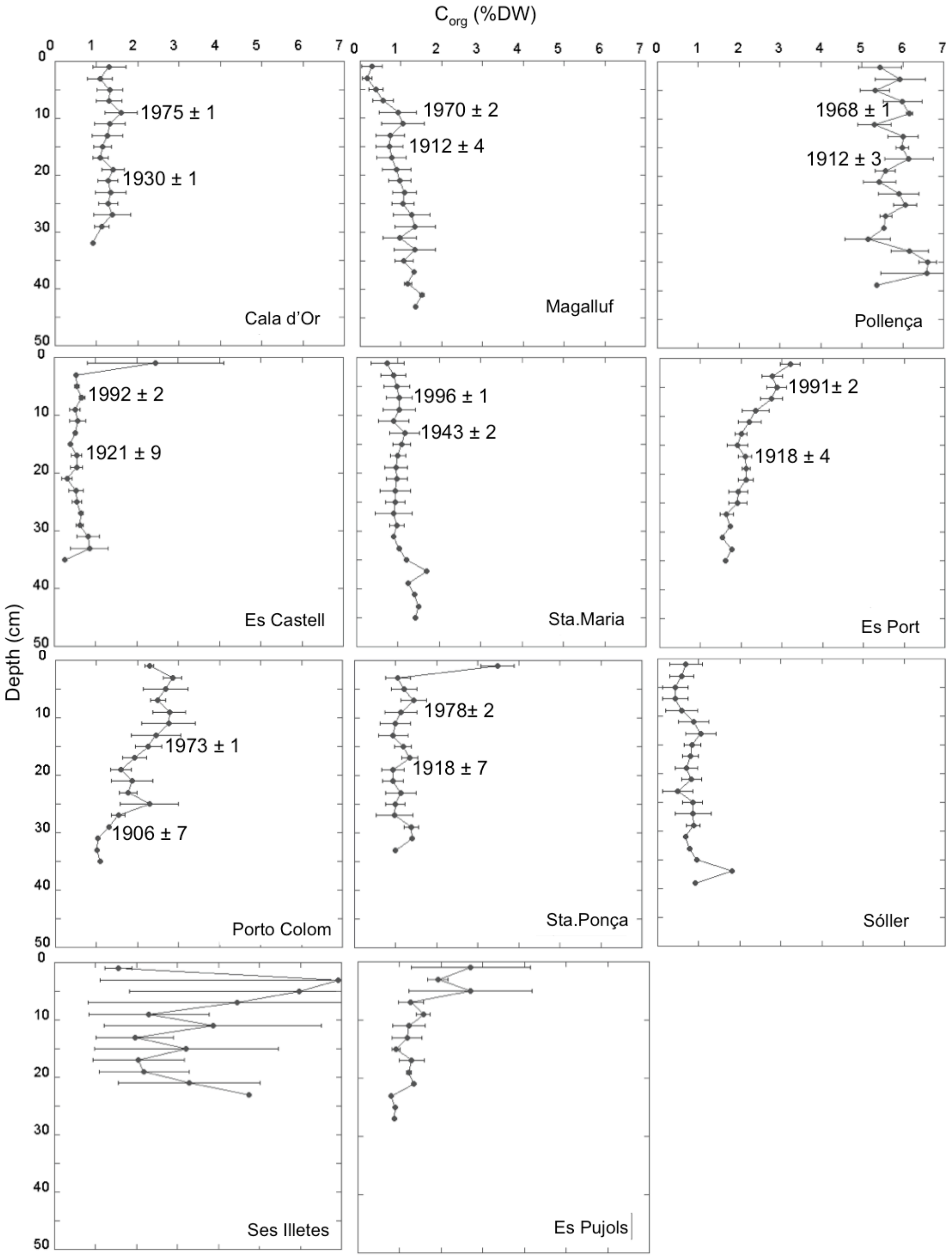


Figure 1.2. Average (±SE) organic carbon concentration (C_{org} %DW) of a minimum of three cores per meadow along the observed depth profile. The years (± SE) of deposition of the oldest sediment layer accumulated since 1900 and since the onset of increase in human pressure are indicated.

The average (± SE) carbon stock stored in the top-30 centimeters sediments of the meadows examined was estimated as 6.1 ± 1.4 kg C m^{-2}, with significant differences between stations (ANOVA, F_{129}=54.58, p<0.0001), ranging from a minimum of 1.94 ± 0.41 kg C m^{-2} in Es Castell to a maximum of 11.3 ± 1.18 kg C m^{-2} in Pollença (Figure 1.3). The two meadows for which decompressed stocks could not be estimated (Sta. Ponça and Sóller) are not included in the statistical analysis.

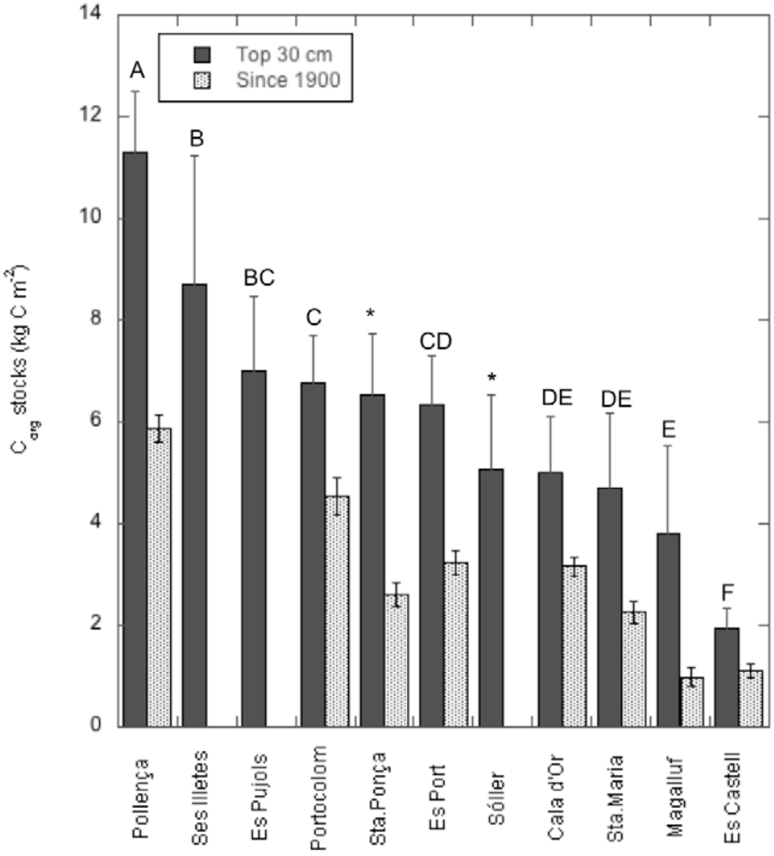


Figure 1.3. Mean ± SE organic carbon (C_{org}) stocks (kg C m^{-2}) stored in the sediments top-30 cm and since 1900 for the *P. oceanica* meadows studied. Different letters indicate significant differences in the top-30 cm carbon stocks (ANOVA, F_{129}=54.58, p<0.0001; Tuckey-Kramer test, α=0.05). The asterisk (*) identifies those meadows for which decompressed stocks are not available and observed stocks are represented but not included in the statistical analysis.

Total ^{210}Pb concentrations showed a clear decline with depth in eight out of the eleven sites (Figure SI 1.5). Excess ^{210}Pb was not detected (or very limitedly) in sediment cores collected at Sóller and Ses Illetes and evidenced intense mixing in Es Pujols, precluding the determination of reliable sediment chronologies at these sites.

Meadows where sediments could be dated (n= 8) accumulated on average 2.97 ± 0.65 kg C m^{-2} between years 1900 and 2012, with an average C_{org} burial rate of 26 ± 6 g C m^{-2} y^{-1}. However, the C_{org} buried in *P. oceanica* meadows since 1900 widely varied across sites, ranging from 0.97 ± 0.23 kg C m^{-2} in Magalluf to 5.9 ± 0.3 kg C m^{-2} in

Pollença (Figure 1.3), with corresponding average burial rates ranging from 9 ± 2 g C m^{-2} y^{-1} in Magalluf to 52 ± 2 g C m^{-2} y^{-1} in Pollença (Table 1.3).

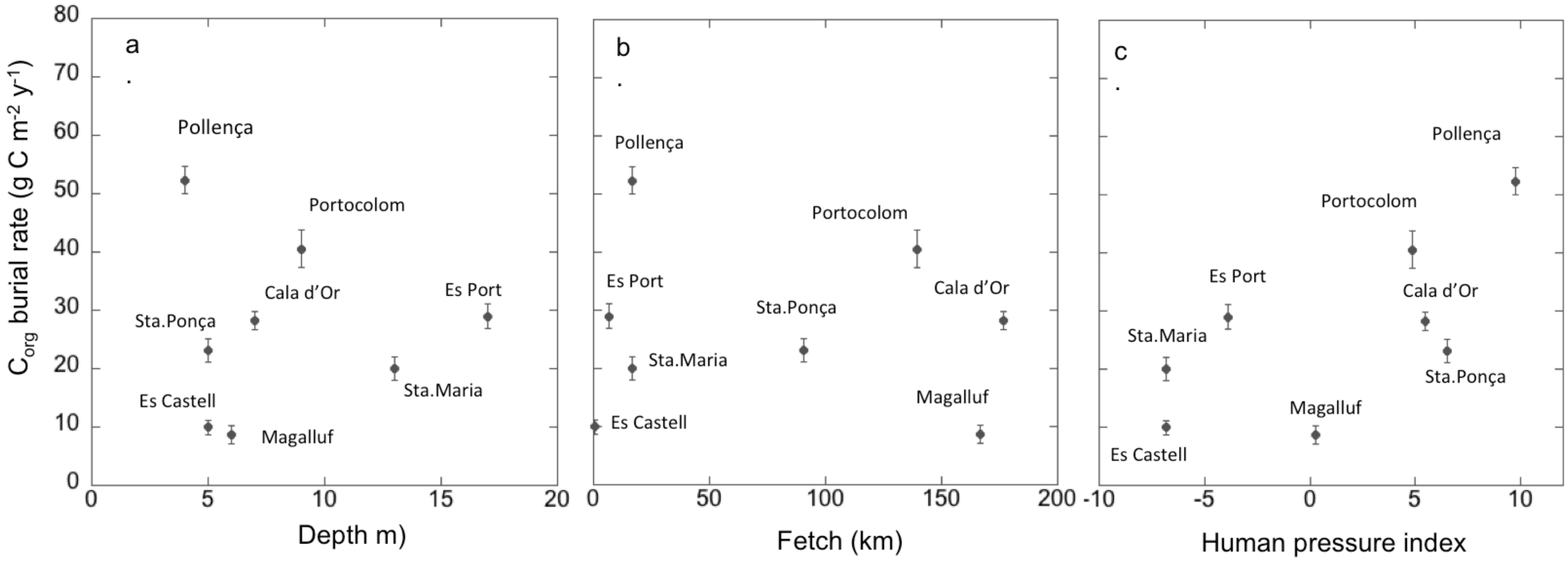
Table 1.3. Organic carbon (C_{org}) burial rate (g C cm^{-2} y^{-1}) since 1900 until present and for the periods before and after the approximate year of acceleration in human pressure at each station ($Year_{HP}$).

Station	Human pressure	$Year_{HP}$	Since 1900		Before $Year_{HP}$		After $Year_{HP}$	
			g C m^{-2} y^{-1}	SE	g C m^{-2} y^{-1}	SE	g C m^{-2} y^{-1}	SE
Pollença	Very high	1960	52	2	58	5	65	4
Sta. Ponça	Very high	1970	23	2	22	4	33	3
Cala d'Or	Very high	1975	28	2	32	3	30	2
Portocolom	High	1970	40	3	30	4	64	4
Magalluf	High	1970	9	2	10	3	10	3
Es Port	Moderate	1991	29	2	30	3	42	6
Es Castell	Low	1991	10	1	8	1	21	3
Sta. María	Low	1991	20	2	18	3	43	6

The effective fetch in the meadows examined ranged from 0.46 km in Es Castell, an enclosed bay, to 187 km in Es Pujols, a highly exposed site (Table 1.1). Sediment grain size in the seagrass examined increased significantly with increasing fetch (linear regression analysis: (F_{229}= 81.9, R^2 = 0.26, p <0.0001; Fig. SI 1.6). On the contrary, there was no significant relationship between water depth nor the effective fetch and the average C_{org} burial rates since 1900 across meadows (Figure 1.4a and 1.4b) (p>0.05).

The C_{org} burial rate in the meadows examined tended to be higher as the index of human pressure in the meadows examined was higher, although this relationship was not significant (Figure 1.4c) (p>0.05). The C_{org} burial rate increased since the onset of the increase in human pressure in all the meadows examined except for Magalluf and Cala d'Or (Figure 1.5 and Table 1.3). Considering all the studied meadows, the average C_{org} burial rate since acceleration of anthropogenic pressure (38 ± 9 g C m^{-2} y^{-1}) was significantly higher than that recorded for the period before (26 ± 7 g C m^{-2} y^{-1}) (Wilcoxon Signed rank test, df=7, S=-15, p=0.039). Correcting the estimates of C_{org} burial rates of the most recent period for the potential remineralization of the C_{org} stocks during the time span between the two periods, assuming a decomposition rate of *P.oceanica* carbon of 0.0005 yr^{-1} (Serrano et al. 2012), resulted in a reduction of the recent C_{org} burial rates of between 2.0 % and 3.1 % depending on the meadow.

Figure 1.4. Organic carbon (C_{org}) burial rate (g C m^{-2} y^{-1}) since 1900 vs. a) depth, b) effective fetch and c) human pressure index in the meadows studied. See Methods for description of calculation of human pressure index.



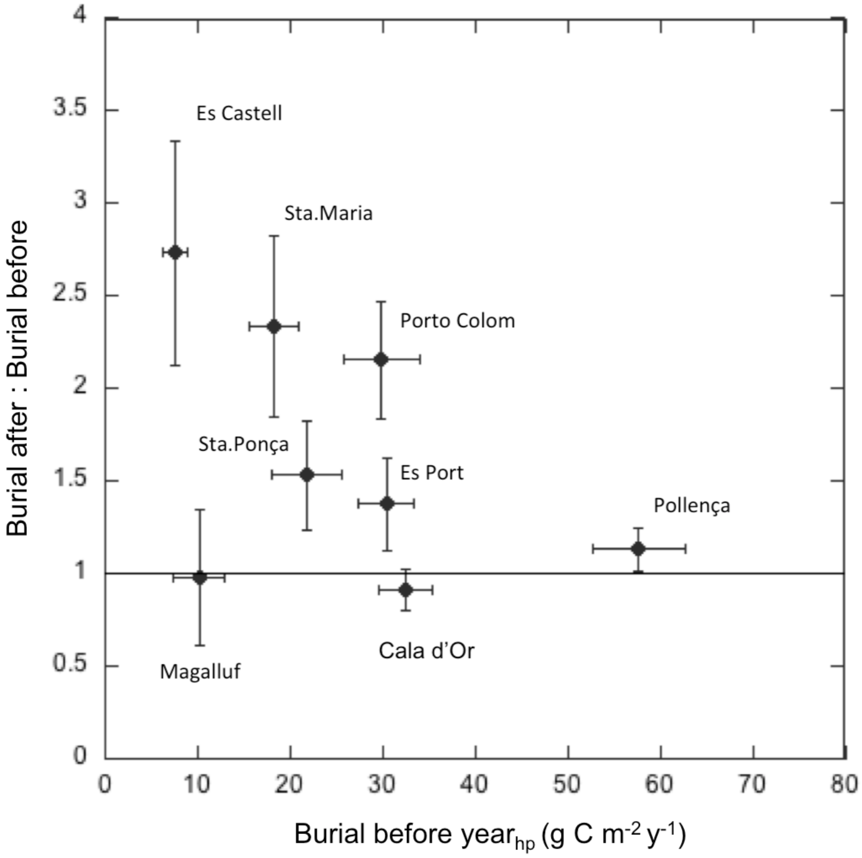


Figure 1.5. Relationship between organic carbon (C_{org}) burial rate (± SE) per meadow for the period between 1900 and the onset of human pressure increase (x axis) and the ratio (± SE) between the C_{org} burial rates for the periods after and before the year of increase of human pressure (y axis) in each of the meadows studied. The transition dates between both periods are reported in Table 1.1.

1.4.Discussion

The *P. oceanica* meadows studied here support relatively low C_{org} sinks compared to *P. oceanica* meadows studied in the past. Estimated top meter sediment stocks, observed and decompressed (202 ± 79 Mg C_{org} ha^{-1} and 167 ± 65 Mg C_{org} ha^{-1} respectively, Table SI 1.3) are around two times lower than those reported in previous studies (372 ± 75 Mg C_{org} ha^{-1}, Fourqurean et al. 2012) whereas burial rates (9 - 52 g C_{org} m^{-2} y^{-1}) ranked among the lowest values reported for other *P. oceanica* meadows studied in the past (6 - 175 g C_{org} m^{-2} y^{-1}, Mateo et al. 1997; Pergent et al. 2012). These results suggest that data hitherto available may have been biased towards meadows supporting very high carbon stocks and burial rates, as sampling was largely directed to *P.oceanica* reefs (e.g. Mateo et al. 1997 and Serrano et al. 2012) that are impressive structures that can reach several meters high (Lo Iacono et al. 2008) but may not be representative of the whole spectrum of existing *P. oceanica* meadows.

Despite the studied seagrass meadows grew at depths encompassing up to 13 m difference, variability in carbon burial rates was uncoupled to that of water depth. Hence, our results do not support the recent findings pointing out that seagrass burial capacity decreases with increasing water depth as a consequence of the decrease of seagrass productivity (Serrano et al. 2014). In fact, not only seagrass production sets the total carbon available to be sequestered since allochthonous carbon has been demonstrated to contribute up to a 50% of the total C_{org} buried in the top seagrass sediments (Kennedy et al. 2010). The loading of allochthonous carbon in the water column depends on local factors, and is usually higher in coastal areas influenced by river discharges and/or nearby urbanized areas (Short and Burdick 1996; Bowen and Valiela 2001). On the other hand, C_{org} burial might increase in deeper meadows, due to a reduction in water flow, that enhances sedimentation and decreases resuspension and sediment aeration, preventing organic matter remineralisation (Lavery et al. 2013). Therefore, the effect of water depth in seagrass C_{org} burial is still unclear and further research is needed taking into account the different local conditions such as the different sources of carbon to seagrass meadows.

Mean grain size tended to increase with fetch, consistent with previous studies conducted with other species (Murphey and Fonseca 1995; Fonseca and Bell 1998). This result demonstrates the suitability of the fetch index used to represent the hydrodynamic conditions of the different sites and the relevance this factor has at determining the sedimentary dynamics in seagrass meadows. Hydrodynamic forces were also expected to control the C_{org} burial rates as more sheltered meadows would favor the sedimentation of allochthonous carbon and prevent resuspension, export and/ or erosion of the sediment carbon deposits. However, this expectation was only partially supported by the results obtained. The highest C_{org} burial rate was found in one of the most sheltered meadows (Pollença, 52 ± 3 g C m^{-2} y^{-1} with fetch = 17 km) and the lowest burial rate was found in one of the most exposed sites (Magalluf, 9 ± 2 g C m^{-2} y^{-1} and fetch= 167 km). However, some sheltered meadows showed very low C_{org} burial rates, such as those in Es Castell and Santa Maria (10 ± 2 and 20 ± 2 g C m^{-2} y^{-1} with fetch equal to 0.5 and 16 km, respectively) and some other meadows with medium and high exposure showed high burial rates, such as Portocolom or Cala d'Or (40 ± 3 and 28 ± 2 g C m^{-2} y^{-1} with fetch = 139 and 176 km, respectively). Thus, fetch was not enough to explain the C_{org} burial rate in the meadows examined.

On the other hand, the C_{org} burial rates observed seemed to be correlated to the level of human pressure in the meadows examined, being higher in sites under a "Very High" human pressure (35 ± 9 g C m^{-2} y^{-1}) compared to meadows under "High" or "Moderate" human pressure (25 ± 2 and 29 ± 2 g C m^{-2} y^{-1}) and especially compared to the most pristine meadows, Es Castell and Sta. Maria (15 ± 1 g C m^{-2} y^{-1}) (Figure 1.4c; Table 1.3). Magalluf constitutes an exception to this general trend as it showed the lowest C_{org} burial rate (9 ± 2 g C m^{-2} y^{-1}) despite being under a "high" human pressure.

As mentioned before, carbon burial rate in seagrass meadows depends on plant productivity (e.g. shoot density) and the presence of allochthonous subsidies for sedimentation and burial in the sediment. However, meadow shoot density does not seem to explain the differences in the C_{org} burial rates observed as shoot densities of the meadows showing the highest burial rates (643 ± 75 and 568 ± 41 shoots m^{-2} in Pollença and Portocolom, respectively) have not been significantly different from that found in the meadows showing the lowest C_{org} burial rates (Es Castell and Magalluf with 515 ± 40 and 479 ± 35 shoots m^{-2}, respectively) (Table 1.1). For the contrary, the load of sestonic carbon in the water column (e.g. phytoplankton) for sedimentation and accumulation in the sediments is expected to vary among the different meadows according to their level of anthropogenic pressure (Bowen and Valiela 2001), and to be lower in pristine meadows compared to those under a relatively higher human pressure (as supported by data on chlorophyll a shown in Table SI 1.1). Despite the lack of long-term data on particle sedimentation in all the meadows examined, this hypothesis is supported by previous studies (Holmer et al. 2004). The second highest C_{org} burial rate in this study (40 ± 3 g C m^{-2} y^{-1}) corresponds to the meadow in Portocolom where a higher C_{org} sedimentation rate compared to other meadows (i.e. Sta.Maria and Magallauf) has been measured in the past and attributed to the high nutrient and organic inputs from the surrounding town, boating activity and fish farming (Holmer et al. 2004; Vaquer-Sunyer et al. 2012). In Es Port bay, signs of eutrophication have been reported during the summer period (Marbà et al. 2006; Table SI 1.1) as well as higher particle sedimentation rates compared to other meadows in Cabrera National Park (e.g. Sta.Maria) (Table SI 1.2; Holmer et al. 2004) which is consistent with the significantly higher C_{org} burial rate found in this meadow (29 ± 2 g C m^{-2} y^{-1}) compared to those measured in the two pristine sites of the park: Es Castell (10 ± 1 g C m^{-2} y^{-1}) and Sta. Maria (20 ± 2 g C m^{-2} y^{-1}).

Hence we hypothesize that the higher C_{org} burial rates identified in meadows under a high human pressure are explained by a higher load of sestonic carbon in the water column. Yet, the magnitude of the deposition of this allochthnous carbon is likely to be determined by fetch, being enhanced in sheltered meadows, with a long seawater residence time (e.g. Es Port and Pollença) and prevented in highly exposed sites, such as Magalluf.

An increase of 69 ± 24 % in the average C_{org} burial was found in the *P. oceanica* meadows examined since the onset of increase in human pressure in coastal areas compared to the period before. Several hypotheses may be formulated to explain the observed increase in C_{org} burial rates. It could be attributable to the age difference of the sediment stocks accumulated during the two periods compared, over which diagenetic processes may have acted. However, the decomposition of organic matter accumulated in sediments is usually reflected as a decay in carbon concentration along the sediment depth profile (Garten et al. 2007) and, in this study, a decay of carbon concentration with increasing sediment depth has been observed only in 3 out of the 6 meadows where C_{org} burial rate significantly increased towards present. In addition, a potential remineralization of the most recent accumulated C_{org} stocks at a decay rate of 0.0005 y^{-1}, (Serrano et al. 2012), for a period of time equal to the age difference of the stocks accumulated during the high and low human pressure periods considered, would lead to an apparent reduction of the recent C_{org} burial rates of between 2.0 % and 3.1 %, depending on the meadow. This apparent reduction is much lower than the average increase in the C_{org} burial observed between periods (69%).

On the other hand, as human pressure increased in the studied region, the input of nutrients into coastal waters has likely increased too, as reported in many other areas worldwide (Short and Burdick 1996; Bowen and Valiela 2001; Nedwell et al. 2002). A nutrient enrichment could have had a fertilization effect and led to an increase in plant biomass productivity during the most recent period, although this is not consistent with the general decay in shoot density observed in the meadows examined for the last decade (Table 1.1). On the contrary, an excess input of nutrients in coastal waters may have enhanced eutrophication (Nixon 1995) and the increase in the load of allocthonous carbon in the water column that sedimentates and accumulates in the sediment, as reported in previous studies (Savage et al. 2010), including studies conducted in seagrass meadows (Macreadie et al. 2012).

Unfortunately, the shorter time period of meadow demographic observations (3-11 years) compared to that encompassed by the sediment record for the most recent period (~21-52 years) and the absence of long-term time series of chlorophyll a concentration in the water column at the study sites prevent further assessment of the role that trajectories of meadow structure and phytoplankton abundance may have on *P.*

oceanica carbon sinks.

In summary, our study contributes to the data available on C_{org} sinks in *Posidonia oceanica* meadows, explores the effect of water depth and wave exposure (fetch) and provides C_{org} burial rates in seagrass meadows during the last century, a period of an intense increase in human pressure in coastal environments. No effect of water depth on C_{org} burial was found and although fetch clearly controlled sedimentation patterns in the meadows examined, it was not enough to explain differences in C_{org} burial rates. Carbon burial rates tended to be higher in meadows under a relatively higher anthropogenic pressure and to rise during the period after human pressure increased in each site. The possible eutrophication associated to the increase of human pressure on coastal areas may have enhanced the contribution of allochthonous carbon to the sediment stocks. However, this hypothesis needs to be further explored to discard other mechanism that may explain the observed increase in C_{org} burial rate.

Acknowledgments

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Supplementary Information

(Chapter 1)

Figure SI 1.1. Radius used for the estimation of the Effective Fetch (Hakanson, 1981), based in 15 distance measurements from the study site to the opposite shore. The central radius is placed in the direction that results in the highest value. The distance (χ_i in km) from the sample site to a shore or to an island is measured for every deviation angle γ_i, where γ_i equals ±6°, ±12°, ±18°, ±24°, ±30°, ±36° and ±42°. The effective fetch (Lf) is calculated from the formula $Lf = (\Sigma\ \chi_i \cos \gamma_i)/(\ \Sigma \cos \gamma_i)$, where χ_i is the measured distance in kilometres and γ_i is the angle between the measured distance and the central radius.

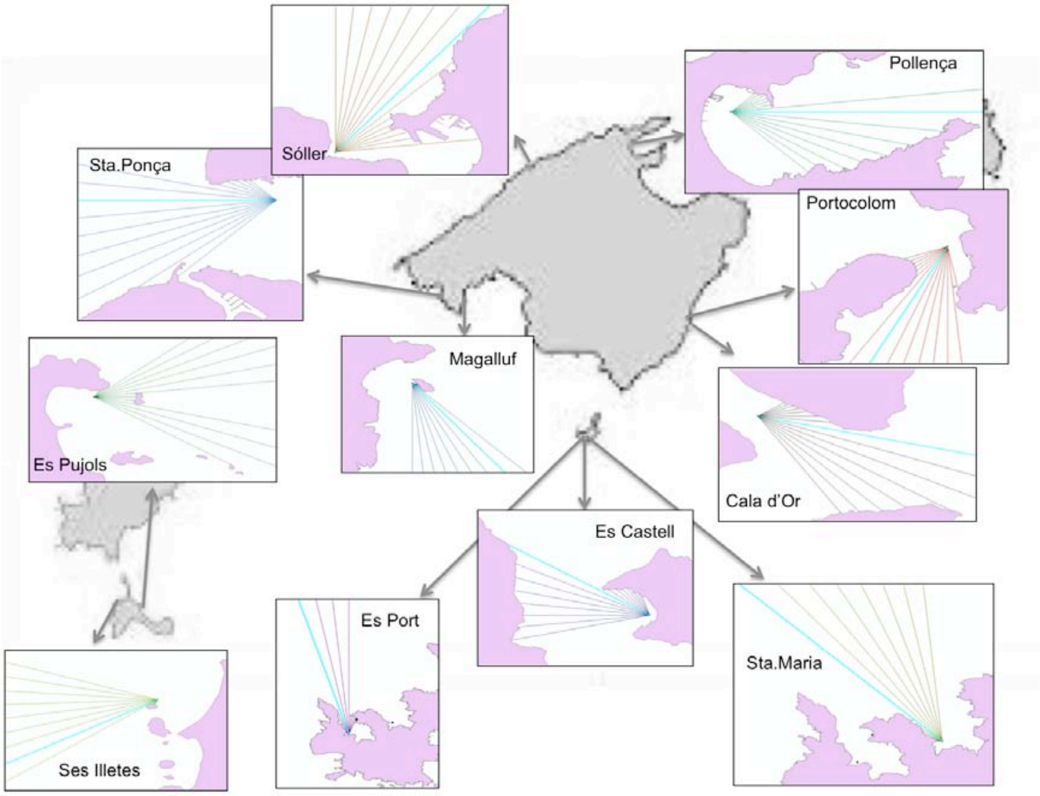

Figure SI 1.2. Cumulative number of hotel places in each of the study sites and dates related to other factors of pressure considered: fish farms and/or the construction of the first yacht club or recreational marinas. The number of hotel beds per year since 1931 to 2010 were extracted from the Spanish Hotels Catalogue (1932-1991) and the Balearics' Tourist Accommodation Catalogue (1996-2010) (Antoni Pons, *personal communication*).

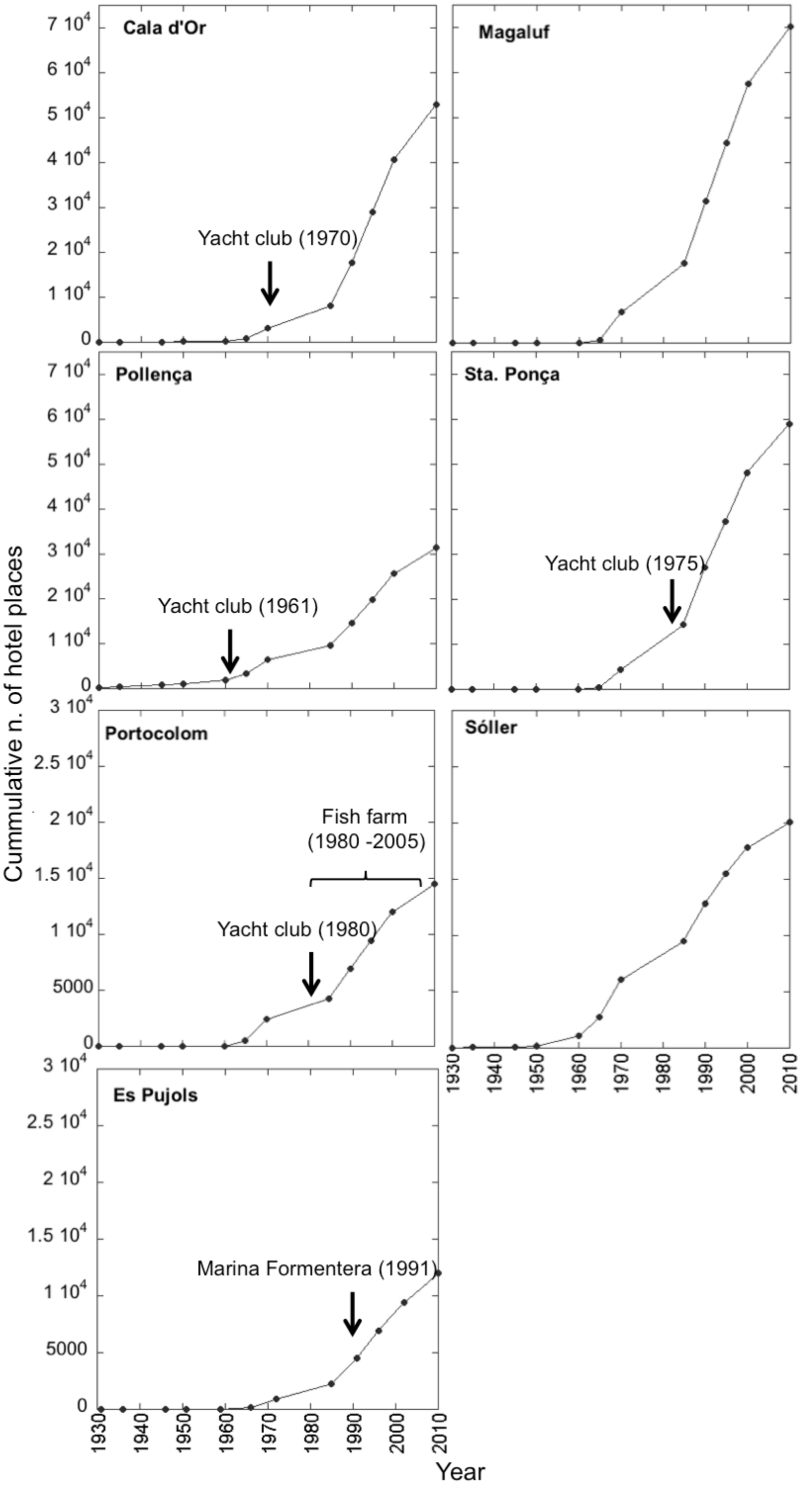

Figure SI 1.3. Organic Matter (OM, %DW) estimated from LOI data vs. Organic Carbon (C_{org}, %DW) measured by Elemental Analyser - Isotope Ratio Mass Spectrometry (EA-IRMS). The linear regression analysis revealed a significant relationship between OM (%DW) and C_{org} (%DW): $y= (-0.61 \pm 0.09) + (0.29 \pm 0.01)\cdot x$ ($F_{100}= 973.8, R^2=0.91, p < 0.001$).

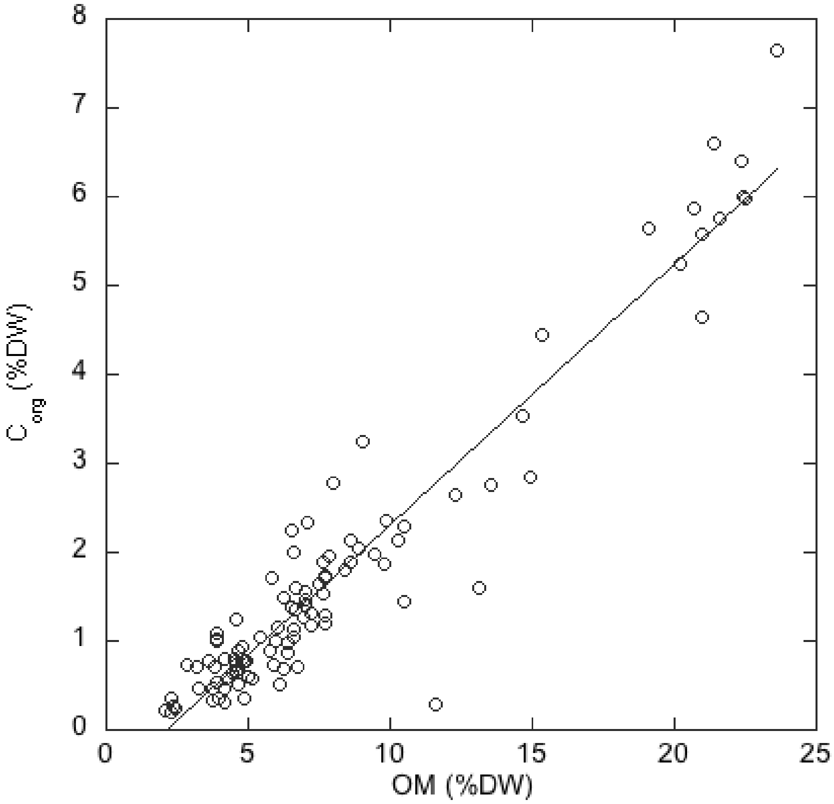

Figure SI 1.4. Contribution (%) of the different sediment grain size classes according to the Udden-Wentworth scale (silt, Ø < 4µm; clay, Ø: 4 -63 µm; sand, Ø: 63 -2000 µm and gravel, Ø: 2000-4000 µm) at each site.

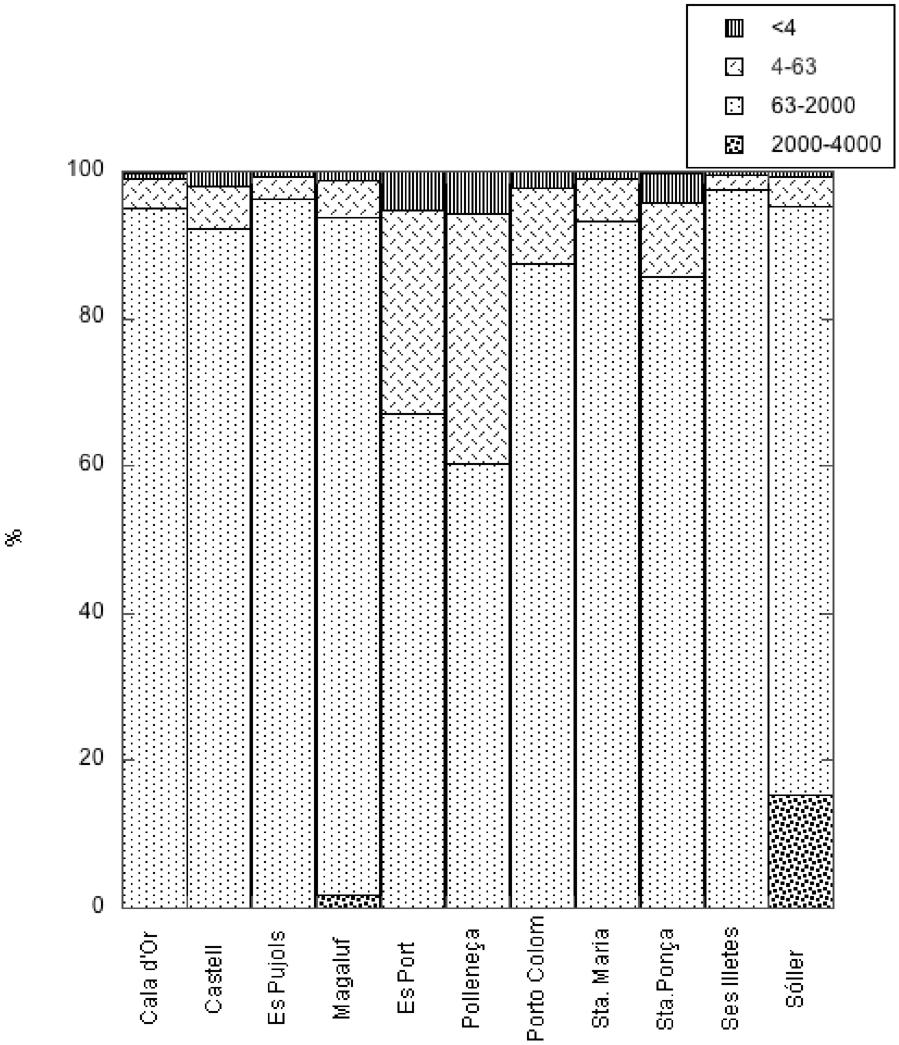

Figure SI 1.5. Total ^{210}Pb concentration profiles in one core per station. The supported ^{210}Pb (^{210}Pb$_{sup}$) concentration is represented by the grey area. The years (±SE), estimated from sediment ^{210}Pb dating, of deposition of the oldest sediment layer accumulated since 1900 and since the onset of increase in human pressure (Table 1) are indicated.

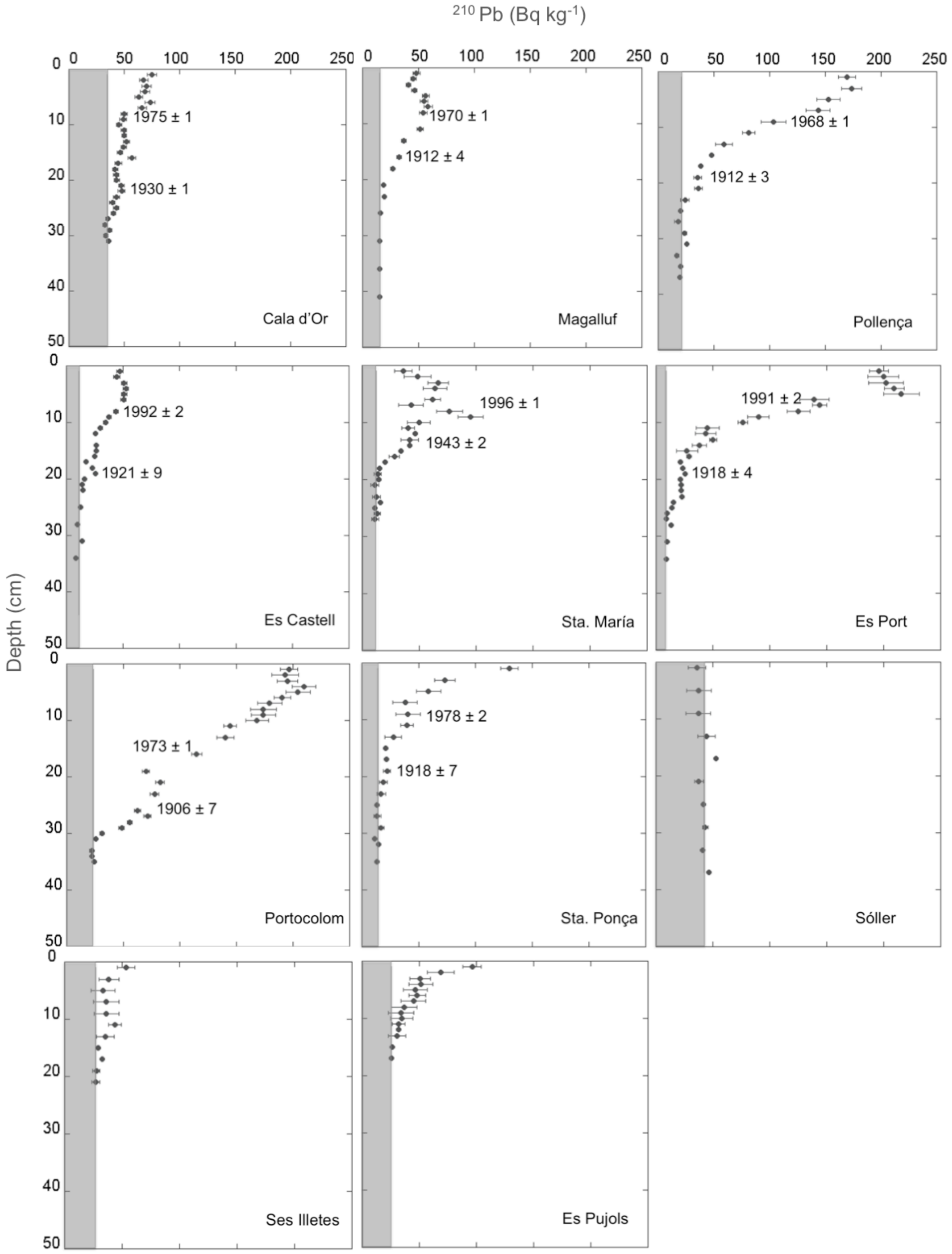

Figure SI 1.6. Mean sediment grain size (± SE) vs. effective fetch in the meadows studied. The solid line shows the fitted linear regression:
$y = 323 (\pm 26) + 2.02 (\pm 0.22)\, x$ (F_{229}= 81.99, R^2 = 0.26, $p < 0.0001$).

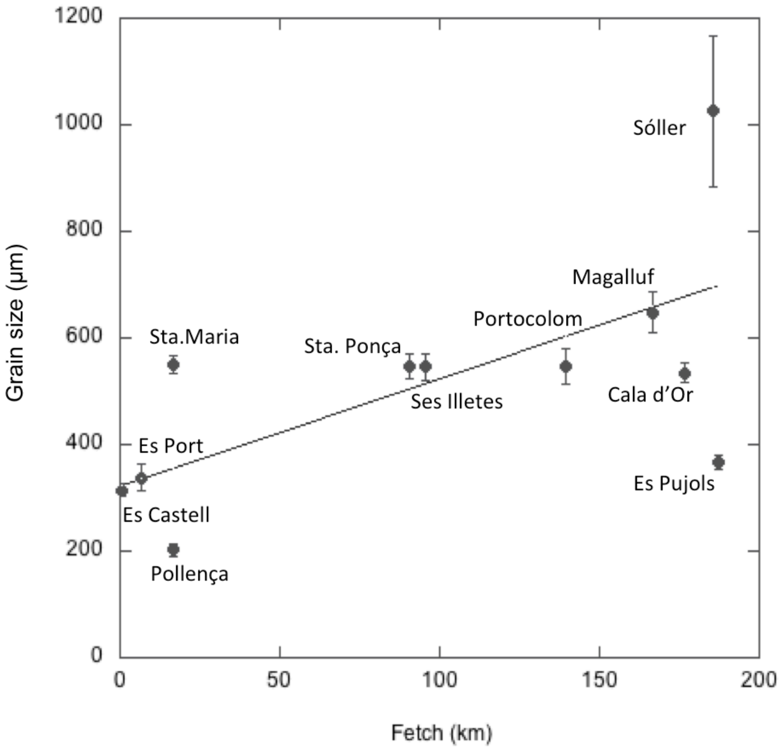

Table SI 1. Compilation of data on the anthropogenic pressures considered in the meadows examined. Population data (2012) was extracted from the National Institute of Statistics of Spain (INE) and from municipal archives. Hotel places (2012) were extracted from Spanish Hotels Catalogue (1932-1991) and the Balearics' Tourist Accommodation Catalogue (1996-2010) (Antoni Pons, *personal communication*). Data on wastewater inputs come from Spatial Data Infrastructure of The Balearic Islands (IDEIB, http://ideib.caib.es/visualitzador/visor.jsp) (IDEIB visor) and only considered those within a 1 km distance from the sampling sites. The number of ports and berths close to the meadow (in the same bay) were extracted from the CITTIB (2008). "Boats sailing in coastal waters" were extracted from Balaguer et al. (2011) for the sites in Mallorca, whereas data for the sites in Formentera are estimates following methodology describe in Balaguer et al. (2011). For water quality, average ± SE chlorophyll a data (Chl a) are extracted from a report by the Environmental Department of the Government of the Balearic Islands (http://www.caib.es/sacmicrofront/archivopub.do?ctrl=MCRST259ZI166880&id=166880) except for the meadows from Cabrera that were extracted from Marbà et al. (Proyectos de Investigación en parques Nacionales: 2003-2006; available at http://www.magrama.gob.es/es/parques-nacionales-oapn/programa-investigacion/oapn_inv_art_0208_tcm7-46337.pdf). *Value for year 2005; ** visitors per day during the high touristic season (Marbà et al. 2002).

Station	**Population (2012)**	**Tourism**	**Waste water inputs**		**Nautical pressure**			**# Fish farms**	**Human pressure index**	**Category of human pressure**	**Water quality**
		# hotel beds	**# Seawage pipes**	**# punctual discharges**	**# Ports**	**# berths**	**# Boats sailing in coastal waters**				**Chl a (µg L-1)**
Pollença	7,074	5,796	2	38	2	923	1303	0	9.7548	Very High	0.19 ± 0.05
Sta.Ponça	11,006	10,671	2	10	1	522	1013	0	6.5247	Very High	0.35 ± 0.18
Cala d'Or	4,322	12,191	1	11	2	563	1365	0	5.4463	Very High	0.24 ± 0.08
Portocolom	4,559	2,500	1	1	1	553	1365	1	4.8712	High	0.22 ± 0.07
Sóller	2,903	2,248	1	11	2	568	242	0	1.2295	High	0.24 ± 0.13
Magalluf	4,397	12,759	1	2	0	0	1013	0	0.2429	High	0.48 ± 0.18
Es Port	380**	0	1	0	1	50	50	0	-3.8933	Moderate	0.15 ± 0.01
Es Pujols	863*	2,545	1	4	0	0	153	0	-4.0219	Moderate	0.15 ± 0.07
Ses Illetes	0	0	0	0	0	0	153	0	-6.5470	Low	0.096 ± 0.017
Es Castell	0	0	0	0	0	0	0	0	-6.8036	Low	0.11
Sta.María	0	0	0	0	0	0	0	0	-6.8036	Low	0.09

Table SI 1.2. Results from linear regression analysis between organic carbon (C_{org} %DW) and observed sediment depth.

Station	**Slope**	**R^2**	**p-value**	**df**	**F Ratio**
Cala d'Or	-0.002 ± 0.007	0.001	>0.05	60	0.06
Es Castell	-0.011 ± 0.008	0.03	<0.05	67	1.88
Es Pujols	-0.079 ± 0.025	0.20	0.003	41	9.99
Magalluf	0.026 ± 0.005	0.27	<0.0001	78	27.8
Es Port	-0.037 ± 0.005	0.47	<0.0001	62	53.6
Pollença	0.008 ± 0.008	0.02	>0.05	66	0.96
Portocolom	-0.052 ± 0.009	0.35	<0.0001	60	32.45
Ses Illetes	-0.092 ± 0.096	0.02	>0.05	42	0.92
Sóller	0.014 ± 0.004	0.16	0.0005	70	13.4
Sta.Maria	0.007 ± 0.005	0.03	0.16	67	1.97
Sta.Ponça	-0.032 ± 0.01	0.14	0.0021	64	10.26

Table SI 1.3. Estimated sediment top meter organic carbon (C_{org}) stocks (Avg. ± SE; Mg C ha^{-1}) observed and decompressed. The extrapolation to a one-hectare has been made considering data on meadow cover (%, mean ± SE) shown in Table 1.

Station	**Top meter stocks (Mg C_{org} ha^{-1})**	
	Observed (Mean ± SE)	**Decompressed (Mean ± SE)**
Pollença	527 ± 74	319 ± 45
Sta. Ponça	171 ± 37	
Cala d'Or	170 ± 41	141 ± 34
Portocolom	270 ± 39	212 ± 31
Sóller	96 ± 39	
Magalluf	87 ± 68	76 ± 59
Es Port	113 ± 18	78 ± 13
Es Pujols	227 ±49	216 ± 46
Ses Illetes	285 ± 82	253 ± 73
Es Castell	84 ± 27	52 ± 17
Sta. Maria	191 ± 60	153 ± 48
Total	202 ± 79	167 ± 65

Propagated errors

To estimate the sediment top meter organic carbon stocks (Mg C ha^{-1}) and burial rates per meadow, the average C_{org} (± SE) stock per slice derived from a minimum of three replicated cores per site examined is used as explained in the methods section.

To estimate the propagated error associated to the C_{org} top meter stocks per meadow we apply the formula:

$$SE_{propagated} = \sqrt{\ [\ (SE_{mean})^2 + ((mean/\sqrt{}n)* \sqrt{}\ ((X/SE_X)^2 + (Y/SE_Y)^2 \ldots)^2\ ]}$$

Where SE_{mean} is the standart error of the mean C_{org} stock along the sediment depth profiles, n is the number of sediment slices and X and SE_X and Y SE_Y are the average C_{org} stock and standart error per slice along the sediment depth profile, respectively.

The propagated error associated to the burial rates were estimated as:

$$SE_{propagated} = \text{Burial rate}* \sqrt{}\ ((SE_{accumulated\ stocks}/\text{accumulated stocks})^2 + (SE_y/y)^2)$$

where $SE_{accumulated\ stocks}$ is the propagated error of the accumulated C_{org} stocks calculated as the sum of the SE of the C_{org} stocks per slice, y is the number of years of each period, and SE_y the estimated error of the number of years for each period (calculated as the sum of the SE of the ages limiting each period, derived from ^{210}Pb chronology).

Chapter 2

Dynamics of carbon sources supporting burial in seagrass sediments under increasing anthropogenic pressure

Abstract

Seagrass meadows are strong coastal carbon sinks of autochthonous and allochthonous carbon. The aim of this study was to assess the effect of coastal anthropogenic pressure on the variability of carbon sources in seagrass carbon sinks during the last 150 years. We did so by examining the composition of the sediment organic carbon (C_{org}) stocks by measuring the $\delta^{13}C$ signature and C:N ratio in ^{210}Pb dated sediments of 11 *Posidonia oceanica* seagrass meadows around the Balearic Islands (Spain, Western Mediterranean) under different levels of human pressure. On average, the top meter sediment carbon deposits were mainly (59 ± 12 %) composed by *P. oceanica* derived carbon whereas seston contribution was generally lower (41 ± 8 %). The contribution of *P. oceanica* to the total sediment carbon stock was the highest (~80%) in the most pristine sites whereas the sestonic contribution was the highest (~60 to 80%) in the meadows located in areas under moderate to very high human pressure. Furthermore, an increase in the contribution of sestonic carbon and a decrease in that of seagrass derived carbon towards present was observed in most of the meadows examined, coincident with the onset of the tourism industry development and coastal urbanization in the region. Our results demonstrate a general increase of total carbon accumulation rate in *P. oceanica* sediments during the last century, mainly driven by the increase in sestonic C_{org} burial, which may have important implications in the long-term carbon sink capacity of the seagrass meadows in the region examined.

2.1.Introduction

Mounting evidence of the capacity of seagrass meadows to capture and store significant amounts of carbon (e.g. Mateo and Romero 1997; Kennedy et al. 2010; Duarte et al. 2013a) ranked these ecosystems among the most important organic carbon (C_{org}) sinks on Earth (Duarte et al. 2005; Nellemann et al. 2009; Mcleod et al. 2011; Lavery et al. 2013) with estimated global C_{org} burial rates comparable to those for saltmarshes, mangroves, and terrestrial forests (Mcleod et al. 2011). Due to the high global loss rate of seagrass meadows (Waycott et al. 2009), protection and conservation actions to preserve seagrass carbon sinks have become a relevant component of strategies to mitigate climate change, termed “Blue Carbon” strategies (Laffoley and Grimsditch 2009; Nellemann et al. 2009; Duarte et al. 2013a).

Kennedy et al. (2010) estimated, based on a synthesis of stable carbon isotopes in seagrass sediments, that around 50% of the C_{org} stored in the top 10 cm of seagrass sediments is derived from seagrass biomass, although a wide variability was found between sites (with 25th and 75th percentiles equivalent to 33% and 62% contributions, respectively). The remaining sediment carbon was of allochthonous origin derived from phytoplankton and/or terrestrial inputs, which is trapped into seagrass sediment due to the effective role of seagrass canopies as filters of sestonic particles (Gacia et al. 2002; Bos et al. 2007; Hendriks et al. 2010). The identification of the sources of C_{org} and their contribution to the sedimentary sink is especially relevant as these sources differ in their persistence in the sediment stock (Holmer et al. 2004). Sestonic matter, particularly phytoplankton, is much more labile than seagrass tissues (Enriquez et al. 1993) as reflected in much faster decomposition rates (K, median $K_{seagrass}$=0.007 d^{-1}, $K_{microalgae}$=0.046 d^{-1}; Enriquez et al. 1993). Therefore, carbon deposits enriched in seston are expected to be more prone to microbial decomposition, conducive to a lower burial efficiency and to the release of much of the sediment C_{org} back to the ocean-atmosphere CO_2 pool. Furthermore, the remineralisation of sediment C_{org} may affect seagrass growth and survival (Holmer et al. 2004) positively by increasing nutrient availability, which enhances seagrass growth in nutrient limited environments (Harvey et al. 1995; Hansen and Kristensen 1998), or/and negatively by supporting high sulphate reduction rates (SSR) that may lead to anoxic conditions (Holmer and Kristensen 1996) that compromise seagrass survival (Calleja et al. 2007). Therefore, assessments of seagrass carbon sinks should not only focus on the total C_{org} deposited but also on the sources of the C_{org} being stored.

A requirement for atmospheric CO_2 sinks to be relevant to climate change mitigation is that the C removed must not return to the atmosphere as CO_2 over time scales longer than approximately 40-150 years (Dobbes et al. 1998; Tipper and Jong 1998; Marland et al. 2001). Seagrass C_{org} has been shown to remain in the sediment deposits over millenary scales (e.g. Mateo et al. 1997) but, since the Industrial Revolution seagrass meadows have experienced major changes due to multiple drivers, including direct impacts from human modification of watersheds and coastal areas, pollution, and anthropogenic climate change (Duarte 2014) that may have altered their C sink capacity for the last century. Eutrophication for instance, usually associated with an increase in human pressure in coastal areas, is the main cause of the widespread decline of seagrass meadows worldwide (Waycott et al. 2009). While seagrass decline with eutrophication is likely to result in a reduced input of seagrass-derived C_{org} to seagrass sediments, the enhanced phytoplankton production is expected to lead to an increased input of seston-derived C_{org} to seagrass sediments (Bowen and Valiela 2001). Hence, the contribution of different carbon sources to sediment C_{org} sinks is expected to differ among seagrass meadows under different levels of human pressure, due to different meadow health status and/or loads of sestonic carbon in the water column. It is also expected that the relative contribution of carbon sources to C_{org} stocks has varied over time, provided the major global increase in anthropogenic pressure that coastal areas have experienced over the past 60 years (Duarte 2014).

Here we assess the sources of C_{org} to dated sediment deposits in *Posidonia oceanica* seagrass meadows during the last 150 years with the aim of resolving the variability among meadows and over time in response to the extent and increase of anthropogenic pressures on the coastal areas. This work is carried out in The Balearic Islands (Western Mediterranean), one of the most important touristic destinations in Europe (Hof and Schmitt 2011) where the development of the touristic industry around the 60's led to a dramatic increase in the population density and urbanization in the coastal areas, with subsequent deterioration of the water quality (Morey et al. 1992; Hof and Schmitt 2011; Roig-Munar et al. 2013). The anthropogenic pressure varies from low at near-pristine, protected and undeveloped sites to very high at strongly human-influenced bays where low water residence time likely amplifies anthropogenic impacts in the coastal ecosystems (Marbà et al. 2002; Garcia-Solsona et al. 2010). *P. oceanica* meadows, a key ecosystem endemic to the Mediterranean Sea, are remarkable in that they support the highest carbon burial rates and sedimentary C_{org} stocks yet reported for any seagrass species (Fourqurean et al. 2012; Lavery et al. 2013). The seagrass meadows around the Balearic Islands are extensive, covering an estimated area of 633 km^2 extending down to 45 m depth (Álvarez et al. 2015). However *P. oceanica* meadows have been declining in this region for at least the last two decades due to the increase in human pressures and water temperature (Marbà and Duarte 2010; Marbà et al. 2014).

We hypothesize that the contribution of different carbon sources to the sediment deposits in the meadows examined will vary depending on exposure to coastal anthropogenic pressures, and specifically, that the contribution of sestonic-derived carbon will increase with increasing human influence in the coastal areas studied.

2.2. Methods

Description of study sites

The sources of buried carbon were assessed in a total of 11 *P. oceanica* meadows in the Balearic Islands: 6 in Mallorca Island, 2 in Formentera Island and 3 in the Cabrera Archipelago National Park (Figure 2.1). Mallorca is the largest and most populated island, with about 50% of its territory transformed by human activities (Balaguer et al. 2008). All meadows from Mallorca selected for this study are under a significant level of human pressure derived from a high urbanization and/or nautical activities. Formentera is the smallest and least populated island of the archipelago. Although there are still some non-urbanized areas (e.g. Ses Illetes beach), its rise as an important touristic destination, with particularly intense pressure from nautical tourism, led to a rapid urbanization of certain sites (e.g. Es Pujols) and to a significant degradation of the coastal areas (Morey et al. 1992; Roig-Munar et al. 2013). The Cabrera Archipelago is a group of small islands that were used as an army station from 1940 until its declaration as a National Park in 1991. Although Cabrera Archipelago does not support resident population, it receives a relatively intense flow of visitors. Santa Maria bay, closed to visitors since 1993, and Es Castell, where anchoring is not allowed, are the two most pristine *P. oceanica* meadows sampled in this study. On the contrary, the meadow at Es Port's bay supports a higher level of human pressure, as contains the main services of the park, receives the visitors arriving by ferries and it is the only location in the park where sailing boats are allowed to overnight in one of the 50 buoys available. The sewage produced by visitors in moored ships, few of which are equipped with holding tanks, is usually released raw to Es Port water along with the outflow of the treated sewage from land (PNMTAC 2009).

Based on the quantification of several factors of anthropogenic pressure in coastal areas, the meadows selected have been assigned to different relative categories of human pressure they support (see Chapter 1): "Very High" pressure (i.e. Pollença, Sta.Ponça, Cala d'Or) and "High" pressure (i.e. Portocolom, Sóller, Magalluf) in Mallorca meadows; "Moderate" pressure in Es Pujols (Formentera) and in Es Port's Bay (Cabrera National Park) and "Low" pressure in Ses Illetes (Formentera) and the two remaining meadows in Cabrera National Park: Es Castell and Sta. Maria (Figure 2.1).

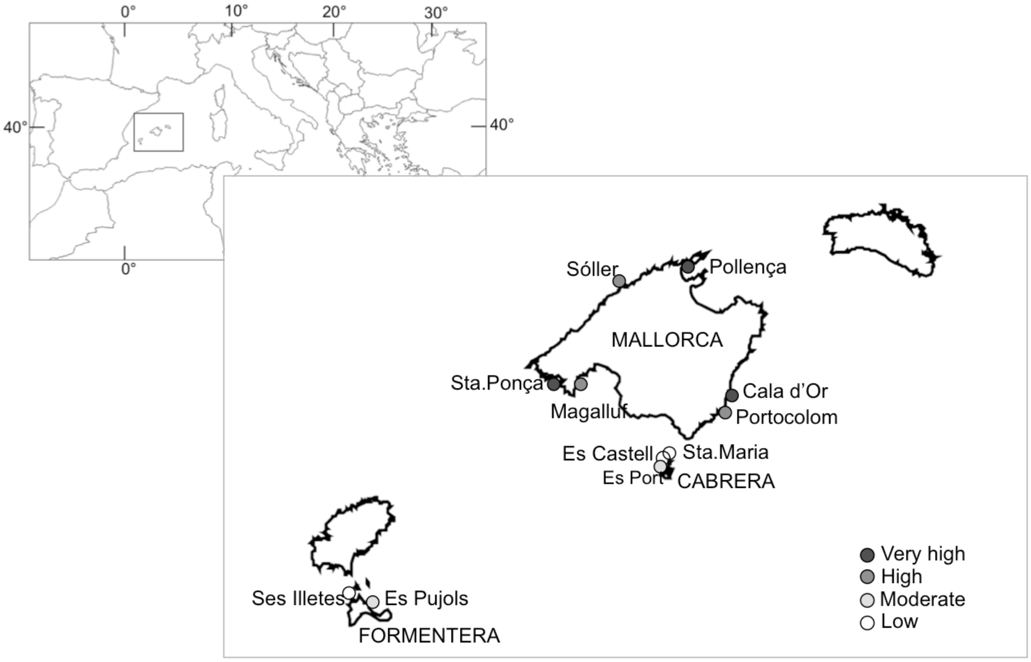

Figure 2.1. Sampling sites in the Balearic Islands. Symbol colour gradient indicates the level of human pressure in each site, from "low" to "very high".

Sediment sampling and analysis

Three sediment cores of 17 - 45 cm long were extracted from each seagrass meadow in July 2012 (except for those at Sta. Maria, which were collected in July 2013) by inserting PVC pipes 60 cm long and 9 cm diameter by SCUBA divers. All cores were stored frozen until processing in the lab.

Sediment accumulation rates were obtained from concentration profiles of ^{210}Pb measured in one of the cores at each site, sliced every 1 cm. Concentrations of ^{210}Pb were determined by alpha spectrometry through the measurement of its granddaughter ^{210}Po, assuming radioactive equilibrium between both radionuclides. About 100 - 200 mg aliquots of each sample were spiked with ^{209}Po and microwave digested with a mixture of concentrated HNO_3 and HF. Boric acid was then added to complex fluorides. The resulting solutions were evaporated and diluted to 100 mL 1M HCl and Po isotopes were autoplated onto pure silver disks. Polonium emissions were measured by alpha spectrometry using PIPS detectors (CANBERRA, Mod. PD-450.18 A.M). Reagent blanks were comparable to the detector backgrounds (i.e. 1-2 x 10^{-5} c s^{-1}). Analysis of replicate samples and reference materials were carried out systematically to ensure the accuracy and the precision of the results. The supported ^{210}Pb was estimated as the average ^{210}Pb concentration of the deepest layers once ^{210}Pb reached constant values. Then, excess ^{210}Pb concentrations were obtained by subtracting the supported ^{210}Pb from the total ^{210}Pb. Age models of the sediment records were obtained by modeling the excess ^{210}Pb concentration profiles along the accumulated mass at each site. The age of the sediment layers along the core profiles was estimated using the Constant Rate of Supply model (CRS, Appleby and Oldfield 1978), except for Cala d'Or and Porto Colom where the Constant Flux : Constant Supply model was applied (CF:CS, Krishnaswamy et al. 1971). Robust sediment age estimates were obtained for eight out of the eleven meadows examined. However, intense mixing processes precluded the establishment of sediment chronologies for the remaining 3 meadows: Sóller, Ses Illetes and Es Pujols (see Chapter 1).

The remaining portions of the dated cores and the other additional cores per site, sliced at 2 cm intervals, were used to determine sediment density, sediment organic matter concentration (OM, in %DW) and sediment organic carbon (C_{org}) content along the depth profile. Sediment density (DBD, g cm^{-3}) was estimated by dividing the dry weight of the sediment sample (g) (dried at 60ºC during a minimum of 48h) by the sample fresh volume (cm^3). OM (%DW) was estimated from loss on ignition, as the weight loss of dry sediment in the samples after combustion at 550 ºC during 4 hours. Sediment organic carbon (C_{org} %DW) was measured in every other slice along the depth profile from one core per site on an Elemental Analyser - Isotope Ratio Mass Spectrometry (EA-IRMS) at Iso-Analytical Laboratory (United Kingdom) and estimated in the rest of the samples applying the relationship between OM (%DW) and C_{org} (%DW) contents measured in the same sample (n=101),

$$C_{org} = -0.601\ (\pm\ 0.09) + 0.290\ (\pm\ 0.009)\ OM\ (F_{100=}\ 973.8, R^2=0.91, p < 0.001)$$

obtained in Chapter 1 (equation 1.2).

Bulk organic carbon (g C cm^{-3}) was estimated as the product of C_{org} (%DW) and the sediment dry bulk density (DBD).

Carbon sources were estimated based on the quantification of the $\delta^{13}C_{org}$. The sediment $\delta^{13}C_{org}$ signature was measured in every other slice along the depth profile from one of the cores collected per site (Table 2.1) using the Elemental Analyser - Isotope Ratio Mass Spectrometry (EA-IRMS) at the Iso-Analytical Laboratory (United Kingdom). We consider two main sources of C_{org} to the sedimentary stocks: seagrass tissue and sestonic matter (which includes living and non-living matter in the water column, such as microalgae and/or terrestrial detritus). The contribution of these sources to the total stock was estimated applying a two-component isotope-mixing model (equation 2.1) as described by Phillips and Gregg (2003)

$$\delta^{13}C_{sediment} = \delta^{13}C_{seagrass}\, f + \delta^{13}C_{seston}\, (1 - f) \qquad (2.1)$$

where $\delta^{13}C_{seagrass}$ (± SE) values correspond to averages of the $\delta^{13}C_{org}$ measured in leaves and rhizomes of *P. oceanica* shoots collected at each site in 2008 and 2009 (except for

Es Port and Porto Colom, where only the leaf $\delta^{13}C_{org}$ was available and the SE considered was the average of the SEs of the other meadows $\delta^{13}C_{seagrass}$ signature) (Table SI 2.1). The $\delta^{13}C_{seston}$ values correspond to the values reported for the same meadows by Papadimitriou et al. (2005) except for Cala d'Or, Porto Colom and Sóller, where the average $\delta^{13}C_{seston}$ reported in Papadimitriou et al. (2005) was used (-22.1 ± 1.7) (Table SI 2.1).

In assessing the contribution of different C_{org} sources along time we also considered the potential variability in the $\delta^{13}C_{org}$ source signatures over time due to the Suess effect, i.e., the decrease in the atmospheric CO_2 and oceanic DIC $\delta^{13}C$ signature towards present derived from the burning of fossil fuels (Keeling 1979). We did so by estimating past *P. oceanica* and seston $\delta^{13}C_{org}$ signatures based on three different rates of oceanic DIC $\delta^{13}C$ depletion with time. The rates applied correspond to the slopes of the linear regression equations resultant from fitting predicted $\delta^{13}C_{org}$ increment values ($\Delta\, \delta^{13}C_{org}$) for different years within three different periods (1850-1900, 1900-1950 and 1950-2010) using the equation described by Schelske and Hodell (1995) and corrected by Verburg (2007), suitable for periods between 1840 – 2000

$$\Delta\, \delta^{13}C_{org} = (4577.8 - 7.343 * Y) + (3.9213*10^{-3} * Y^2) - (6.9812 * 10^{-7} * Y^3) \qquad (2.2)$$

where Y is the year. The rates obtained (-0.0015 ‰ y^{-1} for the period 1850 - 1900, -0.0073‰ y^{-1} for the period 1900 - 1950 and -0.0259 ‰ y^{-1} for the period 1950 - 2010, Figure SI 2.1) were used for adjusting the $\delta^{13}C_{org}$ signature of the sources used in Eq. 2.1 according to the age assigned at each sediment layer.

As sources $\delta^{13}C_{org}$ may have changed along time due to variability in different environmental factors, including the Suess effect, we also measured sediment C:N ratios to further support trends in the contribution of different sources identified based on the isotopic signature, as marine algae usually have lower C:N values (C:N < 10) compared to seagrass (Meyrs, 1994). C:N ratios were calculated only considering the organic fraction of sediment carbon (C_{org}). N was determined for every slice of one core per site using a CN elemental analyser (Truspec CN determinator, LECO). Estimates of $\delta^{13}C_{org}$ and C:N were obtained down to the maximum sediment depth sampled, but they could only be associated to a specific date down to the oldest ^{210}Pb-derived age for each meadow (Table 2.1).

In those meadows where robust dates could be assigned to specific sediment layers, the burial rates of C_{org} derived from *P. oceanica* and seston were estimated using the calculated contribution of each source (%) at each sediment layer and the total C_{org} stock of the corresponding sediment layer divided by the time (i.e. the total accumulation period) encompassed by the segment. C_{org} stock (g C cm^{-2}) was estimated as the product of C_{org} bulk concentration and the sediment slice thickness (2 cm). To estimate the average change in the burial rates of C_{org} derived from *P. oceanica* and seston, both burial rates along time for each meadow were standardized by the burial rate measured in the year closest to 1930, the oldest date encompassed by the sediments sampled in all the meadows examined (Table 2.1).

Statistical analysis

A paired t-test was applied to assess the difference on the average $\delta^{13}C_{org}$ signature of the sediments and seagrass tissues across the meadows examined and to compare the contributions of *P. oceanica* and seston along all the sediments. Analysis of Variance (ANOVA) was used to assess the differences among meadows in sediment $\delta^{13}C_{org}$, C:N and the relative contribution of *P. oceanica* and seston to the sediment C_{org} stock. The Tukey-Kramer HSD test was used to identify the meadows that differed significantly in the properties examined. Linear regression analysis was used to describe the rate of change in $\delta^{13}C_{org}$ and C:N with sediment depth and Ln transformed burial rates with time.

We propagated the standard error (SE) for the average $\delta^{13}C_{seagrass}$ signature across the meadows examined, the average contribution of each source per meadow and the estimated burial rates over time (Supplementary Information).

Table 2.1. Maximum sediment core depths analysed for $^{13}\delta C_{org}$ and C:N and the deepest layer dated by ^{210}Pb and the corresponding age.

Station	**Max. depth for $^{13}\delta C_{org}$ (cm)**	**Max. depth for C:N (cm)**	**^{210}Pb dating**	
			Max depth dated (cm)	**Corresponding year**
Cala d'Or	29	29	21	1930 ± 1
Es Castell	33	33	17	1921 ± 9
Es Port	25	25	21	1868 ± 9
Es Pujols	25	17		
Magalluf	41	41	17	1871 ± 8
Pollença	37	35	21	1844 ± 6
Portocolom	25	25	25	1924 ± 6
Ses Illetes	21	21		
Sóller	33	33		
Sta. Maria	33	31	17	1852 ± 8
Sta. Ponça	29	29	21	1887 ± 12

2.3.Results

Sediment $\delta^{13}C_{org}$ was either uniform across the sediment profile or significantly increased (linear regression analysis, $p < 0.05$) with sediment depth in the meadows sampled at Es Castell, Es Pujols, Magalluf and Santa Maria with mean (± SE) rates of increase of 0.07 ± 0.03 ‰ cm^{-1}, 0.39 ± 0.09 ‰ cm^{-1}, 0.25 ± 0.04 ‰ cm^{-1} and 0.06 ± 0.02 ‰ cm^{-1}, respectively (Figure 2.2, Table 2.2). The sediment C:N ratios increased significantly (linear regression analysis, $p< 0.05$) with sediment depth in Magalluf, Pollença, Portocolom and Santa Ponça at mean rates of 0.29 ± 0.04 cm^{-1}, 0.12 ± 0.04 cm^{-1}, 0.27 ± 0.09 cm^{-1} and 0.20 ± 0.09 cm^{-1}, respectively (Figure 2.2, Table 2.2).

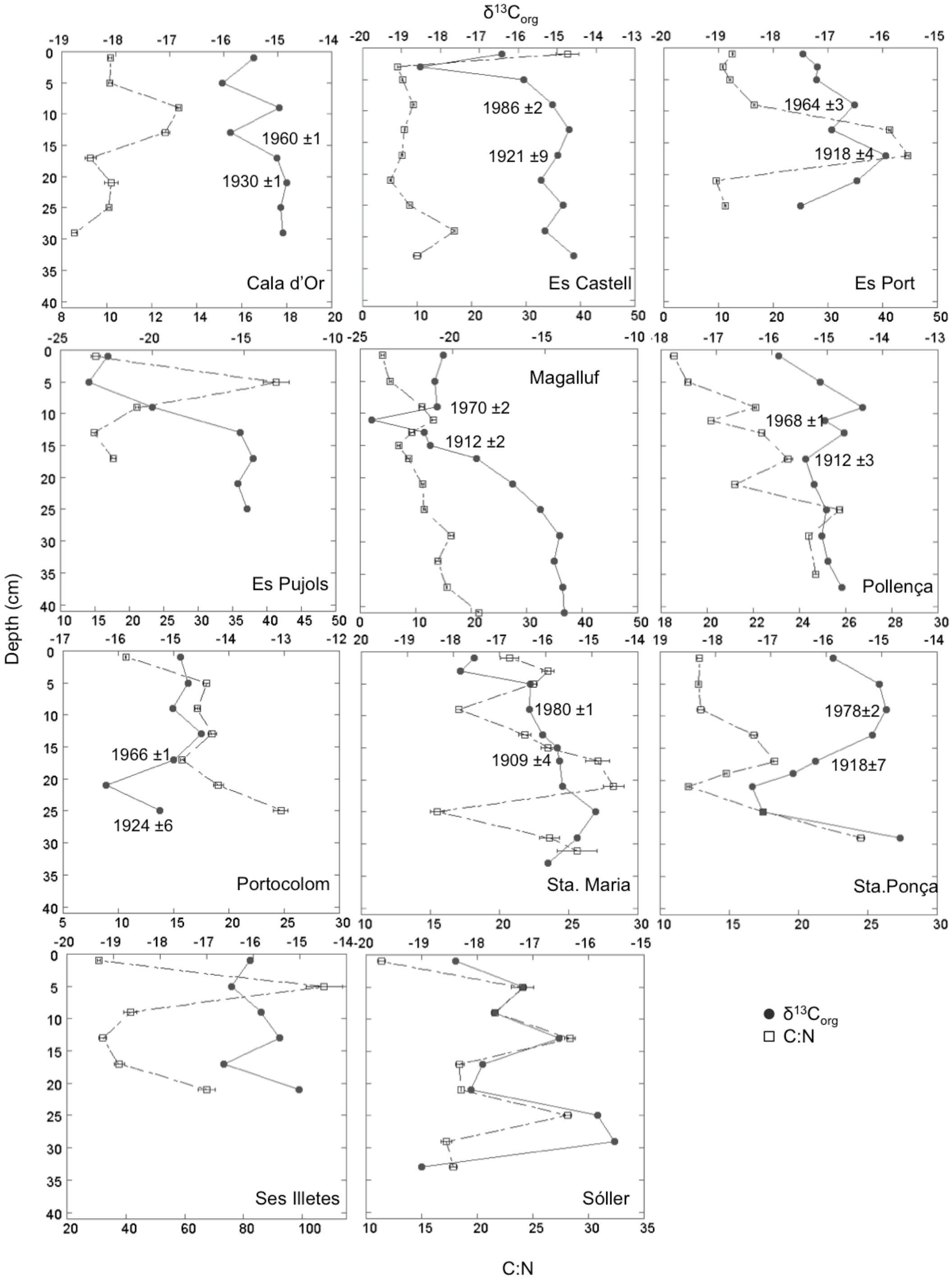

Figure 2.2. $\delta^{13}C_{org}$ and C:N along sediment depth profiles at each station. Dates derived from the ^{210}Pb age models are shown in those meadows where the sediments could be dated.

Table 2.2. Results of the linear regression analysis between $\delta^{13}C_{org}$ signature and C:N ratio with depth along the sediment profile.

Station	$\delta^{13}C_{org}$					C:N				
	Slope	**R^2**	**p-value**	**df**	**F Ratio**	**Slope**	**R^2**	**p-value**	**df**	**F Ratio**
Cala d'Or	0.031 ± 0.015	0.42	>0.05	7	4.42	-0.04 ± 0.07	0.032	>0.05	14	0.44
Es Castell	0.07 ± 0.03	0.41	0.04	9	5.65	-0.15 ± 0.19	0.04	>0.05	16	0.63
Es Pujols	0.39 ± 0.09	0.78	0.008	6	18.1	-0.67 ± 0.55	0.17	>0.05	8	1.48
Magalluf	0.25 ± 0.04	0.76	<0.0001	12	35.3	0.29 ± 0.04	0.73	<0.0001	20	50.32
Es Port	0.02 ± 0.02	0.1	>0.05	7	0.67	0.55 ± 0.66	0.06	>0.05	12	0.68
Pollença	0.01 ± 0.01	0.09	>0.05	10	0.86	0.12 ± 0.04	0.41	0.006	16	10.24
Portocolom	-0.04 ± 0.02	0.32	>0.05	6	2.4	0.27 ± 0.09	0.47	0.0095	12	9.83
Sta. Maria	0.065 ± 0.017	0.62	0.004	10	14.6	0.076 ± 0.099	0.04	>0.05	15	0.57
Sta. Ponça	-0.03 ± 0.04	0.1	>0.05	8	0.8	0.20 ± 0.09	0.26	0.049	14	4.68
Ses Illetes	0.04 ± 0.04	0.2	>0.05	5	0.98	-0.85 ± 1.30	0.05	>0.05	9	0.43
Sóller	0.019 ± 0.04	0.032	>0.05	8	0.23	-0.06 ± 0.11	0.02	>0.05	16	0.28

The average sediment $\delta^{13}C_{org}$ and C:N ratio in the meadows examined varied within the range determined by the average $\delta^{13}C_{org}$ signatures and C:N ratios of the sources considered, *P. oceanica* and seston, except for Ses Illetes, where the sediment C:N ratio exceeded that of *P. oceanica* tissue (Figure 2.3). The average $\delta^{13}C_{org}$ of sediment differed significantly among the meadows examined (ANOVA, $p < 0.001$, $F_{98}= 3.35$), ranging from a maximum of -15.05 ± 0.12 ‰ and -15.08 ± 0.21 ‰ in Pollença and Porto Colom, respectively, to a minimum of -18.21 ± 0.99 ‰ in Magalluf (Figure 2.3). The average $\delta^{13}C_{org}$ of sediment was significantly lower than that for seagrass tissues in all meadows examined (paired t-test, $p<0.0001$, $t = -6.53$, df=10), confirming the contribution of an isotopic lighter carbon source, such as seston (average $\delta^{13}C_{org}$ = -21.65± 0.34 ‰). The C:N ratio also differed significantly across sites (ANOVA, $p<0.0001$, F_{162} =21.28), ranging from a maximum of 53.6 ± 2.8 in Ses Illetes to a minimum of 10.5 ± 0.6 in Cala d'Or (Figure 2.3).

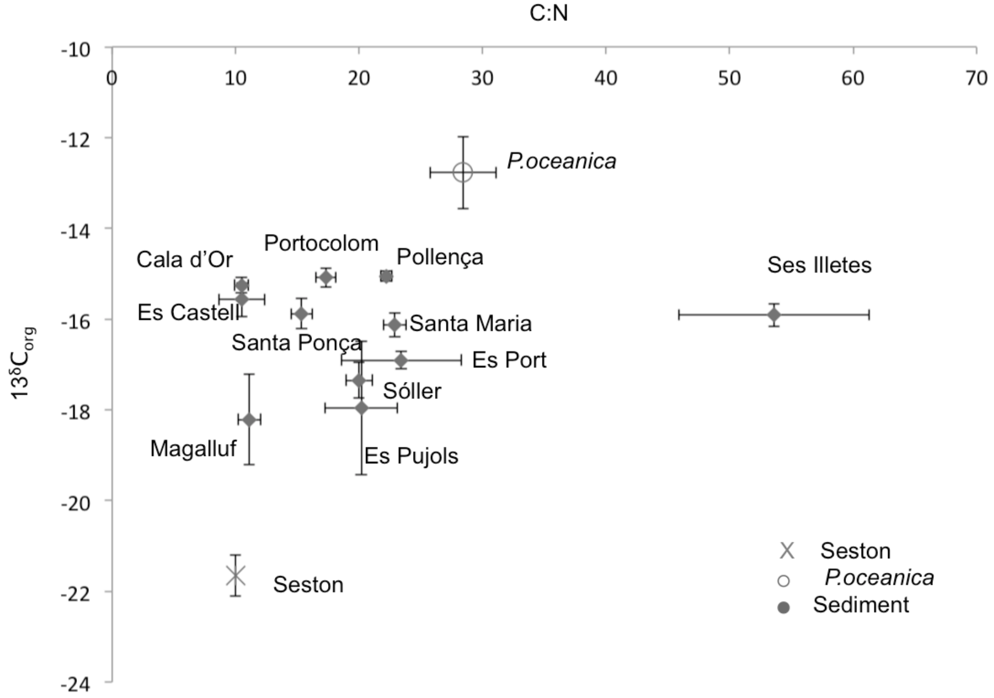

Figure 2.3. Cross plot of C:N elemental ratios and δ^{13} C_{org} of the sources considered in this study (*P. oceania* and seston) and the average values measured along the sediment profile in each meadow. C:N values for *P .oceanica* and seston were taken from Fourqurean et al. (2007) and Meyrs et al. (1994), respectively.

The two-component isotope mixing model revealed that *P. oceanica* had a significantly greater contribution to the total C_{org} stock (59 ± 12 %) than seston (41 ± 8 %) across all sediments examined (paired t-test, $p <0.001$, t =-3.49, df=98). However, the average contributions of each source differed significantly across meadows (ANOVA, $p <0.0001$, F_{98}=9.91; Tuckey- Kramer HSD, α=0.05; Figure 2.4). Ses Illetes and Santa Maria showed the highest contribution of *P. oceanica* (86 ± 13 % and 81 ± 10 %, respectively), while Es Port and Magalluf showed the lowest contribution of *P.*

oceanica (19.6 ± 4.3 % and 33.2 ± 9.4 %, respectively) and the highest contribution of seston (80 ± 11 % and 67 ± 10 %, respectively).

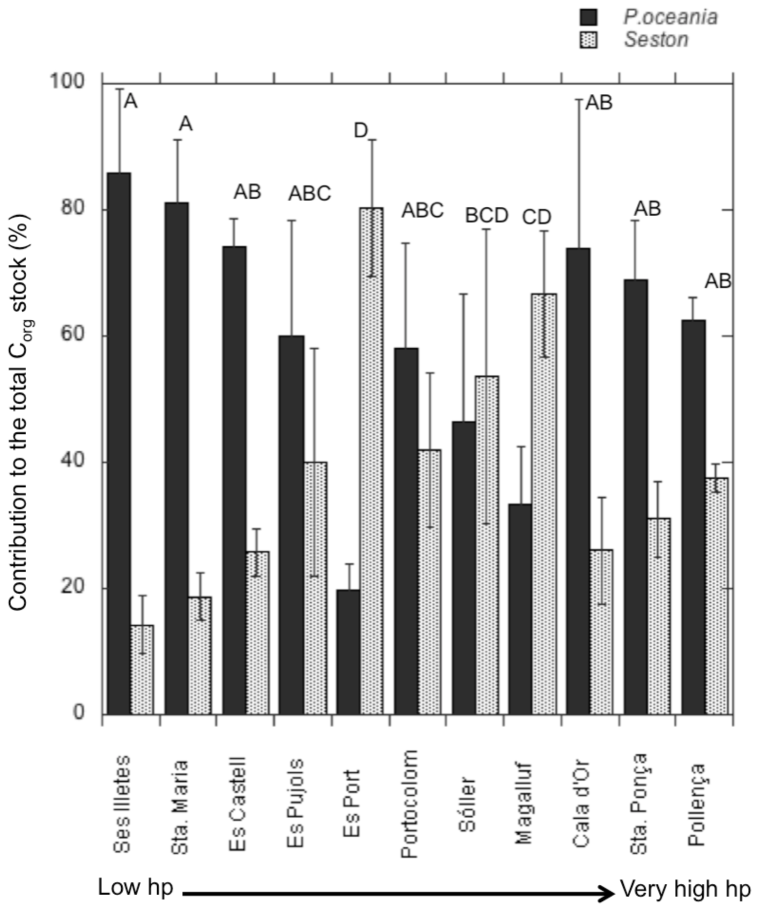

Figure 2.4. Sestonic and *P. oceanica* average (± SE) contribution (%) to the total carbon stock measured in the sediment profiles. X axis is ordered from the meadows under "Low" human pressure (Low hp) towards those under "Very high" human pressure (Very high hp). Stations not connected by same letters are significantly different (Tukey-Kramer HSD, α=0.05).

The contribution of *P. oceanica* and seston to the sediment C_{org} stock changed over time, showing, in general, a decrease in the contribution of *P. oceanica* and an increase in the sestonic carbon towards present (Figure 2.5). For those meadows where seston $\delta^{13}C_{org}$ was not available (Cala d'Or and Porto Colom) and the regional average (± SE) was used, the propagated SEs were very large and precluded the identification of clear trends. The observed trends change when the isotopic mixed model is corrected by considering the Suess effect on the $\delta^{13}C_{org}$ signature of the sources (Figure SI 2.2).

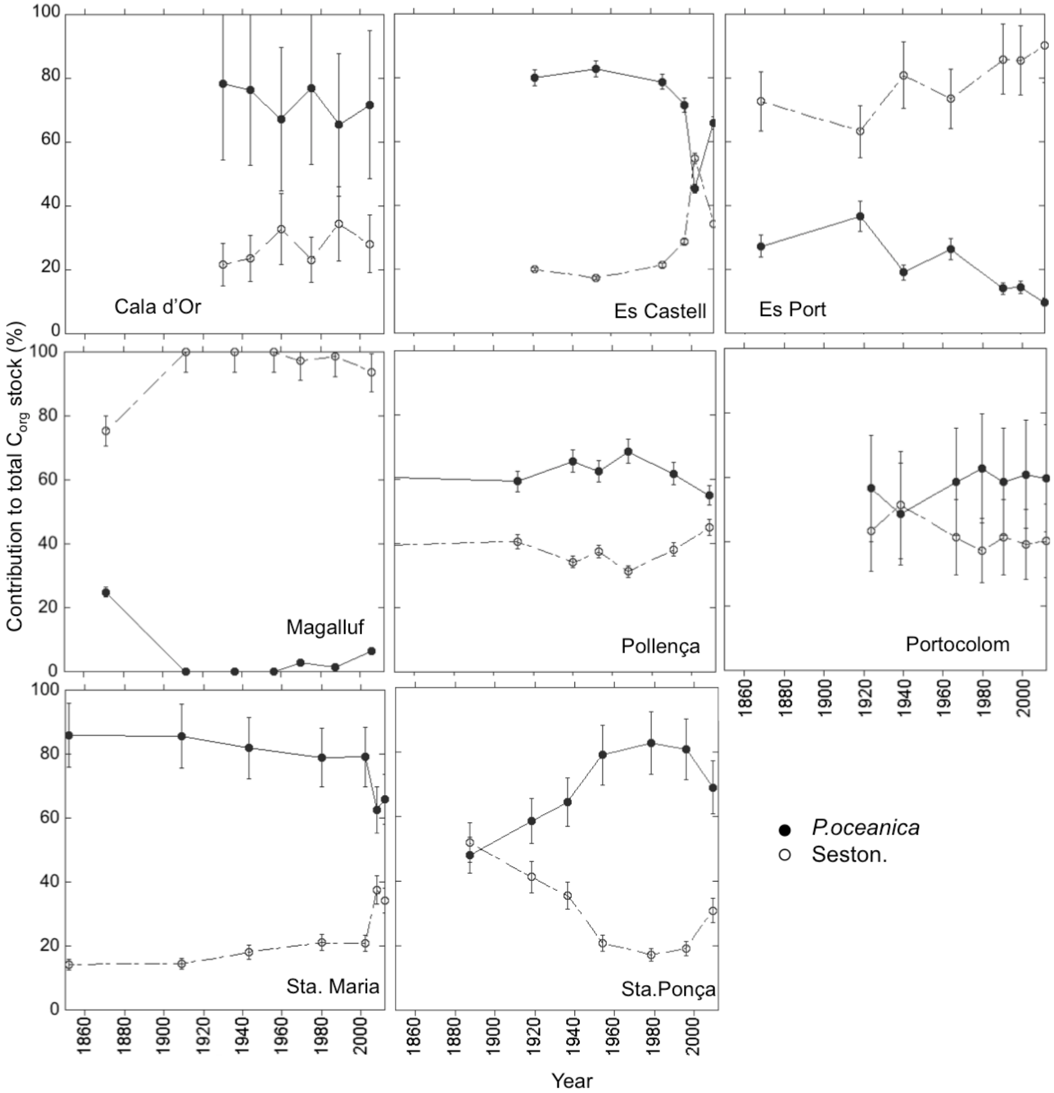

Figure 2.5. Estimated contribution (± SE) of *P. oceanica* and seston to the carbon pool with time at each station for those meadows where the sediment could be dated.

Examination of the average burial rate of each carbon source along time across all the meadows showed that the burial rate of C_{org} derived from seston (2.8 ± 0.6 % y^{-1}; $p<0.0001$, $R^2=0.38$, $F_{38}=22.45$) was higher than that of *P. oceanica* derived C_{org} (2.3 ± 0.6 % y^{-1}; $p<0.0001$, $R^2=0.47$, $F_{33}=29.53$) (Figure 2.6), with similar trends observed in most of the individual meadows examined (Figure SI 2.3).

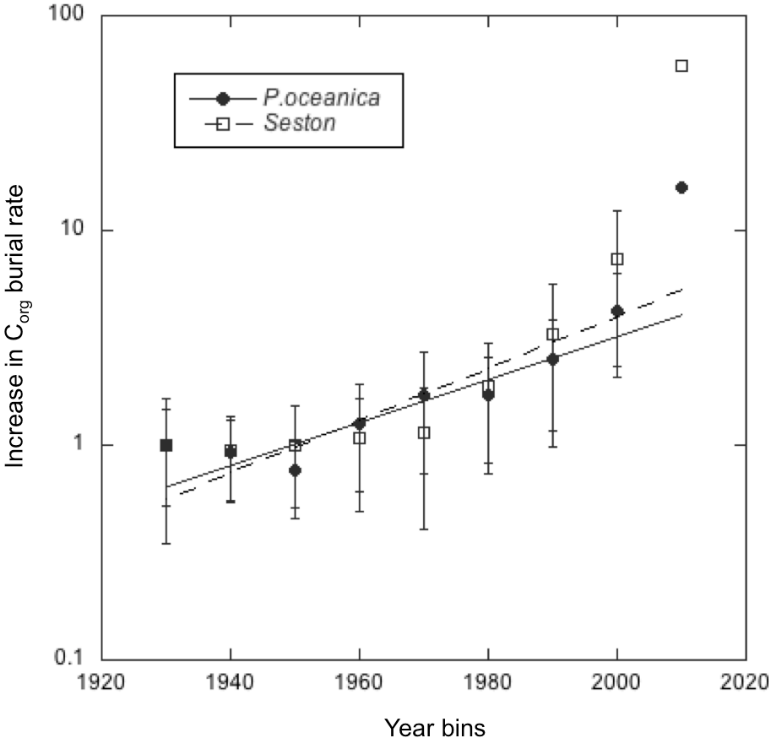

Figure 2.6. Change in burial rates of *P. oceanica* and seston derived-C_{org} since 1930 in the seagrass meadows examined. The values represented are averages (±SE) of C_{org} burial rates along the sediment profile standardized by the C_{org} burial rate estimated for the year ~1930 (the most ancient layer in common for all the meadows examined), grouped by 10 year bins. Solid and dashed lines show the fitted linear regression equations $\ln(y) = -0.44 + 0.023\ (\pm 0.006)*x$ ($R^2=0.47$, $p<0.0001$) and $\ln(y) = -0.57 + 0.028\ (\pm 0.006)*x$ ($R^2=0.37$, $p<0.0001$) obtained by plotting standardized burial rates for *P.oceanica* and seston derived C_{org} respectively, against time (years).

2.4.Discussion

The average sediment $\delta^{13}C_{org}$ and C:N values in the meadows examined fall between the $\delta^{13}C_{org}$ and C:N values characteristic of seston previously measured in the study sites ($\delta^{13}C_{org}$= -21.6 ± 0.4 ‰, Papadimitriou et al. 2005) and reported in the literature (C:N ratio < 10, Meyers 1994) and those of *P. oceanica* carbon measured in the region ($\delta^{13}C_{org}$= -12.8 ± 0.8 ‰; C:N= 28.4, Fourqurean et al. 2007). This is consistent with the assumption that the sediment C_{org} pool in the seagrass sediments examined corresponded to a mixture of carbon derived from *P.oceanica* and seston. Although the sediments from Ses Illetes, in Formentera, showed a higher C:N ratio (54 ± 8), this value is still consistent with maximum C:N ratios reported for *P. oceanica* tissue (47.7, Fourqurean et al. 2007).

The two-component isotope mixing model, considering *P. oceanica* and seston as the two main potential sources of carbon, allowed estimating that 41 ± 8 % of the total C_{org} sediment stock in the meadows studied has a sestonic origin whereas the remaining 59 ± 12 % consists of *P. oceanica* derived carbon. This average contribution of *P. oceanica* to the total carbon stock is comparable to previous global estimates where seagrasses contribute about 50% of the C_{org} in seagrass sediments (Kennedy et al. 2010). However, the contribution of seagrass and seston to the sediment C_{org} varied greatly across meadows.

The highest contribution of *P. oceanica* to the top meter C_{org} stock (about 80%) was found in two of the meadows under "low" human pressure in this study: Ses Illetes, a non-urbanized beach in Formentera Island (Roig-Munar et al. 2013) and Santa Maria, one of the two most pristine sites of the study located in Cabrera National Park. Conversely, the highest contribution of seston (80 ± 3 %) to the sediment C_{org} stock was found in the meadow at Es Port, also in Cabrera National Park, followed by that at Magalluf (67 ± 10 %) under relatively "moderate" and "high" levels of human pressure, respectively. Es Port concentrates most of the human pressure within the Cabrera Archipelago, receiving the sewage produced by visitors in moored ships, usually discharged raw into the water, as well as the treated sewage produced by the visitors on land (Marbà et al. 2002; PNMTAC 2009). In addition, Es Port is a very sheltered bay with a relatively long water residence time (11 days) that enhances the sinking of particles from the water column (Marbà et al. 2002; Holmer et al. 2004), likely leading to the largest contribution of allochthonous carbon to the sediment stock compared to other meadows under higher human pressure. The contribution of seston (26% - 54%) and *P.oceanica* (75% - 45%) to carbon sediment stocks was more similar among the rest of the meadows, all of them supporting a moderate to very high level of human pressure, except for Es Castell (in Cabrera National Park), which is classified under "Low" human pressure. Yet, Es Castell, located at the sheltered entrance of Es Port Bay, is exposed to eutrophied waters from the port of Cabrera, which might explain the slightly larger contribution of seston (26 ± 4 %) to the C_{org} sediment stock relative to that found in Sta. Maria (19 ± 4 %), the other pristine site of the park.

These results suggest that the contribution of different carbon sources to sediment deposits depends on the level of anthropogenic pressure. The highest contribution of seston-derived C_{org} was found in meadows subject to an intense human influence, whereas *P. oceanica* contribution tended to be higher in the pristine and non-urbanized sites. This trend becomes clearer when considering the average C_{org} bulk concentration derived from each source, especially in the case of seston-derived C_{org}, that tended to be low in the most pristine sites (e.g. Es Castell, Sta. Maria and Ses Illetes) and increased in meadows supporting "moderate" to "very high" human pressure (Table SI 2.2). Yet, other factors such as the configuration of coastal areas likely plays a significant role by favoring the accumulation of allochthonous carbon available in the water column in sheltered meadows (Marbà et al. 2002; Van Keulen and Borowitzka 2003).

The results also show a general temporal variability in the contribution of the different carbon sources to the sediment deposits. The $\delta^{13}C_{org}$ signature tended to decrease towards the sediment surface, pointing to a shift in the relative contribution of the sources. Indeed, the results of the two-component isotope mixing model in the dated sediments showed that the contribution of *P. oceanica* decreased over the past 30 to 50 years, whereas the contribution of sestonic carbon tended to increase in most of the meadows examined. The decrease in the C:N ratio towards the surface in four of the meadows examined also suggests an increase in seston, characterized by a lower C:N ratio (C:N <10; Meyers, 1994) compared to seagrass.

However, the results of the mixing model applied assuming constant $\delta^{13}C_{org}$ for the sources, need to be considered with care. The Suess effect tends to attenuate the trends outlined above on the results of the mixing model, although the decreasing rates in the DIC $\delta^{13}C$ signature with time used might be overestimated due to the model applied for their estimation, as explained by Verburg (2007). In addition, other environmental factors and processes could have affected the $\delta^{13}C$ signature of the C_{org} sources over time, hindering the interpretation of these results.

The increase in DIC since Industrial Revolution due to the rise in atmospheric CO_2 (Friedli et al. 1986) is expected to have enhanced the isotopic discrimination in marine plants resulting in a decrease in their $\delta^{13}C_{org}$ signature, especially for those groups known to be CO_2 limited, such as seagrasses (Durako and Sackett 1993; Zimmerman et al. 1997), compared to macroalgae or phytoplankton communities (Falkowski 1994; Beer and Koch 1996). A depletion in the $\delta^{13}C_{org}$ of seagrasses in response to higher DIC concentration has been demonstrated experimentally (Durako and Sackett 1993) and *in situ* observations demonstrated a spatial variability in the $\delta^{13}C_{org}$ of phytoplanktonic communities consistent with different ambient CO_2 concentrations (Rau et al. 1989; Rau 1994). However, both the increase in DIC and the Suess effect would provide a similar signal at a regional scale, resulting in a similar trend of decreasing $\delta^{13}C_{org}$ towards the surface of the sediments. Hence, a dominant role of any or both of these effects is inconsistent with the variable patterns of $\delta^{13}C_{org}$ with sediment depth and age observed among the meadows examined here.

Temperature can also control the carbon isotopic ratio in primary producers by controlling the CO_2 solubility in the water column (Rau et al. 1989; Hemminga and Mateo 1996). Warming of the Mediterranean Sea during the past century (Béthoux 1990; Romano and Lugrezi 2007), particularly intense over the last 3 - 4 decades (Lejeusne et al. 2010), could have decreased the CO_2 solubility, resulting in a higher likelihood of CO_2 limitation and lower discrimination for primary producers, leading to an enrichment in the $\delta^{13}C_{org}$ of the sediment towards present. Moreover, photosynthetic rates are expected to increase with warming (Harris et al. 2006), which would result in enhanced CO_2 limitation and reduced carbon isotopic discrimination. However, the trend expected from warming is opposite to the $\delta^{13}C_{org}$ decreasing trend towards present found in the sediments of some of the meadows examined. In addition, warming, like the Suess effect and the increase in DIC, operates at the regional scale, and thus may contribute to temporal variability in the sediment $\delta^{13}C_{org}$ in the meadows examined but can not explain differences in the trends observed across them.

Light availability also modulates the isotopic discrimination in primary producers by controlling the photosynthetic CO_2 demand (Lepoint et al. 2003; Vizzini and Mazzola 2003; Mateo et al. 2010). Whereas long-term records in the Balearic Islands are not available, water transparency, which depends on the concentration of suspended particles, nutrients supply and subsequent eutrophication process (Short and Burdick 1996), is likely to have decreased during the last century driven by the increase in human pressure and urbanization in coastal areas (Bowen and Valiela 2001; Duarte 2002; Lee et al. 2006). Indeed, the Mediterranean Sea is undergoing a process of eutrophication for at least the last 3 - 4 decades (Mateo et al. 2010), with an intensity likely variable among sites depending on the level of anthropogenic pressure. Eutrophication may lead to a reduction in seagrass photosynthetic rates and to an increase in isotopic discrimination but also to an increase in the load of phytoplankton and seston, expected to lead to an increase in sedimentation rates of these materials in the underlying seagrass sediments.

Finally, even if the contribution of the various sources had been constant over time, a decrease in $\delta^{13}C_{org}$ towards the sediment surface could result from the higher burial efficiency of the seagrass C_{org} compared to that of seston. However, higher remineralization of seston compared to seagrass carbon, which would lead to an increase in $\delta^{13}C_{org}$ with depth, should be accompanied by a decline in the concentration of organic matter with depth (Garten et al. 2007). Such a decline in organic matter along with an increase in $\delta^{13}C_{org}$ with depth was only observed in the meadow at Es Pujols (see Chapter 1) suggesting that a different degree of remineralization of the sources cannot account for the patterns observed in $\delta^{13}C_{org}$ with depth in most of the meadows of this study. In addition, the effect of diagenesis may also be discarded as sediment organic matter, including seagrass tissues, seem to experience, in general, non or little change in the $\delta^{13}C_{org}$ signature due to decomposition (Zieman et al. 1984; Fourqurean and Schrlau 2003; Mateo et al. 2001; Meyers et al. 1994).

Hence, we conclude that the most reasonable explanation for the decrease in $\delta^{13}C_{org}$ of the sediment carbon stock towards present observed in some of the meadows examined is the increase in the input of allochthonous carbon, derived from eutrophication and enhanced particle sedimentation resulting from urbanization and increase in the usage of coastal areas in the study region since the 60's. This hypothesis is supported by the exponential increase in the burial rate of seston-derived C_{org} for the last century in the meadows examined, which is faster than that found for *P. oceanica*-derived C_{org} , especially since the 60's decade (Figure 6). These results are consistent with what has been found in other regions of the world, such as Botany Bay, in Sydney (Australia; Macreadie et al. 2012), Oyster Harbour (SW Australia; Serrano et al. 2016) or Southern Chile (Mayr et al. 2014), suggesting that the shift towards more sestonic enriched carbon deposits in seagrass sediments might be a global phenomenon in areas under a significant recent increase in anthropogenic pressure.

In summary, the contribution of *P. oceanica* and seston to the carbon deposits in the sediments examined varied spatially and temporally due to local differences in anthropogenic pressure and its rise during the last century. Inputs of carbon from both sources have, on average, increased in recent decades. However, the burial of seston derived C_{org} has increased faster than that of seagrass, consistent with a likely general eutrophication associated with the intensification of the anthropogenic pressure in the coasts of the region of study. The general enrichment in sestonic carbon identified towards present might imply the weakening of the carbon deposits (Macreadie et al. 2012), as they become more labile and easier to remineralize, and therefore more vulnerable to disturbances.

Acknowledgments

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Supplementary Information
(Chapter 2)

Figure SI 2.1. Relationships between the time dependent increment of $\delta^{13}C$ (y axis) predicted with the equation by Shelske and Hodell (1995): $\Delta \delta^{13}C = 4577.8 - 7.343 * Y + 3.9213*10^{-3} * Y^2 - 6.9812*10^{-7} * Y^3$, and time (years). The solid line shows the fitted linear regression for each period.

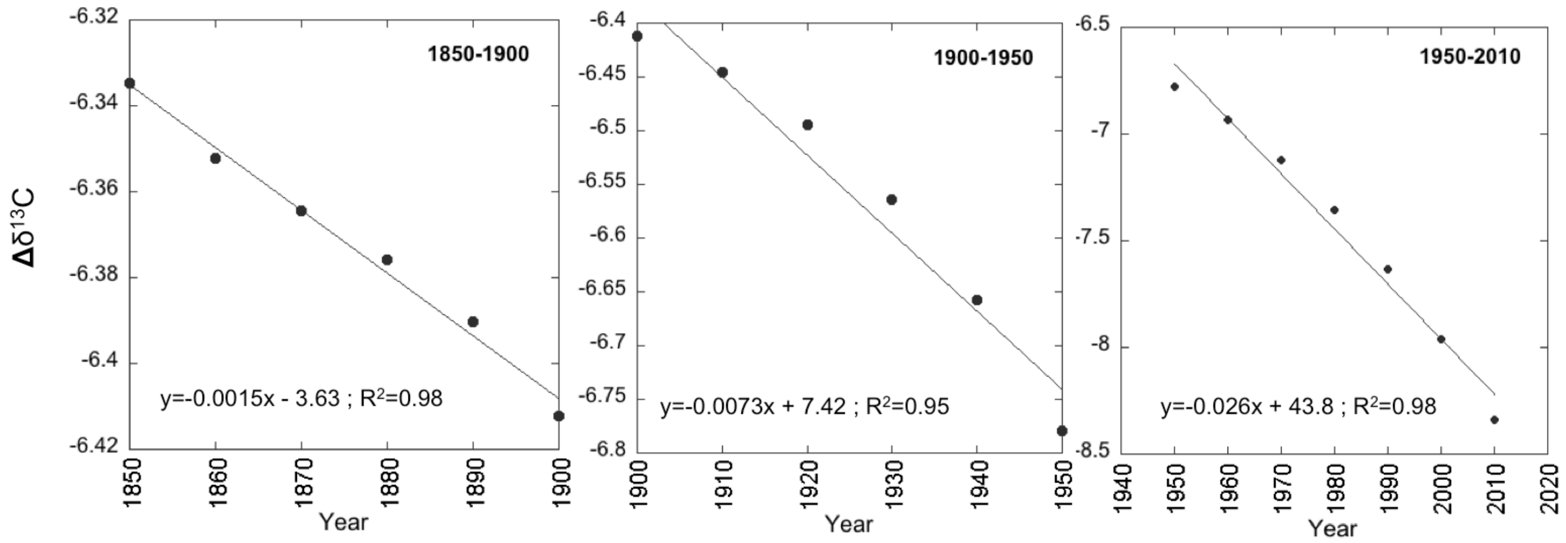

Figure SI 2.2. Estimated contribution of *P.oceanica* to the carbon pool with time in each station applying the model δ $^{13}C_{sediment}$ = δ $^{13}C_{seagrass}$ f + δ $^{13}C_{seston}$ (1 – f) (Phillips and Gregg, 2001) considering a constant value for the sources δ ^{13}C with time and a time dependent Suess effect: -0.0015 ‰ yr^{-1} (1850-1900), -0.0073‰ yr^{-1} (1900-1950) and -0.0259 ‰ yr^{-1} (1950-2010).

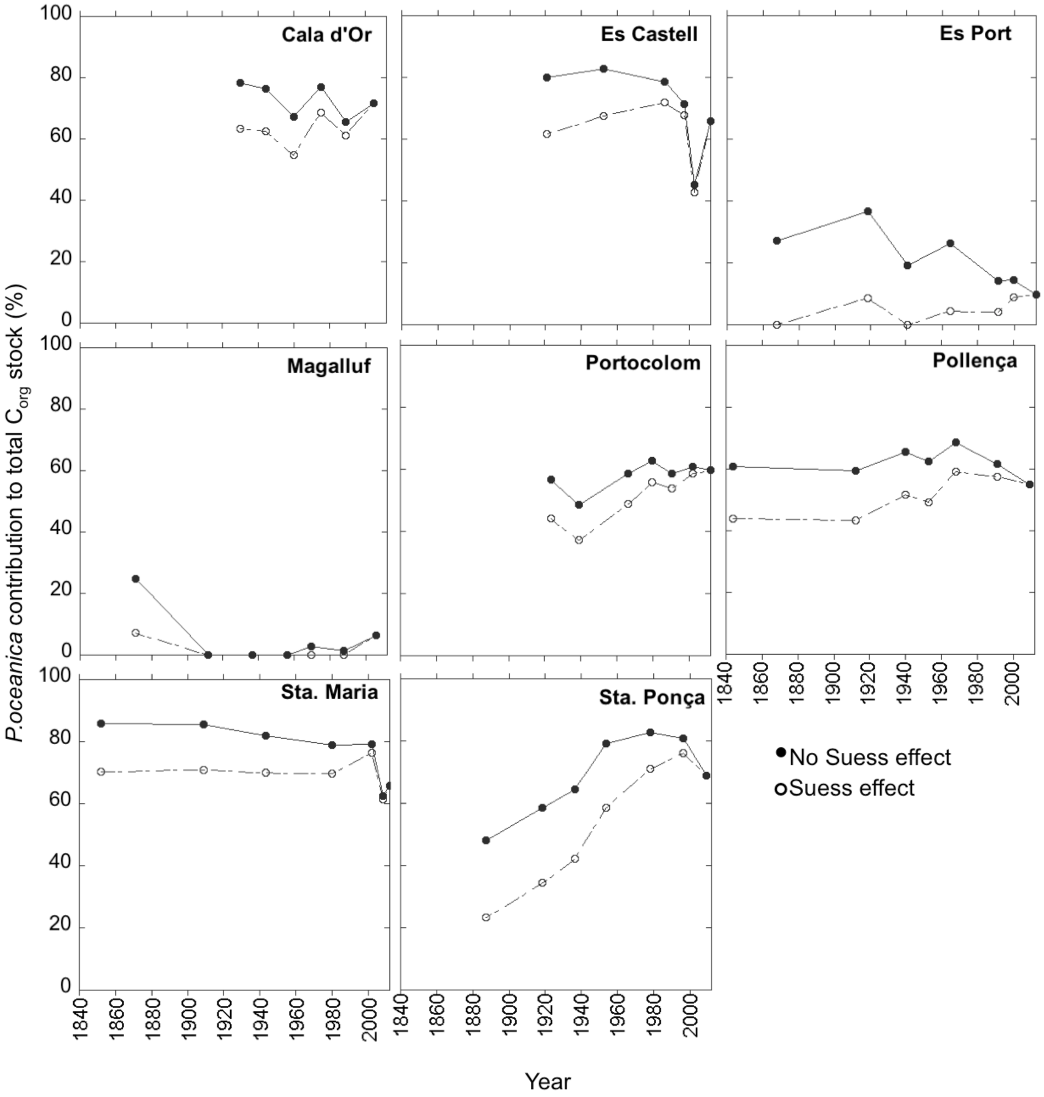

Figure SI 2.3. Change in burial rates of *P. oceanica* and seston derived C_{org} since 1930 in each of the seagrass meadows examined. The values represented are standardized C_{org} burial rates by the burial rate measured in 1930 (or the closest year) in each meadow for *P.oceania* and from seston derived carbon. For the meadow at Magalluf, only sestonic C_{org} burial rate is shown, as no *P.ocean*ica derived C_{org} was detected in the sediments accumulated in 1930 and standardization could not be applied.

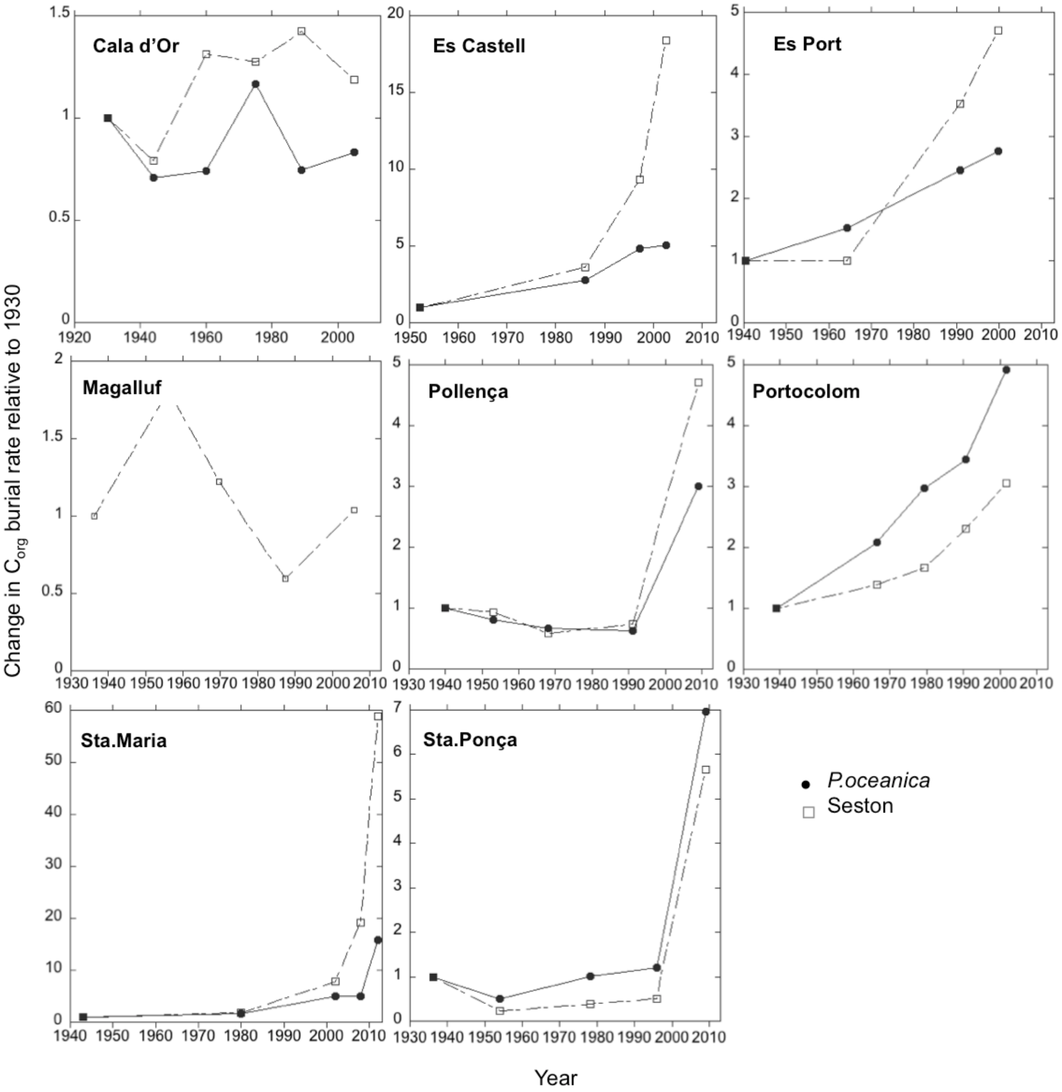

Table SI 2.1. Reference values used for the two-component isotope mixing model. *P.oceanica* $\delta^{13}C_{org}$ reference values correspond to an average of the $\delta^{13}C_{org}$ measured in leaves and rhizomes of plant shoots collected in each site in 2008 and 2009. The seston $\delta^{13}C_{org}$ reference values correspond to a single value obtained by Papadimitriou et al. (2005) at each meadow except for Cala d'Or, Sóller and Porto Colom, where values for these meadows were not available and the value used corresponds to the average ± SE for the region reported in the same study.

Station	***P.oceanica*** **$\delta^{13}C_{org}$ Reference value**	**Seston $\delta^{13}C$ Reference value**
Cala d'Or	-12.83± 0.6	-22.1 ± 1.7
Es Castell	-12.95 ± 0.3	-23.1
Es Port	-12.40 ± 0.7	-18
Es Pujols	-14.85 ± 0.4	-22.4
Magalluf	-11.53 ± 0.6	-21.1
Pollença	-10.94 ± 0.6	-21.90
Portocolom	-10.00 ± 0.7	-22.1 ± 1.7
Ses Illetes	-15.05 ± 0.9	-21.1
Sóller	-11.85± 0.7	-22.1 ± 1.7
Sta. Maria	-14.41± 1	-23.6
Sta. Ponça	-13.71± 0.8	-20.7

Table SI 2.2. Average (± SE) bulk of organic carbon derived from *P.oceanica* and seston in the sediments examined.

Station	**Human pressure**	***P.oceanica* derived C_{org}**	**Seston derived C_{org}**
		mg C cm-3 (avg ± SE)	**mg C cm-3 (avg ± SE)**
Ses Illetes	Low	14.6 ± 4	2.1 ± 0.8
Sta. Maria	Low	8.2 ± 2.2	1.7 ± 0.5
Es Castell	Low	3.9 ± 0.8	1.3 ± 0.3
Es Pujols	Moderate	7.8 ± 2.2	4.1 ± 2.7
Es Port	Moderate	2.9 ± 0.7	12.1 ± 2.4
Portocolom	High	8.8 ± 2.2	6.5 ± 2.1
Sóller	High	4.2 ± 1.7	4.8 ± 1.9
Magalluf	High	4 ± 1.7	4.6 ± 1.5
Cala d'Or	Very high	7.2 ± 4	2.5 ± 0.7
Sta. Ponça	Very high	7.8 ± 2.2	3.4 ± 0.7
Pollença	Very high	17.8 ± 0.8	10.6 ± 1.2

Propagated errors

Propagated SE are reported for average $\delta^{13}C_{seagrass}$ across the meadows examined, the average contribution of each source per meadow and the burial rates along time estimated and was calculated taking in account the C_{org} SE along the depth profile (derive from the use of a minimum of three cores per site), the SE of the sediment age and that of the sources $\delta^{13}C$.

For average estimates Eq. SI. 1.1 was applied

$$SE_{propagated} = \sqrt{\ [\ (SE_{mean})^2 + ((mean/\sqrt{n})^* \sqrt{((x/SE_X)^2 + (y/SE_Y)^2 \ldots)^2}\ ]} \qquad (SI\ 2.1)$$

where SE_{mean} is the standart error of the mean of the estimated variable along the sediment profile, n is the number of sediment slices and X and SE_X and Y SE_Y are the average ± SE of the estimated variable in every sediment slice considered (n).

When multiplication or division were needed in the estimates (e.g. $q = x * y$; $x \pm \delta x$ and $y \pm \delta y$) we applied Eq. SI 1.2

$$\delta q = q^* \sqrt{[[\delta x/x]^2 + [\delta y/y]^2 \ldots]} \qquad (SI\ 2.2)$$

III.2

Inorganic Carbon deposits (PIC): global distribution and environmental controls

Chapter 3

Seagrass meadows as a globally significant carbonate reservoir

Abstract

There has been growing interest in quantifying the capacity of seagrass ecosystems to act as carbon sinks as a natural way of offsetting anthropogenic carbon emissions to the atmosphere. However, most of the efforts have focused on the particulate organic carbon (POC) stocks and accumulation rates and ignored the particulate inorganic carbon (PIC) fraction, despite important carbonate pools associated with calcifying organisms inhabiting the meadows, such as epiphytes and benthic invertebrates, and despite the relevance that carbonate precipitation and dissolution processes have in the global carbon cycle. This study offers the first assessment of the global PIC stocks in seagrass sediments using a synthesis of published and unpublished data on sediment carbonate concentration from 403 vegetated and 34 adjacent un-vegetated sites. PIC stocks in the top 1 m of sediment ranged between 3 and 1660 Mg PIC ha^{-1}, with an average of 654 ± 24 Mg PIC ha^{-1}, exceeding those of POC reported in previous studies by about a factor of 5. Sedimentary carbonate stocks varied across seagrass communities, with meadows dominated by *Halodule*, *Thalassia* or *Cymodocea* supporting the highest PIC stocks, and tended to decrease polewards at a rate of 8 ± 2 Mg PIC ha^{-1} per degree of latitude (general linear model, GLM; $p < 0.0003$). Using PIC concentrations and estimates of sediment accretion in seagrass meadows, the mean PIC accumulation rate in seagrass sediments is found to be 126.3 ± 31.05 g PIC m^{-2} y^{-1}. Based on the global extent of seagrass meadows (177 000 to 600 000 km^{2}), these ecosystems globally store between 11 and 39 Pg of PIC in the top metre of sediment and accumulate between 22 and 75 Tg PIC yr^{-1}, representing a significant contribution to the carbonate dynamics of coastal areas. Despite the fact that these high rates of carbonate accumulation imply CO_2 emissions from precipitation, seagrass meadows are still strong CO_2 sinks as demonstrated by the comparison of carbon (PIC and POC) stocks between vegetated and adjacent un-vegetated sediments.

3.1. Introduction

Calcium carbonate ($CaCO_3$) accounts for about a 25 % of the surface marine sediments (Balch et al. 2005). Contemporary oceanic carbonate sediments are mainly composed of two main mineral forms of calcium carbonate, calcite (including Mg calcite, magnesium-rich calcite) and aragonite, both primarily formed by biogenic precipitation (Smith 2013). The coastal ocean accounts for around 33 % of the global $CaCO_3$ production (Smith 2013), but it is where the highest proportion of carbonate sediment accumulation takes place (nearly two-thirds of its production), whereas in open ocean sediments, only one-third of the $CaCO_3$ produced is accumulated (Milliman and Droxler 1996; Smith 2013). A broad range of communities are involved in the production and subsequent accumulation of $CaCO_3$ in marine sediments, including benthic ecosystems dominated by coral reefs (Chave et al. 1972; Smith 2013), calcareous algae (Milliman 1993) and maerl beds (Bosence and Wilson 2003); and planktonic communities including coccolithophores (Westbroek et al. 1989), foraminifera (Langer et al. 1997), and pteropods (Fabry 1990). More recently the important contribution of echinoderms (Lebrato et al. 2010), mollusks (Chauvaud et al. 2003) and fish (Wilson et al. 2009) to $CaCO_3$ production has been revealed. Relative to other ecosystems, the production of $CaCO_3$ in seagrass meadows ecosystems and its accumulation in the sediments is poorly studied and not explicitly considered in any of the existing assessments of the global ocean carbonate budget (Milliman et al. 1993; Milliman and Droxler, 1996; Lebrato et al. 2010), despite the important load of carbonate often found in their sediments and leaves (Canals and Ballesteros 1997; Gacia et al. 2003; Perry and Beavington-Penney 2005; Serrano et al. 2012; Enríquez and Schubert 2014) and their role as a source of carbonate sand for beach formation and preservation (De Falco et al. 2003; Tigny et al. 2007). Indeed, a global estimate of the carbonate stock in seagrass sediments is not yet available and the potential contribution of these systems to the global ocean carbonate budget remains to be evaluated.

There is considerable interest in quantifying the capacity of the world's ecosystems to trap and store carbon, as this can offset anthropogenic carbon emissions to the atmosphere. To date, most work on the carbon pools in seagrass ecosystems has focused on the amount of particulate organic carbon (POC) stored (Fourqurean et al. 2012; Lavery et al. 2013), whereas, except for *Posidonia oceanica* in the Mediterranean Sea (Serrano et al. 2012), the inorganic component, particulate inorganic carbon (PIC), has not yet been considered in the assessment of carbon deposits in seagrass meadows. Seagrass ecosystems support diverse and active communities of calcifying organisms and through their photosynthetic activity their canopies provide pH environments that facilitate carbonate deposition (Hendriks et al. 2014a). While PIC, in the form of shells and other skeletal remains, represents a substantial carbon stock; the production of PIC through calcification may act as a source of CO_2 to the atmosphere (Frankignoulle et al. 1994; Gattuso et al. 1998; Smith, 2013). Thus, understanding the amount of carbonate in seagrass ecosystems is crucial to determining its role in the global atmospheric carbon cycle. The evaluation of carbonate accumulation rates and stocks in seagrass sediments is also relevant as it may significantly contribute to sediment accretion in coastal areas, a fundamental mechanism supporting the role of seagrass in coastal protection (Duarte et al. 2013b).

Seagrass meadows accumulate PIC through calcium carbonate production by calcifying organisms inhabiting the meadows, such as epiphytes (Frankovich and Zieman 1994; Perry and Beavington-Penny 2005; James et al. 2009; Enríquez and Schubert 2014) and benthic invertebrates (Jeudy de Grissac and Boudouresque 1985) and the deposition of carbonate associated with sedimentation of particles (Gacia et al. 2003). In addition, a recent study demonstrates a direct implication of the seagrass *Thalassia testudinum* in the formation of aragonite needles that accumulate internally in the cell walls and as external deposits on the blades (Enríquez and Schubert, 2014). Other evidence for the existence of active carbonate processes in seagrass beds include calcification and carbonate dissolution in the canopy, associated with the daily cycles of photosynthesis and respiration (Frankovich and Zieman 1994; Barrón et al. 2006; Yates and Halley 2006), and the dissolution of calcium carbonate in the sediment as a result of below-ground release of CO_2 by respiratory processes (Hu and Burdige 2007).

All the processes mentioned (precipitation, dissolution and sedimentation) partially depend on seagrass metabolic activity and plant structural features and thus $CaCO_3$ stocks in seagrass sediments are likely to vary across meadows of different species (Duarte 1991b). In addition, $CaCO_3$ stocks in seagrass meadows will likely vary with latitude, as temperature regulates the seawater saturation state for carbonate minerals that increases with increasing temperature (Zeebe and Ridgwell 2011), thereby favoring biogenic carbonate precipitation in warmer waters (Mutti and Hallock 2003).

Here we provide the first global assessment of the PIC deposits in seagrass ecosystems. We do so through a synthesis of published and unpublished data on carbonate stocks in seagrass sediments. We examine the variability of PIC stocks with biogeographic region, latitude and taxonomic composition of the seagrass community. We also compare the PIC and POC stocks in seagrass ecosystems with those in adjacent un-vegetated sediments, provide a first global assessment of the PIC : POC ratio over sediment depth profiles and discuss its implications for current estimates of CO_2 sequestration in seagrass ecosystems.

3.2. Methods

We compiled published data available on carbonate stocks in seagrass meadows and adjacent un-vegetated sediments. We considered the total pool of $CaCO_3$ reported without distinguishing between the different possible biogenic carbonate mineral forms (calcite, Mg calcite and aragonite). Fourqurean et al. (2012) provided data for 201 sites, and a literature search using both the Web of Knowledge (using the search terms "seagrass*" AND "inorganic carbon*" AND ["calcific* OR sediment* OR $CaCO_3$ OR dissolute* OR dia- genesis"]) and Google Scholar (using the search terms "seagrass carbonate") yielded data for an additional 82 sites. We amended the database with unpublished values for 154 additional sites sampled by the authors. This yielded a total of 437 sites with data on sediment carbonate concentration in coastal areas occupied by seagrasses, of which 34 corresponded to sand patches adjacent to seagrass meadows (Mazarrasa et al. 2015a). The final database comprised estimates for 403 seagrass vegetated sites, of which 219 consisted of values for sediment surface samples (ca. 1–30 cm depth) and 184 consisted of values for sediment cores of variable length (149 cores < 100 cm long, and 35 cores 100 cm long).

The greatest proportion of the sites (46 %) was located in tropical and subtropical regions (20–40 degrees latitude) for both the Southern and Northern hemispheres, whereas the data from higher latitude regions were scarce (Figure 3.1). Data on surface sediment carbonate was broadly distributed, but most (80 %) core data available were from subtropical and temperate seagrass meadows (Figure 3.1).

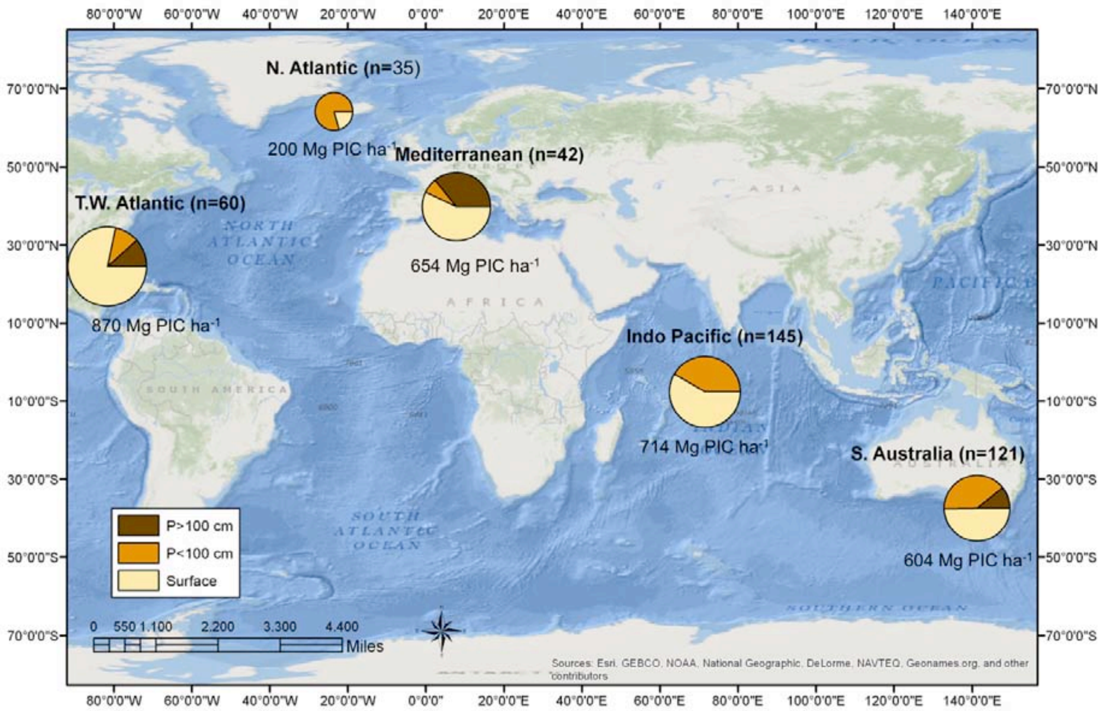

Figure 3.1. Distribution of the data of PIC stocks in seagrass meadows (average top meter; Mg PIC ha^{-1}) compiled in this study by the biogeographic regions described by Hemminga and Duarte (2000). The size of the pie charts is proportional to the top meter of PIC stocks in each region. The fraction of PIC stocks estimated from surface sediments (yellow) and short sediment cores (P < 100 cm, orange) and longer cores than 100 cm (P > 100 cm, brown) is indicated.

Lithogenic characteristics of the sites were not considered in this study, as we assume that carbonate sediment stocks have a biogenic origin. We cannot avoid mentioning that this could lead to an overestimation of carbonate deposition rates in areas where lithogenic carbonate might be important. However, as the biogenic carbonate pool is considered to be dominant in contemporary oceanic sediments (Smith 2013), local geological characteristics might not have a highly relevant impact on the results of this study.

When only one of the variables, $CaCO_3$ or PIC, was reported, the other was estimated assuming that PIC is 12 % of the total molar mass of $CaCO_3$. In most cases, PIC was reported as a percentage of dry weight (% DW). To estimate the PIC concentration (mg PIC cm^{-3}), we multiplied the PIC (% DW) by the sediment dry bulk density (DBD; g cm^{-3}). When DBD was not reported (n = 113 sites), we used the average DBD (1.03 g cm^{-3}) reported by Fourqurean et al. (2012) for seagrass sediments in the calculations. The error introduced by this assumption was small, as a paired *t* test revealed an average deviation of 3.3 % (*T* ratio = 4.32; $p < 0.0001$) when we tested the differences between estimating PIC concentration using the observed DBD and the assumption of 1.03 for the sites where an observed DBD was reported.

Due to the variability in length of the sediment cores available for the study, mean PIC concentration in seagrass sediments was estimated for the top 10 cm of sediment for a total of 385 sampled sites, for which at least one measurement of PIC was reported for this depth zone. To estimate the carbonate stock within the top meter of sediment for the total database available we assumed a constant concentration of PIC in the top meter for those cores where shallower profiles were reported, as almost half (46 %) of the long cores (length > 100 cm, n = 35) showed no significant change in PIC concentration with depth within the first top meter and the remaining long cores showed only a slight increase of 0.011 % DW cm^{-1}, on average.

The sites were classified based on (1) the seagrass biogeographic regions described by Hemminga and Duarte (2000) (North East Pacific, South East Pacific, Tropical Western Atlantic, North Atlantic, South Atlantic, Mediterranean, Indo- Pacific, Western Pacific and Southern Australia), (2) 10º latitude bins and (3) the genus of the dominant seagrass species (*Amphibolis*, *Halophila*, *Halodule*, *Enhalus*, *Thalassia*, *Zostera*, *Posidonia*, *Syringodium*, *Thalassodendron* and *Cymodocea*).

PIC and POC concentrations were compared along the sediment depth profiles when both variables were reported in the same site (n = 392). The depth profile of POC, PIC and POC : PIC within the top meter was explored for the longest cores (length > 100 cm) when at least three different data points were reported within the top meter (n = 26). For those sites from which data for sediments from adjacent vegetated and un-vegetated patches were reported (n = 34), POC and PIC concentrations were also compared.

Statistical analysis

We used a paired sample *t* test to assess the difference between the frequency distribution and average of observed values and estimated values of top meter stocks and the difference between PIC and POC across the data set and between adjacent vegetated and un-vegetated patches. Analysis of variance (ANOVA) and post-hoc Tukey-Kramer HSD tests were applied to compare the PIC stocks among the biogeographic regions and among the dominant genera. We used general linear models (GLMs) to test the effect of latitude on the PIC stocks, the depth variability in the POC and PIC concentrations and their POC : PIC ratio and the variability in POC and PIC concentrations in vegetated and un-vegetated patches. All statistical analysis were conducted using the statistical software JMP 5.01a.

3.3. Results

Particulate inorganic carbon concentrations within the top 10 cm of seagrass sediments ranged between 0.3 and 174 mg PIC cm^{-3}, with an average of 62.5 ± 1.7 mg PIC cm^{-3} and a median of 54 mg PIC cm^{-3} (n=385). The PIC stock in the top meter of sediment in seagrass meadows showed a wide variability, ranging between 3 and 1660 Mg PIC ha^{-1}, with an average ± standard error and a median of 654 ± 24 and 643 Mg PIC ha^{-1} , respectively (n = 403; Figure 3.2). Estimated stocks (mean ± SE, 676 ± 26 Mg PIC ha^{-1}, Table SI 3.1) were significantly higher than those derived from direct measurements (mean ± SE, 423 ± 52MgPIC ha^{-1}, Table SI 3.1, $p > 0.05$); however, estimated and measured paired values did not show a significant difference (Figure 3.2; paired *t* test, $p > 0.05$).

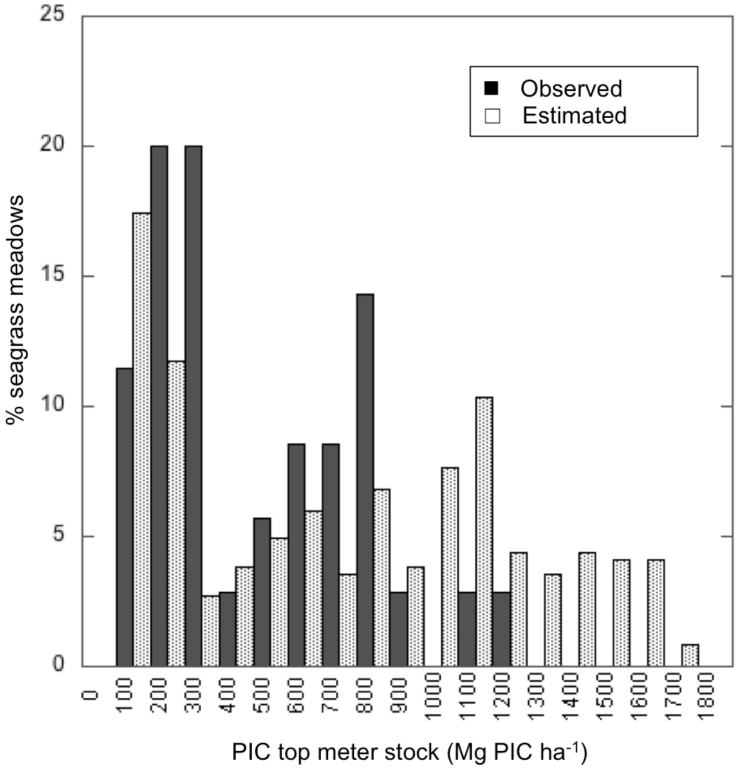

Figure 3.2. Frequency distribution of observed (i.e. sites reporting data to at least 1 m, n = 35) and estimated (i.e. sites where shallower depths were reported, n = 368) PIC stocks (Mg PIC ha^{-1}) in the top meter of seagrass sediments.

The PIC stocks differed significantly among seagrass biogeographic regions (ANOVA, F-ratio = 12.64, $p < 0.0001$). The largest stocks were found in the Tropical Western Atlantic similar to those from the Indo-Pacific and the Mediterranean regions. The North Atlantic PIC stocks were significantly lower (Table 3.1).

Table 3.1. Number of observations, mean ± standard error, median and range of values for the PIC stocks in each biogeographic region (Tropical Western Atlantic, Indo-Pacific, Mediterranean, Southern Australia and Northern Atlantic). The results of the comparison among different regions (Tukey–Kramer HSD test) are shown in the last column where different letters represent a significant difference ($p < 0.05$).

Biogeographic region	**n**	**Mean (Mg PIC ha^{-1})**	**SE (Mg PIC ha^{-1})**	**Median (Mg PIC ha^{-1})**	**Range (Mg PIC ha^{-1})**	**Tukey-Kramer HSD test**
T.W. Atlantic	60	869.5	54.6	891.4	16 - 1660	A
Indo-Pacific	145	713.9	47	795.2	3 - 1611	AB
Mediterranean	42	654.4	71.3	658.2	87 - 1542	AB
S. Australia	121	603.9	34.2	566.5	8 - 1475	B
N. Atlantic	35	204.9	35.4	68.2	8 - 555	C

The largest PIC stocks were found in equatorial and subtropical regions and tended to decrease polewards by 8 ± 2 Mg PIC ha^{-1} per degree of latitude (Figure 3.3; GLM, ChiSquare = 13.43, $p < 0.0002$). The low PIC values found between 10 and 20 in the Southern Hemisphere are derived from Queensland (Australia), and the low values between 50–60 and 60–70 (Northern Hemisphere) correspond to meadows in northern Denmark and south-west Greenland, respectively (Figure 3.3).

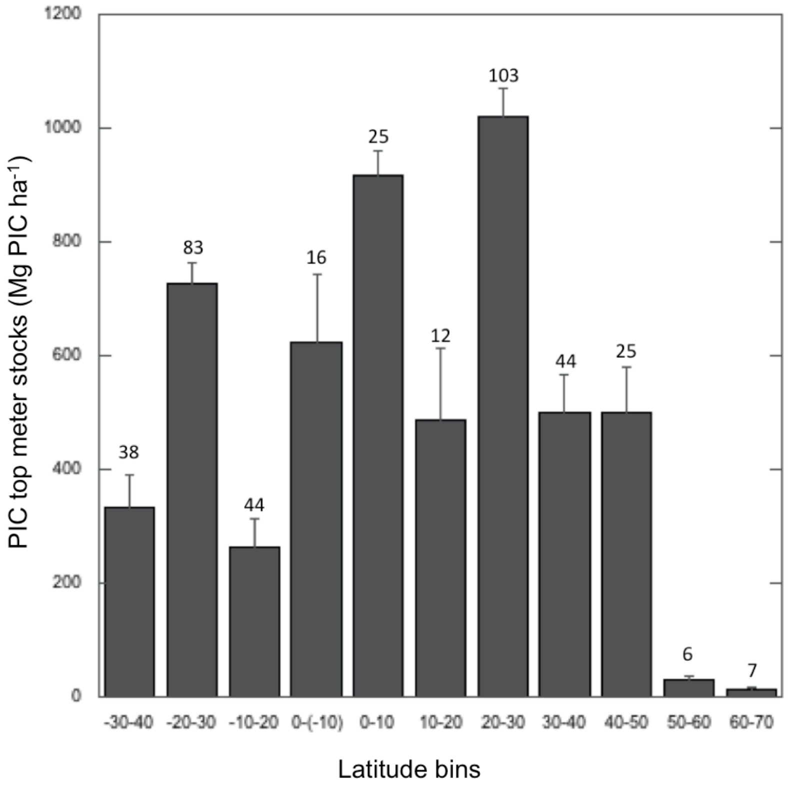

Figure 3.3. Average PIC stocks (Mg PIC ha^{-1}) ± SE by 10 latitude bins. The number above each bar indicates the number of observations reported for each latitude bin.

The PIC stocks also differed among dominant species (ANOVA, F ratio = 13.98; $p < 0.0001$). The highest PIC stocks were found underlying *Halodule*, *Thalassia* and *Cy- modocea* meadows, while the lowest stocks were supported by *Zostera* and *Halophila* meadows (Figure 3.4). *Posidonia* meadows had intermediate PIC stocks.

Where both PIC and POC were measured concurrently (392 sites; n = 3076), mean PIC concentrations tended to exceed mean POC concentrations (paired *t* test: *T* ratio = 64.77, $p < 0.0001$). The POC : PIC ratio ranged from nearly 0 to 108, with an average of 0.74 ± 0.05 and a median of 0.20 (Table 3.2; Figure 3.5). For the longest cores in the database (length 100 cm) which had a minimum of three different observations reported over 1 m depth (n = 26), the POC concentration (mg POC cm^{-3}) along the sediment profile of these cores tended to decrease with depth whereas PIC (mg PIC cm^{-3}) was more variable (Figure SI 3.1). The POC : PIC ratio declined consistently with depth in the top meter of sediment in 69 % of these cores at an average of 0.00054 % cm^{-1}.

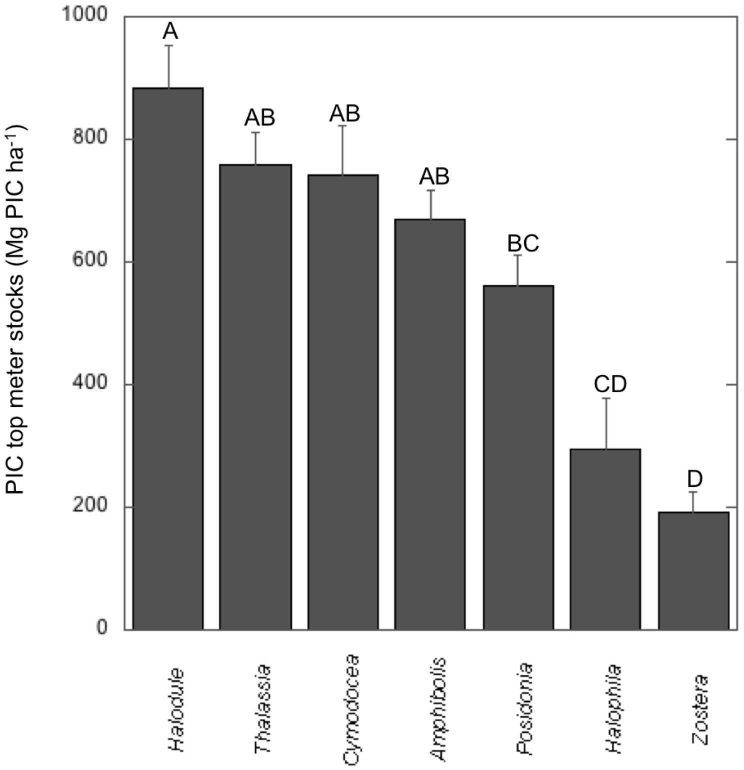

Figure 3.4. Average PIC stocks (Mg PIC ha^{-1}) ± SE across the dominant seagrass genera forming the meadows. Only genera with more than 10 observations are shown. Identical letters indicate no significant differences between dominant species forming the meadows (ANOVA and post-hoc Tukey-Kramer HSD test).

Table 3.2. Mean ± standard error (SE), median, minimum and maximum values of particulate inorganic carbon (PIC), particulate organic carbon (POC) and the estimated POC : PIC ratio for the data set where both POC and PIC were reported (392 sites; n = 3076).

	PIC (mg cm^{-3})	**POC (mg cm^{-3})**	**POC: PIC**
Mean ± SE	72.5 ± 0.8	51.6 ± 0.6	0.74 ± 0.05
Median	68.3	49.4	0.20
Max	325.1	321.0	107.6
Min	0.2	0.4	0.00038

There was a strong relationship between PIC content (% DW) in paired vegetated and un-vegetated sediments ($R^2 = 0.92$, Figure 3.6a), with a slope very close to 1 (0.99 ± 0.02) and an intercept not different from 0 (0.17 ± 0.99), indicating that the PIC content in seagrass sediments did not differ significantly from that in adjacent un-vegetated sediments (paired *t* test, *T* ratio = 1.67, $p > 0.05$; n = 195) (Figure 3.6a). However, no relationship was found between the POC content (% DW) in seagrass sediments and adjacent bare sediments (Figure 3.6b). POC content was significantly higher in vegetated sediments (mean ± SE, 0.66 ± 0.04) compared to adjacent bare sediments (mean ± SE, 0.35 ± 0.017, paired *t* test, *T* ratio = -6.57, $p < 0.0001$; n = 195).

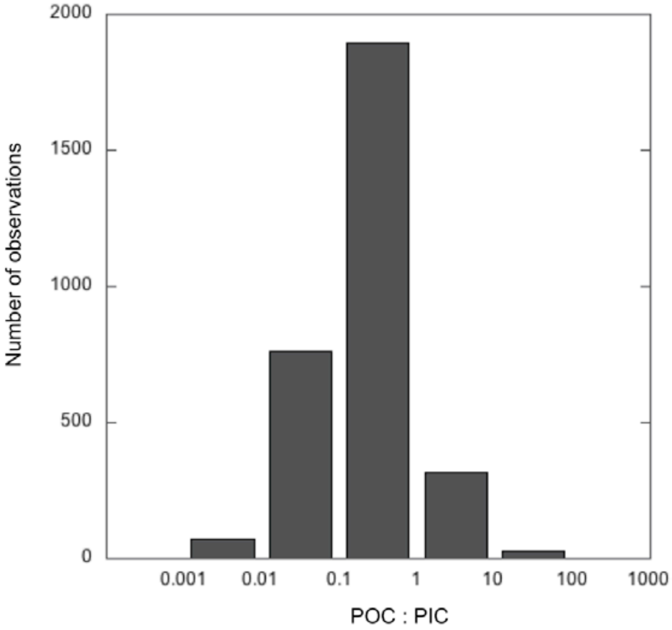

Figure 3.5.
Frequency distribution of the POC: PIC ratio in the seagrass sediments examined
(392 sites; n = 3076).

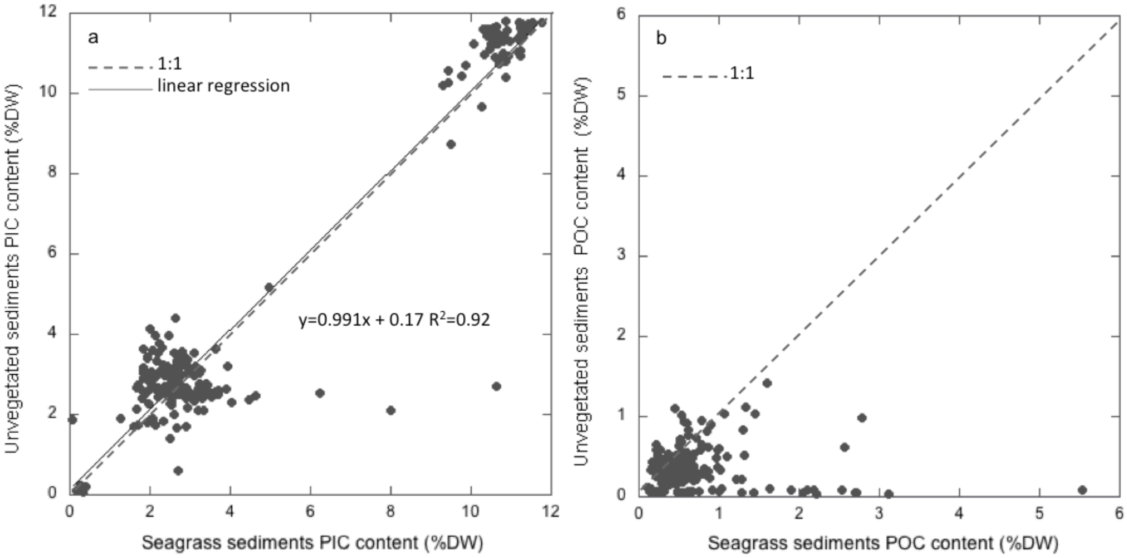

Figure 3.6. Relationship between (a) PIC content (% DW) in seagrass sediments (x axis) and adjacent un-vegetated sediments (y axis) and (b) POC content (% DW) in seagrass sediments (x axis) and adjacent un-vegetated sediments (y axis). The dashed line shows the 1:1 relationship whereas the continuous line in (a) represents the linear regression model between PIC content (% DW) in vegetated patches vs. adjacent un-vegetated patches.

3.4. Discussion

PIC global stocks and the effect of species and latitudinal distribution

Available data on PIC stocks in seagrass meadows showed an important geographic bias. Whereas seagrass meadows are distributed along the coast of all continents except Antarctica (Hemminga and Duarte 2000), data on PIC stocks in seagrass sediments are mostly restricted to tropical and temperate regions, with a particularly important contribution to the data set by meadows in Australia and the Mediterranean, especially for the profiles of at least 1 m deep. Fourqurean et al. (2012) also found a similar bias on the distribution of data available for their review of particulate organic carbon (POC) stocks in seagrass meadows, although the data were more widely distributed. The geographic bias in data availability and the great variability in PIC stocks among the sites included in this study, add uncertainty in the assessment of the global estimates provided here. Even scarcer are data from un-vegetated sediments adjacent to seagrass meadows, with a comparative approach possible in only 34 of the total of 437 sites, limiting the certainty of comparisons of PIC and POC stocks in vegetated versus un-vegetated habitats.

The median PIC sediment top meter stocks of 643 Mg PIC ha^{-1} (n = 403) is nearly 5 times larger than the median stock of POC recently estimated by Fourqurean et al. (2012) at around 140 Mg POC ha^{-1} (n = 89). Based on the available range of estimates of global seagrass area, between 177 000 and 600 000 km^2 (Mcleod et al. 2011), seagrass meadows store globally between 11 and 39 Pg of PIC in the top meter of sediment.

Our results show that the PIC stocks of seagrass meadows vary depending on the seagrass genera. Large genera, with larger leaf size and extended leaf life span (Duarte 1991) were expected to sustain a higher amount of calcareous epiphytes and favor a higher accumulation of PIC. The age of the leaves affects the colonization of seagrass leaves by epiphytes (including calcareous organisms; Heijs 1985; Borowitzka et al. 1990; Cebrián et al. 1994), and the mineral load has been found to increase with increasing leaf age (Gacia et al. 2003). The height of the canopy, which correlates with shoot size, has also been shown to determine the epiphyte biomass and species biodiversity in meadows of *Amphibolis* (Borowitzka et al. 1990). Sedimentation process and particle trapping in a meadow are also linked to canopy height (Gacia et al. 2003) and leaf density (Fonseca and Cahalan 1992), and therefore PIC sedimentation and retention may be also favored in seagrass meadows dominated by larger species, where long leaves effectively slow water currents and increase particle settling. In addition, larger seagrass species may favor carbonate precipitation through their metabolic activity as the leaf area index has been seen to be directly related to maximum and range of carbonate saturation state (Ω) values in seagrass meadows (Hendriks et al. 2014a). Hence, we expected to find high storage of PIC in the sediment of large seagrass genera. However, some large genera, such as *Posidonia*, did not support particularly large stocks, while some small genera, such as *Halodule*, supported large stocks. The lack of a clear effect of the seagrass genera size could be due to other controlling factors on the precipitation and preservation of carbonate in the sediment at regional and local scales not covered by the current study. These may involve differences in geomorphology, salinity, water depth, tidal and current regimes, nutrient and light availability and CO_2 balance (Lees 1975) as well as the presence of nearby ecosystems, such as corals in tropical regions, which may act as sources of carbonates to seagrass sediments.

Latitude also influenced the size of the PIC stocks in seagrass sediments, which tended to decrease with increasing latitude, consistent with the higher epiphyte carbonate loads in seagrass leaves in tropical compared to temperate regions (Gacia et al. 2003). This general trend of decline with increasing latitude has been observed in other carbonate- intense ecosystems, such as reef-building corals (Veron and Minchin 1992; Veron 1995) and encrusting red algae communities, which are more heavily calcified in warm tropical than in cold temperate waters (Lowenstam and Weiner 1989). The latitudinal distribution of carbonate stocks may be explained by temperature and salinity dependence of the saturation state of carbonate minerals (Ω) (Zeebe and Wolf-Gladrow 2001). The saturation of calcium carbonate in seawater is mostly dependent on the availability of $CO^{2-}{}_3$, as Ca^{2+} concentration is 2 orders of magnitude higher than $CO^{2-}{}_3$ concentrations (Gattuso et al. 1998). From a thermodynamic perspective, cold and fresh water generally promotes lower Ω saturation states and prevents $CaCO_3$ precipitation (Mucci 1983). As both salinity and temperature tend to decrease with increasing latitude, the carbonate saturation state decreases polewards with respect to tropical and temperate waters (Hoegh-Guldberg et al. 2007). Hence, the precipitation of biogenic $CaCO_3$ is favored in tropical and subtropical areas compared to temperate regions (Mutti and Hallock 2003). Discrepancies from the general trend, such as the low carbonate stocks reported in the latitudinal bins 10º S to 20º S are probably explained by local factors that alter the Ω saturation states, such as inputs of fresh water and terrigeneous sediments from river discharges in the sites of study (Mellors et al. 2002; Fisher and Sheaves 2003).

PIC estimated accumulation rates in seagrass meadows

Our review of the literature indicated that PIC accumulation in seagrass sediments is high and comparable to other carbonate producing ecosystems. Based on our identified mean PIC concentration of 62.5 ± 1.7 mg PIC cm^{-3} in the top 10 cm of seagrass sediments (sites = 385, n = 802) and a mean rate of sediment accretion in seagrass meadows of 0.2 ± 0.04 cm yr^{-1} (Duarte et al. 2013b), we estimate that the PIC accumulation rates in seagrass sediments would average 126.3 ± 31.05 g PIC m^{-2} yr^{-1}. This rate is somewhat below the range of PIC sedimentation rates reported by Gacia et al. (2003) in seagrass meadows of SE Asia, based on direct measures of daily sediment deposition at eight different sites (145–9443 g PIC m^{-2} yr^{-1}) but higher than the average PIC accumulation rate in sediments of *Posidonia oceanica* meadows (54.3 ± 1.9 g PIC m^{-2} y^{-1}) estimated from sediment stock assessment and sediment dating (Serrano et al. 2012). Extrapolation, assuming an estimated range of global area of seagrass meadows between 177 000 and 600 000 km^2 (Mcleod et al. 2011), suggests a total accumulation of PIC in seagrass sediments ranging between 22 ± 5 and 76 ± 19 Tg PIC yr^{-1}. These estimates are subject to uncertainties derived from the high variability in PIC stocks among regions and species, and the absence of estimates on seagrass extent for each region/system considered in this study. Assuming that tropical seagrass represent two-thirds of the total seagrass, PIC accumulation rates can be calculated separately for tropical (17.6 ± 4.5 and 59.7 ± 15.2 Tg PIC yr^{-1}) and temperate meadows (4.5 ± 1.5 and 15.3 ± 4.9 Tg PIC yr^{-1}, for the low and high global seagrass area estimates, respectively), yielding a range for global PIC sequestration in seagrass meadows from 22 ± 6 to 75 ± 20 Tg PIC yr^{-1}, depending on the global seagrass extent considered.

The rates of PIC accumulation estimated in this study, both globally (22–75 Tg PIC yr^{-1}) and per surface area (126.3 ± 31.05 g PIC m^{-2} yr^{-1}), highlight the importance of seagrass meadows as major sites for $CaCO_3$ accumulation and storage in the ocean. The global PIC accumulation rates of seagrasses are substantially lower than in deep oceans by pelagic communities (100–132 Tg PIC yr^{-1}) but significantly higher when considering their contribution per surface area (0.34–0.45 g PIC m^{-2} yr^{-1}). Seagrass PIC accumulation rates were comparable to those of coral reefs both globally (84 Tg PIC yr^{-1}) and per surface area (140 g PIC m^{-2} yr^{-1}). Relative to *Halimeda* bioherms (20 Tg PIC yr^{-1}), seagrass PIC accumulation showed higher global rates but significantly lower rates per surface area (400 g PIC m^{-2} yr^{-1}) (Milliman and Droxler 1996; Catubig et al. 1998; Table 3.3).

Table 3.3. Estimated area, and PIC accumulation rates globally (Tg PIC yr^{-1}) and per surface area (g PIC m^{-2} yr^{-1}) for different carbonate producing ecosystems including the results found for seagrasses in this study and a global estimation considering neritic, slopes, and pelagic areas along with organism-level data.

Ecosystem	**Area (10^{12} m^2)**	**Global (Tg PIC y^{-1})**	**Per surface area (g PIC m^{-2} y^{-1})**	**Reference**
Planktonic communities	290	100-132	0.34-0.45	Catubig et al. (1998), Milliman and Droxler (1996)
Coral reefs	0.6	84	140	Milliman and Droxler (1996)
Halimeda bioherms	0.05	20	400	Milliman and Droxler (1996)
Bank/Bays	0.8	24	30	Milliman and Droxler (1996)
Seagrass meadows	0.6-0.177	22-75	126.3	Mcleod et al. (2011); This study
Global		1,500		Lebrato et al. (2010)

Implications in the assessment of the CO_2 sink capacity of seagras meadows

While PIC represents a substantial carbon stock, carbonate precipitation results in a rise of the partial pressure of CO_2 (pCO_2), which, can result in CO_2 supersaturation and re- lease of CO_2 to the atmosphere (Ware et al. 1992). The net release of CO_2 with carbonate deposition is defined by the molar ratio of CO_2 flux : $CaCO_3$ precipitation (Ψ), which increases with decreasing temperature and increasing pCO_2 (Frankignoulle et al. 1994). Ψ varies from 0.63 in surface waters in low to mid-latitudes, where carbonate precipitation takes place, to 0.85 below 500 m depth throughout the ocean, where most dissolution takes place (Smith 2013). Due to the vertical variation in Ψ, Smith (2013) identified the pelagic carbonate system as a net sink of CO_2, as most of the surface production (Ψ = 0.63) dissolves as it reaches deep waters (Ψ = 0.85) compensating for the CO_2 emitted by $CaCO_3$ precipitation in surface waters. In contrast, carbonate deposition in shallow ecosystems, such as seagrass meadows, would act as a CO_2 source as approximate two-thirds of the $CaCO_3$ produced in shallow benthic ecosystems accumulates in the sediment, and Ψ has the same value for $CaCO_3$ precipitation and dissolution (Milliman and Droxler 1996; Smith 2013). Given that seagrass meadows are sites of strong net primary production, any pCO_2 increase due to calcification may be more than compensated for, by organic production. Hence, Ψ has been interpreted to imply a POC : PIC production ratio threshold, with a value of 0.63 equivalent to no net change in pCO_2 and values greater or smaller than this value implying a net sink or source, respectively.

The median POC : PIC ratio of seagrass sediments found in this study was 0.2, independent of depth (median of surface sediments 0.17), well below the POC : PIC ratio threshold of 0.63, with only 18 % of seagrass sediments showing POC : PIC ratios > 0.6. Following the rationale above and assuming that organic carbon and calcium carbonate accumulate in the sediment in proportion to their production, these results could be interpreted to imply that CO_2 emissions derived from carbonate deposition may offset the CO_2 sink capacity associated with organic carbon burial in seagrass sediments globally, as discussed before for *Posidonia oceanica* in the Mediterranean (Mateo and Serrano 2012; Serrano et al. 2012). However, such interpretation would be premature. In general terms, the organic and inorganic carbon cycles in the ocean run at very different rates and although organic matter is produced at much faster rates than $CaCO_3$, it is also decomposed more rapidly (Smith 2013). However, the carbonate precipitation in seagrass meadows is intimately regulated by the organic metabolic rates of the ecosystem (Smith and Atkinson 1983; Barrón et al. 2006; Yates and Halley 2006; Hendriks et al. 2014a), and when both organic and inorganic carbon metabolic pathways have been measured *in situ* simultaneously, seagrass meadows have been found to be mainly net CO_2 sinks systems at a yearly scale (Barrón et al. 2006), even despite the underestimated net community production (NCP) rates that may result from the use of confined incubation chambers related to photooxidation processes and subsequent CO_2 increase and O_2 decrease during daytime (Champenois and Borges 2012). In addition to carbon burial, a significant fraction of the net community production of seagrass, supporting a CO_2 sink, is also exported as DOC and POC (Cebrián et al. 1997; Barrón and Duarte 2009). Hence, the comparison of sediment standing stocks would reflect only a fraction of the sink capacity of the seagrass ecosystems but not the net effect of the organic and inorganic carbon metabolic pathways on the net CO_2 flux. Therefore, more research, which takes into account both the organic and inorganic carbon cycles associated with these systems, is needed to better assess the role of seagrass ecosystems as carbon sinks or sources.

Understanding the balance between CO_2 emissions from carbonate deposition and CO_2 sequestration from organic carbon storage in seagrass sediments should not only focus on the POC : PIC ratio, but also on resolving how seagrass affect the POC : PIC ratio compared to adjacent un-vegetated sediments. When comparing the carbon content (%DW) between vegetated and adjacent un-vegetated patches, there was no difference in PIC, whereas the POC content was about two-fold larger in vegetated sediments compared to adjacent un-vegetated sediments as previously observed (Duarte et al. 2010; Kennedy et al. 2010). This result indicates that, despite the significant carbonate sediment deposits identified and that seagrasses favor carbonate precipitation and accumulation by epiphytes and other organisms inhabiting the meadow, sediment PIC largely depends on local environmental conditions that control carbonate precipitation and a significant fraction may derive from external sources, such as adjacent carbonate producer systems (corals). As a consequence, the POC : PIC ratio of seagrass sediments (mean ± SE, 0.28±0.06) exceeded that of adjacent un-vegetated sediments (mean ± SE, 0.19±0.040) in 73 % of the meadows examined. Hence, the organic carbon stock present in seagrass sediments would be expected to be reduced by half if seagrass cover was lost, while the inorganic stock would be comparable, thereby confirming the role of seagrass meadows as intense CO_2 sinks. It is important to point out that the rational above is related to the content (% DW) of both PIC and POC and not to the rate of accumulation, which may be significantly higher in seagrass compared to adjacent sand patches due to autotrophic production and sediment trapping.

In addition there are possible interactions between carbonate and organic carbon deposition that might enhance carbon sequestration in seagrass meadows. One possibility may be that high carbonate deposition rates may promote organic carbon sequestration and storage by enhancing sediment accretion and by rapidly removing organic carbon from surface sediments and away from the oxic zone, thereby enhancing preservation of organic carbon. The accumulation of carbonates in seagrass sediments may also influence below-ground biomass through the stimulation of vertical growth in the sediments, or through alteration of sediment composition and nutrient availability (Short 1987; Ferdie and Fourqurean 2004). In fact, Erftemeijer (1994) found higher below-ground biomass in seagrass meadows growing in carbonate sediments compared to meadows from the same species that develop in terrigenous sediment. Thus, the potentially higher below-ground production in carbonate-rich meadows may enhance organic carbon burial.

Implications in the role of seagrass meadows as coastal protection

Carbonate stocks represented an average of 51 ± 1 % of the dry weight in the top 10 cm (range 0.2 to 100%) of the seagrass sediments examined, therefore contributing significantly to the sediment accretion rate and coastal protection from increased sea level rise and storminess with climate change (Duarte et al. 2013b). The capacity of seagrass meadows to raise the seafloor at speeds that could match or exceed current sea level rise allows them to remain effective in protecting coastal areas (Duarte et al., 2013b). A recent review of coastal ecosystems sediment accretion rates found an average accretion rate of 2 ± 0.4 mm yr^{-1} for seagrass communities (Duarte et al., 2013b; Mazarrasa et al., 2013b), highlighting the important role these ecosystems may play in climate adaptation in coastal areas. Carbonate production and accumulation supports about half of this accretion rate.

This study offers the first global compilation of carbonate deposits in seagrass sediments. Despite some limitations in the geographic distribution of the data available, the scarcity of data from adjacent sand patches and the lack of local sediment accretion rates, we identified the significant role of seagrass ecosystems in the carbonate dynamics of coastal areas, with carbonate stocks and rates relevant at the global scale. Carbonate stocks, markedly higher in tropical and subtropical meadows, play a significant role in supporting the accretion rate of seagrass meadows, and while high carbonate deposition lead to CO_2 emissions, the comparison of vegetated vs. adjacent un-vegetated sediments still identifies seagrass meadows as strong CO_2 sinks. In order to increase understanding of the effect of carbonate accumulation in seagrass meadows on the function they play as CO_2 sinks, further investigation is required, especially on the coupling of the organic and inorganic metabolic processes that take place within the meadows.

Acknowledgements

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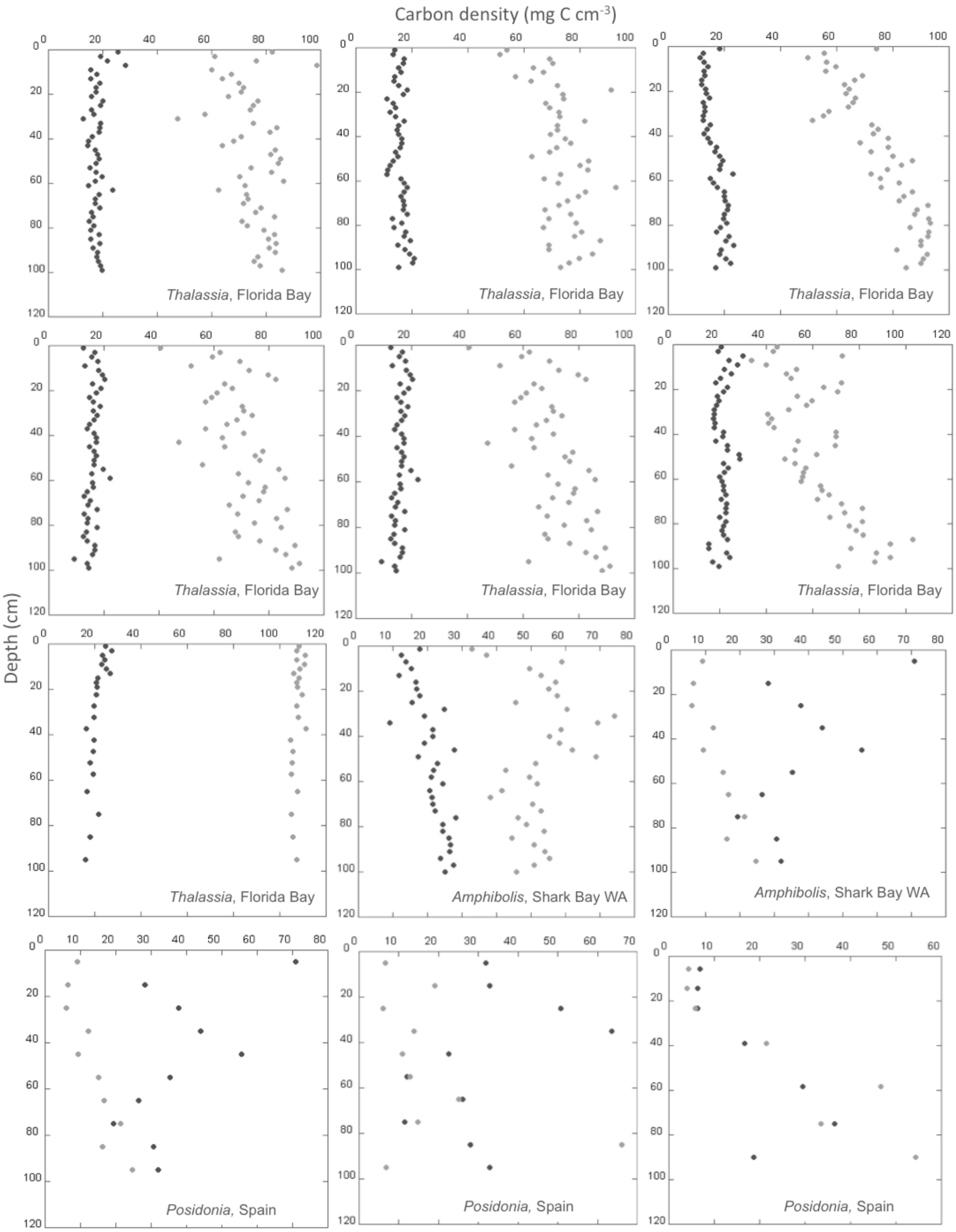
Supplementary Information
(Chapter 3)

Figure SI 3.1. Carbon concentration (mg C cm^{-3}) (black and grey dots for POC and PIC respectively) along the top meter profile of the longest cores in the database (≥100 cm) that had a minimum of three different data reported over one meter depth (n= 26). Meadow dominant genera and region are specified in each panel.



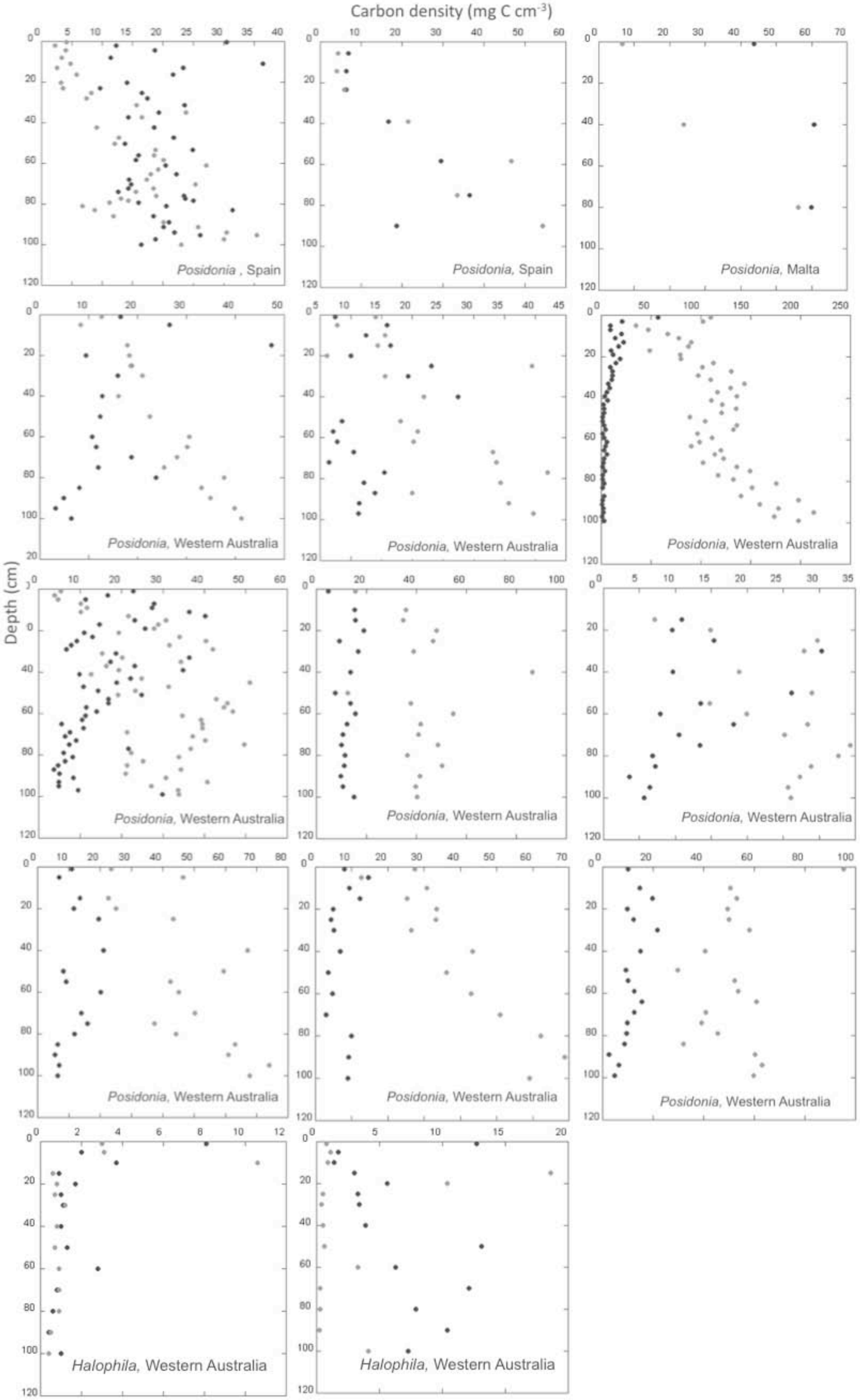

Carbon density (mg C cm-3)
Depth (cm)
0 5 10 15 20 25 30 35 40
0 20 40 60 80 100 120
Posidonia , Spain
0 10 20 30 40 50 60
Posidonia, Spain
0 10 20 30 40 50 60 70
Posidonia, Malta
0 10 20 30 40 50
Posidonia, Western Australia
5 10 15 20 25 30 35 40 45
Posidonia, Western Australia
0 50 100 150 200 250
Posidonia, Western Australia
0 10 20 30 40 50 60
Posidonia, Western Australia
0 20 40 60 80 100
Posidonia, Western Australia
0 5 10 15 20 25 30 35
Posidonia, Western Australia
0 10 20 30 40 50 60 70 80
Posidonia, Western Australia
0 10 20 30 40 50 60 70
Posidonia, Western Australia
0 20 40 60 80 100
Posidonia, Western Australia
0 2 4 6 8 10 12
Halophila, Western Australia
0 5 10 15 20
Halophila, Western Australia

Table SI 3.1. Data descriptors of the observed, estimated and total data available on top meter PIC stocks (Mg PIC ha^{-1}).

Top meter stocks (Mg PIC ha^{-1})	**N**	**Mean**	**SE**	**Median**	**Max**	**Min**
Observed	35	423.5	52.2	289.0	1189.5	17.9
Estimated	367	676.3	25.8	698.2	1660.0	3.1
Total	402	654.3	24.2	647.7	1660.0	3.1

Chapter 4

Latitudinal gradient in carbonate loads on seagrass leaves

Abstract

Seagrass meadows play a significant role in the formation of carbonate sediments, serving as a substrate for carbonate-producing epiphyte communities. The magnitude of the epiphyte load depends on plant structural and physiological parameters that are related to the time lapse available for epiphyte colonization but the carbonate accumulation is likely to also depend on the carbonate saturation state of seawater (Ω) that tends to decrease as latitude increases due to the decrease in temperature and salinity. A decrease in carbonate accumulation with increasing latitude has already been demonstrated for other carbonate producing communities. The aim of this study was to assess the effect of latitude on the epiphyte $CaCO_3$ load and accumulation rate on seagrass leaves. Between 9 and 12 shoots were sampled from 8 different meadows of the *Zostera* genus distributed across a broad latitudinal range (from 27ºS to up to 64ºN) along with measurements of temperature and Ω. The Ω significantly decreased as latitude increased and temperature decreased. The epiphytic $CaCO_3$ load on the seagrass leaves ranged from 13.5 %DW to 31.5 %DW and the associated $CaCO_3$ accumulation rate varied from 0.0004 to 0.088 mg $CaCO_3$ cm^{-2} d^{-1}, both variables showing a clear latitudinal trend decreasing from southern and warmer regions towards northern and cooler latitudes, consistent with the decrease in Ω.

4.1. Introduction

Seagrass meadows play an important role in the formation of biogenic carbonate sediments through a variety of processes such as enhancing mineral sedimentation from the water column (Gacia et al. 2003a), providing habitat to a wide variety of calcifying organisms such as mollusks and echinoderms (Boström and Bonsdorff 1997) and even by producing internal carbonate needles, as it has been recently described for *Thallasia testudinium* (Enriquez and Schubert 2014). More importantly, seagrass blades themselves provide a substrate for the development of calcifying epiphyte communities formed by coralline algae, bryozoans, foraminifera and serpulids (Land 1970; Perry and Beavington-Penney 2005). This carbonate producing community contributes to carbonate accumulation in seagrass sediments when deposited in the sediment with leaf litter, supporting the role of seagrass sediments as globally significant carbonate deposits (Chapter 3, Mazarrasa et al. 2015c).

Epiphyte colonization of seagrasses leaves depends on plant physiological and structural features. Larger species with a large leaf surface area usually have slower leaf turnover rates (Duarte 1991b) and longer leaf life spans, extending the time interval available for epiphyte colonization compared to that of smaller seagrass species (Heijs 1985; Borowitzka et al. 1990; Cebrian et al. 1994; Gacia et al. 2003). However, the development of calcifying epiphytic communities is expected to be also driven by the carbonate saturation state of seawater (Ω), that ultimately sets the conditions for $CaCO_3$ precipitation or dissolution (Feely et al. 2004).

A carbonate mineral's Ω is defined as the ratio between the product of the concentrations of Ca^{2+} and CO_3^{-2} in seawater at the *in situ* conditions of salinity and temperature, and the stoichiometric solubility product (K^*_{sp}) for the particular form of carbonate of interest under the same conditions:

$$\Omega = ([Ca^{2+}_{sw}] * [CO_3^{2-}{}_{sw}]) / K^*_{sp}$$

K^*_{sp} is defined as:

$$K^*_{sp} = [Ca^{2+}_{sat}] * [CO_3^{2-}{}_{sat}]$$

where $[CO_3^{2-}{}_{sat}]$ is the total (free and complexed) carbonate ion concentration in a seawater solution saturated with $CaCO_3$ (Zeebe and Wolf-Gladrow 2001). When $\Omega > 1$, seawater is supersaturated and $CaCO_3$ precipitation is favored whereas under $\Omega < 1$ dissolution of $CaCO_3$ is enhanced. In the ocean $[Ca^{2+}_{sat}]$ does not vary significantly and therefore Ω is mainly conditioned by the carbonate ion concentration (CO_3^{2-}), which depends on environmental conditions such as temperature, salinity and any biogeochemical process that leads to a change in inorganic carbon speciation (Zeebe and Wolf-Gladrow 2001). In general, cold, low-pH and fresh water leads to low CO_3^{2-} concentrations (Mucci 1983; Zeebe and Wolf-Gladrow 2001) and, therefore, low Ω saturation states and low $CaCO_3$ precipitation rates or dissolution.

Among the carbonate minerals with a biogenic origin, calcite is precipitated by most of the seagrass epiphyte groups (corallines, bryozoans, benthic foraminifers; Patriquin 1972; James et al. 2009) whereas aragonite is only precipitated by serpulids, which are usually less abundant among the epiphyte community (Perry and Beavington-Penney 2005). Whereas calcite is the stable form, aragonite is more soluble (i.e. has a higher K^*_{sp} than calcite) and therefore shows lower saturation states compared to calcite

(Ω_{Ca} < Ω_{Ar}) at the same conditions of temperature and salinity (Zeebe and Wolf-Gladrow 2001).

As temperature decreases with increasing latitude, the carbonate saturation state (Ω) decreases poleward (Feely et al. 2009; Jiang et al. 2015; Takahashi et al., 2014), reducing the scope for calcifiers (Lees 1975, Mutti and Hallock 2003). Hence, coral reefs for instance are mostly limited to tropical and subtropical regions (Veron and Minchin 1992; Veron 1995), encrusting algae tend to be more heavily calcified in temperate compared to colder waters (Lowenstam and Weiner 1989), calcification rates of fish bones have been found to decline from temperate and tropical species to arctic species (Moss 1963) and calcium carbonate content of marine invertebrates decreased with latitude (Watson et al. 2012).

However, the latitudinal gradient in Ω may be confounded by processes that act on a more local scale in the coastal waters inhabited by seagrass. These include variation in terrestrial inputs of fresh water, nutrients, organic and inorganic carbon, acids and carbonate alkalinity (Duarte et al. 2013c). Also seagrass meadows themselves, as metabolic-intense ecosystems, are able to alter the carbonate system in the surrounding water and enhance Ω through the removal of CO_2 during the day (Duarte et al. 2013c; Hendriks et al. 2014a) and *vice versa* during night. Hence, it is unclear whether a latitudinal gradient in carbonate loads on seagrass leaves occurs.

Existing studies on carbonate accumulation in seagrass leaves have been conducted at local or regional scales (Patriquin 1972; Canals and Ballesteros 1997; Gacia et al. 2003a; Perry and Beavington-Penney 2005) and therefore have not tested the possible effect of latitude on the carbonate loads in seagrass leaves. Yet, the low $CaCO_3$ production rates on leaves reported for temperate seagrass meadows (Canals and Ballesteros 1997; Perry and Beavington-Penney 2005) compared to the high rates identified in tropical regions (Patriquin 1972; Frankovich and Zieman 1994) suggest the existence of a latitudinal gradient in the carbonate content and accumulation of the seagrass epiphyte load. This hypothesis is consistent with the latitudinal gradient found in the carbonate stocks of seagrass sediments (Chapter 3, Mazarrasa et al. 2015c). In addition, other studies have found a clear effect of salinity on the load of carbonate epiphytes within the same seagrass species and region (Walker and Woelkerling 1988; Bosence 1989; Frankovich and Zieman 1994) further suggesting a potential role of Ω as a driver of carbonate loads on seagrass leaves.

Here we assess the variability of carbonate epiphyte loads and accumulation rates on seagrass leaves and test the hypothesis that these properties decline with latitude as the saturation state for carbonate minerals and the temperature decreases. In order to avoid potential confounding effects derived from structural and physiological features, such as differences in leaf turnover rates among species (Stevenson 1988), and to avoid the known species where carbonate needles accumulate within the leaves (i.e. *Thalassia testudinum*; Enriquez and Schubert, 2014), we used meadows comprised of plants from the genus *Zostera,* which is distributed worldwide across most of the seagrass biogeographic and latitudinal regions (Green and Short 2003).

4.2. Methods

Between 9 and 12 shoots were collected from each of six *Zostera marina* meadows, one *Heterozostera tasmanica* meadow and one *Zostera muelleri* meadow, distributed across a broad latitudinal range spanning from 27ºS in Australia up to 64ºN in Greenland (Figure 4.1, Table 4.1). The leaves from each shoot were separated, ranked by position within the shoot from the youngest to the oldest leaf, and leaf length and width were measured to the nearest mm.

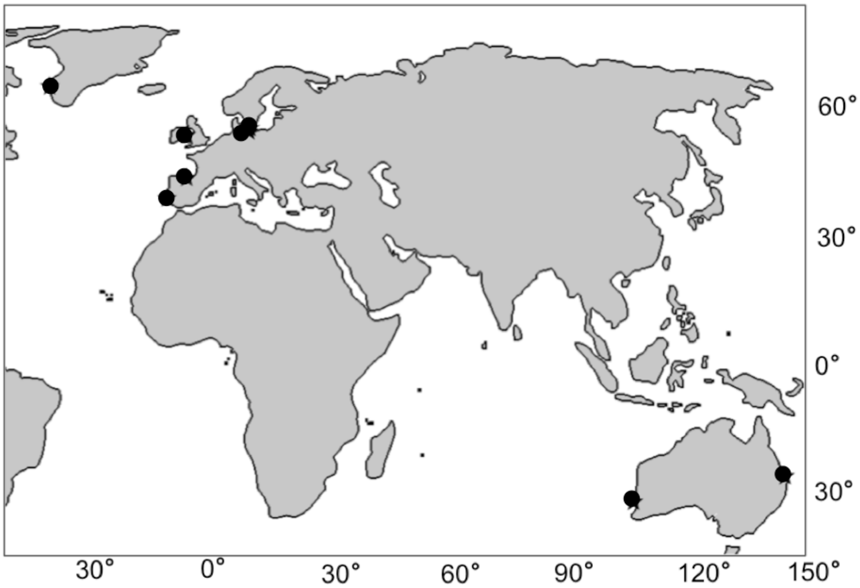


Figure 4.1. Location of the meadows sampled for this study.

For total organic matter content (OM, %DW) and carbonate content ($CaCO_3$, %DW) analysis, leaves from the same shoot were gathered as a single sample to obtain estimates of organic matter and carbonate content per shoot. OM (%DW) was estimated from the weight loss of dried leaves (60ºC for 24 h) following combustion at 450ºC for 2 h and $CaCO_3$ (%DW) was calculated from the weight loss of the ashes resulting from OM determination after combustion at 1000ºC for 1 h (Dean 1974), as done by Gacia et al. (2003). We consider all the $CaCO_3$ load to be derived from the epiphyte load (Gacia et al. 2003). As shoots from different sites showed important differences in their structural features (number of leaves, leaves surface area or shoot weight) (Figure SI 4.1) results on $CaCO_3$ load are provided as %DW and/or normalized to leaf surface area to allow comparison among sites.

$CaCO_3$ load per unit leaf surface area (g $CaCO_3$ cm^{-2}) was estimated as the ratio between the total weight of $CaCO_3$ per shoot (calculated as the product of the shoot $CaCO_3$ in %DW and the shoot weight divided by 100) and the total shoot leaf surface area.

To estimate the carbonate accumulation rate (mg $CaCO_3$ cm^{-2} d^{-1}) per shoot, shoot carbonate load per leaf surface area was divided by the approximate age of the shoot. The approximate shoot age was estimated by taking into account the number of leaves per shoot and the leaf formation rate (leaves $shoot^{-1}$ y^{-1}), which was predicted from the latitudinal position of the sites using the equation (4.1) proposed by Olesen et al. (2015):

$$y = -0.745x + 59.1 \ (R^2= 0.574) \qquad (4.1)$$

for *Zostera marina* meadows in the Northern hemisphere, where y is the number of leaves produced per shoot per year and x is the latitude in degrees. For *H. tasmanica* and *Z. muelleri* shoots, leaf formation rates (30 and 28 leaves shoot y^{-1} respectively) were extracted from Duarte (1991b).

Table 4.1. Sampling sites, location, species and estimates of the carbonate saturation states for calcite (Ω_{Ca}± SD) and aragonite (Ω_{Ar}± SD).

Site	**Latitude/Longitude**	**Species**	Ω_{Ca}± SD	Ω_{Ar}± SD
Moreton Bay (MB, Australia)	27º25' 52.01" S/ 153º.26' 0787" E	*Zostera muelleri*	5.39 ± 0.20	3.49±0.13
Rottnest Island (RI, Western Australia)	32º01'35.23"S/ 115º31'25.59" E	*Heterozostera tasmanica*	4.76±0.02	3.11±0.02
Ria Formosa, (RF, Portugal)	36°59'45.9"N/ 7°49'43.9"W	*Zostera marina*	4.67±0.88	3.06±0.59
Santander, Spain	43º25'.51.10"N /3º48'46.25"W	*Zostera marina*	4.15 ± 0.07	2.67 ± 0.03
Nefyn, UK	52º56' 22.85" N/4º31'21.77" W	*Zostera marina*	3.24 ± 0.11	2.06 ± 0.07
Dragor Strand (DS, Denmark)	55°34'57.55"N /12°39'56.66"W	*Zostera marina*	3.29± 0.13	1.86±0.07
Skaering Strand, (SS, Denmark)	56°14'21.89"N /10°20'24.66"W	*Zostera marina*	3.01±0.16	1.81±0.09
Kobbefjord (KF, Greenland)	64º09'44.80"N/ 51º 33'38.86"W	*Zostera marina*	3.59 ± 0.44	2.25±0.27

For estimation of carbonate saturation state (Ω), samples were taken, when possible, for alkalinity and DIC or pH at the same time as shoot sampling, along with data on salinity and temperature. For the meadow in Moreton Bay (MB, Australia), all variables were extracted from the Integrated Marine Observing System (IMOS: https://imos.aodn.org.au/imos123/home) from Australia, for the meadows from Santander (Spain) and Nefyn (UK), pH was extracted from the Bio-ORACLE database (Tyberghein et al. 2012) and for the meadow from Ria Formosa (Portugal), data correspond to average summer values (Table SI 4.1). Ω was calculated using the CO_2 SYS excel program (Pierrot et al. 2006) with the K1 and K2 constants from Mehrbach et al. (1973), as modified by Dickson and Millero (1987), and the $KHSO_4$ constant from Dickson (1990).

Statistical analysis

Analysis of Variance (ANOVA) and post-hoc Tuckey-Kramer test were used to assess the differences in leaf organic matter content (OM %DW), carbonate content ($CaCO_3$ %DW) and carbonate load per leaf surface area (mg $CaCO_3$ cm^{-2}) in seagrass shoots between sampling sites. Linear regression analysis were used to assess the relationship between latitude and temperature *vs.* Ω, latitude and temperature *vs.* carbonate content ($CaCO_3$ %DW) and accumulation rates in seagrass leaves (mg $CaCO_3$ cm^{-2} d^{-1}) and Ω *vs.* carbonate content ($CaCO_3$ %DW) and accumulation rates in seagrass leaves (mg $CaCO_3$ cm^{-2} d^{-1})

4.3. Results

Shoot $CaCO_3$ and organic matter (OM) content (both as % of DW) differed significantly among meadows (Figure 4.2a and Figure SI 4.2; ANOVA, $p<0.0001$; Tuckey-HSD, $\alpha=0.05$). *H. tasmanica* shoots from Rottnest Island (Western Australia) supported the highest content of $CaCO_3$ (32.7 ± 5.8 %DW) whereas *Z. marina* shoots from Nefyn (UK) had the lowest $CaCO_3$ content (13.5 ± 2.1 % DW) (Figure 4.2a).

Similarly, the highest $CaCO_3$ load per unit leaf surface area (g $CaCO_3$ cm^{-2}) was recorded for *H. tasmanica* shoots from Rottnest Island (2.1 ± 0.4 mg $CaCO_3$ cm^{-2}), one order of magnitude greater than those of *Z. marina* shoots from Nefyn (UK), that showed the lowest $CaCO_3$ load (0.31 ± 0.04 mg $CaCO_3$ cm^{-2}) (ANOVA, $p<0.0001$; Tuckey-HSD, $\alpha=0.05$; Figure 4.2b).

Calcium carbonate saturation state varied from minimum values of 3.01 ± 0.08 and 1.81 ± 0.05 for Ω_{Ca} and Ω_{Ar} respectively in Skaering Bay (Denmark) to 5.39 ± 0.07 and 3.49 ± 0.05 for Ω_{Ca} and Ω_{Ar} respectively, in Moreton Bay (Australia) (Table 4.1). Ω_{Ca} and Ω_{Ar} showed a strong and significant negative relationship with latitude (linear regression analysis), decreasing at rates of -0.06 ± 0.01 ($R^2=0.84$, $p=0.0014$) and -0.044 ± 0.008 per degree latitude ($R^2=0.83$, $p= 0.0015$) (Figure 4.3a; Table 4.2), respectively. Ω_{Ca} and Ω_{Ar} increased significantly with temperature, at rates of 0.11± 0.03 $^{o}C^{-1}$ ($R^2=0.63$, $p=0.02$) and 0.08 ± 0.02 $^{o}C^{-1}$ ($R^2=0.64$, $p=0.017$), respectively (Figure 4.3b, Table 4.2).

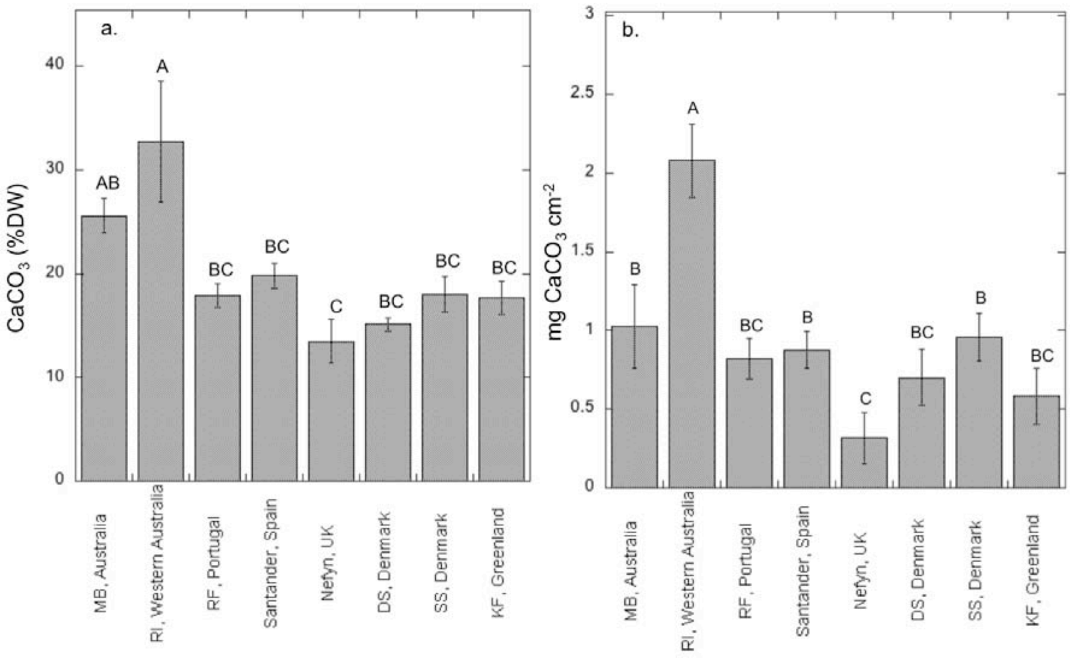


Figure 4.2. Shoot average (±SE) calcium carbonate a) content ($CaCO_3$ %DW) and b) load per leaf surface area (mg $CaCO_3$ cm^{-2}) per site. Different letters indicate significant differences among sites (Tuckey-HSD, α=0.05).

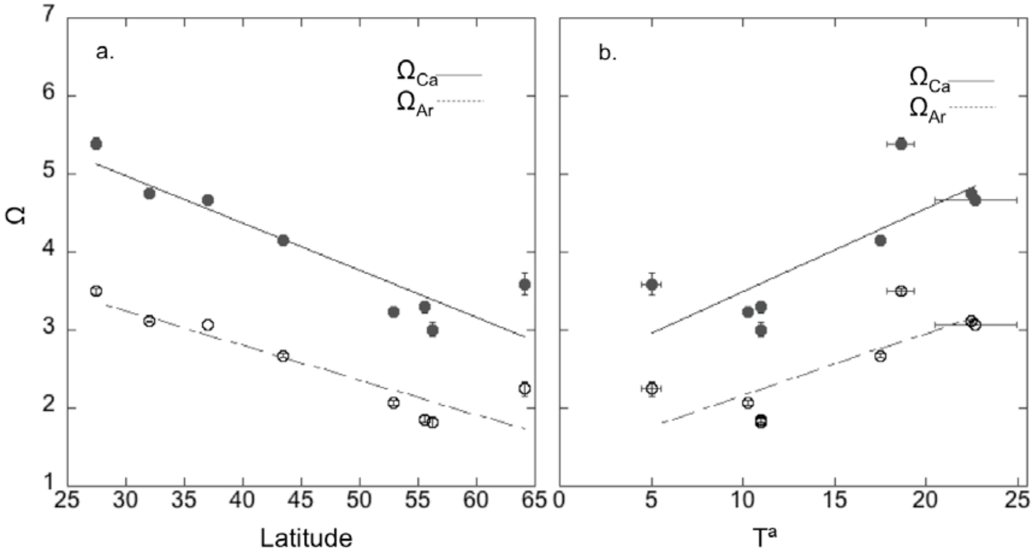


Figure 4.3. Relationship between a) absolute latitude and calcium carbonate saturation state for calcite (Ω_{Ca}) and aragonite (Ω_{Ar}) and b) in situ temperature and calcium carbonate saturation state for calcite (Ω_{Ca}) and aragonite (Ω_{Ar}). In a) solid line shows the fitted linear regression $y= 6.79-0.06\ (\pm0.01)x$ (R^2=0.84, p=0.0014) for calcite and dashed line shows the fitted linear equation $y= 4.59 - 0.04\ (\pm0.008)x$ (R^2=0.83, p=0.0015) for aragonite and in b) solid line shows the fitted linear regression $y= 2.43 + 0.11(\pm\ 0.03)x$ (R^2=0.63, p=0.02) for calcite and dashed line shows the fitted linear equation $y= 1.36 + 0.08\ (\pm0.024)x$ (R^2=0.64, p=0.017) for aragonite.

Table 4.2. Results of the linear regression analysis.

Model	**df**	**F-ratio**	**R^2**	**Slope ± SE**	**p-value**
Latitude vs. Ω_{Ar}	7	30.49	0.83	-0.044 ± 0.008	0.0015
Latitude vs. Ω_{Ca}	7	31.2533	0.84	-0.06 ± 0.01	0.0014
T^a vs. Ω_{Ar}	7	10.7	0.64	0.079 ± 0.024	0.017
T^a vs. Ω_{Ca}	7	10.12	0.63	0.11 ± 0.33	0.019
Latitude vs. $CaCO_3$ content (%DW)	84	19.74	0.19	-0.33 ± 0.07	<0.0001
Latitude vs. $CaCO_3$ accumulation rate (mg cm^{-2} d^{-1})	84	26.18	0.3	-0.0013 ± 0.0002	<0.0001
T^a vs. $CaCO_3$ content (%DW)	84	13.9	0.14	0.57 ± 0.15	0.0004
T^a vs. $CaCO_3$ accumulation rate (mg cm^{-2} d^{-1})	84	34.84	0.29	0.0025 ± 0.0004	<0.0001
Ω_{Ar} vs. $CaCO_3$ content (%DW)	84	19.32	0.19	6.66 ± 1.51	<0.0001
Ω_{Ca} vs. $CaCO_3$ content (%DW)	84	19.77	0.19	4.96 ± 1.12	<0.0001
Ω_{Ar} vs. $CaCO_3$ $CaCO_3$ accumulation rate (mg cm^{-2} d^{-1})	84	24.77	0.23	0.023 ± 0.004	<0.0001
Ω_{Ca} vs. $CaCO_3$ accumulation rate (mg cm^{-2} d^{-1})	84	24.06	0.22	0.016 ±0.003	<0.0001

$CaCO_3$ load (%DW) and accumulation rate (g $CaCO_3$ cm^{-2} d^{-1}) decreased significantly with latitude (linear regression analysis, $p<0.0001$) at rates of -0.33 ± 0.07 % DW ($R^2=0.19$) and -0.0013 (±0.0002) g $CaCO_3$ cm^{-2} d^{-1} ($R^2=0.30$) per degree latitude, respectively (Fig. 4.4a,b; Table 4.2) whereas increased with increasing temperature at rates of 0.57 ± 0.15 $CaCO_3$ %DW $^{o}C^{-1}$ ($R^2=0.14$, $p=0.0004$) and 0.0025 ± 0.0004 g $CaCO_3$ cm^{-2} d^{-1} $^{o}C^{-1}$ ($R^2=0.29$, $p<0.0001$), respectively (Figure 4.5a,b; Table 4.2).

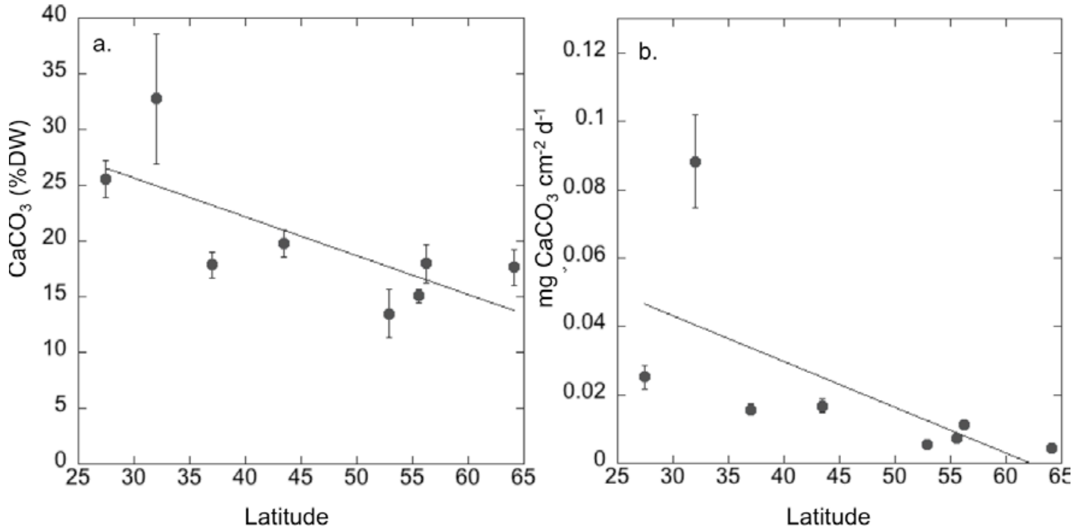


Figure 4.4. Relationship between absolute latitude and shoot calcium carbonate a) content ($CaCO_3$ %DW) and b) accumulation rate (mg $CaCO_3$ cm^{-2} d^{-1}) (avg ± SE). Lines show the fitted linear regressions: y= 35.04 (± 3.6) -0.33 (±0.07)x ($R^2=0.19$; $p<0.0001$) for $CaCO_3$ (%DW) and y= 0.08(±0.01) - 0.0013 (±0.0002)x ($R^2=0.30$, $p<0.0001$) for $CaCO_3$ accumulation rate.

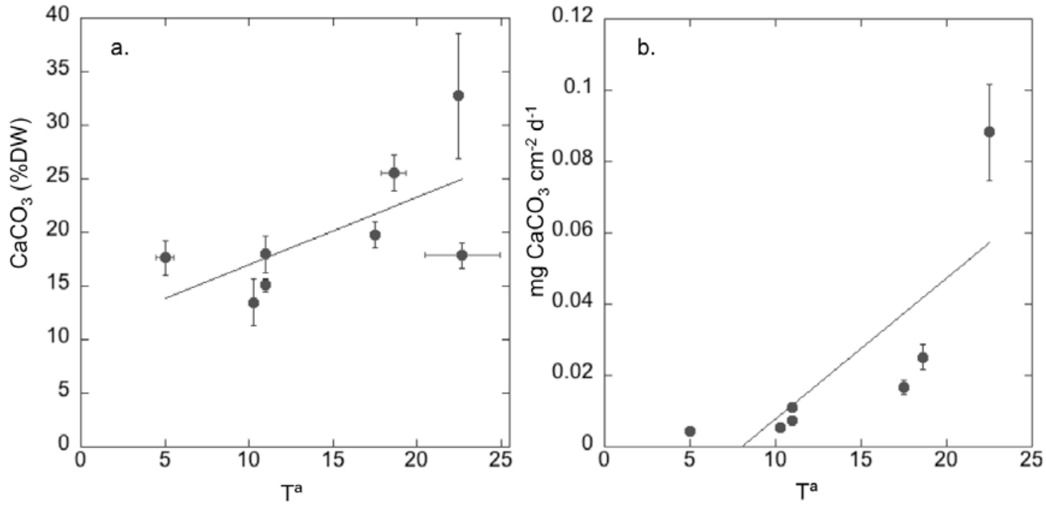


Figure 4.5. Relationship between temperature (avg. ± SD) at the sampling site and $CaCO_3$ a) content (%DW) and b) accumulation rate (mg CaCO3 cm^{-2} d^{-1}) (avg ± SE). Lines show the fitted linear regression equation: a) y= 11.15 + 0.57 (±0.15)x ($R^2=0.14$, $p=0.0004$) and b)y= -0.017 + 0.0025 (±0.0004)x ($R^2=0.29$, $p<0.0001$).

Similar to the response to temperature, $CaCO_3$ load (%DW) and accumulation rate (mg $CaCO_3$ cm^{-2} d^{-1}) increased significantly with increasing Ω_{Ca} and Ω_{Ar} (linear regression analysis, Figure 4.6a,b; Table 4.2).

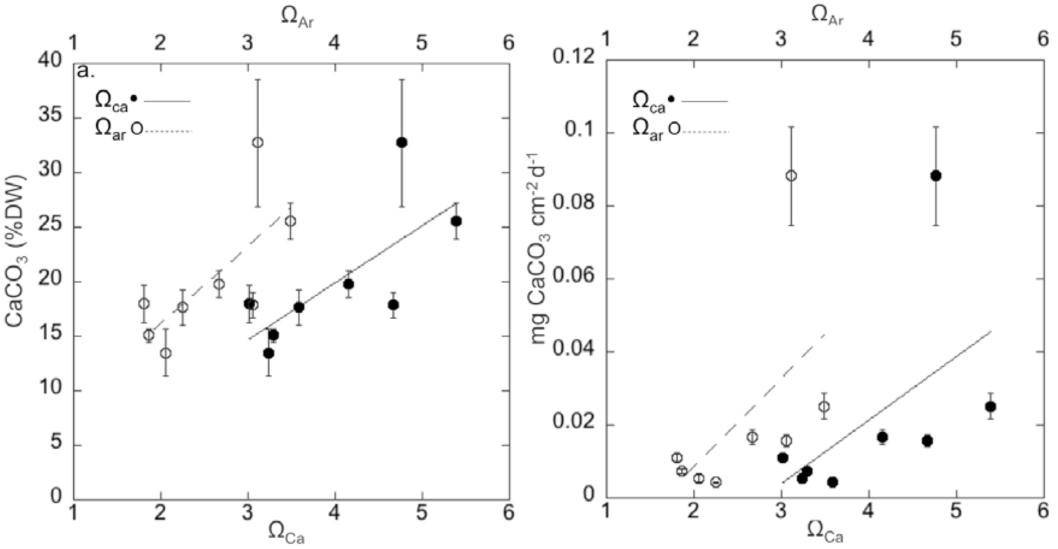


Figure 4.6. Relationship between Ω_{Ca} and Ω_{Ar} and $CaCO_3$ a) content ($CaCO_3$ %DW) and b) accumulation rates (mg $CaCO_3$ cm^{-2} d^{-1}) (avg ± SE). Solid and dashed lines fit the linear regressions equations in a) y=-0.12 +4.96 (±1.12)x (R^2=0.19, p<0.0001) and y=2.86 + 6.56 (±1.51)x (R^2=0.19, p<0.0001) and in b) y=-0.045 + 0.016 (±0.003)x (R^2=0.22, p<0.0001) and y=-0.037 + 0.023(±0.004)x (R^2=0.23, p<0.0001) that predict the responses to calcite and aragonite respectively.

4.4 Discussion

The studied meadows were distributed along a broad latitudinal gradient, from 27ºS to up to 64ºN, largely covering the geographical distribution range of the *Zostera* genus (Green and Short 2003). This broad latitudinal gradient is associated with large differences in water temperature and light regimes, affecting important structural and physiological features in seagrass meadows such as the ratio between above and below-ground biomass, plant phenology (Clausen et al. 2014) and leaf formation rates (Olesen et al. 2015).

The latitudinal gradient encompassed here also comprised a steep gradient in the saturation state (Ω) of the two main forms of biogenic carbonate (calcite and aragonite) in the seagrass meadows sampled, which decreased from warm subtropical regions to colder subarctic latitudes. This result is consistent with the strong temperature-dependency of Ω (Zeebe and Wolf-Gladrow 2001) and with previous global assessments (Feely et al. 2009; Takahashi et al. 2014; Jiang et al. 2015). However, temperature explained a lower percentage of the variability in Ω among the meadows examined (64%) compared to latitude (84%), as latitude integrates other environmental factors that also affect Ω, such as salinity (Mucci 1983), which generally decreases from low to high latitudes (Table SI 4.1) (Antonov et al. 2006).

The hypothesis that the carbonate load and accumulation rate on seagrass leaves decreased with latitude and increased with temperature and calcium carbonate saturation state was supported by our results. The largest $CaCO_3$ content and accumulation rates were found in the subtropical meadows of *Heterozostera tasmanica* and *Zostera muelleri* (32.7 and 25-6 %DW and 0.09 and 0.025 mg $CaCO_3$ cm^{-2} d^{-1}). The $CaCO_3$ content and accumulation rate in the leaves of *H. tasmanica* shoots, sampled in Rottnest Island (Western Australia), a region of intense carbonate precipitation, was particularly high, consistent with the dominance of biogenic $CaCO_3$ in the sediment composition at this site (~96%; Collins 1988; Holmer and Kendrick 2013) and the presence of important scleractinian coral communities (Veron and Marsh 1988). In contrast, high-latitude *Zostera* shoots tended to show lower $CaCO_3$ load and accumulation rates (13.5-19.8 %DW and 0.004-0.017 mg $CaCO_3$ cm^{-2} d^{-1}).

However, the amount of variability in $CaCO_3$ (%DW) and accumulation rates explained differed among the environmental factors considered. Carbonate saturation state (Ω) and latitude were the factors explaining the largest proportion of the variability among sites (~19%) in leaf $CaCO_3$ load (%DW) compared to the variability explained by temperature (~14%). In contrast, the variability in $CaCO_3$ accumulation rates was better explained by temperature and latitude (~30%) than by the carbonate saturation state (~0.22 %). The stronger relationship between $CaCO_3$ accumulation rates and temperature and latitude is likely attributable to the multiple traits, in addition to Ω, associated to these properties, including plant physiological and structural parameters such as the latitude-dependence of leaf turnover (Olesen et al. 2015), which determines the time interval available for leaf colonization by calcifying epiphytes (Heijs 1985; Borowitzka et al. 1990; Cebrian et al. 1994; Gacia et al. 2003). In addition, seagrass meadows in warm waters may be more productive than those in cold, high latitude areas, thereby being able to raise pH further, through their photosynthetic activity, which also increases Ω and facilitates calcification (Hendriks et al. 2014a).

The weak, albeit significant, relationship between seagrass carbonate load and accumulation rate and Ω suggests that the saturation state of carbonate minerals is only indicative, but not the only predictor of biomineralisation processes (Feely et al. 2009), at least when comparing different communities, as the Ω level required for biomineralisation is very different among different organisms (Feely et al. 2009). In addition, the precipitation of minerals by organisms is the result of biologically induced (Lowenstam 1981) or controlled processes (Mann 1983) and therefore it can't be solely explained by environmental conditions (Hendriks et al. 2014b), although these may impose constraints on the magnitude of mineralization and the form of the mineral predominantly precipitated (Lowenstam and Weiner 1989). Hence, other environmental factors affecting the organisms' metabolism play a significant role. Nutrient availability including both organic food and inorganic nutrients (i.e. N and P) may regulate the capacity of carbonate producer organisms to precipitate $CaCO_3$ (Mutti and Hallock 2003; Thomsen et al. 2012), especially in cold and fresh waters, where the amount of energy required for carbonate precipitation increases due to low CO_3^{2-} concentration (Mutti and Hallock 2003, Hendriks et al. 2014b, Ramajo et al. 2016).

In addition, the Ω values used here may not fully describe those prevailing at each of the meadows, as the values, in most cases, were based on a single sampling event whereas the accumulation of carbonate epiphytes on the seagrass leaves takes place for the duration of the whole life span of each shoot (25 to 140 days for this study), a period during which Ω in vegetated environments is relatively variable in response to environmental conditions and plant metabolism (Hendriks et al. 2014a; Krause-Jense et al. 2015). For instance, daily metabolic activity in coastal vegetation meadows leads to the variation of pH and Ω over a daily range that may shift in response to light availability (Hendriks et al. 2014a; Krause-Jense et al. 2015). Additionally, water velocity (Hendriks et al. 2014a) or changes in water masses caused by tides and subsequent changes in salinity and temperature may affect the carbonate saturation state over short time intervals, although local metabolic activity is a more significant controlling factor (Krause-Jense et al. 2015). Hence, a single water sampling event may not fully represent the carbonate saturation state under which the leaf carbonate load was deposited. In addition, the Ω values considered in this study correspond to the water mass surrounding the meadow sampled whereas in benthic macrophytes communities, pH shows differences even within a 1 m^3 scale inside the canopy and even at a micro-scale across the leaf boundary layers where carbonate precipitation by epiphyte communities takes place (Krause-Jense et al. 2015).

However, despite the lack of data describing diurnal or seasonal change, the results show a clear latitudinal trend on the carbonate accumulation on seagrass leaves that tended to decrease towards colder and northern regions consistent to the decrease in the carbonate saturation state (Ω). These results are in agreement with previous findings for calcifier organisms such as stony corals, invertebrates and fish (Moss 1963; Veron and Minchin 1992; Watson et al. 2012) and with the latitudinal gradient identified in the carbonate content in the sediments of seagrass meadows (Chapter 3, Mazarrasa et al. 2015c).

Based on the rate of carbonate accumulation per shoot estimated for the *Zostera marina* meadows examined in this study and the average shoot density reported for this species in the northern hemisphere (718 ± 88.5 shoots m^{-2}; assuming this to be independent of latitude, as reported by Clausen et al. 2014) we estimate that *Zostera marina* meadows studied here could contribute an average of 140 ± 24 g $CaCO_3$ m^{-2} y^{-1} to the sediment, with a 30-fold variability from 9 ± 5 and 327 ± 47 g $CaCO_3$ m^{-2} y^{-1} at the highest (64º09'44.80"N) and the lowest (36°59'45.9"N) latitudes, respectively (Table 4.3). This wide range of $CaCO_3$ production rates is comparable to those reported for meadows in tropical and subtropical regions formed by other species such as *Thalassia testudinum* in Jamaica (Land 1970) and Florida Bay (Frankovich and Zieman 1994), *Amphibolis antarctica* in Western Australia (Walker and Woelkerling 1988) and *Thalassia* meadows in temperate regions (Perry and Beavington-Penney 2005) (Table 4.3), highlighting the potential role of *Zostera marina* meadows in the production of sediment through leaf carbonate load accumulation. The *Zostera* genus considered in this study is relevant due to its broad distribution worldwide, with *Zostera marina* meadows being the most widely distributed and sustaining important ecological functions such as carbon sequestration (Greiner et al. 2013), nutrient retention (Hemminga et al. 1999), stimulation of biodiversity (Duffy 2006) and sediment stabilization (Bos et al. 2007). This study also reveals a potential role of *Zostera* meadows in contributing to biogenic carbonate sediment formation, that declines from subtropical to high latitudes.

Acknowledgements

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Table 4.3. Calcium carbonate production by epiphytes in seagrass meadows per meadow surface area reported in previous studies and those estimate in this study.

Seagrass species	**Location**	**Latitudinal region**	**Production (g CaCO3 m^{-2} y^{-1})**	**Reference**
Thalassia testudinum	Jamaica	Tropical	40-180	Land, 1970
Thalassia testudinum	Barbados	Tropical	2,800	Patriquini, 1972
Thalassia testudinum	Florida Bay, USA	Subtropical	30-303	Nelsen and Gingsburg, 1986
Thalassia testudinum	Florida Bay, USA	Subtropical	81-842	Bosence, 1989
Thalassia testudinum	Florida Bay, USA	Subtropical	1.9-282.7	Frankovich and Zieman, 1994
Amphibolis antarctica	Shark Bay, Australia	Subtropical	50-526	Walker and Woelkerling, 1988
Thalassodendrum ciliatum	Inhaca Is. Mozambique	Temperate	43.9	Perry and Beavington-Penney, 2005
Thalassia hemprichii	Inhaca Is. Mozambique	Temperate	33.4	Perry and Beavington-Penney, 2005
Posidonia oceanica	Balearic Is. Spain	Temperate	60-70	Canals and Ballesteros, 1997
Zostera marina	Northern hemisphere	Temperate- Subarctic	141 ± 24 (9-327)	This study

Supplementary Information
(Chapter 4)

Table SI 4.1. Sampling date and average (± SD) of the variables of the carbonate system sampled along with salinity and temperature measurements used for the calculations of calcium carbonate saturation state for calcite (Ω_{Ca}) and aragonite (Ω_{Ar}): total dissolved inorganic carbon (DIC), total alkalinity (TA) and pH.

Site	**Sampling Date**	**Latitude/Longitude**	**DIC ± SD (mmol/kgSW)**	**TA ± SD (mmol/kgSW)**	**pH ± SD**	**Salinity ± SD**	**T^a ± SD**	**Ω_{Ca}± SD**	**Ω_{Ar}±SD**
MB, Australia	18/02/2014	27º25' 52.01" S/ 153º.26' 0787" E	[b]2,011.6 ± 13.83	[b]2,334.93 ± 0.59		[b]35.68 ± 0.03	[b]18.6 ± 0.77	5.39 ± 0.20	3.49±0.13
RI, Western Australia	11/2/14	32º01'35.23"S/ 115º31'25.59" E		2,288.6 ± 11.23	8.17 ± 0.2	35.31	22.48± 0.015	4.76±0.02	3.11±0.02
RF, Portugal	7/10/14	36°59'45.9"N/ 7°49'43.9"W				[c]35.4 ± 0.63	[c] 22.7 ± 2.21	[c] 4.67±0.88	[c] 3.06±0.59
Santander, Spain	8/10/14	43º25'.51.10"N /3º48'46.25"W	2,072 ± 35.04		[a]8.17	35	17.5	4.15 ± 0.07	2.67 ± 0.04
Nefyn, UK	20/11/2014	52º56' 22.85" N/4º31'21.77" W	1,981.8 ± 71.25		[a]8.184	34.8	10.3	3.24 ± 0.11	2.06 ± 0.07
DS, Denmark	1/10/14	55°34'57.55"N /12°39'56.66"W	1,608.82 ± 6.24	1,748.3		10	11	3.29± 0.13	1.86±0.07
SS, Denmark	3/11/14	56°14'21.89"N /10°20'24.66"W	2,041.15 ± 9.15	2,182.36		20	11	3.01±0.16	1.81±0.09
KF, Greenland	15/06/2014	64º09'44.80"N/ 51º 33'38.86"W	1,726.17± 102.84	1,946.06 ± 121.25		30.8 ± 0.62	5 ± 0.5	3.59 ± 0.44	2.25±0.27

[a] Extracted from Bio-ORACLE database.

[b] Estimated from values reported in IMOS (https://imos.aodn.org.au/imos123/home) for the period between December 2013 and February 2014.

[c] Average summer value

Figure SI 4.1. Average (± SE) number of leaves per shoot (a) leaf surface area per shoot (b) and shoot weight (c) per site. All the features examined showed significant differences among sites (ANOVA, p<0.0001).

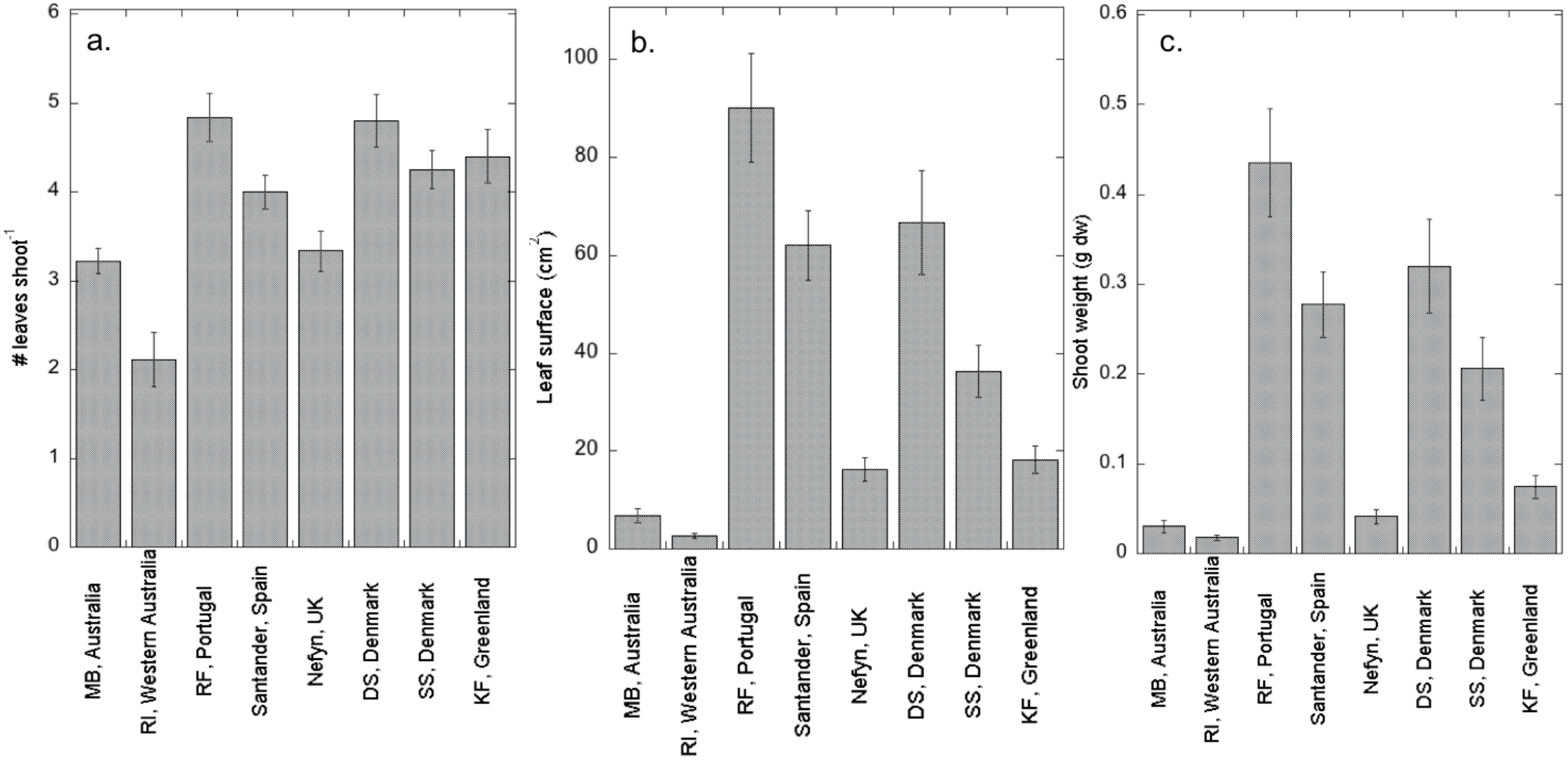
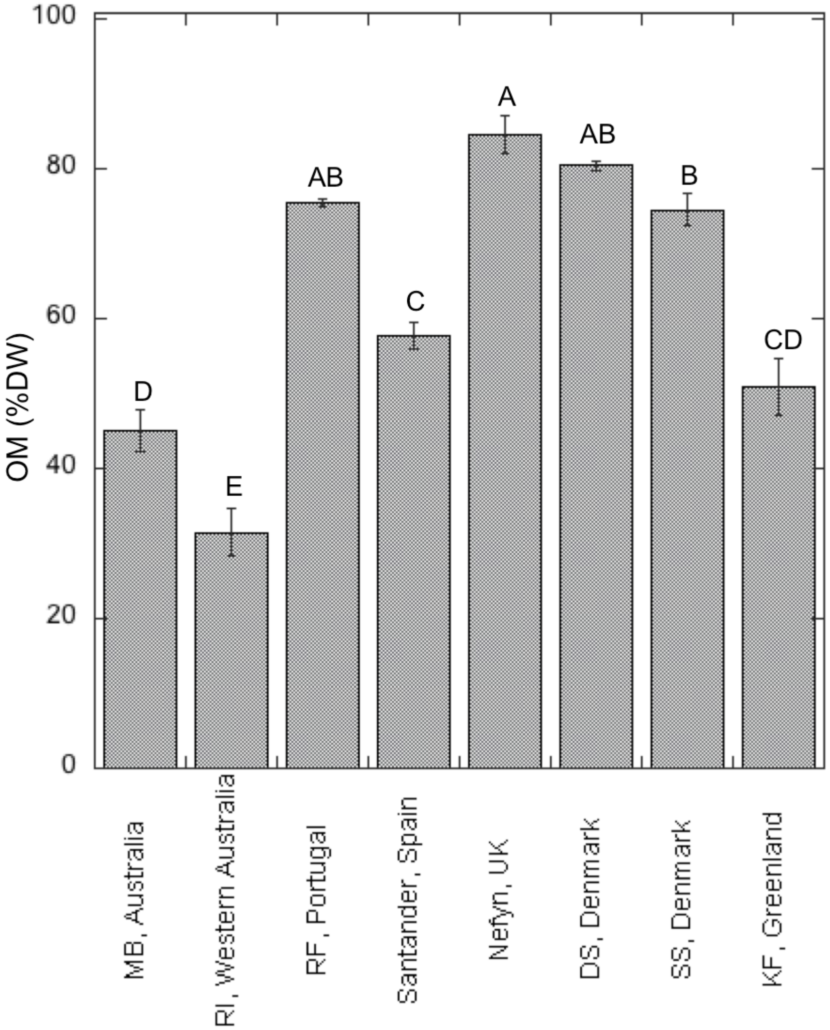


Figure SI 4.2. Shoot average (±SE) organic matter content (OM %DW) per site. Different letters indicate significant differences among sites (Tuckey-HSD, α=0.05).



III.3

Vulnerability of carbon deposits to be lost through remineralization and potential interactions with accumulated $CaCO_3$

Chapter 5

Assessing the vulnerability against remineralization of seagrass carbon deposits following sediment erosion

Abstract

Loss of seagrass meadows may lead to the loss of their carbon sink capacity and also to CO_2 emissions from the remineralization of previously buried organic matter if sediments are eroded and suspended into aerobic conditions. The loss of organic matter (OM) and organic carbon (POC) stocks through remineralization in seagrass sediments was examined experimentally by incubating sediment suspensions from four seagrass (*P.oceanica*) meadows under aerobic conditions. OM significantly decayed with time in three of the meadows examined at rates that ranked from -0.05 ± 0.02 % d^{-1} to -0.20 ± 0.07 % d^{-1}. The decrease in OM was favored as the magnitude of the initial organic carbon stocks, especially those enriched with sestonic derived organic carbon, and nitrogen content in the sediments examined were higher while OM decay was prevented as the sediment initial $CaCO_3$ concentration was higher. Only in two of the four meadows sediment suspensions the POC stocks decayed significantly, at the rates of -0.19 ± 0.07 and -0.22 ± 0.07% d^{-1}, with nearly a year needed for the 50% of the carbon stocks to be remineralized. These results suggest that seagrass sediment C deposits are partially remineralized when sediments get suspended and that the vulnerability to remineralization depends on intrinsic-sediment biogeochemical properties and C deposit characteristics.

5.1. Introduction

The conversion and/or degradation of terrestrial ecosystems that act as natural carbon sinks is a major source of CO_2 to the atmosphere, accounting for 12% of the total anthropogenic C emissions during the last decades (1980-2010; Houghton et al., 2012). Therefore, strategies to mitigate climate change include initiatives to protect and conserve these ecosystems through economic incentives, such as the Reduced Emissions from Deforestation and Degradation (REDD+) program (Scholz and Schmidt 2008). Marine vegetated coastal ecosystems (i.e. mangrove forests, saltmarshes and seagrass meadows) occupy only 0.2% of the ocean surface but contribute 50% of the global C sequestration in marine sediments (Duarte et al. 2005, 2013b), ranking among the most efficient carbon sinks in the biosphere. Yet vegetated coastal ecosystems are also among the most threatened on Earth (Valiela et al. 2001; Waycott et al. 2009), experiencing global loss rates of 1 to 7 % $year^{-1}$ (Duarte et al. 2008; Waycott et al. 2009). Hence, the protection and restoration of these ecosystems is being enhanced through the implementation of different strategies (known as "Blue Carbon Strategies"), similar to those that already exist for terrestrial ecosystems, as a way to mitigate climate change (Nellemann et al. 2009; Mcleod et al. 2011; Duarte et al. 2013a).

The development of "Blue Carbon" strategies has led to significant research in carbon burial in vegetated coastal habitats and has also helped identify a number of gaps in our understanding (Mcleod et al. 2011; Duarte et al. 2013a). In particular, the impact of seagrass loss on the fate of the associated sedimentary carbon stocks is one of the major gaps (Duarte et al. 2013a; Macreadie et al. 2014). Most of the carbon stored in seagrass meadows is located in the sediment compartment (Fourqurean et al. 2012) in the form of roots and rhizomes (both living and death), leaf debris and allochthonous carbon (Kennedy et al. 2010) trapped from the water column thanks to the filtering capacity of seagrass canopies (Hendriks et al. 2008). Loss of seagrass cover reduces the buffering capacity of seagrass meadows against waves and currents, rendering the sediments more vulnerable to erosion and suspension (Ward et al. 1984; Duarte et al. 2013a), which may lead to the partial loss of previously buried carbon stocks, as demonstrated in recent studies (Macreadie et al., 2015; Marbà et al., 2015, Serrano et al. 2016). The erosion of seagrass sediments may cause the export and deposition of the previously buried carbon in adjacent ecosystems (Macreadie et al. 2014) and/or lead to its remineralization once sedimentary organic carbon becomes exposed to aerobic conditions, as observed in other coastal ecosystems (Huang et al. 2010; Lovelock et al. 2011). Therefore, the decline of seagrass meadows may not only imply a loss of their carbon sequestration capacity but could cause the remineralization of sedimentary carbon stocks to CO_2, and hence their shift from being a CO_2 sink to a CO_2 source (Duarte et al. 2013a).

The remineralization of organic carbon previously stored in seagrass sediments has been assumed in calculations of CO_2 emissions after seagrass loss (Pendleton et al. 2012; Fourqurean et al. 2012). Yet, at present, there is no empirical evidence supporting that the erosion and subsequent resuspension of seagrass sediments leads to remineralization of organic carbon and, thus, to CO_2 emissions. The absence of such evidence remains a critical stumbling stone to include conservation of seagrass carbon stocks as part of emissions accounting and, hence, to the development of *Blue Carbon* strategies targeting seagrass meadows (Herr et al. 2012; Duarte et al. 2013a; b).

The risk of remineralization of resuspended sedimentary organic carbon deposits of seagrass meadows is likely to be determined by the nature of the organic matter buried and its refractory condition. Decomposition rates of organic detritus produced by photosynthetic organisms increase with nutrient concentration (especially nitrogen), and decrease with lignin content (Melillo et al. 1984; Enriquez et al. 1993). Sestonic matter, especially microalgae, which typically comprises 50% of the organic carbon in surface seagrass sediments (Kennedy et al. 2010), is much more labile and prone to remineralization than seagrass-derived detritus (Enriquez et al. 1993; Banta et al. 2004), which is relatively recalcitrant due to a high lignin content and low nutrient concentrations (Enriquez et al. 1993; Klap et al. 2000). This typically leads to higher microbial activity and organic matter decomposition in seagrass sediments rich in labile seston-derived organic matter (Hansen and Kristensen 1998; Holmer et al. 2004). Thus, the risk of sediment carbon deposits to be remineralized following erosion and suspension is expected to be higher as the load of sestonic derived organic carbon is higher.

There are other sediment biogeochemical factors that may also influence organic matter remineralization, such as the concentration of $CaCO_3$. In biogenic carbonate-rich sediments, organic matter tends to associate to $CaCO_3$ particles through surface sorption or through intracrystaline interactions, becoming less accessible to microorganisms and decomposers (Suess 1973; Ransom et al. 1998; Bock and Mayer 2000; Ingalls et al. 2004). In addition, the organic matter-mineral interactions likely embed the organic matter in a more anoxic environment (Burdige 2007). Furthermore, nutrient availability is typically reduced in carbonate sediments due to biogeochemical sorption process (Short 1987; Ferdie and Fourqurean 2004), which may also inhibit microbial activity and organic matter decomposition (Harrison 1989).

Here we experimentally assess the remineralization of organic matter (OM) and organic carbon (POC) deposits stored in seagrass sediments when sediments are eroded and get suspended in aerobic conditions. We conducted this study using sediments from four Mediterranean *Posidonia oceanica* meadows in the Balearic Islands (Western Mediterranean). Specifically, we hypothesize that experimental remineralization rates would vary according to differences in the sediment properties of each meadow, being enhanced as the initial sediment POC stocks, especially of seston-derived organic carbon, and the nitrogen content are higher; and reduced, as the initial $CaCO_3$ concentration increases.

5.2. Methods

Sediments used in the experiment were extracted in March 2014 from four different meadows around the Balearic Islands, located at water depths ranging from 5 to 17 meters: Magalluf and Pollença in Mallorca Island and Santa Maria and Es Port in Cabrera National Park (Figure 1.1). The meadow at Santa Maria can be considered pristine, whereas the other meadows sampled for this study are under a significant level of human pressure due to a combination of urbanization, tourism and recreational boating (Holmer et al. 2004; results provided in Chapter 1). Previous studies revealed contrasting sedimentary conditions among the meadows selected (Holmer et al. 2004; Calleja et al. 2007).

Sediments were sampled using sediment cores (9 cm ID and 30 cm long) and directly transported to the laboratory. Only the top 10 cm of the sediment cores were used in the experiment, that correspond to the sediment fraction accumulated during the last 34-50 years (depending on the meadow), as indicated by ^{210}Pb sediment dating results obtained in Chapter 1.

Living seagrass tissues were removed and sediment from each meadow, separately, was mixed and homogenized leading to four different sediment pools. A total of 25 replicate experimental units (sediment suspensions) per meadow sediment pool were prepared in 500 ml plastic containers by suspending 60 ml of sediment in 440 ml of seawater. Five experimental units per sediment pool were processed the first day to measure the initial biogeochemical conditions (t_0). The 20 remaining experimental units per meadow were incubated in a temperature-controlled chamber, at 20ºC (the yearly average temperature in *P.oceanica* meadows in the region; Marbà personal communication) and in dark conditions, to avoid potential primary production by benthic microalgae. The aerobic conditions in the sediment suspensions were maintained by bubbling each experimental unit with air pumps through a silicon tube. Due to the low water volume used and the bubbling, water tended to evaporate, resulting in a trend towards increased salinity, that was corrected by adjusting salinity weekly by adding distilled water as needed.

Experimental units were retrieved from the experiment for biogeochemical analysis at four different sampling times (5 replicate experimental units at each time) encompassing one year of incubation: t_1, 15 days; t_2, ~45 days; t3, ~105 days; t4, ~ 320 days, except for the sediments from the meadow at Pollença, that were incubated for a maximum of 239 days. The sampling times were distributed considering that organic matter remineralization was likely to follow an exponential trend, including a rapid phase of loss of the more labile material followed by a slower loss of more recalcitrant substrates (Enriquez et al.1993; Kristensen, 1994). Once experimental units were retrieved from the experiment after incubation period the following analyses were conducted.

Dissolved organic carbon (DOC) was measured with a Total Organic Carbon analyzer (Shimadzu TOC-V), in samples of the supernatant, acidified with 20 µl of H_3PO_4, obtained after centrifuging 50 ml of the sediment suspensions at 1500 rpm during 15 minutes. Sediments suspensions in each experimental unit were oven-dried at 60ºC during 36-48 h and the dry weight of the remaining sediment pool was measured. The sediment organic matter concentration (OM %DW) was estimated as the percent weight loss of dry sediment sample after combustion at 500ºC during 4 hours. Particulate organic carbon concentration (POC %DW) in three of the five experimental units retrieved at each sampling time was measured in an Elemental Analyzer-Isotope Ratio Mass Spectrometry (EA-IRMS) (Elemental Iso-Analytical Laboratory; United Kingdom). The relationship between OM (%DW) and POC (%DW) concentrations measured in the same sample (Figure SI 5.1), described by the fitted regression equation

$$y = 0.29x - 0.64; \; (R^2=0.98, \; p< 0.0001, \; n=60) \tag{5.1}$$

was used to estimate the POC (y) from OM (x) of all the sediment samples along the experiment. OM and POC stocks along the experiment (mg OM ml^{-1} and mg POC ml^{-1}) were estimated by multiplying the OM and POC (%DW) by the sediment dry weight (mg) remaining in each experimental unit and standardized to the initial volume of sediment used (60 ml) to produce the experimental units.

Nitrogen concentration (N, % DW) was measured with a CN elemental analyzer (Truspec CN determinator, LECO) and N stocks (mg N ml^{-1}) were estimated by multiplying N concentrations (%DW) by the sediment dry weight (mg) in each replicate container and standardized to the initial volume of sediment used (60 ml). Calcium carbonate concentration ($CaCO_3$; % DW) was estimated in three of the five replicate experimental units, as the percentage of weight lost overnight of samples acidified with 1 M hydrochloric acid. Nitrogen and $CaCO_3$ were only measured for experimental units processed in t_0, therefore being only representative of the initial conditions for each meadow sediment pool. Similarly, the contribution of the different carbon sources to the total initial carbon stock in each sediment pool was estimated based on measurements of sediment $\delta^{13}C$ composition in three of the five experimental units processed at t_0, using an Elemental Analyser-Isotope Ratio Mass Spectrometry (EA-IRMS) (Elemental Iso-Analytical Laboratory; United Kingdom); and applying a two-component isotope mixing model (equation 5.2) as used in Holmer et al. (2004).

$$\delta\ ^{13}C_{sediment} = \delta\ ^{13}C_{seagrass}\, f + \delta\ ^{13}C_{seston}\, (1 - f) \qquad (5.2)$$

where $\delta\ ^{13}C_{seagrass}$ represents the $\delta^{13}C$ measured in leaves and rhizomes of plant shoots collected between the years 2008 and 2009 in the meadows tested, and $\delta\ ^{13}C_{seston}$ values correspond to the $\delta^{13}C$ in seston samples obtained by Papadimitriou et al. (2005) (Table SI 5.1).

Statistical analysis

Analysis of Variance (ANOVA) and post-hoc Tuckey-Kramer test were used to assess the differences between initial biogeochemical properties among the different sediment pools examined. Assuming an exponential decay of the sediment organic materials, the OM (mg OM ml^{-1}) and POC (mg POC ml^{-1}) decay rates ($-k$) were estimated as the slope of the curve obtained by fitting a least-square regression analysis between the Ln-transformed OM and POC stocks vs. time (days). Decompositions rates are also reported as the half-life of the sediment organic materials (i.e. $T_{1/2} = k^{-1} * \ln 2$) to provide a more intuitive description of the organic detritus turn over rates (Enriquez et al. 1993). The effect of initial sediment biogeochemical properties (POC stock, sources contribution to POC stocks, N concentration and stock and $CaCO_3$ concentration in t_0) in the OM decomposition along the experiment was assessed by conducting least-square regression analysis using the whole data set resulted from combining data from the 4 meadows sediment pools (n =100) and where the ln-transformed OM stock (mg ml^{-1}) was the dependent variable (y), and time (in days), the initial value (t_0) of the sediment property tested and the interaction between time and sediment properties in t_0 were the model effects.

5.3. Results

The seagrass meadows sediments incubated exhibited significant differences in all the biogeochemical properties examined at the beginning of the experiment (ANOVA, $p<0.0001$; Tukey-Kramer, $\alpha=0.05$; Figure 5.1). Sediments from Pollença had the highest POC stock (55. 4 ± 4.1 mg POC ml^{-1}) approximately three-fold larger than Es Port sediments (13.4 ± 0.6 mg POC ml^{-1}) and more than ten-fold larger than Magalluf and Sta. Maria sediments (3.43 ± 0.73 and 3.48 ± 0.44 mg POC ml^{-1}, respectively, Figure 5.1a). The initial $CaCO_3$ concentration (%DW) was significantly higher in the sediments from Sta. Maria and Magalluf (97.9 ± 0.1% and 96.7 ± 0.1 %, respectively) followed by the sediments from Es Port (93.3 ± 0.4 %DW) and those from Pollença, which showed the lowest initial $CaCO_3$ concentration (76.9 ± 1.2 %DW, Figure 5.1b). The largest initial N concentration and stock were found in the sediments from Pollença (0.23 ± 0.01 % DW and 1.50 ± 0.08 mg N ml^{-1}) followed by those from Es Port (0.20 ± 0.01 % DW and 1.17 ± 0.07 mg N ml^{-1}). Sta. Maria and Magalluf had the most nitrogen-depleted sediments, with initial concentrations and stocks one order of magnitude lower than the other seagrass sediments (0.07-0.08 %DW and 0.86-0.93

mg N ml^{-1}, Figure 5.1 c,d).

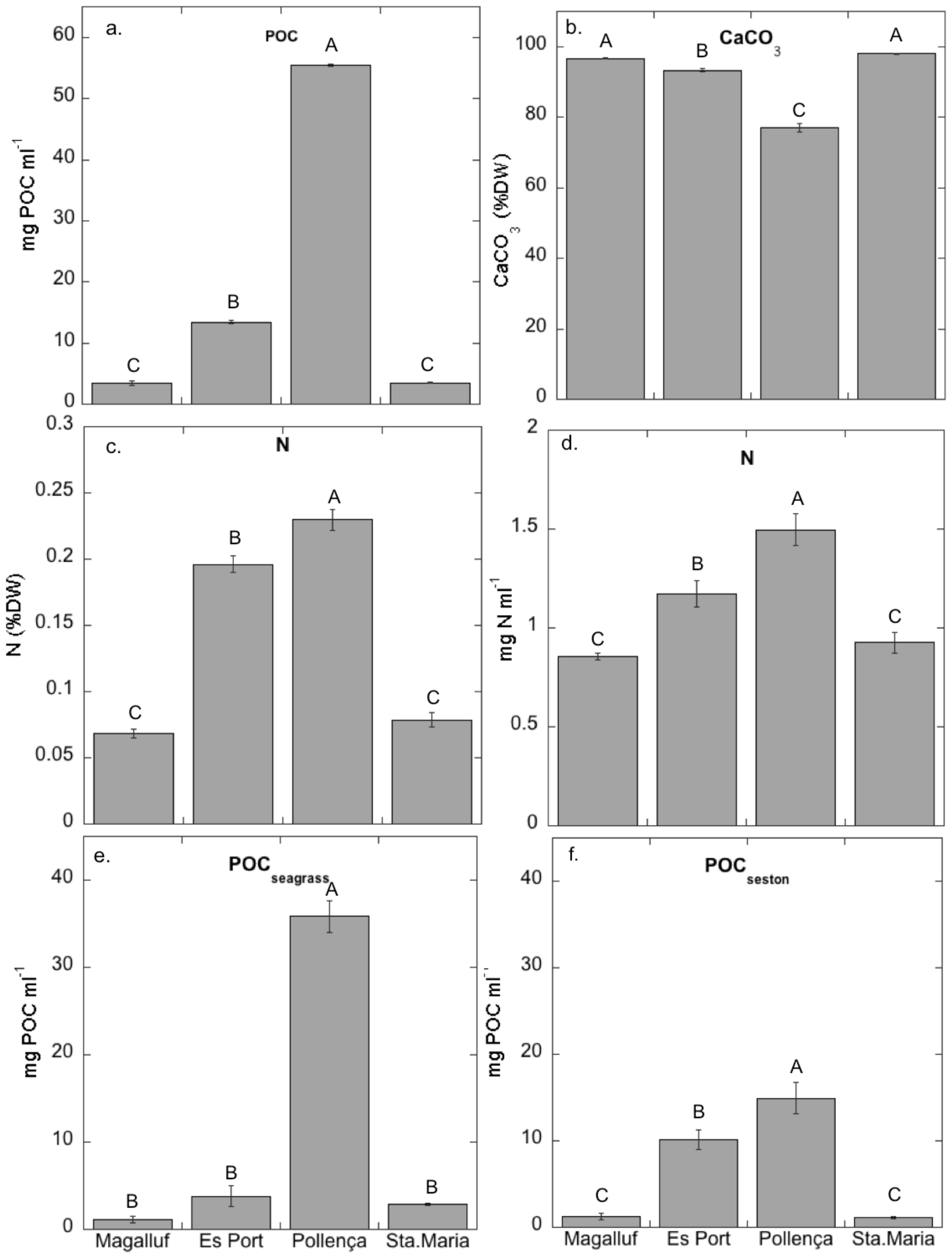


Figure 5.1. Initial biogeochemical conditions (t_0) (Average ± SE) in the sediments used for the experiment. Different letters indicate significant differences between sediment pools (Tuckey-Kramer test, α=0.05). a)POC, particulate organic carbon stock (mg ml^{-1}); b) $CaCO_3$, calcium carbonate concentration (%DW); c)N, nitrogen concentration (%DW); d)N, nitrogen stock (mg ml^{-1}); e)POC$_{seagrass}$, seagrass-derived organic carbon stock (mg ml^{-1}) and f)POC$_{seston}$, seston-derived organic carbon stock (mg ml^{-1}).

The seagrass sediments incubated also differed in their carbon isotopic signature ($\delta^{13}C$), with Pollença sediments showing a significantly heavier $\delta^{13}C$ signature (-14.2 ± 0.2 %) compared to the other meadows that ranged from -16.5 ± 0.3 % in Magalluf to - 17.3 ± 0.1 % in Sta. Maria (ANOVA, $p<0.0001$, Figure SI 5.2a). The isotope-mixing model revealed significant differences (ANOVA, $p<0.0001$) in the contribution of carbon sources to the initial sediment carbon stocks across meadows (Figure SI 5.2b,c). The largest contribution of seagrass-derived organic carbon to the total POC stock was found in the sediments from Sta. Maria and Pollença (70.9 ± 6.1% and 70.7 ± 3.5 % respectively), whereas the largest contribution of seston-derived organic carbon was found in Es Port (72.3 ± 9.1 %). Sediments at Pollença had the largest initial stock of POC derived from seagrass (35.9 ± 3.4 mg $POC_{seagrass}$ ml^{-1}) compared to that in the other meadows, which were not significantly different among each other (1.16 – 3.82 mg $POC_{seagrass}$ ml^{-1}, ANOVA, $p<0.0001$; Figure 5.1e). The largest initial sestonic POC stock was also found in Pollença sediments (14.9 ± 1.8 mg POC_{seston} ml^{-1}), followed by those at Es Port (10.2 ± 1.1 mg POC_{seston} ml^{-1}), being ten times larger than the stocks in sediments from Magalluf and Sta. Maria (1.30 ± 0.4 and 1.18 ± 1.12 mg POC_{seston} ml^{-1}) (ANOVA, $p<0.0001$; Figure 5.1f).

The organic matter stock (mg OM ml^{-1}) measured in the experimental units tended to decrease with time in all the sediment pools examined, except for that from Es Port, which did not show a significant OM decay over time (Figure 5.2, Table 5.1). The highest OM decay rate was found in the sediments from Pollença (-0.20 ± 0.07 % d^{-1}), followed by those in Sta. Maria (-0.06 ± 0.02 % d^{-1}) and Magalluf (-0.05 ± 0.02 % d^{-1}) (Figure 5.2, Table 5.1). The decay rates of POC stocks with time were statistically weaker than those of OM, as only the sediments from Pollença and Sta.Maria showed a statistically significant POC decay rate with time (-0.22 ± 0.07 % d^{-1}, $R^2=0.30$, $p=0.0044$; and -0.19 ± 0.07 % d^{-1}, $R^2=0.24$, $p=0.0139$, respectively, Table 5.1).

DOC values along the experiment (0.03-0.29 mmol C L^{-1}) were at least two orders of magnitude lower than POC values (5-342 mmol C L^{-1}), indicating that leaching of POC as DOC was negligible and that changes in POC were due to remineralization.

The least-square regression analysis revealed a significant effect ($p<0.0001$) of the initial sediment biogeochemical conditions considered, in the decay of the OM stock along the experiment, except for the contribution of the carbon sources to the total initial C sediment stock (i.e.% seston and % seagrass; $p > 0.05$, Table 5.2). The model that explained the largest variability in the change in the OM stock with time was that considering the N initial stock (mg N ml^{-1}, $R^2=0.83$) followed by those considering initial $CaCO_3$ concentration (%DW) ($R^2=0.82$) and the total and seston-derived initial organic carbon stocks (POC and POC_{seston}, $R^2=0.81$). The models considering the initial N concentration (%DW) and seagrass-derived POC stock explained a lower percentage of the variability in OM over time and across meadows ($R^2=0.74$).

The significant ($p=0.01$) and negative interaction term between time and initial POC stock indicates that the OM stock decayed faster with time with increased initial sediment POC stock (Table 5.2). Similarly, the OM stock declined faster over time for sediments with high initial stocks of seston-derived POC (Table 5.2). The significant ($p=0.017$) and negative interaction term between time and initial N stock indicate that the OM stock declined faster with time with increased initial N stock in the sediment ($p=0.017$; Table 5.2). In contrast, the OM stock declined slower with time with increased initial $CaCO_3$ concentration (% DW), as indicated by a significant ($p=0.0096$) and positive effect of the interaction term between time and initial $CaCO_3$ concentration (%DW, Table 5.2).

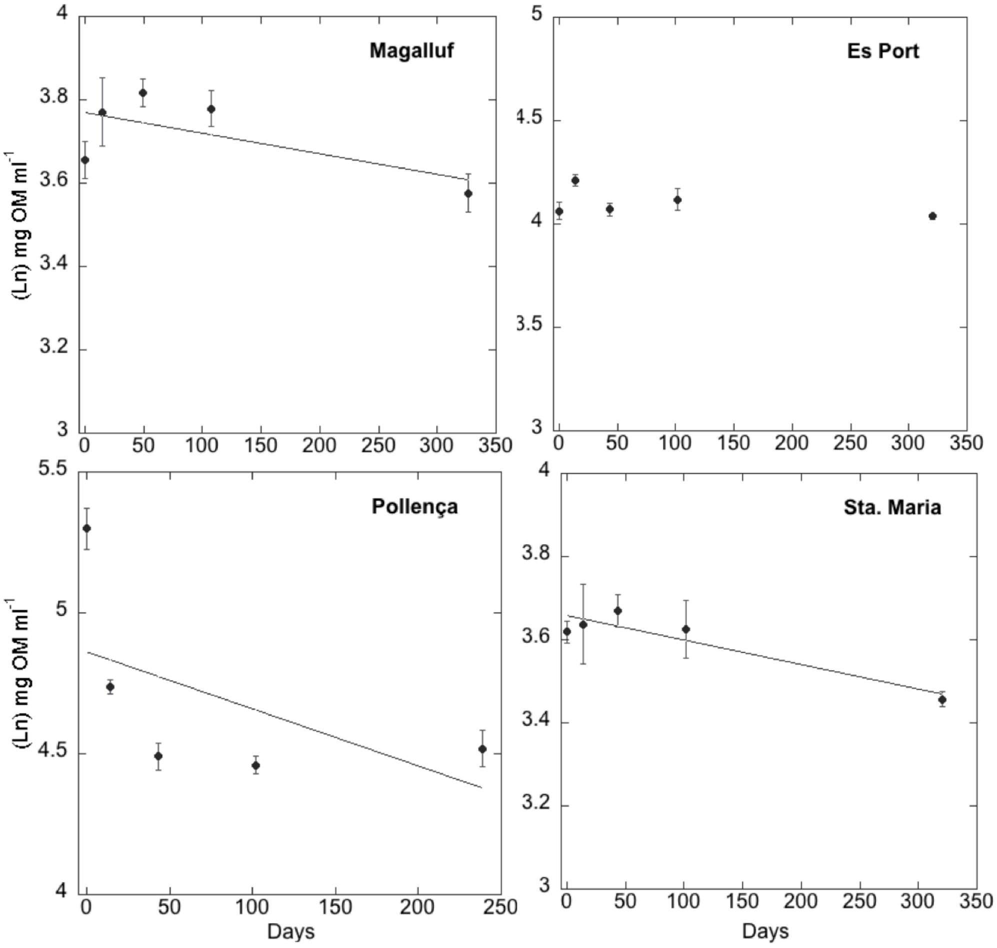


Figure 5.2. Organic matter (mg OM ml^{-1}), Ln transformed, against time (days) per meadow sediment suspensions. Solid lines represent the fitted equations obtained by least square analysis for sediments where a significant change in OM stock along time was found. Model parameters are provided in table 5.1.

Table 5.1. Model parameters estimates, coefficient of determination (R^2) and significant level (p-value) obtained by least-square regression analysis between OM and POC stocks (mg ml-1), Ln-transformed, and time (days) per station. Models are of the form of Ln(OM or POC)= Intercept (± SE) – Slope (± SE) x days. (*) indicates a significant change ($p < 0.05$) of OM or POC with time.

Station	**n**	**(Ln) mg OM ml^{-1}**				**(Ln) mg POC ml^{-1}**			
		Intercept	**Slope**	**R^2**	**p-value**	**Intercept**	**Slope**	**R^2**	**p-value**
Magalluf	25	3.77 ± 0.034	-0.00049 ± 0.00022	0.18	0.034*	1.64 ± 0.12	-0.00066 ± 0.00082	0.029	0.42
Es Port	25	4.12 ± 0.024	-0.00026± 0.00016	0.11	0.11	2.69 ± 0.029	-0.00029 ± 0.00019	0.094	0.14
Pollença	25	4.86 ± 0.08	-0.0020 ± 0.0007	0.28	0.006*	3.54 ± 0.083	-0.0022 ± 0.0007	0.3	0.0044*
Sta.Maria	25	3.656 ± 0.03	-0.0006 ± 0.0002	0.25	0.01*	1.35 ± 0.109	-0.00193 ± 0.0007	0.24	0.0139*

Table 5.2. Results of the models obtained by least-square regression analysis between OM stock (Ln mg OM ml^{-1}) vs. time (days), the initial value of different biogeochemical variables and the interaction between days*biogeochemical variable. Models are of the form of: Ln(OM) = a +b_1*days+b_2*biogeoch.variable +b_3*(days * bigeoch.variable). (*) indicates a significant effect ($p < 0.05$) in the OM stock.

Biogeochemical variable tested	**Overall model effect**	**Term**	**Effect estimate**	**p-value**
Initial POC stock (mg POC ml^{-1})	R^2=0.81	intercept	3.74 ± 0.034	<0.0001*
	p<0.0001*	days	-0.00081 ± 0.0002	<0.0001*
		POC stock	0.0187 ± 0.00099	<0.0001*
		days* POC stock	-0.00003 ± 0.00001	0.01*
Initial CaCO3 (%DW)	R^2=0.82	intercept	8.48 ± 0.22	<0.0001*
	p<0.0001*	days	-0.00081± 0.00019	<0.0001*
		CaCO3 (%DW)	-0.048 ± 0.0025	<0.0001*
		days*CaCO3 %DW	0.00007 ± 0.00003	0.0096*
Initial $POC_{seagrass}$ (%)	R^2=0.076	intercept	3.97 ± 0.15	<0.0001*
	p>0.05	days	-0.00098 ± 0.00042	0.0219*
		% $POC_{seagrass}$	0.27 ± 0.25	0.29
		days* %$POC_{seagrass}$	-0.0031 ±0.0023	0.185
Initial POC_{seston} (%)	R^2=0.076	intercept	4.24 ± 0.13	<0.0001*
	p>0.05	days	-0.00098 ± 0.00042	0.0219*
		% POC_{seston}	-0.27 ± 0.25	0.29
		days*% POC_{seston}	0.0031 ± 0.0023	0.18
Initial $POC_{seagrass}$ stock (mg ml^{-1})	R^2=0.74	intercept	3.81 ± 0.04	<0.0001*
	p<0.0001*	days	-0.00084 ± 0.00023	0.0004*
		$POC_{seagrass}$ stock	0.026 ± 0.0017	<0.0001*
		days*$POC_{seagrass}$ stock	-0.00005 ± 0.00002	0.014*
Initial POC_{seston} stock (mg ml^{-1})	R^2=0.81	intercept	3.63 ± 0.04	<0.0001*
	p<0.0001*	days	-0.0008 ± 0.0002	<0.0001*
		POC_{seston} stock	0.07 ± 0.004	<0.0001*
		days*POC_{seston} stock	0.00007 ± 0.00003	0.0474*
Initial N (%DW)	R^2=0.74	intercept	3.31 ± 0.06	<0.0001*
	p<0.0001*	days	-0.0008 ± 0.0002	0.0005*
		N (%DW)	5.55 ± 0.34	<0.0001*
		days* N (%DW)	-0.0052 ± 0.0032	0.1091
Initial N stock (mg N ml^{-1})	R^2=0.83	intercept	2.27 ± 0.09	<0.0001*
	p<0.0001*	days	-0.0008 ± 0.0002	<0.0001*
		N stock	1.65 ± 0.08	<0.0001*
		days*N stock	-0.0019 ± 0.0008	0.0174*

5.4. Discussion

The experimental results obtained demonstrate that seagrass sediments organic matter and carbon deposits are vulnerable to remineralization and to be released as CO_2 due to sediment erosion and subsequent suspension. Yet, the results of the least square regression analyses reveal that remineralization rates are dependent on intrinsic-sediment biogeochemical properties and carbon deposits characteristics.

In particular, the decrease in the stock of OM in the suspended sediments was accelerated in sediments with high N stocks according to the general pattern towards increased detritus remineralization rates with increased nitrogen content described by Enriquez et al. (1993). High sediment POC stocks, and particularly high seston-derived POC stocks, also led to faster OM remineralization rates, consistent with the higher lability of seston-derived detritus compared to that of the generally more refractory seagrass detritus (Enriquez et al. 1993; Banta et al. 2004). Previous studies conducted in mangroves and seagrass sediments also reported higher organic carbon remineralization rates in organic-rich sediments and in those with a high contribution of seston-derived carbon, respectively (Holmer et al. 2004; Lovelock et al. 2011). For the contrary, remineralization rates of sediment organic matter declined with increased sediment carbonate content, suggesting a possible role of carbonate deposition in enhancing the burial efficiency of organic carbon in seagrass sediments, which might be explained through different mechanisms. Calcium carbonate crystals may inhibit remineralization by physically protecting OM particles (Burdige 2007) that, in biogenic carbonate sediments, tend to be associated to carbonate minerals through intracrystalline interactions or surface sorption processes, reducing the accessibility to microorganisms and decomposers (Suess 1973; Bock and Mayer 2000; Ingalls et al. 2004). In addition, nutrient availability for microorganisms tend to be limited in sediments with a high carbonate content due to biogeochemical sorption processes between the nutrients and the mineral particles (Short 1987; Ferdie and Fourqurean 2004), which may slow down microbial activity and therefore organic matter remineralization (Melillo et al. 1984; Harrison 1989).

The loss of seagrass C deposits through remineralization due to erosion and subsequent suspension had not been described before. Hence, previous estimates on potential CO_2 emissions from sediments following seagrass loss were based on top meter POC stocks and chained two assumptions: (1) that the POC stock within the top meter of the sediment was vulnerable to loss, and (2) that a fraction (25-100%) of the POC lost would be eventually remineralized (Pendleton et al. 2012; Fourqurean et al. 2012). The partial erosion of the sediment carbon stocks following seagrass loss has already been proved, although it is likely to affect over less than one meter (Macreadie et al. 2015; Marbà et al. 2015). However, the assumption that the lost stock would be partially or entirely remineralized had not been tested to date. The decay rate of POC obtained in the resuspended sediments from Pollença (-0.0022 d^{-1}) and Sta. Maria (-0.0019 d^{-1}), the only two sediments where a significant decay of POC over time could be detected, indicate a minimum half-life of particulate organic carbon in suspension of 315 and 364 days respectively, which means that only approximately 50% of the POC initial stocks were remineralized and emitted as CO_2 after nearly one year of being suspended in aerobic conditions. Taking into account that the suspension of particles in coastal systems is likely to be limited to a much shorter time period (i.e. 30 days for the open ocean, Lande and Wood, 1987), these results suggest that organic carbon particles suspended from disturbed seagrass sediments are likely to re-deposit elsewhere after experimenting little decomposition.

Hence, the results presented in this study indicate that the erosion and suspension of seagrass sediments leads to the remineralization of the previously buried carbon deposits but that the loss of carbon stocks through remineralization is limited to a fraction of the sediment deposits (maximum of 50%) and depends on intrinsic-sediment biogeochemical properties, with remineralization rates being higher in highly organic sediments (especially if enriched with seston-derived carbon) with high nitrogen content and low carbonate concentration.

The input of nutrients and allochthonous carbon into seagrass sediments is enhanced in coastal areas under a high human influence (Bowen and Valiela 2001; Duarte 2002; Holmer et al. 2007). In addition, several studies have reported an increase in total and seston-derived organic carbon (i.e. microalgae) buried in seagrass sediments during the last century associated with an increasing trend in anthropogenic pressure in coastal areas (Macreadie et al. 2012; Mayr et al. 2014). Hence, the risk of remineralization of resuspended seagrass C deposits may be increasing in areas under a high human influence, where, in addition, seagrass loss and physical impacts to the seabed (e.g. anchoring and trawling) are more frequent (Short and Wyllie-Echeverria 1996; Duarte 2002). In this context, the development of "*Blue Carbon*" strategies targeting the conservation of seagrass meadows and their sedimentary carbon stocks become even more urgent.

Acknowledgments.

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Supplementary Information
(Chapter 5)

Figure SI 5.1. Organic Matter (OM, %DW) estimated from LOI data vs. POC (%DW) measured by Elemental Analyser - Isotope Ratio Mass Spectrometry (EA-IRMS). The linear regression analysis revealed a significant relationship ($p < 0.0001$) between MO (%DW) and POC (%DW): $y = 0.29\ (\pm\ 0.005)x - 0.64\ (\pm\ 0.61)$, n=60, ($R^2$=0.98).

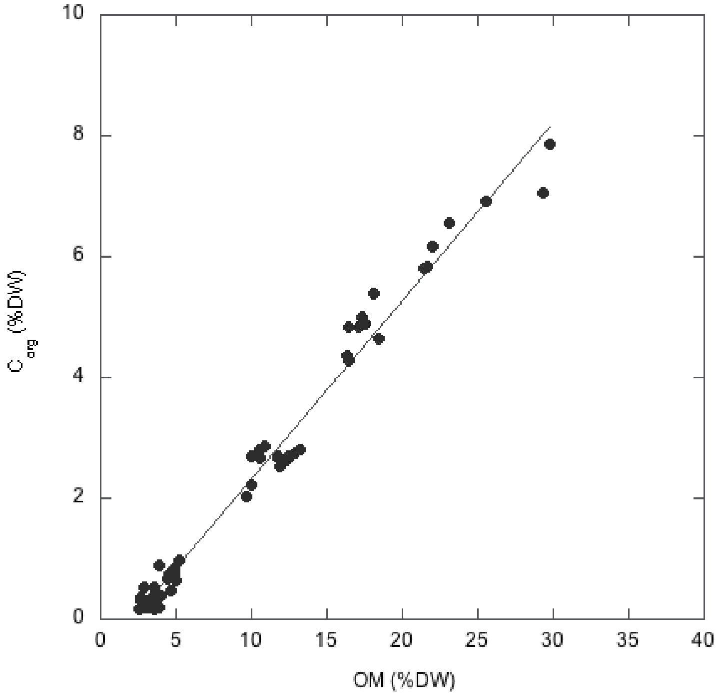

Figure SI 5.2. a) Organic carbon isotopic signature ($\delta^{13}C$), and source contribution (%) of b) seagrass-derived organic carbon and c) seston-derived organic carbon, to total POC stock at the beginning of the experiment in the different meadows sediment pools.

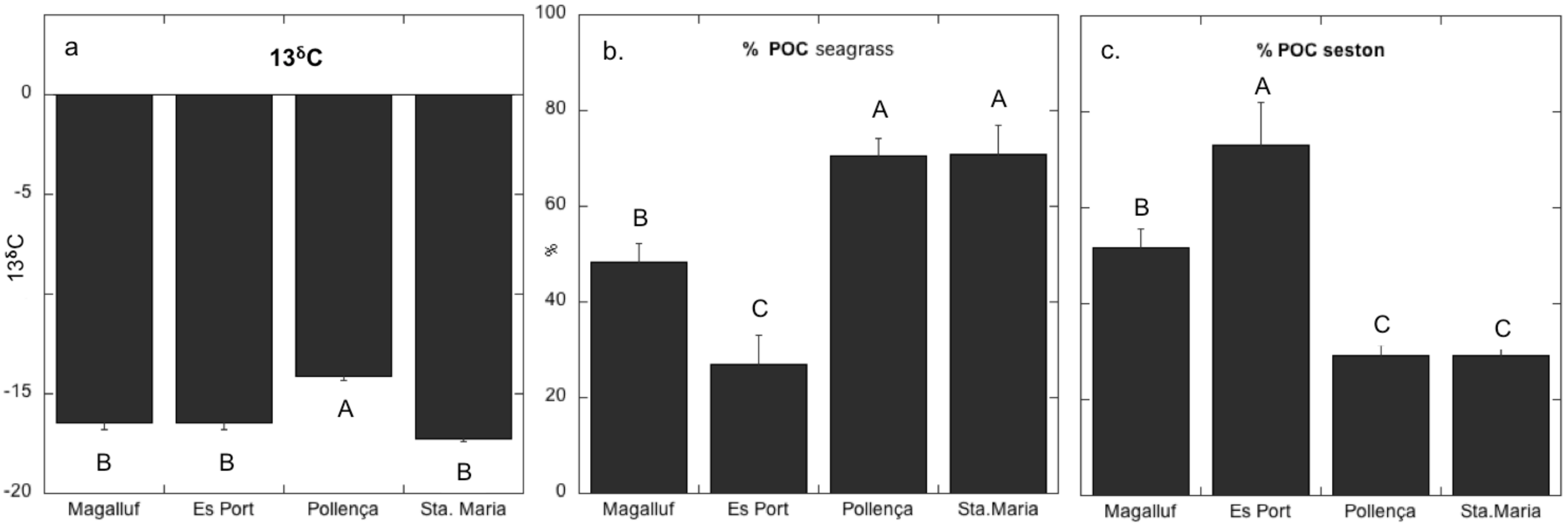

Table SI 5.1. Organic carbon isotopic signature ($\delta\ ^{13}C$) reference values of the carbon sources used for the two-component isotope mixing models. . The $\delta^{13}C_{seagrass}$ values correspond to the average ± SE $\delta^{13}C$ measured in 4 replicated leaves and 3 replicated rhizomes sampled in each meadow during the years 2008 and 2009 whereas the $\delta^{13}C_{seston}$ values correspond to single measurements from the study by Papadimitriu et al. (2005).

Meadow	**Seagrass** **$\delta^{13}C$ ± SE**	**Seston** **$\delta^{13}C$**
Es Port	-12.4 ± 0.7	-18
Magalluf	-11.53 ± 0.6	-21.1
Pollença	-10.94 ± 0.6	-21.90
Sta.Maria	-14.41 ± 1.08	-23.6

Chapter 6

Carbonate deposition increases carbon burial efficiency in seagrass sediments

Abstract

Seagrass meadows are active ecosystems in calcium carbonate ($CaCO_3$) precipitation and dissolution and have been identified as significant carbonate sedimentary reservoirs. As $CaCO_3$ precipitation leads to CO_2 release, the accumulation of $CaCO_3$ in the sediment has been interpreted to constrain the role as carbon sinks that seagrass meadows are considered to play. For the contrary, carbonate accumulation may enhance organic carbon burial efficiency in seagrass meadows by physically protecting organic matter from the action of decomposer organisms and by enhancing anoxic conditions, therefore preventing organic matter remineralization. This study assess the effect of carbonate accumulation in the decay rates of seagrass derived organic matter through a nine-months experiment where fresh seagrass leaves were incubated in aerobic conditions under four different treatments representing a gradient of $CaCO_3$ concentration from 17% to 85 %DW. Organic matter decay rates ranged between -0.07 ± 0.02 %DW d^{-1} to -0.003 ± 0.02 %DW d^{-1} and tended to decrease as the initial $CaCO_3$ (%DW) concentration in the different treatments increased, supporting the hypothesis that carbonate accumulation in seagrass meadows enhances organic carbon burial efficiency by preventing organic matter remineralization.

6.1. Introduction

Seagrass meadows have been identified as key ecosystems for climate change mitigation due to their capacity to act as globally-relevant natural carbon sinks, by sequestering and storing large amounts of organic carbon (C_{org}) in sedimentary stocks (Duarte et al. 2005, Fourqurean et al. 2012; Duarte et al. 2013a). Based on the results obtained in Chapter 3, seagrass meadows have also been identified as globally-significant reservoirs of carbonate ($CaCO_3$), that accounts for about half of the dry weight in surface seagrass sediments (Mazarrasa et al. 2015c).

Carbonates in seagrass sediments are predominantly of biogenic origin, deposited from water column calcification or that by seagrass epiphytes (Frankovich and Zieman 1994; Gacia et al. 2003; Perry and Beavington-Penney 2005). Carbonate precipitation in shallow waters leads to the release of CO_2 to the atmosphere in a molar ratio (Ψ), CO_2 flux: $CaCO_3$ precipitation, of about 0.63 (Smith 2013). Hence the accumulation of $CaCO_3$ in seagrass sediments has been interpreted as a reduction of the capacity of seagrass meadows to act as carbon sinks (Mateo and Serrano 2012; Serrano et al. 2012). However, this interpretation is inconsistent with the net CO_2 sequestration balance measured in seagrass meadows when both organic and inorganic metabolic cycles are taken into account (Barron et al. 2006; Maher and Eyre 2011; Tokoro et al. 2014) and with subsaturated CO_2 concentrations measured in surface water over seagrass meadows (Gazeau et al. 2004).

As carbonates are an important component of the carbon cycle and represent a major contribution to the carbon stocks in seagrass meadows, assessing the capacity of seagrass as carbon sinks demands an improved understanding of the role carbonates may play in the storage and preservation of organic carbon in seagrass sediments. Whereas C_{org} and $CaCO_3$ accumulation in seagrass sediments are generally examined as separate, parallel processes, these two components of the carbon budget of seagrass meadows may interact. In particular, organic matter tends to get closely associated with $CaCO_3$ particles either during the mineralization process or by surface sorption (Suess 1973; Bock and Mayer 2000; Ingalls et al. 2004), becoming less accessible to microorganisms (Bock and Mayer 2000; Ingalls et al. 2004). The organic matter-mineral interactions seem to involve a physical protection in small mesospores where organic matter gets protected from bacteria exoenzymes (Mayer 1994; Burdige 2007) which may prevent its remineralization and enhance its preservation. This hypothesis is consistent to some observational and experimental evidences provided by previous studies. For intance, Mayer (1994) reported a significantly greater C_{org} concentration and preservation in carbonate-rich sediments compared to a broader spectrum of sediments and soils from different environments. Kenworthy and Thayer (1984) found a slightly higher decay in seagrass material buried in organic rich sediments compared to that buried in sandy sediments towards the end of a long-term litter-bag incubation experiment, although this difference was not significant likely due to short incubation times. In addition, results in Chapter 5 showed that the remineralization of resuspended sedimentary seagrass C_{org} deposits decreases with increasing $CaCO_3$ sediment concentration. The anoxic conditions existing within the surface or inter-mineral mesospores would also reduce organic matter remineralization rates (Burdige 2007) as, although both aerobic and anaerobic decay in seagrass tissues have been documented, (Harrison 1989) refractory components of organic matter, such as lignin, are not known to decompose under anaerobic conditions (Hackett et al. 1977; Ziekus 1981). Moreover, carbonate deposition supports the bulk, 51 %DW on average, of sediment accretion in seagrass meadows (Chapter 3, Mazarrasa et al. 2015c), helping bury C_{org} faster below the oxic sediment layer and thereby enhancing its preservation.

Here we test the hypothesis that high carbonate loads enhance C_{org} burial efficiency in seagrass meadows. We did so through a nine-month experiment where leaf material collected from the same *Posidonia oceanica* meadow was incubated in oxic conditions under different carbonate loads.

6.2. Methods

We prepared four different treatments using a mixture of fresh seagrass (*P.oceanica*) leaves sampled from the beach of S'Estanyol (39°21'21.47"N 2°54'59.97"E) and sand from Sa Rapita beach (39°21'43.19"N, 2°57'35.02"E) in Mallorca (The Balearic Islands, Western Mediterranean) where sediments are dominated by biogenic carbonate (Alonso et al. 1988).

Sand was burned at 550ºC during 4 h to remove organic matter material and keep the $CaCO_3$ fraction. Three subsamples of seagrass leaves were dried at 60 ºC to estimate the conversion factor between dry weight/fresh weight (DW/FW=0.22 ± 0.01).

Four treatments (Control, Medium Carbonate, High Carbonate and Very High Carbonate) were prepared by mixing wet seagrass leaves, cut in small pieces (~2-3 cm), and burned sand, in the following proportions (Table SI 6.1): Control (C, 100 %DW seagrass leaves), Medium Carbonate (MC, ~11 %DW seagrass leaves + 89 %DW burned sand), High carbonate (HC, ~4% DW seagrass leaves + 96 %DW burned sand), and Very high carbonate (VHC, 0.76 %DW seagrass leaves + 99.24 % DW burned sand). Three subsamples per treatment were kept for analysis of initial (t_0) OM (%DW) and $CaCO_3$ (%DW) concentration. Then, twenty experimental units per treatment were prepared by filling 20 falcon tubes up to approximately 10 ml with 7.23 ± 0.13 g (for the VHC), 5.34 ± 0.018 g (for the HC), 3.57 ± 0.061 g (for the MC) and 1.74 ± 0.02 g (for the Control) of sediment mixture, thereby containing the same volume of initial material and therefore the same height of sediment in the experimental container, with different concentration of OM and $CaCO_3$.

Falcon tubes were filled with seawater from S'Estanyol beach, individually closed with a net to avoid loss of material, and placed vertically in a seawater tank where aerobic conditions were maintained by bubbling through two silicon tubes. The experimental units were maintained in dark conditions for a maximum of 280 days. Five experimental units per treatment were sampled at geometrically-distributed time intervals, following a logarithmic distribution: t_1: 25 days, t_2: 88 days, t_3: 176 days and t_4: 280 days, as an exponential decay of the organic matter was expected (Enriquez et al. 1993; Kristensen 1994).

After sampling, the dry weight (after 36-48 h at 60ºC) of the material remaining in each experimental unit was measured. OM (%DW) was estimated through LOI techniques as the fractional weight loss of the dry sample after combustion at 500ºC during 4 hours. $CaCO_3$ (%DW) for the MC, HC and VHC treatment replicates was estimated from PIC (% DW) measurements assuming that PIC is 12% of the total $CaCO_3$ molar mass. PIC was estimated as the total C (%DW) measured with a CHN analyzer in samples pre-combusted at 500ºC during 4 hours (Fourqurean et al. 2014). For the Control replicates (containing seagrass leaves only), $CaCO_3$ (%DW) was estimated from the weight loss of the resulting ashes obtained from LOI after combustion at 1000ºC for 1 hour (Gacia et al. 2003).

Statistical analysis

We conducted ANOVA and Tukey-Kramer HSD analysis to test significant differences among treatments initial conditions of $CaCO_3$ (%DW) and OM (%DW). Organic matter decay rate was estimated as the slope of the curve obtained by fitting the OM concentration (%DW) vs. time (days). The relationship between initial concentration of $CaCO_3$ (%DW) and OM (%DW) and the OM decay rate identified in each treatment was estimated through linear regression analysis.

6.3. Results & Discussion

The treatments differed significantly with respect to OM (%DW) and $CaCO_3$ (%DW) at the beginning of the experiment (t_0) (ANOVA, $p<0.0001$, Table 6.1). Control treatments showed a significantly higher OM content (%DW) (73.4 ± 1.8 % DW) followed by the MC treatment (10.8 ±2.1) that showed the second significantly largest OM content (%DW) compared to HC (5.4 ± 0.8 %DW) and VHC (2.2 ±0.3 %DW). Conversely, the Control treatment showed the lowest $CaCO_3$ concentration (17.8 ± 0.6 %DW) followed by the MC treatment (76.5 ± 2.53 %DW) and the HC and VHC treatments (81.72 ± 0.65 and 86.5 ± 0.09 %DW, respectively) (Table 6.1).

Table 6.1: Mean ± SE initial organic matter (OM %DW) and carbonate ($CaCO_3$ %DW) content measured in the treatments at the beginning of the experiment. Different letters indicate significant differences among treatments (Tukey-Kramer test α= 0.05).

Treatments	**OM (%DW)± SE**	**$CaCO_3$ (%DW)**
VHC, Very high $CaCO_3$	2.19 ± 0.29$_{C}$	86.55 ± 0.09$_{A}$
HC, High $CaCO_3$	5.4 ± 0.79$_{BC}$	81.72 ± 0.65$_{AB}$
MC, Medium $CaCO_3$	10.79 ± 2.13$_{B}$	76.55 ± 2.53$_{B}$
Control	73.39 ± 1.75$_{A}$	17.82 ± 0.65$_{C}$

Organic matter tended to decrease with time in all the treatments at average decay rates of -0.07 ± 0.02 % OM d^{-1}, $^{-}$0.0054 ± 0.0059 % OM d^{-1}, $^{-}$0.005 ± 0.002 % OM d^{-1}, and -0.0027 ± 0.02 % OM d^{-1} for the control, MC, HC and VHC treatments, respectively (Table 6.2, Figure 6.1). Only for the Control and HC treatments the decay was statistically significant (Table 6.2) whereas for the MC the decay was not significant due to high variability of the data at t_3 (Figure 6.1).

Table 6.2. Results of the linear regression analysis between OM (%DW) and days per treatment.

Treatments	**Slope ± SE**	**R^2**	**p-value**	**F-ratio**	**df**
VHC, Very high $CaCO_3$	-0.0027 ± 0.002	0.05	0.28	2.28	22
HC, High $CaCO_3$	-0.005 ± 0.002	0.24	0.0185*	3.12	22
MC, Medium $CaCO_3$	-0.005 ± 0.006	0.039	0.4	0.96	22
Control,	-0.07 ± 0.02	0.45	0.0015*	27.99	18

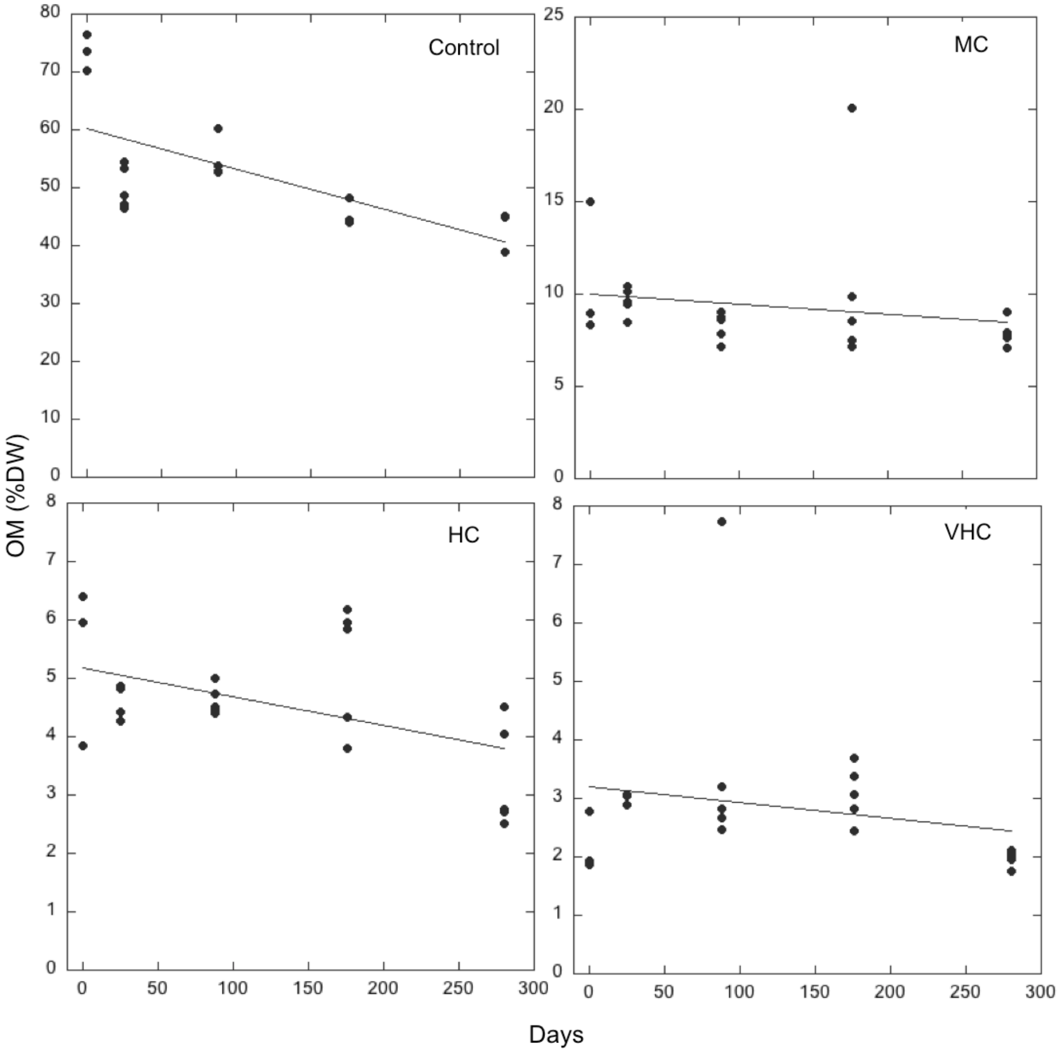

Figure 6.1. Change of organic matter concentration (OM %DW) vs. time (days) in the different treatments: Control; MC, Medium $CaCO_3$; HC, High $CaCO_3$ and VHC, Very High $CaCO_3$. Solid lines represent the fitted equations obtained by linear regression analysis (results reported in Table 6.2).

The decay rate of organic matter (*k*, %DW d^{-1}) measured in the different treatments significantly increased with increasing initial OM concentration (linear regression analysis; R^2= 0.99; p-value=0.0029, Figure 6.2a) and decreased significantly with increasing initial carbonate concentration (linear regression analysis; R^2=0.99; p-value=0.0045, Figure 6.2b).

These results support the hypothesis that the accumulation of $CaCO_3$ inhibits seagrass organic matter remineralization. In particular, organic carbon decay rates were 25 times slower under high carbonate load compared to the decay rate of the seagrass carbon in the absence of carbonates. This effect is likely explained by the physical protection that carbonate mineral particles exert to organic matter, which becomes less accessible to decomposer microorganisms and embedded in a more anoxic environment (Suess 1973; Bock and Mayer 2000; Ingalls et al. 2004; Burdige 2007). In addition, high rates of carbonate sedimentation in seagrass meadows may shorten the time needed for accumulated organic matter to reach the anoxic layer of the sediment, therefore favoring C_{org} burial efficiency. In fact, in marine sediments C_{org} burial rates tend to be enhanced in areas of high sedimentation rates as sediments become anoxic within few centimeters from the sediment surface (Henrichs 1992; Burdige 2007). For instance, considering the density of $CaCO_3$ and that of organic matter in *P. oceanica* sediments in the Baleraric Islands (90.4 ± 2 and 9.0 ± 1.6 %DW, respectively) their contribution to the volume of *Posidonia oceanica* sediments is, on average, 20 ± 0.9 % and 28 ± 4.5 %, with water representing the rest of the volume (52 ± 8 %). Assuming carbonate and organic sediments to have the same relative water volume, the sediment accretion in the absence of carbonate deposition (i.e. that supported by organic carbon deposition with the corresponding water) would be only 2.1 ± 0.1 mm year^{-1}, compared to the observed 3.64 ± 0.2 mm year-1, therefore demonstrating that carbonate deposition accounts 43.5 % of the sediment accretion in the *Posidonia oceanica* sediments studied here. This implies that organic matter would remain in the oxic layer 1.7 times longer than at the carbonate load observed in seagrass sediments in the Balearic Islands. A high load of carbonates in the sedimentation pool may also facilitate C_{org} burial by reducing bioturbation by feeding animals and nutrient availability (Short 1987; Ferdie and Fourqurean 2004) therefore reducing microbial activity and OM remineralization, opposite to what would be expected under a situation of organic rich sedimentation pool (Burdige 2007; Fanjul et al. 2015; Melillo et al. 1984).

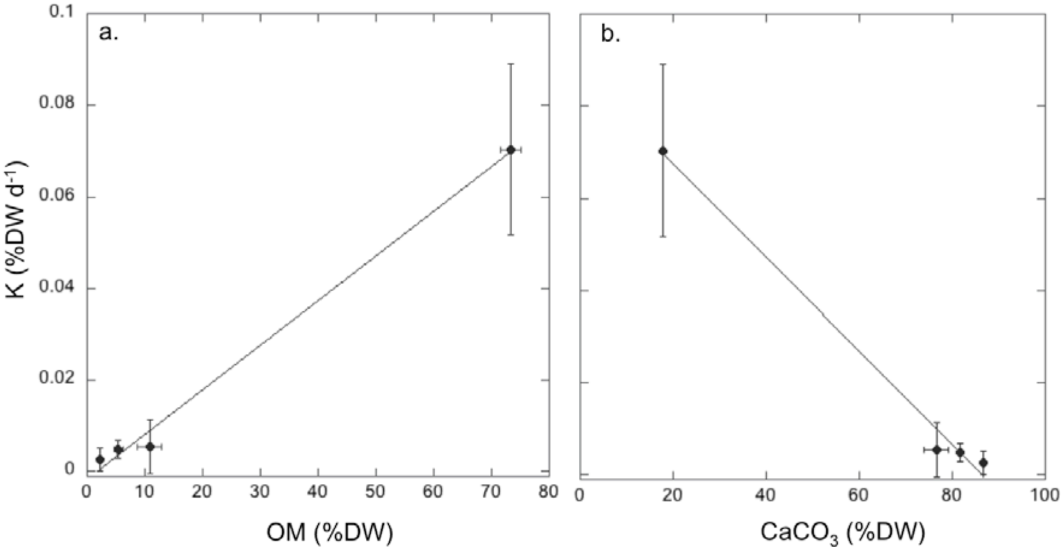

Figure 6.2. Relationship between a) initial OM (%DW) and b) initial $CaCO_3$ (%DW) and OM decay rate (k, %DW d^{-1}). Solid lines represent the fitted equations obtained by linear regression analysis: a) $y=-0.0015(\pm0.0019)+0.001(\pm5.22*10^{-5})x$ ($R^2=0.99$; p-value=0.0029) and b) $y=0.088(\pm0.005)-0.00102(\pm6.9*10^{-5})x$ ($R^2=0.99$; p-value=0.0045).

In conclusion, the results presented in this study support the presence of interactions between carbonate and organic carbon burial in seagrass sediments, suggesting that $CaCO_3$ precipitation is not only a source of CO_2 to the water-atmospheric pool, but may also enhance organic carbon burial. Specifically, our experiment showed a very large, 25-fold, effect of high carbonate on reducing organic carbon decay. Although this is the first such experimental evidence available, it is consistent with observations of increased organic carbon preservation in carbonate-rich sediments (Mayer, 1994). The results presented here suggest that the role of carbonate deposition on sediment carbon cycling should be reconsidered to address its potential effects in the burial efficiency of organic carbon.

Acknowledgments

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Supplementary Information
(Chapter 6)

Table SI 6.1. Treatments preparation: proportions and material used. Wet leaves mass was estimated using the DW/FW conversion factor measured for the same leaves pool (0.22 ± 0.01).

Treatments	**Proportions**		**Weight of material used**	
	Seagrass leaves (%DW)	**Burned sand (%DW)**	**Wet leaves (g)**	**Sand pre-burned at 500ºC (g)**
VHC, Very high $CaCO_3$	0.76	99.24	3.61	100.85
HC, High $CaCO_3$	4	96	17.87	90.04
MC, Medium $CaCO_3$	11	89	44.66	75.95
Control	100	0	178.50	0.00

IV

General Discussion

By the time this thesis was planned, the carbon sequestration capacity of seagrass meadows had been already recognized (Gacia et al. 2002; Duarte et al. 2005; Kennedy et al. 2010) and their burial capacity identified to be significant and comparable to that of terrestrial forests. Especially relevant were the C_{org} deposits described in *Posidonia oceanica* meadows in the Mediterranean Sea that may be, in some cases, thousands of years old (Mateo et al. 1997; Lo Iacono et al. 2008). Seagrass meadows are one of the most threaten ecosystems on earth, but since they play an important role acting as a natural carbon sink, there has been an enhanced willingness to promote their inclusion in conservation strategies for climate change mitigation, similar to those that already exist for land ecosystems. Yet, the suitability of seagrass meadows as candidates for the application of such strategies was constrained due to a lack of scientific understanding regarding certain aspects related to their function as carbon sinks (Duarte et al. 2013a). The three results sections of this thesis have contributed to fill some of these main gaps of knowledge.

IV.1. Carbon sinks: the effect of environmental factors and anthropogenic pressure on coastal areas during the last century

The first goal of this thesis was to "*assess the effect of human pressure and two environmental factors, wave exposure and meadow depth"*. The research conducted to address this first goal was based on an extensive survey of *Posidonia oceanica* sediment cores around the Balearic Islands, encompassing a broad range of meadow depths, wave exposures and levels of human pressure. Although focused on a single species, the research conducted to address this first goal (presented in Chapters 1 and 2) has led to important findings, likely extrapolated to meadows formed by other species, as it is discussed further bellow.

Through the results provided in Chapter 1, this thesis significantly increases the data on carbon stocks and burial rates that, especially in the case of burial rates, were scarce at the time this thesis was planned (Table I.1). During the last 5 years, the list of direct measurements of C_{org} burial rates in seagrass sediments has been significantly lengthened (Table IV.1). Yet, the number of total C_{org} burial rates is still scarce and biased towards meadows formed by *Posidonia species* and from Western Australia (WA) and the Western Mediterranean Sea (WM). This scarcity of burial rates is in contrast to the significant increase during the last years of the amount of data on C_{org} stocks in seagrass sediment worldwide (Fourqurean et al. 2012; Lavery et al. 2013; Alongi et al. 2015; Campbell et al. 2014; Miyajima et al. 2015; Samper-Villarreal et al. 2016), but it is likely explained by the increased cost of sediment dating.

More importantly, this thesis provides C_{org} burial rates for a more recent period (~ the last 100 years) compared to previous studies, which is particularly interesting as it is when major changes in coastal areas due to human activity have taken place (Duarte 2014). This has allowed for the assessment of the extent to which anthropogenic pressure affects the functioning of carbon sinks in seagrass meadows, especially relevant considering the declining status of seagrass meadows worldwide as a consequence of different impacts derived from human activities (Orth et al. 2006; Waycott et al. 2009).

Table IV.1. Compilation of direct measurements of C_{org} burial rates in seagrass sediments to date (WA: Western Australia; WM: Western Mediterranean).

Species	**Location**	**C_{org} burial rate (g C m^{-2} y^{-1})**	**Time scale (y)**	**Reference**
Posidonia oceanica	Ischia, Italy	19-191	~ 100-1000	Romero et al. 1994
Posidonia oceanica	Ischia, Italy	30	~ 3370	Mateo et al. 1997
	Culip, WM	9		
	Port Lligat, WM	75		
	Campello, WM	112.1		
	Tabarca, WM	61.7 - 104.2		
	Medas, WM	12.6		
Posidonia oceanica	Port Lligat, WM	21	~ 4300	Serrano et al. 2012
Zostera marina	Virginia, USA	36.4 ± 2.8	10	Greiner et al. 2013
Posidonia sinuosa	Cockburn Sound, WA	2.1-12.61	~ 500-900	Serrano et al. 2014
Posidonia oceanica	Talamanca, Balearic Is. WM	85.6		
	Es Pujols, Balearic Is. WM	140.1		
Posidonia australis	Oyster Harbour, WA	16.2 ± 2.4 - 25.2 ± 4.7	~ 120	Marbà et al. 2015
Zostera marina	Seto Inland Sea, Japan	3.13-10.14	~ 220-810	Miyajima et al. 2015
Enhalus acoroides	Ishigaki Island, Japan	5.37		
Enhalus acoroides / Thalassia hemprichii	Southern Thailand	2.44		
Posidonia australis	Oyster Harbour, WA	3.45	~ 3100	Rozaimi et al. 2016
Posidonia australis	Oyster Harbour, WA	12.3 ± 4.9	~ 600	Serrano et al. 2016
Posidonia oceanica	Pollença, Balearic Is. WM	52 ± 2	~ 100	This thesis (Chapter 1)
	Sta.Ponça, Balearic Is. WM	23 ± 2		
	Cala d'Or, Balearic Is. WM	20 ± 2		
	Portocolom, Balearic Is. WM	40 ± 3		
	Magalluf, Balearic Is. WM	9 ± 2		
	Es Port, Balearic Is. WM	29 ± 2		
	Es Castell, Balearic Is. WM	10 ± 1		
	Sta.Maria, Balearic Is. WM	20 ± 2		

The effect of human pressure

The *Posidonia oceanica* meadows considered in this thesis are, in most cases, located in areas under a relatively high level of anthropogenic pressure derived from a high urbanization and usage of the coast, intensified since the development of the tourism industry around the 60's (Rullan 1998; Murray et al. 2008). All the meadows considered have shown a decline in their shoot density for at least the last decade, likely resulting from the combination of enhanced eutrophication, usually derived from an intense human activity in coastal areas, and an increase in water temperature (Marbà and Duarte 2010; Marbà et al. 2014). In such a context, the carbon sink capacity of *Posidonia oceanica* meadows was expected to be negatively affected by human pressure, as it does negatively affect the shoot population. Yet, surprising and interesting results were found. As shown in Chapter 1 the highest C_{org} burial rates were measured in meadows under a significant level of human pressure whereas the lowest burial rates were found in the most pristine meadows. In addition, the C_{org} burial rates in most of the meadows tended to increase since human pressure in the region of study increased. Based on these results, a possible interpretation could have been that the carbon sink capacity in the seagrass meadows examined was enhanced under a high and increasing human pressure in coastal areas, although the general decay in the meadows shoot density for the last decade did not support this hypothesis.

The examination of the sources of C_{org} contributing to the sediment deposits presented in Chapter 2 shed some light in the results found in the previous chapter and raised new concerns about the consequences of human pressure in seagrass meadows carbon sinks. The main results of Chapter 2 show that the contribution of the two main sources of carbon to the sediment stocks considered, seagrass and seston derived organic matter, was related with the level of human pressure at each site. The largest contribution and total amount of sestonic derived- carbon was found in meadows under a relatively high human pressure whereas *Posidonia oceanica* derived organic matter was the main source of carbon to the sedimentary stocks in the most pristine meadows. In addition, a more rapid increase in the sestonic-derived C_{org} burial rate was found compared to that of *P.oceanica* during the last century, evidencing that the accumulation of seston was the main driver in the observed increase in C_{org} burial rate found in the meadows examined since human pressure increased, reported in Chapter 1.

Based on these results, the most parsimonious explanation to the trends observed seemed to be the eutrophication and increase in sestonic load in coastal waters derived from an increase in urbanization and human usage of the coast, as a consequence of the tourism industry development. Despite not having long-term data on water quality (i.e. chlorophyll *a* or seston concentration), eutrophication is known to be a usual phenomenon in areas under a high incidence of human pressure, including the Mediterranean Sea (Nixon 1995; Short and Burdick 1996; Bowen and Valiela 2001; Mateo et al. 2010). The hypothesis proposed to explain the trends observed is also supported by other studies that report an increase in the accumulation of microalgae in coastal sediments towards present associated to an increase in nutrient inputs derived from urbanization and/or human activities (Savage et al. 2010; Mayr et al. 2014). Similar observations have been recently reported in sediments from seagrass meadows along the East and Western Australian coasts (Macreadie et al. 2012; Serrano et al. 2016).

As suggested in the discussion of Chapter 2, an increase in the contribution of an allochthonous source of carbon, more labile than seagrass (e.g. seston), may decrease the long-term resilience of the sediment C_{org} deposits, especially when meadows are subject to disturbance. Macreadie *et al*. (2012) suggested that the increase in sestonic contribution towards present leads to a "weakening" of the seagrass sediment carbon deposits as they may become more prone to remineralization. Yet, this remained as a hypothesis that needed to be tested and that was resolved in Chapter 5 of this thesis.

The effect of environmental factors: water depth and wave exposure

Neither of the two environmental factors considered showed a clear effect on the C_{org} burial rates of the meadows examined. The C_{org} burial rates were expected to decrease as the meadow depth increases, consistent with the findings of Serrano et al. (2014), due to an expected reduction in seagrass primary production associated to a decrease in light availability with depth. Yet, as demonstrated in previous studies (Kennedy et al. 2010) and by the results in Chapter 2 of this thesis, the C_{org} burial rate in seagrass meadows does not only depend on seagrass derived organic carbon inputs but also, and significantly so, on allochthonous carbon that accumulates from the water column, which is independent of meadow depth. In addition, hydrodynamic forces tend to attenuate as meadow depth increases, leading to a more depositional-favorable environment, as sedimentation is enhanced and erosion and export are prevented. Recent studies found opposite results regarding to the effect of meadow depth in C_{org} stocks, that tended to increase (Lavery et al. 2013) or decrease (Samper-Villarreal et al. 2016) as the depth of the meadows increased. Therefore the effect of depth on the C_{org} burial rates remains unclear and needs to be further explored taking into account the potential interactions with other factors, such as the availability of other sources of carbon in the environment.

The second environmental factor assessed, exposure to wave energy, was a determinant factor in the patterns of sedimentation and erosion in the meadows examined as demonstrated by its relationship with the sediment grain size. Yet, it was not enough to explain the variability in the C_{org} burial rates observed across the studied meadows.

The characterization of the effect of environmental factors on the carbon sink capacity of seagrass meadows is highly relevant, especially from a management perspective, as it may help to identify the most suitable areas for Blue Carbon restoration and conservation projects (Mcleod et al. 2011). The first section of results of this thesis indicates that human influence, by triggering sestonic carbon availability in the water column, is a key factor driving carbon burial rates in seagrass meadows and seems to be more determinant than environmental factors, at least in regions under high levels of anthropogenic pressure.

IV.2. Inorganic Carbon deposits (PIC): Global distribution and environmental controls

The second main goal of this thesis was "*to quantify seagrass inorganic carbon (i.e. PIC) deposits globally and to examine the latitudinal variability of one of the main sources of PIC to seagrass sediment: the carbonate epiphyte load on seagrass leaves*".

The results presented in Chapter 3 reveal that seagrass meadows are significant sedimentary reservoirs of particulate inorganic carbon in the form of $CaCO_3$. PIC sediment deposits were found to be 5 times larger than the sedimentary POC deposits quantified in a previous global assessment by Fourqurean et al. (2012), and to vary across species and latitudes, tending to decrease from southern to northern regions. Similarly, the results presented in Chapter 4 demonstrate that the carbonate load and accumulation rate on leaves of *Zostera* genus is also latitude-dependent and tends to decrease polewards, consistent to the decrease in the carbonate saturation state of seawater when moving from southern, warmer regions to northern, colder latitudes.

As carbonate precipitation leads to the release of CO_2, the existence of such a magnitude of $CaCO_3$ deposits in seagrass meadows could be interpreted as an offset of the capacity these ecosystems have to act as carbon sinks (Mateo and Serrano 2012; Serrano et al. 2012). However, this thesis provides results that offer alternative interpretations of the role that $CaCO_3$ may play in the carbon sink capacity of seagrass meadows.

First of all, the comparison between the POC and PIC stocks in sediments from seagrass vegetated and adjacent un-vegetated sand patches showed that whereas PIC concentration (% DW) was comparable among patches, POC concentration (% DW) was around two times larger in vegetated than in adjacent un-vegetated patches. These results suggest that, although $CaCO_3$ accumulation rate in sediments is likely to be favored in seagrass patches through particle trapping and epiphyte accumulation, the formation of $CaCO_3$ sedimentary deposits depends on environmental factors, such as the carbonate saturation state of seawater and/or the presence of other carbonate producer communities, other than seagrass epiphytes, that usually coexist with seagrass meadows, especially in certain regions (i.e. corals). On the contrary, the formation of POC stocks in seagrass sediments is directly dependent on the presence of seagrass vegetation cover. Thereby, the ratio POC: PIC tended to be higher in vegetated versus un-vegetated patches, indicating that seagrass meadows indeed act as carbon sinks.

More importantly, a potential role of sediment $CaCO_3$ for enhancing the carbon sink capacity of seagrass meadows was proposed based on the fact that, in biogenic carbonate rich sediments, organic matter tends to associate with mineral particles reducing its exposure to decomposing organisms and to aerobic conditions (Suess 1973; Ransom et al. 1998; Bock and Mayer 2000; Ingalls et al. 2004; Burdige 2007). This hypothesis was subsequently verified with the two experimental studies conducted to address the third goal of this thesis, presented in Chapters 5 and 6.

IV.3. Vulnerability of carbon deposits to be lost through remineralization and potential interactions with accumulated $CaCO_3$

The third main goal of this thesis was to "*characterize the vulnerability of C_{org} sediment deposits to be lost through remineralization when sediments are disturbed and exposed to oxic conditions, as well as to assess the role that $CaCO_3$ may have in C_{org} burial efficiency*".

Land use change and degradation of terrestrial ecosystems, such as forests, is one of the most important sources of CO_2 to the atmosphere, contributing 12% of the anthropogenic emissions during the last two decades (Houghton et al. 2012). Hence, climate change mitigation strategies include mechanisms that enhance the protection of these ecosystems. The potential emission of CO_2 derived from the degradation of Blue Carbon ecosystems has been a keystone for the consideration of these ecosystems in the carbon emissions accounting frameworks and to enhance their protection as part of the climate change mitigation strategies.

Recent studies have demonstrated that the loss of seagrass meadows leads to a partial erosion of the sedimentary C_{org} deposits (Macreadie et al. 2015; Marbà et al. 2015). Yet, the fate of the carbon could not be resolved. The experiment presented in Chapter 5 demonstrates that sediment C_{org} deposits in seagrass meadows are vulnerable to remineralization and to be lost as CO_2 when suspended and exposed to oxic conditions. Furthermore, it demonstrates that this vulnerability varies across seagrass meadows depending on biogeochemical properties of the sedimentary carbon stocks. Organic matter remineralization was enhanced in sediments enriched with organic matter, particularly seston derived organic carbon, and with higher nitrogen content. On the contrary, the decay rate of organic matter was inhibited, as the carbonate content of the sediment was higher.

Beyond the demonstration that, indeed, seagrass sediment C_{org} deposits can be remineralized and released as CO_2 after erosion, the research presented in Chapter 5 is particularly useful to interpret some of the findings in previous chapters. It experimentally supports the hypothesis suggested in Chapter 2 and by Macreadie et al. (2012), that the enrichment of the seagrass sedimentary carbon stocks with sestonic organic matter leads to a "weakening" of the sediment carbon sinks, as they become more prone to remineralization. In addition, the results provided in Chapter 5 warn that not only the quantity but also the quality of the C_{org} accumulated matters in order to assess the role seagrass meadows play as long-term carbon sinks. For instance, in the particular case study of the *Posidonia oceanica* meadows around the Balearic Islands, the increase in the C_{org} burial rate observed since the rise in human pressure in the region, should not be interpreted as an enhancement of the carbon sink capacity of these meadows but as a sign of weakening and increased vulnerability. The weakening of seagrass carbon sinks associated to water eutrophication is likely to be occurring in many areas around the world considering the global distribution of seagrass ecosystems and the high human population and incidence of human activities in coastal areas, that are, in addition, expected to increase in the near future (Duarte 2014). Moreover, in coastal areas exposed to anthropogenic activities, the physical erosion of the seabed through boat anchoring, dredging or coastal construction is a frequent impact to seagrass meadows (Short and Wyllie-Echeverria 1996; Marbà et al. 2014), increasing even more the likelihood of loosing C_{org} deposits through remineralization as they are exposed to oxic conditions.

Additionally, the results obtained in Chapter 5 also suggest that sedimentary $CaCO_3$ suppresses organic matter remineralization. This hypothesis was further supported by results obtained in the experiment presented in Chapter 6 of this thesis, where organic matter was better preserved, as the concentration of $CaCO_3$ was higher. Based on these results, the accumulation of $CaCO_3$ in seagrass sediments should not be simply considered as an offset in their carbon sink capacity but, to the contrary, as a mechanism that enhances C_{org} burial efficiency and the resilience of sedimentary C_{org} deposits in the case sediments are eroded and exposed to oxic conditions.

IV.4. New advances and further challenges

During the last five years there has been a boom in the research efforts addressed to assess the role that seagrass meadows play as Blue Carbon ecosystems as well as to identify or develop mechanisms to enhance their protection and conservation within the current framework of climate change mitigation strategies (Hejnowicz et al. 2016; Thomas, 2014). This growing interest is well illustrated when conducting a simple search in Google Scholar using the terms "seagrass blue carbon" by year, that shows an increase in the number of result from 564 in 2008, before the publication of the Blue Carbon report by Nelleman et al. (2009), to up to 1110 in 2015 (Figure IV.1).

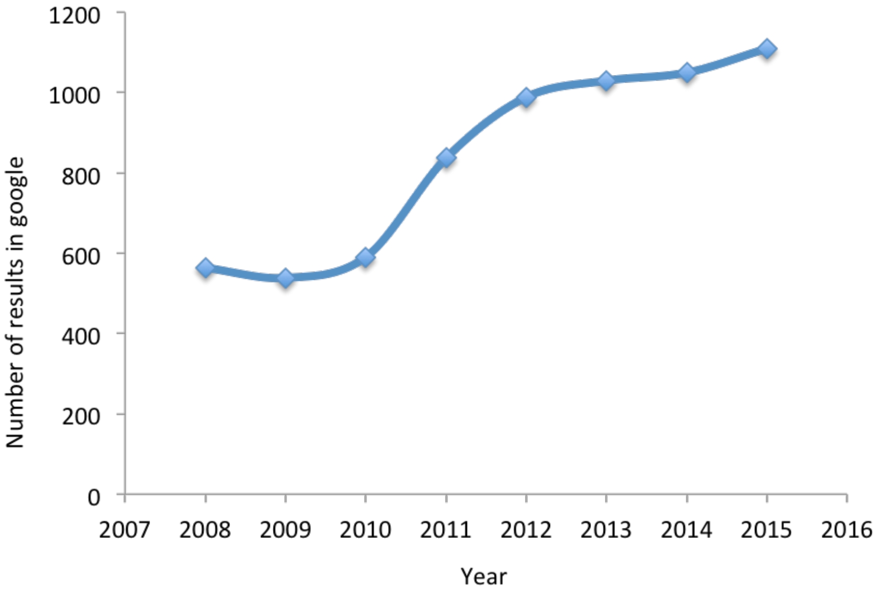

Figure IV.1. Number of results obtained by searching in Google Scholar using the terms “seagrass blue carbon” by year.

In addition to the factors considered in this thesis, there has been other subjects of interest examined by the scientific community that have significantly contributed to the knowledge on the carbon sink capacity of seagrass meadows and their potential role in climate change mitigation. These subjects include the effect of other environmental factors such as long-term and near-term nutrient availability trends (Armitage and Fourqurean, 2016; Howard et al. 2016), changes in the food web structure driven, for instance, by shifts in predator populations (Atwood et al. 2015) and CO_2 enrichment and ocean acidification (Russell et al. 2013; Garrard and Beaumont, 2014). In addition, and particularly relevant, are the studies evaluating the role of seagrass restoration projects, that have demonstrated to have an enormous potential as climate change mitigation strategies (Duarte et al. 2013a; Marbà et al. 2015; Garnier et al. 2013).

Yet, the effect of the environmental factors assessed in other studies and in this thesis remains still poorly explored and lead, in some cases, to contradictory or unclear results (Atwood et al. 2015; Russell et al. 2013; Apostolaki et al. 2014; Howard et al. 2016). Moreover, most of the studies report below ground biomass or C_{org} sediment stocks (Armitage and Fourqurean, 2016; Howard et al. 2016; Samper-Villareal et al. 2016; Russell et al. 2013) but not C_{org} burial rates, which, as mentioned before, are essential to actually assess the role of an ecosystem as a carbon sink (Macreadie et al. 2014; Hejnowicz et al. 2016). In addition, there are still other relevant gaps of knowledge that need to be covered for a better assessment of the role of seagrasses as carbon sinks and their relevance at a global scale. First of all, the estimates of global seagrass meadows cover, that currently range two fold (177,000-600,000 km^2, Duarte et al. 2013b), need to be improved, with special emphasis on mapping areas that have received less attention, either for being far away from human population or due to a lack of resources in developing nations (Duarte et al. 2013a; Macreadie et al. 2013). Mapping seagrass meadows has been a difficult task due to the limitation of remote sensing to 5 m depth, but the application of acoustic side sonar, useful for up to 25 m depth, has been demonstrated to be adequate for seagrass meadows and offers a new opportunity to improve current seagrass cover estimates (Montefalcone et al. 2013; Hejnowizc et al. 2016). On the other hand, the measurements of C_{org} burial rates (not only sediment stocks) need to be increased including a much broader spectra of segrass species (Duarte et al. 2013a) as, as shown before (Table IV.1), the scarce data on C_{org} burial rates available mainly come from meadows formed by *Posidonia* species. Finally, the effect of climate change on the carbon sink capacity of seagrass meadows should be urgently addressed (Mcleod et al. 2011). The rise in water temperature is one of the most critical impacts, considering global warming and the sensibility of seagrass meadows to increasing temperature and heat waves (IPCC, 2014; Marba and Duarte 2010; Collier and Waycott, 2014). In addition, increasing temperature may cause shifts in species distribution and usually leads to higher metabolic rates, especially respiration, driving seagrass meadows to a more heterotrophic status (Short and Neckels, 1999). Microbial metabolism is also enhanced under high temperatures leading to higher organic matter decomposition rates (Melillo et al. 1984). Hence, temperature increase is expected to lower the capacity of seagrass meadows to accumulate and bury C_{org} in the long term (Pedersen et al. 2011).

Hence, while the consideration of seagrass meadows as good candidates for the implementation of climate change mitigation strategies is becoming more realistic, there is still a need to improve and strengthen the knowledge of their carbon sink capacity to be included in the available UNFCCC conservation mechanisms that already exist for terrestrial ecosystems (i.e. REDD+ or NAMA's) (Hejnowicz et al. 2016). Yet, important advances have been made, such as the publication of the "Methodology for Tidal Wetlands and Seagrass Restoration" (VCS, 2015), the first applicable greenhouse gas accounting methodology for coastal wetland restoration, that will allow seagrass restoration projects to earn carbon credits that can be sold in the voluntary carbon market (www.thebluecarbonportal.org). This means that communities could benefit from the protection and conservation of seagrass meadows by selling the carbon credits to individuals, companies or NGO's, willing to compensate for their emissions. By guaranteeing the protection and conservation of seagrass ecosystems, these communities will also benefit from the multiple ecosystems services seagrass meadows provide, that rank from food and livelihood to cultural benefits (Wyllie-Echeverria and Cox, 2000; de la Torre-Castro and Rönnbäck, 2004; Shokri et al., 2009; Unsworth and Cullen, 2010) leading to a sustainable management of these ecosystems, as demonstrated by several mangroves restoration projects (Wylie et al. 2015).

Although the most efficient response to the threat of climate change would be modifying the way we live, trying to lower the amount of CO_2 we produce, the protection of natural carbon sinks contributes to reducing the excess CO_2 that is already in the atmosphere and helps to prevent further emissions through the degradation of these ecosystems. Particularly, the protection and restoration of seagrass ecosystems based on Blue Carbon initiatives would not only contribute to climate change mitigation but enhance the conservation of one of the most valuable ecosystems on earth upon which coastal communities around the world relay on (Constanza et al. 1997; Cullen-Unsworth et al. 2013).

V

General Conclusions

1. Carbon sinks in *Posidonia oceanica* are highly variable among meadows in terms of the magnitude of the sediment deposits, burial rates during the last century and the contribution of different sources to the sediment stocks.

2. The differences in C_{org} burial rates observed across meadows in the Balearic Islands could not be explained either by the meadow depth or by the exposure to wave energy. On the contrary, the level of human pressure in the meadows examined was determinant in the C_{org} burial rates.

3. The C_{org} burial rates tended to increase during the last century, coincident with the raise in anthropogenic pressure in the region of study (Balearic Islands).

4. The contribution of *Posidonia oceanica* derived organic matter to the sediment deposits tended to be higher in the most pristine sites whereas sestonic derived organic matter had a larger contribution to the sediment C_{org} stocks in the meadows under a high human pressure. The burial rate of sestonic derived carbon increased faster than that of *P.oceanica* derived carbon during the last century.

5. The close relationship between the spatial and temporal variability in C_{org} burial rates and contribution of different C_{org} sources, with the spatial and temporal variability in human pressure in the region of study (i.e. The Balearic Islands), points at eutrophication of coastal waters derived from increased anthropogenic activity during the last century, associated to the boom in the tourism industry and urban development, as the most likely driver of the increase in the carbon burial rates observed in the meadows examined.

6. Seagrass sediments represent significant deposits of particulate inorganic carbon, in the form of $CaCO_3$, at a global scale. PIC seagrass sediment stocks are 5 fold larger than the POC stocks identified in previous studies, vary among species, tend to decrease with latitude and show accumulation rates comparable to those identified in intense carbonate-producing systems such as coral reefs.

7. The carbonate ($CaCO_3$) epiphyte load and accumulation rates in seagrass leaves of *Zostera* genus showed a clear latitudinal gradient decreasing from southern and warmer regions towards northern and colder latitudes, consistent with the decrease in the seawater carbonate saturation state (Ω) and with the latitudinal gradient found in the PIC sediment stocks of seagrass meadows globally.

8. Sediment organic carbon deposits in seagrass meadows are vulnerable to remineralization and to be released as CO_2 due to erosion and subsequent suspension. Yet, this vulnerability depends on intrinsic-sediment biogeochemical properties, with carbon remineralization rates being enhanced in organic matter enriched sediments, especially with sestonic derived organic matter, with high nitrogen content and low carbonate concentration.

9. The enrichment with sestonic-derived organic carbon towards present observed in the sediments of the *Posidonia oceanica* meadows around the Balearic Islands is likely leading to the weakening of the carbon sink capacity of these meadows,

as C_{org} deposits become more vulnerable to remineralization. This phenomenon is likely to be occurring at a global scale especially in areas under a high and increasing human pressure, as suggested by other studies reporting similar trends in allochthonous carbon accumulation in seagrass sediments.

10. Despite the large PIC deposits identified in seagrass sediments and that the accumulation of PIC through $CaCO_3$ precipitation leads to CO_2 emissions, the comparison between POC and PIC stocks between vegetated and un-vegetated patches still identifies seagrass meadows as strong carbon sinks. Furthermore, the $CaCO_3$ content in seagrass sediments reduces the vulnerability of the organic carbon stocks to remineralization when sediments are exposed to oxic conditions and enhances the C_{org} burial efficiency in seagrass sediments.

VI

Conclusiones Generales

1. Los sumideros de carbono de *Posidonia oceanica* son muy variables entre praderas en relación a la magnitud de los depósitos del sedimento, a las tasas de enterramiento de carbono de los últimos 100 años y a la contribución de las distintas fuentes de carbono.

2. Las diferencias en las tasas de enterramiento de C_{org} observadas en las praderas de las Islas Baleares no han podido ser explicadas por diferencias ni en la profundidad de las praderas ni en su grado de exposición al oleaje. Por el contrario, el nivel de presión antrópica en las praderas estudiadas es determinante en las tasas de enterramiento de C_{org}.

3. Las tasas de enterramiento de C_{org} muestran una tendencia a incrementar durante el último siglo, coincidiendo con el aumento de la presión antrópica en la zona de estudio (Islas Baleares).

4. La contribución de la materia orgánica derivada de *Posidonia oceanica* a los depósitos de sedimento tiende a ser mayor en las praderas más pristinas mientras que la contribución de la materia orgánica de origen sestónico a los depósitos de C_{org} del sedimento es mayor en praderas bajo una elevada presión antrópica. Durante el ultimo siglo, las tasas de enterramiento de carbono de origen sestónico se han incrementado más rápido que las de carbono derivado de *Posidonia oceanica*.

5. La relación entre la variabilidad espacial y temporal en las tasas de enterramiento de carbono y en la contribución de las distintas fuentes de carbono a los depósitos sedimentarios, y la variabilidad espacial y temporal en la presión humana en la región de estudio (Islas Baleares), apunta a la posible eutrofización de las aguas costeras, consecuencia del incremento de la actividad antrópica y urbanización de la costa derivada del boom turístico en la zona, como la causa más probable del incremento del enterramiento de C_{org} identificado en las praderas estudiadas.

6. Los sedimentos de las praderas de fanerógamas marinas constituyen un importante depósito de carbono inorgánico particulado (PIC), en forma de $CaCO_3$, a escala global. Los depósitos sedimentarios de PIC en fanerógamas marinas son 5 veces mayores que los depósitos de POC cuantificados en estudios previos, varían entre especies, tienden a disminuir con la latitud y muestran tasas de acumulación comparables a las de ecosistemas productores de $CaCO_3$, como los corales.

7. La carga de carbonato ($CaCO_3$) asociada a los epífitos foliares y la tasa de acumulación en las hojas del género *Zostera* muestran un claro gradiente latitudinal, decreciendo desde regiones cálidas en latitudes bajas a regiones frías en latitudes altas, coincidiendo con la disminución en el estado de saturación del carbonato del agua de mar (Ω) y con el gradiente latitudinal detectado en los depósitos de PIC del sedimento de praderas de fanerógamas marinas a nivel global.

8. Los depósitos de carbono de las praderas de fanerógamas marinas son vulnerables a la remineralización y a ser emitidos en forma de CO_2 como

consecuencia de la erosión del sedimento y su suspensión. Sin embargo, la vulnerabilidad a la remineralización depende de características biogeoquímicas intrínsecas del sedimento, siendo mayor en sedimentos ricos en materia orgánica, especialmente si tiene origen sestónico, con un alto contenido en nitrógeno y baja concentración de carbonato.

9. El incremento en la contribución de carbono derivado de seston, observado en los sedimentos de las praderas de *Posidonia oceanica* en las Islas Baleares, da lugar al debilitamiento de los sumideros de carbono de estas praderas debido a un aumento en la vulnerabilidad de los depósitos de C_{org} a ser remineralizados. Este fenómeno es muy probable que esté teniendo lugar a escala global, especialmente en zonas bajo una elevada y creciente presión antrópica, como sugieren otros estudios que reportan tendencias similares en la acumulación de carbono alóctono.

10. A pesar de la magnitud de los depósitos de PIC identificados en los sedimentos de las praderas de fanerógamas marinas y de que la acumulación de PIC derivada de la precipitación de $CaCO_3$ lleva consigo la emisión de CO_2, la comparación entre los depósitos de POC y PIC entre parches vegetados y sin vegetación demuestra que las praderas de fanerógamas son importantes sumideros de carbono. Por otro lado, el $CaCO_3$ en sedimentos de praderas de fanerógamas marinas reduce la vulnerabilidad de los depósitos de carbono orgánico a ser remineralizados cuando los sedimentos son expuestos a condiciones óxicas y aumenta la eficiencia en el enterramiento de carbono en el sedimento de las praderas.

VII

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