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Blue carbon sink capacity of degraded ecosystems: the potential role of disturbed seagrass meadows in climate change mitigation

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1. Introduction

The anthropogenic emission of carbon dioxide (CO₂) into the atmosphere has rapidly grown in the last 200 years reaching alarming levels (NOAA, 2016), and has been mainly driven by fossil fuels burning (i.e. petroleum and coal) and land-use conversion (Solomon et al., 2007; Van Der Werf et al., 2009). The increase of CO₂ concentration is contributing to climate change with rise of temperature and sea level, and ocean acidification negatively affecting the health of ecosystems and living organisms (IPCC, 2007). Mitigating climate change is a global priority and requires strategies that drastically reduce CO₂ emissions and enhance carbon sequestration through conservation and restoration of natural carbon sinks (Agrawal et al., 2011; Duarte et al. 2013; Rogelj et al., 2016). Despite the small area occupied by seagrasses, saltmarshes and mangroves, these coastal vegetated ecosystems are among the most significant carbon sinks, defined as blue carbon, on the biosphere playing an important role in climate change mitigation (Mcleod et al., 2011; Duarte et al., 2013). Also, their carbon sequestration rate is up to 10-fold higher than in terrestrial ecosystems because of the ability of seagrasses, saltmarshes and mangroves to trap particles from the water column and store them in the soil (McLeod et al., 2011; Macreadie et al., 2014).

The importance of blue carbon ecosystems in reducing climate change mitigation has attracted high scientific interests, especially in the last decades. Despite that, there are still important gaps of knowledge that must be addressed and, recently, summarized in 10 points (Macreadie et al., 2019). Mainly, gaps regarding the effects of natural and anthropogenic disturbances on carbon accumulation in these ecosystems are still pending questions (Macreadie et al., 2019; Spivak et al., 2019). Considering uncertainties on climate change rate and relative drivers (i.e. sea level rise, warming, extreme events, and acidification), future researches have to be addressed on how these disturbances will affect blue carbon accumulation, especially, in mature ecosystems and during their restoration (Macreadie et al., 2019). Furthermore, it is fundamental to understand the fate of blue carbon stock once the ecosystem is degraded or lost, and, especially, to quantify if carbon is released by and the extension of the disturbance in the depth soil profile (Lovelock et al., 2017; Macreadie et al., 2019; Spivak et al., 2019). Filling these knowledge gaps is fundamental to better understand how to develop and implement protection and conservation plans for these important

ecosystems that are rapidly disappearing with an annual lose rate of about 7 % (McLeod et al., 2011).

Seagrass meadows are one of the most threatened ecosystems in the world with 29% of their area already lost (Waycott et al., 2009). Climate changes as sea level rise and warming have deeply altered distribution, productivity and community composition of these ecosystems (Jordà et al., 2012; Koch et al., 2014; Lovelock et al., 2017; Orth et al., 2006; Saunders et al., 2013; Waycott et al., 2009). Furthemore, anthropogenic disturbances as coastal develpoment and mechanical impacts (dregding, mooring, trawling, etc.) contributed directly and indirectly to the seagrasses decline and loss of their ecosystem services (Boudouresque et al., 2016a; Serrano et al., 2015; Short et al., 2009, Kendrick et al., 2002). Once the meadows is lost, the soil remains exposed to disturbances, both anthopogenic and natural, and to oxygen penetration and microbial activities with possible realease of carbon prevoiusly accumulated to to the adjacent environment.

In the Mediterranean Sea, one of the major contributors to carbon sink, the seagrass *Posidonia oceanica*, is marked by decline within the entire basin and, and this may lead to a shift from being sink to CO₂ sources (Marbà et al., 2014). In this context, the aim of the PhD project is to contribute to increase the knowledge on the capacity of blue carbon ecosystems, especially *P*. *oceanica* meadows, to act as sinks of carbon, nitrogen and trace elements and to evaluate effects of human and natural impacts on nutrients burial rate and stocks.

The project attempts to contribute to overcome gaps of knowledge focusing on blue carbon ecosystems impacted by anthropogenic disturbance and climate change. Special focus has been given to the fate of the carbon sink after the loss of vegetation and the carbon sink capacity of blue ecosystems characterized by an increasing decline of seagrasses. In details the main aims are:

- to summarize the previous findings on blue carbon ecosystems and the effects of anthropogenic and natural pressures, with a particular focus on the important role of *Posidonia oceanica* and future perspectives of blue carbon sciences – Chapter 2;
- to assess the capacity of seagrass ecosystems under natural and anthropogenic impacts to sequester carbon and to act as blue carbon sinks. In particular, the effort is on the differences between the carbon stock capacity in *C. nodosa* meadows and *P.*

oceanica patches in a semi-enclosed environment characterized by high environmental variability – Chapter 3;

to focus on the capacity of degraded seagrass ecosystems characterized by dead *P. oceanica* mat to act as sinks of biogenic and trace elements – Chapter 4.

2. Coastal vegetated ecosystems as natural carbon sinks: the role of *Posidonia* oceanica

Abstract

The term "blue carbon" refers to the organic carbon sequestered and stored by the ocean and, by coastal vegetated ecosystems as seagrasses, saltmarshes and mangroves (Nellemann et al., 2009). These ecosystems are able to sequester large quantity of carbon in the soil acting as natural sink and mitigate climate change (McLeod et al., 2011). Considering the importance of reducing CO₂ emissions in the near future, these ecosystems represent an important tool to reach this goal. Concurrently, they are vulnerable to anthropogenic and natural impact that may reduce the blue carbon sink and point the focus on the protection and the conservation of these ecosystems (Duarte et al., 2013; Orth et al., 2006). Considering the available studies (Source Scopus; keywords used during the web-searching: blue carbon, coastal vegetated ecosystems, carbon stock, mangroves, seagrass and saltmarshes), here, we proposed briefly the main knowledge on blue carbon ecosystems with a special focus on the endemic Mediterranean *Posidonia oceanica*.

2.1 Coastal vegetated ecosystems: distribution and ecosystem services

Coastal vegetated ecosystems are characterized by the presence of submerged or partially emerged plant as seagrasses, mangroves and saltmarshes. Despite Antarctica, these ecosystems are globally distributed from the upper intertidal zone to major depth (max. 40m) along the coastal zone. Especially, mangroves occur only in tropical and subtropical zones while seagrasses and saltmarshes are wider disseminated from tropical to temperate zone. Their extension is full of uncertainties due to few available data. Considering recent estimations, mangroves occupy a total area of 83945 km² (Hamilton and Casey, 2016), saltmarshes a total area of 55000 km² (Mcowen et al., 2017) while seagrasses cover an area of 350000 km² (UNEP-WCMC, Cambridge, 2016).

Despite the small fraction of ocean surface (0.2%) covered by them, these are one of the most valuable ecosystems supporting high productivity, representing important nursery area and hot spot of biodiversity (Barbier et al., 2011; Costanza et al., 2014). Moreover, their canopies and roots act as mechanical barrier against water current, storms and dampen wave energy reducing coastal erosion. With the same mechanism, they promote settling of suspended particles improving water quality, sea floor raising and soil stabilization (Alongi, 2008; Ondiviela et al., 2014). These ecosystems build up large deposit of carbon playing a fundamental role as natural carbon sink and climate change mitigation (Alongi, 2014; Duarte et al., 2013b; Mazarrasa et al., 2017a).

2.2 Blue carbon sinks and key factors regulating carbon deposition

To distinguish from the carbon stored in terrestrial ecosystems (Green), the one sequestered by coastal vegetated ecosystems and, generally, by the ocean is defined "blue carbon" (Nellemann et al., 2009). In these ecosystems, carbon sequestration rate is up to 10 fold-time than the corresponding in terrestrial ones (McLeod et al., 2011). Mainly, thus is related to their ability to promote the deposition of suspended particles (allochthonous carbon) that are fixed into the soil by the roots (Kennedy et al., 2010). The above and below-ground biomass of these plants and their detritus (autochthonous carbon) contribute to increase the quantity of carbon stock in these ecosystems. Furthermore, due to the anoxic conditions of their sediments and refractory trait of organic matter trapped in that reduced possible microbial activities (Fourqurean and Schrlau, 2003), the organic carbon sequestered is preserved for long period (Filho et al., 2006; Howard et al., 2017;

Mateo et al., 1997; Serrano et al., 2012; Ward et al., 2008). Considering all the available data, the magnitude of carbon buried vary among the ecosystems (Pairwise t test, P< 0.01), and (Fig.2.1), mangroves showed the greater amount of organic carbon stock in the top 1m (318 \pm 242 Mg ha⁻¹) compared to saltmarshes (191 \pm 187 Mg ha⁻¹) and seagrasses (101 \pm 113 Mg ha⁻¹).





Due to the importance of these ecosystems in climate change mitigation, in the last two decades, scientific community has tried to understand the factors and processes regulating the deposition of blue carbon (source: Scopus, Fig. 2.2). The deposition of those sinks is affected by multiple abiotic and biotic factors as environmental characteristics, soil properties, and vegetation composition and/or morphology (Kelleway et al., 2016; Lavery et al., 2013; Xiong et al., 2018)

Sediment grain size is one of major abiotic factors that affects carbon sequestration. In fact, fine sediments are positively correlated with organic carbon concentration because they are marked by greater superficial area than coarser and enhanced organic matter adsorption (Kelleway et al., 2016; Serrano et al., 2016b). Consequently, the concentration of fine sediments, especially mud, could be used as a suitable indicator of organic carbon stock in the

soil (Dahl et al., 2016; Serrano et al., 2016b). In submerged ecosystems as saltmarshes and mangroves, where subsequently to tidal excursion the soil remained exposed to oxygen, fine sediments characterized by low porosity and, so on, by low oxygen exchange, avoiding possible remineralisation of organic carbon (Kelleway et al., 2016). Also, water salinity and temperature deeply affect the carbon stock capacity of these ecosystems. It has been proven as the quantity of organic carbon in the soil is negatively affected by an increment of salinity (Van De Broek et al., 2016). Furthermore, increase of water temperature promote microbial activities and remineralisation of organic matter stock in the sediment with a diminution of the carbon sink (Serrano et al., 2018; Penderson et al., 2012).



Figure 2.2: Scientific studies published in the last 20 years on blue carbon ecosystems, source Scopus (25th August 2019).

The hydrodynamics conditions (water current, wave actions and tides) modify the transport and the sedimentation of particles that could represent up to 50% of carbon sequestered in those ecosystems (Kelleway et al., 2013; Kennedy et al., 2010). Mangroves and saltmarshes that occupied sheltered areas depending more on the tidal currents that regulating nutrient exchange, sedimentation and their distribution (Kelleway et al., 2017). Meanwhile other features as water depth and turbidity influence mostly seagrasses regulating carbon accumulation and primary production (Lavery et al., 2013; Mazarrasa et al., 2018; Serrano et al., 2014).

Mangroves, saltmarshes and seagrasses are characterized by large variation in terms of size, biomass, shoots or plant density and growth rate (Lavery et al., 2009; Herminga and Duarte, Chumura, 2016). Larger species with greater biomass, especially the below-ground, are more efficient to stabilize the sediment preserving the carbon stock against erosion and reducing hydrodynamic energy (Kristensen et al., 2008; Sousa et al., 2017) (Kristensen et al., 2008; Sousa et al., 2017). Meanwhile the presence of each ecosystems and species distribution is mainly affected by environmental conditions as temperature, both water and air, salinity and pH that control their presence or absence and, consequently, the possible carbon deposition.

2.3 Blue carbon loss

These ecosystems are vulnerably to anthropogenic and natural impacts and rapidly disappearing with an annual rate up to 7% (Pendeleton et al., 2012). The decline is related to the increasing of population and human activities across the coasts strongly affecting the presence and the health of blue carbon ecosystems (Halpern et al., 2008). Mainly, mangroves and saltmarshes are threatened by land use conversion for agriculture and aquaculture, deforestation and draining (Gillis et al., 2017; Lovelock et al., 2017). Meanwhile, seagrasses are mostly impacted by depletion of water quality, eutrophication, run off and dredging (Barañano et al., 2018). Furthermore global changes as sea level rise, warming and acidification deeply alteret distribution, productivity and vertical accretion of these ecosystems (Saunders et al., 2013; Waycott et al., 2009). Especially, sea level rise will contribtue to the loss of intertidal forest and marshes; and deep water seagrasses meanwhile it is expected a landward expansion gaining new submerged areas, where is no limited by human settlements, and possible increase of soil organic carbon (Ewers Lewis et al., 2017; Howard et al., 2017). Furthermore, an exchange between ecosystems are expected with the expantion of seagrases into mangroves and saltmarshes areas. Especially, increasing of sea level exposes mangroves and saltmarshes to large floading period and salinity intrusion that may lead plant death and/or reduction of primary production (Ward et al., 2016). Extreme events as storms are more frequently and causing canopy damages, loss of vegetation and sediment erosion (Forsberg et al., 2018; Macreadie et al., 2013). Furthemore, increasing of

temperature is expected to alter the distribution of these ecosystems that will migrate towards the pole and will, concurently, exposed to heat stress with die-off event and increasing soil remineralization (A. Arias-Ortiz et al., 2018; Thomson et al., 2015). Despite the ability of these ecosystems to absorb large quantity of CO₂ by photosynthesis and convert it in biomass, making them winners with future level of CO₂, new studies underlined as the acidification is reducing the capacity to store carbon and to growth vertically (Saderne et al., 2018; Vizzini et al., 2019).

The effects of those impacts on both vegetation and soil compartments are related to the interaction of multiple stressors and to their magnitude (Saunders et al., 2013; Macreadie et al., 2019). Basically, the exposure of these ecosystems to distrubance casues the loss of vegetation and destabilization of sediment that is exposed to erosion, microbial activity and oxydation. As consequences, large quantity of carbon are released and exported to the adjacent habitats or otherwise is oxidesized to CO₂, (Spivak et al., 2019 and the references herein).

2.4 Blue carbon ecosystems in the Mediterranean Sea: the importance of Posidonia oceanica

In the Mediterranean Sea, the endemic seagrass *Posidonia oceanica* (L.) Delile is one of the most abundant species forming extensive meadows from shallow water to down 40m (Boudouresque & Meinesz, 1982). It is a long-lived species and ranking the slowest growing rate across plants (1-6cm y⁻¹; Marba et al., 1998). *P. oceanica* is able to form biogenic reef called "matte" consisting of complex network of both living and old tissue, especially rhizome and roots, buried within sediment under anoxic conditions (Boudouresque et al., 2016; Mateo et al., 1997). The mat contributes to the rise of sea floor and is an important substratum on which *P. oceanica* growths itself. Additionally, this biogenic reef is a key carbon sink preserved for long period and representing a natural archive to reconstruct environmental changes (Romero *et al.*, 1996; Mateo *et al.*, 1997). Despite the important role of *P. oceanica* as blue carbon sink and the wide area occupied by (500000 km², Borum et al., 2004), only few data are available on *P. oceanica* stock and mainly related to three country: Spain, Italy and Greece (Fig. 2.3 and the reference herein). Considering the data reported, *P. oceanica* shows large variability in carbon stock capacity with major stock in Spain (Fig. 2.3) where forms dense

meadows and mat up to 5m (Serrano et al., 2012). Also, in site as Portlligat in Spain, *P. oceanica* showed high carbon stock comparable (376 Mg ha⁻¹; Mazarrasa et al., 2017b; Fig. 2.3) with the mean value found for mangroves.

As the other blue carbon ecosystems, *P. oceanica* is vulnerable to anthropogenic impacts that caused the loss of 73% of its shallow limits (Holon et al., 2015). Major causes of this declined are ascribed to coastal development, industrial pollution and eutrophication events reducing the available area and decreasing water quality improving turbidity and, so, reducing light availability for primary production (Boudouresque et al., 2009). Also, mechanical disturbances as trawling and anchoring are contributed to loss of *P. oceanica* meadows (Marbà et al., 2014).



Figure 2.3: Box plots of organic carbon (Mg ha⁻¹) in *P. oceanica* at 1 m. Observations are available only for 3 countries: Italy (n obs= 2; Mateo et al., 1997); Greece (n obs = 9; Apostolaki et al., 2019, 2018) and Spain (n obs= 13; Mazarrasa et al., 2017b; Ricart et al., 2016; Serrano et al., 2016b, 2012), respectively. Thick central line represents median, lower and upper whiskers of the box represent respectively the lowest and the highest values of total range of distribution. Outliers are marked by filled dots.

Furthermore, the Mediterranean Sea is warming faster under climate change and P. oceanica is exposed to heat wave and increasing of salinity that negatively affected the primary production and the survival of this species (Jordà et al., 2012; Marbà and Duarte, 2010). As results of these impacts, 11-52% of *P. oceanica* ecosystem has been lost with depletion of its capacity to act as carbon sink (Marbà et al., 2014) and the species might be functionally extinguished before 2050 (Chefaoui et al., 2018).

The loss of *P. oceanica* is exposing large carbon stock to possible switch to source of CO_2 .folowing erosion and oxidation of the buried organic matter. The potential estimation of CO_2 released from this ecosystem is full of uncertainties because there is a lack of data about the real extension of *P. oceanica* meadows and the magnitude its soil stock.

2.5 Blue carbon future perspectives

Despite the increase of scientific interests on blue carbon ecosystems in the last decade, and their importance in climate change mitagation, lack of knowledges characterized their global extension and related carbon stock (Macreadie et al., 2019; Ruiz-Frau et al., 2017). Gaps regarding the effects of natural and anthropogenic disturbances on carbon accumulation in these ecosystems are still pending questions (Macreadie et al., 2019b; Spivak et al., 2019). Especially, considering uncertituds on climate change rate and relative factors (sea level rise, warming, extreme events, acidification), future researches have to address their efforts to understand how these disturbances will affect blue carbon accumulation, especially, in mature ecosystems and during their restoration (Macreadie et al., 2019). Efforts have to focus on understanding the fate of blue carbon stock once the ecosystems is impacted or lost, and, especially, to quantify the carbon released by and the extension of the disturbance in the depth soil profile (Lovelock et al., 2017; Macreadie et al., 2019; Spivak et al., 2019). Filling those knowledge gaps are fundamental to better understand how to develop and implent protection and conservation plans for these important ecosystems that rapidly disappered with an annual lose rate of about 7 % (McLeod et al., 2011).

3. Seagrass meadows as potential carbon sinks in a semi-enclosed basin: evaluating the stock capacity of *C. nodosa* and *P. oceanica*

Abstract

Seagrass ecosystems are important natural carbon sink that store large amount of carbon in the sediments. In the Mediterranean Sea, major organic stocks are associated to *Posidonia oceanica* that is fast declining due to global changes (heat-wave and increase of salinity) and may be replaced by *Cymodocea nodosa*. Here we assess the carbon stock capacity of a Mediterranean semi-enclosed basin characterized by decline of *P. oceanica* and expansion of *C. nodosa*. Our findings showed that in semi-enclosed basin like Stagnone di Marsala, *C. nodosa* is able to store large amount of carbon, with values comparable to *P. oceanica*. However, despite the stress condition determining the regression of *P. oceanica*, this species stores large quantity of carbon. Moreover, due to the combination of peculiar characteristic of hydrodynamic conditions, high input of seagrass detritus and the presence of seagrass meadows, Stagnone di Marsala seems to be an important sink of blue carbon giving indication about the important ecological role of this kind of ecosystems.

3.1 Introduction

Marine costal ecosystems as seagrass meadows have been recognized to play a key role in global carbon cycle (Duarte et al., 2013b; Fourgurean et al., 2012a). Together with mangroves and saltmarshes, seagrass meadows are among the most significant natural carbon sinks enhancing climate change mitigation (Duarte et al., 2013). Large deposits of organic carbon, defined blue carbon, are principally located in the soils and account for 20% of that total carbon sequestered by marine sediments (Fouruerean et al., 2012; Kennedy et al., 2010; Nellemann et al., 2009). The carbon stock capacity is mainly related to: accumulation of autochthonous carbon plant-derived (excess of primary productivity, seagrass biomass and detritus), settling of suspended organic matter from water column endorsed by seagrass canopy, and anaerobic sediment conditions (Duarte et al., 2013a; Kennedy et al., 2010). As a consequence, large quantities of blue carbon stock are accumulated for long-periods (Serrano et al., 2011). Additionally, many recent studies have pointed out how biotic and abiotic factors regulate this fundamental process (Lavery et al., 2013; Macreadie et al., 2014; Mazarrasa et al., 2017b; Samper-Villarreal et al., 2018; Serrano et al., 2016b, 2014). The potential storage of carbon is likely affected by morphologically differences among seagrass species, habitats characteristics and distribution (Mazarrasa et al., 2018 and references herein). Generally, large and persistent species (Posidonia spp., Thallassia spp., Enhalus spp.) are marked by higher biomass production, potentially increasing the quantity of autochthonous carbon storage. Moreover, these species characterized by tall canopy efficiently reduce the hydrodynamic forces promoting particle sedimentation from water column (Kilminster et al., 2015; Lavery et al., 2013; Rozaimi et al., 2016). Also, landscape configuration influences carbon stock capacity of these ecosystems. Continuous meadows are more efficient at carbon sequestration than patchy as consequence of both larger deposition of seagrass-detritus and suspended particles (Oreska et al., 2017; Ricart et al., 2017). Furthermore, seagrass meadows occur from shallow to depth waters with different environmental conditions that may enhance or reduce the carbon stock capacity (Mazarrasa et al., 2018 and references herein). Higher net productivity found in shallower ecosystems seems to suggest larger accumulation of carbon stock compared to deeper meadows (Collier et al., 2007; Dahl et al., 2016; Serrano et al., 2014). Contemporary, shallow ecosystems are more exposed to hydrodynamic energy that may reduce carbon deposition, increasing sediment erosion and export while deeper

meadows are characterized by higher accumulation (Lavery et al., 2016, Serrano et al., 2016). Thus, the combination of these factors contributes to sequester and store large quantity of carbon in these ecosystems and mitigate climate change (4.2-8.4 Pg C; Fourquran et al., 2012).

Additionally, seagrass ecosystems are able to sequester inorganic carbon, but little information of the quantity of stocks, possible differences among the species, factors regulating the deposition and possiible effects of disturbances are avaiable (Apostolaki et al., 2019; Fourqurean et al., 2012b; Howard et al., 2017.; Mazarrasa et al., 2015; Santos et al., 2019). Especially, inroganic carbon deposition depends on accumulation of suspending particles, precipitation of carbonate plant-derived and calcification associated to epyphytes (Enríquez and Schubert, 2014; Frankovich et al., 1997; Howard et al., 2018; Kinderbergen et al 2018). Additionally, environmental factors (pH, water temperature and salinity) and species features (canopy structure and shoot density, etc.) regulate the inorganic carbon deposition and its relative stock (Mazarrasa et al., 2015; Saderne et al., 2019). This process is important because contributes to carbonate accumulation in sediment that promote seafloor elevation and the accretion of below-ground biomass and may enhanche organic carbon sequestration (Mazarassa et al., 2015 and herein references).

Seagrass meadows occupy coastal areas that are marked by high anthropogenic and natural impacts contributing to their decline (Halpern et al., 2008; McLeod et al., 2011). These ecosystems, among others, are one of the most threatened in the world and rapidly disappearing at the annual rate of 7% (Mcleod et al., 2011; Short et al., 2011; Waycott et al., 2009; Borum et al., 2004). Coastal environenments have been modified by urbanization, industrial development and agriculture, and thereafter promoting land degradetion, water quality decrease, erosion, pollution and eutrophication (Saunders et al., 2013; Waycott et al 2009; Halpern et al., 2008). Thus, seagrass meadows are exposed to increasing input of contaminants as trace elements with possible negatitive effects on their photosynthetic systems, hence on growth rate, once accumulated in their tissues. Despite the essential role of some trace elements (i.e. Fe, Zn, Mn, etc.,) and their natural avaiability, above certain level all are potentially toxic for marine organisms such as seagrasses (Bonanno and Orlando-Bonaca, 2018; Campbell et al., 2005; Gray, 2002; Monperrus et al., 2007, Rainbow et al., 2007). Furthermore, trace elements are biomagnifying through the trophic web and their bioaccumulation in seagrass tissue is a potential risk for this plant and, also, for other oganisms

(Rainbov et al., 2007; Signa et al., 2017). Cuncurrently, seagrasses promote the deposition of fine-organic particles that have strong binding affinity with trace elements and, hereby, sequestring large quantity of pollutants (Turner and Millward, 2002). Accordingly, sediments associated to these ecosystems are marked by higher concentration of trace elements than water column with a ratio of 3:1 and act as natural sinks (Bonanno and Raccuia, 2018; Lafabrie et al., 2007; Malea et al., 2019; Serrano et al., 2013). These ecosystems play, also, a key role in nutrient removal, especially nitrogen, that are buried in the soil where oxygen released by roots stimulate coupled nitification-denitrification (Aoki et al., 2019). Despite that as consequences of antrhopogenic impacts, eutrhophication events occur in coastal waters and cause seagrass decline due to nutrient loading exceed followed by reducing of light and oxygen avaiability, stimulation of phytoplankton and macroalgae growth (Touchette et al., 2000; Campbell et al., 2018, Ontaria et al., 2019). Additionally, global changes as heat-waves, sea level rise and intensification of extrem events (i.e. drouguht, storms and precipitations) are contributing to alter segrasses distribution, primary prduction and their carbon stock capacity (Jordà et al., 2012; Koch et al., 2014; Orth et al., 2006; Saunders et al., 2013). Considering possible future scenarios (IPCC, 2013), these ecosystems will face radical changes of environmntal condition as water depth, pH, temperature and salitity that will modify their global distribtuion. It is expected a landwardexpasion of their upper limit towards new submerged area and towards higher latitude with the creation of new blue carbon sink (Krause-Jensen and Duarte, 2014; Saunders et al., 2013). Meanwhile, global warming is increasing seagrass die-off event and promoting microbial activties in the soil with consequent decline of carbon sequestration (Arias-Ortiz et al., 2018; Marbà and Duarte, 2010). Togheter antrhopogenic and natural impacts have already caused the loss of 29% of seagrass meadows and consequently realeased of 300 Tg C y⁻¹ from the soil (Pendleton et al., 2012; Rozaimi et al., 2016; Waycott et al., 2009). In fact, as seagrass meadows disappear, the soil is exposed to future disturbances (i.e erosion) and environmental factors (i.e. oxygen and microbial activity) with potentially shift from sink to source of carbon. Mainly, the response of these ecosystems to possible impacts seems to be species-specific and depending on the duration and magnitude of distrubances (Mazarrasa et al., 2018; Spivak et al., 2019).

Among seagrass ecosystems in the Mediterranean Sea, *Posidonia oceanica* L. Delile is facing a remarkable regression across the entire basin (Bouduresque et al., 2009; Marbà et al 2014).

P. oceanica is an endemic seagrass species which is able to form dense meadows and biogenic reef known as "matte" and support the largest carbon stocks ever found (Boudouresque et al., 2016; Mateo et al., 1997; Mazarrasa et al., 2015; Serrano et al., 2019). Mostly, this decline is related to eutrophication, physical impacts as anchoring, coastal development and climate changes with a substatial decrease of its capacity to sequester carbon in the last 50 years and possible functional extintion in the next 100 years (Chefaoui et al., 2018; Marbà et al., 2014). Concurrently, other species such as Cymodocea nodosa Ucra Archenson seem to tolerate better environmental changes, especially increses in temperature and salinity (Chefaoui et al., 2016). Thus, under future scenario of climate change, *C.nodosa* would repleace the declining P. oeanica, as already confirmed by recent findings (Burgos et al., 2017). Additionally, seagrass replacement may affect the carbon sink associated to those ecosytems depending of the different carbon stock capacity of each species. Moreover, both seagrass species often colonize shallow-water coastal bodies where are exposed to strong fluctuation of enviromental factors (Agostini et al., 2003; Rismondo et al., 1997; Serrano et al., 2019) that may affect carbon sequestration. Also, these areas are remarkably vulnerable to climate changes (Brito et al., 2012; Mazarrasa et al., 2018) and, here, the seagrass meadows are extremly exposed to distrubances for the reasons above-mentioned and may fast decline or being replaced by other opportunistic species. Once the meadows decline, the carbon stock is exposed to possible disturbances that could promote erosion and carbon release. Despite the prevolus studies on blue carbon ecosystems, the fate of these distrubed stocks needs be explored thoroughly (P. I. Macreadie et al., 2019b; Spivak et al., 2019).

The present study assesses the capacity of seagrass ecosystems under natural and anthropogenic impacts to sequester carbon and to act as blue carbon sinks. In particular, the focus is on the differences, between the carbon stock capacity in *C. nodosa* meadows and *P. oceanica* patches in a semi-enclosed environment characterized by high environmental variability. For this reason, the investigations have been performed in the Stagnone di Marsala (Sicily-Italy), a basin with lagoon-like features and peculiar geomorphological characteristics, marked by the presence of extended *C. nodosa* meadows, *P. oceanica* patchy distributed and various macroalgal species. Aim of the study was to compare carbon storage in *P. oceanica* to that in *C. nodosa* meadows among different sectors of the basin characterized by diverse biogeochemical and hydrodynamics conditions. Also, the isotopic composition of seagrass sediment was examined to identify the possible source of the sedimentary organic carbon.

Furthermore, ²¹⁰Pb dating was performed to detect any change recorded by the sediments. Considering the capacity of seagrass ecosystems to act as nitrogen sink and limited data availability, we estimated and compared the nitrogen storage in both species. Lastly, due to high anthropogenic pressure along the coast of the Stagnone di Marsala and role of seagrass as natural filter for contaminants, trace element analyses have been performed across the basin.

3.2 Material and Methods

3.2.1 Study site



Figure 3.1: Maps of Stagnone di Marsala with sampling stations (1-4). The dotted line marks the separation between the different sectors of the basin.

The Stagnone di Marsala is a hypersaline basin with lagoon-like features, located in the northwestern part of Sicily (Italy) with an extension of 20 km² (Fig.3.1). It is delimited to the west by Isola Grande, a calcarenitic platform, and to the east by the Sicilian coast. The basin is oligotrophic and marked by large annual variations in water temperature and salinity (Pusceddu et al., 1997; Sarà et al., 1999; Vizzini and Mazzola, 2008). It is characterized by shallow waters (0.5-3 m depth) and water exchange with the adjacent open-sea is assured by the presence of two channels: a narrow and shallow to the North (40 m wide, 0.3-0.4 m deep) and a broader and deeper to the South (1200 m wide, 1-2 m deep). As a result of this morphology together with wind action, the presence of small islands (S. Maria, Is. Schola, and S. Pantaleo; Fig.3.1) and submerged vegetation, acting as mechanical barriers, the northerncentral part of the Stagnone di Marsala is characterized by turbulent hydrodynamic conditions and lower water exchange, while the southern part presents greater water exchange (de Marchis et al., 2012; La Loggia et al., 2004). According to that, the basin is mainly divided in two subsectors: North, and South. The sand-muddy bottom is covered by extensive meadows of Cymodocea nodosa (Ucria) Archenson among all the basin and patches of Posidonia oceanica (L) Delile, especially, in the central part. Seabottom is covered by extensive meadows of Cymodocea nodosa (Ucria) Archenson among all the basin and patches of Posidonia oceanica (L) Delile, especially in the central part. Additionally, different macroalgal species are widely distributed all over the basin such as Cystoseyra spp., Caulerpa prolifera (Forskkål), Chaetomorpha linum (Müller), Gracilaria sp., Jania sp., Laurencia sp, (Bellissimo and Orestano, 2014; Vizzini and Mazzola, 2008). The presence of these seagrass species largely contributes to the ecological value of the lagoon (Mazzola et al., 2010). The Stagnone di Marsala represents one of the most important transitional ecosystems in Italy and is widely recognized as an important biotype for its great naturalistic value and is included in the Sites of Community Importance (SCI, Directive 92/43/CEE) and Natura 2000 Sites. However, the Stagnone di Marsala is under threat due to the increase in temperature and salinity and anthropogenic pressure (intensive cultivations, urbanisation, salt works, tourism, etc). as results, all the area is experiencing the regression of important species such as the seagrass P. oceanica. Additionally, due to the reducing of the north opening caused by strong sedimentation rate and bottom uplift, in the last decades the Stagnone di Marsala is suffering a decrease in water exchange, especially in the northern sector (de Marchis et al., 2012).

3.2.2 Sampling strategy and analytical methods

Sampling was carried out in October 2017 and, according to geomorphological subdivision and the presence of different macrophyte species, four stations were selected with similar depth (0.5-1.2 m) and salinity (38-44%): Station 1 in the northern part, Station 2 and 3 in the central part and Station 4 in the southern part of Stagnone di Marsala (Fig. 3.1, Table 3.1). Moreover, different vegetation coverage marked the selected stations: Station 1 was covered with *C. nodosa*, *C. prolifera* and *Gracilaria* sp., Station 2 with *P. oceanica* and *C. prolifera*, Station 3 with *C. nodosa* and *C. prolifera*, *Cystoseira* sp., *Jania* sp. and Station 4 with *C. nodosa*, *C. prolifera*, sp., *Halimeda tuna*, *Laurencia* sp.

Stations	Coordinates (WGS 84)	Habitat type	Depth (m)	Salinity (‰)	Water Temperature (°C)	Granulometry (%DW, Gravel- Sand-Silt/Clay)
Station 1	37° 53.780'N 12° 27.388'E	C. nodosa	0.45	38.41	23.95	3-91-6
Station 2	37°52.440'N 12°27.636'E	P. oceanica patches	0.5	44.1	23.18	12-40-48
Station 3	37° 51.645'N 12° 27.055'E	C. nodosa	0.5	44.86	23.86	10-85-5
Station 4	37°50.063'N 12°28.018'E	<i>C. nodosa</i> mixed with macroalgae	1.2	40.05	21.76	2-93-5

Table 3.1: Sampling design and site characteristics at each station in Stagnone di Marsala.

At each station, macrophyte and sediment were collected by scuba divers. For macrophyte sampling, triplicate of quadrats (20x20cm) were randomly tossed on the meadows and vegetation was harvested. Sediment samples were collected using PVC cores (I00 cm long and \emptyset 40 mm) in one replicate per station. After collection, all the samples were frozen at -20C°. In the laboratory, each sample was processed to investigate species composition and estimate seagrass shoot density (shoots m⁻²), biomass of each component (detritus, seagrass, algae, epiphytes, g DW m⁻²), and leaf area index (LAI). Leaf area index (LAI) was determined as product of leaf surface area (total leaf length per mean leaf width) by shoot density. Sediment cores were sliced into 1 cm section using a stainless-steel handsaw, freeze-dried and powdered.

For grain-size analysis, wet samples were previously left in H_2O_2 solution (3-10%) to eliminate the organic matter. Subsequently, sediment fractions (gravel, sand, silt/clay) were separated by wet sieving at 63 µm, oven dried at 60°C and weighed. To estimate dry bulk density (DBD, g cm⁻³), sediment dry weight was divided by the volume of the wet samples.

Aliquots of dry sediment were weighed in tin capsules to perform total carbon (C%) and total nitrogen (N%) analysis and in silver capsules for organic carbon (C_{org}) analysis using an elemental analyser (Thermo Flash EA 1112). Exclusively for organic carbon, after weighing, sediments samples were acidified with HCl (18%) in order to eliminate carbonates.

Stable isotope composition (δ^{13} C and δ^{15} N) was obtained analysing dry sediment by mean of an isotope ratio mass spectrometer coupled with an elemental analyser (Thermo IRMS Delta Plus XP coupled with Thermo EA-1112). Sediment samples used for δ^{13} C analyses were previously acidified with a solution of HCl (2N) to dissolve carbonates potentially influencing δ^{13} C signature.

Organic and inorganic carbon and nitrogen stocks were estimated as C stock = Σ (C_i x b_i x d_i), where C_i is the concentration of carbon or nitrogen (% dry weight), b_i is the dry bulk density (g cm⁻³) and d_i is the depth (cm) of the sediment slide (_i), and converted from g cm⁻² to kg m⁻² and to Mg ha⁻¹. In order to have a real estimation of carbon stocks presented in the studied area, compression was considered according to Serrano *et al.*, 2012.

To quantify trace elements (aluminium [AI], arsenic [As], cadmium [Cd], chrome [Cr], copper [Cu], iron [Fe], mercury [Hg], led [Pb], manganese [Mn], nickel [Ni], phosphorous [P], zinc [Zn]), dry sediment sub-samples (0.2 g each) were mineralised using an automatic microwave

digestion system (MARS 5, CEM) with a solution of 67–70% HNO₃, 30% HF, 30% H₂O₂ and Milli-Q water at a ratio of 6:2:0.4:1.6. Thereafter, mineralized samples were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 8000, PerkinElmer). Trace element concentrations were determined using a hydride generation system linked to ICP-OES with a reductant solution, consisting of 0.2% sodium (Na) borohydride and 0.05% Na hydroxide. Analytical quality control was performed using Certified Reference Materials (CRMs): Marine sediment NIST 2702 (National Institute of Standards and Technology). All the analyses were performed in each 1 cm-slice for the first 10 cm.

All the above-mentioned analyses were performed in each 1 cm slice up to the maximum depth reached by each sediment core.

²¹⁰Pb activity was determined through the analysis of its alpha-emitting granddaughter ²¹⁰Po by alpha spectrometry after addition of ²⁰⁹Po as an internal tracer and digestion in acid media using an analytical microwave (Sanchez-Cabeza et al., 1998). The concentrations of excess ²¹⁰Pb used to obtain the age models were determined as the difference between total ²¹⁰Pb and ²²⁶Ra (supported ²¹⁰Pb). Gamma spectrometry measurements were conducted in selected samples from various depths along each core. Supported ²¹⁰Pb was taken as the concentrations at depth and/or from gamma spectrometry analyses that allowed determining the concentrations of ²²⁶Ra conducted in selected samples of each core. To estimate sediment calculation rates the Constant Flux: Constant Sedimentation model (CF:CS) was applied (Ariane Arias-Ortiz et al., 2018; Krishnaswamy et al., 1971).

3.2.3 Data analysis

Shapiro-Wilk test was used to check if data were normally distributed, while Cochran's test to check the heterogeneity of variance of vegetable composition (detritus, algae, seagrass biomass), seagrass (density and L.A.I) and sediment (Water, Porosity, DBD, C_{org}, C_{inog}, N, δ^{13} C, δ^{15} N, trace elements) variables prior to perform one way-Analysis of Variance (ANOVA) to detect differences among stations. Post-hoc pairwise tests were performed when significant differences were detected. Principal Component Analysis (PCA) was performed on trace element normalized data to detect distribution patterns of the stations. The aforementioned analyses were performed using R version 3.6.1.

3.3 Results

Total biomass values differed between stations, with higher value in Station 2 (16207.08 ± 5162.79 g DW m⁻²; Tab.3.2; Fig. 3.2) that was from 4 up to 23-fold higher compared to Station 1, Station 3 and Station 4, respectively (Tab.3.2; Fig. 3.2). Mostly, detritus was responsible for more than 91% of the biomass in Station 1 and Station 2 while the rest was assembled by seagrass with values between 7 and 8%. Moving towards the southern part of Stagnone di Marsala, the contribution of detritus to the total biomass decreased, whereas increased the percentage of seagrass and algae as main contributors in Station 4 (40 and 48%, respectively). *C. nodosa* was found in all the stations while *P. oceanica* was only in Station 2. Greater density (2283.33 ± 325.32 shoot m⁻²) and LAI were related to *P. oceanica* in Station 2 and were both up to 7 and up to 26 than the corresponding values of *C. nodosa* among all the stations (Tab.3.2; Fig. 3.3).



Figure 3.2: Biomass (g DW m⁻²; mean \pm SD) of each vegetal component at each station. Different vegetal composition characterized each station: Station 1 with *C. nodosa*, Station 2 with *P. oceanica*, Station 3 with *C. nodosa* and Station 4 with *C. nodosa* mixed with macroalgae. Capital letters indicate significant differences between stations (Pairwise test, P < 0.05).



Figure 3.3: Shoot density and leaf area index (mean \pm SD) of seagrass species at each station. Different vegetal composition characterized each station: Station 1 with *C. nodosa*, Station 2 with *P. oceanica*, Station 3 with *C. nodosa* and Station 4 with *C. nodosa* mixed with macroalgae. Significant difference (Pairwise t-test, P < 0.05) among stations is given by capital letters.

Table 3.2: Mean values (\pm SD) of biomass (g DW m⁻²) and percentage values within parenthesis of each component shoot density and Leaf Area Index (m² m⁻²; LAI) at the four stations. Different vegetal composition characterized each station: Station 1 with *C. nodosa*, Station 2 with *P. oceanica* patches, Station 3 with C. *nodosa* and Station 4 with *C. nodosa* mixed with macroalgae. Significant difference (Pairwise t-test, P < 0.05) among stations is given by capital letters.

	Station 1	Station 2	Station 3	Station 4
Algae biomass	20.83 ± 29.46	148.56 ± 133.66	2.37 ± 0.97	454.31 ± 30.84
(g DW m ⁻²)	(0.49 %)_A	(0.92 %) A	(0.34 %) A	(47.61 %) B
Seagrass	341.11 ± 63.64	1115.80 ± 109.66	417.64 ± 11.20	385.56 ± 163.03
m-2)	(8.02 %) A	(6.90 %) B	(59.69 %) C	(48.06 %) C
Detritus biomass	3889.86 ± 1838.67	14850.19 ± 2783.08	279.72 ± 80.92	105.42 ± 40.66
(g DW m⁻²)	(91.48 %) A	(91.63 %) B	(39.89%) C	(11.05%) C
Total Biomass (g DW m ⁻²)	4252.19 ± 1804.07	16175 ± 2661.2	701.29 ± 92.88	954.19 ± 177.54
	А	В	С	С
Seagrass Density	642.66 ± 153.60	2283.33 ± 325.32	538.66 ± 62.92	1029.33 ± 236.59
(shoots m ⁻²)	А	В	А	С
Seagrass LAI	1.14 ± 0.28	10.73 ± 1.63	0.88 ± 0.17	1.05 ± 0.38
(m ² m- ²)	А	В	AC	AC

Granulometry analyses revealed that sediments were mainly sandy with low contribution of gravel and silt except for Station 2 where 48 % was silt/clay and showed significant lower dry bulk density (Tabs 3.1 and 3.3; Fig.3.4). The vertical distribution of sediment variables in core profiles was not consistent. Mean carbon and nitrogen content in the first 50 cm of sediments differed among the stations. Station 2 showed the highest content of C_{org} (4.32 ± 1.48 %DW) and N (0.34 ± 0.11 %DW) while C_{inorg} was highest in Station 4 (5.49 ± 0.86 %DW). Both C_{org} and N in Station 2 were from 2 to 3.7-fold compared to the other 3 stations (Tab. 3.4; Fig. 3.5). C_{inorg} content was within the same range between both Station 3 (3.23 ± 0.05 %DW) and Station 2 (3.94 ± 1.55 %DW) while Station 1 was the less enriched (1.69 ± 0.80 %DW; Tab.3. 4; Fig. 3.5).

Table 3.3: Mean value (\pm SD) of sediment variables in the first 50 cm of each station. Different vegetal composition characterized each station: Station 1 with *C. nodosa*, Station 2 with *P. oceanica* patches, Station 3 with *C. nodosa* and Station 4 with *C. nodosa* mixed with macroalgae. Significant difference (Pairwise t-test, P < 0.05) among stations is given by capital letters.

	Station 1	Station 2	Station 3	Station 4
Water (%DW)	33.50 ± 8.60 A	60.10 ± 5.21 B	28.04 ± 5.56 C	29.40 ± 7.19 C
Porosity (%DW)	0.59 ± 0.22 A	0.71 ± 0.07 B	0.46 ± 0.09 C	0.51 ± 0.13 D
DBD (g DW cm ⁻³)	1.14 ± 0.13 A	0.46 ± 0.08 B	1.15 ± 0.14 A	1.24 ± 0.18 C
C _{org} (%DW)	1.79 ± 0.83 A	4.32 ± 1.48 B	0.86± 0.18 C	1.15 ± 0.34 C
C _{inorg} (%DW)	1.69 ± 0.80 A	3.94 ± 1.55 B	3.23 ± 0. 02 B	5.49 ± 0.86 C
N (%DW)	0.11 ± 0.05 A	0.41 ± 0.16 B	0.06 ± 0.2 C	0.08 ± 0.03 AC
C _{org} /N	17.5 ± 8.96 A	11.5 ± 3.52 B	15.0 ± 4.46 B	14.9 ± 4.49 B
δ ¹³ C (‰)	-13.7 ± 0.64 A	-12.3 ± 0.94 B	-12.4 ± 0.85 B	-13.0 ± 0.71 C
δ ¹⁵ N (‰)	2.57 ± 0.56 A	1.91 ± 0.39 B	0.59 ± 0.27 C	2.51 ± 0.62 A
C _{org} stock (kg m ⁻²)	0.20 ± 0.08 A	0.19 ± 0.04 A	0.09 ± 0.01 B	0.14 ± 0.03 C
C _{inorg} stock (kg m ⁻²)	0.19 ± 0.10 A	0.19 ± 0.10 A	0.36 ± 0.05 B	0.70 ± 0.18 C
N stock (kg m ⁻²)	0.013 ± 0.005 A	0.033 ± 0.017 B	0.008 ± 0.002 C	0.010 ± 0.003 AC



Figure 3.4: Vertical profile of mean sediment characteristics, organic carbon (C_{org}), inorganic carbon (C_{inorg}), nitrogen (N) content, C_{org} /N ratio and stable isotope composition (δ^{13} C and δ^{15} N) in sediment at each station. Different vegetal composition characterized each station: Station 1 with *C. nodosa* and seagrass detritus, Station 2 by *P. oceanica* and seagrass detritus, Station 3 by *C. nodosa* and seagrass detritus while Station 4 by *C. nodosa*

 C_{org}/N showed similarity between Station 2, Station 3 and Station 4 (11-15) and slightly higher in Station 1 (18.56 ± 9.77). Considering the isotopic composition, Station 1 was most enriched in $\delta^{13}C$ (-13.7 ± 0.64‰) while the other stations showed comparable values. Additionally, greater $\delta^{15}N$ were associate to Station 1 and Station 4 (2.57 ± 0.56 ‰DW and 2.51 ± 0.62 ‰DW, respectively) and lower to Station 3 (0.59 ± 0.27 ‰DW; Tab. 4; Fig.4).

Table 3.4: One-way Analysis results of sediment variables among stations in Stagnone di Marsala. Different vegetal composition characterized each station: Station 1 with *C. nodosa*, Station 2 with *P. oceanica* patches, Station 3 with C. *nodosa* and Station 4 with *C. nodosa* mixed with macroalgae.

	df	Residual	MS	F value	P value		df	Residual	MS	F value	P value
Water	3	196	11283	247.4	<0.001	Ρ	3	36	23034	36.53	<0.001
Porosity	3	196	0.57	29.41	<0.001	Cu	3	36	0.149	15.84	<0.001
DBD	3	196	6.36	6.362	<0.001	Cd	3	36	833.3	99.71	<0.001
C _{org}	3	196	124	164.5	<0.001	Cr	3	36	5804	62.32	<0.001
C _{inorg}	3	196	132.63	122.4	<0.001	Ni	3	36	1227.9	78.31	<0.001
Ν	3	196	1.33	169.7	<0.001	Pb	3	36	408.5	11.66	<0.001
C _{org} /N	3	196	300.46	9.05	<0.001	Zn	3	36	3320	191.9	<0.001
δ ¹³ C	3	196	20.7	32.81	<0.001	Hg	3	36	0.005	38.83	<0.001
$\delta^{15} N$	3	196	42.18	181.3	<0.001	As	3	36	229.49	13.57	<0.001
C _{org} stock	3	196	0.11	42.72	<0.001	Mn	3	36	187427	455.2	<0.001
Cinorg stock	3	196	2.86	195	<0.001	Fe	3	36	113773195	62.52	<0.001
N stock	3	196	0.007	80.67	<0.001	AI	3	36	930692342	32.4	<0.001

Stocks of C_{org}, C_{inorg} and N in the top 50 cm of sediment differed among stations (Fig. 3.5). Comparable mean C_{org} and C_{inorg} stock characterized both Station 1 ($0.21 \pm 0.08 \text{ kg C}_{org} \text{ m}^{-2}$ and $0.19 \pm 0.10 \text{ kg C}_{inorg} \text{ m}^{-2}$) and Station 2 ($0.19 \pm 0.04 \text{ kg C}_{org} \text{ m}^{-2}$ and $0.14 \pm 0.05 \text{ C}_{inorg} \text{ m}^{-2}$). Especially, C_{org} stock was 1.5-2-fold higher and C_{inorg} up to 4.5-fold lower than that in Station 4 ($0.14 \pm 0.03 \text{ kg C}_{org} \text{ m}^{-2}$ and $0.66 \pm 0.16 \text{ kg C}_{inorg} \text{ m}^{-2}$) and Station 3 ($0.09 \pm 0.01 \text{ kg C}_{org} \text{ m}^{-2}$ and $0.36 \pm 0.05 \text{ kg C}_{inorg} \text{ m}^{-2}$), correspondingly. Additionally, Station 2 supported the highest N stock $(0.025 \pm 0.006 \text{ kg N m}^{-2})$ whereas lower values were ascribed to the other three stations with value from 0.008 to 0.014 kg N m⁻² (Fig. 3.5).



Figure 3.5: Box plots of organic and inorganic carbon and nitrogen stocks (kg m⁻²) at each station of Stagnone di Marsala. Different vegetal composition characterized each station: Station 1 with *C. nodosa*, Station 2 with *P. oceanica*, Station 3 with *C. nodosa* and Station 4 with *C. nodosa* mixed with macroalgae. Thick central line represents median, lower and upper whiskers of the box represent respectively the lowest and the highest values of total range of distribution. Outliers are marked by filled dots.

Based on ²¹⁰Pb analyses, sediment accumulation rates were estimated only for Station 2 and Station 4 (Fig. 3.6). Moreover, in Station 2, it was about $3.2 \pm 0.2 \text{ mm y}^{-1}$ in the upper 30 cm while in the Station 4, below the first 3 cm considered as mixed layer, the accumulation rate was about $0.9 \pm 0.3 \text{ mm y}^{-1}$ for up to first 10 cm. Low concentration of ²¹⁰Pb characterized the first 15 cm of Station 1 sediments and no excess of ²¹⁰Pb was detected indicating a negligible net sedimentation that could not be determinate. Furthermore, Station 3 was characterized by low excess ²¹⁰Pb concentration along the upper 19 cm suggesting intense mixing of this sediment avoiding any possible estimation of accumulation rate.



Figure 3.6: ²¹⁰Pb activity (Bq kg⁻¹) and supported ²¹⁰Pb concentration (shaded area) with sediment depth for only Station 2 with *P. oceanica* and Station 4 with *C. nodosa* mixed with macroalgae.

According to ²¹⁰Pb activities, in the first 30 cm of sediment in Station 2, corresponding to the last 91 years, *P. oceanica* was buried carbon, organic and inorganic, and nitrogen with rate of 65.75 g C_{org} m⁻² y⁻¹, 48.94 g C_{inorg} m⁻² y⁻¹ and 8.41 g N m⁻² y⁻¹, respectively.

Table 3.5: Mean stock values (\pm SD) of trace element concentration in the first 50 cm of each station. Different vegetal composition characterized each station: Station 1 with *C. nodosa*, Station 2 with *P. oceanica* patches, Station 3 with C. *nodosa* and Station 4 with *C. nodosa* mixed with macroalgae. Significant difference (Pairwise t-test, P < 0.05) among stations is given by capital letters.

	Station 1	Station 2	Station 3	Station 4
P (kg DW m ⁻²)	8.25 ± 3.39 A	18.1 ± 3.98 B	3.47 ± 1.03 C	2.26 ± 1.05 C
Cu (kg DW m ⁻²)	1.22 ± 0.38 A	2.35 ± 0.30 B	0.40 ± 0.06 C	0.89 ± 0.11 D
Cd (kg DW m ⁻²)	0.05 ± 0.01 A	0.04 ± 0.01 B	0.04 ± 0.01 B	0.01 ± 0.001 C
Cr (kg DW m ⁻²)	2.95 ± 1.55 A	5.69 ± 1.11 B	1.23 ± 0.25 C	1.21 ± 0.27 C
Ni (kg DW m ⁻²)	0.83 ± 0.43 A	2.71 ± 0.46 B	0.51 ± 0.13 C	1.32 ± 0.23 D
Pb (kg DW m ⁻²)	2.50 ± 0.89 A	1.37 ± 0.25 B	1.77 ± 0.31 B	1.26 ± 0.33 B
Zn (kg DW m ⁻²)	15.50 ± 5.43 A	46.60 ± 5.48 B	4.73 ± 1.80 C	13.90 ± 2.54 D
Hg (kg DW m ⁻²)	0.004 ± 0.001 A	0.007 ± 0.001 B	0.002 ± 0.0005 C	0.004 ± 0.0006 A
As (kg DW m ⁻²)	1.30 ± 0.54 A	1.33 ± 0.21 A	0.47 ± 0.08 B	1.30 ± 0.40 A
Mn (kg DW m ⁻²)	4.68 ± 2.67 A	30.40 ± 1.54 B	5.62 ± 0.77 A	10.30 ± 1.60 B
Fe (kg DW m ⁻²)	418 ± 125 A	745 ± 200 B	139 ± 14.5 C	111 ± 18.8 C
AI (kg DW m ⁻²)	3227 ± 389 A	2961 ± 546 A	2057 ± 346 C	1384 ± 561 D

Within the Stagnone di Marsala, trace element concentrations were different across stations. Station 2, characterized by the presence of *P. oceanica*, showed the highest levels of trace element concentrations and stocks (P, Cu, Cr, N, Zn, Mn, Fe, Hg, Al, Fig. 3.7 and Table 3.6) compared to the other 3 stations. Two principal components of PCA explained almost 85% of total variance between stations and gathered together Station 1, 3 and 4 while Station 2 differed most (Fig. 3.8). The first component that described 63.7 % of the total variance was highly correlated to P, Cu, Cr, N, Zn, Mn, and Fe, Hg (r > 0.80 and p < 0.05). On the contrary, the second component describing 21.6% was correlated to Cd, Pb and Al (r> 0.66 and p<0.05).



Figure 3.8: Principal component analysis (PCA) of trace element concentrations in sediment for each station in the Stagnone di Marsala. Station 1 with *C. nodosa*, Station 2 with *P. oceanica*, Station 3 with C. *nodosa*, Station 4 with *C. nodosa* mixed with macroalgae.

3.4 Discussion

Our results demonstrated that this semi-enclosed basin is an important blue carbon sink, which is supported by the presence of *P. oceanica* and *C. nodosa* meadows. Additionally, the variation of C_{org} stock among the basin depends on both species-specific features of seagrass (distribution, biomass, density, etc.) and spatial variation of environmental factors as water depth and hydrodynamic regime. These outcomes are coherent with previous findings that underline the importance of abiotic and biotic factors in carbon accumulation (Serrano et al., 2016, Samper-Villareal 2017, Mazarrasa et al., 2018).

In the Stagnone di Marsala, the two species showed different spatial distribution with *C. nodosa* largely distributed among the entire basin while *P. oceanica* is presented in patches, only in the central area. Both seagrass species in the Stagnone di Marsala showed higher sedimentary C_{org} content compared to previous findings probably due to the high input of organic matter that characterizes coastal area (Apostolaki et al., 2018; De Lacerda, 1994; Serrano et al., 2016a) Generally, sediments associated to *P. oceanica* were more enriched in sedimentary C_{org} compared to *C. nodosa* as recently reported in Greece (Apostolaki, 2018; 2019). In the Stagnone di Marsala, mean C_{org} content associated to *P. oceanica* was higher than that lately found in Greece (0.49 % DW; Apostolaki et al., 2019) and in Spain (3.91 % DW;

Serrano et al., 2016a). Moreover *P. oceanica* supported lower carbon stock than in Spanish sites (31-32.5 Kg Corg m⁻²; Serrano et al., 2016), where it forms denser meadows and thicker sediment deposit at major depths.

Both factors, seagrass density and water depth, are positively affecting the deposition of C_{org} and the carbon stock capacity. Additionally, burial rates of C_{org} were slightly lower than the mean estimated for *P. oceanica* (84 ± 20 g C_{org} m⁻² y⁻¹, Serrano et al., 2016) but fell inside the range reported in the same study (6-202 g C_{org} m⁻² y⁻¹).

Despite the wide distribution of *C. nodosa* across the Mediterranean Sea and the potential role in climate change mitigation, few data are available of carbon content and even less on carbon stock. Considering the future expansion of this seagrass that will replace *P. oceanica* following increasing temperature and water salinity, further studies have to focus on its carbon stock capacity (Chefaoui et al., 2018). In the Stagnone di Marsala, C_{org} content associated to *C. nodosa* sediments was up to 4.5-fold higher then available estimations for Greece (0.35-0.4 % DW; Apostolaki et al., 2018, 2019). Relative mean stocks were lower than the only existing data reported for this species in Greece (Apostolaki et al., 2019). Surprisingly, in the northern sector, *C. nodosa*, showed C_{org} stock value similar to *P. oceanica* in the central part, despite the difference in stock capacity between the two species reported in the literature (Apostolaki et al., 2019). The low hydrodynamic regime in the northern area (La Loggia et al., 2004) may promote the deposition of large quantity of organic matter, as seagrass detritus, enhancing the C_{org} stock capacity of *C. nodosa* in this sector.

Generally, the Stagnone di Marsala supports lower C_{inorg} stock than the global estimation reported for seagrass ecosystems (32.7 Kg C_{org} m⁻²; Mazarrasa et al., 2015). Probably, the differences between our results and previous findings may be ascribed to potential effects of local environmental factors such as salinity, temperature and water depth in the deposition of carbonates (Howard et al., 2018). Moreover, C_{inorg} stocks followed a spatial pattern with increasing values from the northern to the southern sector. Mainly, this is related to the increase in water exchange in the southern sector, which enhances particle transport. Additionally, higher C_{inorg} stock associated to stations characterized by *C. nodosa* compared to the station with *P. oceanica* followed the global trend reported for both genus (Mazarrasa et al., 2015). This difference could be ascribed to a higher efficiency of *C. nodosa* in trapping and retaining suspended particles (Hendriks et al., 2010). Moreover, this effect is amplified in the southern sectors by the higher density of *C. nodosa* compared to the other sectors.

Marine coastal ecosystems are subjected to anthropogenic activities due to the geomorphological continuity with terrestrial ecosystems and are characterized by large accumulation of nutrients and trace elements in the sediment (Bonanno and Orlando-Bonaca, 2018; Pereira et al., 2010; De Lacerda, 1994). Seagrasses are natural filters for nutrients and trace elements, and with their roots they stabilize the soils, preventing possible release to the water column and consequently oxidation and transformation into toxic forms that may cause detrimental effects on organisms.

The entire Stagnone di Marsala was characterized by the presence of different trace elements, and relative concentrations were comparable with the previous data reported for Mediterranean marine sediments (Bonanno and Orlando-Bonaca, 2018). Additionally, some trace elements (Cd, Ni, As, Ni) exceeded the threshold reported for marine sediments by Italian Law (G.U.R.I. D.M. 367/03, 2004). The coastline of this basin is characterized by the presence of several anthropogenic activities such as an international airport (Birgi airport), agriculture and industrial activities and of an urban area (Marsala city), which can contribute to contaminant accumulation. In particular, the highest concentrations and stocks were found in the central sector, where the presence of *P. oceanica* together with higher sedimentary C_{org} content, that showed high affinity with these contaminants, and reduced hydrodynamic conditions probably promote large deposition of contaminants. Generally, all the other stations characterized by *C. nodosa* were marked by comparable trace elements distributions.

Nitrogen content and stock followed the same pattern, with higher values associated to the central sectors with *P. oceanica*. However, N stock and burial rate associated to *P. oceanica* in Stagnone di Marsala were lower than estimations reported for this species (Gacia et al., 2002; Mateo et al., 1997; Romero et al., 1994). N stock associated to *C. nodosa* sediments, especially in the northern and central sectors, showed comparable values to Greece (Apostolaki et al., 2019).

In the last decades, the Mediterranean area has been subjected to widespread regression of *P. oceanica* due to the synergistic effects of global change and anthropogenic pressures (Chefaoui et al., 2018; Marbà et al., 2014). Under this scenario of increasing temperature and salinity, species as *C. nodosa* seems to be able to cope with these changes expanding their distribution. Considering the difference reported for carbon stock capacity between these two species and the large amount of organic carbon sequestered by *P. oceanica* meadows, the loss of *P. oceanica* more likely affects the ability of these ecosystems to mitigate climate change

effects. Our findings showed that in semi-enclosed basins like Stagnone di Marsala, *C. nodosa* is able to store large amount of carbon, with values comparable to *P. oceanica*. However, despite the stress condition determining the regression of *P. oceanica*, this species stores large quantities of carbon. Moreover, due to the combination of peculiar characteristic of hydrodynamic conditions, high input of organic matter and the presence of seagrass meadows, Stagnone di Marsala seems to be an important sink of blue carbon giving indication about the important ecological role of this kind of ecosystems. Despite the growing interest of the scientific community on blue carbon science, there is still lack of information about distribution of blue ecosystems and their relative stock capacity. Studies like the present one can provide useful information for future investigation in a perspective of conservation management plan.
4 Carbon stock capacity of high degraded ecosystems: the potential role of dead *Posidonia oceanica* mat as carbon sink

Abstract

Seagrass ecosystems store significant amount of blue carbon contributing to mitigate climate change. These ecosystems are vulnerable to natural and anthropogenic impacts that potentially reduce the stock capacity and enhance the release of CO₂. This study examined the role exerted by dead *Posidonia oceanica* as carbon sink in a highly degraded area (Augusta Bay, Italy). Carbon stock of dead mat was compared with that of unvegetated sediments. Also, due to the large contamination of the environment, nitrogen and mercury stocks were compared. Results provide the role of dead mat as important carbon sink and its capacity to retain large quantity of nitrogen and mercury. Despite its condition, the dead mat still preserved carbon contributing to mitigate climate change and avoiding any released of contaminants.

4.1 Introduction

Blue carbon ecosystems as seagrass meadows are globally recognized for their significant role in mitigate climate change (Boudouresque et al., 2016; Costanza et al., 1997; Duarte et al., 2013b; Kennedy et al., 2010). In fact, these ecosystems sequester high quantity of carbon in the soil that remains buried in for long period, over millenia, and acting as blue carbon sink (Alongi, 2012; Fourqurean et al., 2012; McLeod et al., 2011; Kennedy et al., 2010; Nellemann et al., 2009). This is related to the interection of multiple factors as seagrass species composition, meadows density and productivity, hydrodinamism, nutrient availability and water depth (Mazarrasa et al., 2018; Serrano et al., 2016, Serrano et al., 2014).

Despite the increase of scientific interests on blue carbon in the last decade, since this term was firstly used (Nellemann et al., 2009), the effects of natural and anthropogenic disturbances on carbon accumulation in these ecosystems are still pending questions (P. I. Macreadie et al., 2019b; Spivak et al., 2019). Especially, efforts have to focus on understanding the fate of blue carbon stock once the ecosystems is impacted or lost, and, quantifying the carbon released by and the extenstion of the disturbance in the depth soil profile (Lovelock et al., 2017; P. I. Macreadie et al., 2019b; Spivak et al., 2019). Filling those knowledge gaps are fundamental to better understand how to develop and implent protection and conservation plans for these important ecosystems that rapidly disappered (McLeod et al., 2011). Seagrass meadows, among the others, are one of the most threated ecosystems in the world with 29% of their area already lost (Waycott et al., 2009). Especially, sea level rise, acidification, warming and extreme events (storms) deeply alteret distribution, productivity and community composition of these ecosystems (Jordà et al., 2012; Koch et al., 2014; Lovelock et al., 2017; Orth et al., 2006; Saunders et al., 2013; Waycott et al., 2009). Furthemore, anthropogenic disturbances as coastal development, agricultaral activities, land conversion, mechanical disturbances contributed directly and inderectly to the seagrass decline and loss of their ecosystem services (Boudouresque et al., 2016a; Serrano et al., 2015; Short et al., 2009, Kendrick et al., 2002). Especially, major impacts related to human activities are eutrhophication, increase of water turbidity and anchoring that improve seagrass meadows disaperances (Marbà et al., 2012, Orth et al., 2006, Serrano et al., 2015). Once the meadows is lost, the soil remains exoposed to disturbances, anthopogenic and natural, and to environmental factors, as oxygen, microbial activities, and the carbon accumulated could be relased to the adjacent environment

contributing to increase CO₂ content. The fate of the oganic matter and, generally, of the carbon accumulated in these ecosystems sediments after seagrass disapperance still not well understood. Especially, the superficial soil without vegetation is easily exposed to erosion or bioturbation. Consequently, the organic matter is relased and exported to the adjacent habitas or otherwise is oxidesized promoting aerobic decomposition (Spivak et al., 2019). The effects of impacts on both vegetation and soil compartments are related to the interaction of multiple stressors and to their rates (Saunders et al., 2013; Macreadie et al., 2019).

As consequences of antrhopogenic disturbances, marine coastal ecosystems are potential sink of pollutant such as trace elements (Halpern et al., 2008). Trace elements are naturally avaiable in low content: some are toxic, other are essential for living processes and may be toxic above certain contents (Stevenson and Cole, 2009). Additionally, trace elements are persistant contaminants in the envrionment and rapresent a potential risk for marine organism due to their capacity to be bioaccumulated and biomagnificated (Bonanno and Orlando-Bonaca, 2018; Campbell et al., 2005; Gray, 2002; Monperrus et al., 2007; Signa et al., 2013). Mostly, these contaminants tend to be accumulated in marine sediments with a ratio of 3:1 rather than water coulmn (Bonanno and Borg, 2018; Turner and Millward, 2002). Cuncurrently, seagrasses meadows enhance the deposition of fine-organic particles that have strong binding affinity with trace elements and, hereby, sequestring large quantity of pollutants in their soil (Bonanno and Raccuia, 2018; Lafabrie et al., 2007; Malea et al., 2019; Serrano et al., 2013). Additionally, seagrasses tend to accumulate great quantity of trace elements in roots and leaves and reducing their avaiability (Kaldy, 2006; Lewis and Richard, 2009). Above certain content, trace elements, as mercury, interfers with the photosynthetic systems and affects seagrass productivity (Clijster and van Assche, 1985; Macinnis-Ng and Ralph, 2002). Moreover, the presence of mercury in the marine envirnoment is associated to natural input as volcanic explosion and to industrial activities (Nriagu, 1988) and different studies have shown high mercury content associated to seagrass sediment and relative tissue (Sánchez-Quiles et al., 2017; Sanz-Lázaro et al., 2012; Serrano et al., 2013).

In the Mediterranean Sea, the endemic seagrass *Posidonia oceanica* (L.) Delile is one of the most abundant species, which form dense meadows and biogenic reef known as mat (Boudouresque & Meinesz, 1982). The mat is an important substratum on which *P. oceanica* grows, consisting of complex network of both living and old tissues buried within sediment under anoxic conditions

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(Boudouresque et al., 2016; Mateo et al., 1997). This represents an important carbon sink preserved for long period and reaching up 8m thick (Romero *et al.*, 1996; Mateo *et al.*, 1997). Additionally, the mat represents an important archive on anthropogenic impacts and pollutants accumulation that helps to reconstruct environmental changes and disturbances along time (Serrano et al., 2012).

The fast decline of *P. oceanica* is threatened this carbon stock with potential shift from sink to source of carbon. In fact, *P. oceanica* meadows are following the negative global trend likewise the other blue carbon ecosystems with an annual decrease of 6.9 % due to climate change and, especially, to the high anthropogenic pressure along the coast (Benoit *et al.*, 2005; Holon et al., 2015). It has been estimated that up to 52% of *P. oceanica* ecosystems been lost in the last 50 years with depletion of its capacity to act as carbon sink (Marbà et al., 2014, Chefaoui et al., 2018). Especially, following the seagrass loss, the soil remains exposed to possible disturbances and the fate of the carbon stock is a matter of speculation.

Here, we assess the capacity of degraded seagrass ecosystems to act as sinks of biogenic and trace elements. In particular, we reconstruct the recent storage and burial in dead and alive *P. oceanica* mats and in adjoining unvegetated sediment in Augusta Bay in Sicily (Italy) by determining the vertical distribution and accumulation of elemental and isotopic carbon and nitrogen, as well as mercury along sediment profiles.

4.2 Materials and Methods

4.2.1 Study area

The study was performed in Augusta Bay, a semi-enclosed basin located in eastern Sicily (Italy), and delimited to the north by Santa Croce Cape and to the south by Santa Panagia Cape (Fig.4.1). The north-eastern side of the bay is occupied by Augusta Harbour, an important industrial area and commercial port, hosting the largest European petrochemical complex and the most important chloro-alkali plat of Italy (Le Donne *et al.*, 2008). Moreover, the harbour is delimited by Augusta town and two breakwaters built during the 1960s and connecting to the open sea through to Levante (400m wide and 40 m deep) and Scirocco Inlets (300m wide

and 13m deep, ICRAM, 2008). High contamination level and eutrophication has characterized Augusta Harbour due to industrial and domestic discharges, uncontrolled sewage discharges

and dredging and defined as one of the most polluted area in Europe (Bellucci et al., 2012; De Domenico et al., 1994; ICRAM, 2008). Before the built of demercurisation and waste treatment plant in 1983 in according to the national law (G.U.R.I, L.319/1976), the chloroalkali plant has discharged waste waters without treatments with a consequently high accumulation of mercury (Hg), a toxic metal, in the marine environment (Bellucci et al., 2012). Therefore, due to its peculiar water circulation, Augusta Harbour represented an important Hg source not only for this area but also for the entire Mediterranean Sea (Sprovieri et al., 2011). In fact, the presence of the two inlets together with wind and tidal actions influenced water exchanges and inner circulation (ICRAM, 2008; ISPRA, 2010, Fig. 1). Especially, there are two main currents that are originated by the incoming water from Scirocco and Levante Inlets. Mainly, the water exchange is low with cyclonic circulation preventing high water exchange and allowed the content of pollutions inside the bay. Outcoming bottom flow exported the to the rest of the bay and, additionally, are intercepted by Levantine Intermediate Water and entering in the Mediterranean Sea (Sprovieri et al., 2011).

The north-western sector of Augusta Bay is covered by an extensive and abundant *P. oceanica* meadows in Xifonio Gulf with a mean depth of 13m and mean density of 363.1±20,4 shoot/m² (Costantini et al., 2015). On the other side, the southern sector is defined by the coastal area of Priolo Bay, a Site of Community Importance (SCI) by the European Commission Habitats Service (94/43/EEC) and its seabed is characterized by a dead mat of *P. oceanica*. According to previous studies, *P. oceanica* meadows in Priolo Bay has disappeared around the 70's, most probably following the increase in turbidity during the construction of the construction of artificial breakwaters (Giaccone & Di Martino, 1995; Costantini et al., 2015). Additionally, due to the direct connection between Augusta Harbour and Priolo Bay through Scirocco Inlet and to the presence of bottom water currents that facilitate the dissemination of contaminants along this coastal area, high contents of mercury and other trace elements as cadmium in the surface sediments have been detected (Di Leonardo et al., 2014). According to the national law (G.U.R.I, L. 426/1998), Priolo Bay and Augusta Harbour have been classified as National Important Site (SIN) and, since 2002, it has been included in the National Remediation Plan by the Italian Environmental Ministry for the high state of environmental degradation of the area.

4.2.2 Sampling strategy



Figure 4.1: Map of Augusta Bay with main currents and sampling sites location.

Sediment samples were collected in duplicate by professional scuba-divers using 40 cm long PVC hand-cores (Ø 40 mm) in three stations; dead *P. oceanica* mat (hereafter called MAT-D) and bare sediments (hereafter called SED) in Priolo Bay and living *P. oceanica* mat (hereafter called MAT-L) in Xifonio Gulf. After collection, sediment samples were immediately frozen at -20°C (Table 1 and Fig. 1).

Table 4.1: Coordinates of sampling station and environmental variables and grain size at each station.

Area	Station	Coordinat es	Depth (m)	Granulom etry (Gravel- Sand- Silt/Clay %DW)	рН	Salinity (%)	Chl a (mg I ⁻¹)
Priolo Bay	MAT-D	37°9'17" N 15°13'6"E	12	5-65-31	8.18	37.94	0.91
	SED	37°9'32"N 15°13'21" E	12	5-81-14	8.23	37.86	0.86
Xifonio Gulf	MAT-L	37°13' 58" N 15°13'59 E	13	34-62-4	8.10	37.73	0.84

4.2.3 Analytical methods

In the laboratory, sediment cores were sliced into 1 cm section using a stainless-steel handsaw, freeze-dried (for 48 h) and powdered. For grain analyses, wet samples were previously left in H_2O_2 solution (3-10%) to eliminate the organic matter. Subsequently, sediment fractions (gravel, sand, silt/clay) were separated by wet sieving at 63µm, overdried at 60°C and weighted. To estimate dry bulk density (DBD, g cm⁻³), the dry weight of sediment was divided by the volume of the wet samples.

Dry sediment aliquots were weighed in tin capsules to perform total carbon, total nitrogen analyses and in silver capsules for organic carbon analyses using an elemental analyser (Thermo Flash EA 1112). Exclusively, for organic carbon, after weighing, sediments samples were acidified with HCl (18%) in order to eliminate carbonate contents.

Stable isotope composition (δ^{13} C and δ^{15} N) was obtained analysing dry sediment aliquots in tin capsules, after weighting, by an isotope ratio mass spectrometer coupled with an elemental analyser (Thermo IRMS Delta Plus XP coupled with Thermo EA-1112). Only for δ^{13} C

analyses, sediment samples were acidified with a solution of HCI (2N) before weighting aliquots.

To quantify mercury (Hg) content, dry sediment sub-samples (0.2 g each) were mineralised using an automatic microwave digestion system (MARS 5, CEM) with a solution of 67–70% HNO₃, 30% HF, 30% H₂O₂ and Milli-Q water at a ratio of 6:2:0.4:1.6. Thereafter, mineralized samples were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 8000, PerkinElmer). Mercury (Hg) contents were determined using a hydride generation system linked to ICP-OES with a reductant solution, consisting of 0.2% sodium (Na) borohydride and 0.05% Na hydroxide. Analytical quality control was performed using Certified Reference Materials (CRMs): Marine sediment NIST 2702 (National Institute of Standards and Technology). All the analyses were performed in each 1 cm-slice for the first 10 cm and every 2 cm up to 20 cm of sediment.

Stocks were estimated as X stock = Σ (X_i * b_i * d_i), where X_i is the content of the element (% dry weight), b_i is the dry bulk density (g cm⁻³) and d_i is the depth (cm) of the sediment slide (*i*), and converted from g cm⁻³ to kg m⁻². Sediment decompression was assessed considering the total length of the corer and the retrieved (Serrano *et al.*, 2012).

²¹⁰Pb activity was determined in selected core sub-samples through the measurement of its granddaughter ²¹⁰Po, using alpha spectrometry. This method is based on that outlined in Flynn (1968) and using double acid leaching of the dried sediment sample followed by autodeposition of the Po in the leachate onto silver polished discs. ²⁰⁹Po was added as an isotopic yield tracer. Samples were counted over a three-day period on a Canberra Alpha spectrometry Quad system at the National Oceanography Centre, Southampton, UK. The limit of detection was 0.1 Bq kg¹. Sediment accumulation rates were calculated from the ²¹⁰Pb data using the "simple" model (or Constant Flux : Constant Sedimentation, CF:CS model, Robbins, 1978) of ²¹⁰Pb dating, which gives an average sediment accumulation rate over the datable core depth, and via the Constant Rate of Supply (CRS) model (Appleby & Oldfield, 1978). For the simple or CF:CS model, sedimentation rate was calculated by plotting the natural logarithm of the unsupported ²¹⁰Pb activity (²¹⁰Pbxs) against depth and determining the least-squares fit. The CRS model compares the cumulative unsupported ²¹⁰Pb activity below any given depth in a core to the total unsupported ²¹⁰Pb activity in the core, and allows (a) ages to be calculated for any particular core depth, and (b) varying sediment accumulation rates to be modelled using the calculated age-depth curve. ²¹⁰Pb inventories (in Bq m⁻²) were calculated as the sum

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of unsupported ²¹⁰Pb activity * sediment dry bulk density * sample thickness in each core layer.

4.2.4 Data analysis

Linear regression analysis was used in order to detect any possible trend of the distribution of sediment variables with the depth. Shapiro-Wilk test was used to check if data were normally distributed and Cochran's test for the heterogeneity of variance in sediment stock before the performance an Analysis of Variance (ANOVA). One way-Anova was used to detect possible statistical differences between stations. All the statistical analyses were performed using R version 3.6.1. The SEQUENTIAL REGIME SHIFT DETECTION software (significance level = 0.05; Rodionov and Overland, 2005) was used to detect discontinuities in the sediment properties related to the time series.

4.3 Results

4.3.1 Dead mat and bare sediment in Priolo Bay

Granulometry analyses showed that most of the sediments were predominantly sandy with major contribution of silt/clay in MAT-D (Table 4.1). Additionally, all the sediments sampled were characterized by similar values of porosity within the range of 0.17–0.19 % DW. Only few sediment variables showed significant variability along the depth profile. Especially, C_{org} and N in bare sediment ($R^2 = 0.60$, P < 0.05 and $R^2 = 0.79$, P < 0.05, respectively) decreased with the sediment depth while Hg content and relative stock both increased ($R^2 = 0.63$, P< 0.05 and $R^2 = 0.74$, P<0.05, respectively). Additionally, positive trends were detected for Hg content and stock in dead mat ($R^2 = 0.75$, P<0.05 and $R^2 = 0.65$, P<0.05, respectively). Mainly, despite porosity, all the variables significantly differed between the two stations of Priolo Bay (P< 0.001, Tab. 2).

Table 4.2: Mean value (\pm SD) of sediment variables, elemental and isotopic composition in the first 20 cm for each station. Total stock in the top 20 cm are indicated between brackets. P value is referred only to the differences between MAT-D and SED.

	MAT-L	SED	MAT-D	P value
Water (%DW)	21.47± 7.50	27.53± 5.828	23.68± 8.65	< 0.001 ***
Porosity (%DW)	0.19± 0.03	0.17± 0.04	0.16± 0.05	0.097
DB (gcm ⁻³)	0.97 ± 0.27	0.64 ± 0.09	0.77 ± 0.26	< 0.001***
C _{org} (%DW)	0.30 ± 0.08	3.22± 0.81	2.35 ± 0.48	< 0.001***
C _{inorg} (%DW)	11.03 ± 1.28	8.52 ± 1.31	8.27 ± 1.13	< 0.001***
N (%DW)	0.03 ± 0.01	0.13 ± 0.02	0.06 ± 0.02	< 0.001***
C _{org} /N	11.35 ± 2.59	25.99 ± 7.04	40.65 ± 15.66	< 0.001***
δ ¹⁵ N (‰DW)	0.72 ± 0.74	2.25 ± 0.60	2.33 ± 1.32	< 0.001***
δ ¹³ C (‰DW)	-18.63 ± 0.31	-15.09 ± 0.61	-15.02 ± 0.66	< 0.001***
Hg (mg DW kg⁻¹)	0.36 ± 0.19	1.84 ± 0.91	0.07 ± 0.05	< 0.001***
C _{org} stock (kg DW m ⁻²)	0.18 ± 0.05 (2.76)	0.03 ± 0.01 (0.43)	0.21 ± 0.04 (3.23)	< 0.001***
C _{inorg} stock (kg DW m ⁻²)	0.65 ± 0.21 (9.91)	1.05 ± 0.25 (16.23)	0.54 ± 0.03 (8.25)	< 0.001***
N stock (kg DW m ⁻²)	0.004 ± 0.001 (0.069)	0.002 ± 0.0004 (0.048)	0.008 ± 0.001 (0.124)	< 0.001***
Hg stock (kg DW m ⁻²)	0.00005 ± 0.00001 (0.006)	0.0004 ± 0.0003 (0.001)	0.001±0.0006 (0.018)	< 0.001***



Figure 4.2: Vertical profile of water content, porosity and dry bulk density of in sediment at each station. Shaded area referes to the period of seagrass decline in Priolo Bay.

Dead mat sediments were characterized by lower dry bulk density (0.64 \pm 0.08 g cm⁻³) and higher C_{org} and N contents compared to bare sediment, with C_{org} content at MAT-D (3.22 \pm 0.81 % DW) 11-fold higher than SED (0.30 \pm 0.08 %DW) and N content 4 times greater (MAT- D 0.13 \pm 0.02 %DW) than at bare sediment (0.03 \pm 0.01 %DW). Likewise, MAT-D showed the highest content of mercury with a mean of 1.84 \pm 0.92 mg kg⁻¹ that was 5-fold than SED (0.36 \pm 0.19 mg kg⁻¹). Larger contents of inorganic carbon were observed in SED with an average of 11.03 \pm 1.28 %DW, while in MAT-D of 8.52 \pm 1.31%DW (Fig. 4.3).



Figure 4.3: Vertical profile of organic carbon (C_{org}), inorganic carbon (C_{inorg}) and nitrogen (N) content in sediment at each station. Asterisk indicates linear regression trend along the depth profile. Shaded area referes to the period of seagrass decline in Priolo Bay.



Figure 4.4: Vertical profile of C_{org}/N ration and stable isotope composition ($\delta^{13}C$ and $\delta^{15}N$) in sediment at each station. Shaded area referes to the period of seagrass decline in Priolo Bay.

Considering C_{org}/N ratio, it resulted much lower in bare sediment (11.35 ± 2.59; Fig. 4.4) than in sediment associated to dead *P. oceanica* mat with an average of 25.99 ± 7.04. Comparable results were obtained for $\delta^{15}N$ and $\delta^{13}C$, where bare sediments were less enriched than dead mat. Principally, δ^{15} N values in SED often fluctuated along the depth with a range from 0.28‰ to 1.68 ‰. On the contrary, results for MAT-D (2.25 ± 0.60 ‰) were almost 3-fold times higher than bare sediment. Additionally, the δ^{13} C showed negligible differences between MAT-D (-15.09 ± 0.61 ‰) with values in the range from -14 ‰ to -16‰ while SED showed more depleted values (on average -18.60 ± 0.30 ‰; Fig.4.4).



Figure 4.5: Vertical profile of C_{org}, C_{inorg} and N stock in sediment at each station. Blue lines indicate possible regime shifts and shaded area the period of seagrass decline in Priolo Bay.



Figure 4.6: Stock in the top 20 cm of sediment at both stations in Priolo Bay.



Figure 4.7: Vertical profile of ²¹⁰Pb activity in sediment at each station.

Considering the total stock estimations in top 20 cm, there were significant differences among the station for all the stock calculated (Table 4.2; Fig.4.5). Greater C_{org} stock (mean stock 0.21 \pm 0.04 kg C_{org} m⁻² and total 3.23 kg Corg m⁻², respectively) value was associated to dead *P.oceanica* mat and resulting 7-fold higher than in SED (mean stock 0.03 \pm 0.01 kg C_{org} m⁻² and total stock 0.43 kg C_{org} m⁻², respectively) (Fig.4. 5-4.6). Furthermore, dead mat supported higher N (mean stock 0.008 \pm 0.001 kg N m⁻² values and total 0.12 kg N m⁻² values) and Hg stocks (mean stock 0.001 \pm 0.0006 0.12 and total 0.018 kg Hg m⁻²) compared to that in bare sediment. Accordingly, N and Hg stock in MAT-D resulted both 3 times higher than those in SED. Conversely, bare sediment showed the highest inorganic stock (mean stock 1.05 \pm 0.25 and total 16.23 kg C_{inorg} m⁻², respectively) while lower values were observed in dead mat.

Based on ²¹⁰Pb activities (Fig. 4.7), MAT-D showed a broadly exponential decline in ²¹⁰Pb activity with depth to near-constant (i.e. supported) activities of ca. 12 Bq kg⁻¹ at 20 cm depth. Lack of large inflections in the profile indicated that there have been no major changes in sediment input or erosion, and relatively uniform sediment accumulation over time. On the contrary, highly disturbed sedimentation recorded in bare sediment preventing the dating. Application of CF:CS model (Robbins, 1978) to MAT-D yielded an average sediment accumulation rates of 1.9 mm y⁻¹ and, accordingly, the top 20 cm sediments corresponded to 98 y.

According to ²¹⁰Pb activities, in the last century dead mat has buried nutrient and mercury at rates of 35.60 g C_{org} m⁻² y⁻¹, 81.47 g C_{inorg} m⁻² y⁻¹ and 1.26 g N m⁻² y⁻¹ and 0.19 g Hg m⁻² y⁻¹, respectively (Fig. 4.8-4.9). Considering the regime shifts results, discontinuities were found around 1965, after which C_{org} , N and Hg stocks increased towards upper depth level. Lastly, C_{inorg} showed discontinuity at 1980 with content decreased toward the upper levels.



Figure 4.8: Vertical profile of C_{org}, C_{inorg} and N burial rate in sediment at each station. Shaded area refers to the period of seagrass decline in Priolo Bay.



Figure 4.9: Vertical profile of C_{org}, C_{inorg} and N burial rate in sediment at each station. Asterisks indicate significant linear trends with depth. Blue lines indicate possible regime shifts and shaded area the period of seagrass decline.

4.3.2 Living mat in Xifonio Gulf

Living mat characterized the seabed of Xifonio Gulf with mostly sandy sediments. Several variables as N (R² = 0.87, P < 0.05) and Hg (R² = 0.82, P < 0.05) showed evident decrease following the depth profile (Fig. 4.3-4.6). The sediment of this station was characterized by C_{inorg} content of 8.27± 1.13 %, C_{org} of 2.35 ± 0.48 % DW, N of 0.06 ± 0.02 % DW and Hg of 0.0004 kg Hg m⁻². Moreover, MAT-L showed enriched values of δ^{13} C 15.09 ± 0.61 ‰ and high C_{org}/N ratio (15.09 ± 0.61). Considering the accumulation of biogenic and trace element (Fig.4.8-4.10), living mat sediment showed greater value of C_{inorg} (9.95kg C_{org} m⁻²) stock followed by C_{org} (2.74kg C_{org} m⁻²) and relative lower stock of nitrogen and mercury (0.07 Kg N m⁻², 0.0004 kg Hg m⁻²). These stocks have been accumulated in 91 y with a sediment accumulation rate of 2.1 mm y⁻¹ and corresponding burial rate of 30.16 g C_{org} m⁻² y⁻¹, 109.32 g C_{inrg} m⁻² y⁻¹, 0.76 g N m⁻² y⁻¹, 0.001 g Hg m⁻² y⁻¹.



Figure 4.10: Stock in the top 20 cm of sediment at living mat in Xifonio Gulf.

4.4 Discussion

4.4.1 Dead mat

Our results demonstrated that despite the disappearance of *P. oceanica*, the remaining mat continues to preserve the carbon stock in the soil and corroborate by the absence of any evident erosion through ²¹⁰Pb analyses. Additionally, the dead mat is an efficient sink of nitrogen and mercury accumulated during the last century as a result of the anthropogenic activities. Dead mat sediments trapped in the dead mat have been accumulated in the last 98 years with a rate similar to the average reported for this biogenic reef in Mediterranean Sea (2.1 ± 0.4 mm y⁻¹, Serrano et al. 2016). The sediment associated to dead mat was Corg-riched compared to bare sediment and, despite the absence of living materials in dead mat, the Corg stock accumulated in the first 20 cm resulted comparable to that reported elsewhere for P. oceanica mat as in Balearic Island-Spain (3.32 kg Corg m⁻², Serrano et al., 2012). Moreover, burial rates of C_{org} were 2 to 3-fold lower than mean estimated for *P. oceanica* mat (84 ± 20 g C_{org} m⁻² y⁻¹, Serrano et al., 2016) but felt inside the range (6-202 g C_{org} m⁻² y⁻¹) reported in the same study. Meanwhile, according to previous findings, unvegetated sediments were characterized by low organic carbon content and stock (Apostolaki et al., 2019; Gullström et al., 2017; Jankowska et al., 2016). However, despite its condition and the absence of living meadows that are promoting the settling of organic matter, Corg content in dead mat sediment was comparable to value reported for Balearic Island (3.21% DW, Serrano et al., 2014). This is an indication of the capacity of the mat to trap and store carbon for long time (Mateo et al., 1997) even after the death of the meadow.

According to C_{org}/N ratio and $\delta^{13}C$ value, most of organic carbon sequestered in the dead mat in Augusta Bay derived from seagrass detritus. Conversely, bare sediments were characterized by poor organic carbon and depleted $\delta^{13}C$, indicating a different source, probably microphytobenthos and/or phytoplankton (Pocklington et al., 1979). Both stations support lower stocks than values reported for seagrass and unvegetated sediments (Mazarrasa et al., 2015). Unfortunately, our data do not allow to detect the precise moment of seagrass disappearance. Until 1958, it has been reported the presence of *P. oceanica* in this area (Moliner and Picard, 1953) and, in the same study, the authors already hypnotized a forthcoming decline of this seagrass following the increasing of water turbidity caused by the built of two breakwaters (early 1960s). Additionally, during 1970s, frequently eutrophic crises have been reported in Augusta Bay connected to wastewater discharges and dredging

activities, and consequently fish die-off (De Domenico et al., 1972; Genovese, 1981). Turbidity water condition and eutrophication have important consequences on seagrass meadows and are considered as major causes of their decline, especially, for slow growing species as P. oceanica (Ceccherelli et al., 2018; Marbà et al., 2015; Serrano et al., 2014; Wylie et al., 2016). The intensification of anthropogenic impacts is reflected by dead mat sediments that showed higher amount of nutrient and trace elements, especially increasing from 1965. Since 1950s, many industrial activities have been set along the coasts of Augusta Bay with negative effects on the environment, principally, due to uncontrolled waste-water discharges, agricultural activities, nutrients input and dredging followed by eutrophication and fish die-off events (De Domenico et al., 1972; Genovese, 1981). The disparity of N and Hg stocks between unvegetated sediments and dead mat underlined the role of the mat as sink for those elements. Both nitrogen stocks estimated for dead mat and unvegetated sediments were comparable with relative stock reported for Greece (0.16 ±0.18 kg N m⁻² in *P. oceanica* soil and 0.04 ± 0.01 kg N m⁻² in unvegetated sediments; Apostolaki et al., 2019). Furthermore, N burial rate in dead was comparable with data reported before for *P. oceanica* in NW Mediterranean Sea and within the wide range of 0.2-13.4 g N m⁻² y⁻¹ (Mateo et al., 1997 (b); Pendersen et al., 1997; Romero et al., 1994; Gacia et al., 2002).

Mercury is one of the major sources of contamination in Augusta Bay caused by industrial uncontrolled discharge of waste water between 1950s and 1980s (Bellucci et al., 2012; Sprovieri et al., 2011). So far, anthropogenic input of mercury has been reduced since the beginning of 80s whit the activation of a demercurisation plant. This is consistent with the decrease of Hg content been recorded in the upper 7 cm of dead mat sediments corresponding to 1983-2011 period.

Both stations in Priolo Bay supported high levels of mercury that were over than the corresponding limit for marine sediments (0.30 mg Hg kg⁻¹,G.U.R.I., 2011). Furthermore, mean Hg content in dead mat was 2-fold higher that other Mediterranean coastal areas (Cap Canari-France, Livorno and Porto Torres-Italy) characterized by the presence of *P. oceanica* and similar industrial activities (from 0.02 ± 0.01 to 0.56 ± 0.14 mg Hg kg⁻¹, Lafabrie et al., 2007). The mat, in this case, prevents the release of Hg in the environment and possible oxidation to more toxic chemical forms as dimethylmercury that could affect the biological systems of marine organisms and the biomagnification along the foo web.

4.4.2 Living mat

Sediment accumulation rate in living *P. oceanica* mat was comparable to previous estimations for *P. oceanica* mat (2.1 \pm 0.4 mm y⁻¹, Serrano et al. 2016). Additionally, C_{org} and C_{inorg} stocks supported by this living mat were both comparable with available data for Balearic Island (3.32 kg C_{org} m⁻² and 8.54 kg Ci_{org} m⁻², Serrano et al., 2012). C_{org} burial rate was included in the range of 6-202 g C_{org} m⁻² y⁻¹ (Serrano et al., 2016), while C_{inorg} burial rate was similar to the globally average of 126.3 \pm 0.7 g C_{inorg} m⁻² y⁻¹ estimated for seagrass ecosystems (Mazarrasa et al., 2015). No evidences of erosion or sediment release has been detected by ²¹⁰Pb analyses that confirmed the important role of *P. oceanica* mat as carbon sink. Despite the role of seagrass ecosystems as sink of nitrogen, very few data are available on the quantity of N stock in seagrass soils and, especially, in P. oceanica sediments. Seagrass remove and promote the deposition of nitrogen by absorption through leaving and accumulation into the biomass, by the action of canopies as a physical filter that allow sediment settlement or burring in its soils seagrass tissue N-riche and promoting microbial denitrification by realising oxygen or carbon from the roots (Aoki et al., 2019; Santos et al., 2019.; van der Heide et al., 2011). In this way, seagrasses are able to improve their productivity and growth reducing suspended particulate, increasing water quality and, so, transparency and limiting the N availability for the growth of other organism as algae (Gurbisz et al., 2017). Comparing our results with recent findings, N stocks was lower than data reported for P. oceanica in Greece (0.16 ± 0.18 kg N m⁻²kg, Apostolaki et al., 2019). N burial rate reported here was similar to previous findings for P. oceanica in NW Mediterranean Sea within the wide range of 0.2-13.4 g N m⁻² y⁻¹ (Mateo et al., 1997; Romero et al., 1994). So far, these are the first estimations of the role of this living mat as carbon and nitrogen sink and contribute to amplify the global map and data of blue carbon ecosystem.

In Augusta Bay the presence of mercury in the soil is not only ascribed to the southern sector where major industrial activities took place and have discharged major quantity of pollutants but also is spread to the rest of the area. In fact, the sediments in Xifonio Bay showed similar Hg content to that reported for other impacted area as Porto Torres (Sardinia-Italy, Lafabrie et al., 2007). The spread of mercury contamination among the basin is related to the illegal discharge of polluted materials detected in different spots of Augusta Bay (Bellucci et al., 2012) and to bottom water outflow that export pollutants from the southern sector toward the adjacent open sea (Sprovieri et al., 2011). Our findings showed that the dead matte supports higher stocks than unvegetated sediments and still plays an important role as carbon sink and in climate change mitigation. Additionally, in the last century, the dead mat has been contributed to remove high quantity of nitrogen and mercury. Despite the loss of *P. oceanica* and the high anthropogenic pressures among the area, the remaining mat keeps accumulating nutrients and contaminants preventing any release in the adjoining environment. Concurrently, the dead mat represents a potential risk to shift from carbon and mercury sink to source following future disturbance, especial mechanical one as dredging or dumping. Moreover, anthropogenic contamination is extended to the entire Augusta Bay, based on mercury accumulation in living mat, but without negative effect on carbon accumulation. In fact, living *P. oceanica* supports high stock, contributing to blue carbon budget. *P. oceanica* is fast declining among the entire Mediterranean Sea and mainly caused by the high anthropogenic pressure as registered in Augusta Bay. Thus, a better understanding of the role of degraded seagrasses in climate change mitigation is required together with the improvement of restoration programs that may help the natural recovery of environment fostering the blue carbon sequestration.

5. Conclusion

Seagrass meadows are important natural carbon sinks contributing to mitigate climate change. These ecosystems are vulnerable to natural and anthropogenic impacts, and depending on the magnitude and the duration of the impacts, the carbon stored in seagrass soils may be negatively affected. Despite the increasing knowledge on seagrass carbon sinks, little attention has been given to fundamental species as *Posidonia oceanica*. This species is rapidly disappearing due to coastal development, erosion and global change. Considering the high quantity of carbon sequestered by *P. oceanica*, this study has focused to understand if disturbed ecosystems may still act as carbon sinks. Investigations were focused on two different ecosystems exposed to anthropogenic and natural disturbances that have caused large regression of *P. oceanica*, or even to the dead of the meadows.

In the first study, environmental stressful conditions (warming and high salinity) together with anthropogenic impacts are contributing to the decline of *P. oceanica* meadows. Despite that, the carbon stock capacity of *P. oceanica* is not affected and this species still accumulates large quantities of carbon comparable with previous findings. The sink capacity of the area is enhanced by the expansion of *C. nodosa* that supported high carbon stock comparable with that of *P. oceanica*. Also, the peculiar hydrodynamic conditions that promote the accumulation of a large amount of organic matter in this ecosystem underlines the importance of abiotic factors for carbon accumulation.

In the second study, despite the dead of *P. oceanica*, the remaining mat supports higher stocks than unvegetated sediments and still plays an important role as carbon sink. Additionally, in the last century, the dead mat has been contributed to the removal of high quantities of nitrogen and mercury. Despite the loss of *P. oceanica* and the high anthropogenic pressure in the area, the remaining mat keeps storage of nutrients and contaminants preventing any release to the adjoining environment.

Overall, outcomes obtained from this research contributed to deepen the knowledge on the role of degraded seagrass ecosystems as blue carbon sinks. These results highlight the capacity store carbon also if the ecosystems are impacted and the importance of future initiatives to protect, restore and implement the natural recovery of degraded seagrass meadows fostering the blue carbon sequestration.

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