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DELIVERABLE C1.2: RESULTS REPORT

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ENTREGABLE C1.2: INFORME DE RESULTADOS

LIFE BLUE NATURA
LIFE14CCM/ES/000957

Group of Aquatic Macrophyte Ecology
Centre for Advanced Studies of Blanes
Spanish Council for Scientific Research

Blanes
31 May 2019 (updated September 2019)

Art by Angela Rossen

Natural Monument of *Posidonia oceanica* Reef, Roquetas de Mar, Almería, Spain
Source: Group of Aquatic Macrophyte Ecology, GAME

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Table of Contents

Acknowledgements

Abbreviations

LIFE BN	Life Blue Natura
AMAYA	Agencia de Medio Ambiente y Agua de Andalucía
CMAOT	Consejería de Medio Ambiente y Ordenación del territorio
GAME	Group of Aquatic Macrophyte Ecology (CEAB-CSIC)
CEAB-CSIC	Centro de Estudios Avanzados de Blanes - Consejo Superior de Investigaciones Científicas
IUCN	International Union for the Conservation of Nature
HyT	Hombre y Territorio
UNEP	United Nations Environment Program
UNFCCC	United Nations Framework Convention on Climate Change
IPCC	Intergovernmental Panel for Climate Change
COP	Conference of the Parties
SMEs	Small and Medium Enterprises
VCS	Verified Carbon Standard
BC	Blue Carbon
BCE	Blue Carbon Ecosystem
POM	Particulate Organic Matter
POC	Particulate Organic Carbon
TOM	Total Organic Matter
TOC	Total Organic Carbon
SOM	Sediment Organic Matter
PIC	Particulate Inorganic Carbon
TIC	Total Inorganic Carbon
GHG	Greenhouse Gasses
tCO_2	Ton of Carbon Dioxide
tCO_{2-e}	Ton of Carbon Dioxide equivalents

Figures

Fig. 2.1. Map of the sampling stations distribution.

Fig. 2.2. Cover, shoot height above the sediment and shoot density evolution at the POSIMED station of Terreros, between 2012 and 2018. a) landscape cover of the meadow (circles) and dead matte (squares) interannual change. Dead matte cover shows a slightly significant increasing trend (R^2= 0.26, p< 0.05); b) Shoot (vertical rhizome) height above the sediment surface (cm); c) shoot density evolution within permanent demography plots, showing no consistent temporal trend. d) global shoot density within transects, showing a significant reduction trend (R^2= 0.26; p< 10-3).

Fig. 2.3. a) Landscape meadow cover, b) vertical rhizome height above the sediment. c) Global shoot density significant reduction at Villaricos Posimed station (12m depth), between 2012 and 2014 (R^2= 0.21 p< 10-4).

Fig. 2.4. Coring on the matte of Life Blue Natura degraded meadow station of Villaricos (15 meters depth).

Fig. 2.5. Meadow phenologic parameters variability in Aguamarga POSIMED station. a) Meadow cover (white dots) and dead matte cover (black dots), and significant linear regression line of dead matte cover with time (R^2= 0.21 p< 0.05); b) vertical rhizome height above the sediment, and evolution of shoot density within permanent demography plots (c) and within transect plots (d).

Fig. 2.6. Meadow phenologic parameters variability in Roquetas POSIMED station. a) Meadow cover (white dots) and dead matte cover (black dots), and significant linear regression line of dead matte cover with time (R^2= 0.21 p< 0.05); b) vertical rhizome height above the sediment, and evolution of shoot density within permanent demography plots (c) and within transect plots (d).

Fig. 2.7. Two views of Punta Entinas meadow. In the top picture, the meadow has developed a thin (around 30cm) matte. It was difficult to install the permanent demography quadrat, because the rock was soon hitted. In the bottom picture, we observe the P. oceanica meadow growing directly on rock, with no matte development. Source: Diego Moreno, Programa de Gestión Sostenible del Medio Marino, Junta de Andalucía.

Fig. 2.8. Meadow phenologic parameters variability in Punta Entinas POSIMED station. a) Meadow cover (white dots) and dead matte cover (black dots), and significant linear regression line of dead matte cover with time (R^2= 0.21 p< 0.05); b) vertical rhizome height

above the sediment, and evolution of shoot density within permanent demography plots (c) and within transect plots (d).

Fig. 2.9. Meadow phenologic parameters variability in Melicena POSIMED station. a) Meadow cover (white dots) and dead matte cover (black dots), and significant linear regression line of dead matte cover with time (R2= 0.21 p< 0.05); b) vertical rhizome height above the sediment, and evolution of shoot density within permanent demography plots (c) and within transect plots (d).

Fig. 2.10. Cala Burras growing on rock

Fig. 2.11. Calculus diagram

Fig 3.1.: age and depth distribution on TE.S_A core (manual core from a healthy, shallow, *P. oceanica* meadow) of soil density, sediment accretion rate, total organic matter, total organic carbon, total organic carbon flux, total inorganic carbon, total inorganic carbon flux and grain size distribution (coarse, >2mm; very coarse sands, 1-2 mm; coarse sands, 1-0.5 mm; medium sands, 0.5-0.25; fine sands, 0.25-0.063; and mud, <0.063mm).

Fig 3.2. age and depth distribution on TE.D_C core (vibrocore from a healthy, deep, *P. oceanica* meadow). Acronymes as Fig 3.1.

Figure 3.3. age and depth distribution on DE.I_A core (manual core from a degraded, intermediate, *P. oceanica* meadow) Acronymes as Fig 3.1.

Figure 3.4. age and depth distribution on RO.S-C19 core (manual core from a death mat, shallow, *P.oceanica* meadow). Acronymes as Fig 3.1.

Figure 3.5. age and depth distribution on BA.S_A core (manual core from a healthy, intermediate, *C. nodosa* meadow). Acronymes as Fig 3.1.

Figure 3.6. age and depth distribution on SA.ZN-S_A core (manual core from a healthy, intertidal, *Z. noltii* meadow). Acronymes as Fig 3.1.

Fig. 3.7. Boxplots of a) Annual Leaf Production (LPY, in leaves per year) and b) Vertical Rhizome Growth (VRG in cm per year) at each P. oceanica station. The median, 25% and 75% quartiles are represented. Top and bottom of each violin boxplot represent the minimum and maximum

Fig 3.8. Average annual leaf production (triangles, LPY, leaves per year) and Average annual Vertical Rhizome Growth (circles, VRG, cm per year). Dotted straight line and curved lines represent the linear regression and its 95% confidence intervals.

Fig. 3.9. a) Average Vertical Rhizome Growth (VRG, black bars) and Average Sediment Accretion Rate (SAR, white bars) in the same period ecompassed by rhizome internodes. b) Semilog regression of VRG with SAR

Fig. 3.10. a) Sediment carbon stocks (t TOC / ha) accumulated in the first sediment meter in all the seagrass stations (vegetated and un-vegetated; b) Average carbon flux to the sediment (t TOC / ha yr) in the last century in these stations. Empty columns represent "no data", with the exception of CA.S (*Posidonia* directly on rock, where it means "0 sediment TOC stock".

Fig. 3.11. Carbon stock in the canopy biomass compartment of stations. Empty columns correspond to "no data" with the exception of un-vegetated stations (no seagrass nor algae) BA.S-C, ME.I-C, RO.I-E and TE.I-C, which correspond to "0 values".

Fig. 3.12. Significant relationship between global shoot density and canopy TOC stock: R2= 0.35, p< 0.05. The model curve and its 95% confidence interval bands are plotted. TOCstockcanopy = 0.021($\pm$0.008SE) ·e0.004($\pm$0.001SE)·Shoot density. CI95% of the exponent: (0.002; 0.007); CI95% interval of Y0: (0.010; 0.040).

Fig. 3.13. Carbon stock (in 1m sediment thickness) and average flux (last century) variability with depth in Andalusian meadows

Fig. 3.14. a) Average carbon flux variability in the last century in Western Mediterranean P. oceanica meadows. Data from Santa Maria and shallow Es Port come from project PALEOPARK, while data from deep Es Port meadow comes from Mazarrasa et al, 2017. Error bars correspond to standard error of the mean. b) Linear regression of the % of flux in meadows (as compared to the flux in its respective shallowest part of the meadow) with respect to depth. Error bars are obtained dividing the standard error of the flux by the total flux in its respective shallowest part of the meadow. The solid line represents the significant linear regression (R2= 0.74, p< 10-3 ; y= - 4.043($\pm$ 0.795(SE)·X + 119($\pm$10.56(SE)) where y = % of Carbon flux with respect to 5m depth and X is depth, expressed in meters. The dotted lines represent the 95% confidence interval of the slope (Table. 3.X).

Fig. 3.15. % of Average carbon flux wih respect to the 0 to 10 depth range, suggesting that the decline would not be constant, but would accelerate with meadow depth

Fig. 3.16. a) Decline trend of sediment Bulk density in the upper 80 cm of Villaricos matte. b) reduction of TOC flux since 1980 in Villaricos declining meadow.

Fig. 3.17. Vertical profile of TOC density (g cm-3) along the RO.S-C recent dead matte (open circles) and the RO.S live matte (open triangles) consensus cores. A detail of the upper 20 cm is shown in the upper-right corner, showing that the only difference in TOC content is in the top 3cm, probably due to the absence of live belowground P. oceanica organs..

Tables

Table 2.1. Location sampled: coordinates, species present and codes.

Table 2.2. TOM and TOC% on each station. Al correlation had a $p < 0.001$

Table 3.1. Bathymetric distribution of the rocky bottoms bearing Posidonia oceanica (ha)

Table 3.2. Systematic difference between P. oceanica vertical rhizome growth and Sediment Accretion Rate (cm yr-1)

Table 3.3. Correlation between TOM and grain size distribution for each specie

Table 3.4. Estimates used for the model of TOC stock decline with time since loss of seagrass cover

Table 3.5. CO_2 stocks and fluxes per depth and area for each Type of bottom

Table 3.6. Areal stocks and fluxes determined for some special types of P. oceanica meadow.

Glossary of terms and definitions

The report you have in your hands is intended to be clear and useful to readers outside the discipline such as policy makers, students, technicians, economists, managers, etc. We have therefore prepared a specially detailed and comprehensive Glossary of Terms to improve the accessibility of the text.

^{14}C age = radiocarbon age	Time since and organic material stopped being biomass and started to be necromass, estimated through its remaining content in the radioactive ^{14}C isotope. This method is is used for determining the age of an object containing organic material by using the properties of radiocarbon, a radioactive isotope of carbon, that decays regularly with time. Given that the half life of ^{14}C is 5730 years (± 40), this technique allows us to date organic materials usually between 100 and 50.000 years of age.
^{210}Pb age	Age of a sediment layer estimated from its excess content in the radioactive isotope ^{210}Pb. its half-life of 22.3 years, allows to date sediments from present to 150 years ago.
Above ground storage/stock	Carbon stored in above-ground biomass (e.g. trunks, stems, leaves) or other above-ground carbon sinks.
Accumulation rate	The rate at which atmospheric CO2 is sequestered. Usually reported as a mass per unit area per year.
Activity	An action undertaken to reduce anthropogenic GHG emissions; or an action undertaken to increase anthropogenic GHG removals by sinks.
Additional/Additionality	The effect of a project activity to reduce anthropogenic GHG emissions below the level that would have occurred in the absence of the project activity; or
Approved methodology	A methodology for undertaking a project activity that has been approved by the appropriate authority for projects or activities.
Autochthonous carbon	Carbon (organic or inorganic) formed at a site distant to that where it is found.
Below ground storage	Carbon stored below ground level as biomass (e.g. roots and rhizomes) or sedimentary/soil carbon.
Biomass	The total quantity (usually weight) of organisms in a given area or volume.

Biospheric carbon sink	A carbon sink is any compartment of the biosphere that captures a net amount of carbon and locks it for a long period of time, relevant to global change. Oceans, forest and soils are the main biospheric carbon sinks. When a sink stops adding net carbon, it no longer is a sink but turns into a in steady state stock, in stationary stock, or in a slow source .
Blue Carbon	Term coined in 2009 by Nellemann et al., (2009) that typically refers to the organic carbon captured by coastal vegetated ecosystems, mainly mangrove forests, tidal saltmarshes, and seagrass meadows. Both the organic carbon in the living tissues and buried in the sediments are considered BC. Whether the carbon contained in the form of carbonates is to be considered Blue Carbon, is still a matter of debate within the scientific community. The organic carbon accumulated in other areas of the ocean, in a chemical form or in the sediments, would also be a part of the BC but not typically included in the global inventories.
Blue Carbon	The carbon stored and sequestered in coastal ecosystems such as mangrove forests, seagrass meadows or tidal marshes.
Cap and trade system	Consists in measurably reducing national GHG emissions below certain levels (cap) in strategic economic activities. Flexibility mechanisms allow entities to compensate their GHG emissions excess from these caps, by purchasing carbon credits, which consist in certified carbon emission reductions (carbon offsets), or un-used carbon emission permissions from other countries.
Carbon credit	Generic term for any tradable certificate or permit representing the right to emit one tone of carbon dioxide or the mass of another greenhouse gas with a greenhouse effect equivalent to that of one ton of carbon dioxide.

Carbon market

Markets where carbon credits/carbon offsets are traded, directly or indirectly between entities seeking to compensate for their carbon emissions and enterprises that have reduced their carbon emissions below a certain quantity assigned (under the Kyoto protocol) and have the permission to sell their carbon offsets (cap and trade scheme), or entities implementing projects to produce a net reduction in global GHG emissions. The carbon markets can be regular, where clients are enterprises obliged to maintain their GHG emissions under certain thresholds, and where the carbon credits/offsets that can be traded are regulated, both things under the Kyoto Protocol. There are also voluntary carbon markets, for enterprises and projects producing carbon credits are not regulated by the Kyoto Protocol.

Carbon offset

Reduction in emissions of carbon dioxide or greenhouse gases made in order to compensate for or to offset an emission made elsewhere. Carbon offsets are produced by projects that carry out on-the-ground emissions reduction activities, and are typically measured in metric tonnes of carbon dioxide equivalents, or tCO_{2e}.

Carbon pools

Above-ground biomass, below-ground biomass, litter, dead wood and soil/sediment organic carbon.

Carbon sequestration rate = carbon flux = carbon long-term burial rate

Pace at which the fraction of organic or inorganic carbon is buried in the sediments of seagrass meadows to stay for long periods of time. Not to be confused with photosynthetic carbon fixation by primary producers. Only a small fraction of the photosynthetically fixed carbon will be derived by some types of macrophytes to the long-term compartment in the sediments (namely, saltmarshes, mangrove forests, and seagrass meadows).

Carbon stock

Mass of organic or inorganic carbon accumulated by seagrass ecosystems. The organic forms can be living or dead debris of the seagrass, both from above or belowground. The inorganic fraction is basically represented by carbonates, largely calcium carbonate.

CO_2

Carbon dioxide, a gas composed of one carbon and two oxygen atoms. It is a major component of the global carbon cycle and a key greenhouse gas

CO_{2-eq}	Measure of the environmental impact of one tonne of any greenhouse gases in comparison to that of one tonne of CO_2.
Conference of the Parties	Is the supreme decision-making body of the UNFCCC. Its main task is to review the implementation progress made in reducing GHG emissions by the nations having joined the Conference (Parties).
Corer, core, coring	Cylinder that can be made of various materials, have different diameters and be driven into de soils or sediments manually or using different percussion and rotation devices. The core is the soil/sediment sample within the corer which, a priori, conserves its chronological sequence of sedimentation. Coring is the action of sampling cores using corers.
C_{org}	Organic carbon
Dating methods	The various methods used to age sediments/soils or carbon within sediments/soils, thereby allowing the accumulation rate to be determined. The most common methods involve the use of the radioisotopes Carbon-14 or Lead-210.
Emissions	An amount of a substance (usually a gas) that is released into the environment (usually the atmosphere). Here, the most commonly considered emissions are CO_2, CH_4, N_2O.
GHG (greenhouse gas)	A greenhouse gas listed in Annex A to the Kyoto Protocol, unless otherwise specified in a particular methodology. With respect to blue carbon ecosystems, the most commonly considered GHGs are carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O)

Grain size analysis

Were mechanical coring techniques cannot be used, typically in shallow waters, manual coring is the choice. It consists on slowly hammering PVC corers down in the sediments while rotating them to minimize core compression. Depending on the grain size of the sediments being cored, the pipe will require to be fitted with a core catcher to retain lose sediments. Core lengths of up to 3 m can be obtained using this technique. Both the penetration and removal of the manual cores can be a very arduous work, especially when it has to be performed underwater in SCUBA. Retrieval usually requires the participation of several divers and a lift air balloon.

Grain size analysis	Measurement of the abundance of different sediment grain-size classes. It is performed by successive sieving through decreasing size-mesh, or analyzing laser diffraction patterns.
Kyoto Protocol	The protocol to the Convention adopted in Kyoto, Japan on 11 December 1997, which entered into force on 16 February 2005. The Kyoto Protocol, among other things, sets binding targets for the reduction of GHG emissions by Annex I Parties.
Labile carbon	Forms of carbon relatively easily degraded or remineralised.
LIFE Blue Natura	Project funded within the EU LIFE Programme entitled "Andalusian Blue Carbon for Climate Change Mitigation Quantification and Valorisation Mechanisms" (LIFE14CCM/ES/000957). It aims at providing the scientific knowledge on the distribution and size of the blue carbon carbon sinks associated to seagrass meadows and saltmarshes in the region of Andalusia, as well as providing the instruments to make possible its inclusion in the inventories of the national emission compensation systems as well and its monetization in the voluntary carbon markets.
LIFE Programme	LIFE is the EU's financial instrument supporting environmental, nature conservation and climate action projects throughout the EU. Since 1992, LIFE has co-financed more than 4500 projects. For the 2014-2020 funding period, LIFE will contribute approximately €3.4 billion to the protection of the environment and climate.

Manual coring

Were mechanical coring techniques cannot be used, typically in shallow waters, manual coring is the choice. It consists on slowly hammering PVC corers down in the sediments while rotating them to minimize core compression. Depending on the grain size of the sediments being cored, the pipe will require to be fitted with a core catcher to retain lose sediments. Core lengths of up to 3 m can be obtained using this technique. Both the penetration and removal of the manual cores can be a very arduous work, especially when it has to be performed underwater in SCUBA. Retrieval usually requires the participation of several divers and a lift air balloon.

Mat

Complex combination of belowground parts of seagrasses (mainly roots and rhizomes) that intermingle in a matrix of sediment of various sizes (from mud to coarse sand and rock). In the larger species such as *Posidonia oceanica* in the Mediterranean, mats can achieve several meters in thickness representing massive carbon deposits. Their aspect and dynamics are very analogous to those of terrestrial peat.

Organic carbon

Carbon, both particulate and dissolved, found in an organic compound, including living organisms, detritus, litter, and dissolved compounds

Paris Agreement

Iinitiative of the UNFCCC that aimed at bringing for the first time "all nations into a common cause to undertake ambitious efforts to combat climate change and adapt to its effects, with enhanced support to assist developing countries to do so". (UNFCCC).

***Posidonia oceanica* 'reef'**

In some special cases, the massive accumulation and preservation of organic carbon in *P. oceanica* meadows, results in the formation of a peat-like deposit that can be directly observed and reach several meters in height. In Roquetas de Mar, Almería, Spain, there is an outstanding example of this formations that has been declared 'Natural Monument of the Posidonia Reef'. The area protected as natural monument occupies 108 ha.

Project	A coordinated action by a private or public entity which coordinates and implements any policy/measure or stated goal (i.e. incentive schemes and voluntary programmes) that leads to GHG emission reductions or net anthropogenic GHG removals by sinks that are additional to any that would occur in the absence of the coordinated action.
Project boundary	The physical delineation and/or geographical area of a project activity and the specification of GHGs and sources under the control of the project participants that are significant and reasonably attributable to the project activity, in accordance with the applied methodologies and, where applicable, the applied standardized baselines
Remineralization	The process in which organic carbon is transformed into inorganic forms, such as carbon dioxide (CO_2)
Saltmarsh, tidal (or salt marsh):	Coastal ecosystem in the upper coastal intertidal zone between land and open saltwater or brackish water that is regularly flooded by tides. It is dominated by dense stands of salt-tolerant plants such as herbs, grasses, or low shrubs. These plants are terrestrial in origin and are essential to the stability of the saltmarsh in trapping and binding sediments. Saltmarshes play a large role in the aquatic food web and the delivery of nutrients to coastal waters. They also support terrestrial animals and provide coastal protection.
SAR	Sediment accumulation rate – the net rate of vertical accumulation of sediment at a site.
Seagrass	Flowering marine vascular plants (angiosperms) that grow in marine waters along the coasts of the world with the exception of subpolar and polar areas. There are around 60 species that belong to four families coming from several ancestors: *Posidoniaceae*, *Zosteraceae*, *Hydrocharitaceae*, and *Cymodoceae*.
Sediment	Naturally occurring material broken down by weathering and erosion, and subsequently transported to a place where it accumulates. In contrast to soils, sediments are relatively unstructured and are not formed by interaction of biological, physical and chemical processes.
Sedimentary carbon	Organic and inorganic carbon stored within sediments

Sequestration	The capture and long-term storage of atmospheric carbon dioxide.
Shoot/shoot density	
Sink	Reservoir that accumulates and stores carbon-containing chemical compounds. Use of the term sink usually implies that the storage is long-term (or semi-permanent).
Soil	A complex, structured mixture of organic matter, minerals gases, liquids and living organisms formed by the interaction of the parent material, organisms, climate and relief.
Soil carbon	Organic and inorganic carbon stored within soils
Stocks (of carbon)	The total amount of, in this case, carbon stored in an area or volume. Used interchangeably with 'store'.
Verification	The periodic independent evaluation and retrospective (ex post) determination of monitored GHG emission reductions that have occurred as a result of a project activity; or
Verified Carbon Standard (VCS)	Standard for Certifying Carbon Emissions Reductions. "The VCS Program is the world's most widely used voluntary GHG program. More than 1300 certified VCS projects have collectively reduced or removed more than 200 million tonnes of carbon and other GHG emissions from the atmosphere". (Verra)
Vibrocoring	Coring method that uses a metal barrel lodging a PVC or polycarbonate pipe inside and uses gravity and vibration to penetrate in the sediments. On its top end, the barrel is fitted with an electrical vibrating 1ton-head. Because of its length and weight, it is usually operated from a research vessel or platform and hence cannot be used in waters shallower than 10 m. Continuous cores of up to 6 m can be obtained using this technique.

Units & math terms

kt   Kiloton, 1000 tons, 10^9 grams.

Mt   Megaton, 1 million tons, 10^{12} grams.

Pg = Gt   Petagram = Gigaton, 10^{15}. Common unit for the global carbon cycle.

C to CO_2 1 g of Carbon equals 3.67 grams of CO_2.

Yr   Years

Km, ha, m^2 Square kilometers, hectares and square meters. Common units to express carbon stocks per unit surface.

SE   Standard Error of the Mean. A stadistic used to show how consistent or dispersed are the values of the various replicates of a certain variable with respect to the average value.

1. Introduction

1. Introduction

A relentless CO_2 rise in the atmosphere is also increasing the interest in the conservation and promotion of biosphere carbon sinks to attenuate that trend (Howard et al. 2017). Coastal ecosystems such as mangroves, tidal saltmarshes, and seagrass meadows can remove and lock significant amounts of carbon for relevant periods of time resulting in very large carbon stocks (C M Duarte et al., 2004). These "Blue Carbon Ecosystems" (BCE) are considered an asset to reduce the CO_2 concentration in the atmosphere and therefore mitigating climate change (Fourqurean et al., 2012b; Hiraishi et al., 2013; Lavery et al., 2013a). While the global impact of this ability is a current matter of controversy, the potential for monetization of this carbon is a fact (O'Sullivan et al., 2011).

In the same way, as each additional ton of CO_2 captured or not emitted by terrestrial forests can be traded within the regular or voluntary carbon markets (cap and trade approach), the CO_2 sequestered by BCE could be turned into tradable carbon credits via offsetting projects (Hamrick and Gallant, 2018; O'Sullivan et al., 2011). The path to monetization is not easy. First, a detailed quantification of sinks size and dynamics is imperative. These are important challenges, both conceptual and technical, given the high complexity of the processes involved, the extension and diversity of the BCE, and the overall scarcity of information available. Then, the tons of carbon captured or emissions avoided have to be certified through labyrinthine numerical approximations and, finally, integrated in the current environmental legislations (Herr et al., 2017).

The Paris Agreement acknowledges the importance of the gas sinks and encourages the Parties to take measures to conserve and enhance them and to provide a national inventory report of their magnitude and distribution (UNFCCC, 2016). Furthermore, the interest in CO_2 trading, the inclusion in 2013 of a Supplement for wetlands to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, (Hiraishi et al., 2013) and the momentum of the research in BC, sets a favorable scenario to stimulate inventorying and quantifying these BC sinks, and to design mechanisms to include them in climate change mitigation strategies, because it can play a role in this challenge, as well as in local economic development (Barbier et al., 2011; Constanza, 2008; Nellemann et al., 2009).

As mentioned above, the first key step in order to bring the BC to climate change mitigation strategies is to assess, with the best possible detail, the abundance, distribution, and dynamics of the carbon stocks and fluxes associated to these ecosystems. It is time to do so.

The accumulation of organic Carbon (C_{org}) in seagrass meadows results from several processes: accretion (autochthonous plant and epiphyte production, and trapping of allochthonous C_{org}; Kennedy et al., 2010), erosion (e.g., export; Hyndes et al., 2014; Romero

et al., 1992) and decomposition (Mateo et al., 1997). Previous studies demonstrate that both autochthonous (e.g., plant detritus and epiphytes) and allochthonous (e.g., macroalgae, seston and terrestrial mat) sources contribute to the C_{org} pool in seagrass soils (Kennedy et al., 2010; Watanabe and Kuwae, 2015).

Plant net primary productivity is a key factor controlling the amount of C_{org} potentially available for sequestration in seagrass ecosystems (Serrano et al., 2014), but the past and present depositional environment is an important factor controlling C_{org} storage in coastal habitats (De Falco et al., 2004; Lavery et al., 2013a). There is large variation in C_{org} stores among morphologically different seagrass species (Lavery et al., 2013b; Trevathan-Tackett et al., 2017), because that morphology influences both: seagrass production and particle trapping capacity. C_{org} accumulates more in estuaries compared to open-coast environments (Nellemann et al., 2009), because estuaries usually are highly depositional environments, receiving fine-grained particles from terrestrial and coastal ecosystems. Fine sediments have also shown to enhance C_{org} accumulation (Burdige, 2007; Keil and Hedges, 1993). All factors affecting chemical stabilization of organic matter trapped in the sediment are also determining blue carbon stock and sink capacity (Burdige, 2007): factors like organic matter C/N/P ratios, microbial biomass (Danovaro and Fabiano, 1995; Sparling, 1992), and soil temperature (Pedersen et al., 2011).

Therefore, there are many factors which natural or human-induced change, may enhance or reduce the blue carbon habitat sink capacity, or even transform them in net GHG emitters. Such factors have to be taken into account in order to make an inventory of blue carbon sinks and emissions, as well as to determine additionally in an eventual Blue Carbon mitigation project.

The structure of the Project LIFE Blue Natura is a faithful reflection of the reality exposed above. Very briefly, its preparatory actions have provided the cartographic information on the distribution of blue carbon sinks of the Andalusia Autonomous Community (Action A1: Cartography and Characterization of habitats); performed and extensive coring survey of blue carbon habitats growing under various environmental settings (geographic, depth, substrate, and degradation gradients; Action A2: Design and sampling needed to assess the stocks and fluxes associated to Andalusia seagrass meadows, and the corresponding action A3 for saltmarshes), and has determined the stocks and fluxes of organic carbon associated to those different settings (Action C1: Estimate of stocks and fluxes associated to Andalusia seagrass meadows, of which this report is the action deliverable. The corresponding results for saltmarshes will be addressed in deliverable C3).

In this report we present the results for this last Action (C1). They include all the variables planned in the proposal for the action (see Material and Methods section), most of them leading to obtain the variables to answer three key questions:

1. How much organic and inorganic carbon (or CO_2 equivalents for other GHG) is stored/stocked in the biomass and sediments of the four Andalusian seagrass species? (stock).

2. How much does this stock differ among the various environmental settings along the Andalusian coasts? (Distribution).

3. At what rate does this stock accumulate? (Sequestration rates) and how it is affected by habitat degradation/recovery?

The answers to these questions are crucial to determine the potential of this carbon as a monetizable asset through conservation or reforestation projects, in a first stage, in the voluntary markets.

This report also contains a description of the methodology used in the field, in the laboratory, as well as the numerical procedures applied.

In the Results and Discussion section, the results are presented and commented, and the uncertainties and limitations associated to these results are discussed.

Inventorying the stocks and fluxes of the organic carbon accumulated by the seagrass meadows growing along 945 km of coastline, from 0 to 20 m depth, is not a minor endeavor. The immensity of the area under study was therefore one of the main challenges to be tackled.

To obtain adequate representativeness of the variability of the sink stocks and fluxes in Andalusian coasts, 88 cores from 1 to 5 m in length were taken at 32 stations (10 locations) using, when necessary, diving operations both from the shore and from a research vessel (R/V).

The R/V proved to be indispensable for the coordination of the dives in the deep stations (around 20 m depth), and to operate the heavy coring devices (vibrocore and multicorer). These long cores allowed estimating a potential total size of the sink (maximum stock until steady state) to a very high level of accuracy, impossible to achieve with just manual cores. Modeling observed (standing) stocks along a 5 m-core takes us closer to understanding what happens with the carbon that a meadow accumulates over all its life as a net sink until it turns into a steady state stock.
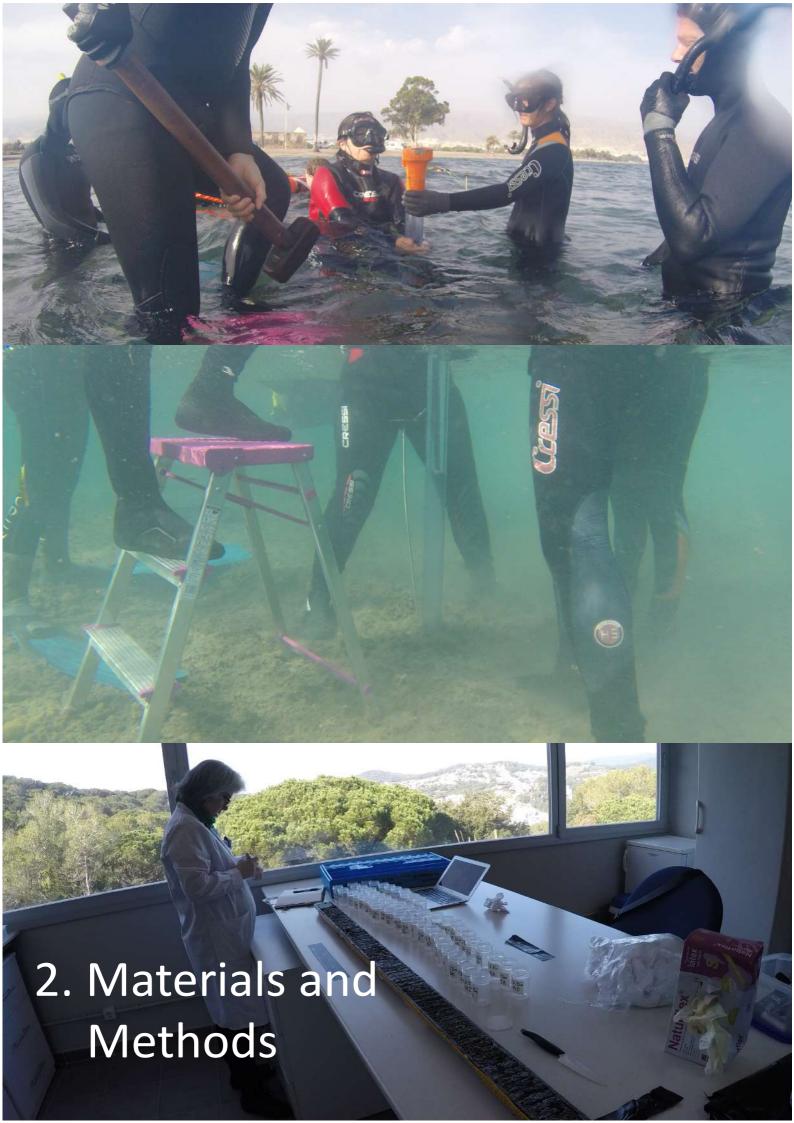
In the present report, in addition to improving results responding to questions 1 and 2, we have addressed question 3, examining in detail several case studies of seagrass degradation at various degrees, as well as of seagrass recolonization. We focus in quantifying the consequent changes in C stocks and fluxes, and in devising ways of estimating the potential liberation/sequestration of carbon, associated to seagrass meadow degradation and recolonization.

Finally, this report provides some background information and recommendations of interest to the elaboration of compensation projects and suggests future lines of action to improve our knowledge of the phenomenon of organic carbon refractory accumulation under seagrass meadows. A sound knowledge is, after all, the foundation that (1) policy makers need to take safe steps on the path to the inclusion of the coastal carbon in the national emissions and sink inventories, and in order to evaluate blue carbon economic value, and (2) what the SMEs and NGOs need to assess to decide whether Blue Carbon offset projects are or not financially viable (Herr et al., 2011; O'Sullivan et al., 2011).

The results presented in this report provide key elements for the actions to follow in the project LIFE Blue Natura: specially implementation Actions C3 to C7, as well as dissemination actions, mainly for E3 and E4. These actions are aimed at facilitating the issuance and retirement of carbon offset projects based on restoration of vegetated coastal ecosystems, by elaborating a seagrass and saltmarsh-derived carbon credits certification standard for Andalusia (C4), a handbook to guide the certification for offset projects based on seagrass meadows and saltmarshes (C5), among others, as well as to reach different sectors of the society, like SME and NGO technicians, and public officials (E3), and public and private decision-makers (E4), as well as journalists (E5) and the general public (E1 and E6). These results will also contribute to the growing international effort to bridge key knowledge gaps for inclusion of blue carbon in climate change mitigation strategies, mainly through actions E2 and E7.

Overall, LIFE Blue Natura aims are contributing to broader international climate policy discussions and sharing knowledge with other countries with a wealth of BC resources, willing to conserve or restore vegetated coastal ecosystems, while promoting local economic development.

Summarizing: This report aims at providing an inventory of the blue carbon stocks and fluxes in Andalusia, to characterize its spatial variability and to identify some of the main factors influencing carbon sequestration, preservation, and past/future dynamics which may be relevant for carbon offsetting projects. The potential loss/recovery of the carbon sink function and stocks, following seagrass meadow degradation/restoration. The basis for the monetization of the resource, is set.

2. Materials and Methods

2. Materials and methods

2.1. **Field sampling strategy**

The great geographic extension of the Andalusian coast, as well as the large variability of environmental conditions, makes a full factorial sampling design totally out of reach and of scope of this project, we sought to capture as much as possible of the variability in seagrass carbon stocks and fluxes associated to species (*Posidonia oceanica, Cymodocea nodosa*, *Zostera noltei* and *Zostera marina)*, geography (Atlantic, Mediterranean and Alboran seas), environmental factors (depth, sea bottom) and plant health status, and focusing in a few special case studies. In a post-hoc discussion, it was decided to consider 'Seagrass Type or Stable meadows' all those that were not notoriously degraded (see later).

In total we sampled seagrass habitats at 10 sites and 32 stations, from Almería to Cadiz. We collected 88 cores, of which 14 were vibrocores, and the rest were manually collected. We also collected 75 biomass samples and 78 *P. oceanica* vertical shoots, as well as samples of the various dominant plant species growing in each area, in order to measure the primary producers isotopic signal (to try establishing the main total organic carbon – TOC – contributors to the sink). The results for the isotopic signals and lepidochronological analyses are not part of this deliverable. They will be used in future elaborations of the data in the form of scientific manuscripts and additions to this deliverable.

Below we provide a summary of the sampling strategy. Exhaustive information on the sampling methods used, the sites and stations sampled, are available in LIFE BN Deliverable A2 (A2 LIFE BLUE NATURA: Results of Sampling in Andalusian Seagrasses, 2017).

Table 2.1. Location sampled: coordinates, species present and codes.

Region /Province	Natura 2000 SAC	Location	Coordinates		Depth (m)	Species	Categories	Code
Mediterranean, Almería	ES6110010	Terreros	37º21´0.12´´N	1º40´36.37´´W	5.5	*Posidonia oceanica*	Shallow, type	TE.S
Mediterranean, Almería	ES6110010	Terreros	37º20´58.02´´N	1º39´32.10´´W	12.5	*P. oceanica*	Intermediate, type	TE.I
Mediterranean, Almería	ES6110010	Terreros	37º20´59.70´´N	1º39´32.40´´W	12.5	*P. oceanica*	Intermediate, unvegetated	TE.I-C
Mediterranean, Almería	ES6110010	Terreros	37º20´37.00´´N	1º39´10.60´´W	18.5	*P. oceanica*	Deep, type, living matte	TE.D
Mediterranean, Almería	ES6110010	Villaricos	37º15´18.93´´N	1º45´29.53´´W	15.9	*P. oceanica*	Intermediate, chemically degraded, dead matte	DE.I
Mediterranean, Almería	ES6110010	Palomares	37º12´27.13´´N	1º47´30.20´´W	10.2	*Cymodocea nodosa*	Intermediate, type	PA.I
Mediterranean, Almería	ES0000046	Aguamarga	36º56´15.66´´N	1º56´0.42´´W	4.8	*P. oceanica*	Shallow, type	AG.S
Mediterranean, Almería	ES0000046	Aguamarga	36º56´10.20´´N	1º55´55.32´´W	10.8	*P. oceanica*	Intermediate, healthy	AG.I
Mediterranean, Almería	ES0000046	Aguamarga	36º55´59.40´´N	1º55´55.32´´W	18	*P. oceanica*	Deep, type	AG.D
Mediterranean, Almería	ES0000046	El Alquian	36º49´39.10´´N	1º23´14.00´´W	6.8	*C. nodosa*	Shallow, vegetated	BA.S
Alboran, Almeria	ES0000046	El Alquian	36º49´38.54´´N	2º23´13.36´´W	7.1	*C. nodosa*	Shallow, unvegetated	BA.S-C
Alboran, Almeria	ES6110019	Roquetas	36º47´15.50´´N	2º35´20.10´´W	1.5	*P. oceanica*	Shallow, type, living matte	RO.S
Alboran, Almeria	ES6110019	Roquetas	36º47´11.35´´N	2º35´24.10´´W	0.7	*P. oceanica*	Shallow, chemically degraded, dead matte	RO.S-C
Alboran, Almeria	ES6110019	Roquetas	36º47´12.2´´N	2º35´24.20´´W	1	*C. nodosa*	Shallow, type	RO.S-Cn
Alboran, Almeria	ES6110019	Roquetas	36º47´25.92´´N	2º34´50.58´´W	10.5	*P. oceanica*	Intermediate, relatively healthy	RO.I-Shallow
Alboran, Almeria	ES6110019	Roquetas	36º47´16.08´´N	2º34´39.06´´W	14.8	*P. oceanica*	Intermediate, colonization-recolonization	RO.IM60
Alboran, Almeria	ESZZ16003	Roquetas	36º47´16.08´´N	2º34´39.06´´W	14.8	*P. oceanica*	Intermediate, type	RO.I
Alboran, Almeria	ESZZ16003	Roquetas	36º47´9.24´´N	2º34´47.64´´W	14.7	*P. oceanica*	Intermediate, mechanically degraded (trawled)	RO.I-E
Alboran, Almeria	ESZZ16003	Roquetas	36º47´9.24´´N	2º34´47.64´´W	14.8	*P. oceanica*	Intermediate, recolonizing patch	RO.I-R
Alboran, Almeria	ESZZ16003	Roquetas	36º47´9.24´´N	2º34´47.64´´W	14.5	*P. oceanica*	Intermediate, chemically degraded (border trawling mark, silted)	RO.I-B

Alboran, Almeria	ESZZ16003	Almerimar	36º41´51.72´´N	2º49´32.46´´W	4.5	*P. oceanica*	Shallow, live matte on rock + sand (patchy)	AL.S
Alboran, Granada	-	Melicena	36º44´53.99´´N	3º14´3.78´´W	4.2	*P. oceanica*	Shallow, in decline (70% dead matte)	ME.S
Alboran, Granada	-	Melicena	36º44´51.10´´N	3º14´12.10´´W	11.8	*P. oceanica*	Intermediate, unvegetated	ME.I-C
Alboran, Granada	ES6170030	Calaburras	36º29´22.88´´N	4º41´37.02´´W	3	*P. oceanica*	Shallow, rock	CA.S
Alboran, Málaga	ES6170030	Calaburras	36º29´26.60´´N	4º41´36.40´´W	0.5	*P. oceanica*	Shallow, dead matte on rock	CA.S-C
Alboran, Málaga	ES6170030	Calaburras	36º29´25.9´´N	4º41´37.25´´W	0.5	*C. nodosa / P. oceanica*	Shallow, dead matte colonized with *C. nodosa*	CA.S-Cn
Atlantic, Cadiz	ES0000140	Santibañez	36º28´5.96´´N	6º15´5.99´´W	0	*Zostera noltei*	High intertidal, lagoon	SA.Zn-S
Atlantic, Cadiz	ES0000140	Santibañez	36º29'3.66´´N	6º12'18.70´´W	0	*Zostera noltei*	Intertidal, lagoon	SA2.Zn-S
Atlantic, Cadiz	ES0000140	Santibañez	36º28´7.99´´N	6º15´4.59´´W	0.3	*Z. noltei*	Low intertidal, lagoon	SA.Zn-D
Atlantic, Cadiz	ES0000140	Santibañez	36º27´59.16´´N	6º14´40.75´´W	0	*Z. noltei*	Intertidal, in recovery, lagoon	SA.Zn-C
Atlantic, Cadiz	ES0000140	Santibañez	36º28´9.41´´N	6º15´2.81´´W	0.5	*C. nodosa*	Subtidal, lagoon	SA.Cn
Atlantic, Cadiz	ES0000140	Santibañez	36º28´10.45´´N	6º15´1.40´´W	1.5	*C. prolifera*	Subtidal, lagoon (algae)	SA.Cp

2.1.1. *Variability of the Posidonia oceanica meadows sampled*

We sampled in 7 *P. oceanica* sites and 21 stations (see Fig. 2.1 and Table 2.1). Following the design of action A2 and background information from action A1, we studied *P. oceanica* stocks and fluxes variability depending on the following factors:

a. Geography

Posidonia oceanica meadows and dead mats or sand patches were sampled in the Mediterranean (Terreros = TE, Villaricos = DE and Aguamarga = AG sites) and Alboran seas (Roquetas = RO, Almerimar = AL, Melicena = ME, Calaburras = CA). Five sites were in the coasts of Almería (TE, DE, AG, RO, AL), 1 in Granada (ME) and 1 in Málaga (CA). In all the sites but Almerimar (which substituted Punta Entinas due to bad weather), there is background information from the Andalusian monitoring network POSIMED-Andalucia (AMAyA-CMAOT, 2018).
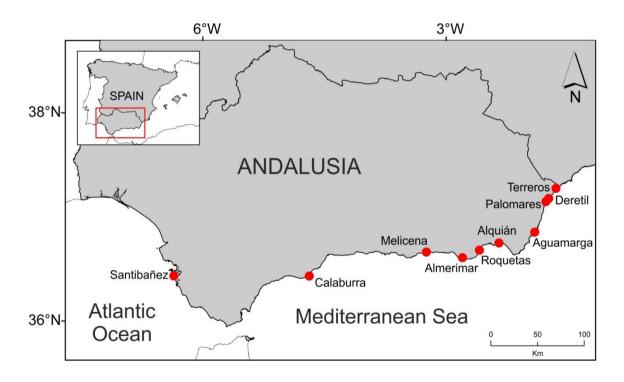
Fig. 2.1: Map of the sampling stations distribution

Terreros: the POSIMED station at 10.1 m shows a 60% to 70% *P. oceanica* coverage (average mesoscale and landscape cover, respectively), on a mixed bottom of sands and rocks. Less than 10% dead mat is observed, which indicates a good Conservation Index.

Between 2012 and 2018, dead mat landscape cover has slightly increased (R^2= 0.26, p< 0.05), while landscape meadow cover shows no trend (Fig. 2.2.a). Shoot density shows contradictory results: fixed plots show no consistent trend in 5 years (Fig. 2.2.c), while transect plots show a significant shoot density reduction of 25 ± 6.2(SE) shoots m^{-2} yr^{-1} in the same period (R^2= 0.26, p< 10^{-3}; Fig. 2.2d). On average, vertical rhizomes are not too high above the sediment, and appeared stable (Fig. 2.2b). All this suggest that no erosion is occurring within the meadow, which can be considered as STABLE during the period monitored (2012-2018). Nevertheless, it has to be closely followed, to see if the slight decline trend in shoot density continues with time or not, given the increasing coastal pressures in the area, from urban sprawl.
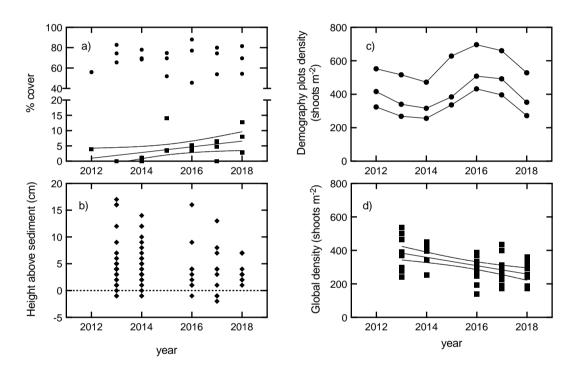
Fig. 2.2. Cover, shoot height above the sediment and shoot density evolution at the POSIMED station of Terreros, between 2012 and 2018. a) landscape cover of the meadow (circles) and dead matte (squares) interannual change. Dead matte cover shows a slightly significant increasing trend (R2= 0.26, p< 0.05); b) Shoot (vertical rhizome) height above the sediment surface (cm); c) shoot density evolution within permanent demography plots, showing no consistent temporal trend. d) global shoot density within transects, showing a significant reduction trend (R2= 0.26; p< 10-3).

Villaricos: This meadow, also installed on mixed sand and rocky bottoms, is very degraded by the chemical effluents of the Deretil factory, installed in 1961, which has affected the *P. oceanica* meadow in 2 km coastline until 10-12m depth (Moreno et al, 1999, 2001). Most of

this loss occurred between the 70's and the 90's. Waste spills have been reduced since 2000, but the POSIMED station, at 12 m depth shows that meadow regression was still advancing between 2012 and 2014: shoot density significantly decreased (R^2= 0.21 p< 10^{-4} ; Fig. 2.3c) at a rhythm of 13.4 $\pm$ 2.8 shoots m^{-2} yr^{-1}, while landscape meadow cover remained low but stable, around 27 $\pm$ 5% 8Fg. 2.3a). Dead mat cover was very high in 2012 (45 $\pm$ 8%). In the two subsequent years it was not observed. Maybe it is being buried below sand. Nevertheless, Shoot height above the sediment was low but did not increase during the 3 years observed (Fig. 2.3b). The area sampled in this study was 3 meters deeper, but presented similar characteristics and qualitatively similar meadow cover, although it showed dead mat reefs, tall
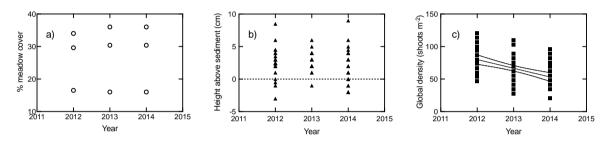
Fig. 2.3. a) Landscape meadow cover, b) vertical rhizome height above the sediment. c) Global shoot density significant reduction at Villaricos Posimed station (12m depth), between 2012 and 2014 (R2= 0.21 p< 10-4).

Fig. 2.4: example of the coring process over a chemically degraded *Posidonia oceanica* meadow Villaricos (Source: Diego Moreno, Programa de Gestión Sostenible del Medio Marino, Junta de Andalucía)

of 1-2m, with plant tufts interspersed (Fig. 2.4). Therefore, we consider this meadow UNDER REGRESSION.

Aguamarga: the POSIMED station is in a meadow border at 12 m depth, installed on a soft sediment bottom (fine sand and even mud), and fringed by a *C. nodosa* meadow, which is better adapted to this type of sediment. Therefore, the conditions are not easily extensible to the stations cored in this study. In the POSIMED station, between 2012 and 2017, landscape meadow cover has been relatively stable (Fig. 2.5a). Dead mat cover is low (around 5%), but with a marginally significant progression rate of 0.4 ± 0.2 % yr^{-1} ($R^2= 0.21$ $p< 0.05$; Fig. 2.5a). In this period, shoot density in demography plots and in transects, as well as shoot burial have not significantly changed (Fig 2.5c, d and b, respectively). Therefore, the Aguamarga meadow has been apparently STABLE for the period 2012-2018.
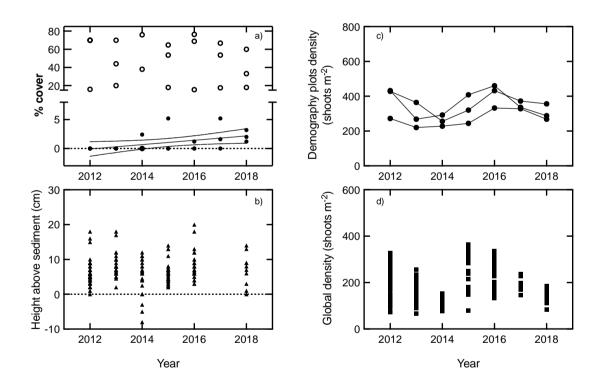
Fig. 2.5. Meadow phenologic parameters variability in Aguamarga POSIMED station. a) Meadow cover (white dots) and dead matte cover (black dots), and significant linear regression line of dead matte cover with time (R^2= 0.21 p< 0.05); b) vertical rhizome height above the sediment, and evolution of shoot density within permanent demography plots (c) and within transect plots (d).

Roquetas: the POSIMED station is at 11 meters depth, in the deep limit of the Roquetas barrier reef SEC, so that relatively protected from illegal trawling and dredging activities which have severely degraded the deeper meadow area, but near RO.I. shallow station in this study.

Nevertheless, it is not protected from wastewater influence in the area. Plants are settled on sediments rich in shells, sand and also mud, similar to the one observed in the stations cored in this study. In the POSIMED station the meadow cover has remained stable between 2012 and 2017 (around 40% and 70%, mesoscale and landscape covers, respectively; Fig. 2.6a), but dead mat landscape cover has significantly increased at a rate of 1.5 ± 0.4 % yr^{-1} ($R^2=$ 0.38 $p<$ 0.01 ; Fig. 2.6a). Shoot burial at this station seems adequate and stable (3 to 3.5 cm, on average, between 2012 and 2018, Fig. 2.6b). No significant net decrease was detected in the permanent demography quadrats (Fig.2.6c), but 100% cover and global shoot densities measured in transect quadrats, significantly decreased, at an average rate of $26 \pm 3(SE)$ shoots m^{-2} yr^{-1} ($R^2=$ 0.29 $p<$ 10^{-4} ; Fig. 2.6d). Given these results, we would consider the meadow as IN DECLINE at 10 meters depth. However, given the landscape information on large dead matte extensions in the meadow reef barrier as well as of extensive trawling marks in the deep meadow, we consider the whole Roquetas meadow as UNDER REGRESSION.
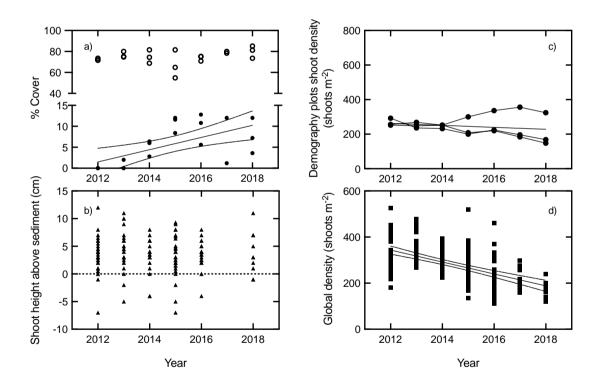
Fig. 2.6. Meadow phenologic parameters variability in Roquetas POSIMED station. a) Meadow cover (white dots) and dead matte cover (black dots), and significant linear regression line of dead matte cover with time ($R^2=$ 0.21 $p<$ 0.05); b) vertical rhizome height above the sediment, and evolution of shoot density within permanent demography plots (c) and within transect plots (d).

**Punta Entinas:** despite we couldn't sample in this POSIMED station, at 11.5m depth, it is an area close to the cored station of Almerimar, and shares some characteristics with it. Punta Entinas is characterized mainly as *P. oceanica* meadow growing on rocks in the cartography. So it is worth to expose some information about it, which will be useful in the discussion of the scaling up of carbon stock estimates. These meadows are characterized by AMAyA-CMAOT (2017) as "extensive and growing on sediments of different granulometries (sands and gravels), or on rock plateaus". Landscape pictures of the area show mats of around 30-50 cm leaning out of the substrate (Fig. 2.7). The area has very low human pressure in its southernmost part, where the potential nutrient inputs from the El Ejido-Roquetas agricultural pole is possibly being buffered by the saltmarsh system of Punta Entinas-Sabinar Natural park and by the open sea currents.

The POSIMED station shows high and stable landscape cover (83.5% $\pm$ 1.0% (SE); Fig. 2.8a). The dead mat cover is small (3.4% $\pm$ 0.6% (SE); Fig. 2.8a), but shows a marginally significant increase with time of +0.6% $\pm$ 0.3%(SE) per year (R^2= 0.23; p< 0.03). Vertical rhizome height above the sediment is low and shows great spatial variability (4.3 $\pm$ 0.3 (SE); Fig. 2.8b), although, on average, it has significantly increased between 2012 and 2018 (R^2= 0.1; p< 0.01), at a rate of +0.4 $\pm$ 0.2 cm yr^{-1}. Shoot densities in the transects and within permanent demography plots were consistent and showed no clear trend (Fig. 2.8c and d). Global shoot density was, on average, 325 $\pm$ 8 shoots m^{-2}. Therefore, we consider it as STABLE, although the significant increase of its dead mat is an early warning indicator of a possible decline, and should be closely supervised in the following years.

The shallow reef of Almerimar, at 4.5 meters depth, grows also on rock, but seems has a larger mat development, as one of the cores reached 1.1m sediment thickness (see pictures in Annex A2 deliverable Life Blue Natura: results of sampling in Andalusian seagrasses.pdf). Almerimar is not a meadow monitored by POSIMED, despite it has great interest: its landscape suggests that it could be a receding barrier reef.
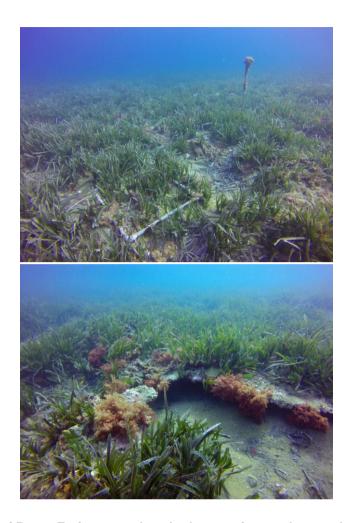
Fig. 2.7. Two views of Punta Entinas meadow. In the top picture, the meadow has developed a thin (around 30cm) matte. It was difficult to install the permanent demography quadrat, because the rock was soon hitted. In the bottom picture, we observe the *P. oceanica* meadow growing directly on rock, with no matte development. Source: Diego Moreno, Programa de Gestión Sostenible del Medio Marino, Junta de Andalucía.

Melicena: the area monitored by POSIMED, at 6 m depth, has greater cover and density values (75% landscape cover, 50% mesoscale cover, around 10% dead mat) than those observed in the station cored at this study, which was a bit shallower (4.2 m depth), and showed great extensions of dead mat (visually around ⅔ of the area). Nevertheless, the sediment characteristics seem similar (sands). At the POSIMED station, meadow and dead mat cover did not significantly change along time, although its spatial variance appeared to decrease, registering less low values for meadow cover and less high values for dead mat cover, with time (Fig. 2.9a and b). Shoot density at 100% cover and global shoot density
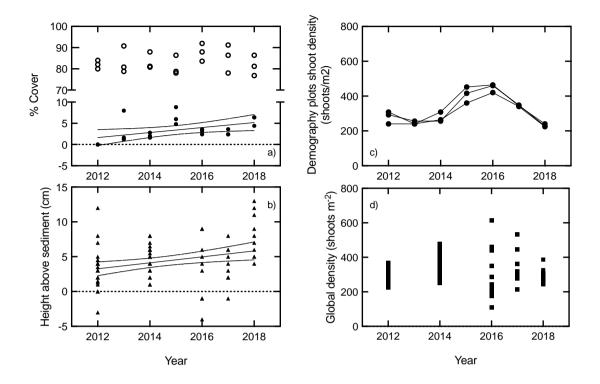
Fig. 2.8. Meadow phenologic parameters variability in Punta Entinas POSIMED station. a) Meadow cover (white dots) and dead matte cover (black dots), and significant linear regression line of dead matte cover with time (R^2= 0.21 p< 0.05); b) vertical rhizome height above the sediment, and evolution of shoot density within permanent demography plots (c) and within transect plots (d).

showed low, but significant positive correlations with time (global shoot density grew at an average rate of +15.5 $\pm$ 4.9(SE) shoots m^{-2} yr^{-1} (R^2= 0.10; p< 0.005, Fig. 2.9d) shoot burial is high and seems stable between 2010 and 2018 (on average 3.7 $\pm$ 0.2(SE) cm above the sediment, Fig. 2.9c). These results, combined with the landscape observation, suggests that the meadow may have suffered a strong decline in the near past, although now it would be stable or even starting to recover. Therefore, we will consider it to be IN RECOVERY.

Calaburras: the POSIMED station is a 3-4m deep patchy meadow growing directly on rocky bottom, close to the station CA.S sampled in this study. Landscape meadow cover has significantly increased between 2004 and 2017, at an average rate of 1.3 $\pm$ 0.5% per year (R^2= 0.19; p< 0.02; Fig. 2.10a), while dead mat has remained close to nihil. Shoot density at 100% cover did not significantly increase (p= 0.07), but global shoot density increased at an average rate of 12.7 $\pm$ 1.5 shoots m^{-2} yr^{-1} (R^2= 0.31; p< 10^{-4}; Fig. 2.10c). This indicates that the seagrass patches are growing, and could eventually coalesce to form a more continuous
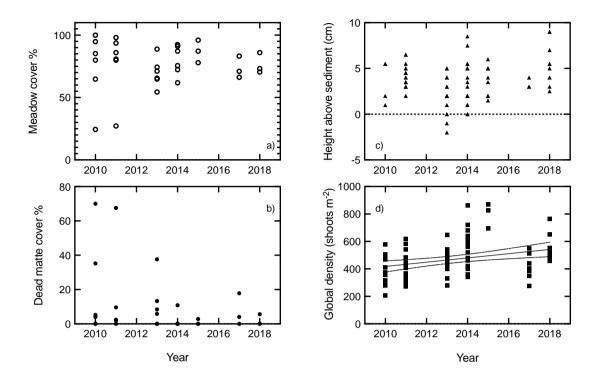
Fig. 2.9. Meadow phenologic parameters variability in Melicena POSIMED station. a) Meadow cover (white dots) and dead matte cover (black dots), and significant linear regression line of dead matte cover with time (R^2= 0.21 p< 0.05); b) vertical rhizome height above the sediment, and evolution of shoot density within permanent demography plots (c) and within transect plots (d).

meadow. Nevertheless, vertical rhizomes height above the sediment significantly increased with time (0.6 ± 0.1 cm yr^{-1}; R^2= 0.22; p< 10^{-4}, Fig. 2.10b), which indicates that no burial, and thus, no sediment accumulation is taking place there. Thanks to POSIMED data, and not having precise background information about the long-term existence and evolution of this meadow, we could classify this area of seagrass patches as IN PROGRESSION or IN RECOVERY.

As for the area of dead mat, there are no quantitative data, but marine technicians from AMAyA report that this mat is long dead, since at least the 1960s.

b. Depth

Natural depth variability of *P. oceanica* carbon stocks and fluxes were studied in two relatively healthy meadows in Almería: Aguamarga, in Cabo de Gata (South-Eastern Almeria), and San Juan de los Terreros (North-Eastern Almeria). Three stations were selected at each site: shallow (TE.S, and AG.S) at 5-6 m depth, intermediate at 10-13 m depth (TE.I and AG.I), and
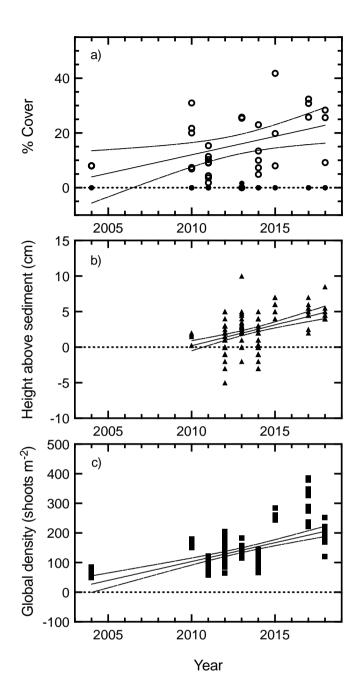
Fig. 2.10. Meadow phenologic parameters variability in Calaburras growing on rock POSIMED station. a) Meadow cover (white dots) and dead matte cover (black dots), and significant linear regression line of dead matte cover with time (R^2= 0.21 p< 0.05); b) vertical rhizome height above the sediment, and evolution of shoot density within permanent demography plots (c) and within transect plots (d).

deep, at 18-20 m depth (AG.D, TE.D). Few meadows in Andalusia surpass 20 meters-depth, and they are in the Eastern of Almeria. None of the Andalusian meadows in Alboran surpass the 20 meters depth, and most of them do not surpass 10 meters depth, either. In Roquetas (Almeria Bay, Alborán sea), we sampled relatively healthy meadow areas at 1.5m depth (RO.S), 10.8 m depth (RO.I-Shallow) and 14.8 m depth (RO.I, Table 2.1).

c. Presence-Absence of vegetation

Determining the places historically lacking *P. oceanica* was not possible, *a priori,* in the same environmental conditions than the healthy (stable) meadows. Therefore, we focused in unvegetated areas in the standing and the recent-past, neighbouring the standing vegetated areas. We added a -C to their sample code ("control") as they may represent the difference in carbon stocks and fluxes between currently vegetated and unvegetated areas. In Terreros, we selected a sandy area, adjacent to the healthy intermediate meadow of Terreros (TE.I-C, at the same depth), and one un-vegetated, sandy area at 10 meters depth (ME.I-C), near the shallow Melicena meadow (ME.S, at 4.2 m, Granada, Table 2.1).

d. Bottom type

All cores were collected on *P. oceanica* meadows growing on soft bottoms. Additionally, we sampled 3 biomass samples in shallow (3 m depth, CA.S station, Málaga) *P. oceanica* large patches growing on rocks, which had not developed a mat yet (or will never develop it at all, Table 2.1). The dead mat stations CA.S-C and CA.S-CN consisted in shallow, not very thick mats settled on rocky bottom.

The different meadows sampled on soft bottoms showed a range of fractions of silt, clay and sand, as well as of mat development. Such variations were revealed after opening the cores, and through grain size distribution analyses of sediment core subsamples in the lab (see Materials & Methods section "Laboratory analyses".

e. Mat development and living- vs dead mat

There may be differences in carbon stocks between a *P. oceanica* meadow with a well-developed mat (i.e. organic 'peat-like' reef) and one without such structure underneath (and among an infinite number of meadows with infinite different degrees of mat development, as *P. oceanica* meadows tend slowly to develop a mat, Sintes et al., 2005). During the LIFE BN surveys we sampled a well-developed shallow mat in the Natural Monument of the Roquetas *Posidonia* barrier-reef (Almeria). The development dynamics, size of the sink, and carbon sequestration rates could be compared with those measured in TE.S and AG.S, taking into account that there may be also differences due to geography (RO.S in the Alboran sea, TE.S and AG.S in the Mediterranean sea).

Moreover, the mat was sampled in 2 conditions: mat with and without a living overlying *P. oceanica* canopy (RO.S and RO.S-C, respectively). The dead mat area is relatively recent,

and could have originated by the recurrent freshwater discharges occurring in the area since 2005 (D. Moreno, *pers. comm.*). The mat sampled was a peat-like formation leaning out of the sediment, and covered by diverse algae and encrusting animals. A core was also inserted in a sandy patch at the bottom of that mat reef (core RO.S-C-bottom), in order to see if there was also mat underneath. Also the sandy patch of Terreros (TE.I-C) incidentally showed to have a mat buried under 50 cm of sand, but this will be accounted for in the results section. Finally, the manual core RO.IM60 (Table 2.1) was extracted from an area with a large proportion of horizontal shoots, while vertical shoots were very short. After the core opening and analyses, we could see that the organically enriched sediment horizon was very thin and was situated at the core top. These characteristics indicated that the plants were colonizing bare mineral sediment, which is credible, given that the area has been severely trawled.

We also sampled very shallow (0.5-1 m depth) dead mat in Calaburras (Málaga), old of several decades: CA.S-C (covered with algae) and CA.S-CN (covered with algae and the smaller seagrass *Cymodocea nodosa*). This dead mat looked thin, and settled on rocks and boulders. The shallow meadow of Melicena (Granada, ME.S, Table 2.1) also showed large extensions of dead mat.

Finally, the deep Terreros (TE.D, 18.5 m depth, Table 2.1) *P. oceanica* meadow had also a well-developed mat. The degraded-intermediate depth meadow sampled in Villaricos (DE.I, 15 m depth) also showed a thick dead mat.

f. Degraded meadows

We sampled 2 severely degraded meadows, due to different disturbance types, which could affect the sediment carbon stock in different ways:

- *Mechanically degraded meadows* (by illegal trawling and/or dredging)**:** we expected that, in addition to a reduction in C fluxes, they could have lost not only its living plant carbon stock, but also a portion of the top part of its sediment C stock. One of those areas was sampled at 14.8 m depth in Roquetas, in the center of a trawling-mark (RO.I-E, Table 2.1) as well as in its edge (RO.I-B, 14.5 m). RO.I-E was mainly devoid of *P. oceanica* plants and consisted of bare sediment with few algae, while RO.I-B was partially covered with the small seagrass *C. nodosa* and the invasive algae *Caulerpa cylindracea*, and showed the symptoms of degratation by the siltation subsequent to trawling (Sánchez-Lizaso et al., 1990), but no erosion. So, in terms of the fate of the carbon stocks, RO.I-B would fit in the following category*.*

- *Chemically degraded meadows:* apart from RO.I-B, two stations matched this category. The meadow of Villaricos (station DE.I), very close to the chemical factory of Deretil (see above, Fig. 2.3). We sampled a dead mat with some surviving shoots in a severely degraded

area, at 15 m depth (Fig. 2.4). On the other hand, the shallow dead mat of Roquetas reef (already described in the e. subsection), is likely related to nutrient-rich freshwater effluents.

2.1.2. *Meadows in decline*

Two of the meadows sampled fit in this category: a barrier reef in Almerimar bay, which seems to be undergoing erosion or at least it is currently patchy (AL.S), and the meadow of Melicena (ME.S), which is representative of the scarce meadows occurring in the coast of Granada. They are shallow, patchy and in decline, with around 60-70% of dead mat, although the causes of the decline are uncertain. Effluents from the numerous adjacent legal and illegal agriculture greenhouses, as well as recent land movements made for their construction in the hillsides may have impacted meadow's health through a reduction in water transparency or nutrient excess. Siltation is not detected, following stable vertical rhizomes height above the sediment detected by POSIMED-Andalucía. There is also evidence that illegal trawling also has destroyed the deeper areas of the Granada meadows. In the Alboran sea, the meadow depth limits are also naturally shallower (Table 2.1).

2.1.3. *Meadows in colonization-recolonization*

Two stations were established which seemed to be recolonizing, both at intermediate depths in Roquetas. One was the intermediate station RO.IM60, neighboring several trawling marks (around 150 m away from disturbed stations RO.I-E and RO.I-B). We deduced an horizontal colonization dynamics for this meadow from its low cover and density, together with its great proportion of horizontal (colonizing) shoots and the thin organic sediment horizon in the top (see above section e.). The vertical shoots found were very young and short. In contrast, the other recolonizing station (RO.I-R) consisted on *P. oceanica* patches interspersed in the bare sediment of the RO.I-E station (i.e. the trawling mark), and extremely long vertical shoots sprouting from the sediment revealed that these patches probably consisted in plants having survived trawling and subsequent siltation, which would have re-emerged to the surface (Table 2.1).

2.1.4. *Sampling small seagrass meadows: Cymodocea nodosa and Zostera noltei*

In the action A2 design, it was planned to characterize Blue Carbon stocks and fluxes of the small seagrass meadows occurring in 3 natural parks (El Estrecho, Cadiz Bay and Odiel Bay), and to study uniquely their variation with depth and among species (*Cymodocea nodosa*,

Zostera marina and *Z. noltei*). Once in the field, it was found that *Z. marina* presented an extremely low spatial cover in these natural parks, so its presence is to be considered negligible and relict. The meadows of El Estrecho and Odiel Natural Parks could not be sampled due to the severe weather conditions that occurred during the field surveys. To compensate for this, two *C. nodosa* meadows were studied in the province of Almería. Background information from A1 action was also taken into account. Due to the small depth range and patchy distribution of *C. nodosa* meadows, the study of the stocks and fluxes in this species along a depth gradient was not feasible at any particular site.

C. nodosa meadows were studied in 5 sites, presenting a diversity of environmental settings (Table 2.1: Palomares, in the the North-Eastern Almerian coast (Mediterranean sea), at 9 and 12 m depth (PA.I station). El Alquian, in Almeria Bay (Eastern Alboran sea), 7 m depth (BA.S station and the unvegetated adjacent station of BA.S-C). The Roquetas barrier reef lagoon, in Almeria bay (Eastern Alboran sea), at 1 m depth (station RO.S-CN). The shallow dead mat colonized by *C. nodosa* at Calaburras, at 0.5 m depth (Málaga, Western Alboran sea, station CA.S-CN). The shallow subtidal area of the Santibáñez lagoon (Cádiz, Atlantic sea, 0.3-1 m depth, station SA.CN).

The carbon associated to *Zostera noltei* sink was studied in Santibáñez bay, in the high (SA.ZN-S station) and low (SA.ZN-D) intertidal areas, as well as in a low intertidal area which has experienced intensive clam harvesting and cultivation activities until 2 decades ago. These activities are still present but to a much lesser extent (SA.ZN-C station, Table 2.1). Furthermore, a fourth station was taken on the 2018 field mission in another intertidal area of Santibañez, which appeared to have a larger fine sediment accumulation (SA2.ZN).

2.1.5. *Additional sampling: Caulerpa prolifera meadow*

At the Santibañez lagoon (Cádiz), a *C. prolifera* meadow was studied in order to compare its carbon stocks and fluxes with those of adjacent *C. nodosa* meadow. As *C. prolifera* and *C. nodosa* often compete for space and come one after another in a typical ecological succession, it was considered relevant to measure their carbon stocks and fluxes, and see if they could be discriminated.

2.1.6. *Other carbon compartments*

The vast majority of the Blue Carbon in seagrass meadows accumulates in their sediments (>98%; C. M. Duarte et al., 2004). Nevertheless, the IPCC protocols for carbon sinks and emissions recommend the inclusion of the carbon pool sequestered in the plant living standing stocks (both above and belowground), as well as surface, drifting necromass stocks. To keep

up with this standard, at each selected station we sampled a minimum of 3 replicate cores (18 to 590 cm long) and 3 replicate biomass samples (within 20 x 20 cm quadrats, up to 2 cm thick down the sediment). Although we evaluated the necromass and belowground biomass compartments, we only took into account the aboveground biomass compartment (leaves + epiphytes canopy) for the carbon inventories, because we considered that the other compartments were included in the top core sediment.

2.2. **Laboratory analyses**

2.2.1. *Sub-sampling, parameters and analyses on sediment cores*

Two out of three cores per station were subsampled in the field, at 6 to 8 levels, through 3-cm holes pre-made along the cores, at 5-10 cm intervals for the top samples and 25 for the bottom ones. The third core was brought to the lab, where it was cut open longitudinally. One hemicore was subsampled in 1 to 2 cm-thick slices, which were dried at 50ºC and subsequently weighted (for full details on subsampling procedures see LIFE BN, 2017 Deliverable A2). The other hemicore was kept as a backup and to perform other determinations in the future.

Each dry-weighted subsample was disaggregated and sieved through a 2 mm-mesh sieve. Coarse Organic Matter (COM), coarse shells and stones and gravel were separated and weighted. Part of the COM was used for radiocarbon dating (see below). The sediment fraction below 2 mm plus the COM were the ones included in chemical analyses.

One core per station, the accretion rates of the sediment for recent times (<100 years) were estimated in the top 30 cm, using the ^{210}Pb technique. The rest of the core was dated at one or two levels, selected between the top 50 cm and the bottom.

Total Organic Matter (TOM) was measured in all the sediment subsamples, from 0 to 30 cm core depth, and in every other sample thereafter. Total Organic Carbon (TOC) and Total Inorganic Carbon (TIC) were measured on at most 12 subsamples, evenly selected along the core (See Table. 2.2).

a. Geochemical and biomass analysis

A 3-4 g fine (< 2mm) sediment aliquot was digested with 35 % H_2O_2 in order to remove Sediment Organic Matter (SOM), then dried and sieved through a 1 mm, and analyzed in a Mastersizer 2000 analyzer, to obtain the small grain size distribution, through laser diffraction, for the following fractions: <0,063 mm (silt and clay), 0,063-0,25 (fine sand), 0,25-0,5 (medium sand), 0,5-1 (coarse sand). TOM was determined as the weight lost in another aliquot of ca. 3g sediment sample, combusted at 450ºC during 5 hours (see Annex C1-C2_ analysis protocols.docx for more details). In 10 to 12 subsamples per core, around 1 g of fine sediment (< 2mm) was digested by adding HCl 1M until cessation of bubbling. The digestate was centrifuged and rinsed with MQ-water until pH 7 before drying at 50ºC. Weight difference before and after the digestion plus the weight of the shells in the coarse (> 2mm) fraction were used to estimate the subsample total carbonate content. From this, TIC was calculated, knowing the molar weight of Calcium Carbonate ($CaCO_3$). Accuracy and precision were monitored using a certified TOC and carbonate standard sediment (SETOC 776 from WEPAL). The digested sediment aliquot was used to measure Total Organic Carbon (TOC) at the IATC-CSIC center in Granada, using a mass-spectrometer and a IRMS (Isotopes Ratio Mass Spectrometer) for subsequent isotopic analysis and the station sampled on the 3º mision was send to Hilo lab. The SETOC 776 standard was used to intercallibrate samples sent to both labs.

The 3 biomass replicates for each station were washed and sorted into necromass (largely leaf litter) and biomass (leaves, rhizomes and roots). Those fractions and subfractions were dried at 60ºC during 3-4 days, and weighed.

Average and standard error of each biomass compartment, and of necromass, for each vegetated station, were calculated from the 3 replicates.

Average plant TOC content (in %) from isotopic analyses performed on the plants collected at different stations in this study, combined with data from Cresson et al. (2019) on differences in %TOC content among *P. oceanica* plant parts, were used to transform biomass estimates (gDW m^{-2}) to TOC mass estimates (gTOC m^{-2}).

b. Lead (^{210}Pb) and radiocarbon (^{14}C) dating

From the 30 first cm from each replicates A core (cores subsampled in full at each station), aliquots of 5 g of ground sediment samples were sent to the Unit of Physics of Radiations from the Autonomous University of Barcelona (UAB), to estimate recent sediment accretion rates from ^{210}Pb in excess. In the same core, we selected 2-3 ancient plant debris (preferably leaf

rests) for carbon dating. Carbon datings were performed by accelerator mass spectrometry (DirectAMS - Accium BioSciences), using a NEC Pelletron 500 kV AMS. See Annex C1-C2_ analysis protocols.docx for more details.

Description of plant growth reconstruction techniques

a) Lepidochronology:

The sheaths of some seagrass species (*Thalassia testudinum, Enhalus acoroides, Posidonia sp*) remain attached to the rhizomes after the leaf blade has fallen. Similarly to the rings in the wood of trees (dendrochronology), the sheaths thickness presents an annual periodicity cycle, beginning in autumn, depending on depth and locality and progressively decreasing in winter, until it increases again in late winter or early spring (Pergent, 1987). The study of these cycles has been termed *lepidochrononology* (Pergent, 1990). The parameters which temporal evolution have been described so far using this technique are the following:

(i) Growth rate and rhizome production (which seem to be mainly coupled with fluctuations in sediment accretion; Marba et al., 1996, Cebrián et al., 1994)), by the determination of the age of a particular section of rhizome and the removal of segments corresponding to one or several years (Pergent and Pergent-Martini, 1990).

(ii) Number of leaves formed during an annual cycle (what it is suggested to reflect variations in the characteristics of the water column; Dawes and Tomasko, 1988; Neundorfer and Kemp, 1993), which corresponds to the number of sheaths per cycle.

b) Plastochrone interval:

The insertion points of the leaves on the rhizome are referred to as nodes and are identifiable by the scars left after leaf abscission. Accordingly, the rhizome pieces in between 2 consecutive leaf scars are termed rhizome internodes. These are produced in the time interval between the formation of 2 successive leaves, which is termed plastochrone Interval (Erickson and Michelini, 1957). This architecture ensures a close (1:1) relation- ship between the production of rhizome internodes and leaves. and vertical rhizomes, are reduced to small bracteae (Tomlinson and Vargo, 1969). The close (1:1) relationship between the production of rhizome internodes and leaves is the basis for age determinations of seagrass shoots and rhizomes, which can be estimated as the number of leaf scars (or internodes) plus standing leaves produced since the appearance of the shoot or rhizome of interest. The basic time units

of the resulting age estimates are plastochrone intervals, which have been proposed to represent the internal growth rhythm of the plants, thereby providing useful estimates of 'biological tune' (Erickson & Michehi 1957).

Here we present data for the number of Leaves Produced per Year (LPY) and Rhizome Vertical Elongation (RVE) data for 10 years of reconstruction. Shoots were collected at 7 different locations along the Andalusian coast (13 stations; N= 80 total rhizomes processed).

2.3. **Numerical procedures**
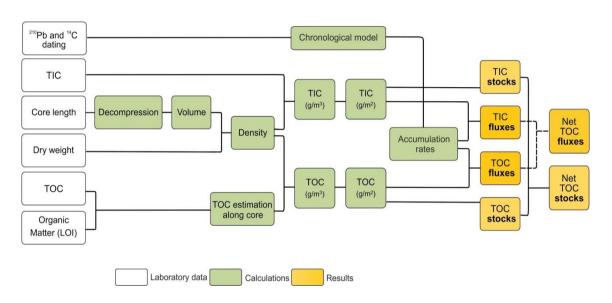
Fig. 2.11. Calculus diagram

2.3.1. *Corrections for core compression*

A decompression factor was applied to all cores presenting less than 30 % compression. The factor was obtained using a simple exponential function ($y=a \cdot e^{b \cdot x}$) under the assumption that compression factor increases linearly from the bottom to the top of the core, that is, that the first sediment entering in the pipe has suffered the compression of all the subsequent sediment that entered thereafter, and so on (Morton and White, 1997). The function is fitted between two points, (y_1, x_1) and (y_2, x_2), being y_1 the length of the core minus the penetration depth of the corer, $x_1=y_1$; y_2 is set to 0.1 (virtually no compression in the core bottom, as it is the last sediment to have entered the pipe) and x_2 is again the penetration depth of the corer. The resulting equation is then used to calculate a correction factor where 'x' is the observed sediment distance to the top. Then: the corrected (or decompressed) sample depth is the observed depth minus the correction factor. Cores with compressions between 30-40 % were decompressed as the above but using 1 for y_2 instead of 0.1. Those cores with a compression

exceeding 40 % were decompressed following a linear model (y=*mx+b*)*,* that is, considering a constant compression factor along the core.

It is relevant to mention that the volume of the subsamples (slices) taken from the cores was calculated after the mathematical core decompression. For the cores subsampled in the field through pre-made holes, the volume taken in the field was corrected applying the % volume increase that the slice shall have experienced at the level were each hole was located.

2.3.2. *Estimating TOC from TOM*

Because it is well established that TOM and TOC in seagrass sediments are highly correlated (R^2 = 0.96; *p*<0.001; (Fourqurean et al., 2012a), OC analysis were only performed on some of the samples, allowing us to fit a linear regression, and infer the remaining samples using the equation obtained (Table 2.2.). The error by applying this indirect method for estimating C_{org} is around 4%, therefore, well within field and experimental errors involved in the direct estimates of TOC.

Estimating sediment subsample bulk density - Bulk density is a basic parameter, necessary to express the contents of any element or compounds, usually measured as a percentage of the sample, in absolute spatially scalable units. Bulk density (*BD*, g/cm^3) was calculated by dividing the subsample dry weight *DW* (in grams) by its volume (*V*, in cm^3), that's: *BD* = *DW* / *V*.

Subsample volume (*V*, in cm^3) was calculated using the formula of the semicylinder, that is ½ of the corer circular cross section (in cm^2, estimated from the tube radius *R* (in cm), using the area of the circle), multiplied by the de-compressed thickness of the slice *L* (in cm), that's *V* = $\Pi R^2/2L$

Organic carbon stocks per unit area - The *TOC density* in each subsample was calculated from its bulk density (*BD*, in *g/cm³*) and *TOC content* (in %): *TOC density (g/cm³) =BD*TOC/100*. This value was then multiplied by the corresponding section thickness, to estimate the average *TOC stock* per area at that sediment depth (cm², m², ha, etc.). The total stock per core was computed by adding the TOC stock per unit area of all core subsamples. The TOC stock in 1 m sediment was estimated summing up the TOC stocks of the core

Table 2.2: TOM and TOC% on each station. Al correlation had a p < 0.001

Station	Total amount of samples	Samples analyzed for TOC	Equation	R^2
TE.S	44	25	y = 0.5293x - 0.0974	0.88
TE.I	52	31	y = 0.9683x - 0.6424	0.86
TE.I-C	46	20	y = 1.36x - 1.446	0.82
TE.D	44	28	y = 2.9873x - 2.1419	0.64
DE.I	245	36	y = 0.5558x - 0.3616	0.77
PA.I	64	19	y = 0.2847x - 0.0641	0.66
AG.S	88	51	y = 0.604x + 0.2417	0.66
AG.I	65	16	y = 0.639x - 0.465	0.8
AG.D	40	22	y = 0.8703x - 1.2006	0.68
BA.S	51	39	y = 0.1888x + 0.0006	0.55
RO.S	56	29	y = 0.536x + 0.3725	0.92
RO.S-C	110	45	y = 0.5167x + 1.4978	0.68
RO.S-CN	30	6	y = 0.0789x + 0.168	0.63
RO.I	63	33	y = 0.344x + 0.8934	0.88
RO.I-B	30	10	y = 0.983x - 1.3397	0.7
RO.I-E	25	10	y = 0.4279x + 0.0748	0.79
RO.I-R	34	21	y = 0.5931x - 0.4145	0.75
RO.I-Shall	30	20	y = 1.7568x + 0.1134	0.6
AL.S	33	10	y = 0.409x + 0.2772	0.94
ME.S	30	19	y = 0.5396x - 0.4307	0.96
ME.I-C	17	4	y = 1.2685x + 0.123	0.82
CA.S-CN	27	13	y = 0.81x - 1.2155	0.86
CA.S-C	33	16	y = 0.6505x - 0.7115	0.94
SA.ZN-S	28	13	y = 0.3593x - 0.3713	0.92
SA.ZN-D	37	24	y = 0.2624x - 0.036	0.92
SA.ZN-C	49	24	y = 0.3239x - 0.1717	0.85
SA.CN	40	18	y = 0.0964x + 0.235	0.7
SA.CP	30	14	y = 0.2825x + 0.8944	0.73
SA.ZN2	34	13	y = 0.1981x - 0.5458	0.67

subsamples until 1m decompressed core depth, for inter-stations and inter-studies comparation purposes, following the IPCC guidelines for carbon stocks inventories. For those cores subsampled in the field (i.e, those for which only a few subsamples were taken along the core through the premade holes), the average TOC between successive subsamples was integrated along the core length between both subsamples. The TOC content per unit area was added along the whole core and also to 1m sediment thickness, for the abovementioned comparative purposes.

Average (± SD) carbon stock for each station was estimated by constructing a "consensus core" from the three replicates. For that, average and standard deviation of *TOC density* was calculated from replicated subsamples. We considered as such, the sediment subsamples from the three replicate cores that had been taken at similar sediment depth levels. In the levels without replicated values, the unique *TOC density* value was adopted. Standard deviation of the averaged sediment levels was also calculated. These standard deviations were combined through their coefficients of variation, to estimate standard deviations of the carbon stock per unit area, at each replicated level. The average of the coefficients of variation of TOC density, conceptually equivalent to the variability among groups in an ANOVA, was multiplied by the carbon stock, to obtain an estimate of TOC stock standard deviation within the station. In most of stations, the consensus core reached or surpassed the targeted 1m sediment thickness. When it was not the case, we normalized TOC stock estimates to 1 m of sediment thickness, by extrapolating the TOC decay rate with sediment depth, if it was significant, or by summing a core average TOC density value until 1m sediment depth, if there was not a significant decay pattern.

2.3.3. *Chronological models, accretion, stocks and fluxes*

c. *Chronological models*

Replicates A for each station were used to calculate the TOC flux to the sediment (i.e., the rates of organic carbon sequestration). Radiocarbon ages were used primarily for the models and were combined with the ^{210}Pb technique to fine tune the chronology of the sediments for the last 100 years. The models were elaborated using the "rbacon" package for R software (Blaauw and Christeny, 2011). The age of the top most subsample of the core (the year of sampling: 2016 or 2017), was also considered in the model. Radiocarbon dates are expressed as calibrated years before present (marine curve 13; Reimer et al., 2013). Dates were corrected for isotopic fractionation (^{13}C/^{14}C), for the reservoir effect and for the local anomaly (ΔR=2 ± 26 years, Siani et al., 2010).

d. Accretion rates

The rhythm at which the sediment is accreted in the meadow was calculated as the length of core A accreted per year, following the age model constructed for each core, from Pb and C dating.

The long-term carbon fluxes into the sink were estimated by multiplying the carbon content of each subsample by its accretion rate. The fluxes have been estimated for the whole core and for the last 100 years. The sequestration rates for any specific period of time (in a context of e.g., the elaboration of compensation projects) can be easily calculated by multiplying the average of the accretion rates for the desired period. Nevertheless, see the discussion on the method limitations, explained in Results and Discussion.

The rhythm at which the sediment is accreted in the meadow was calculated as the length of core A accreted per year, following the age model constructed for each core, from Pb and C dating.

The long-term carbon fluxes into the sink were estimated by multiplying the carbon content of each subsample by its accretion rate. The fluxes have been estimated for the whole core and for the last 100 years. The sequestration rates for any specific period of time (in a context of e.g., the elaboration of compensation projects) can be easily calculated by multiplying the average of the accretion rates for the desired period. Nevertheless, see the discussion on the method limitations, within the Results and Discussion section.

e. Statistical analyses for comparisons within and among stations

Variability in carbon stocks and fluxes among species, sites and depths were graphically explored.

When required, differences in carbon stocks between paired stations (for example between the meadow and bare sediment at Terreros, TE.I vs TE.I-C), were tested by comparing TOC density values of their consensus core, in 1m sediment thickness, using un-paired t-tests.

Un-paired t-tests were also used to compare carbon flux differences along RO.S before and after the putative date of settlement of an impact, or of meadow loss, taking the same number of annual estimates before and after that date.

Regression analyses:

Last-century average TOC flux to the sediment with depth was also tested through type II regression analysis, for the sites in which a depth gradient had been explored (TE, AG and RO), but in this case, we added data on TOC fluxes in 2 Cabrera Island sites (Balearic Islands, Spain) for which there are also data along a depth gradient (Paleopark project, unpublished data, and Mazarrasa et al., 2017). Local variability was removed by transforming the flux data with respect to the value in the shallowest station of each site (all were at around 5 meters depth), which was considered as 100% flux.

Type II regression was also used to model the relationship between shoot density and canopy biomass, and thus with TOC stock, in order to use shoot density data from POSIMED to upscale TOC stocks in the meadow canopy compartment in Andalusia.

Type I regression was also applied to estimate if there had been a significant decline in TOC flux since Deretil settlement, at Villaricos.

2.3.4. *Upscaling: from areal to global estimates*

During the course of Action A1 (Cartography and characterization of habitats), a very complete cartography has been compiled, integrating a number of sources and observations taken during the multiple surveys conducted in this project, including spatial information on seagrass distribution, bathymetry and substrate type (AMAyA, Action A1; see further). GIS software allowed us to obtain the total areas covered by the various seagrass typologies for which the areal carbon stocks and fluxes have been calculated in this study (Table 3.5).

Global estimates were therefore calculated by multiplying the representative organic carbon stocks and fluxes of each typology by the area occupied by that typology. A detailed description and discussion of the distribution of these global estimates is out of the scope of this deliverable. The analysis in detail of the TOC stock and flux variability with meadow depth, health and substrate, has allowed us to propose a strategy and criterium to refine the scaling-up of the CO_2 carbon sink and stocks, which are summarized in annex I.

First discussions on board the R/V García del Cid during a LIFE BN mission. October 2017 Source: GAME

3. Results and Discussion

Discussing LIFE Blue Natura results on a global context at the EU Parliament, Brussels. November 2018 Source: IUCN

3. Results and Discussion

The assessment of the organic carbon stocks and sequestration rates made for coastal soils in this study represents the most detailed study performed on seagrass Blue Carbon in the European Union and probably in any other region of the world.

From Almería to Cádiz, over 122 linear meters of coastal soils (91 cores), from 10 sites and 33 stations ranging from 1 to 20 m depth, bearing or not a vegetation cover, have been scrutinized visually, physically and chemically.

As grand summary, the average stock accumulated in the top meter of seagrass soils in Andalusia amounts to 761.7 tCO_2/ha, ranging from 93.8 to 2077 tCO_2/ha (*Z. noltei,* Cádiz Bay to *P. oceanica* in healthy conditions above 5m of depth, respectively; table 3.5, Fig. 3.5). In the last century, this stock has been accumulating at an average rate of 0.85 tCO_2/ha yr, ranging from 0.05 to 2.48 tCO_2/ha yr (for small seagrasses to *P. oceanica* in healthy conditions above 5 m depth, respectively). Scaling up this numbers by assigning the corresponding stocks and fluxes obtained in the field to the total area occupied by each bottom type (table 3.3), the weighted total stocks at 1 m and fluxes for Andalusian seagrasses are of 8889.6 $ktCO_2$ and 9.9 $ktCO_2$/yr, respectively.

Therefore, the suspected (hypothesized) variability associated to species specificities, geographic setting, depth, sea bottom type, and state of degradation of the meadows, used as criteria for the sampling design, have proved to be correct and were ranked in their degree of importance as substrate (hard vs soft bottom) = species *(Zostera noltei < Cymodocea nodosa < Posidonia oceanica)* > degradation (*he*althy vs degraded) > depth (1 m vs 20 m).

The depth effect could be observed for fluxes but not for stocks as those respond better to geographic variability, linked to the local sedimentary regime (see discussion below). The effect of geographical location could not be assessed overall as not all of the species were present in each one of the regions of Andalusia.

Table 3.1. Bathymetric distribution of the rocky bottoms bearing *Posidonia oceanica* (ha)

Type of bottom	Surface covered in rock at each bathymetric range (ha)						
	0-5	5-10	10-15	15-20	20-25	25-30	Total
Dead matt	3.21	2.66	0.5		0.7	0.05	
Posidonia oceanica	79.37	543.35	718.41	494.9	294.77	28.29	
P. oceanica mixed bottom		0	6.21	35	1.67		
P.oceanica in regression		0.23	7.66	6.48	19.93	8.41	
P. oceanica and *Cymodocea nodosa*	5.83	15.51	9.6	15.79	0.59		
Totals	88.41	561.75	742.38	552.17	317.66	36.75	**2299.12**

A gigantic number of uncertainties and difficulties still prevail, most notably i) the always limited mapping effort, specially of dead mat and potential thickness, ii) the possible role of carbonates as a source of CO_2, iii) other possible emissions of GHG off the meadows such as methane of nitrous gasses; iv) the corrections that should be made for meadow cover, v) the dynamic condition of the sink (growing vs steady-state), vi) the complexity of determining the loss of service following meadow death or habitat destruction or vii) the very high cost of monitoring changes in the sink, to mention some. Nevertheless, the various typologies assessed here, constitute an important step forward in providing the grounds for establishing criteria for meadow management and for deciding the best settings to guarantee the success of eventual compensation projects, both in restoration and in emissions avoidance initiatives. Moreover, a number of recent scientific papers have provided some clues to tackle some of these uncertainties.

These and other matters are discussed in more detail below.

3.1. **Main geochemical variables estimated**

For each station bulk density, sediment accretion rate, total organic matter, total organic carbon, total organic carbon flux, total inorganic carbon, δ13C and δ15N isotopes and grain size distribution were characterized (see Annex II). Examples representative of the main typologies and sampling techniques are shown and briefly discussed below, as difference among stations would be further discussed on following sections.

On a well-developed meadow, it was expected to find a time-related reduction in TOM and TOC down-core that could be adjusted to an exponential curve with time, according to a fast degradation on the topmost part of the core and slower below it. However, from the 30 stations we only found a clear pattern in 6 of them (BA.S, DE.I, SA.ZN.S, RO.S-C, RO.I-R, and TE.I), all from different typologies and health status.

On TE.S, a well preserved shallow *P. oceanica* meadow (Fig 3.1), we can see a curve-fitting trend until 200 cal. yr BP below which TOM and TOC contents are irregular. This is due to the intrinsic temporal variability of the meadows, as an exponential decay curve would require high stability on the organic matter input (i.e, plant production). TOC fluxes reach a maximum on the top most layers, as fresh, non-degraded matter is present, and compaction has not occurred yet. TIC distribution seems to follow the fine fractions content (Fig. 3.1). An increase in fine fractions on the last 500 yr has been detected in TE.S, which could also alter the degradation pattern of TOM on the meadow, as more fine sediments reduce the porosity and hinder oxygen penetration. The TE.S sediment accretion rate (SAR) is very high compared to that of the deep meadow (Fig 3.2, TE.D, well preserved deep *P. oceanica* meadow), as

proximity to the emerged land would imply a higher inorganic input that would promote the accretion of the mat.

In the deep Terreros meadow (TE.D, Fig 3.2), sediment bulk density and SAR, estimated only
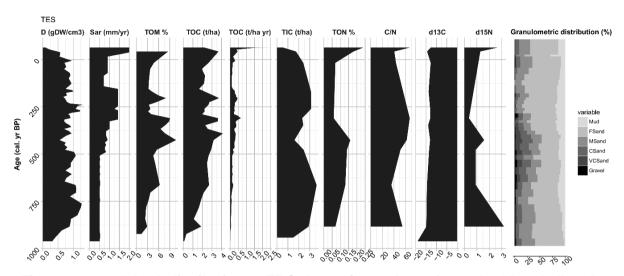
Fig 3.1.: age and depth distribution on TE.S_A core (manual core from a healthy, shallow, *P. oceanica* meadow) of soil density, sediment accretion rate, total organic matter, total organic carbon, total organic carbon flux, total inorganic carbon, total inorganic carbon flux and grain size distribution (coarse, >2mm; very coarse sands, 1-2 mm; coarse sands, 1-0.5 mm; medium sands, 0.5-0.25; fine sands, 0.25-0.063; and mud, <0.063mm).

Fig 3.2: age and depth distribution on TE.D_C core (vibrocore from a healthy, deep, *P. oceanica* meadow). Acronymes as Fig 3.1.

with ^{14}C at TE.D are very stable, while TIC decreased with core depth. There is a marginally significant decrease in TOC density towards the core top, in the topmost 50 cm (≈ 500 yr, $p<$ 0.05, R^2= 0.60). This reduction in carbon density seems to coincide with a higher percentage of fine fractions, that would point to more turbidity of the water column and lower light

penetration, which could translate into lower plant productivity and carbon burial. Such TOC density pattern is not observed in the shallow, nor in the intermediate stations of Terreros, despite fine sediments also have increased in the last 500 years. Light penetration becomes more important as a production factor with depth, as it determines the depth limit for seagrass survival (Duarte, 1991). Moreover, common trends of decline of deep *P. oceanica* meadow have been detected and could be linked to reduction in Mediterranean water transparency (Astruch et al., 2017). However, our results are not conclusive: in the deep core of Aguamarga we also detect an increase in fine materials and a concomitant reduction in coarse materials in the last 1000 years, but sediment TOC density only decreases in the top 10 core cm (in the last 24 years).

An example of chemically degraded *P. oceanica* meadow is station DE.I (Fig 3.3). Density and SAR follow similar trends than the two examples above. The main change in carbon stocks occurs around 80 cm from the top (>1000 cal. yr BP), 950 yr before the start of the chemical disturbance, and mainly driven both, by a reduction of the mat bulk density and a reduction in the %TOC. It could be expected that a chemical degradation could not affect only the soil being formed at the moment of the perturbation but also the extant soil. A more specific study would be needed to clarify the causes and consequences of this degradation, but comparison between this and healthy stations is discussed on following sections.

The flux values estimated correspond to the performance of the meadow before dying, although they might be influenced by an eventual degradation of TOC in the degraded
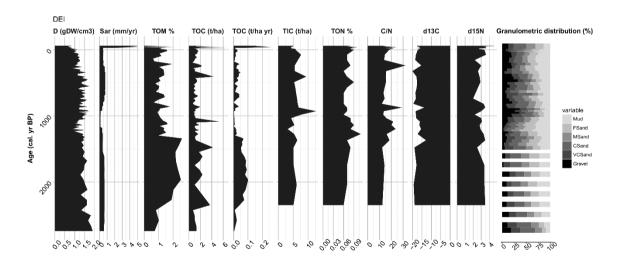
Figure 3.3: age and depth distribution on DE.I_A core (manual core from a degraded, intermediate, *P. oceanica* meadow) Acronymes as Fig 3.1.

meadow dead mat. This is due to the methodology used to estimate carbon flux, based on the measurement of TOC stock in each sediment layer and the core age model, which tells us how much time it took to accumulate that TOC stock. Such core analysis does not allow us to measure instantaneous fluxes, but an average carbon accumulation rate over the last years

(depending on the resolution on sediment accretion rates and sub-sampling frequency). However, it provides valuable information, as we can follow the degradation pattern that ended up with the loss of the sequestration capacity. Granulometric distribution in DE.I cores follow cyclical changes that could reflect temporal ecosystem variability.

Cymodocea nodosa cores show the expected TOC decay curve with core depth and time since sediment deposition (e.g. Fig. 3.5, Fig. 6 in Annex II), which suggests that we sampled the entire sediment carbon stock. These and other variables, like grain size distribution and sediment bulk density also suggest a relative stability of sedimentary conditions over the period encompassed by the sediment core, which is shorter than in *P. oceanica meadows*, due to higher Sediment Accumulation Rates (SAR), which also determines larger TOC fluxes.

Z. noltei cores (Fig 3.6), also showed maximum values of TOM and TOC richness in the upper sediment layers, very likely due to the presence of the living plant parts, and fast TOC stock
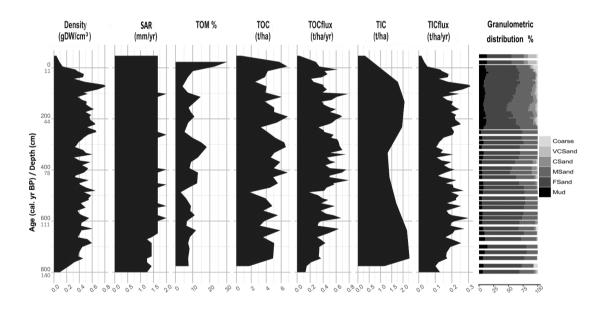
Figure 3.4: age and depth distribution on RO.S-C19 core (manual core from a dead, shallow, *P. oceanica* meadow). Acronymes as Fig 3.1.
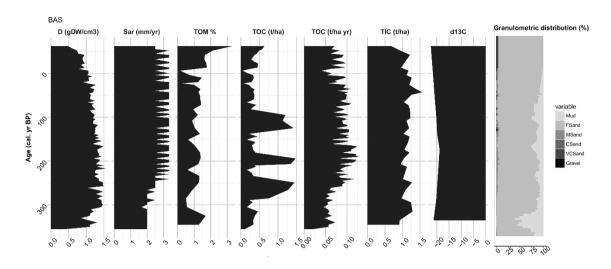
Figure 3.5: age and depth distribution on BA.S_A core (manual core from a healthy, intermediate, *C. nodosa* meadow). Acronymes as Fig 3.1.

decay rates with sediment depth, indicating that *Z. noltei* organic matter buried is more labile than that of *P. oceanica* and is not well conserved on the soil. Despite this decay, the TOM showed a significant positive correlation with the sediment mud content, something already described by Serrano et al (2016) .and attributed to a better preservation of organic matter, due to the higher compaction and more difficult spread of oxygen in the pore water.
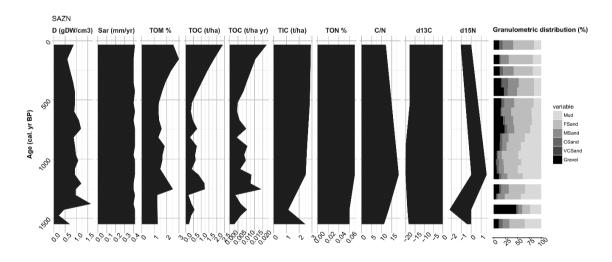
Figure 3.6: age and depth distribution on SA.ZN-S_A core (manual core from a healthy, intertidal, Z. noltii meadow). Acronymes as Fig 3.1.

3.2. **Sediment Accretion Rate and Rhizome Elongation in *P. oceanica* meadows**

Depending on the station, we could reconstruct the rate of rhizome vertical growth (VRG) and the mumebr of leaves produced every year (LPY) for a period ranging between 16 and 8 years. LPY for each station fluctuated with time, and in most of sites it did not show clear temporal trends. Highest values were recorded in ME.S (9.46 ± 0.26 leafs yr $^{-1}$) and lowest in AG.D (7.01 ± 0.14 leafs yr $^{-1}$; Fig. 3.7; Table 3.2). The average LPY among sites per year showed a marginally significant increasing trend with time. (R^2= 0.02 p< 0.09). The regression was not better because LPY in 2017 decreased abruptly. We could consider it as stable along the 10 years analyzed (8.1 ± 0.4 leaves year $^{-1}$; Fig. 3.8; Table 3.2).
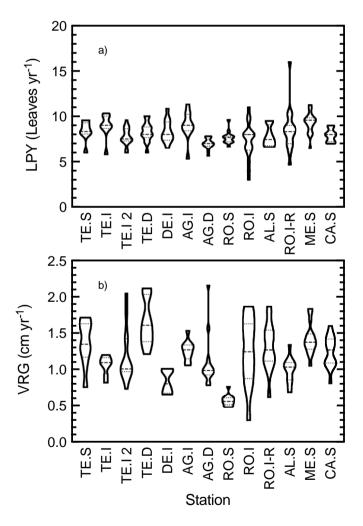
Fig. 3.7. Boxplots of a) Annual Leaf Production (LPY, in leaves per year) and b) Vertical Rhizome Growth (VRG in cm per year) at each P. oceanica station. The median, 25% and 75% quartiles are represented. Top and bottom of each violin boxplot represent the minimum and maximum.

RVE for each station fluctuated along those periods differently but did not show any specific pattern. Stations AG-I, DE-I, RO-S and TE-I showed the most stable temporal pattern. Highest values were recorded in TE-D (1.6 ± 0.09 cm year $^{-1}$) and lowest in RO-S (0.57 ± 0.02 cm year $^{-1}$) (Fig. 3.7; Table 3.2). Considering the period for which we had 7 to 13 stations (2004-2017), the average RVE decreased until 2016, while in 2017 it increased abruptly (Fig. 3.8; Table 3.2). Despite that 2017 increase in vertical growth, when considered globally, there was a significant reduction of vertical growth with time (R^2= 0.03, p< 0.03). LPY and RVE temporal variability were opposite (Fig. 3.8). This suggests that the years when the plant produced larger internodes, it also produced less leaves.
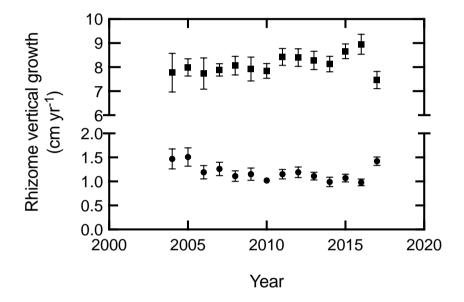
Fig 3.8. Average annual leaf production (triangles, LPY, leaves per year) and Average annual Vertical Rhizome Growth (circles, VRG, cm per year). Dotted straight line and curved lines represent the linear regression and its 95% confidence intervals.

Table 3.2. Systematic difference between P. oceanica vertical rhizome growth and Sediment Accretion Rate (cm yr-1)

	Average	SD	SE	95% CI	Median
VRG	1.125	0.296	0.1	(0.88 ; 1.37)	1.25
SAR	0.581	0.284	0.1	(0.34 ; 0.82)	0.545
SAR-VRG	-0.54	0.21	0.08	(-0.72 ; -0.37)	-0.54

RVE was always greater than SAR estimated for the period encompassed by shoot growth reconstruction (Wilcoxon signed rank paired test of difference of medians: p< 0.01; median of the differences: 0.535; paired t-test: p< 0.001, difference of the means: 0.544; Fig. 3.9a, Table
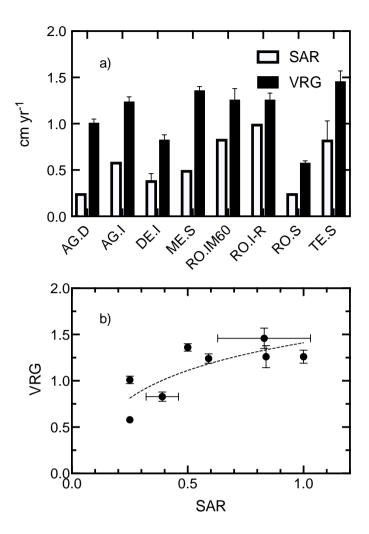
Fig. 3.9. a) Average Vertical Rhizome Growth (VRG, black bars) and Average Sediment Accretion Rate (SAR, white bars) in the same period ecompassed by rhizome internodes. b) Semilog regression of VRG with SAR.

3.2). Neverthelss, SAR and VRG were positively correlated (R^2= 0.57; p< 0.04 ; Fig. 3.9b), and thus, SAR during the last decade could be predicted from VRG in Andalusian *P. oceanica* meadows, using an exponential model (Eq. 3.1). Therefore, RVE lower or equal to 1.1 cm yr^{-1}) could be used, instead of costly ^{210}Pb measurements, to estimate recent sediment accretion rates, using equation 3.1.

$$\textbf{SAR = 0.93 e}^{0.46\textbf{VRG}}\textbf{ -1}\qquad\qquad\textbf{eq. 3.1}$$

Standard error of the slope: ± 0.16

Standard error range of the Y intercept: (0.7697 ; 1.1223)

RVE when SAR approaches 0: 0.16 cm yr^{-1}.

3.3. **Variability in carbon stocks and fluxes:**

3.3.1. *Long- vs medium-term stocks and fluxes –*

It is relevant to remind here that carbon densities were estimated with more resolution for the longest cores of each station and in a smaller number of samples for the other 2 replicate cores, and that the carbon stock for each station was estimated by combining subsample TOC density values in a consensus core, with average and standard deviations of TOC densities for the paired sediment layers in 2 or more cores. In most of the stations, the consensus core reached or surpassed the targeted 1m sediment thickness. When it wasn't the case, we normalized TOC stock estimates to 1 m of sediment thickness by extrapolating TOC density estimates, in order to allow us for comparisons among meadows (within this study and to other studies following IPCC recommended methodology).

We estimated the average organic carbon flux for all core sections as well as for the sections corresponding to the last 100 years of sediment accretion (medium-term average). This latter estimation fulfils two objectives: 1) make comparisons among meadows, and 2) this carbon flux includes the total carbon being deposited in the meadow sediment, with the medium-term degradation (i.e. less than 100 years) already discounted. Once organic matter is buried, aerobic and anaerobic degradation continues to transform it into CO_2. In the "fresh" organic matter there is a fraction of labile organic matter which is readily mineralized. There is also a fraction of more recalcitrant organic matter that will last much longer within the sediment (or it is formed during the diagenetic process). As aerobic degradation is usually many times faster than anaerobic degradation (Fenchel et al., 1998), the oxidation of the organic matter will depend on the sediment accretion rate and the depth reach of the oxygen within the sediment (which depends on hydrodynamism, water oxygen concentration and sediment porosity, among other factors).

Carbon fluxes estimated for the last century were higher than average fluxes calculated with the whole sediment core, because the top sediment layers are less compacted than the bottom ones, and because average carbon flux for the last 100 years is much more influenced by the carbon-rich top sediment sections. Even though part of this carbon flux estimation is not the one to feed long-term accumulation estimates, it is relevant for conservation carbon offset projects, as it will be discussed in section 3.5.

Last, for the station where *Cymodocea nodosa* was growing on dead mat (CA.S-CN), stocks were calculated as usual, but TOC fluxes were assumed to be those of *Cymodocea nodosa* growing over sediment. If we had estimated TOC fluxes with the usual reconstruction method they would have been overestimated, as they would integrate old organic matter accumulated by *P. oceanica* in the past, not only the current TOC flux produced by *C. nodosa*.

3.3.2. *Spatial and species variability in TOC stocks and fluxes*

The overall variation of the carbon stocks in the top sediment meter of Andalusian seagrasses was very large, as much as two orders of magnitude (Fig. 3.10, Table 3.1). The station showing the highest carbon stocks in 1 m sediment was the *P. oceanica* deep meadow of Aguamarga (0-5 m, Almeria, AG.S), with more than 1234.5 tTOC/ha. The lowest sediment carbon stock was found in BA.S-C station, a sub-surface dead *C. nodosa* matte, with 24.5 tTOC/ha.
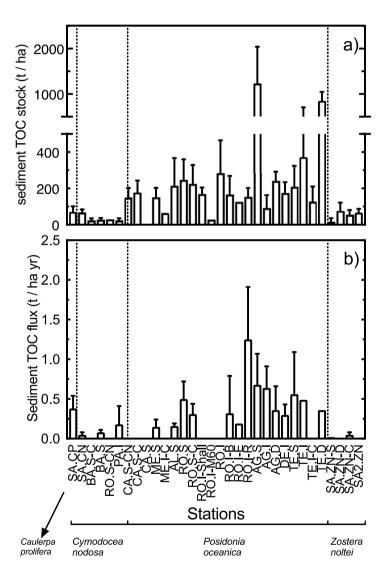

Fig. 3.10. a) Sediment carbon stocks (t TOC / ha) accumulated in the first sediment meter in all the seagrass stations (vegetated and un-vegetated; b) Average carbon flux to the sediment (t TOC / ha yr) in the last century in these stations. Empty columns represent "no data", with the exception of CA.S (Posidonia directly on rock, where it means "0 sediment TOC stock".

The average carbon stock$_{1m}$ in healthy *P. oceanica* meadows was of 500.1 ± 182.2 tTOC/ha in the Mediterranean Sea (Eastern Almeria), while in the Alboran sea (Western Almeria, Granada and Malaga), carbon stocks were halved: 224.6 ± 25.2 tTOC/ha. As for *C. nodosa*, carbon stocks in Alboran and Mediterranean seas were similar (28.1 ± 9.1 tTOC/ha and 24.5 ± 10.5 tTOC/ha, respectively), while at the Santibañez lagoon (Cadiz), the carbon stock under *C. nodosa* meadow was twice as high (66.2 ± 17.8 tTOC/ha) as in the Alboran and Mediterranean open seas.

Carbon fluxes in Andalusian seagrasses in the last century also varied two orders of magnitude (Fig. 3.10): The station with the highest C fluxes in the last century were the recolonization patches of Roquetas meadow (RO.I-R, 1.25 ± 0.66 tTOC/ha yr). Excluding this area in recovery, the highest C fluxes were found on the shallow areas of the Aguamarga meadow, at 5 m depth (0.68 ± 0.39 tTOC/ha yr), while the station showing the lowest TOC flux on a *P. oceanica* meadow was that of Melicena, at 4 m depth (0.15 ± 0.09 tTOC/ha), which presents a large proportion of dead mat. The highest TOC fluxes on *C. nodosa* meadows were found at Palomares meadow, 10.2 m depth, but with a high SD (0.18 ± 0.23 t TOC/ha) and the lowest at Santibañez lagoon (0.05 ± 0.03 t TOC/ha).

a. *Variability in sediment TOC stocks and fluxes among species –*

The three different species sampled in this project, can be considered as representative of the carbon stock diversity of Andalusian meadows: *Posidonia oceanica*, *Cymodocea nodosa* and *Zostera noltei* (additionally, a *Caulerpa taxifolia* core was taken in Santibañez Bay for comparison purposes). *Posidonia oceanica* is a large slow-growing seagrass, the two others being small, fast growing species. *Zostera noltei* was only sampled in Cadiz (Santibañez) bay and *P. oceanica* along all its range, which is the Eastern Andalusian coast, but not in Cadiz, where it is absent. *C. nodosa* was studied in both systems: open sea (Mediterranean and Alboran), and the coastal lagoon of Cadiz (Santibañez) bay. Consequently, *C. nodosa* and *Z. noltei* could be compared between them at Santibañez bay. The best stations to discuss differences in accumulation between *P. oceanica* and *C. nodosa* were those from Almeria bay, where we found both species in very developed and differentiated meadows. Direct comparison between *P. oceanica* and *Z. noltei* were not possible, but the data from both species may be compared using *C. nodosa* as pivotal for the comparison.

Posidonia oceanica TOC in the top meter of sediment holds an average of 343.1 ± 99.2 tTOC/ha, one order of magnitude more than the small seagrass *C. nodosa* (37.2 ± 19.5 tTOC/ha) and the even smaller *Z. noltei* (53.6 ± 26.2 tTOC/ha). The stocks under the sediments of the seaweed *C. prolifera* were even larger than those of the smallest seagrass (70.7 ± 30.2 tTOC/ha, Fig. 3.10). The only sampled *C. nodosa* meadow that was growing on a *P. oceanica* mat, dead of many decades (Calaburras, CA-S-Cn), showed carbon stocks comparable to those of Alboran live *P. oceanica* meadows. The TOC stock$_{1m}$ was indeed not significantly different from that measured in a dead mat of the same site and depth, covered only with algae (CA.S-C). The areas dominated by dead *P. oceanica* mats, often present a secondary dense colonization by algae and smaller seagrasses, and it has been hypothesized that this vegetal cover could act as a 'lid' for the extant carbon stock, by reducing erosion, while a spontaneous or induced succession of recolonization of *P. oceanica* might begin. Our results do not allow us to be conclusive about that, since carbon stocks to 1m in the dead matte covered only with algae and with algae + *C. nodosa* are not significantly different (see section 3.3. for further discussion on this).

Over the last century *P. oceanica* meadows accumulated organic carbon in their sediments 3 times faster than *C. nodosa* (Fig. 3.10). The small seagrass species and *C. prolifera* had comparable carbon stocks and fluxes among them.
P. oceanica TOC stocks and fluxes measured in Andalusia were within the range measured in other Mediterranean *P. oceanica* meadows (i.e. 47 to 755 tTOC/ha in Ibiza and Formentera islands, and 0.2 to 1.9 tTOC/ha yr in Portlligat and Formentera islands, respectively; Serrano et al., 2012, 2014).

b. TOC stock in canopy (living) biomass: variability among species and with meadow shoot density in P. oceanica *meadows* –

Biomass measurements within the stations, combined with TOC content data of plant samples, allowed us to estimate the carbon stock in the seagrass canopies of most of the stations. With the exception of the plant patches growing directly on rock, at Calaburras (CA.S), canopy biomass TOC stocks were between 43 and 1543 times lower than sediment TOC stocks$_{1m}$ (high intertidal *Z. noltei* in Santibañez, SA.ZN-S, and deep Terreros *P. oceanica* meadow, TE.D, respectively, Fig. 3.11 and Table 1, Annex I). Therefore, they added very little to the C$_{org}$ stocks inventories. Seagrass canopies are important from the functional point of view as they act as sediment traps thus increasing sediment accretion and retention (Gacia and Duarte, 2001).
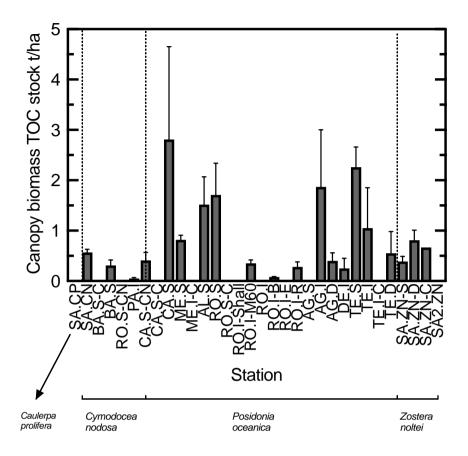
Fig. 3.11. Carbon stock in the canopy biomass compartment of stations. Empty columns correspond to "no data" with the exception of un-vegetated stations (no seagrass nor algae) BA.S-C, ME.I-C, RO.I-E and TE.I-C, which correspond to "0 values".

The largest canopy TOC stocks were observed in shallow *P. oceanica* meadows, while in deep and intermediate meadows, canopy TOC stocks were comparable to those measured in small seagrass species (Fig. 3.11, Table 1, Annex I).

Finally, combining shoot density data from POSIMED stations (action A1, materials and methods section) very close or coinciding with coring stations (action A2), as well as literature data on canopy biomass, density and carbon content (Apostolaki et al., 2009; Gobert, 2002), we could establish a quantitative, exponential relationship between *P. oceanica* meadow global shoot density and canopy TOC stock (R^2= 0.35, p< 0.05; Fig. 3.12). This quantitative relationship is useful, because it allows to estimate canopy TOC stock in other meadows from the POSIMED network, as well as around the Mediterranean, because shoot density is an important parameter, commonly measured in meadow descriptions and monitoring. Moreover, shoot density has a well-known light-induced exponential decline with depth (Pergent-Martini et al., 1994). Therefore, we can easily translate this into an exponential model of decline in

TOC Stock$_{canopy}$ with meadow depth, which can be useful to escalate C$_{org}$ stock estimates for an inventory.
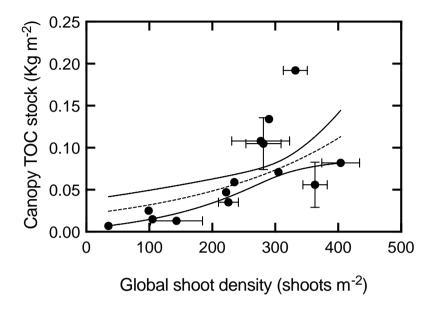
Fig. 3.12. Significant relationship between global shoot density and canopy TOC stock: R^2 = 0.35, p< 0.05. The model curve and its 95% confidence interval bands are plotted.

TOCstock$_{canopy}$ = 0.021($\pm$0.008SE) $\cdot$e$^{0.004(\pm0.001SE)\cdot Shoot\ density}$. CI95% of the exponent: (0.002; 0.007); CI95% interval of Y0: (0.010; 0.040).

c. **Variability of sediment TOC stocks and fluxes with depth in *P. oceanica* meadows –**

Despite shallow meadows are far more productive than deep ones (Pergent-Martini et al., 1994), carbon stocks in the top 1m sediment of *P. oceanica* meadows do not show a clear tendency with depth, and show great variability among sites: TOC stock in the top meter of Terreros seems to increase with depth, while in Aguamarga it appears to decrease with meadow depth, and in Roquetas, it seems not to change with bathymetry (Fig. 3.13a).

Within Aguamarga and Terreros (Roquetas is not considered here as the core age models at intermediate stations are only based on ^{14}C and are not comparable with the age model obtained in the shallow meadow, based on ^{210}Pb and ^{14}C), TOC fluxes seem to decrease with depth (Fig 3.13b). We have found the same pattern in two other *P. oceanica* meadows from

Cabrera National Park (Balearic islands, from project Paleopark (Fig. 3.14a). The global analysis of Paleopark and Life Blue Natura data, comparing the percentage of (100 yr)TOC flux in deep meadows with their respective shallow counterpart (set to 100%), showed a significant reduction in carbon flux with depth, of $4 \pm 0.8(SE)\%$ ($R^2 = 0.74$, $p < 0.001$) per meter increase in depth (Fig 3.14b). The graphs from figures 3.12b, 3.13b and 3.14 suggest that the flux reduction rate with depth may not be constant, but could decrease with depth. Thus, we tried a quadratic model, but it was not significant. More data points are needed to define a significant quadratic model of flux reduction with depth. Our results in Andalusia are consistent with the significant reduction in TOC flux found by Serrano et al. (2016) in a shallow *P. sinuosa* meadow from Australia, with respect to its deeper counterpart.

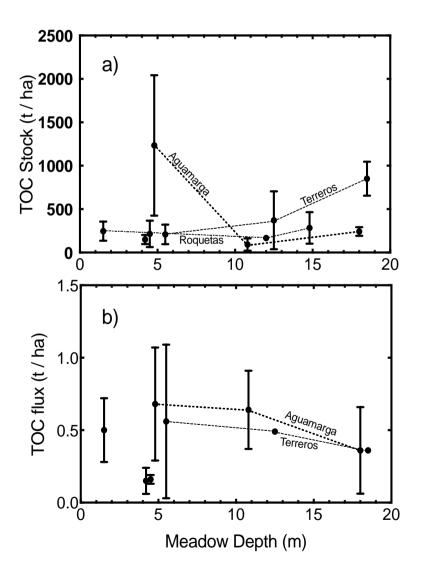**Fig. 3.13. Carbon stock (in 1m sediment thickness) and average flux (last century) variability with depth in Andalusian meadows**

Given that the TOC flux to the sediment decreases with depth, the finding of a greater 1-m TOC stock in Terreros is probably due to a higher sediment TOC concentration, together with the convention of measuring the carbon on the top meter of the soil, without taking into account how long it took to accumulate that 1-m stock. Therefore, TOC concentration and thus the stock, seem to be more influenced by sedimentation rate in the area than by meadow production: higher sediment inputs would dilute sinking organic matter, and this factor, may or may not follow a trend with depth, depending on the particular geomorphology and hydrodynamics of each meadow. The dilution sedimentary effect would reduce the top meter TOC stock, although probably not the global TOC stock, in the shallow meadow. In contrast, in the deep meadow, TOC is more concentrated, implying higher carbon stocks at equal sediment thickness. Therefore, a larger 1 m-thick carbon stock in the deep Terreros meadow (TE.D) would result from slow inorganic sedimentation, combined with long-term stability and burial, rather than from higher sink capacity.

In contrast, in Aguamarga, carbon stocks seem to be more influenced by the depth-light induced productivity gradient, thus showing a reduction of the top meter TOC stock with meadow depth. The Aguamarga meadow is adjacent of a larger watershed and potential sediment input than that of Terreros, which reinforces the hypothesis that the geomorphology and hydrodynamics influence can largely vary at the landscape scale.

In addition, several authors have found evidences supporting that deep meadows are older than shallow ones, as the long-term sea-level rise would generate a long-term coastward meadow colonization-migration (Astruch et al., 2017). Therefore, we may expect deep meadows to have accumulated great TOC stocks, despite being less intensive carbon sinks at present. Thus, those at deep meadows could constitute particularly sensitive areas of the meadows where larger carbon pools could be released if degraded and eroded by the present pressures of water transparency reduction and illegal trawling.

Fig. 3.14. a) Average carbon flux variability in the last century in Western Mediterranean *P. oceanica* meadows. Data from Santa Maria and shallow Es Port come from project PALEOPARK, while data from deep Es Port meadow comes from Mazarrasa et al, 2017. Error bars correspond to standard error of the mean. b) Linear regression of the % of flux in meadows (as compared to the flux in its respective shallowest part of the meadow) with respect to depth. Error bars are obtained dividing the standard error of the flux by the total flux in its respective shallowest part of the meadow. The solid line represents the significant linear regression (R^2= 0.74, p< 10^{-3} ; y= - 4.043($\pm$ 0.795(SE)·X + 119($\pm$10.56(SE)) where y = % of Carbon flux with respect to 5m depth and X is depth, expressed in meters. The dotted lines represent the 95% confidence interval of the slope (Table. 3.14).

Fig. 3.15. % of Average carbon flux wih respect to the 0 to 10 depth range, suggesting that the decline would not be constant, but would accelerate with meadow depth

d. Sediment TOM stock variability with substrate type and grain size distribution –

Carbon sequestration is not a direct effect of grain size, although both can be influenced by seagrass production (Serrano et al., 2016a). Seagrass canopy density is also linked to grain size sorting (van Katwijk et al., 2010).

Silt and clay (particle sizes < 63 μm) have been shown to retain more C_{org} compared to sands or greater sized sediments (Burdige, 2007; Keil and Hedges, 1993). This greater C_{org} content would be due to lower redox potentials and slower remineralization rates in the low-porosity, fine-grained sediments (Burdige, 2007; Hedges and Keil, 1995; Pedersen et al., 2011). However, large inputs of seagrass-derived C_{org} in the sedimentary pool have been shown to break the linear relationship among mud content and C_{org} contents, typically found in marine sedimentary environments (Bergamaschi et al., 1997; De Falco et al., 2004; Serrano et al., 2016a). Moreover, the amount of C_{org} that can be associated with mud particles is limited (Hassink, 1997), and this could also weaken a relationship between mud and soil C_{org} contents.

Table 3.3 Correlation between TOM and grain size distribution for each specie

	R correlation TOM % - Grain size distribution % (p-value<0.002)					
	Mud **<0.063 mm**	**FSand** **0.063-0.25 mm**	**MSand** **0.25-0.5 mm**	**CSand** **0.5-1 mm**	**VCSand** **1-2 mm**	**Coarse** **>2 mm**
Posidonia		0.4349818	0.249188888	-0.349644256	-0.454562577	-0.27858936
Cymodocea		-0.346567164	**0.253815345**	0.563740212	**0.529833118**	**0.4971302**
Zoostera						
Caulerpa	**0.797729618**		**-0.797729618**	**-0.812177503**	**-0.827052249**	**-0.799793602**

A significant relationship between mud and C_{org} contents would allow mud to be used as a proxy for C_{org} content, thereby enabling robust scaling up exercises at a low cost as part of blue carbon stock assessments, given that most countries have conducted extensive geological surveys in their coastal areas to determine sediment grain size.

In this study, the correlation matrices of the 6 grain size fractions defined with TOM along cores (Table 3.3) do vary among species, sites and stations.

In 53% (9 out of 16 live and dead mat stations) of *P. oceanica* meadows there was a significant positive correlation of TOM% and mud content, ranging from R= 0.38 to 0.78 (Table 3.2). In 29% of these cores, we also found significant correlations of TOM with fine sand content. In the cores where we found a significant correlation between TOM and fines, we observed the concomitant negative correlations with coarse material (as the sediment grain-size fractions are not independent variables within each other. AG.I station (Aguamarga intermediate depth, Table 3.3). showed a different pattern, with positive correlations of TOM with mud (< 0.063 mm) and materials with grain sizes above 0.5mm, and negative correlations with fine and medium sands (between 0.063 and 0.5mm).

No significant correlation between TOM and any sediment fraction content was detected within *C. nodosa* meadow cores. The only exception was the sediment under the *C. nodosa* plants of Santibañez, for which we detected a significant positive correlation between TOM and fine sand sediment contents (R = 0.51; Table 3.3).

Santibáñez lagoon, with high depositional dynamics and small seagrasses, showed the highest covariance between grain size and TOM. Sediment under *C. prolifera* had the TOM content most dependent on a narrow grain size range: it positively co-varied with mud content (R = 0.80) and negatively with the rest of sediment fractions (R between 0.83 and 0.71). The

cores under *Z. noltei* plants growing in the low-intertidal stretch showed positive correlation with mud and sand content, and negative with coarse material content. Grain size seems to be the main driver of *C. prolifera* organic matter accumulation. This could be explained by its physiology: as an algae, *C. prolifera* lacks roots or any other buried organ that could directly modify or interact with soil., In contrast, both *C. nodosa* and *Z. noltei*, have roots and rhizomes that can stay buried as organic matter when the plant dies, interacting with soil bacteria, and pumping O_2 into the soil when they are alive, in order to avoid sulfide formation (Borum et al., 2006), thus influencing sediment organic matter mineralization, at least in the upper sediment layer. On the other hand, during the sampling at Santibañez, we observed that *C. nodosa* and *C. prolifera* formed patches of a few squared meters, and that the surface sediment where *C. nodosa* was growing was sandier, while the patches were *C. prolifera* grew, were muddier.

Our results show that there is too much inter-site variability and correlation coefficients are too low in most of the stations, making it unreliable to apply models to predict TOM (and thus TOC) content from sediment grain size distribution, in any of the meadow habitats examined. Nevertheless, granulometric information helps understanding geochemical processes, which play a key role in carbon accumulation, and hence allow better-informed predictions and management decisions (when choosing or trying to improve a BC 'friendly' habitat).

e. Sediment TOC stocks and fluxes of unvegetated areas: comparison with vegetated ones –

It is established that the carbon susceptible of being certified as a carbon credit from an emission offset project, is only that which represents an addition to what it was already in the system, reduces GHG emissions or enhances their sequestration with respect to its present dynamics in the "business as usual" scenario, which includes present legal regulations (additionality; (Mason and Plantinga, 2013).

In the case of seagrass meadows, a way of comparing scenarios between presence and absence of the habitat in a particular site, is to sample cores in sandy sediments adjacent to the meadow, as a reference to determine the content and the rate of carbon sequestration, taking place without blue carbon habitat mediation.

In this study, the 'control cores' were taken at intermediate depths in a bare sandy bottom adjacent to *P. oceanica* meadows at two sites (Terreros and Melicena, although in the latter, the vegetated meadow, ME.S, was sampled at shallower depth) but with no patches of any seagrass in a radius of at least 30 m.

In the case of the sand patch of Terreros (TE.I-C) we found dead mat under 55 cm of sand (Deliverable A2). The Organic carbon in its top had settled 1603 years BP (in the year 352 AD). The 1m-TOC stock of that sand patch sediment (which includes 45 cm of dead *Posidonia* mat) was 66% of that found in the adjacent meadow (TE.I). When we compared only the TE.I-C sand pack TOC stock with the live mat TOC stock in TE.I (top 55 cm of sediment in each station), the TOC stock in the sand patch was 28% (49.7 ± 35.5(SD) tTOCha^{-1}) of the TOC stock in the same thickness of live meadow sediment (179.3 ± 158.0(SD) tTOCha^{-1}). The Addition of the TOC stock$_{biomass}$ contained in the meadow canopy (1.1 ± 0.8(SD) tTOCha^{-1}) did not alter this proportion.

In Melicena, the un-vegetated ME.I-C station (11.8 m depth) sediment was dominated by coarse sand, although it contained fragments of leaf sheaths (Deliverable A2). The sediment from the vegetated *P. oceanica* meadow to be compared with (a patchy meadow at 4.2 m depth, ME.S), consisted in a mat sediment (Deliverable A2) but with a peculiarity: its topmost 30 cm, which usually are rich in organic matter, had low TOC densities, similar to those in ME.I-C. Below that depth, it increased, while it decreased in ME.I-C (Fig. 3.15b). The average TOC stock in ME.S was estimated in 118.1 ± 40.3 t TOC ha^{-1} in the 78 cm of sediment thickness measured, and would have accumulated in 464 years. The TOC stock in the ME.I-C sandy patch at the same sediment thickness was of 53 t TOC ha^{-1}, which supposed 45% of the stock at ME.S.

The ^{210}Pb decay models could not be obtained for cores from the bare sediments, because they did not show a coherent stratigraphic sequence, suggesting dynamics of removal and sediment mixing, which is common in sandy bottoms. Therefore, direct comparison of TOC fluxes between seagrass habitat and adjacent bare sediment was precluded. Therefore, a different approach is proposed to estimate the effect of meadow sediment retention (Gacia and Duarte, 2001) in carbon sequestration for those cases where the accretion rate cannot be determined in non-vegetated areas: the average carbon density from bare sediments next to the meadow can be subtracted from the carbon density of the seagrass meadow sediment, so that the carbon that was going to be accumulated anyway will not be considered when

calculating the TOC fluxes. This is a current highly controversial topic of discussion in the community of BC specialists.

In Terreros, this procedure indicated that in the meadow (TE.I), TOC flux$_{100yr}$ was nearly 9 times larger than in the sand patch. In contrast, for ME.S, meadow TOC flux$_{100yr}$ was only of 13% larger than in ME-I-C. This is because the section of sediment accumulated in the last century encompassed the top 22 cm, with the abovementioned low carbon densities. The ME.S station consists in a patchy meadow with many sandy and dead mat areas, and the ^{210}Pb and ^{14}C-based chronological model for ME.S sediment suggests that there has been an acceleration of sediment accretion rate in the top 5 cm, since 1999 (Annex II). Altogether these evidences suggest that the shallow meadow of Melicena may be submitted to accelerated burial of mineral sediments, which may be reducing meadow productivity, or at least meadow C$_{org}$ inputs.

All these approaches comparing carbon stocks and fluxes in the meadow and adjacent bare sediments underestimate the positive effect of seagrass habitat presence on sedimentary TOC stock, because part of the carbon buried in the bare sediment consists in necromass laterally exported from the adjacent blue carbon habitat. Such organic matter would not have been produced, nor buried in the bare sediment, in the absence of the habitat, and therefore should also be discounted from a local estimate of sediment carbon stocks and fluxes in the absence of habitat. This last reflection provides indication of the complexity of the estimates when it comes to accommodate the premise of additionality. Fortunately, δ^{13}C and δ^1N isotopic signals can help us to partially discriminate which proportion of buried C$_{org}$ comes from seagrass production and which comes from other habitats (plankton, terrestrial, algae), despite methodological and conceptual limitations (Papadimitriou et al., 2005). Sediment C and N isotopic signatures are being analyzed and will hopefully help us to estimate the fraction of C$_{org}$ sequestration in sand patches that we could expect without meadow presence.

3.4. **Conservation status, meadow dynamics, and dead vs living mat**

So far, we have examined regional variability of seagrass blue carbon stocks and fluxes, as well as among species, with depth, and sediment substrate characteristics. We have also compared seagrass sediments carbon stocks with those of adjacent bare sediments, and we have established a quantitative model to predict carbon stocks of the meadow canopies, from meadow shoot density, a variable widely used in *P. oceanica* surveys. We have also

established another quantitative model to estimate short-term sediment accretion rate (determinant to TOC flux into the sediment) from vertical rhizome growth. All this information is useful to elaborate a blue carbon inventory of Andalusian seagrass meadows.

But in order to calculate the eventual additionality of a climate change mitigation project based in meadow conservation or restoration actions, we also need to know how seagrass carbon stocks and fluxes do change with meadow decline and recovery. This will allow us to estimate the potential net carbon sequestration that a particular project may produce, with respect to leaving "business as usual". This is a very difficult task we usually do not know with precision when a particular meadow started to decline or to recover. Information collected in action A1, combined with a close-up examination of temporal and vertical changes along cores have helped us to obtain some insight in this subject.

3.4.1. *Effects of Villaricos meadow decline in sediment TOC stock and flux:*

The Villaricos station DE.I, situated at 14.8 m depth, is in an area affected by the Deretil factory effluents, which was installed 1961, as explained in section 2.1.1.a. Plant cover and biomass was low, and so was Canopy TOC stock (Table 1, Annex 1). When compared with TOC stock found in Aguamarga and Terreros between 10 and 18 meters depth (average of AG.I. TE.I, AGD. And TE.D; Annex I),TOC stock$_{canopy}$ was 74% lower, while the sediment TOC stock$_{1m}$ was 55% lower, than in the nearby healthy meadows at the same depth range.

A close examination of the sediment parameters profiles, showed a significant reduction of sediment bulk density, in the upper 80 cm (1000 years BP; R^2= 0.76, p< 10^{-4}; Fig. 3.16a), at a rate of -0.0100$\pm$0.0008 g cm^{-3} per cm, towards the top, with no qualitative changes in mineral grain size distribution (Fig. 3.3), nor changes in the age model, associated to such change (Annex II). The TOC density of the upper 80 cm (median TOC dens = 0.0085 gTOC cm^{-3}) was significantly lower than in the rest of the core (Median TOC dens = 0.010 gTOC cm^{-3} ; Mann-Witney test; p< 0.02), which produced the relatively low TOC stock$_{1m}$ of this meadow.

The core age model situated year 1961, the year of the factory installation, at cm 45, and showed a significant decline in carbon flux since year 1980, at a mean rate of -0.024$\pm$0.01 tCO$_2$ ha^{-1} yr^{-1} each year (R^2= 0.42; p< 0.05; Fig. 3.16b). This suggests that the factory effluents would have started to impact meadow carbon flux in the 80's. Moreno et al. (2001)reported

the decimation of the seagrass meadow from 0 to 10 meters depth, in a 2 km coastline stretch. DE.I station is beyond this area, but the meadow appearance is that of a tall mat reef with very low plant cover (Fig. 2.4). Also, biomass and sediment parameters show that in 2016, the
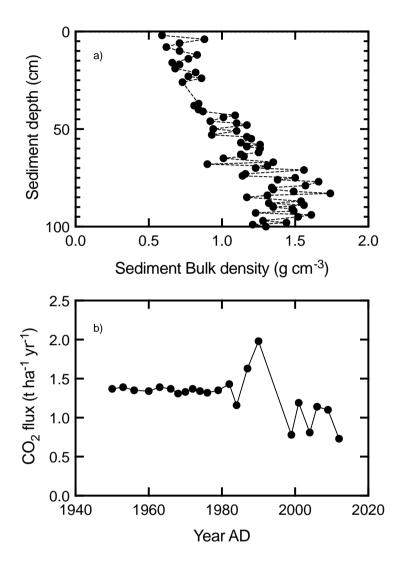
Fig. 3.16. a) Decline trend of sediment Bulk density in the upper 80 cm of Villaricos matte. b) reduction of TOC flux since 1980 in Villaricos declining meadow.

Villaricos meadow at this depth was also heavily impacted. Moreover, the TOC density reduction measured in the top 80 cm, suggests that part of the carbon stock accumulated in the last 1000 years has been affected by meadow loss: 44.4 t CO_2 ha^{-1} are likely to have been lost since 1980, representing an average emissions rate of around 1.22 t CO_2 ha^{-1} yr^{-1}.

Preliminary analyses of FTIR (Fourier Transform InfraRed spectroscopy) data on sediment matter along the Villaricos core indicate that they are impoverished in fresh organic matter (polysacharids), with respect to cores from healthy meadows like AG.I and TE.I (Carmen Leiva, data not shown), this would suggest that organic matter mineralization processes within the mat of Villaricos would be faster, which is consistent with the lower carbon stock observed.

The decline pattern in sediment bulk density in the upper 80 cm towards the mat surface is not observed in healthy meadows (eg. TE.D, Fig. 3.2). Interestingly, it partially coincides with the height of the mat above the sediment (around 1m). This suggests a possible mechanism for loss of the mat once the meadow dies and is not buried: current erosive forces and/or enhanced organic matter mineralization would result in the reduction of sediment bulk density, from the top, downwards, and this would make it more susceptible to erosion by hydrodynamism. This is a conceptual model based on our observation at Villaricos-Deretil, and would need further testing.

An eventual project of water and sediment quality restoration and fixation of the weakened mat by re-vegetation, could generate carbon credits from reversal of the net emissions to net sequestration, and from protection of the remaining stock,

3.4.2. *Recent dead mat and reduced Corg flux in the Roquetas barrier reef*

The shallow (1 m depth) meadow of Roquetas seems in decline due to intermittent sewage and freshwater inputs from a nearby wad (CMAOT, 2018), since the Roquetas sewage treatment plant construction in 1969. Part of the barrier reef is dead since around 2005 (Diego Moreno, *pers. Comm.* RO.S-C station). The living part of the meadow is apparently healthy, with high canopy biomass (Annex I, RO.S station).

The TOC density profiles of live and dead mat are very similar in their coinciding lengths (Fig. 3.17). Only in the top 3 cm, the TOC density of the dead mat is lower than the TOC density of the living meadow (Fig. 3.16, detail). Consequently, the TOC stock$_{1m}$ of both stations are not significantly different. The dead barrier reef was cored 3 meters deep (2496 yr BP - that is year 541 BC) in the sediment, and TOC density and *P. oceanica* organic debris did not decline. Therefore, we can expect the TOC stock to continue deeper, at least 1 meter more (unless it hits a rocky bottom).

From the 3.2 meters cored, we have estimated stock of the barrier reef of Roquetas the Mar in 2685.2± 275.1 (SE) tCO_2 ha^{-1}. Two meters of this reef blue carbon stock (1846.3 ± 189.2 (SE) t CO_2 ha^{-1}), accumulated since 1829 yr BP (year 126 AD), emerges above the sediment,
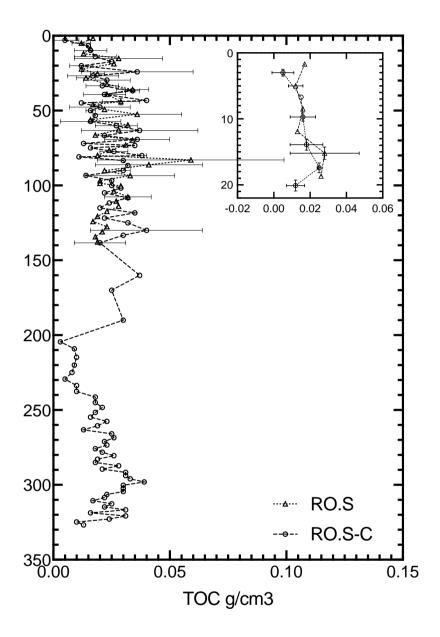
Fig. 3.17 Vertical profile of TOC density (g cm^{-3}) along the RO.S-C recent dead matte (open circles) and the RO.S live matte (open triangles) consensus cores. A detail of the upper 20 cm is shown in the upper-right corner, showing that the only difference in TOC content is in the top 3cm, probably due to the absence of live belowground *P. oceanica* organs.

at a very shallow depth. The trimmed edge of this dead mat reef indicates that it is suffering an erosive process. Therefore, this dead mat barrier reef is a potential source of significant amounts of CO_2 and it is highly recommended to restore and re-vegetate it, in order to maintain

that ancient carbon stock in the soil. Analysis of successive satellite imagery since 2005, and/or the installation of milestones around 5 meters inside the dead barrier reef border, would allow to measure if and at what rate erosion is taking place.

As for TOC flux, despite ^{210}Pb profile in the upper RO.S-C sections do not reveal sediment erosion of the top dead mat, we could not estimate its present CO_2 flux, as the organic material of the mat consisted in *P. oceanica* rhizome and root debris, and therefore, produced before meadow death.

The CO_2 flux$_{100yr}$ of the living mat (RO.S) showed a significant reduction after 1969, the year when the intermittent inputs of fresh partially treated sewage waters started, of $1.23 \pm 0.23(SE)$ t CO_2 ha^{-1} yr^{-1} (Fig. 3.18). Therefore, we estimate that since 1969, the sewage pressure has prevented the remaining part of the barrier reef meadow to bury $58 \pm 11(SE)$ t CO_2 ha^{-1}.

All these observations indicate that the shallow Roquetas barrier reef is in decline. The
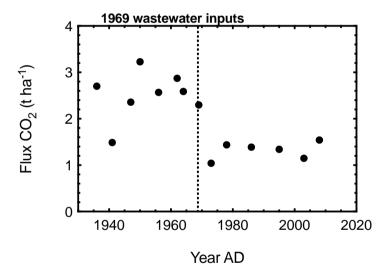
Fig. 3.18. Carbon flux of RO.S station in the last decades.

POSIMED station at 10 m depth and around 500 meters away, which shows a significant decline in shoot density and an increase in dead mat cover (Fig. 2.6), indicates that the meadow decline is extensive.

3.4.3. *Effects of illegal trawling / dredging on TOC stocks of the deep Roquetas meadow*

The intermediate-deep meadow of Roquetas has suffered intensive illegal trawling and dredging, especially during the nineties (Diego Moreno, *Pers. Com.*). This pressure has been reduced since the first decade of 2000, thanks to the deployment of protection barrier reefs.

At both sides of the selected trawl mark (RO,I-E and RO,I-R stations) there was a slope around 50 cm high, in which *P. oceanica* was also dead. The sediment was partially colonized by the alga *Caulerpa racemosa* and by *C. nodosa* (RO.I-B station). The control live meadow RO.I was located around 100 meters away from this station.

The average 1-m sediment C_{org} stock of RO.I was of $1042 \pm 664(SE)$ t CO_2 ha^{-1}. In addition, we estimated the Canopy biomass Corg stock in $1.3 \pm 0.3(SE)$ t CO_2 ha^{-1}. Therefore, the meadow C_{org} stock$_{1m}$ in RO.I was $1043 \pm 665(SE)$ t CO_2 ha^{-1}.
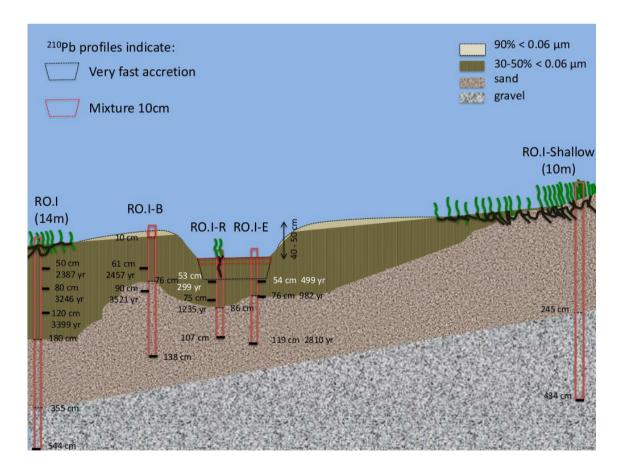
Fig. 3.19. Diagram of the sampling desing in deep Roquetas station

The ^{210}Pb profile in the first 36 cm of core RO.IM60 revealed a stable and moderate accretion rate during the last century, of 0.15 to 0.18 cm yr^{-1}, depending on the model applied. [210]Pb stabilized at 17 cm down the sediment. Grain size distribution analysis revealed that the sediment contained 20-42% of mud (Annex II).

The ^{210}Pb profile of RO.I-B revealed mixing in the top 16 cm sediment. The mud content was very high (around 90%) in the top 7cm, and then decreased sharply to 23-45%. The ^{210}Pb profile did not stabilize until cm 35-37. These patterns indicate that siltation has taken place and added 22 to 25 cm of sediment very fast, which could be the cause of *P. oceanica* disappearance in the border of the trawling mark, a phenomenon already described for other meadows impacted by bottom-trawling, like in El Campello (Sánchez-Lizaso et al., 1990). In addition, ^{14}C dating of RO.I and RO.I-B, yielded ages of 3246 $\pm$ 32 yr BP and 3521 $\pm$ 31 yr BP at 80 and 76 cm, respectively. This unevenness of 11 cm suggests that the edge of the trawl mark area, may have also suffered an erosion of 33 cm and a posterior partial refilling of 22 cm.

Ignoring the possible sediment movements, and only taking into account the C_{org} contained in the first meter sediment thickness, the average stock at the dead mat edge (RO.I-B) for the top was of 608 $\pm$ 379(SE) t CO_2 ha^{-1}), the 58% of the C_{org} stock$_{1m}$ in RO.I (control/reference site).

If we consider that trawling has only produced siltation in the trawled edge, we have to measure the C_{org} stock contained in the top 122 cm of sediment: the 22 cm added, which probably come from sedimentation of part of the materials re-suspended during the trawling event (Sánchez-Lizaso et al., 1990), plus the supposedly intact 1m dead mat buried below. The top 22 cm of sediment contained a C_{org} stock of 64 $\pm$ 43 t CO_2 ha^{-1}, and the 100 cm of sediment below contained 695 $\pm$ 398 t CO_2 ha^{-1}. In total, this supposes a new C_{org} stock of 759 t CO_2 ha^{-1}, the 73% of the inferred original C_{org} stock.

Finally, if we consider that both, siltation and erosion, processes have occurred, we have to discount the net loss of 11 cm, and sum up only the carbon stock of the first 89 cm, that is, 518 $\pm$ 323(SD) t CO_2 ha^{-1}, 50% of the original 1 m C_{org} stock.

Therefore, the trawling/dredging impact on the meadow would have produced the direct emission of 27% to 50% of the 1 m meadow C_{org} stock in the borders of the trawling area, that is, between 282 $\pm$ 179(SE) and 521 $\pm$ 332(SE) t CO_2 ha^{-1}.

As for the carbon stock in the trawling/dregding paths (station RO.I-E), the edge slope indicated that, at least, 50 cm of sediment had been removed and not refilled. The ^{210}Pb and grain size distribution profiles of RO.I-E and RO.I-R cores (Annex II), indicate that erosion may have gone deeper, and then been partially refilled with 47 to 69 cm of fine-grained sediments (58 cm on average). So, to estimate the impact on carbon stock in the trawling/dregding mark, we counted a net emission of the top 108 cm (50 cm of the present slpe + the 58 cm recently refilled sediments) of the RO.I C_{org} stock, plus its canopy biomass, and then discount the C_{org} stock of the top 58 cm from RO.I-E. We estimate that the erosive activity of trawling/dregding removed $1102 \pm 702(SD)$ tCO_2 ha^{-1}, accumulated in the last 4382 ± 31 years BP, and the subsequent siltation process would have buried 303 tCO_2 ha^{-1}, of unknown residence time. This yields a negative balance of $799 \pm 702(SD)$ tCO_2 ha^{-1} removed, and potentially emitted to the atmosphere (Fig. 3.20a).
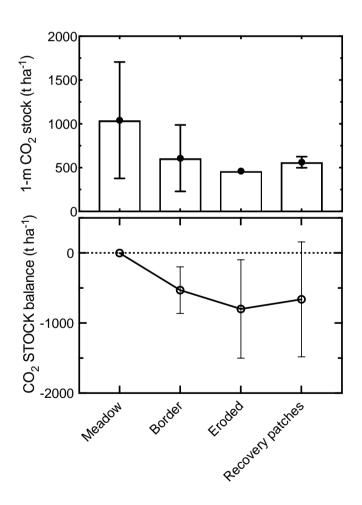

Fig. 3.20. a) differences on stocks among the deep roquetas meadow stations; b) C=2 released after degradation.

82

RO.I-R ^{210}Pb profiles showed intensive sediment accretion rates, greater than in RO.I-B, as the core ^{210}Pb did not stabilize until reaching 69 cm depth. The sediment accretion rate was between 0.5 and 2.5 cm yr^{-1} (Annex II). The vertical rhizome growth registered within this station between 2004 and 2017 was also large to cope with it (1.5 cm yr^{-1} ; Figs. 3.7 and 3.9, Fig. 3.19a).

Despite the small plant patch sizes in RO.I_R, their sediments were slightly enriched in organic matter with respect to RO.I-E core (TOC densities 0.013 ± 0.001 and 0.017 ± 0.002 g cm^{-3}; $p < 0.05$).

Even taking into account the greater sediment accretion (which added 15 cm of sediment thickness to the RO.I-R stock accumulated since the erosive event), the carbon balance is still negative: 662 ± 820 t CO_2 ha^{-1} emitted to the atmosphere (Fig. 3.19a).

In order to obtain the total carbon balance of the trawling meadow degradation, we would need to compare present carbon fluxes in the control and impacted areas. Unfortunately, we do not have these kind of data. Our carbon flux estimates are based on reconstructive methods, and besides, we do not have data for the RO.I station.

3.4.4. *Meadows in re-colonization*

We have studied two meadow areas showing recolonization dynamics, both in the trawled area of Roquetas.

The first meadow was already considered in the initial sampling design: RO.I-R consisted in 3 re-colonization patches within the trawling/dredging path (RO.I-E). The seagrass patches were apparently survivors from the trawling erosion, because they had deep-long vertical shoots sprouting from the sediment. Details about this station have been given in the previous section. Here we would just like to outline that the C_{org} stock$_{1m}$ of RO.I-R was 122% greater than in RO.I-E (Fig 3.20a), while C_{org} flux was between 6 and 7 times greater in RO.I-R (4.59 ± 2.41 t CO_2 ha^{-1} yr^{-1}) than in RO.I-E (0.71 ± 0.01 t CO_2 ha^{-1} yr^{-1}, Fig. 3.10, Annex II).

The manual core RO.IM60 was initially collected as part of the RO.I station, at the same depth and in the nearby of RO.I-E. The meadow consisted in coalescing patches, with many rhizome runners, and no long vertical shoots were found. When subsampled and analyzed, we

detected that this core was different to the other two cores from RO.I station: only the upper 5 cm sediment contained significant amount of organic matter to the point that bulk sediment ^{14}C dating did not yield any result. However, the ^{210}Pb profile in the upper 25 cm sediment gave a smooth, monotonous and robust accretion rate. Given that this Roquetas meadow has been trawled extensively, we interpreted that it also was a re-colonization area, but in a more advanced stage (patch coalescence). The narrow upper horizon of organic sediment, together with ^{210}Pb allowed us to estimate carbon flux for this station in 5.19 t CO_2 ha^{-1} yr^{-1}.

Therefore, we can see that meadow re-vegetative projects may obtain carbon credits by enhancing carbon sequestration.

3.4.5. *Erosion of a C. nodosa meadow at El Alquian*

The sandy patch (BA.S-C) adjacent to a *C. nodosa* meadow reef in El Alquian (BA.S) was 30-40 cm lower than the reef was sampled as control station (no habitat type). But when opening the core, we discovered that the layer of sand in BA.S-C was very thin and, below it, there was a dead mat of *C. nodosa*. The TOC stocks for the top meter of sediment were similar. Nevertheless, while the ^{210}Pb profile of BA.S showed a cumulative profile, that of BA.S-C indicated an erosive dynamics. The coastline of El Alquian is also suffering erosion, and recently has been the object of coastal works to stop it. This suggests that the erosion dynamics in the *C. nodosa* meadow at BA.S-C may be part of a wider process in the area. 30 to 40 cm of *C. nodosa* mat would have been eroded, liberating around 32 ± 6.4(SE) tCO_2 ha^{-1}.

3.4.6. *TOC stock in the ancient dead mat of Calaburras*

The area of dead mat in Calaburras seems to be relatively recent. The mat is reported to have been dead during many decades, at least since the 70's (Jose Miguel Remón, Pers. Comm.). Before dying, the meadow may have started to grow, according to CA.S-C ^{14}C dating, 2 centuries ago, around the French Revolution year.

The bulk density profiles of five of the six cores collected, showed consistent decline trends towards the top, starting between 80 and 40 cm, depending on the station and the core (Annex II). This pattern is also present in the severely degraded meadow of Villaricos (section 3.4.1),

and contrasts with those observed in the mats of healthy meadows, which do not show any consistent trend in their bulk density vertical profile (Annex II).

The TOC density profile of CA.S-C also showed a significant decline towards the surface, starting above cm 34 (year 1972), at an average rate of -0.18 $\pm$ 0.06 t CO_2 ha^{-1} yr^{-1} (R^2= 0.47; p< 0.02; Fig. 3.21a).

The TOC density profile of CA.S-Cn also showed a significant decline towards the dead mat surface, starting above cm 66 (year 1931), at an average rate of -0.10 $\pm$ 0.03 t CO_2 ha^{-1} yr^{-1} (R^2= 0.70; p< 0.04; Fig. 3.21b).

Such TOC density declines toward the top dead mat could indicate the onset of significant meadow decline in the past, which would have affected carbon burial rate, before the total loss of the plant. It also could correspond to a vertical profile of a mat undergoing erosion. The ^{210}Pb profiles in the upper 30 cm indicate that the dead mat is currently not under erosion, though. The present dense *C. nodosa* and/or algal turf cover may be protecting the remaining dead mat from erosion. The decline in TOC density since the 1972 in CA.S-C, would have reduced its TOC stock$_{1m}$ in 40%. As for CA.S-Cn, TOC density decline, which would have
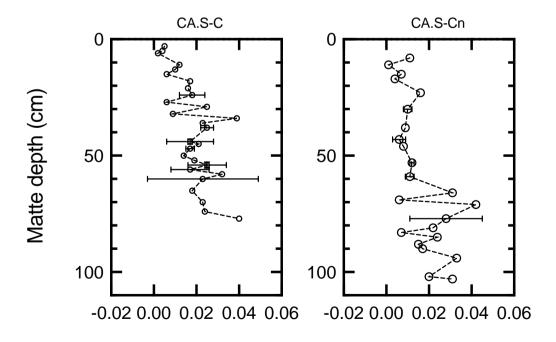

Fig. 3.21.TOC density profile on Calaburras station.

started in 1931, would have reduced its TOC stock$_{1m}$ only a 21%. This could indicate that *C. nodosa* could be more efficient in protecting the remaining dead mat TOC stock from erosion, but many uncertainties arise in this exercise, and further studies would be necessary to be able to disentangle TOC stock erosion from past flux decline.

CA.S-C and CA.S-Cn had not significantly different TOC densities between each other (un-paired *t*-test, $p > 0.08$). The sediment TOC density of the two stations combined (0.017 ± 0.001(SE) g TOC cm^{-3}) was significantly lower than the sediment TOC density of the Roquetas recent dead mat (0.023 ± 0.002(SE) g TOC cm^{-3}). This yielded significantly lower TOC stocks$_{1m}$ (189 ± 22(SE) t TOC ha^{-1}) (RO.S-C, 226 ± 24(SE) t TOC ha^{-1}). This slight difference

Table 3.4. Estimates used for the model of TOC stock decline with time since loss of seagrass cover

Estimate of stock difference:	Estimate of year	Years	% TOC stock$_{1m \text{ left}}$
RO.S-C vs RO.S	Local expert knowledge	11	*0.91*
RO.I-B vs RO.I	Local expert knowledge	27	*0.67*
DE.I analysis of the vertical profile	TOC flux decline onset	36	*0.49*

(translated in tones of CO$_2$: 135 to 212 t CO$_2$ ha^{-1}) could also be derived from geographic differences rather than by the loss of part of the dead mat TOC stock. Nevertheless, the bulk and TOC density decline trends towards the top suggests that there has been a partial loss of the TOC stock over the last decades.

3.4.7. *A tentative, simple model of TOC stock loss in dead mat without erosion*

We used the estimated change in TOC stock$_{1m}$ with the total or virtual loss of seagrass cover, together with the known or estimated years passed since that loss (Table 3.4). We did not add Calaburras, for which too many uncertainties remained about the time of meadow death, but we used Villaricos, for which we have a narrower period for the onset of the human pressure on the meadow, despite not having a control station to compare TOC stocks.

We fitted a linear decline model ($R^2 = 0.98$; $p < 0.02$; N = 4 points Fig. 3.22):
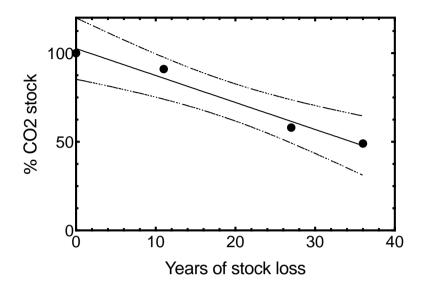
Fig. 3.22. linear model of TOC loss.

% TOC remaining = -1.42 ($\pm$ 0.15SE) · T(years since vegetation cover loss) + 103.5 ($\pm$ 3.1SE)

This simple and well correlated model relies in very few points and would therefore need more data to increase its robustness, accuracy and resolution.

POSIMED-Andalucia data shows that, globally, *Posidonia* meadows have remained stable between 2012 and 2018 (Deliverable A1). The landscape plant and dead mat cover have not significantly changed with time. The small scale dead mat cover has significantly increased (1.3 $\pm$ 0.2(SE)% per year, R^2= 0.03 p< 10^{-4}), but so has also done the small scale plant cover (2.6 $\pm$ 0.4(SE)% per year, p< 10^{-4}). Therefore, the global inventory of Andalusian seagrass blue carbon stock, can be expected to be globally stable for the moment. Nevertheless, the vertical rhizome height above the sediment has significantly increased, at a global average rate of +0.17 $\pm$ 0.03(SE) cm per year (R^2= 0.01 p< 10^{-4} ; Fig. 3.22; regression of global data). This indicates a global temporal trend of decline of sediment inputs, which would be expected to concomittantly reduce global carbon fluxes of Andalusian seagrasses, both by receiving less inorganic and organic sedimentary inputs, and by reducing its own vertical accretion of belowground organs, the main source of carbon inputs in this species (Papadimitriou et al., 2005), as we have demonstrated that sediment accretion rate is positively correlated with vertical shoot growth (Fig. 3.9).

On the other hand, the POSIMED seagrass monitoring has detected some meadows with a significant decline in shoot density and cover, like Roquetas, Villaricos, or Cala Cocedores, or a significant increase in landscape dead mat cover in some apparently healthy meadows, like Terreros, Aguamarga or Punta Entinas (Figs. 2.2, 2.5 and 2.7). These meadows have a greater potential to generate carbon credits from seagrass mitigation projects, as their carbon stocks and fluxes may be in decline, as it has been shown here for Roquetas and Villaricos.
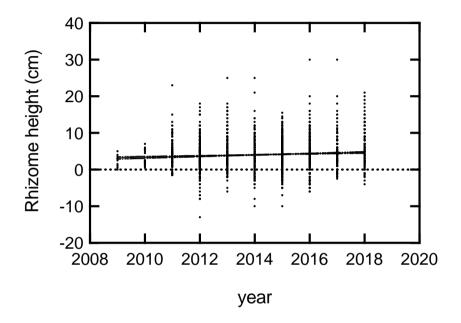
Fig. 3.23. Evolution of rhizome elongation

3.4.8. *Advancing the upscaling of the estimates: from areal to regional*

The up-scaling of the areal results to global estimates is not a primary goal for this report but the new results advanced here, together with new information from A1 action, has allowed us to provide a series of recommendations to up-scale and chart Andalusian seagrass carbon stocks and fluxes. Such recommendations are exposed in Annex 3 to this report. Here we just update the global figures advanced in Deliverable C1.1, with the definitive areal estimates of carbon stocks and fluxes (Table 3.5).

Table 3.5: CO$_2$ stocks and fluxes per depth and area for each Type of bottom

Type of bottom	Depth (m)	Area (ha)	tCO2/ha	se	tCO2/ ha yr	se	ktCO2/ Andal.	ktCO2/ Andal. Yr
P. oceanica stable	<5	183.2	2077	1227.3	1.6	0.6	380.5	0.3
	5-10	706.2	2030.9	1249.9	1.7	0.6	1434.2	1.2
	10-15	841.1	840.9	227.5	1.2	0.4	707.3	1
	15-20	658.2	2003.9	1115.8	1.3	0.5	1319	0.9
	>20	665.3	2003.9	1115.8	1.3	0.5	1333.2	0.9
C. nodosa stable (open sea)	<5	48.4	100.9	5.8	0.5	0.2	4.9	0
	5-10	897.6	100.9	5.8	0.5	0.2	90.5	0.4
	10-15	1560.4	100.9	5.8	0.5	0.2	157.4	0.7
	15-20	989.6	100.9	5.8	0.5	0.2	99.8	0.5
	>20	356.2	100.9	5.8	0.5	0.2	35.9	0.2
C. nodosa semi-enclosed	0.1	249.6	243	-	0.2	-	60.6	0.1
C. nodosa and *Z. noltei*	<5	0.3	93.8	32.8	0.1	-	0	0
P. oceanica regression	<5	2.2	1204.7	711.8	1.3	0.4	2.7	0
	5-10	48.1	1177.9	724.9	1.3	0.4	56.7	0.1
	10-15	170	487.7	132	0.9	0.3	82.9	0.2
	15-20	61	1162.3	647.2	1.7	0.4	70.9	0.1
	>20	287.7	1162.3	647.2	1.7	0.4	334.4	0.5
P. oceanica and *C. nodosa*	<5	70.7	2077	1227.3	1.6	0.6	146.8	0.1
	5-10	94.2	2030.9	1249.9	1.7	0.6	191.3	0.2
	10-15	410.7	840.9	227.5	1.2	0.4	345.3	0.5
	15-20	280.4	2003.9	1115.8	1.6	0.5	561.9	0.5
	>20	36.3	2003.9	1115.8	1.6	0.5	72.7	0.1
P. oceanica, C. nodosa and *Zostera spp.*	<5	1.7	2077	1227.3	1.6	0.6	3.5	0
	5-10	35.2	2030.9	1249.9	1.7	0.6	71.5	0.1
	10-15	19.5	840.9	227.5	1.2	0.4	16.4	0
P. oceanica and *Caulerpa cylindracea*	10-15	1.4	840.9	227.5	1.2	0.4	1.2	0
	15-20	40.8	2003.9	1115.8	1.6	0.5	81.8	0.1
P. oceanica mixed bottom	5-10	7.9	2030.9	1249.9	1.7	0.6	16	0
	10-15	21.1	840.9	227.5	1.2	0.4	17.7	0
	15-20	26.8	2003.9	1115.8	1.6	0.5	53.7	0
	>20	1	2003.9	1115.8	1.6	0.5	2	0
P. oceanica dead mat	<5	3.8	1537	947.5	0	0.4	5.8	0
	5-10	15.6	1502.9	957.7	0	0.4	23.4	0
	10-15	51.2	622.2	226	0	0.3	31.9	0

89

	15-20	24.6	1482.9	1115.8	0	0.4	36.5	0
	>20	29	1482.9	1115.8	0	0.4	43	0
P. oceanica on rock	<5 to >20	2299.2	395.4	-	0.6	-	909	1.3
C. nodosa on dead mat	<5	0.1	551	-	0.5	0.2	0.1	0
Z. noltei stable (semi-enclosed)	<5	473.7	93.8	32.8	0.1	-	44.4	0
Zostera marina stable	<5	0.1	93.8	32.8	0.1	-	0	0
	10-15	1.2	93.8	32.8	0.1		0.1	0
Total / Mean		11671.3	1133.6	658.3	1	0.4	8847.2	9.8

Once the tCO_2 stocks and sequestration rates per unit surface have been calculated for the various meadow typologies, the next step is assigning to each one of those typologies the area they represent in the Andalusian coasts. These kind of estimates are key as they set reference values for the natural resource/ecological service under study, that are the basis for establishing management plans or for assigning them an instrumental value. In this case, the value of a ton of CO_2 is well defined in the regular carbon markets (EU Emissions Trading System - EU ETS; Fig. 3.24) or in the voluntary markets (Ullman et al., 2013).

As an example, if a rate of meadow loss has been determined, then the global economic loss in terms of CO_2 not captured or emitted can also be estimated. Reciprocally, if a compensation project reduces that rate or stops it, the stocks and sequestration capacity that have been saved could be certified as carbon credits and the potential global benefit of conservation actions could be estimated (e.g., Ullman et al., 2013).

The task of upscaling discrete field observations to a large area of ecosystem is hampered by a number of challenges. On the one hand, delimiting the total area the values from a core (or 3 replicates) are representative of, is particularly complicated. The simple fact that seagrass meadows are largely subtidal, makes it impractical the use of aerial imagery techniques. So if even determining the presence of the seagrass is already complicated, characterizing limits or transitions between typologies is almost impossible. Only direct expert observations (SCUBA) can provide reliable information. Owing to the slow and costly nature of such observations, they are always very limited.

Fortunately, the large experience and knowledge accumulated by the AMAyA team through the various projects they have led on Andalusian seagrasses, and the monitoring programs funded by the Andalusian Autonomous Government, helped to significantly overcome this limitation. This expertise has led to the elaboration of a series of GIS layers compiling and integrating all the cartographic information on sea bottoms available for the Andalusian coasts

(see Action A1 deliverables). This integration of the mapping efforts yields a total area occupied by seagrass meadows of 11,539.9 ha (not including dead mat areas) and distinguishes all 4 seagrass species growing on different sea bottoms and in the 3 status of conservation: normal, in regression, and dead mat (Table 3.3).

The categories presented on table 3.3 have been stablished using the information obtained by both action A1 and C1 and the information from AMAyA.

Data on *P. oceanica* stable was obtained from those stations with good developed healthy meadows. For *C. nodosa* meadows on open sea we combine three available stations, on semi-enclosed areas the station from Santibañez bay was used as reference. For mixed meadows of *C. nodosa* and *Z. noltei* data was obtained from Santibañez bay stations. In mixed meadows of *P. oceanica* and small seagrasses the values from healthy *P. oceanica* meadows were used as those were the closed data available according to personal observations and the literature, as well as for *P. oceanica* stablished above mixed bottom. *C. nodosa* over dead mat was calculated with data from the reference station of Calaburras. All *Zostera spp.* categories were calculated using Santibañez bay stations. For those areas where *P. oceanica* meadows is in regression, the stations we obtain over degraded meadows were used to compare differences in stock and fluxes with the healthy ones and the correction factor obtained was applied to the values on the healthy meadow category to obtain the final values of meadow in regression. A similar approach was followed to calculate the values for *P. oceanica* dead mat category. *P. oceanica* on rock was assumed to be half the stock of the nearest stable P ocenica meadow, as mat depth was approximately of 50 cm (personal observation, AMAyA).

The classification above described was followed for stocks encompassing the top meter of soil. For average fluxes from the last 100 years, the same classification was used but for 3 cases: fluxes on *P. oceanica* growing on rocky bottom were consider the same fluxes as the nearest station. Fluxes of *C. nodosa* over dead mat were consider equal as those of *C. nodosa* over sediment. Fluxes on dead mat were consider to be null.

3.4.9. *Distribution, areal stocks and fluxes*

Cymodocea nodosa and *P. oceanica* in stable status occupy similar areas as monospecific meadows, but they can also be found sharing a meadow together, with algae, or with *Zostera spp.* The area occupied by *P. oceanica* in regression or dead (dead mat), adds up 742.9 ha,

representing about 15% of the monospecific stable *P. oceanica* meadows. To be noted are the 939.8 ha occupied by mixed meadows of *P. oceanica* and *C. nodosa.*

The small species *Z. noltei* is fundamentally found in semi-confined environments (bays and saltmarsh channels) adding up 473.7 ha.

It is already known that *Z. marina* is actually a relict species in the Mediterranean (Pergent et al., 2014), clearly reflected by the low area occupied (1.3 ha). The impact of this species in the global stocks and fluxes is therefore negligible.

<u>Stocks</u> - As hypothesized, the largest stocks of carbon were those associated to *P. oceanica*, with an average maximum of 2077 tCO_2/ha recorded for meadows growing above 5 m of depth. This value has been assigned to all those types of bottom at that depth bearing *P. oceanica* (as it is hypothesized that the main stock is that stored by *P. oceanica* irrespectively of the other kind of vegetation or feature on the shared area). The stock held by *P. oceanica* dead mats was 26% lower than in stable living *P. oceanica* meadows, revealing an important feature with key implications for emissions compensation projects (see later). This decrease in the stocks is to be attributed to non-erosive loss of the sink.

Deciding on a value for the stocks accumulated under meadows growing on a rocky substrate, is controversial. The identification of this type of bottom is only possible when rocks are directly visible under the vegetation. Whether there is rock or not under the sediments (and at which depth is it) can only be assessed by probing the bottom manually or using high resolution surface seismic (Lo Iacono et al., 2008; Monnier et al., 2019). This is a gigantic endeavor that was way out of scope of this project. As an approximation, following expert observations, in this report the meadows on rocky bottoms have been assigned ½ of the stock of the meadows growing in soft bottom (i.e., 50 cm; let us keep in mind the stock values in this report are given for the top meter of sediment).

In summary, the sea bottom presenting *P. oceanica* adds up 6,969 ha, most of it (5,353 ha) belonging to monospecific stable *P. oceanica* meadows, and 33% growing on rocky substrate. This result is an important one to take into account when it comes to decide where to plan restoration interventions or to quantify extant stocks susceptible of protection.

As expected, the small seagrasses *C. nodosa* and *Z. nolteii* accumulated 3 times less than *P. oceanica* (i.e., 32% of that by *P. oceanica*). The reason is well known: *P. oceanica* belowground organs represent a massive entry of organic matter 'by default' that becomes rapidly buried in anoxic conditions. Moreover, its molecular composition includes highly recalcitrant carbon fractions (Kaal et al., 2016). This combination of factors leads to an unusually massive burial of carbon. In the small seagrasses most of their belowground

material decomposes within the year. It therefore becomes obvious that when planning restoration or protection plans with seagrass meadows, the choice should *P. oceanica* (see later).

Fluxes - Again, the large *P. oceanica* presented much higher average carbon sequestration rates than the smaller ones (1.6 vs 0.1 tCO_2/ha yr; 3 times higher; 31.9 % of the large seagrass).

In *P. oceanica*, maximum fluxes were recorded at shallow depths (<5m), what is consistent with a preference by the species of well illuminated areas with a moderate hydrodynamism (Infantes et al., 2009).

Posidonia oceanica dead mat 'historical' sequestration rates were 4 times lower than stable *P. oceanica* meadows. Strictly, a dead mat does not accumulate carbon, it simply represents a stock and a 'slow' source of CO_2. The fact that the calculated rates are clearly lower, may be indicating that the faster sequestration rates of the overlying layers of mat are not computing in the calculation due to the decomposition of the remains of the plant (living plant is accounted for in flux calculations). A lower illumination in deeper areas of the meadow would explain the decline in the sequestration rates of *P. oceanica* from shallow to deeper areas (1.6 to 1.3 tCO_2/ha yr; 1.2 times higher in shallow areas).

3.4.10. *Global stocks and sequestration rates for Andalusia*

The upscaling for the entire Andalusia reveals valuable information. As a combination of abundance, stocks and fluxes, the largest stocks of *P. oceanica* occur shallow areas of the meadow (<5 m), adding up 13,1 $MtCO_2$. A faster sediment accretion area results in a 'dilution effect' of the carbon stock in a thicker portion of mat, resulting in an overall lower carbon density. The smaller seagrasses contribution to that total stock is only 493.9 $ktCO_2$, what is to

Table 3.6. Areal stocks and fluxes determined for some special types of *P. oceanica* meadow.

Special types	Depth (m)	Area (ha)	tCO2/ha	SE	tCO2/ha.yr	SE	ktCO2/Andal.	tCO2/ Andal.yr
P. oceanica recolonization	14.5	n/a	187.4	18.4	0.17	0.03	-	-
P. oceanica siltation trawling	14	n/a	183.5	47.8	0.02	0.01	-	-
P. oceanica eroded trawling	14.5	n/a	118.6	n/a	0.18	0.02	-	-
P. oceanica degraded chemically Pre-disturbance*	15	0	-	-	0.69**	n/a	-	-
P. oceanica degraded chemically Present *	15	40	143.9	13.9	0.51**	n/a	5756	20.4

say, a 5.58 % of the total. In other words, *C. nodosa* and *Zostera spp.* represent an almost negligible contribution to the Andalusian Blue Carbon.

Global entries to the long-term sink in Andalusian seagrass meadows were higher at shallower areas in both of the dominant species. In *P. oceanica*, the global carbon sequestration rates were found to be around 1.2 times higher at shallow-intermediate than at the deeper areas of the meadow (4.8 vs 1 $ktCO_2$/yr), as a consequence of the also higher overall productivity and sedimentation rates of the system in that areas. The value for *P. oceanica* growing on rock stands out (Table 3.1). As explained somewhere else, a 50 cm-thick mat has been assigned to this type of bottom. It has been considered that this assumption is conservative because it may account for many patches of *P. oceanica* growing in rocky areas that do present a well-developed mat underneath and for the small mat of variable thickness observed for patches growing directly on rock.

The contribution of the small Andalusian seagrasses to the total flux of organic carbon the long-term sink was about a 20 % of the total, due to the large extent of their meadows.

3.5. **Implications of these results for carbon offsetting projects and other project actions**

LIFE Blue Natura is an Andalusian Blue Carbon 'accounting effort' pursuing the implementation of BC initiatives in the carbon markets in the form of conservation (emissions avoided) and restoration (reforestation) projects. In the recent agreements reached in Paris, the Parties committed to 'conserve and enhance, as appropriate, sinks and reservoirs of greenhouse gasses'. The article refers to another specific article (4.1, d) of the UNFCCC that a list, referring to the natural carbon sinks 'biomass, forests, and oceans, as well as other terrestrial, coastal and marine ecosystems'.

The reason why seagrass meadows, saltmarshes and mangroves exhibit a notorious incomplete status at the national inventories is fundamentally the lack of i) sufficiently accurate and extensive quantifications of the carbon stocks and fluxes and ii) the lack of a clear path to monetization and accounting rules.

3.5.1. *Regenerating or increasing carbon fluxes and stocks*

Apart from works to conserve and restore the meadows that have proven to be impacted by human pressures or in decline, another strategy to restore or improve seagrass carbon fluxes,

would be to take measures to restore terrestrial sedimentary inputs to the coastal system and correct human altered hydrodynamics. This would in addition allow blue carbon sink habitats to cope with sea-level rise, enhancing their coastal protection ecosystem service (Duarte et al., 2013; Gattuso et al., 2018; Spencer et al., 2016).

The results shown here indicate potential meadows were seagrass restoration projects may produce a significant amount of carbon credits, like Villaricos or Roquetas. The areas where higher carbon burial rates take place are shallow *P. oceanica* meadows. Projects sustainably increasing sediment carbon fluxes and stocks through replanting or reducing coastal erosion, for example, would generate more carbon credits in shallow meadows.

On the other hand, the distribution of large carbon stocks has been proven to be more site dependent than depth dependent. The thickness of the seagrass sedimentary mat is probably the most determinant factor. Acoustic techniques (e.g., multi-beam scan sonar and /or seismic reflection, Monnier et al., 2019) may help to quantify that thickness for a better estimate of the actual size of the carbon sink under the extant meadows.

3.5.2. *Quality estimates: basis for a efficient implementation of Blue Carbon initiatives*

It is therefore pertinent to remind here that the main goal of this study is to provide numbers of stocks and fluxes of organic carbon associated to the sink of Andalusian seagrass meadows to serve as the basis for the elaboration of i) an economical valorization of the Blue Carbon in Andalusia (Deliverable C3 - IUCN), ii) elaboration of the Certification Andalusian Standard for conservation projects of seagrass meadows (Deliverable C4 - CMAOT), iii) elaboration of a manual for the certification of Blue Carbon projects derived from conservation and restoration actions on seagrass meadows (Deliverable C5 - IUCN), iv) dialog and elaboration of carbon compensation projects for the conservation and restoration of seagrass meadows (Deliverable C6 - IUCN), and v) elaboration of a catalogue of conservation projects for Posidonia oceanica (Deliverable C7 - CMAOT).

3.5.3. *A 'big push' by the Andalusian Government*

 In October 8th this year, the Andalusian Parliament approved the Law for Climate Change with unprecedented additions. In its Title V (Emissions mitigation), Chapter 1 (Objectives and measures for the mitigation of emissions), the Article 37, Item 2 says: 'It will be considered as

carbon emissions offset projects, all those dealing with afforestation, reforestation, restoration, and conservation of the extant forests and wooded lands, littoral ecosystems, those dealing with the conservation or restoration of wetlands, seagrass meadows and analogous areas'. This parliamentary initiative opens the door for seagrass meadows to be object for conservation and restoration projects in that autonomous community.

3.5.4. *News from the European Parliament*

The 27th November this year, the European Parliament Intergroup on 'Climate Change, Biodiversity, and Sustainable Development, organized the session 'Blue Carbon in EU Policy'. During this session, the results of LIFE Blue Natura were shown demonstrating that the conservation and restoration of EU Blue Carbon ecosystems could be economically sustainable, given the large stocks they accumulate (7.7 $GtCO_2$/EU BC; Blue Carbon in EU Climate Policy, 27 November 2018). The Members of the Parliament hosting the event acknowledged that, *"The role of Blue Carbon is a key in reducing emissions and supporting climate action, as we are now discussing about implementing the Paris Agreement and the Paris rulebook. According to the MEP, legislation is a driving force and we have the responsibility to move from reflection to action, including Blue carbon in the EU climate agenda. Destroying ecosystems contributes to the release of CO_2 they absorbed for years or centuries. Although the role of blue carbon ecosystems is well recognized by scientists, a gap analysis is needed to identify research and financial needs, as well as to identify priorities and the way to transfer knowledge across sectors. With reference to the latter, the **Life Blue Natura** project serves as an example to examine the missing knowledge, moreover as a pilot to transfer the experience to the wider region. There is a need to improving the dialogue within the EU, to jointly find solutions and share good practices. At the European level, the **Mediterranean region offers plenty of best practices"**.* Maria Spyrakis (MEP).

The conclusions made direct references to the project LIFE Blue Natura, as a example to follow at the EU level, on how to clear the path for implementation of BC initiatives.

3.5.5. *Stock vs sequestration rate*

The results of this project confirm the widely recognized higher importance of seagrass stocks with respect to the sequestration rates. Andalusian seagrass ecosystems have been burying

organic carbon since at least 4.4 thousands of years (maximum carbon age registered within the cores obtained in Life Blue Natura).

Stocks - The values for the stocks correspond to the top meter of soil. Considering that the thickness of the mat under seagrass canopies in the region, i.e., that containing organic carbon, can extend for several meters, the figures above could be several fold higher, so the ones presented can be taken as conservative estimates. As mentioned somewhere else, emission compensation projects can be based on emissions avoidance or on additional carbon capture. In the first instance, a major and hot issue is determining how much of that stock can be lost following habitat degradation or destruction, as this stock would be the one susceptible to be monetized in the markets (see below). Experimenting or modeling this rate of loss is a complex endeavor and completely out of the scope of this project, where implementation actions are the focus. However, the sampling was designed with the idea of providing some guidance numbers. On the one hand, the highly degraded *P. oceanica* meadows (chemical degradation) accumulated from 30 to 18% less organic carbon than the reference ones (Villaricos vs Terreros and shallow Roquetas). Finally, from the area impacted by trawling that was sampled in Roquetas in this study (mechanical degradation), it was directly observed that the erosion of an average of 50 cm of mat resulted in a direct loss of around 234.4 tCO_2/ha.

Flux - The annual amount of CO_2 captured and derived to the long-term carbon sink in seagrasses is not of a high quantitative and, therefore, economical relevance. As shown above, the carbon sequestration annual rate is only of 0.2-1.6 tCO2/ha yr. As a reference, an ongoing restoration and conservation project involving 117 ha of mangroves in Kenya is yielding around 26 tCO_2/ha annually with a total price in the voluntary markets of 12,500 USD (after factoring in a 30% risk buffer). This project does not account for the carbon stored in the sediments, only that stocked in the wood. A simple comparison clearly evidences that the economic potential of restoring seagrass meadows shall rely more on its effect in protecting the carbon stock than in the annual rate of CO2 sequestration.

3.5.6. *Regular and voluntary carbon markets*

In 1992, the Declaration of Rio set out as a prime objective to stabilize GHG atmospheric concentrations under levels dangerous for climate. In its 4.2 article, the 190 signing countries committed to limit their anthropogenic GHG emissions and to protect and enhance their GHG sinks and reservoirs. In 1997, the UNFCCC adopted the Kyoto Protocol, which established an international cap and trade system and regular offset markets, where legally bound entities could compensate their (temporarily irreducible) excess GHG emissions by purchasing other

country's unused emission permissions, or certified carbon emission reductions contemplated by the protocol. In 2005, the first voluntary carbon markets appeared where people, companies, organizations, and governments not legally obliged by the Kyoto protocol could compensate their carbon footprint by purchasing carbon offsets generated by emission reductions not included in the Kyoto Protocol.

Voluntary markets have developed rules and procedures, including official project registries and standard methodologies to estimate measure and verify net carbon emissions reductions generated by projects, and to translate them to carbon offsets. Project developers can then issue and transact these offsets directly to end buyers, who can claim the emissions reductions as their own if they retire and remove the offsets from circulation. Intermediary retailers or brokers can also resell or charge a fee for finding end buyers.

All voluntary standards require that offsets be real, measurable and verifiable by an independent third-party, and prove that those emissions reductions would not occur without those project activities (additionality). Many projects also have additional positive impacts on sustainable economy, known as "co-benefits", (local job training and creation, safeguarding environmental services and biodiversity). In many cases, co-benefits are one of the main reasons that suppliers and many buyers are engaged in voluntary carbon markets, increasing the monetary value of the carbon offsets. In contrast to compliance markets, where offsets typically sell at a relatively consistent price, offset prices on voluntary carbon markets can range widely, from US$ 0.1/tCO$_{2e}$ to just over $ 70/tCO$_{2e}$ ($ 3-$ 6/tCO$_{2e}$; UNFCCC, 2016, Fig. 3.23).
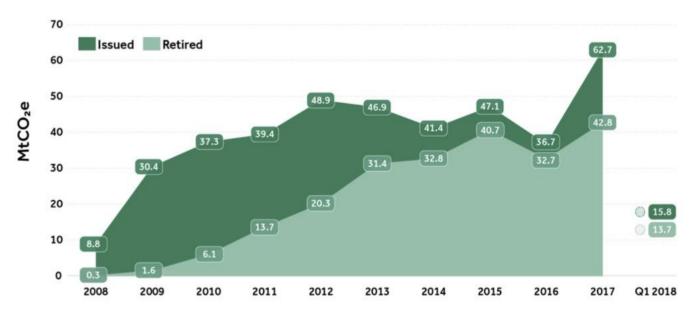

Fig 3.24: MtCO$_2$ credits issued and bought from voluntary carbon markets.

Since trading of voluntary carbon offsets first took off in 2008, voluntary carbon projects have helped to reduce, sequester, or avoid over 437.1 $MtCO_{2e}$. But this is still far away from the total action needed on a global scale. Even with countries' new pledges under the Paris Agreement, at least an 11,000 $MtCO_{2e}$ emissions reduction gap remains to keep the world from warming above two degrees Celsius (UNFCCC, 2016).

It would be easy to dismiss the climate impact of the voluntary carbon markets as insignificant, in comparison with compliance carbon markets like the EU-ETS, which are still un-accessible to most of blue carbon projects. But voluntary markets are growing fast. In the last decade, demand for voluntary carbon offsets has grown by over 140-fold, from just 0.3 $MtCO_{2e}$ in 2008 to 42.8 $MtCO_{2e}$ in 2018 (Fig. 3.24). In 2017, issuances (62.7 $MtCO_{2e}$) and retirements (42.8 $MtCO_{2e}$) reached record-highs. Moreover, offsetting projects based on forestry and land use are a growing source of offset issuance and retirement (59% and 49%, respectively, came from these project types in the first trimester of this year; EMP, 2018).

This uptick in 2017 coincides with the Paris Agreement. The signing countries are implementing more and more domestic carbon pricing schemes (like the Spanish Footprint registry and the Andalusian SACE, including voluntary and compliance schemes). Moreover, the Paris Agreement articles 6.2 and 6.4 establish a unit of emissions reductions (called Internationally Transferable Mitigation Outcomes, ITMOs) that could be traded between countries and propose the creation of a centralized, global mechanism to trade ITMOs, to be supervised by an international governing organization. The mechanism may increase the market actors, including companies from new sectors or individuals to purchase and retire offsets on a voluntary and/or compliance basis, in order to enhance demand and supply of emissions reductions ensure that they occur above what countries have promised.

Also in Paris, countries agreed to collaborate in reduction of emissions occurring across borders (and thus not easily accountable to any country), namely from international aviation and shipping, through two United Nations (UN) sector-specific agencies: International Civil Aviation Organization (ICAO) and International Maritime Organization (IMO). ICAO is opening of the international aviation CORSIA market, the first-ever sector-wide cap-and-trade program, which will start in 2021 on a voluntary basis. CORSIA could increase the carbon offset demand in 1.6-3.7 billion tonnes for emissions reductions from 2021-2035. This would dwarf any other operational or planned market, including the European Union's Emissions Trading Scheme and China's cap-and-trade program. Which standards will be eligible in this market is still to be decided. All EU countries will participate in the CORSIA program.

There are still few blue carbon offset projects (mostly on mangroves), and just one on seagrasses. The Ocean Foundation has developed the carbon offset program Seagrass

Grow!, which will soon adopt the wetlands blue carbon offset methodology developed by Restore America's Estuaries (RAE), which is undergoing its final revisions under the Verified Carbon Standard (VCS). SeaGrass Grow! has developed a Blue Carbon Offset Calculator which allows to calculate annual individual, family, company or organization carbon dioxide emissions, and then calculates the amount of Blue Carbon habitat restoration required to fully offset those emissions (https://www.oceanfdn.org/calculator). They are also undergoing a number of seagrass restoration programs in USA saltmarshes, mangroves and seagrasses. (http://www.gefblueforests.org/tof-blue-carbon-initiatives/)

About compensation projects

There are several factors hampering the development of seagrass offset projects:

Until recently, there were no standard methodologies available for calculating and verifying the carbon credits generated by seagrass restoration (since 2015, there is an VCS methodology approved), and there is still no standard available just for conservation activities

The techniques for seagrass restoration, especially for seagrass planting, are still not well developed, and their success rate still needs to be improved for the slow growing species *P. oceanica*.

The low seagrass carbon fluxes into the sediments make that the potential carbon emission reductions that can be obtained through increasing seagrass carbon fluxes are limited, and the project scale needed for financial feasibility may be too large and not viable. The greatest potential for carbon offset generation would come therefore from avoiding the erosion of their large sedimentary carbon stocks.

In order to increase their financial feasibility, seagrass restoration carbon offset projects should then ensure that the restoration will help avoiding the destruction of the large carbon stocks. Restoring declining meadows with large mats, and large *P. oceanica* dead mats risking erosion should be a priority. Many of these mats are deep and remain uncharted.

Beyond planting - There has been a good number of low-scale transplantation experiences in *P. oceanica* and *C. nodosa* (see OCEANA, 2010, and references there in). These experiences have allowed defining some good practices, and the effectiveness of reforestation of these species, which is relatively low and expensive in comparison with terrestrial ecosystems. Nevertheless, there are other possible restoration measures that could increase the scale of the restoration project, and thus its cost-effectiveness.

For example improving water quality through increasing the efficiency or size of water treatment plants, as seagrass health and dynamics is strongly correlated to water quality (e.g.,

Montefalcone, 2009). This well-established relationship would probably allow developing tier 2 estimates of the carbon credits generated.

Another effective action in many Mediterranean meadows could be restoring the coastal sedimentation regime, for example through restoring river sediment fluxes using industrial aerial cables to transport the sediments retained in dams. Substituting or improving coastal promenades and other coastal infrastructures in order not to disrupt land-sea sediment inputs and coastal flow, or sustainably managing of beach seagrass litter. Improving sedimentation regime would reduce both meadow and sediment erosion, thus producing direct and indirect positive effects on sediment carbon stocks and sequestration. This well-established relationship would probably allow developing at least tier 2 estimates of the carbon credits generated.

Restoring meadows eroded by illegal trawling and intensive anchoring, should include using passive and active surveillance systems, as well as ecological moorings. These may be cost-effective measures with high impact in ensuring reforestation success and avoidance of sediment stock losses.

Beyond carbon - Given the many important environmental services provided by seagrasses (coastal protection, water transparency, biodiversity, fisheries), it is clear that seagrass carbon offset projects should exploit all these co-benefits in order to obtain higher prices for their carbon offsets in the voluntary markets, in order to improve their financial viability (Herr et al., 2017).

Potential areas for restoration - In Andalusia, following the cartography generated by the A1 action, there are 124.1 ha of dead mat identified, although the undetected area is probably larger, as many dead mats at intermediate and deeper depths remain buried. The cartography also defines 569 ha of *P. oceanica* meadow in regression.

There are also 892.5 ha of *C. nodosa* and *P. oceanica* mixed meadows. Such typologies are most often indicators of *P. oceanica* meadow regression, and thus potential candidates for restoration. There are also 42.2 ha of *P. oceanica* with *Caulerpa cylindracea*, although the presence of this alga only indicates meadow regression when it is intermingled with meadow shoots (not when it is only on the meadow sand patches), and the cartography does not distinguish between both types. Therefore, there are at least 1627.8 ha candidate for carbon offset projects based on *P. oceanica* meadow restoration, that is, 12% of Andalusian *P. oceanica* meadows.

Nevertheless, given the high carbon stocks and vulnerability detected in the degraded meadows and dead mats of Roquetas and Villaricos-Deretil, we think that these two areas would be ideal candidates for carbon offset projects. Such projects should consist first in

improving water discharge management in the case of Villaricos-Deretil and the Roquetas shallow barrier-reef. Then, seagrass recolonization could be accelerated through reforestation activities.

In the case of the intermediate meadows of Roquetas, which have been mechanically degraded by trawling, the restoration project should include sediment remediation in the silted borders of the grooves, and enhancing effective protection against illegal trawling in order to completely stop this pressure. Accelerating plant recolonization through reforestation activities could also be convenient, given the slow growth-rate of the species *P. oceanica*.

4. Conclusions

4. Conclusions, recommendations and future work

Posidonia oceanica should be the target seagrass species, at large, for any conservation or restoration carbon offset project. It holds 94.4 % of all the CO_2 stored in the sediments of Andalusian seagrasses.

The annual input of CO_2 to the sink is 9.2 ktCO_2 for all Andalusian seagrasses, of which 78.8 % is attributed to *P. oceanica.* In a context of carbon offset projects, this amount is little relevance.

From the above, an overall conclusion is that carbon offset projects in Andalusian seagrasses should focus on the conservation of the millenary stocks of *P. oceanica*, that is to say, on efforts leading to avoid the loss of healthy meadows and on the recovery of meadows in regression.

A sound methodology to determining the CO_2 emissions resulting from the degradation or destruction of seagrass habitat is still lacking. Assuming that a 2 % of annual loss of Andalusian meadows led in the erosion and complete remineralization of the top meter of sediment, the resulting CO_2 emissions would be 261,995 tCO_2 (including the loss of sequestration capacity). The potential revenues from avoiding that emissions in the regular carbon markets, assuming a price around 20 € /tCO_2, would be about 5,250,000 € / year.

The POSIMED monitoring network results indicate that there is not a general decline trend on Andalusian *P. oceanica* meadows, at least for the period 2012-2018, although there may be carbon flux decline, linked to the observed increased rhizome un-burial. Large regional or national projects, aiming at improving coastal sedimentary regime, could benefit from mitigation funds, as much as from adaptation funds, if we are able to measure its effects on increasing general carbon fluxes into the sediments, from coastal systems.

Seagrass decline is not general but concentrated in some localities, which calls for local restoration projects to be developed. A good candidate for climate-mitigation restoration projects would be the Almeria bay-Puntan Entinas area, recently included in a new SCI (Bahia de Almeria y Seco de los Olivos), and included the meadows of Roquetas (a part of it already included in the SAC of Arrecife Barrera de Roquetas de Mar), which regression impacts on carbon stocks and fluxes have been extensively studied here. Another site, the meadow of Villaricos, also studied here. In general, projects improving water and sediment quality as well as re-vegetating exposed dead mats, could produce a good amount of carbon credits.

Carbon offset projects based on seagrass meadows should focus on two primary actions: 1. promoting measures to prevent the mechanical destruction of the habitat (anchoring, trawling, siltation) and 2. improving the quality of the waters hosting seagrass meadows (chemical and organic pollution).

The main obstacles to bring the carbon locked by seagrass meadows to the carbon markets are: 1. lack of quality scientific information (mapping, stocks and fluxes quantification, quantification of the real impact of habitat loss in the stock), 2. lack of a sound certification standard and verification method for carbon offset projects, and 3. from the two first, lack of clear policies for the implementation of the Blue Carbon in the national inventories.

Future efforts in should therefore be directed to remove the barriers above: 1. ensuring a good knowledge of the extension of the seagrass meadows habitat and its different characteristics, 2. quantifying the stocks and fluxes of the different types identified. Using aerial imagery where practicable, side scan sonar, high resolution surface seismic, and ground trothing surveys would be the necessary fields methods to combine, 3. performing in situ or mesocosms experiments to determine the impact of mechanical and chemical degradation of the habitat in the loss of stock. 4. put all the knowledge and methods above to establish effective monitoring programs and verification methods, 5. with sound knowledge and methods, implementing Blue Carbon in the national policies will be smoother, 6. advertise the Blue Carbon resource as an attractive marketable asset among carbon trading companies and emitters, with emphasis in those looking for an added value (voluntary markets).

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Annexes

Annex I. Stocks and fluxes of each station

Station	Sediment TOC Stocks at 1m			Leaf canopy stock		Sediment TOC fluxes the last 100 years			SAR last 100 yr	SAR	Age max.	
	t/ha	SD	Age (yr)	t/ha	SD	t/ha	SD	Length 100 yr (cm)				
TE.S	210.2	114.0	1181	2.26	0.40	0.56	0.53	16	3.5	1.3	950	[210]Pb and [14]C
TE.I	372.5	334.8	1538	1.05	0.80	0.49	-	-	0.6	0.6	9422	[14]C
TE.I-C	127.5	83.2	2767	0		0.01	0.00	4	0.4	0.4	4052	[14]C
TE.D	850.1	196.7	1009	0.55	0.43	0.36	-	2	0.9	0.7	3766	[14]C
DE.I	173.9	60.0	1341	0.25	0.20	0.30	0.13	27	3.8	0.9	3620	[210]Pb and [14]C
PA.I	24.5	10.4	122	0.05	0.02	0.18	0.23	58	5.9	5.3	219	[14]C
AG.S	1234.5	807.9	445			0.68	0.39	30	2.9	1.6	1256	[210]Pb and [14]C
AG.I	91.7	71.4	295	1.87	1.13	0.64	0.27	37	3.7	2.2	763	[210]Pb and [14]C
AG.D	242.0	50.7	2455	0.40	0.16	0.36	0.30	24	2.4	0.6	3806	[210]Pb and [14]C
BA.S	28.1	9.1	270	0.31	0.11	0.08	0.03	31	3.1	2.9	355	[14]C
BA.S-C	24.5	10.3	-	0		-	-	-				[210]Pb and [14]C
RO.S	247.9	111.7	758	1.71	0.63	0.50	0.22	27	3.1	1.5	1084	[210]Pb and [14]C
RO.S-C	225.8	103.4	530	0		0.31	0.13	17	1.7	1.7	800	[14]C
RO.S-CN	29.9	-	-			-	-	-				

Site												Dating
RO.I-M60	27.92	-	-	0.35	0.07	1.41		1.60				
RO.I	283.8	181.0	-			-	-	-				
RO.I-Shall	168.5	36.7	-			-	-	-				
RO.I-E	125.6		1028	0		0.19	0.00	12	1.7	1.0	1409	^{14}C
RO.I-R	153.2	48.6	919	0.28	0.10	1.25	0.66	18	8.4	3.9	980	^{210}Pb and ^{14}C
RO.I-B	165.6	103.4	3899	0		0.32	0.47	34	3.0	0.8	5793	^{210}Pb and ^{14}C
AL.S	215.5	151.6	1138	1.52	0.55	0.16	0.03	9	0.8	0.8	1228	^{14}C
ME.S	151.4	51.7	504	0.82	0.09	0.15	0.09	17	1.7	1.8	406	^{210}Pb and ^{14}C
ME.I-C	63.4	-	-	0		-	-	-				
CA.S	-	-	-	2.81	1.84	-	-	-				
CA.S-C	177.1	66.0	116			-	-	-	5.8	5.6	70	^{210}Pb and ^{14}C
CA.S-CN	150.1	53.0	146	0.41	0.16	-	-	-	7.1	4.7	158	^{210}Pb and ^{14}C
SA.ZN-S	19.1	21.8	-	0.39	0.10	0.02	0.00	4	0.4	0.4	1551	^{14}C
SA.ZN-D	76.4	45.7	-	0.81	0.20	-	-	-				^{210}Pb and ^{14}C
SA.ZN-C	54.5	26.0	1862	0.67	0.00	0.05	0.03	5	0.5	0.5	2124	^{14}C
SA2.ZN	66.9	20.3	-	-	-	-	-	-				^{210}Pb and ^{14}C
SA.CN	66.2	17.9	2101	0.57	0.06	0.05	0.03	8	0.8	0.6	1991	^{14}C
SA.CP	*143.1*	*61.2*				0.38	0.16	17	2.2	1.9	212	^{210}Pb

Annex II. Graphs of 210Pb profile, Age model, Density, SAR, TOM, TOC, TOC fluxes, TIC, δ13C and δ15N isotopes and grain size distribution per station

Age and depth distribution of bulk density (gDW/cm³), sediment accretion rate (SAR, mm/yr), total organic matter (TOM, %), total organic carbon (TOC, t/ha), total organic carbon flux (TOCflux, t/ha yr), total inorganic carbon (TIC, t/ha), total inorganic carbon flux (TICflux, t/ha ry), δ13C and δ15N isotopes and grain size distribution, from the longest core of each station.

Station TE.S (Terreros, shallow, healthy)
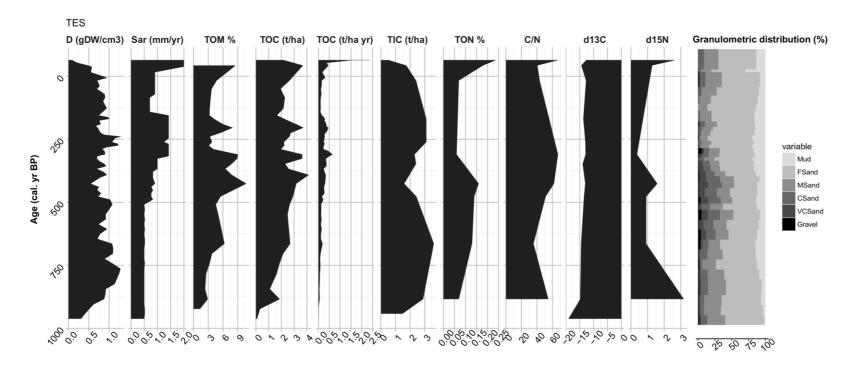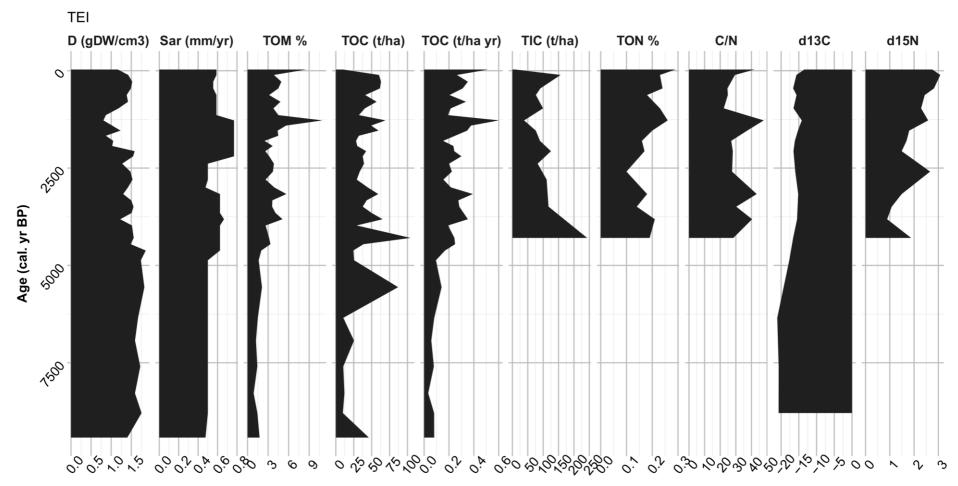
Fig 1: age and depth distribution on TE.S_A core (manual core from a healthy, shallow, *P. oceanica* meadow) of soil density, sediment accretion rate, total organic matter, total organic carbon, total organic carbon flux, total inorganic carbon, total inorganic carbon flux and grain size distribution (coarse, >2mm; very coarse sands, 1-2 mm; coarse sands, 1-0.5 mm; medium sands, 0.5-0.25; fine sands, 0.25-0.063; and mud, <0.063mm).

Fig 2: age and depth distribution on TE.I_A core (virbocore from a healthy, intermediate, *P. oceanica* meadow). See acronymes in Fig. 1.
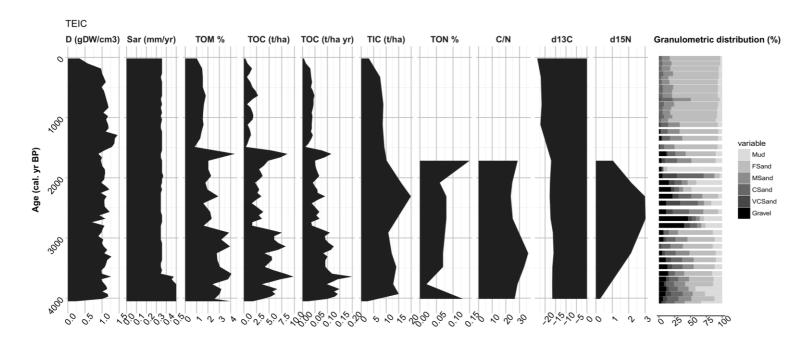
Fig 3: age and depth distribution on TE.I_C core (manual core from a control area, death mat below, intermediate, *P. oceanica* meadow) See acronymes in Fig 1.
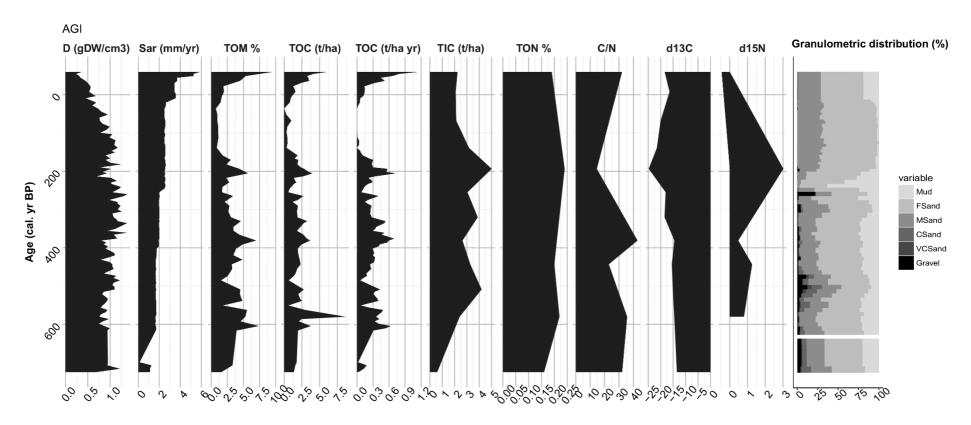
Fig 4: age and depth distribution on TE.D_A core (vibrocore from a healthy, deep, *P. oceanica* meadow) See acronymes in Fig 1.
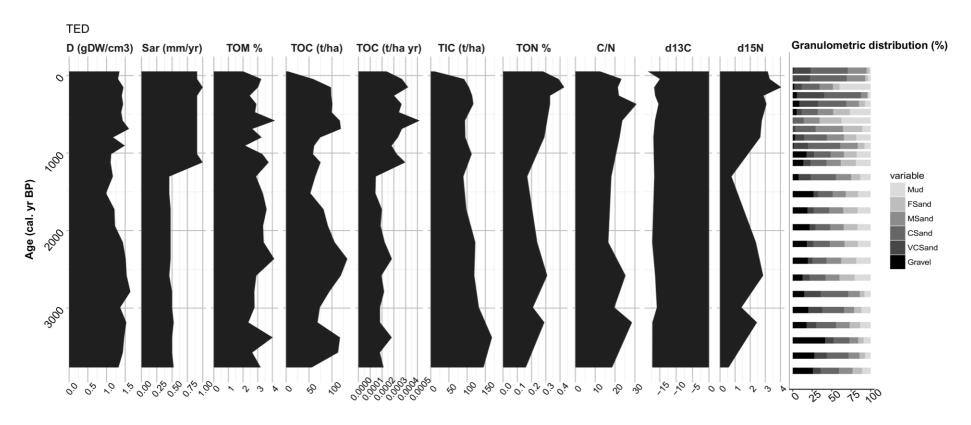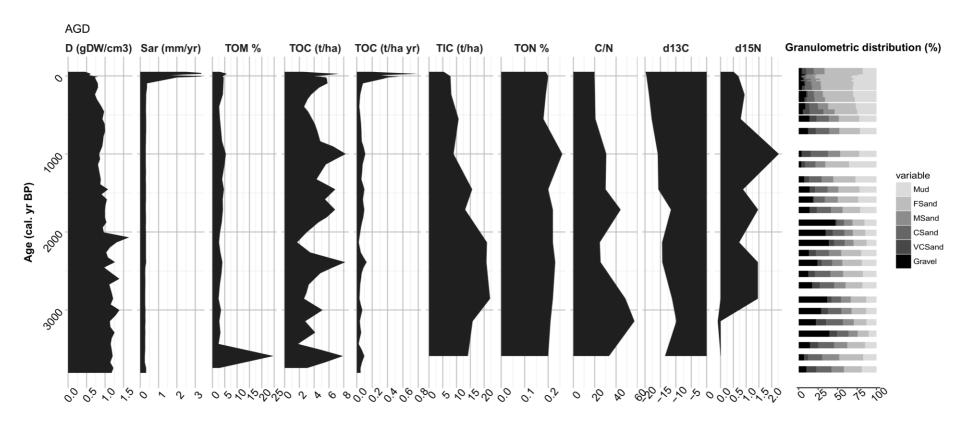
Fig 5: age and depth distribution on DE.I_A core (manual core from a degraded, intermediate, *P. oceanica* meadow) See acronymes in Fig 1.
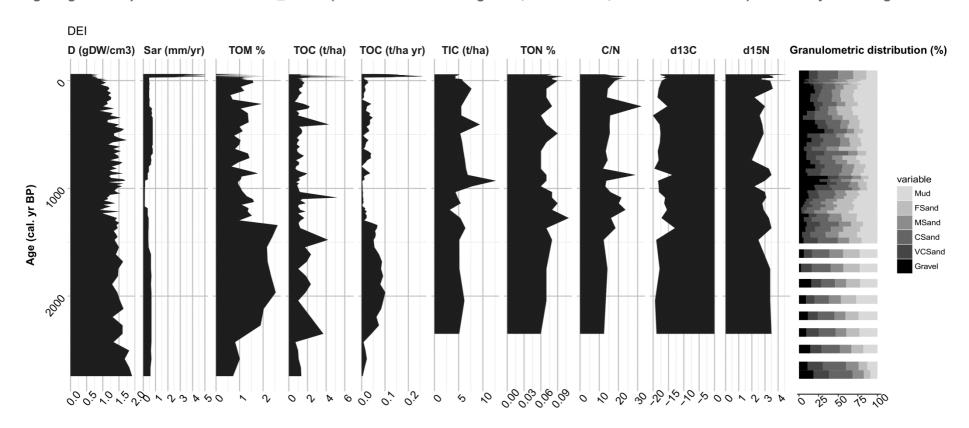
Fig 6: age and depth distribution on PA.I_A core (manual core from a healthy, intermediate, *C. nodosa* meadow) See acronymes in Fig 1.
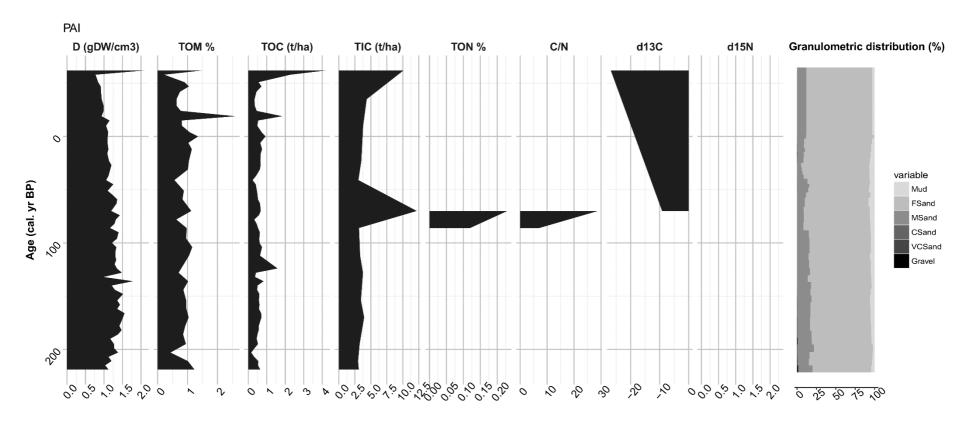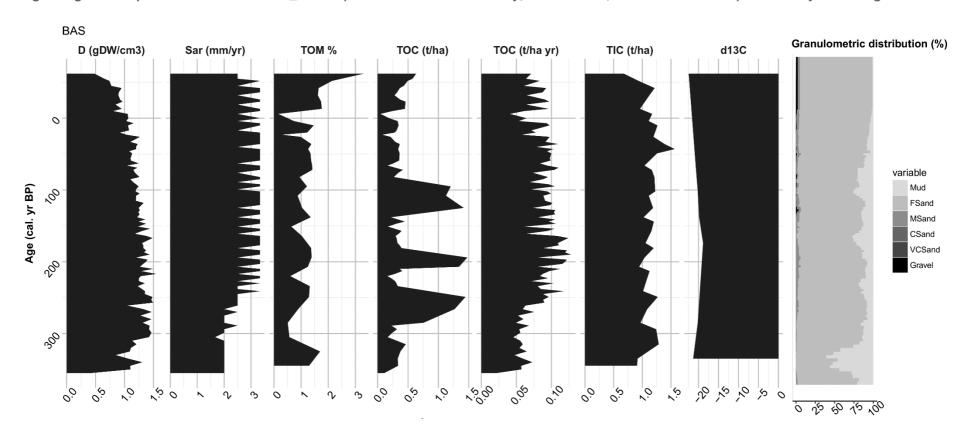
Fig 7: age and depth distribution on AG.S_A core (manual core from a healthy, shallow, *P. oceanica* meadow) See acronymes in Fig 1.
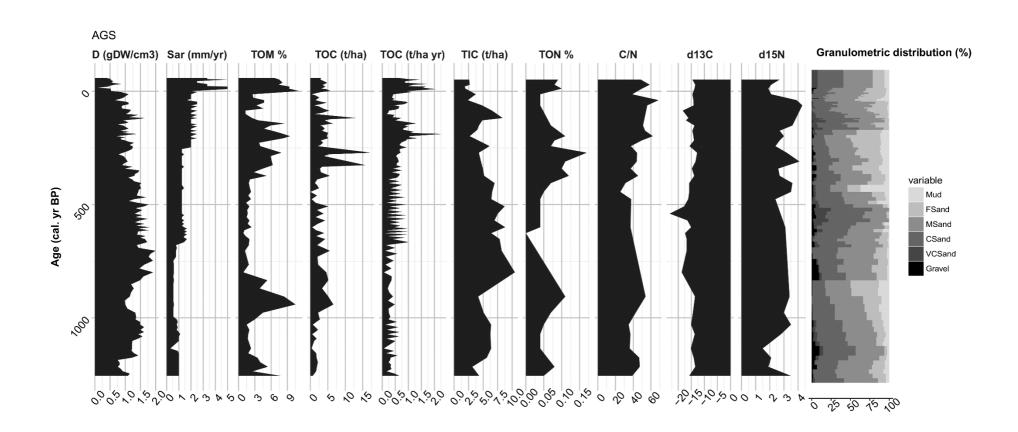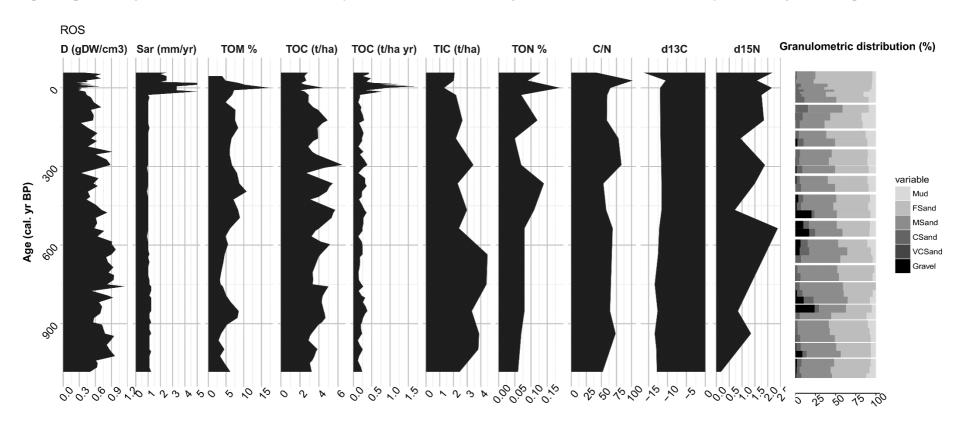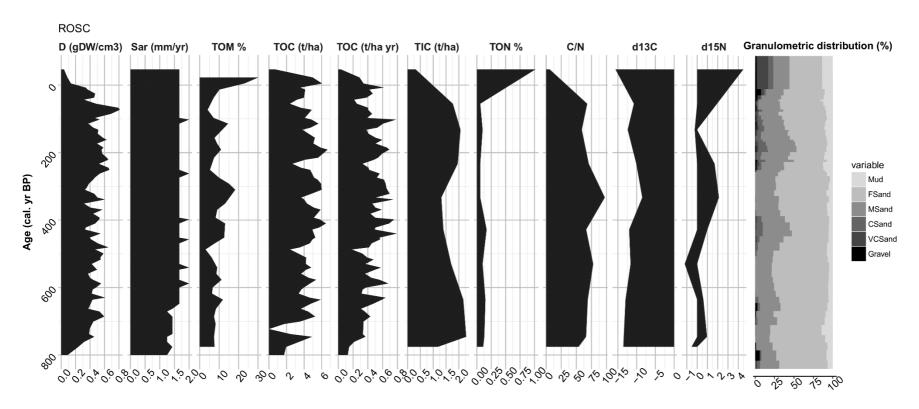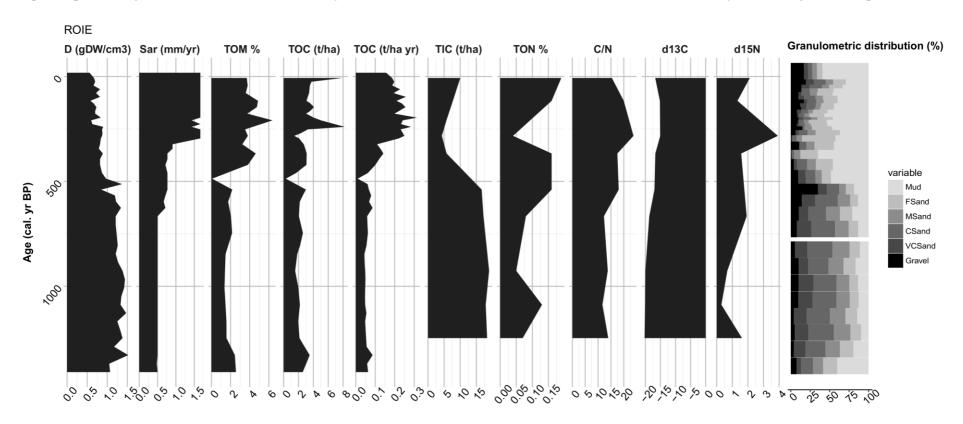
Fig 8: age and depth distribution on AG.I_A core (manual core from a healthy, intermediate, *P. oceanica* meadow) See acronymes in Fig 1.

Fig 9: age and depth distribution on AG.D_A core (manual core from a healthy, deep, *P. oceanica* meadow) See acronymes in Fig 1.

Fig 10: age and depth distribution on BA.S_A core (manual core from a healthy, intermediate, *C. nodosa* meadow) See acronymes in Fig 1.

Fig 11: age and depth distribution on RO.S_A core (manual core from a healthy, shallow, *P. oceanica* meadow) See acronymes in Fig 1.

Fig 12: age and depth distribution on RO.S_C19 core (manual core from a dead, shallow, P. oceanica meadow) See acronymes in Fig 1.
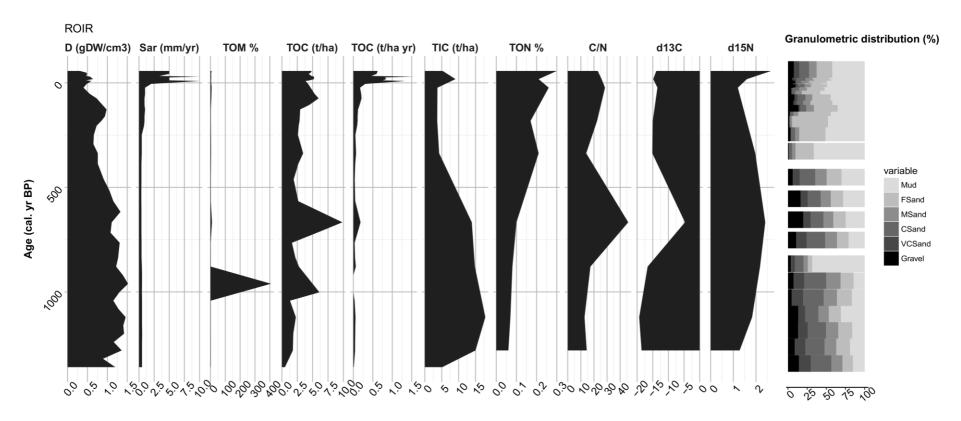
Fig 13: age and depth distribution on RO.I-E core (manual core from a eroded, intemediate, *P. oceanica* meadow) See acronymes in Fig 1.
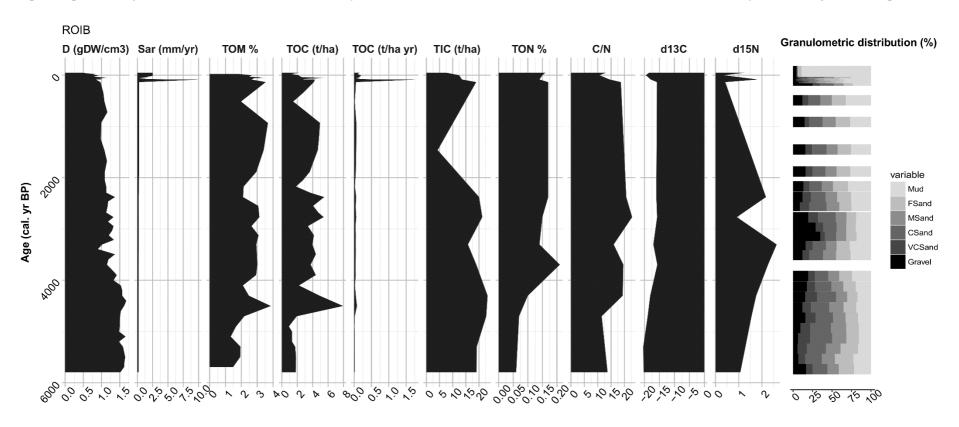
Fig 14: age and depth distribution on RO.I-R_A core (manual core from a recolonized, intermediate, *P. oceanica* meadow) See acronymes in Fig 1.

Fig 15: age and depth distribution on RO.I-B_A core (manual core from a eroded, intermediate, *P. oceanica* meadow) See acronymes in Fig 1.

Fig 16: age and depth distribution on AL.S_A core (manual core from a healthy, shallow, *P. oceanica* meadow) See acronymes in Fig 1.
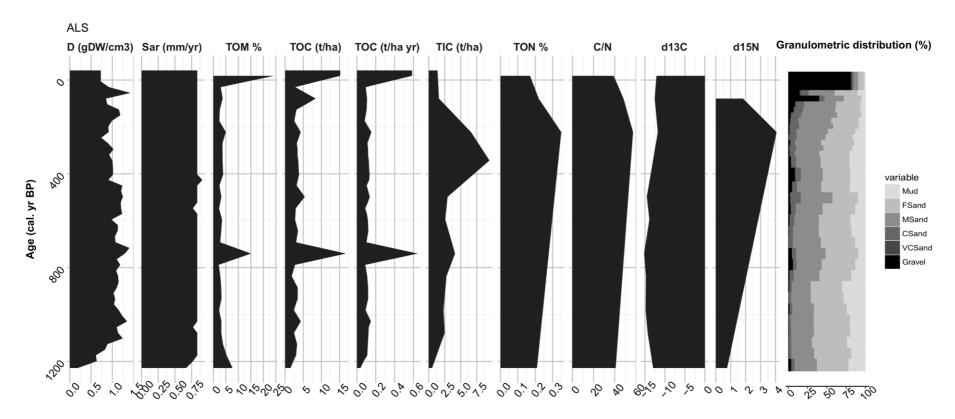
Fig 17: age and depth distribution on ME.S_C core (manual core from a healthy, shallow, *P. oceanica* meadow) See acronymes in Fig 1).
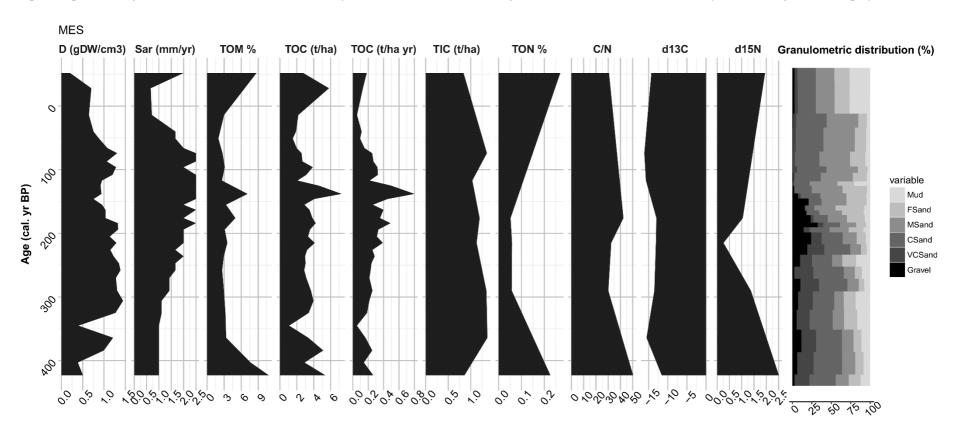
Fig 18: age and depth distribution on CA.S-C_A core (manual core from a dead, shallow, *P. oceanica* meadow) See acronymes in Fig 1.
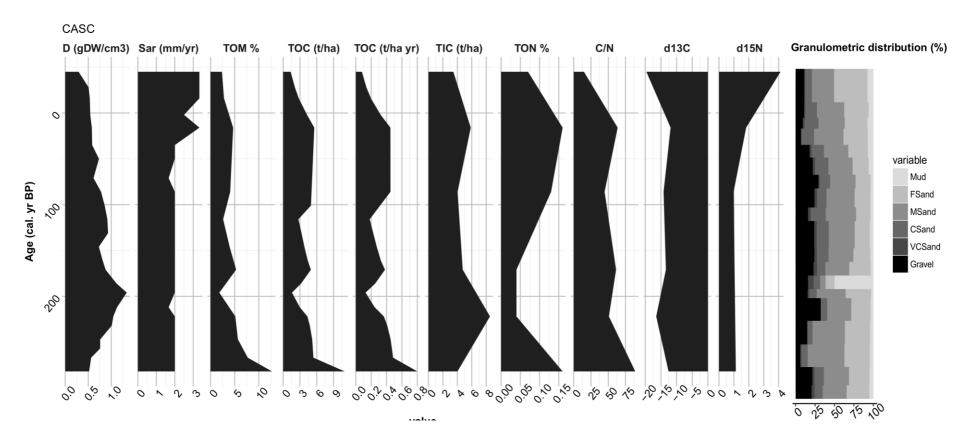
Fig 19: age and depth distribution on SA.ZN-S_A core (manual core from a healthy, intertidal, *Z. noltei* meadow) See acronymes in Fig 1.
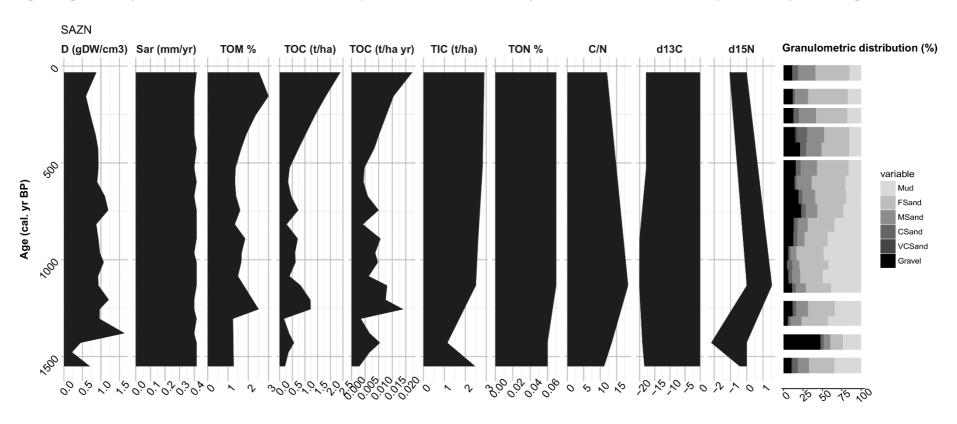
Fig 20: age and depth distribution on SA.ZN-C_A core (manual core from a control area, shallow, *Z. noltei* meadow) See acronymes in Fig 1.
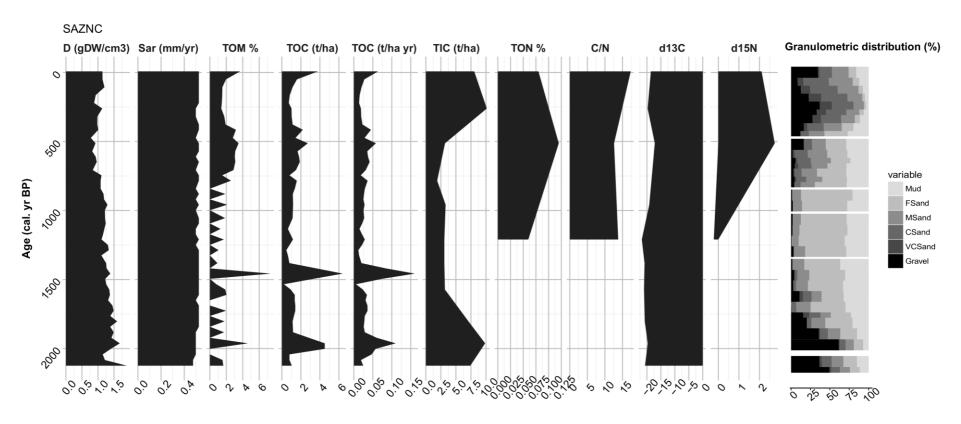
Fig 21: age and depth distribution on SA.CN_A core (manual core from a healthy, shallow, *C. nodosa* meadow). See acronymes in Fig 1.
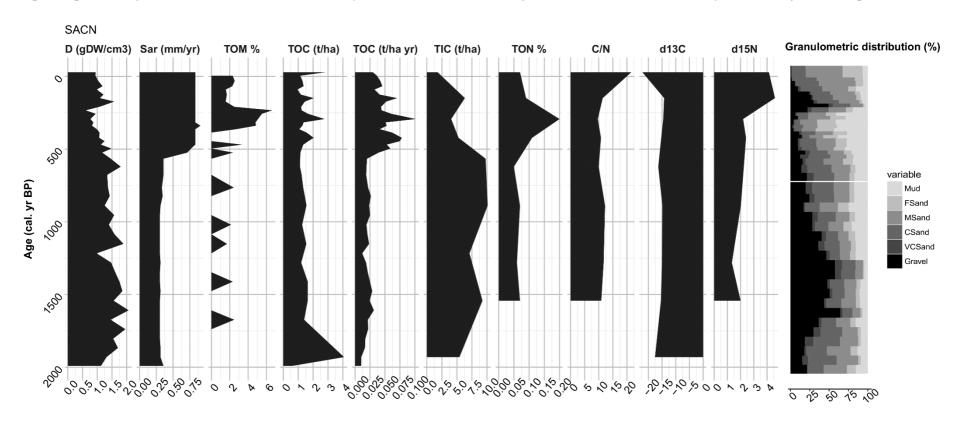
Fig 22: age and depth distribution on SA.CP_A core (manual core from a healthy, shallow, *Caulerpa spp.* meadow). See acronymes in Fig 1.
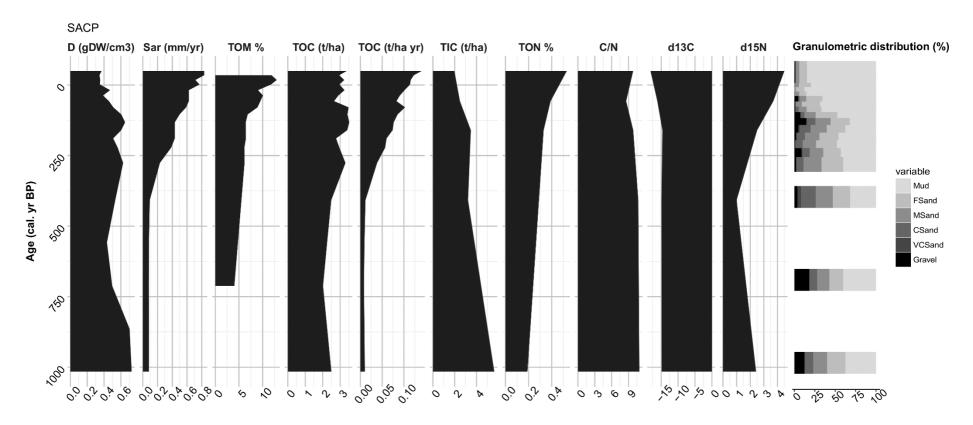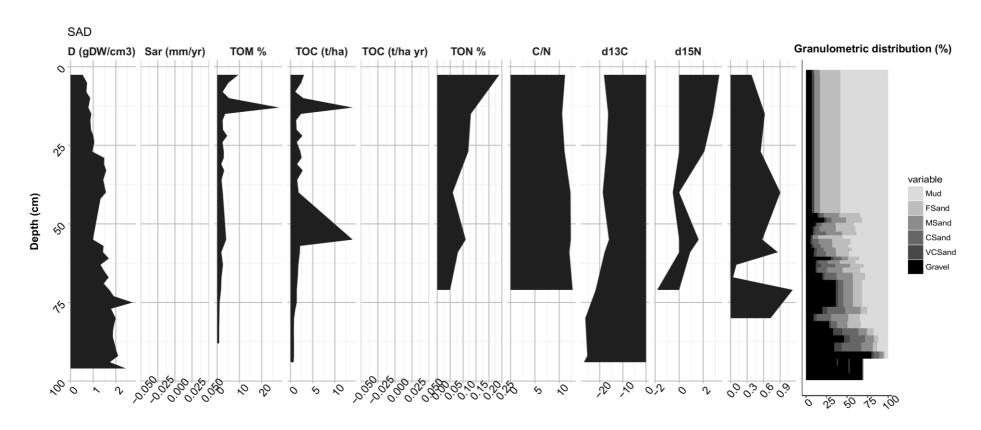
Fig 23: age and depth distribution on SA.ZD-D_A core (manual core from a healthy, shallow, _Zostera noltei_ meadow). See acronymes in Fig 1.

Annex III: Issues affecting the reliability of the estimates

Although the estimates of Blue Carbon stocks and fluxes performed in this study are probably the most detailed ones in the EU, there is a number of important limitations and uncertainties that affect them. They are related to the sample size, to field sampling and laboratory techniques, and even to conceptual aspects. Although some of them have been already introduced above, some of the most critical are listed and commented in this annex.

a. Sampling effort –

The huge extension and complexity of seagrass ecosystems implies simplifications to adjust the sampling effort to the material and human resources available. Sample size limitations have to be overcome through (1) a detailed analysis and interpretation of the local conditions and temporal variation across key cores and stations and (2) the knowledge of experts with a large history of personal direct observations in the study sites. Not all environmental conditions or meadow categories were present at every region, site or station. Also, small seagrasses, could not be sampled on all its geographical range. So very often, any attempt to perform a complete factorial analysis is simply not feasible. On average, the within station horizontal variability in TOC density was already large, with a coefficient of variation of CV= 41% $\pm$ 34%(SD).

In distributing the sampling effort, there is a trade-off to be seriously considered, between sampling horizontal and vertical (sediment layers) variability. The solution of this trade-off depends on the questions to be addressed. Given the scarcity of data on seagrass and saltmarsh carbon variability in both dimensions, and our need to understand temporal changes in carbon stocks analyzing their own vertical distribution (because historical data are nonexistent, we had to make an intensive sampling effort, of both directions, vertical and horizontal.

b. Dating techniques limitations –

Dating the soil samples is key to estimate the long-term carbon sequestration rates of the meadows. The two techniques used in this study were radiocarbon ^{14}C and lead ^{210}Pb.

Radiocarbon dating is used for material that is expected to be older than 200 yr. As the date we need to estimate is that when the soil layer was deposited, we have to assume that organic matter from the layer we are using for the dating, must have been produced at that time. Particularly in the lowest sections of the cores, it is not rare that macro-debris of seagrass cannot be found. In these cases, an alternative is to send an aliquot of bulk sediment to the laboratory for a dating of the total organic carbon contained in the sample.

It is known that the choice of the material to be dated has repercussions on the radiocarbon ages (Belshe et al., 2017).

To check for this, we dated a layer of the core BA.S_A using both plant debris and bulk sediment. The dates obtained differed nothing less than ~2500 years!

Generally, dating made on pieces of coarse organic matter are more reliable, because it belongs entirely to a plant or animal, which used to live in a particular time. On the contrary, bulk sediment organic matter is a mixture of materials from different sources and ages (e.g.

land-produced organic matter, remobilized after erosion, live bacteria feeding on dissolved organic matter that migrates vertically). These considerations are even worsened in the case of shells, as there are geoologically-based inter-basin differences in the isotopic signals of dissolved carbonates, as well as posterior ion interchanges (Teichberg et al, 2017). Given than in most seagrass sediments there were enough pieces of ancient seagrass material, dating bulk samples and shells in this study was discarded and the dating of those deep layers of the sediment not showing enough macro-debris of plant material was abandoned. From best to worst material choice: leaves > rhizomes > roots > bulk sediment organic matter > shells.

The ^{210}Pb dating technique is used for the top-most layers of the soil, i.e., for the recent history of the meadow (0-200 yr). This technique depends on there being a good chronological sequence in sediment and ^{210}Pb inputs, allowing us to get a consistent ^{210}Pb radioactive decay curve. This technique is finer than the ^{14}C and fails when the top cm of the core under study is lacking, or has suffered a stratigraphic alteration. This alteration can mainly happen as a consequence of i) inadequate sampling techniques or ii) alterations during core transport (in SCUBA, once on board, once on the road, etc.), but they can also be diagnostic of recent natural processes, like iii) *in situ* re-suspension of the sediments due to currents or wave action or bioturbation and iv) an erosion dynamic. In this study, the ^{210}Pb curves showed incoherencies in the top layers, not allowing for a proper elaboration of the chronological model in some samples. For these cores, the chronological model was elaborated based only on radiocarbon ages.

Fluxes calculated by combining chronological methods (^{210}Pb in the upper 30 cm and ^{14}C beneath) or only ^{14}C dating were not equivalent: comparing the Carbon fluxes estimated applying sediment accretion rates with both chronological models, within the cores where we were able to perform both dating methods, we observed that using only ^{14}C dating resulted in underestimates of fluxes between 8 and 85 %. The underestimation was more severe in sediments in which the mean core sedimentation rate was high, that is, in rapidly accreting sediments. Whether such under-estimation of recent accretion rates based only on ^{14}C dating also occurs in sediments with recent mixing dynamics in their top (and so that didn't allow us to estimate recent carbon accretion rate from ^{210}Pb), we cannot say, but this was not an objective of the project and our sampling method does not allow us to further develop de issue. The authors recommend future efforts to be devoted to this on following projects. Our finding of a good correlation between recent sediment accretion rates and Posidonia vertical shoot growth, may help when ^{210}Pb indicates a mixing pattern or is just not affordable (75 € per sample, 15 to 30 samples = 1155 to 2250 € per core).

c. Core decompression correction –

As explained in Materials and Methods, two different coring methods were used: vibrocoring, which does not produce core compression, and manual coring, which does. Although compression does not affect the estimation of global core carbon stock or flux, knowing the actual amount of material up to the first meter depth is essential to standardize carbon stock estimates, and to follow the 2013 IPCC guidelines on wetlands GHG accounting.

Some mathematical methods have been pondered to better estimate the real depth of the material. The Coastal Blue Carbon Manual (Howard et al., 2014) recommends us calculating a "compaction correction factor" that distributes the compression equally through the entire core. Although this is a very straightforward solution, there are at least two ways in which the material may not compress homogeneously. First, changes in the material with depth may lead to differential, idiosyncratic resistance to compression. Second, the topmost layers are subject to the compacting force for a longer time during the coring operation than the deeper ones (which entered the last and were not pushed by further material), so the compaction would often decrease from the surface to the deeper layers.

To detect the first case, it would be necessary to measure core-shortening several times during coring into the sediment. This significantly increases sampling effort, and when extracting many cores, it may not be feasible, especially when scuba diving at deep stations. So in this study, as in any other BC quantification study, compression was measured based on the final compression parameters (i.e., once the corer has reached its maximum depth, also called "reject depth"). As for the second factor, Morton and White (1997)showed that logarithmic core shortening is the most common on wetland sediments. Thus, instead of the linear decompression recommended by Howard et al. (2014), we decided to follow Morton and White (1997) and fit a logarithmic curve to decompress our cores by default. However, we found problems decompressing in this way the highly compressed cores, because logarithmic models assume, un-realistically, that the material can be infinitely compressed, while in reality, any material can reach a maximum beyond which it does not compress further. So our method tends to overestimate compression in the shallowest core layers. Therefore, after some tests, we decided to use a logarithmic correction with $y_2 = 0.1$ below 30 % of compression, from 30-40 % we also fitted a logarithmic correction, but with $y_2 = 1$ (which moderates the logarithmic curve slope growth towards the core top, reducing decompression in those sections); while for compressions equal or above 40 %, we decided to apply linear decompression.

Compression is a limiting factor on the methodology, especially when we do not measure the complete soil stock profile. Carbon stocks capped at 1m, without compression correction, can be overestimated up to 60%, according to our data.

d. Carbonate precipitation: CO_2 source or sink?

Calcification process release 0.63 net molecules of CO_2 for each mol deposited at the average sea pH (Smith, 2013). In our study, the highest carbonate production was found on Santibáñez bay, where there is shellfish exploitation. Although presence of seagrass favor shellfish communities, these higher carbonates production was more related to the closure of the bay and local lithogenic inputs. than with any characteristic depending on the seagrass. The average CO_2 content from carbonates in the sediments of *P. oceanica* healthy meadows analysed in this study was of 1371 ± 334 tCO_2/ha, in the top m of sediment, accumulated at a rate of 1.24 tCO_2/ha yr. If these carbonates were precipitated as a consequence of the habitat

presence, and without any further considerations, this would imply a 'sink offset' of about 0.74 tCO_2/ha yr or 2269 tCO_2/yr for the entire Andalusia.

However, recent studies show that seagrass *in situ* calcification is low with respect to C_{inorg} burial (e.g. for P. oceanica meadows in Barrón et al, 2006). This and other evidences indicate that most carbonates stored in seagrass soils have actually been precipitated elsewhere (i.e., a large fraction has been imported from adjacent waters or from the land), and that Seagrass meadows actually promote dissolution of carbonates, increasing total seawater alkalinity, and thus increasing the capacity of atmospheric CO_2 dissolution into sea (Saderne et al., 2019a). This trait would actually increase seagrass potential role as carbon sinks, instead of offsetting it.

Nevertheless, the sea-land alkalinity system is really complex, and there are still too many uncertainties to quantify the role of seagrass meadows in the chemical sea carbon pump. For example, how much of this allocthonous carbonate inputs come from emerged, "fossil" carbonates (i.e. inputs to the seawater carbon pump system), and how much comes from adjacent sea ecosystems (relatively recent, and already within the seawater carbon pump system), may have differential net effects in this chemical system. As for *in situ* Carbonate precipitation, the canopy water pH increase produced by photosynthetic plant uptake of HCO_3^- would alter the stoichiometric molar relationship between carbonate precipitation and CO_2 release, reducing the 0.63 molar ratio of CO_2 emission (Smith, 2013). Moreover, this effect could transform seagrass meadows in potential refugia for calcifier organisms, in a context of seawater acidification (Gruner et al, 2018), increasing the relative importante of *in situ* calcification with global change. Therefore, despite recent data point to Ci burial as a potential enhancer of the seagrass carbon sin, rather than an offset, it still cannot be quantified (Lovelock et al., 2017; Saderne et al., 2019b).

This is why in the present study, we offer estimates of TIC stocks and fluxes, but we do not include them in any sense, in the carbon sink and stock estimates.

e. Scaling-up for global estimates –

Most of obstacles to achieve a satisfactory global estimate for the Andalusian carbon stocks and fluxes associated to seagrass meadows, have been discussed in several instances over this report. One limitation to add has to do with the lack of a marked bathymetric gradient in carbon stocks and fluxes observed. It was initially planned to obtain a function describing the changes in stocks and fluxes associated to the depth gradient. But a modelleable pattern was not found (what, as a matter of fact, is one very valuable outcome of this study). In the absence of such a gradient, stocks and fluxes found for a core at a certain depth was used as representative of the closest range of depths distinguished in this study.

f. Seagrass cover –

Areas with different seagrass cover are all taken equally as if they were homogeneously and totally occupied by the seagrass. This is probably leading to one of the greatest overestimations of the carbon sink associated to seagrass meadows. The spaces 'inter-patches' can arguably be considered to hold or to have held a well developed mat in the sediment, but not necessarily. In this study, we have observed that the 2 patches adjacent to the seagrass observed had seagrass matte beneath, and retained between 87% (the *C. nodosa* stations BA.S-C with respect to BA.S) and 34% (the P. oceanica stations TE.I-C with respect to TE.I) of the carbon stock$_{1m}$, probably depending on the erosive dynamics an time since sand patch formation.

For patch arease, at least, the totality of the sequestration capacity should be subtracted together with a substantial part of the stock. If the average cover of *P. oceanica* in Andalusia has been reported to vary from 25 % to 70 % (Posimed Project Andalusia 2009) of the total stocks and fluxes determined in this study should be subtracted. This procedure is not currently a standard one in the BC scientific community but should probably begin to be considered in the near future. Because the mapping in A1 has few entries of seagrass cover, an estimate of this potential wane is not attempted.

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The GAME team coring the intermediate *Posidonia oceanica* meadow in Terreros (Sample ID: TE_S). October 2016. Source: GAME

Blanes 31 May 2019 (updated September 2019)

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