# METHODOLOGY FOR THE ESTIMATION OF CARBON STOCKS AND EMISSION FACTORS IN TIDAL SALTMARSHES AND SEAGRASS MEADOWS

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# INDEX

# ACRONYMS, UNITS, AND GLOSSARY OF TERMS

C: carbon.

%C: percentage of the biomass that is carbon.

$CO_2$: carbon dioxide.

DOC: dissolved organic carbon.

AUs: absorption units.

ha: hectare ($10^4$ $m^2$).

$m^2$: square metre.

mm: millimetre.

mmol: millimoles ($10^{-3}$ moles).

$N_2O$: nitrous oxide.

FW: fresh weight.

t $CO_2$ eq: ton of carbon dioxide equivalent.

t: ton ($10^6$ g).

µmol: micromoles ($10^{-6}$ moles).

**Biomass:** the total mass of a quantity of biological matter, usually expressed in unit of weight per substrate area.

**Allochthonous carbon:** organic carbon originating outside the baseline project zone or ecosystem and which is carried to the system by means of hydrodynamics and trapped by aboveground biomass structures.

**Autochthonous carbon:** organic carbon that is produced and deposited in the baseline project zone or ecosystem.

**Blue carbon**: in the case of saltmarshes and seagrass meadows, carbon captured through photosynthetic activity and stored in soil or sediment and in biomass, either above or belowground, both living and dead.

**Organic carbon:** carbon derived from photosynthetic activity associated with living or dead biological matter and which, in the case of soil or sediment, can be found in different degrees of degradation.

**Core**. Cylindrical sample of a soil or sediment obtained by driving a cylinder into the ground.

**Apparent density**: the ratio between the mass of soil or sediment and its volume. It provides an indication of its compaction, that is, its role as a structural support, its capacity to transport water or solutes within itself or their oxygenation. It is expressed in g $cm^{-3}$.

**Detritus or debris:** organic material of soil or sediment in an advanced stage of decomposition.

**Ecosystem**: the baseline system for studies in Ecology, consisting of the biological communities together with the physical environment in which it develops.

**Epiphyte**: applies to the alga that lives on a marine macrophyte (seaweed or seagrass) without removing any nutrients, but using it exclusively as a physical support.

**Carbon stock:** the amount of organic carbon stored in an ecosystem. It is usually measured in units with dimensions $M \cdot L^{-2}$ referring to the thickness of the soil or sediment.

**Tidal saltmarsh**: a type of coastal ecosystem dominated by woody halophytic plant species of the family of Chenopodiaceae which is highly dependent on the influence of the tides, being either wholly or partly flooded during the high tide.

**Seagrass meadow**: a type of coastal ecosystem dominated by seagrasses which, depending on species, are adapted to living in both the intertidal and subtidal areas under saline conditions typical of sea water.

**Carbon sink**: reservoirs of autochthonous or allochthonous organic carbon in different compartments of the ecosystem.

# 1. INTRODUCTION

## 1.1. Purpose of the manual

The purpose of this document is to provide the baseline methodology for field estimates and the analysis of **blue carbon** stocks and carbon fluxes in **tidal saltmarshes** and **seagrass meadows**. The manual is intended for those managing or participating in the implementation of blue carbon projects relating to the improvement and restoration of these **ecosystems** in order to quantify over time the stocks and storage of **organic carbon** in the ecosystem.

The principal reference used in the production of this manual is the excellent document produced by *The Blue Carbon Initiative* (Howard et al. 2019), coordinated by Conservation International, the UNESCO Intergovernmental Oceanographic Commission and the International Union for the Conservation of Nature (IUCN). Additional and more detailed information can also be found in the manuals and documentation produced by the Life Blue Natura project (http://life-bluenatura.eu/en/home/) or in the manual for the creation of blue carbon projects recently published by IUCN (IUCN 2021).

## 1.2. Blue carbon stocks and sinks

The calculation of **carbon stocks** in saltmarshes or seagrass meadows requires the summation of all **organic carbon sinks (reservoirs)** in the area in which the project is to be developed. These sinks comprise two essential components of total organic carbon, one autochthonous and one allochthonous. Autochthonous carbon sinks comprise the soil or sediment, the aboveground (or epigean) and belowground (or hypogean) plant **biomass**, both living and dead, of saltmarsh plants or seagrasses, the biomass of accompanying macroalgae and the biomass of **epiphytes**. To these sinks must be added the biomass of organisms originating from ecosystems outside the field of study and which reach the project area by different routes, generally by the action of the movement of water masses or as a result of being trapped by the three-dimensional aerial structure of the species that thrive in the ecosystem. These contributing elements make up the **allochthonous carbon** sinks.

In general, estimates should be carried out for those sinks that represent a significant proportion of the total organic carbon (> 5% of organic carbon) in the tidal saltmarsh or marine angiosperm meadow in question. However, a more accurate and detailed analysis should include the quantification of all the sinks.

# 2. ESTIMATION OF CARBON SINKS IN TIDAL SALTMARSHES AND SEAGRASS MEADOWS

## 2.1. Definition of project boundaries and characteristics

Firstly, one must define the spatial boundaries within which the project is to be carried out in a tidal saltmarsh or a seagrass meadow, taking into account the typology of the project that is to be carried out, and therefore whether its objective will be the recovery, restoration, conservation, or creation

of new communities, etc. Similarly, it is important to consider in which homogeneous environmental units carbon sinks are to be estimated.

In the case of tidal saltmarshes (figure 1), for practical purposes, three main zones can be distinguished by virtue of their proximity to the water mass and their uniformity, easily recognisable by the dominant plant species (Biogeos 2016):

- **Low marsh**. This is the zone closest to the water lamina at low tide and which becomes covered by water due to the daily tidal effect. Species of the genus *Spartina* (*S. maritima*, *S. densiflora*) predominate as well as some Chenopodiaceae annuals such as *Salicornia ramosissima* or perennials such as *Salicornia perennis* in areas with a minimum elevation above the low water level.

- **Mid marsh**. This represents the marsh zone that is partially flooded during high tides with coefficients greater than the average high tide and sits on partially developed soils. Chenopodiaceae such as *S. perennis* or *Halimione portulacoides* predominate together with some species of Plumbaginaceae belonging to the genus *Limonium* such as *L. angustifolium*. Other common species in this zone are *Puccinellia maritima*, *Suaeda vera*, *Cistanche phelypaea* or *Sarcocornia fruticosa*.

- **High marsh**. This represents the marsh zone that is very rarely inundated, only during the peak high tides of the lunar cycle. In this zone there are abundant larger-sized Chenopodiaceae such as *Sarcocornia fruticosa* and *Arthromnemum macrostachyum* or the composite *Irula crithmoides*. Other typical species are *Limoniastrum monopetalum*, *Suaeda vera*, *Frankenia boissieri*, *Salsola brevifolia* or *Puccinellia maritima.*

Figure 1. Image of a saltmarsh during high tide. During these hours the entire mid marsh and much of the high marsh are partially flooded by the effect of the tide.

One might also add a zone of brackish marsh, located inland, in areas where freshwater runoff modifies the salinity of the water towards values lower than those of sea water. In these cases vegetation may include plant species of the genera *Typha*, *Phragmites*, *Arundo*, *Juncus*, etc.

In the case of seagrasses (figure 2), three zones can be distinguished according to their state of emersion/immersion during the daily tidal range and the depth they inhabit, taking as a reference the point of low tide:

- **Intertidal meadows.** Represent those located in areas where there is daily alternation of periods of immersion and emersion. This zone is bounded in the top part by the mean level of the spring tides and towards the bottom by the mean level of the equinoctial low tides. *Zostera noltei* usually predominate, although smaller-sized stands of *Z.marina and Cymodocea nodosa* can be found.

- **Shallow subtidal meadows.** Represent those located in shallow areas, approximately between -0.3 m and -7 m in relation to the tide at its lowest ebb. *Z. noltei*, *Z. marina* as well as *C. nodosa* abound in them. Small-sized populations of *Posidonia oceanica* can also be found.

- **Intermediate and deep subtidal meadows.** Represent those thriving in areas below the shallow subtidal. These are the meadows where especially *P. oceanica* predominate, although it is common to find meadows where large-sized *C. nodosa* or *Z. marina* predominate and very rarely *Z. noltei*. Depending on the locality, separate zones can be defined according to the depth at which they are found, as intermediate subtidal (-7 m to -15 m) and deep subtidal (>-15 m).

Figure 2. Image of a mixed subtidal meadow of *Posidonia oceanica* and *Cymodocea nodosa*.

## 2.2. Principal organic carbon sinks

In the different areas or zones of the two types of ecosystems, the most important carbon reservoirs or sinks are as follows:

**- Aboveground or epigean biomass**: biomass of shrubs (small trunks, green or dry branches, leaves), marsh plants or shoots of seagrass; all living material found above the sediment.

- **Belowground or hypogean biomass:** composed mainly of the roots and rhizomes of shrubs, marsh plants or shoots of seagrass; all living plant material that is buried in the sediment.

- **Organic carbon from soil or sediment:** carbon consisting of organic matter and the degraded remains of organic matter found in soil or sediment and which cannot be visually separated from the inorganic matrix.

- **Dead matter or driftweed (or necromass):** composed mainly of non-living plant remains, both autochthonous and allochthonous, which are trapped amongst marsh plants or shoots of seagrass.

- **Macroalgae:** organic fraction made up of the accumulation of living macroalgae that either develop in the zone or remain trapped amongst the marsh plants or shoots of seagrass (Fig. 3).

**- Dissolved organic carbon (DOC)**: organic carbon fraction produced and released into the water column by these ecosystems and which cannot be weighted or measured directly, since it is of a size less than 0.7 µm. From the organic carbon thus produced, only the recalcitrant fraction is considered to be a long-term sink.

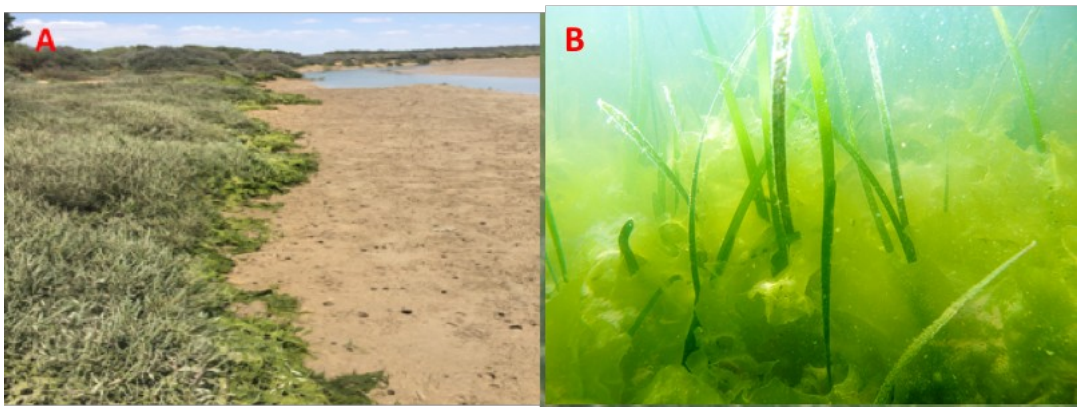


Figure 3. A) Band of macroalgae (*Ulva* sp., *Blidingia marginata*, *Bostrychia scorpioides*) at the foot of the mid marsh. B) *Ulva rotundata* intermingled amongst shoots *of Cymodocea nodosa*.

- **Net primary production exported:** consisting mainly of annually-produced epigean biomass that is not retained at the project site, but is lost from the baseline system when carried away by water currents and remains trapped in other communities. This sink, despite its importance within these systems, poses difficulties of quantification and therefore it will not currently be considered a sink when it comes to calculating carbon absorptions/emissions due to the initiation of a project. However, it is important to expand research into this type of sink because of its relevance and importance.

## 2.3. Sampling in the project area

In order to assess organic carbon stocks in each of these sinks, it will be necessary to undertake sampling in the project area. The number of sampling stations represents a compromise between the sampling time, the budget allocated and the level of accuracy in the estimates for each sink.

Ideally, fixed plots[1] should be selected that are sampled periodically rather than temporary plots that change throughout the project monitoring period. In either case, each of these two strategies has its advantages and disadvantages.

The way in which sampling plots are identified can be based on homogeneity criteria once the area has been surveyed. This homogeneity is usually determined by the dominant species of flora identified in the various project areas, in order to ensure a minimum number of sampling plots in each type of homogeneous environmental unit identified (e.g. high or mid marsh, intertidal or subtidal zone, etc.). This number will depend on the budget allocated to the project and must be determined by the project manager. For saltmarshes or seagrass meadows, there are no fixed rules regarding either the minimum number of samples to be taken in each plot of each of the homogeneous zone identified (for example, a minimum of 3 to 6), or the surface area required for each sample (for example, the internal diameter of cylinders for the analysis of organic carbon in sediment, the surface area of the grids for biomass, etc.), which in addition will depend on the carbon sink being analysed and the characteristics of the vegetation found there (figure 4). Typical values used are cylinders with a diameter of 5-15 cm and 1 metre depth for soil or sediment, and areas of up to 50 x 50 cm for biomass, necromass or macroalgae sinks (e.g. Sousa et al. 2008, García-Marín et al. 2013, Matthew et al. 2019, Morant et al. 2020; IUCN 2021). When selecting the sampling zones within each homogeneous unit, one can either begin with a linear transect from the water lamina outwards or choose the sampling points at random, but always ensuring that the homogeneity of the project area is reasonably well covered (figure 5). For more details on sampling strategies, refer to the Blue Carbon Initiative manual (Howard et al. 2019).

Estimates of carbon sinks should be repeated over a period of time, from initial estimates up until the end of the project period, at frequencies that generally increase the time gap between successive samplings and with the aim, during the initial periods, of identifying any significant deviations over the development of the project that might require the implementation of urgent corrective measures. A baseline recommendation would be to take samples at time zero, at 1 year, at 2 years, at 5 years, at 10 years, at 25 years and at 50 years, although each project will require a tailor-made design that will be extended throughout its lifetime. It is definitely advisable to always take the samples in the same season of the year and during the period of maximum values of aboveground biomass, which is usually during the summer season.

1 *The term 'p* *h homogeneous unit.*

Figure 4. Obtaining a mid-marsh sediment core (*Spartina maritima* meadows ).

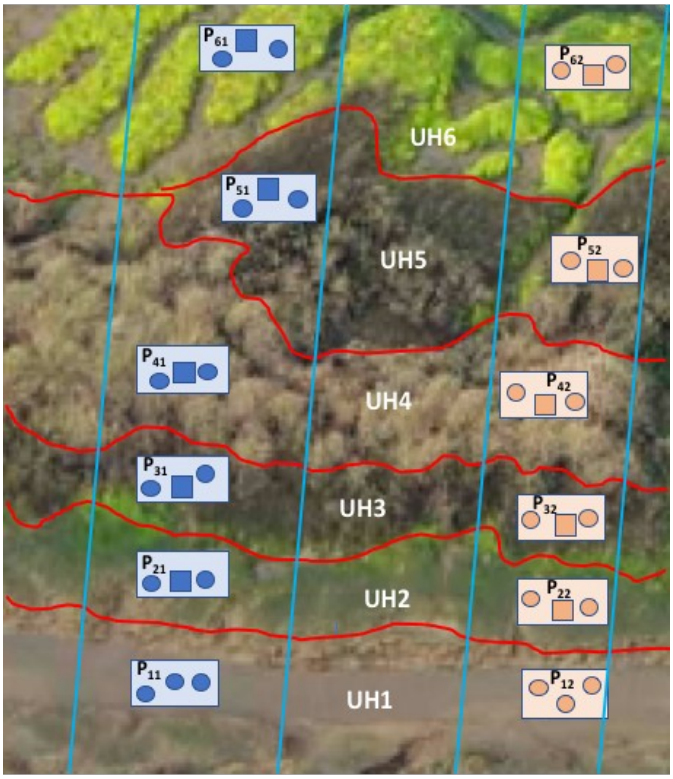


Figure 5. Simple diagram of a sampling design for blue carbon. In the sampling area, 6 homogeneous environmental units (UH) have been identified and 2 transects have been drawn. In each homogeneous unit, 2 plots (P) have been selected and in each plot 3 samples have been taken, either by inserting a cylinder of known diameter into the sediment (circle) or by taking biomass samples in a grid (square). With the aid of a computer program, it will be possible to work out the surface area of each homogeneous unit identified.

## 2.4. Biomass as an organic carbon sink: plant biomass, necromass and macroalgae in tidal saltmarshes and seagrass meadows

In the case of each sink, whether for plant biomass, necromass or macroalgae biomass, both the weight and average organic carbon content will be estimated for each of the selected sampling grids which, once related to the surface area of each homogeneous zone under consideration, will be continually summated in order to thereby obtain the overall value of each of the reservoirs. The way in which the biomass is estimated will depend on the species under consideration.

### 2.4.1. Tidal saltmarshes

In order to estimate the plant biomass in tidal saltmarshes, a transect perpendicular to the coastline or tidal water channel should be drawn. For each homogeneous unit identified (figure 6) one or more plots parallel to the coastline are selected and taken as fixed baseline plots. Within each transect plot, several randomly arranged replicate grids (from 3 to 6) should be chosen. Fixed plots are

usually rectangular and can have dimensions of several tens of metres per side, while the dimensions of the sampling grids are usually less than 1 m per side, from 10 cm (e.g. Cured et al 2013) to 1 m (e.g. Castle et al. 2008). The essential thing is to always follow the same sampling strategy whenever each transect is to be sampled, as the chosen size of the grid influences the final values of normalized biomass per surface area and, therefore, the C value for the sink (Castillo et al. 2008). The same is applies to the season of the year in which the samples are taken. As indicated above, sampling is recommended in the summer period, which is when aboveground biomass usually registers its maximum values in the saltmarshes of the Iberian Peninsula (e.g. Benito and Onaindia 1991, Palomo and Niell 2009, Sousa et al. 2017).

Figure 6. Bands easily discernible in a tidal marsh ecosystem: water lamina, un-vegetated sediment, band of *Spartina maritima* and band of *Halimione portulacoides*.

In order to determine the aboveground biomass, the choice of strategy used will depend on the species to be analysed, and may either consist in the collection of all living aboveground biomass within the selected sampling grid, which will subsequently be dried to a constant dry weight (DW) in an oven at 60 $^{0}$C and standardized by grid surface area (kg DW $m^{-2}$); or else in the calculation of this biomass through indirect methodologies (e.g. allometric relationships), which will avoid the destruction of the existing vegetation in the grid. If then the average content of carbon in the dry biomass of each species is estimated using a CNH Elemental Analyser (%C; see sample processing in section 2.2.) we can calculate the organic carbon content in the aboveground biomass by baseline surface area (kg C $m^{-2}$), by multiplying the biomass in each sampling grid by its carbon content. In cases where there is more than one plant species, one should consider the proportion of biomass per species contained in the total biomass of the grid to the average content of the carbon measured in each species (table 1).

In the event of a CNH autoanalyser not being available, a reference laboratory should be consulted. Nowadays there are numerous companies and universities that provide this certified external analysis service at a very reasonable price; around 5 € per sample, including the measurements of total N.

The calculation of aboveground biomass using allometric relationships will mainly depend on the species and the size of that species, and it should be borne in mind that there will always be a margin of error, given that they are estimates of the true value in each grid. For example, in the case of reeds and *Cyperaceae*, the number of shoots of each species can be counted along with their height in each grid. The sum total of lengths per species is then calculated, taking into account that in those shoots that are partially senescent only the green part is counted. Subsequently, a large number of shoots of different lengths are collected and weighted individually (fresh –FW– and dry –DW–), and a calibration curve is constructed covering a wide range of length values (L, cm or m) of the green fragments with their associated biomass (B, kg DW). The equation of type $B = a + bL$, or else $B = aL + bL^2$ that best fits the point cloud of the calibration curve will enable the calculation of the biomass values for the reference grid (kg DW $m^{-2}$), taking into account the sum total of the lengths arrived at earlier. The average shoot length could also be measured for each species present and, by using the straight-line calibration, estimate the average weight of each shoot, which would be multiplied by the number of shoots counted in each grid (shoots $m^{-2}$). The sum of the resulting biomass for each species will again provide the aboveground biomass in the sampling grid (kg DW $m^{-2}$).

Table 1. Example of the calculation for a sample analysed containing two species of Chenopodiaceae in the mid marsh. The content of C for the grid sampled would be 0.127 kg C $m^{-2}$.

| **Species in the sample** | **Aboveground biomass (Kg DW $m^{-2}$)** | **% Biomass in the total biomass** | **% C** | **Relative C content (Kg $m^{-2}$)** |
|---|---|---|---|---|
| *Halimione portulacoides* | 0.364 | 58.6 | 41.1 | 0.150 |
| *Sarcocornia fruticosa* | 0.257 | 41.4 | 37.2 | 0.096 |

For species such as *Spartina*, the number of patches in each grid (patches $m^{-2}$) can be counted and, as above, a wide number of patches that are representative of the population collected, and these are then weighted individually (in FW and in DW) and the patch length (L, cm or m) measured. From here, the average weight of the patch can be estimated by calculating the average value of the obtained weights (kg DW $patch^{-1}$), a value that will then be multiplied by the average density of the patches in the grid (patches $m^{-2}$), thereby obtaining the average biomass in the grid (kg PS $m^{-2}$). Another possible indirect method is to measure the length of a group of patches *in situ* in order to obtain an average patch size (cm $patch^{-1}$). Using an equation to link L and DW values, the average dry weight value of the patch obtained is multiplied by the density of the patches in the grid (patches $m^{-2}$), thereby obtaining the average biomass in the grid (kg DW $m^{-2}$).

Another possibility consists in calculating the aboveground biomass from equations that link the size of a plant or the circumference of its crown to its biomass. This may be useful for plant species of a more bushy size such as *Limoniastrum monopetalum* (Figure 7). In these cases, plants of different sizes should be sampled, both their height and their dry-weight biomass estimated, and the biomass value subsequently calculated as an equation-dependent variable. Fortunately, the different homogeneous zones of a marsh are often dominated by only a few species, allowing for the standardization of methodologies in each of these homogeneous units. In addition, it is to be

recommended that, right from the outset of the project, direct methodologies should be used in calculating the aboveground biomass in the different homogeneous units, since these can then be used to work out the various allometric or L-DW relationships that will allow indirect methodologies to be applied in estimating these values in successive monitoring activities. Finally the biomass values per unit of surface area obtained (kg DW $m^{-2}$) should be multiplied by the average carbon content in the dry biomass of each species (% C), which will allow the organic carbon content in the aboveground biomass per baseline surface area (Kg C $m^{-2}$) to be calculated. Some authors

differentiate between green shoots, woody shoots and dead shoots (e.g. Palomo and Niell 2009) or between leaves and shoots (e.g. Sousa et al. 2008). This may further refine the C values in the aboveground sink. However, for simplicity's sake, the entire aboveground biomass of a species can be gathered together in one plot and the carbon content estimated for this entire collection of dry biomass.

Figure 7. Image of *Limoniastrum monopetalum*, a species present in the high marsh that can sometimes present a bushy appearance.

As regards belowground biomass, there are some equations that link aboveground biomass values to belowground biomass values (e.g. Gross et al. 1991 for *Spartina alterniflora*). However, given the variability of both compartments, it is much more advisable to estimate biomass values directly through the use of core cylinders whose diameter will depend on the size of the species concerned. In Iberian marshes, most of the belowground biomass lies in the first 30 cm (Caçador et al. 2003, Castillo et al. 2008, Sousa et al. 2008, Palomo and Niell 2009), although other authors sample the belowground biomass up to a depth of 50 cm (Curcó et al. 2002). The cores obtained should be separated into horizontal fragments for ease of working. Each fragment can be washed in a sieve to separate fragments attributable to roots, rhizomes and debris. From the upper surface area, the fraction corresponding to leaf litter (necromass) or macroalgae should be removed and these will be assessed separately. The fragments are subsequently dried in an oven at 60 $^{0}$C until they reach constant weight (kg DW $m^{-2}$) and the carbon content in the dry belowground biomass is then estimated (% C), thereby allowing the average organic carbon content for that compartment in each homogeneous unit (kg C $m^{-2}$) to be calculated. Average carbon value conversion factors can be used in the belowground biomass, but it is always best to calculate these values for each sampling zone, thereby increasing the degree of confidence in the results. By this means we can obtain an average

carbon value for the cores analysed in each plot along with an error assessment (standard deviation or standard error).

For an estimation of the dead matter (or necromass) and macroalgae, values are obtained from grid (e.g. 50 x 50 cm) samples by separating out the dead plant debris (leaves, twigs, etc.) and macroalgae. In both cases the material is dried and weighed (kg DW $m^{-2}$), and the average carbon content (% C) of the dry tissue in both compartments then analysed. The multiplication of both values will eventually provide us with the organic carbon content (kg C $m^{-2}$) of each of the two compartments. With the values from each plot, the average value per homogeneous area can be determined along with an error assessment.

Finally, it is possible that we will encounter in the marsh the debris of sticks, branches and other larger materials swept along by tides or storms. This is a component that is usually of very minor significance and can be sampled along a transect in each chosen plot. In this case we can collect these fragments in several grids in a band 0.5 m wide and 50 m long perpendicular to the water channel in each plot. By this means we can obtain an average dry-weight biomass value for this compartment and an average biomass C value for the compartment in a similar way to the calculations made previously.

### 2.4.2. Seagrass meadows

The biomass of seagrass can have significant seasonal variations, almost disappearing during the winter period even in the smallest species. It is therefore advisable to carry out biomass estimates at the time of the year of maximum production; at the beginning of the summer on the Spanish coast (Ruiz et al. 2015), and then repeating the sampling at the same time of the year in successive periods. In shallow and intertidal areas, sampling can be done on foot at low water on the days of highest tidal coefficient. For subtidal areas, special diving equipment will be required with the support of a boat. Seagrass meadows in temperate zones are usually monospecific or limited to a reduced number of species (2 species, 3 at most), although one of them usually dominates. The selection of homogeneous environmental units in the project area will mainly be carried out on the basis of the bathymetric gradient, which in any one area usually determines both the distribution of the species and the structural characteristics of the meadows, elements that affect their ability to capture carbon (Serrano et al. 2014). Consequently, the homogeneous sampling units should be aligned according to depth in accordance with the classification provided above. In each of these, biomass will be randomly determined in different grids.

The aboveground (epigean) and belowground (hypogean) biomass will be collected with the aid of a sampler (a parallelepiped or cylinder) of known area (figure 8), which is introduced into the sediment (taking care not to cut the leaves) to reach depths of about 40 cm, thereby guaranteeing the collection of the belowground biomass. The sampler size will be variable and dependent on population characteristics (e.g. patch density and canopy size), using smaller areas (e.g. 100 $cm^2$) in the case of very dense or reduced canopy populations, and increasing the sampling area (1,600 $cm^2$) for species with a larger canopy size. After the sampler has been extracted and the material inside

collected from the sampled area, following initial in-situ washing using a mesh or sieve (0.5 mm mesh size) in order to remove sediment debris, the material is kept in cold storage and transported to the laboratory where the different fractions are separated for analysis: epigean biomass (patches), hypogean biomass (roots and rhizomes), dead material (necromass) and macroalgae. In all cases, each of the separated fractions is dried in an oven at 60 $^{0}$C to reach a constant weight (kg DW m$^{-2}$). Once properly ground, the samples are processed for analysing the organic C content of the dry tissue (%C), so that the organic C content can be calculated in each of the grids analysed (kg C m$^{-2}$).

Figure 8. Biomass sampling in shallow subtidal meadows in the Bay of Cadiz. The biomass collected is placed in a net for washing and further processing.

In most seagrass species, the patches can contain a significant amount of epiphytic algae that must be extracted and analysed separately. There are two techniques for achieving this: washing with acid in the case of encrusted calcareous algae or gently scraping the leaves with a scalpel in the case of other epiphytic algae. The biomass of epiphytic macroalgae collected is dried and weighted (kg DW m$^{-2}$) and a fraction is processed to determine the organic C content of the dry tissue (%C). If amongst these epiphytic algae there is a predominance of calcareous forms (e.g. *Hidrolithon farinosum*, *Titanoderma pustulatum*), as may be common in *Posidonia oceanica* leaves, the total carbon content has to be corrected following analysis of the inorganic carbon, as we will see later (section 2.2).

Finally, a fraction of the gross primary production of seagrass meadows or macroalgae can be released into the system as dissolved organic carbon (DOC), part of which is highly refractory and would therefore lead to an additional carbon sink, a long-term sink that can represent quite a significant amount since it results from a percentage of the annual production of aboveground biomass (Egea et al. 2019, Egea et al. 2020).

The estimation of this DOC is complex and is based on the filtration of water samples from a benthic chamber that encloses the community under analysis (figure 9). Water samples should be taken both initially and at various other times in a 24-hour cycle to obtain integrated daily measurements of DOC production or consumption in the community dominated by marine angiosperm (Egea et al. 2019). The samples are filtered through pre-combusted filters (0.7 µm) and the filtered water is stored in vials of diluted phosphoric acid for high-temperature catalytic oxidation in a high-sensitivity total organic carbon analyser. The values obtained represent daily DOC fluxes (mmol C m$^{-2}$ day$^{-1}$) that have to be transformed to t C ha$^{-1}$ which are released into the environment each year as DOC, part of

which will remain in the aquatic environment as a refractory carbon sink. It is important to note that DOC production depends on the status of conservation in which the meadows are found, and even in some sparsely vegetated systems they can begin to behave as consumers of DOC (Egea et al. 2019).

**Example**: A meadow of *C. nodosa* produces a value of 30 mmol C $m^{-2}$ $day^{-1}$ as DOC. The value in units of t C $ha^{-1}$ $year^{-1}$ is obtained as:

30 mmol C $m^{-2}$ $day^{-1}$ x $10^{-3}$ mol µmol$^{-1}$ x 12.011 g mol C x $10^{-6}$ t $g^{-1}$ x $10^{4}$ $m^{2}$ $ha^{-1}$ x 365 days $year^{-1}$ = 1.315 t C $ha^{-1}$ $year^{-1}$.

If we consider that 35% of this carbon is highly refractory, the baseline meadow would sequester every year 0.46 t C $ha^{-1}$ or in other words 1.69 t $CO_2$ $ha^{-1}$.

One should always take into account that a precise value is not representative of the system, since DOC production follows a seasonal dynamic (Egea et al. 2020). Extrapolation of a single value taken in one month of the year is subject to great uncertainty given that DOC production varies both seasonally as well as according to the aboveground biomass of the meadow or the environmental conditions. It is therefore advisable to take measurements at least once in every season of the year in order to present more reliable estimates of annual DOC flux.

For more details on the general methodology in saltmarshes and seagrass meadows, one should refer to the manual prepared by *The Blue Carbon Initiative* (Howard et al. 2019), an A2 deliverable available on the Life Blue Natura project page (http://life-bluenatura.eu/es/resultados/), also the manual developed for South-West Asia (Rahmawati et al. 2019), and the recently published IUCN manual on blue carbon project creation (IUCN 2021) , or the document on meadow techniques developed for the ECOLAGUNES project (Hernández et al. 2011). In the latter, there are also maximum baseline values for biomass and carbon value ranges for seagrasses.

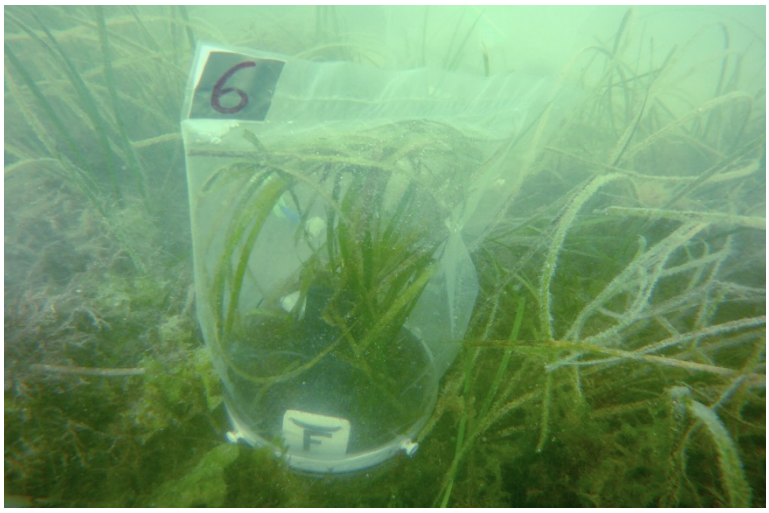


Figure 9. Example of a benthic chamber used for the estimation of DOC fluxes in a meadow dominated by *Cymodocea nodosa*.

## 2.5. Soil or sediment as an organic carbon sink

Organic carbon in soils or sediments constitutes the largest fraction of total carbon stocks in tidal saltmarshes and seagrass meadows. Ultimately it originates from biomass and is made up of plant fragments in different degrees of decomposition derived from roots, rhizomes, plant debris, etc. as well as sedimented organic material of diverse origin. To minimize any double accounting of organic carbon associated with living belowground biomass, it is best to estimate this carbon using cylinders inserted into the sediment in small visible clearings between the saltmarsh or the marine angiosperm meadow.

In general, in order to obtain mutually comparable data, the information on organic carbon content in these ecosystems is limited to the first metre of depth, although in some cases, especially in seagrass meadows (figure 10), cores of up to 5-6 m in depth can be sampled, as has been done in the Life Blue Natura project (http://life-bluenatura.eu/es/resultados/). Should you wish to obtain edaphic material using cylinders inserted into the soil or sediment, the methodology for which is not one of the purposes of this document, you are recommended to consult the manual of *The Blue Carbon Initiative* (Howard et al. 2016), available as A2 and A3 deliverables on the Life Blue Natura project page (http://life-bluenatura.eu/es/resultados/) or else the recently published IUCN handbook (IUCN 2021).

It is very important to remember that soils or sediments can be compacted inside the cylinders during extraction, therefore it is necessary to calculate the compaction correction factor in each case, a factor that is calculated by dividing the length of the sample obtained inside the cylinder (core) by the penetration length of the cylinder. This factor, of value ≤1, divides the value of the length of the core (from the uppermost surface) by the value we wish to find for the depth of the sample of soil or sediment for analysis.

**For example**: If the penetration length of the cylinder is 1.2 m and the core length obtained is 85 cm, the correction factor is 0.708. This means that a subsample of the first 20 cm from the core surface actually represents 28.25 cm of soil or sediment.

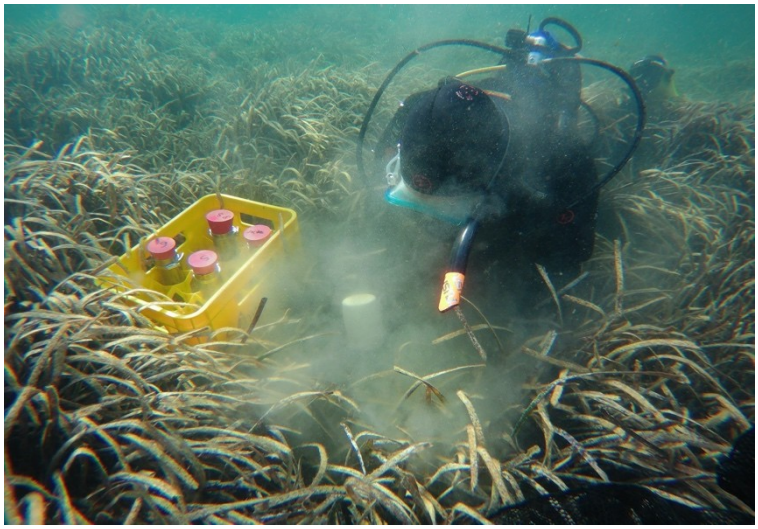

Figure 10. Collection of sediment cores in meadows of *Posidonia oceanica*.

### 2.5.1. Analysis of soil or sediment cores

Once cylinders have been collected from the soil or sediment, they should be transported to the laboratory for analysis. If this is not possible, samples from each cylinder should be taken directly on site from different intervals of depth. Ideally subsamples should be taken throughout the core, although due to difficulty, cost or convenience more samples are usually taken from the surface section and less from the deeper sections. In tidal marshes and seagrass meadows the greatest variations in organic carbon are usually found in the first 20-50 cm from the surface and therefore these depths are the ones that should be sampled most. In fact, in some work carried out in saltmarshes, cylinders of less than 1 m depth have been sampled (e.g. Sousa et al. 2017, Jiménez-Árias et al. 2020). It is essential always to record the volume taken from the subsample for subsequent calculations.

The most common and practical way to sample soil or sediment cylinders is to first estimate **the apparent density** at each depth interval analysed, homogenize each subsample and determine the organic carbon content in that subsample. One possibility is to take subsamples directly at specific depths, usually at the midpoint of each depth range considered. For example: if we wish to estimate the organic carbon content between a depth of 15 and 30 cm, we take the subsample at a depth of 22.5 cm.

Once the subsample is placed in a bag or box, it must be properly labelled, both on its container and in a notebook: code, place, depth, appearance, colour, date and any other relevant details. This information must be properly recorded in a computer file with a copy saved to the cloud. Samples should be kept in cold storage and analysed as soon as possible (the following day). Otherwise, they should be frozen for later analysis.

Once in the laboratory, the calculation of the density of edaphic carbon can be undertaken. This will involve both an estimate of the apparent density of the soil or sediment and its organic carbon content.

#### *2.5.1.1. Estimation of apparent density*

The apparent density (dimensions $M \cdot L^{-3}$) is calculated as a result of the dry weight of each sample or subsample taken (g) divided by its volume ($cm^{-3}$). Shells or fragments that are present should not be removed from the sample at this point. The dry weight of the sample is obtained after drying the sample at 60 $^{0}$C for a minimum of 24 h or for sufficient time to ensure a constant weight, a factor that will depend on the size of sample. The sample can be chopped up to in this way reduce the drying time. Before weighing each sample, it is advisable to keep it in a desiccator long enough for it to reach room temperature. The apparent density values are usually between 0.1 and 0.9 g $cm^{-3}$.

#### 2.5.1.2. Estimation of organic carbon

Estimating the organic carbon content of a soil or sediment sample is a laborious process since each will contain an organic carbon fraction as well as an inorganic fraction. First of all we have to grind the sample to homogenize it. Prior to this, we have to remove the small stones, twigs, shells, etc. The grinding can be done manually in a mortar or automatically in a ball mill.

As already noted above, carbon content is usually determined using a CNH autoanalyser. However, it is important to remember that the autoanalyser provides the value of the total carbon content of the sample and therefore, as we will see later, in the case of soils and sediments we will have to estimate the content that is inorganic (and does not count as blue carbon) in order to be able to subtract it from the total carbon in the sample. Another simple, lesser-used method of organic carbon analysis is the combusting of the sample in a muffle furnace and the subsequent use of empirical relationships between the organic matter content and the organic C content of the sample. Finally, Walkley-Black's method of wet digestion of the sample is the least recommended because it requires a special laboratory and has several limitations.

The methodology based on sample combustion and the calculation of empirical relationships between organic carbon and organic matter is relatively straightforward. However, this method is generally seldom used as it is not the most recommended method because of its use of indirect estimates and because the costs for analysing samples on a CNH autoanalyser have been gradually falling as new equipment is introduced.

In any case, this technique, known as percentage of loss on ignition (LOI), consists of estimating the mass of the sample that is lost (oxidized and emitted as gas, volatilized, etc.) when heated to high temperatures, usually at about 450-550 $^{0}$C for several hours (up to 8). This temperature ensures that the entire organic fraction of the sample is oxidized and what remains is the inorganic fraction. However, this loss on ignition represents the organic matter in the sample, where there are C, N, H, P, S, etc., so it is necessary to calculate a relationship between the percentage of organic matter and the percentage of organic carbon. This can be done by producing a straight-line (or curve) calibration from soils or sediments at the site with different organic matter, in which we estimate the organic carbon on the one hand (by the first method described based on the CNH autoanalyser) and the organic matter through loss on ignition. This is much better than relying on relationships described in the scientific literature for specific places of the following types:

For saltmarsh soils:

%C organic = 0.40 %LOI + 0.0025 $(\%LOI)^2$ (Craft et al. 1991; North Carolina)

%C organic = 0.52 %LOI - 1.17 (Ouyang and Lee 2020; overall values)

Although this second equation is based on measurements in numerous different ecosystems, it is always better to work with relationships between both variables that are developed for the site of the project.

For sediments in seagrass meadows:

%C organic = 0.298% LOI - 0.25 (Postlethwaite et al. 2018; *Zostera marina*; Canada)

%C organic = 0.43% LOI - 0.33 (Fourqurean et al. 2012; overall values; LOI >20%)

%C organic = 0.40% LOI - 0.21 (Fourqurean et al. 2012; overall values; LOI <20%)

Again, although the equations are based on measurements in seagrass meadows worldwide, it is always better to work with relationships between both variables produced for the site of the project itself (e.g. Marbá et al. 2015 or those calculated for tidal marshes, seagrass meadows and saltmarshes and presented in the various reports produced by the Life Blue Natura project, http://life-bluenatura.eu/en/results/).

In any case, in addition to being based on a relationship between variables, this method has certain limitations that have already been described in the literature, such that it may overestimate the organic carbon content in samples with a high content of inorganic carbon or clays.

#### *2.5.1.3. Estimation of inorganic carbon*

Inorganic carbon in coastal soils and sediments is generally found in the form of carbonates such as calcium carbonate ($CaCO_3$) from certain calcareous algae or shells from shellfish. Estimation of $CaCO_3$ can be done by acidification of the sample or using an elemental analyser.

If we choose acidification for the decalcification, it is best to use gentle acidification. First, a small fraction of the sample to be analysed (already homogenized) is taken and placed on a Petri dish or on a watch glass to which a few drops of hydrochloric acid (HCl) 1N are added. If $CO_2$ bubbles appear in the subsample in the form of effervescence, this is indicative of a significant inorganic carbon presence. In this case, the recommended procedure is as follows:

- Weigh 1-5 g of subsample of the dry soil or sediment that is to be analysed. Record the weight, adjusted to the nearest milligram. Bear in mind that we already know the total carbon content of the original sample.
- Add the subsample to a 125 ml flask or a 50 ml glass centrifuge tube (in case we wish to separate the dissolution sample via centrifugation).
- Cover the subsample with HCl 1N and shake for about 15 minutes, either manually or using a bath or ultrasound probe to cause evaporation of inorganic C as $CO_2$. Leave to stand for one day.
- Add a little more of the HCl dissolution. Leave to stand for a few hours until the sediment in the subsample settles.
- Separate the HCl from the subsample by decanting, centrifugation or the use of a pipette, depending on the appearance of the liquid fraction.
- Cover the subsample with distilled water. Shake gently.
- Separate the two fractions once again, as before.
- Repeat the washing of the subsample with distilled water twice more.
- Dry the subsample at 60 $^0$C for 24 h and weigh it.

The difference in weight of the subsample before and after acidification is an estimate of the inorganic carbon content. However, only 12% of the weight difference can be attributed to carbon as this percentage is that represented by this element in $CO_3CA$. For this reason, the weight difference is multiplied by 0.12 and this should now represent the actual value of inorganic carbon in the subsample. Finally, this value is subtracted from the value previously obtained for total carbon, and this provides the organic carbon content of a sample.

For example, suppose we have analysed a sediment sample in which 40% of its dry weight is carbon. We have analysed its inorganic carbon content, which results from the subtraction 3.5 - 3.1 = 0.4 g; 0.4 x 0.12 = 0.048 g = 48 mg. This means that 1.37% of that sample [(0.048/3.5) x 100] should be inorganic carbon, consequently the organic carbon in the sample will be 38.63% of its dry weight.

If we choose to use the elemental analyser, the procedure necessarily requires the prior removal of all organic C. This is achieved as follows:

- Weigh approximately 1-5 g of subsample of the dry soil or sediment to is to be analysed. Record the weight adjusted to the nearest milligram. Bear in mind that we already know the total carbon content of the original sample.
- Place the subsample in a ceramic crucible (previously weighed) and take it to a muffle furnace to be heated to 500 $^{0}$C for a minimum of 3 h to achieve volatilization of the organic compounds.
- Cool the sample to room temperature in a desiccator and weigh the crucible with the sample (adjust the weight to the nearest milligram).
- Take the sample to a CNH autoanalyser. The reading will now correspond solely to the inorganic carbon in the original sample.

Consequently, if we start with a sediment sample in which 35% of its dry weight is carbon, we have now analysed a sample of 4.352 g of that same sediment, which when taken to the muffle furnace leaves an ash residue of 1.124 g. From this residue we analyse its inorganic carbon and this turns out to be 13%. Therefore, the inorganic carbon content of the sample will be 13 x 1.124/4.352 = 3.36%. The organic carbon content of the sample will thus be 31.64%.

# 3. CALCULATION OF TOTAL ORGANIC CARBON STOCKS

The total organic carbon (TOC) stored in the baseline system is the result of the summation of the various biomass reservoirs and of the soil or sediment:

$$TOC_{system} = TOC_{biomass} + TOC_{soil}$$

Since we have estimated each of the reservoirs, it is possible to calculate the stocks in both the biomass as well as in the soil or sediment of the saltmarshes or seagrass meadows.

## 3.1. Total organic carbon stocks associated with biomass

Once all the necessary measurements and estimates have been obtained from the variables involved in the calculation of carbon stocks in the various sinks or compartments of biomass (aboveground, belowground, necromass and macroalgae), we can proceed to calculate the organic C content of each of them and its associated uncertainty.

For each fraction:

1. The average carbon content (kg C $m^{-2}$) of each biomass fraction ($F_i$) shall be determined in each of the baseline plots ($P_i$) of each homogeneous unit (HU) of tidal marsh or marine angiosperm meadow (see figure 5).

2. The units (kg C $m^{-2}$) are converted to t C $ha^{-1}$, therefore we will have to multiply the above value by 10.

$$Kg\,C\,m^{-2} \times 10 = t\,C\,ha^{-1}$$

3. Add all the values of t C $ha^{-1}$ obtained for the different $F_i$ fractions in each baseline plot of each homogeneous unit. From this we will obtain how many t C $ha^{-1}$ there are in each plot.

$$C\,organic\,P_i = \sum_{F=1}^{F=n} tC \times ha^{-1}$$

4. Find the mean organic carbon value and the error (standard deviation; $\boldsymbol{\sigma}$ or standard error; SE) in the n plots sampled in each homogeneous unit HU.

$$\overline{t\,C\,organic \times ha^{-1}}\,HU_i = \frac{\sum_{P=1}^{P=n} Carbon\,P_i}{n} \pm Error$$

5. Calculate the surface S in hectares that each homogeneous unit occupies (for example from information provided by a remote sensor, a drone, a digitally processed aerial photograph, information available on maps, etc.). Multiply the surface area occupied by each homogeneous unit by its C content and add up all the values in order to thereby work out the number of tons of organic C that represent all these compartments in the project area.

$$t\,TOC_{biomass} = \sum_{i=1}^{i=n} \overline{t\,C \times ha^{-1}}\,HU_i \times S_i$$

6. The calculation of the uncertainty associated with this estimate in the project area is obtained as the square root of the sum total of the product squared of the value of the standard deviation of each homogeneous zone (or failing this, the standard error) calculated in step 4 by its surface area in hectares:

$$\boldsymbol{\sigma}_{total} = [(\boldsymbol{\sigma}_{HU1}S_1)^2 + (\boldsymbol{\sigma}_{HU2}S_2)^2 + (\boldsymbol{\sigma}_{HU3}S_3)^2 + ..... + (\boldsymbol{\sigma}_{HUn}S_n)^2)]^{½}\ ,$$

where $\boldsymbol{\sigma}_{HU1}$ ... $\boldsymbol{\sigma}_n$ y $S_1$ … $S_n$ indicate the standard deviations and the surface areas in hectares, respectively, of each of the homogeneous units identified in the project area.

7. Present the results as t C organic ± $\boldsymbol{\sigma}_{total}$ or t C organic ± $SE_{total}$.
8. To express the value in terms of potential $CO_2$ emissions, convert the above value to t $CO_2$ ± $\boldsymbol{\sigma}_{total}$ or t $CO_2$ ± $SE_{total}$, by multiplying this value and the estimated error by the conversion factor of 3.67 which is the ratio between the molecular weight of $CO_2$ (44) and carbon (12).

$$t\,CO_{2biomass} = t\,TOC_{biomass} \times 3.67$$

## 3.2. Total organic carbon stocks associated with the soil or sediment

Since we already have all the necessary measurements and estimates of the variables involved in the calculation of carbon stocks, we can proceed to the following calculations:

1. For each interval (I) of the soil or sediment core (Co) analysed, the organic carbon density (dimensions $M \cdot L^{-3}$) is calculated as:

   Organic carbon density (g C $cm^{-3}$) = apparent density (g $cm^{-3}$) x C % organic/100.

2. Multiply this value by the length of the core interval (L; in cm) from which the previous sample comes (interval $I_i$ of the core Co).

$$g\,C\,cm^{-3}\,x\,L_i = \left(g C\,cm^{-2}\right)_{Ii}$$

3. Add together all values obtained in intervals of the same core, always indicating the total length of each core, since this is a fundamental datum for comparing estimates between zones or during the performance of a project. The units of organic carbon will therefore now be in g C $cm^{-2}$.

$$g\,C\,organic\,cm^{-2} = \sum_{I=1}^{I=n} \left(g\,C\,x\,cm^{-2}\right)_{Ii}$$

4. Convert the units to t C $ha^{-1}$, whereby we will have to multiply this value by $10^2$.

$$g\,C\,cm^{-2} \times 100 = t\,C\,ha^{-1}$$

5. Perform the same calculation for each cylinder of the single homogeneous unit HU and calculate the mean and an estimated error (standard error; SE or standard deviation; $\boldsymbol{\sigma}$) of the organic carbon values in each homogeneous unit.

$$\overline{t\,C\,organic \times ha^{-1}}\,HU_i = \frac{\sum_{Co=1}^{Co=n} C_{Coi}}{n} \pm Error$$

6. Calculate the surface area (S; in ha) of the homogeneous unit to obtain the data for tons of organic C in that zone.

$$t\,TOC_{soil} = \sum_{i=1}^{i=n} \overline{t\,C\,organic \times ha^{-1}}\,HU_i \times S_i$$

7. Perform these same operations on the remaining homogeneous units. Add up all the values and obtain the total estimate of tons of organic C in the soil or sediment in the project area.
8. The calculation of the uncertainty associated with this estimate in the project area is obtained as a sum total of the product of the standard deviation value for each homogeneous unit, or failing this the standard error (calculated in step 5) for its surface area in hectares.

$$\boldsymbol{\sigma}_{total} = [(\boldsymbol{\sigma}_A S_A)^2 + (\boldsymbol{\sigma}_B S_B)^2 + (\boldsymbol{\sigma}_C S_C)^2 + ..... + (\boldsymbol{\sigma}_n S_n)^2)]^{½}$$

   where $\boldsymbol{\sigma}_A$ ... $\boldsymbol{\sigma}_N$ and $S_A$ … $S_n$ indicate the standard deviations and surface areas in hectares, respectively, for each of the homogeneous units identified in the project area.

9. Present the results as t C organic ± $\boldsymbol{\sigma}_{total}$ or t C organico ± $SE_{total}$.
10. To express the value in terms of potential $CO_2$ emissions, convert the previous value to t $CO_2$ ± $\boldsymbol{\sigma}_{total}$ or t $CO_2$ ± $SE_{total}$, multiplying this value and the estimated error by the conversion factor of 3.67, which is the ratio between the molecular weight of $CO_2$ (44) and carbon (12).

$$t\,CO_{2soil} = t\,TOC_{soil} \times 3.67$$

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# 4. ESTIMATED CARBON DIOXIDE EMISSIONS

To understand how blue carbon ecosystems contribute to mitigating climate change, it is necessary to know not only what amount of carbon is sequestered in the soil or sediment, but the amount emitted into the atmosphere in a unit of time and in each baseline system. This value resulting from the net sequestration or emission of $CO_2$ can be directly calculated through methods that evaluate the exchange of gases or, more commonly, from the exchanges in carbon stocks as an indicator of gas exchange. However, other greenhouse gases (GHG) such as methane ($CH_4$) or nitrous oxide ($N_2O$) can only be estimated through gas exchange. The calculation of changes in carbon stocks and GHG emissions serves to establish the baseline for monitoring both sequestration rates and the trend in the emissions of these gases.

As to what approach should be followed for measuring carbon and GHG emissions, we would refer you to the recommendations made in the *Blue Carbon Initiative* document (Howard et al. 2019). Nevertheless, projects are likely to follow the methodology based on the difference in organic carbon stocks at two different points in time: during the pre-operational or zero (initial evaluation) stage, and at various time intervals measured on a scale of years up until the performance of the project. For these subsequent measurements, it is advisable to ensure that the soil or sediment has not modified its strength (or height) through accretion or erosion. This can be determined by establishing a baseline level for the soil or sediment core cylinders taken at the zero stage (for a net change in colour, for example, at a given depth), through measurements taken using a surface elevation table (Cahoon et al. 2002, Callaway et al. 2013), through information provided by government agencies or through research work in which erosion/accretion rates have been directly analysed.

In saltmarshes or seagrass meadows in a good state of conservation the emissions of $N_2O$ are generally very much reduced. They can reach a level of greater significance in degraded systems or in areas where there are significant nitrate inputs into the system, as for example through activities such as aquaculture or due to runoff. The production of $CH_4$ is conditioned by salinity, so it is generally assumed that at salinity levels greater than 18 psu the emissions of this gas are non-existent (IPPC 2014). However, the importance of the emissions of these two GHGs in tidal marshes and seagrass meadows may not be so insignificant and it may require a much deeper evaluation, as has been pointed out in various recent reviews and works of research (García-Bonet & Duarte 2017, Alongi 2020, Oreska et al. 2020, Rosentreter et al. 2021).

The estimation of $CH_4$ or $N_2O$ emissions is made using flux measurements. This is achieved using benthic chambers that enclose a surface area of the system, inside which the increases in these gases dissolved in indoor air can be estimated by means of a gas chromatograph. The use of these benthic chambers requires the standardization of various parameters such as volume, surface area

and incubation time. The flux (dimensions $M \cdot L^{-2} \cdot T^{-1}$) can be expressed in µmol gas $m^{-2}$ min-$^{1}$ and then converted to mg gas $ha^{-1}$ $year^{-1}$.

For example, if the rate of increase of $N_2O$ in the incubation chamber according to the chromatograph reading has been 4.56 µmoles $min^{-1}$ and the surface area of the chamber is 0.5 $m^2$, the rate will be 9.12 µmoles $m^{-2}$ $min^{-1}$. And therefore:

9.12 x $10^4$ $m^2$ $ha^{-1}$ x $10^{-6}$ mol µmol$^{-1}$ x 44.013 g mol $N_2O^{-1}$ x $10^{-6}$ t $g^{-1}$ x 5.256 $10^5$ min $year^{-1}$ = 2.109 t $N_2O$ $ha^{-1}$ $Year^{-1}$.

It remains for this value to be converted to $CO_2$ equivalents released into the atmosphere. For this we use the conversion factor proposed by the Intergovernmental Panel on Climate Change (IPPC) which is 265 Kg $CO_2$ Kg $N_2O^{-1}$ (IPPC 2014, www. climatechangeconnection.org/emissions/co2-equivalents/).

Therefore 2.109 t $N_2O$ $ha^{-1}$ $year^{-1}$ is equivalent to 558.89 t $CO_2$ $ha^{-1}$ $year^{-1}$.

In the case of $CH_4$, the conversion value for potential global warming proposed by this body for a period of 20 years is 84 Kg $CO_2$ Kg $CH_4^{-1}$. This conversion factor for a period of 100 years is approximately 28 Kg $CO_2$ Kg $CH_4^{-1}$, which, despite the fact that $CH_4$ persists in the atmosphere for just over a decade, it is the one used to calculate $CO_2$ equivalents (www.climatechangeconnection.org/emissions/co2-equivalents).

In any case, it has to be borne in mind that the value extrapolated per year is subject to great uncertainty since the emission rates vary both seasonally and according to environmental conditions, therefore it would be advisable to take measurements at different times of the year and by so doing present average emission values throughout the year.

It is also important to take into account the way in which carbon is lost, since in both saltmarshes and belowground seagrass meadows some carbon can be lost in the form of dissolved inorganic carbon, the dissolved or particulate organic carbon that is exported to other adjacent ecosystems and is therefore not registered by flux measurements. The degree of importance of these additional fractions is one of the main topics of interest in research into carbon dynamics in saltmarshes and seagrass meadows (Egea et al. 2019).

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