



PEMSEA Regional Methodology for Blue Carbon Project Accounting

A Harmonized Guide for Estimating Carbon Stocks, Sequestration, and GHG Reductions in Coastal Ecosystems

Annex B

Seagrass Ecosystems – Carbon Stock and Sequestration Assessment Methods



This annex is part of the publication entitled “PEMSEA Regional Methodology for Blue Carbon Project Accounting: A Harmonized Guide for Estimating Carbon Stocks, Sequestration, and GHG Reductions in Coastal Ecosystems. It benefitted from intensive consultations, discussions, and inputs from PEMSEA Country, Non-Country Partners and the PEMSEA Resource Facility supported by Kristina Di Ticman, Blue Carbon consultant. However, the contents of this publication do not necessarily reflect the views or policies of PEMSEA Country and Non-Country Partners and its other participating organizations. The designation employed and the presentation do not imply expression of opinion, whatsoever on the part of PEMSEA concerning the legal status of any country or territory, or its authority or concerning the delimitation of its boundaries.

About Us

Partnerships in Environmental Management for the Seas of East Asia (PEMSEA) is a regional coordinating mechanism for the sustainable coastal and marine development in the East Asian Seas Region.

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B.1 Introduction

Seagrass Ecosystems

Seagrasses are marine flowering plants (angiosperms) that form highly productive and ecologically significant habitats along shallow coasts worldwide. With 76 recognized species distributed across boreal, temperate, and tropical waters, Southeast Asia stands out as a global hotspot, harboring nearly 30% of global diversity (Hejnowicz et al., 2015; Sudo et al., 2021; Douglas et al., 2024). These meadows occupy intertidal to subtidal zones down to ~40 m depth, with distribution shaped by light, temperature, salinity, nutrients, and hydrodynamic energy.

Seagrass meadows provide ecosystem services of exceptional ecological and economic value, often exceeding those of terrestrial forests on a per-hectare basis. Provisioning services include their role as nurseries and feeding grounds for fish, crustaceans, and invertebrates that support both commercial and subsistence fisheries. Regulating services include improving water quality by filtering nutrients and particulates, stabilizing sediments, and buffering coasts against erosion and storm surges. Seagrass canopies dissipate wave energy while root–rhizome systems consolidate sediments, offering natural infrastructure for disaster resilience. Cultural services extend to supporting local livelihoods, food security, recreation, and heritage values.

The economic worth of these services is substantial: seagrass and algal beds have been valued at nearly US\$29,000 per hectare per year (Costanza et al., 2014), far surpassing the value of tropical forests. This highlights their importance not only to coastal communities but also to global ecological stability.

Seagrass and Their Role in Carbon Storage and Sequestration

Seagrass ecosystems are central to the concept of “Blue Carbon” due to their remarkable ability to capture and store organic carbon (C_{org}). They sequester both autochthonous carbon from their own biomass and allochthonous carbon transported from surrounding systems. Most of the carbon is stored belowground, where refractory roots, rhizomes, and trapped organic matter accumulate in anoxic sediments. These conditions allow carbon to be locked away for millennia, making seagrass soils long-term and highly stable carbon sinks.

Although seagrasses cover less than 0.1% of the ocean floor, they account for roughly 10% of annual organic carbon burial in the ocean (Hejnowicz et al., 2015) and up to 15% of marine sediment carbon stocks (Dahl et al. 2023). Local burial rates average around 1.2–1.4 tonnes C_{org}

per hectare per year (Hejnowicz et al., 2015), with stocks often exceeding 140 Mg Corg ha⁻¹ in the top meter of sediment (Serrano et al, 2021). In some regions, such as the Caribbean and Hainan Island, values are even higher, underscoring the significance of local variability and the vulnerability of these stores. Global estimates suggest that seagrasses may be up to 40 times more efficient at long-term carbon burial than terrestrial soils.

Loss of seagrass cover translates directly into lost carbon sequestration capacity and the potential release of centuries' worth of stored carbon, with global economic costs estimated in the billions of dollars annually. As such, their conservation is critical for climate mitigation and adaptation.

Threats to Seagrass

Despite their importance, seagrass meadows are among the most threatened coastal ecosystems, declining globally at a rate of ~7% per year since 1980, with even higher rates in Southeast Asia (~10.9% annually) (Sudo et. al., 2021). Drivers of decline include:

- Human activities: coastal development, dredging, destructive fishing, aquaculture expansion, nutrient enrichment, and plastic pollution.
- Physical and biological stressors: boat propeller scarring, invasive species, and disease outbreaks.
- Climate change: ocean warming, marine heatwaves, sea-level rise, and ocean acidification, all of which reduce productivity and accelerate remineralization of buried carbon.
- Extreme events: typhoons, tsunamis, and El Niño—associated disturbances can devastate entire meadows and their carbon stores.

The cumulative impact of these stressors is widespread habitat loss, degradation of ecosystem services, and erosion of blue carbon stocks.

Programs to protect, conserve and restore seagrass

Recognition of the ecological and climate importance of seagrass has driven conservation and restoration initiatives worldwide:

Seagrass Restoration Projects: Scientific restoration efforts aim to re-establish meadows to recover lost services and carbon sinks. Models suggest restored meadows can capture between 177 and over 1,300 tonnes of CO₂ per hectare within 50 years under optimal conditions. While restoration costs vary widely, the long-term benefits to fisheries, coastal protection, and carbon sequestration often outweigh the investment, especially when linked with carbon tax or credit schemes.

Blue Carbon Projects: Seagrasses are increasingly integrated into Blue Carbon strategies under voluntary carbon markets, with methodologies under development to standardize monitoring, reporting, and verification. These initiatives complement compliance frameworks and highlight opportunities for payments for ecosystem services (PES), ecotourism financing, and integration into marine protected areas. Prominent programs such as the Blue Carbon Initiative and regional PES schemes are advancing both the scientific basis and policy frameworks for sustainable management.

Despite challenges in scaling restoration and embedding seagrass in regulated carbon markets, momentum is growing. Strengthened protection, innovative financing mechanisms, and cross-sectoral policy integration are needed to ensure these ecosystems continue to support biodiversity, livelihoods, and global carbon mitigation.

B.2 Carbon Pools in Seagrass

Seagrass ecosystems contain several carbon pools that vary in size, stability, and contribution to total carbon stocks. These pools include living biomass (aboveground and belowground), soil organic carbon, non-living biomass (litter), associated epiphytes, and inorganic carbon within sediments.

Aboveground Biomass (AGB)

Aboveground biomass comprises the living above-sediment tissues of seagrass, primarily leaves and shoots. Epiphytes growing on seagrass blades are treated as a separate carbon component where included in project accounting. This pool represents the most visible component of the meadow and is subject to strong seasonal and interannual variation, particularly in temperate and boreal systems where leaf biomass may be lost during winter. The aboveground pool generally stores a relatively small proportion of the total carbon compared to soils but plays an important role in productivity, carbon turnover, and sediment trapping.

Belowground Biomass (BGB)

Belowground biomass is composed of living roots and rhizomes. While critical for sediment stabilization and long-term carbon inputs, living belowground biomass typically represents only a small fraction (~0.3%) of the total organic carbon pool stored below the surface.

Soil Organic Carbon (SOC)

Soil organic carbon is the largest and most significant pool in seagrass ecosystems. It accumulates through both:

- Autochthonous inputs, derived from the decomposition of seagrass tissues (particularly refractory roots and rhizomes) under anaerobic conditions.
- Allochthonous inputs, consisting of organic material transported from adjacent marine or terrestrial ecosystems and trapped by seagrass structures.

In many meadows, up to half of the SOC may be of allochthonous origin. Anoxic conditions within sediments allow this carbon to be stabilized and stored for centuries to millennia, making SOC the dominant long-term carbon reservoir.

Non-living Aboveground Biomass (Litter)

This pool includes detached or senescent material such as dead leaves, rhizomes, fruits, and associated algae. In most seagrass systems, litter biomass is small because plant material is rapidly decomposed or exported by tidal and wave action.

Epiphytes

Epiphytes—such as algae, diatoms, and other encrusting organisms—grow on the surfaces of seagrass blades. Although their contribution to total carbon stocks is relatively minor, they play an important role in primary productivity, nutrient cycling, and the overall functioning of the seagrass canopy.

Inorganic Carbon

Inorganic carbon in seagrass sediments is primarily present in the form of calcium carbonate (CaCO_3) from shells, coral fragments, and other biogenic sources. Seagrass beds typically contain a higher proportion of carbonate material than mangrove soils, reflecting their sandy and reef-associated environments. Accounting for inorganic carbon is particularly important in seagrass systems, as high carbonate content can complicate the accurate quantification of organic carbon stocks.

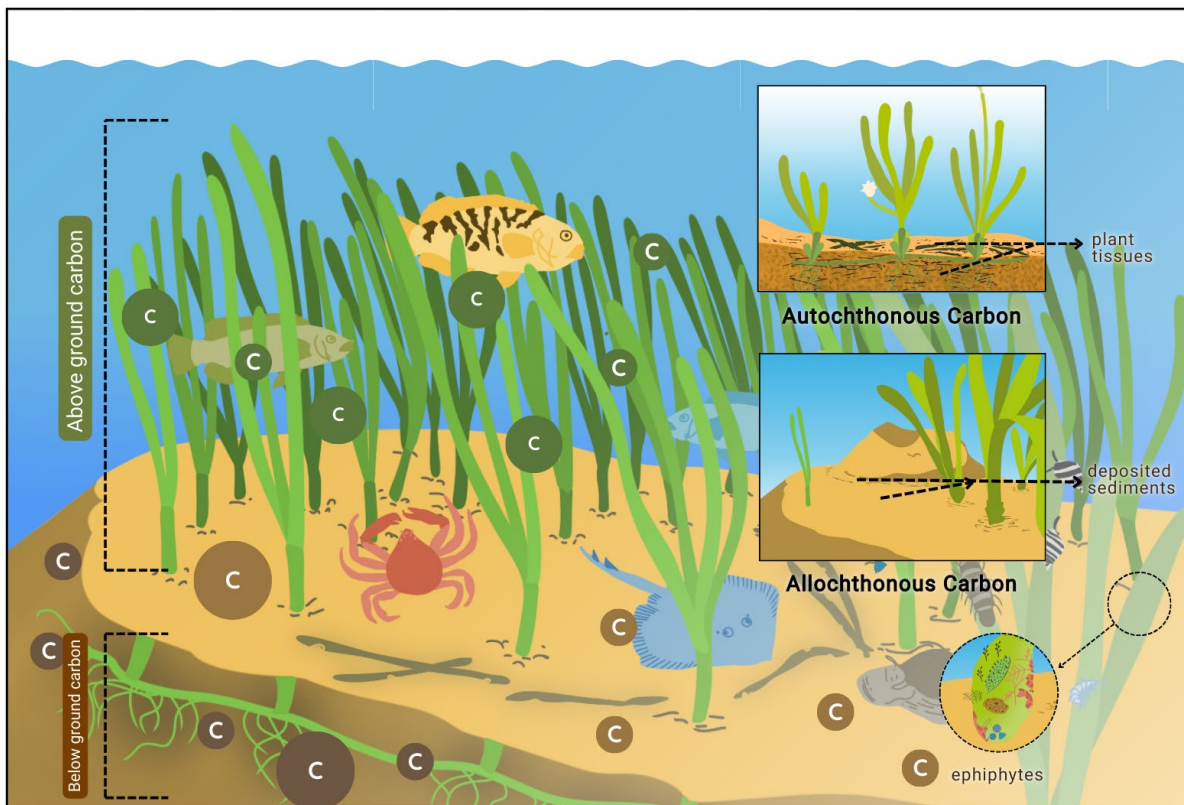


Figure 1: Carbon Pools in Seagrass

B.3 Project Design and Sampling Framework

Designing a seagrass carbon accounting project requires clear delineation of project boundaries, careful stratification of the area, and a robust sampling design. These steps ensure that estimates of carbon stocks are accurate, representative, and repeatable for long-term monitoring.

B3.1 Defining Project Area Boundaries

The first step in seagrass carbon accounting is to establish project boundaries. These boundaries define the scope of assessment and monitoring and must be determined systematically to reflect ecological representativeness while excluding adjacent ecosystems, such as mangroves or salt marshes, unless specifically included in the project. Once defined, boundaries should remain fixed to allow comparability over time; any adjustments must be documented and accounted for in carbon stock estimates.

Project boundaries are typically delineated using a combination of tools and data sources. High-resolution satellite images and aerial or drone-based photography provide baseline maps of meadow extent, while GIS platforms allow integration of these datasets with environmental layers. Ground-truthing through snorkeling, SCUBA diving, or underwater video surveys validates remote-sensing outputs, ensuring accurate identification of seagrass cover, species composition, and sediment type. Local knowledge and existing scientific literature can further refine project area mapping, particularly in regions with long-term monitoring records.

B3.2 Project Area Stratification

Seagrass meadows are rarely homogeneous. Dividing a project area into smaller, more ecologically uniform units, or strata, improves the accuracy and precision of carbon stock estimates and reduces the sampling effort required. Depth is often the primary stratification factor in seagrass systems, since species distribution, cover, and productivity are strongly influenced by light availability along bathymetric gradients.

Other important criteria for stratification include species composition and percent cover, sediment type, hydrodynamic conditions, and patterns of erosion or accretion. Meadows dominated by large, persistent species such as *Enhalus acoroides* or *Thalassia hemprichii* differ significantly in carbon storage from those dominated by smaller, short-lived species like *Halophila* spp. Similarly, high-cover meadows tend to trap more sediments and accumulate greater soil carbon compared to patchy or low-cover beds. In tropical regions, carbonate-rich sediments associated with coral reef environments also affect carbon storage and must be considered when defining strata.

Common strata in seagrass projects therefore include shallow, mid-depth, and deep meadows; areas with low, medium, or high cover; and zones dominated by different species groups. Condition-based strata may also be defined, for instance, distinguishing between natural seagrass meadows and those established through restoration. Remote sensing and GIS are essential for stratification, but validation through field surveys remains critical for confirming cover, density, and species distribution.

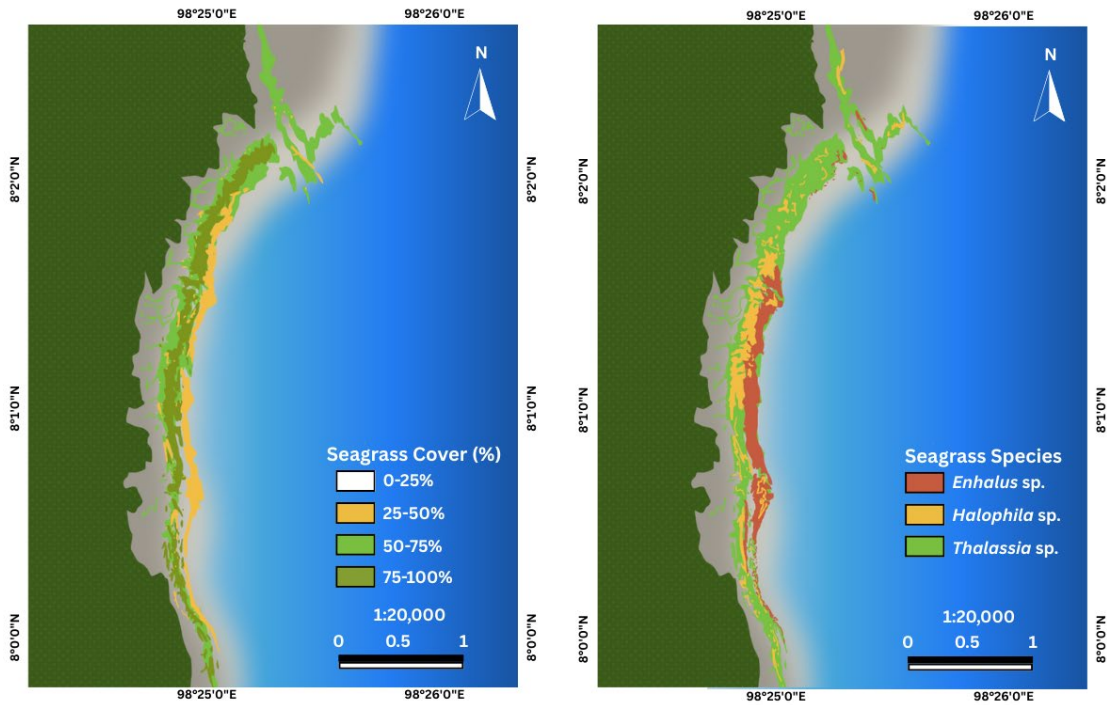


Figure 2: Sample seagrass stratification: by %cover and by dominant species

B3.3 Sampling Design

Within each stratum, representative sampling sites are selected to capture variability in biomass and soil carbon. Because it is impractical to sample an entire meadow, sampling design must balance precision with efficiency and minimal disturbance. Randomly distributed plots are often used to avoid bias, while systematic grid approaches ensure even coverage across large areas. In some settings, transect-based designs are more appropriate, particularly where clear gradients exist, such as from the shoreline to deeper parts of the meadow. For example, a set of transects laid perpendicular to shore, with samples collected at fixed intervals, can capture structural variation effectively.

B3.4 Plot Layouts

Plot size and layout depend on the carbon pool being measured. For aboveground and belowground biomass, small quadrats ranging from 0.25 to 1 m² are generally sufficient. Smaller quadrats are suited to species such as *Halophila* or *Zostera*, while larger quadrats are preferred for persistent species like *Enhalus* or *Posidonia*. Sample soil organic carbon using a sediment corer

appropriate to the substrate, sample-volume requirements and assessed depth, consistent with B4.2. Distinguish the sediment corer from the device used for destructive biomass sampling. Record its internal diameter, penetration depth, recovered length and any refusal or compression. Cores should extend to at least one meter depth, with deeper sampling up to three or five meters where feasible. Subsampling of cores at regular depth intervals provides depth profiles of carbon storage, with particular attention to the upper 20–50 cm where carbon concentrations are often highest.

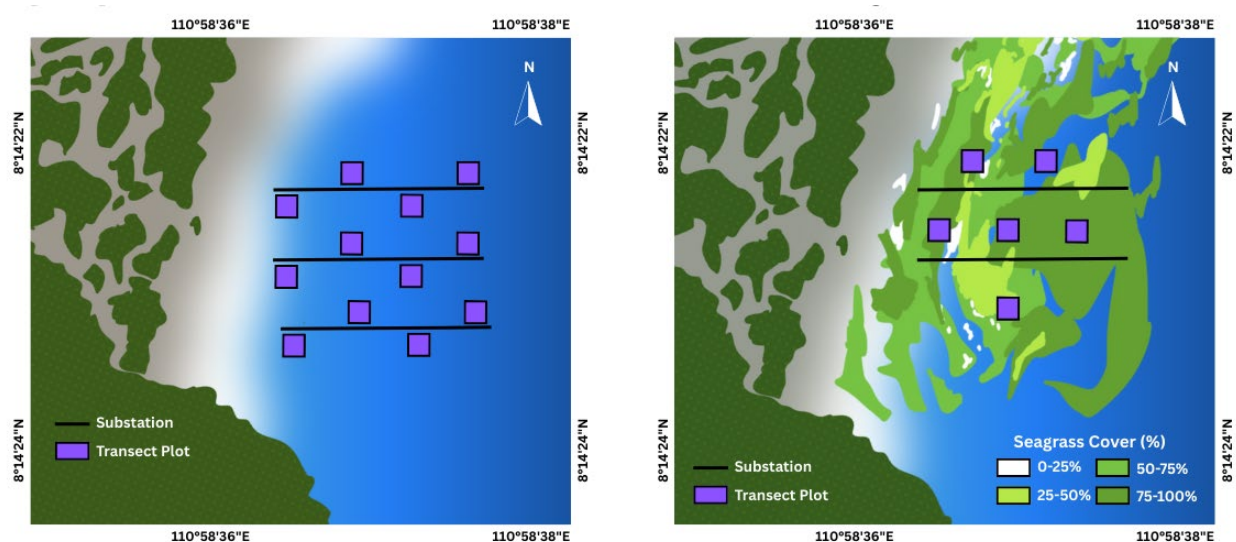


Figure 3: Sample seagrass plot layouts

B3.5 Number of Plots and Precision Targets

The number of plots required is determined by the variability of biomass and soil carbon within each stratum and the desired level of precision. At least three plots are recommended per stratum, although more may be required in heterogeneous areas. Before determining sample size, document the adopted precision target for each relevant carbon-pool estimate, including its confidence level and relative confidence-interval half-width. Determine the final sample size from preliminary variability and that target, subject to any applicable certification requirements. Preliminary surveys of 6–10 plots are useful for estimating variance and informing final sample size. To reduce the risk of data loss, it is advisable to increase the total sample size by about 10 percent. For soil carbon, aggregate samples from several systematically spaced cores within each plot can improve representativeness.

B3.6 Monitoring Schedule and Operational Considerations

Seagrass biomass and productivity vary seasonally, with patterns differing among species, geographic regions, and environmental conditions. To support comparison across monitoring periods, biomass surveys should be conducted under comparable, site-appropriate seasonal and phenological conditions. Where peak biomass is the intended reference condition, its timing should be established from local observations or relevant ecological information rather than assumed to occur during a particular season. Sampling dates, tidal conditions, and relevant environmental observations should be documented. Monitoring intervals shall be established according to the carbon pool and monitoring objectives, consistent with Section B9.2.

B.4 Data Collection and Analysis Methods

Accurate quantification of seagrass carbon stocks requires standardized methods for sampling, processing, and analyzing both biomass and soil. This section outlines recommended procedures for field data collection, laboratory analysis, and calculation of carbon stocks in seagrass ecosystems.

B4.1 Above and Belowground Biomass

Reliable estimates for quantifying seagrass biomass require standardized field methods for sampling and careful laboratory procedures for drying and analysis. Because seagrass grows in both intertidal and subtidal environments, sampling design and equipment must accommodate different site conditions, from shallow wading depths to deeper meadows accessed by boat and SCUBA.

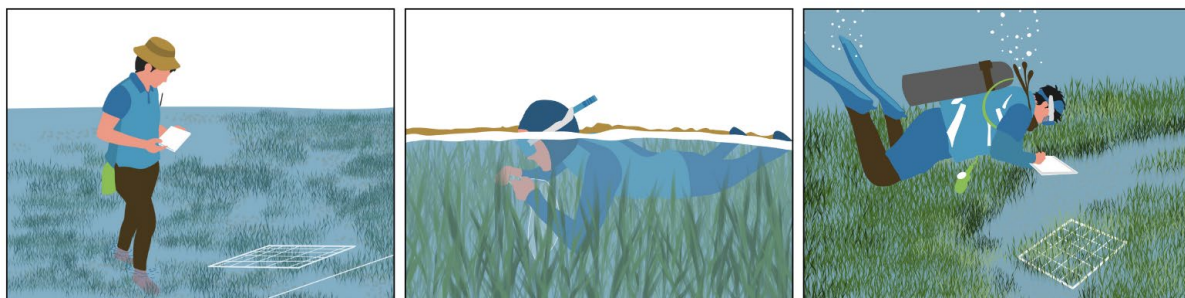


Figure 4: Seagrass assessment and monitoring methods: walk, swim, dive

B4.1.1 Equipment for Biomass Collection and analysis

Accurate biomass assessment involves both field equipment for documentation and sampling and laboratory equipment for processing and carbon analysis.

Field Equipment for Biomass Collection

- **Coring Devices**

Large-diameter core tubes (10–25 cm) are used to extract relatively undisturbed samples that include aboveground shoots and the underlying root-dominated rhizosphere, typically down to 40 cm depth. A corer with an internal diameter of 10 cm (approximately 0.00785 m² cross-sectional area) is often sufficient for small and medium-sized species such as *Halophila* spp. or *Zostera* spp., while larger cores may be required for persistent species like *Enhalus acoroides*. PVC corers of ~120 cm length are widely used in shallow meadows.

- **Tools for Driving and Removing Cores**

In firm or compact sediments, a sledgehammer or post-pounder is used to drive the corer. In soft or deep sediments, corers may require a tripod and pulley system or ladder-crank system for extraction. PVC caps and duct tape are used to seal cores immediately upon retrieval, creating suction to prevent disturbance of the core or loss of material.

- **Sample Separation and Handling Tools**

Mesh bags or sieves allow rinsing of sediment from seagrass material without losing biomass. Sharp knives or spatulas are used for trimming or subsampling cores.

- **Measurement and Location Tools**

Quadrat frames (0.25–1 m²) are placed on the seabed for visual assessment and for destructive sampling in quadrat-based harvests. A meter stick or range pole measures water and sediment depth. A handheld GPS or GPS-enabled camera records location.

- **Sample Storage and Documentation**

Plastic sample bags or vials, waterproof labels, and permanent markers are required for storing and labeling samples. Cameras or smartphones should be used to document site conditions, quadrat placement, and collected samples.

- **Logistics and Safety**

Boats, snorkeling gear, or SCUBA diving equipment are essential for subtidal meadows. Standard field safety equipment (life jackets, first-aid kits, communication devices) should be always carried.

Laboratory Equipment for Biomass Analysis

- **Drying and Storage**

Drying ovens (60–70 °C, typically ~72 h at 60 °C) are used to bring samples to constant weight. Desiccators are required for cooling dried samples without moisture gain.

- **Weighing and Preparation**

Analytical balance (± 0.001 g precision) measure dried biomass. Mortar and pestle or automated mills homogenize samples for carbon analysis.



Figure 5: Sample tools and equipment used for seagrass data collection and analysis

B4.1.2 Visual Assessment

Before biomass samples are collected, a visual assessment of seagrass meadows should be conducted within designated quadrats or census points. This non-destructive step provides baseline information on meadow structure, species composition, and site characteristics that support interpretation of carbon stock estimates.

How to assess

1. Select Census Points

Representative census points are chosen within the mapped seagrass bed. Selection should capture variations in density, configuration, and species distribution identified through remote sensing or preliminary surveys. Points may be placed randomly, along transects, or at fixed intervals to ensure spatial coverage.

2. Record Visual Parameters

Within each census point, usually defined by a quadrat (0.25–1 m²) or a radius of 3–5 meters, detailed measurements are recorded. These include:

- *Total Cover*: Estimate the percentage of area occupied by seagrass, either as exact values (in 10% increments) or using cover classes (e.g., bare, low, medium, high). Training with reference photographs can help reduce observer bias.
- *Species Composition*: Identify seagrass species present and, where possible, estimate individual species' cover. If species-level identification is challenging, categorize into functional size groups (large, medium, small species). Photographs can assist with later identification by specialists.
- *Canopy or Shoot Height*: Measure the average and maximum canopy height from the sediment surface to the leaf tip using a measuring stick, excluding outliers such as the tallest 20% of shoots.
- *Shoot Density*: Count the number of shoots within the quadrat for each species. In branching species, record whether counts refer to shoots, ramets, or apical meristems.
- *Substrate Type*: Note the dominant bottom type (e.g., mud, sandy mud, sand, coral rubble, or rock).
- *Water and Sediment Depth*: Measure depth at the quadrat using a meter stick, dive stick, or depth gauge.
- *Additional Observations*: Record the presence of macroalgae, evidence of grazing (e.g., leaf tip removal), reproductive structures such as flowers or fruits, and any signs of disturbance.

3. Photographic Documentation

At each census point, take vertical photographs directly above the quadrat or survey area to document seagrass cover, density, and substrate. These images provide visual records that can later support percent cover estimates or be used for external validation.

4. Record Location and Environmental Data

Record site information including GPS coordinates, depth, time of sampling, and tidal stage. Environmental variables such as water clarity, salinity, or temperature may also be noted if equipment is available.

B4.1.3 Sample Collection

How to Collect Data:

1. Core Insertion

Biomass samples are collected by inserting a large-diameter core tube (10–25 cm) vertically into the sediment, cutting through the aboveground shoots and into the root-dominated rhizosphere, typically to a depth of 40 cm. Care must be taken not to cut or shear the leaves at the sediment surface during insertion.

2. Sediment Removal

After extraction, the sample is transferred into a mesh bag and gently rinsed in seawater to remove loose sediment. This step minimizes contamination while preserving the integrity of roots, rhizomes, and epiphytes.

3. Sample Storage and Labeling

Cleaned material is placed in labeled plastic bags with site information, transect number, and replicate details clearly marked. Samples must be kept in a cool, moist environment, ideally in a cooler with ice packs or a damp cloth, to prevent decomposition or desiccation during transport.

4. Timing of Processing

To maintain sample integrity, laboratory processing should begin within 24 hours of collection. If delays are unavoidable, samples should be refrigerated or stored in a cold, moist environment for no more than 48 hours.

B4.1.4 Sample Processing

1. Initial Preparation

In the laboratory, samples are removed from storage bags and placed in shallow trays of clean water. This allows further sediment to settle and makes handling easier.

2. Separation of Components

Seagrass plants are separated from epiphytic algae and other material attached to leaf surfaces. Epiphytes are collected, stored, and analyzed separately to account for their biomass contribution.

3. Species Sorting

Where multiple species occur in a sample, shoots and belowground material are sorted by species. This step is important for species-level carbon accounting, as biomass-to-carbon conversion factors may differ.

4. Division into Aboveground and Belowground Parts

Samples are divided into aboveground (leaves and stems) and belowground (roots and rhizomes) fractions. Each fraction is placed in a pre-weighed drying container or envelope, ready for oven-drying.

B4.1.5 Biomass (Dry Weight) Determination

1. Oven Drying

Separated biomass fractions are dried in an oven at 60 °C until constant weight is reached, usually within 72 hours.

2. Weighing

Once dried, samples are cooled in a desiccator to avoid moisture reabsorption and then weighed using an analytical balance to the nearest 0.001 g. Dry weight values are recorded separately for aboveground biomass, belowground biomass, and epiphytes.

3. Data Entry

Recorded dry weights are entered into standardized datasheets. These values are later used in biomass-to-carbon stock calculations by applying area conversions and carbon conversion factors.

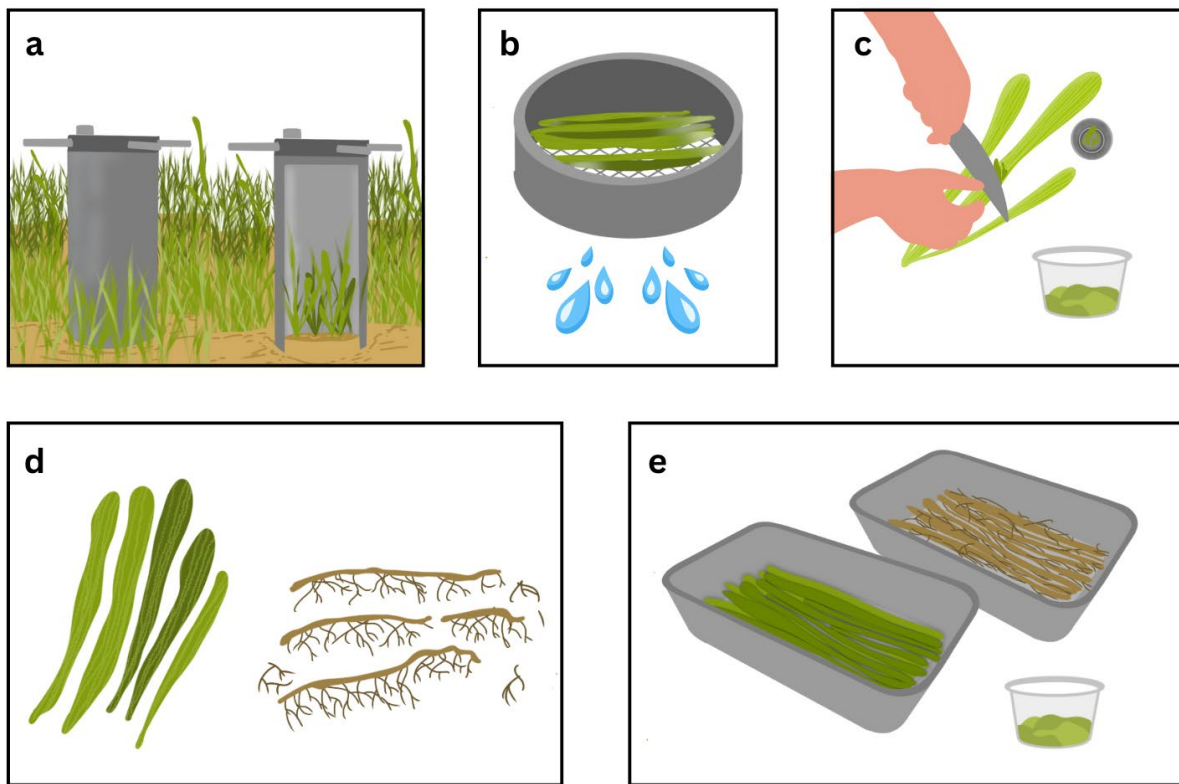


Figure 6: Step-by-step workflow for seagrass biomass sampling and processing: (a) Extract an intact sediment plug using a large-diameter core tube ($\approx 10\text{--}25\text{ cm } \varnothing$), inserting through the canopy into the rhizosphere, then cap and label. (b) Rinse the core contents on a mesh sieve to remove loose sediment while retaining plant material. (c) Scrape epiphytes from seagrass leaves into a separate, labeled container for independent quantification. (d) Sort the plant material and separate aboveground biomass (leaves/shoots) from belowground biomass (rhizomes/roots); identify by species if required. (e) Place epiphytes, aboveground, and belowground fractions in labeled trays and oven-dry at $\sim 60\text{ }^{\circ}\text{C}$ to constant mass for dry-weight determination.

B4.1.6 Total Carbon from vegetative biomass for the core

The living vegetative component is determined by multiplying the biomass of a sample of plant material for a given area by a carbon conversion factor.

a. Calculate Biomass per Core Area (g/cm^2):

The first step is to express biomass in relation to the sampled core area. Aboveground biomass is calculated as the dry mass of leaves and stems divided by the corer's surface area. Belowground biomass is similarly calculated using the dry mass of roots

and rhizomes, while epiphyte biomass is based on the dry mass scraped from seagrass leaves.

$$AGB_{core}(g/cm^2) = \frac{\text{dry mass of aboveground tissues from core (g)}}{\text{Area of core (cm}^2\text{)}} \quad \text{Equation 1}$$

Where:

$$\text{Area of core (cm}^2\text{)} = \pi \times \left(\frac{\text{diameter}}{2}\right)^2 \quad \text{Equation 2}$$

$$BGB_{core}(g/cm^2) = \frac{\text{dry mass of roots + rhizomes from core (g)}}{\text{Area of core (cm}^2\text{)}} \quad \text{Equation 3}$$

$$EPI_{core}(g/cm^2) = \frac{\text{dry mass of epiphytes scraped (g)}}{\text{Area of core (cm}^2\text{)}} \quad \text{Equation 4}$$

b. Standardize to Megagrams per Hectare (Mg/ha)

Core-based biomass values are then scaled up to a per-hectare basis. The conversion factor of 100 accounts for the difference between cm² and hectares.

$$AGB_{core}(Mg/ha) = AGB_{core}(g/cm^2) \times 100 \quad \text{Equation 5}$$

$$BGB_{core}(Mg/ha) = BGB_{core}(g/cm^2) \times 100 \quad \text{Equation 6}$$

$$EPI_{core}(Mg/ha) = EPI_{core}(g/cm^2) \times 100 \quad \text{Equation 7}$$

c. Apply Carbon Conversion Factors

Biomass is then converted to carbon stock by multiplying by a carbon fraction. A default factor of 0.34 (Howard et al., 2014) is commonly applied unless species- or site-specific values are available. For epiphytes, use a measured or justified applicable carbon fraction and document its basis.

$$C_{AGB}(Mg\ C/ha) = AGB_{core}(Mg\ \text{dry biomass}/ha) \times \text{Carbon factor} \quad \text{Equation 8}$$

$$C_{BGB}(Mg\ C/ha) = BGB_{core}(Mg\ \text{dry biomass}/ha) \times \text{Carbon factor} \quad \text{Equation 9}$$

$$C_{Epiphytes}(Mg\ C/ha) = EPI_{core}(Mg\ \text{dry biomass}/ha) \times \text{Carbon factor} \quad \text{Equation 10}$$

d. Compute Total Vegetative Carbon

The total carbon stock of the sampled vegetative components is calculated by summing seagrass aboveground biomass carbon, belowground biomass carbon, and epiphyte carbon where included. Epiphyte carbon shall be recorded separately and shall not be included within the seagrass aboveground biomass term when added as a separate component.

For multiple biomass cores or quadrats within a plot, first calculate the design-consistent mean carbon density for each pool; sum those plot-level pool densities.

$$\text{Total biomass carbon in a plot (Mg C/ha)} = C_{AGB} + C_{BGB} + C_{EPI} \quad \text{Equation 11}$$

B4.2 Soil Organic Carbon (SOC)

Soil organic carbon represents the largest and most stable carbon pool in seagrass ecosystems, often exceeding 98% of the total ecosystem carbon stock. Because biomass coring requires washing and separating plant tissues, it is essential to treat soil organic carbon sampling as a separate procedure. This ensures sediments remain intact and prevent double counting of carbon already allocated to aboveground and belowground biomass pools. In practice, biomass is harvested for AGB and BGB estimates, while SOC cores are collected, sectioned, and analyzed separately.

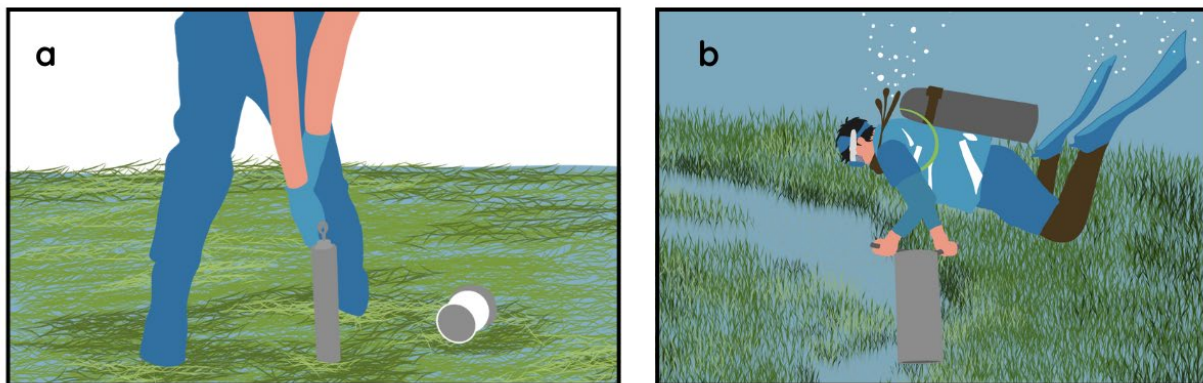


Figure 7: Seagrass coring methods: (a) Intertidal coring, (b) subtidal coring

B4.2.1 Field Equipment for Soil and Sediment Sampling and Analysis

Soil Sample Collection Equipment

Field sampling of SOC in seagrass meadows requires coring devices and handling tools designed to minimize disturbance and preserve soil structure.

- **PVC Core Tubes:** Thin-walled PVC pipe for fine sediments and thick-walled PVC pipe for coarse sediments, typically 30–120 cm in length and approximately 50–80 mm inner diameter. Corer dimensions should be selected according to sediment conditions and the required sampling depth; for SOC stock assessment, the coring system should be capable of reaching ≥ 1 m where feasible.
- **Piston Mechanism:** Rubber bung with eye bolt, washers, and nuts to assist coring in saturated soils.
- **Driving Tools:** Sledgehammer or post-pounder for inserting corers into sediment.
- **Extraction Tools:** Winch, sturdy tripod with pulley, or crank ladder for retrieving corers from soft, saturated soils.
- **Core Sealing Materials:** Rubber caps, stoppers, and duct tape to seal top and bottom of cores, maintaining suction and preventing mixing.
- **Sample Containers:** Plastic bags or rigid containers labeled with site, core ID, depth, device, and date.
- **Cool Box/Ice Box:** For transporting cores and subsamples while keeping them cool until laboratory processing.
- **GPS Device:** For precise location of sampling sites (alternatively GPS-enabled cameras or smartphones).
- **Measuring Tape/Ruler:** For measuring soil depth, core sections, and subsampling intervals.
- **Wooden or Dive Stick:** To estimate sediment depth before coring.
- **Field Documentation Tools:** Waterproof notebooks or slates, pencils, pre-prepared worksheets, and tally sheets.
- **Camera/Smartphone:** For photographic documentation of cores, seagrass cover, and site conditions.
- **Auxiliary Instruments:** pH meter, conductivity/salinity meter, and thermometer for associated water sampling.

Laboratory Equipment for SOC Analysis

SOC analysis requires precise preparation of samples, measurement of bulk density, and determination of organic carbon content using combustion or proxy methods.

- Drying Ovens: For drying sediment samples to constant weight (typically 60 °C for 48–72 hours).
- Mortar and Pestle / Mechanical Grinder: For homogenizing dried soil into a fine powder.
- Sieves: 1-mm mesh for removing coarse debris; finer mesh ($\leq 180 \mu\text{m}$) for detailed analysis.
- Desiccators: For cooling dried samples to room temperature without moisture absorption.
- Precision Balances / Microbalances: For weighing dried samples and subsamples (to 0.001 g accuracy).
- Elemental Analyzer (CHN Analyzer): For determining total carbon, hydrogen, and nitrogen via dry combustion.
- Silver Capsules and Tin Foil: For holding subsamples during combustion analysis.
- Hydrochloric Acid (HCl, 1 M): For in-situ acidification to remove inorganic carbon.
- Hotplate: For drying samples after acid treatment.
- Muffle Furnace: For Loss on Ignition (LOI) analysis, combusting samples at 450–550 °C.
- Glass Crucibles / Ceramic Boats: For LOI procedures.
- Carbonate Analyzer (optional): For measuring carbonate fractions in sediments.

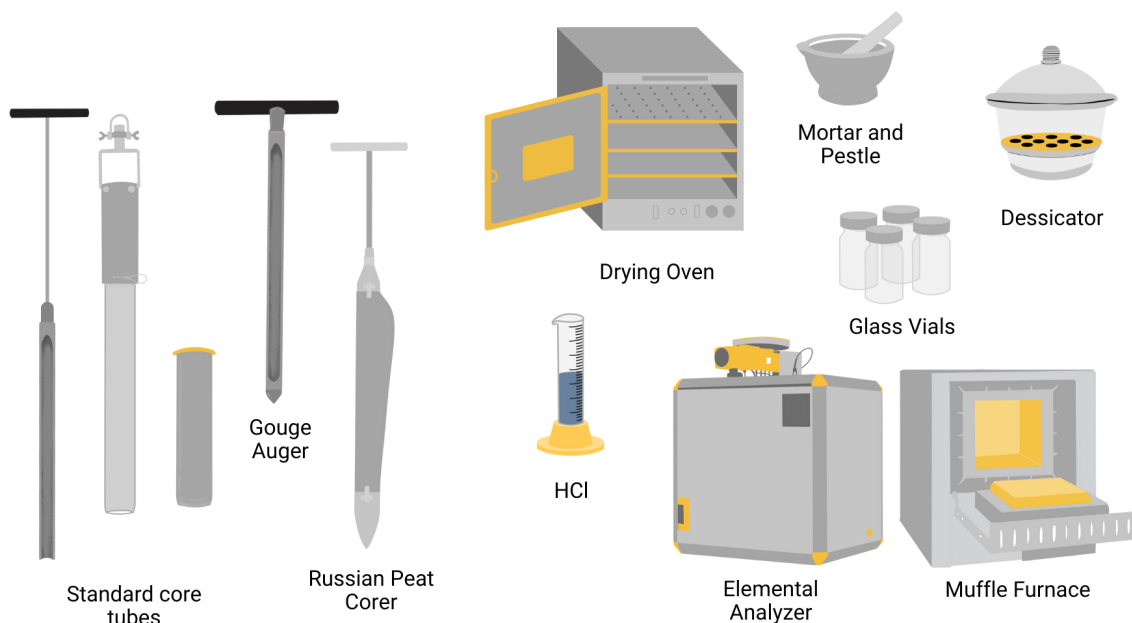


Figure 8: Tools and equipment for sediment collection and analysis

B4.2.2 Soil Sample Collection and Laboratory Analysis

1. Core Collection

Before coring, remove any loose organic litter or living leaves from the sediment surface. Insert the coring device vertically at a steady pace to minimize compaction, driving it with a sledgehammer or post-pounder if necessary, in coarse or deep sediments. Once the desired depth is reached, twist the corer to cut fine roots and seal the top with a rubber stopper or duct tape to create suction and prevent loss of material. Withdraw the corer while maintaining the seal, and immediately cap the bottom. Cores should be kept upright to preserve stratigraphy during transport. Record the penetration depth and recovered length of each soil core, noting any compression, incomplete recovery, or disturbance. Where compaction is evident, appropriate corrections shall be applied to the affected depth intervals and associated calculations. Cores with substantial disturbance or incomplete recovery that cannot be reliably corrected should be rejected and recollected. The assessment and treatment of core compaction shall apply regardless of methodological level.

2. Subsampling and Storage

Cores are sectioned into depth intervals (e.g., 0–15 cm, 15–30 cm, 30–50 cm, 50–100 cm). Subsamples are placed in labeled containers noting site, core ID, depth, and date. Samples should be transported on ice to the laboratory and frozen if processing is delayed.

3. Laboratory Processing

During laboratory preparation, separate identifiable living roots and rhizomes assigned to the belowground biomass pool from the sediment fraction. Record the removed material and retain the original sampled volume. Dry the sediment fraction at 60 °C to constant mass and calculate dry bulk density as follows:

$$DBD (g/cm^3) = \frac{\text{Dry soil mass (g)}}{\text{Volume sampled (cm}^3\text{)}} \quad \text{Equation 12}$$

Use the dry mass of the sediment fraction remaining after living-root and rhizome removal in the bulk-density calculation. Determine organic carbon concentration on a representative aliquot of that same fraction. Homogenize the sediment and document any sieving or removal of coarse material, ensuring that the mass basis used for bulk density remains consistent with that used for organic carbon concentration. Retain dead root material where it falls within the defined SOC pool.

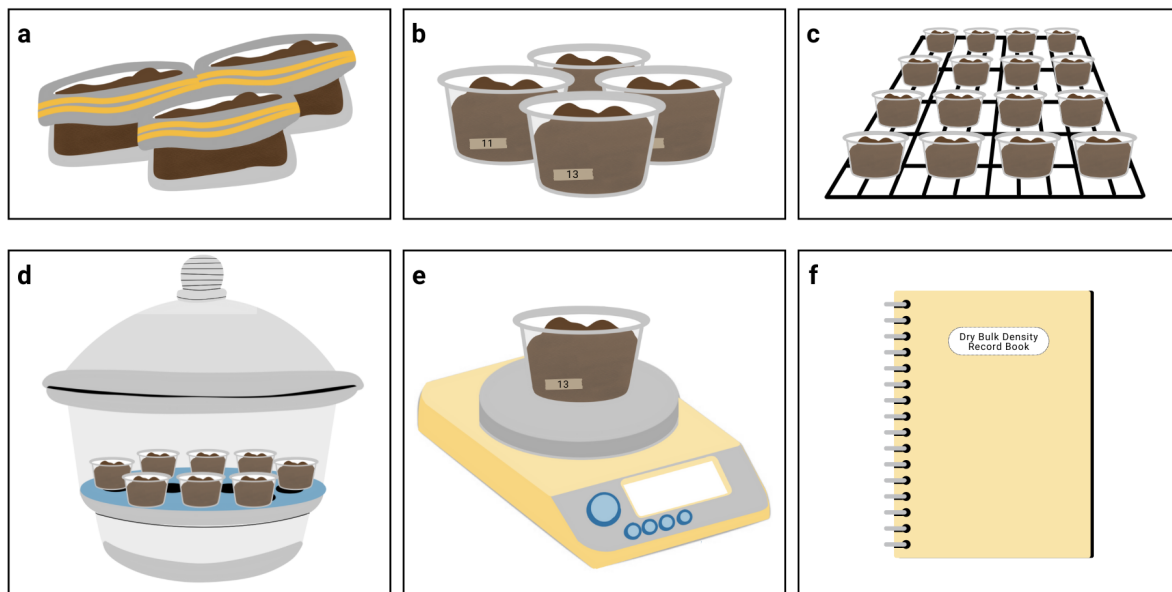


Figure 9: Workflow for determining dry bulk density of soil samples. (a) Samples are first air-dried for 3–7 days before processing. (b) Air-dried material is transferred into labeled, microwave-safe cups and weighed. (c) The samples are then oven-dried at 60 °C overnight, followed by (d) cooling in a desiccator to prevent moisture reabsorption. (e) Each cup is weighed with the dried soil sample, and the drying–cooling–weighing steps are repeated until a constant weight is achieved (defined as $\leq 4\%$ difference from the previous measurement). (f) Results are recorded in a dedicated dry bulk density logbook.

4. Testing for Carbonates

To confirm whether inorganic carbon is present, apply a few drops of 1N HCl to a small subsample on a glass plate. If bubbles of CO_2 appear, carbonate is present and acidification or correction must be applied prior to organic carbon analysis.

5. Inorganic Carbon Removal by Gentle Acidification

When carbonates are present, inorganic carbon (IC) must be removed to avoid overestimating organic carbon during LOI. A slow acidification procedure is recommended to minimize loss of organic carbon.

Procedure:

- a. Subsample preparation
Weigh approximately 5 g of dried, homogenized soil into a glass beaker or 50-mL conical tube.
- b. Initial acid test and treatment

Add enough 1N HCl to fully submerge the sample. Agitate gently (manual shaking or ultrasonic bath) for 15 minutes to break up clumps and allow uniform reaction.

c. Overnight soaking and verification

Allow the sample to sit 18–24 hours until effervescence subsides. Add additional 1N HCl and repeat agitation. If no bubbles appear, carbonate removal is complete.

d. Rinsing

Once soil settles (or after centrifugation), decant the acid (or remove the liquid with a pipette). Rinse with distilled water, allow settling or centrifuge again, and decant. Repeat three times.

e. Drying

Dry the acid-treated sample at 60 °C overnight, cool in a desiccator, and reweigh. Determine organic carbon on an aliquot using a validated carbonate-correction or removal procedure. Express organic carbon relative to the original dry-sediment mass basis used in the stock calculation, documenting mass changes, recovery and applicable corrections. Determine bulk density on a separate untreated aliquot of known volume. Do not assume that acid-treatment mass loss consists entirely of carbonate or use that loss as a direct SOC measurement.

After drying, the decalcified sample is ready for LOI combustion.

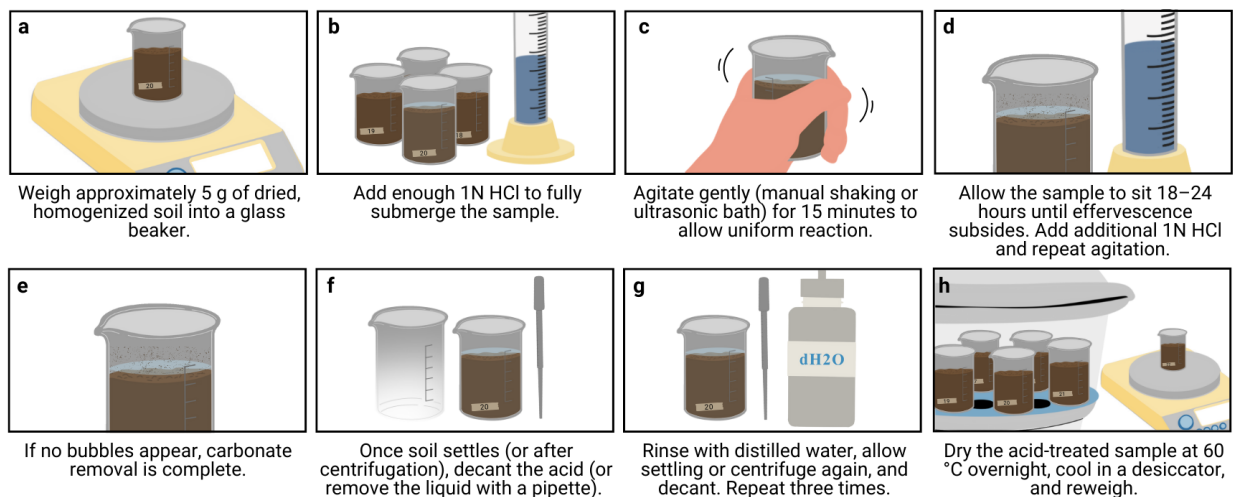


Figure 10: Acidification steps

B4.2.3 Estimating Organic Carbon Content (%C_{org}) using Loss on Ignition

LOI estimates organic matter content by measuring mass loss during high-temperature combustion.

Procedure:

1. Dried and homogenized sediment samples are weighed into pre-weighed crucibles.
2. Samples are combusted in a muffle furnace at 450 °C for 4–8 hours, or at 550 °C for at least 3 hours.
3. The percentage mass loss is calculated as:

$$\%LOI = \frac{W_{pre} - W_{post}}{W_{pre}} \times 100 \quad \text{Equation 13}$$

4. Conversion to %C_{org} is made using empirical equations developed for seagrass soils (Howard et. al.):

- For seagrass sediment samples with LOI below 20%:

$$\%C_{org} = -0.21 + (0.40 \times \%LOI) \quad \text{Equation 14}$$

- For samples with LOI of 20% or greater, use the full-range relationship:

$$\%C_{org} = -0.33 + (0.43 \times \%LOI) \quad \text{Equation 15}$$

Enter LOI as a percentage value—for example, 20 for 20%, not 0.20. The calculated organic carbon concentration is also a percentage value. These empirical relationships require assessment of their suitability for local sediments. Negative predicted concentrations indicate that the relationship is unsuitable for that sample and require direct organic-carbon analysis or a validated alternative.

LOI may overestimate organic carbon if carbonate or structural water is lost during combustion. To reduce uncertainty, calibration with a subset of samples analyzed by elemental analyzer is strongly recommended.

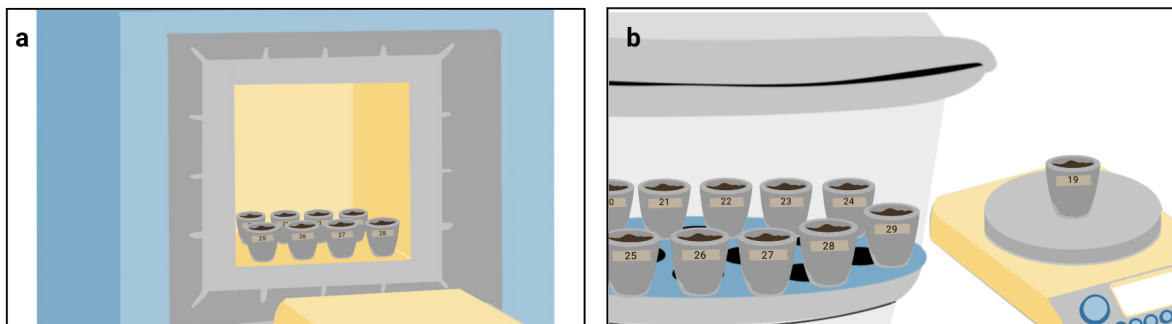


Figure 11: Subsample processing for Loss on Ignition (LOI): (a) Soil subsamples are placed inside a muffle furnace and combusted at 450–550 °C for 4–8 hours to oxidize organic matter. (b) After combustion, samples are allowed to cool to room temperature inside a desiccator to prevent moisture reabsorption. Each crucible is then weighed to determine post-combustion mass for organic matter and carbon content calculations.

B4.2.4 Estimating Organic Carbon Content (%C_{org}) using Elemental Analysis

Elemental analysis via a CHN analyzer is considered a more accurate method for determining soil organic carbon.

Procedure:

1. Dried, homogenized subsamples (2–200 mg depending on sediment type) are weighed into pre-combusted silver capsules.
2. Samples containing carbonate are acidified (in-situ HCl addition) and dried before combustion.
3. Capsules are wrapped in tin foil and analyzed in an elemental analyzer, which combusts samples at high temperature and quantifies C, H, and N by gas chromatography or infrared spectroscopy.
4. Following removal of, or correction for, inorganic carbon, the resulting percentage of carbon by dry weight represents the organic carbon content.

$$\%C_{org} = \text{Measured \%C from CHN}$$

Equation 16

B4.2.5 Total SOC for the core

- a. Determine SOC per Soil Layer

For each layer, the SOC content is calculated by combining bulk density, organic carbon concentration, and layer depth.

$$SOC_{layer} (Mg\ C/ha) = Depth\ (cm) \times DBD\ (g/cm^3) \times \frac{\%C_{org}}{100} \times 100 \quad \text{Equation 17}$$

The factor 100 converts from g C/cm² to Mg C/ha.

- b. Sum Across Soil Depths

The SOC stock for an entire core is calculated by summing the SOC values from all depth intervals. This produces an estimate of the soil organic carbon pool per unit area.

$$SOC_{core} (Mg\ C/ha) = \sum (SOC_{layer\ 1} + SOC_{layer\ 2} + SOC_{layer\ 3} + \dots) \quad \text{Equation 18}$$

Where $SOC_{layer\ 1}$, $SOC_{layer\ 2}$, $SOC_{layer\ 3}$... are the SOC for each layer/depth.

Where multiple cores represent one plot, calculate plot SOC density as the design-consistent mean of representative core SOC densities on the same assessed depth basis. Apply sampling weights where required. Treat cores within a plot as subsamples unless the sampling design defines them as independent sampling units.

- c. SOC Reporting Depth

SOC stocks are normally reported for the upper 1 m of soil. Where sampling extends beyond 1 m, calculate and report the standardized stock using only the measured intervals within 0–100 cm; deeper stocks may be reported separately. Where sampling reaches less than 1 m, report the stock for the actual sampled depth and state that depth explicitly. Do not extrapolate a shallower measured stock to 1 m using a simple depth ratio. Comparisons between monitoring periods shall use a consistent depth basis.

B4.2.6 Sediment Accretion Monitoring Using RSET and Marker Horizon

Sediment accretion represents the rate at which new soil layers accumulate and is fundamental for calculating the Carbon Accumulation Rate (CAR)—the combination of sedimentation rate, soil dry bulk density, and organic carbon content (%C_{org}).

Accretion can be measured using a Rod Surface Elevation Table (RSET) in tandem with a Marker Horizon (MH). The RSET, a portable mechanical leveling device, is mounted on a permanent benchmark pole driven deeply into stable substrate, providing a fixed reference for repeated elevation measurements. The Marker Horizon comprises a visually distinct, inert layer—such as feldspar or clean sand—placed on the sediment surface within a marked quadrat adjacent to the RSET benchmark. These structures become permanent monitoring stations revisited seasonally or annually to track surface elevation change (via RSET) and vertical sediment deposition (via MH).

Surface elevation change shall not be substituted for sediment accretion rate in the carbon accumulation calculation. Accretion measurements combined with sediment carbon density estimate carbon accumulation over the monitoring interval; they do not alone demonstrate long-term net burial.

Deployment and Monitoring Protocol:

1. Site Selection and Setup:

Choose representative shallow seagrass areas (e.g., inner bay, mid-bed, meadow edge). Record GPS coordinates and install visible markers for site relocation.

2. Benchmark Pole and RSET Installation:

Drive a stainless-steel rod into firm substrate beneath the meadow—ideally into consolidated sediment or underlying substrate—leaving the top exposed. The RSET arm, mounted on this rod during each visit, uses its pins to measure sediment surface elevation relative to the fixed benchmark.

3. Marker Horizon Application:

Adjacent to the RSET, lay a $\sim 0.25 \text{ m}^2$ quadrat and apply a $\sim 1 \text{ cm}$ layer of inert marker (feldspar, clean sand). Clearly mark the quadrat for consistent relocation.

4. Initial Baseline Measurement (T_1):

Using the RSET arm, lower each pin to gently contact the sediment surface and note the elevation. Record the Marker Horizon depth as zero.

5. Repeat Monitoring ($T_2, T_3, \dots$):

At consistent intervals, revisit the station, repeat RSET measurements, and extract small cores (5–10 cm dia.) down to the marker horizon. Measure depth of new sediment since the marker horizon and process samples for bulk density and carbon content to quantify carbon associated with accreted material.

Seagrass-Specific Constraints & Alternative Methods

Although the RSET–MH methodology works well in intertidal wetlands, its application in submerged seagrass meadows faces practical challenges. Marker horizons frequently wash away under persistent submersion, while installing stable benchmarks in soft, mobile seabed sediment is logistically difficult. Moreover, elevation data may be affected by root dynamics, subsidence, or compaction, complicating interpretation.

Recent advances offer more suitable alternatives. The subsurface sediment plate method has been developed specifically for seagrass systems. This non-destructive technique involves inserting a plate beneath the surface and re-inserting the sediment plug above it. Repeated measurements of sediment depth above the plate can accurately capture both short-term and long-term accretion and erosion dynamics in submerged meadows. It has been shown to align well with traditional ^{210}Pb dating methods and offers real-time tracking, even capturing episodic events such as storm-related erosion or deposition (Ewers Lewis & McGlathery, 2023).

Another seagrass-adapted method involves Surface Elevation Change Pins (SECP) coupled with marker horizons, but studies suggest marker horizon persistence is limited due to tidal flow and weak retention in soft sediments (Ewers Lewis & McGlathery, 2023; Potouroglou et al., 2017).

B4.2.7 Isotope Sampling for Burial Rates

Isotope dating, particularly using Lead-210 (^{210}Pb), complements soil organic carbon (SOC) analysis by extending the temporal scale of sediment carbon assessments from decades to centuries. While SOC approaches measure present-day carbon content and accumulation rates from soil cores, ^{210}Pb dating reconstructs historical sedimentation trends.

By analyzing ^{210}Pb decay profiles in sediment cores, sedimentation rates (e.g., cm yr^{-1}) can be derived. Combined with bulk density and $\%C_{\text{org}}$ data from the same samples, this method enables reconstruction of long-term Carbon Accumulation Rates. Thus, the same core collection, preparation, and carbon analysis workflow used for SOC can also support isotope-based burial estimates—streamlining logistics by integrating laboratory and sampling procedures.

Field Procedure (Sediment Core Collection and Subsampling):

1. Core Extraction

Select representative sites across the strata. Using a gouge auger, Russian peat corer, or vibracorer, extract undisturbed sediment cores to at least 1 m depth (or until refusal), ensuring vertical insertion and gentle rotation to preserve sediment structure.

2. Orientation and Labeling

Mark the "top" and "bottom" clearly on each core. Log GPS coordinates, date, and core ID in field records for traceability.

3. Core Description

In situ or in the lab, document core stratigraphy (e.g., color, grain size, layering, root presence) and photograph each core with a scale for future reference.

4. Subsampling Intervals

Section cores at high resolution near the surface (e.g., every 2 cm for the upper 10 cm) and at coarser intervals (e.g., 5 cm) deeper down or follow a project-specific design.

5. Preservation

Transfer subsamples into sealed, labeled containers and store at ~4 °C or freeze within 24 hours. When cold storage is impractical (e.g., in remote field settings), consider air-drying or vacuum-sealing as temporary preservation.

Laboratory Analysis:

6. Bulk Density (BD)

Dry each subsample at 60 °C to a constant weight. Compute bulk density by dividing the dry mass by the subsample volume.

7. Organic Carbon Content (% C_{org})

Analyze dried samples using a CHN elemental analyzer. Where that is unavailable, Loss on Ignition (LOI) may be used with appropriate calibration and carbonate corrections.

8. Isotope Analysis:

Measure the total ^{210}Pb activity using alpha spectrometry with PIPS detectors. Derive excess ^{210}Pb ($^{210}\text{Pb}_{\text{xs}}$) by subtracting supported ^{210}Pb from the total. Construct age–depth models using either the Constant Flux–Constant Sedimentation (CF–CS) or

Constant Rate of Supply (CRS) model. Calculate sedimentation rates (Z) from the slope of the age–depth profile.

B.5 Carbon Stock Estimation for Seagrass Project Area

The total carbon stock within a seagrass project area is calculated by systematically aggregating carbon stocks from individual plots to strata and then to the entire project area. This hierarchical approach ensures consistency in reporting at multiple scales and provides estimates both as Mg/ha (per unit area) and as total Mg C (absolute carbon stock).

B5.1 Compute Total Carbon Stock per Plot (Mg/ha)

For each plot, calculate the carbon stock for each measured pool (e.g., aboveground biomass, belowground biomass, epiphytes, and soil organic carbon). The calculations follow the methods described for each pool, expressed in Mg/ha.

Where epiphytes are included as a separate carbon component, seagrass aboveground biomass estimates shall exclude epiphyte material to avoid double counting.

- a. Measure Carbon Stock for Each Pool:

$C_{AGB}(MgC/ha)$: Aboveground biomass carbon stock per hectare.

$C_{BGB}(MgC/ha)$: Belowground biomass carbon stock per hectare.

$C_{EPI}(MgC/ha)$: Epiphyte biomass carbon stock per hectare.

$C_{SOC}(MgC/ha)$: Soil organic carbon stock per hectare.

- b. Sum Carbon from All Pools

Use plot-level pool densities derived from the applicable sampling and averaging procedures, not the sum of replicate core densities.

$$C_{Plot}(Mg\ C/ha) = C_{AGB} + C_{BGB} + C_{EPI} + C_{SOC} \quad \text{Equation 19}$$

B5.2 Aggregate Carbon Stock to Stratum Level

For stratified seagrass project areas, carbon stock is aggregated to the stratum level to account for spatial variation (e.g., depth zones, species dominance, or cover categories).

- a. Calculate Average Carbon Stock per Stratum:

Average carbon stock for all plots within a stratum:

$$C_{Stratum}(Mg\ C/ha) = \frac{\sum C_{Plot}(Mg/ha)}{Number\ of\ Plots\ in\ the\ Stratum} \quad \text{Equation 20}$$

- b. Calculate Total Carbon Stock for the Stratum:

Multiply the average carbon stock by the total area of the stratum:

$$C_{Stratum}(Mg\ C) = C_{Stratum}(Mg\ C/ha) \times Stratum\ Area\ (ha) \quad \text{Equation 21}$$

B5.3 Compute Total Carbon Stock for the Project Area

- a. Sum Carbon Stocks Across All Strata:

Total carbon stock for the project area is the sum of carbon stocks across all strata:

$$C_{Project\ Area}(Mg\ C) = \sum C_{Stratum}(Mg\ C) \quad \text{Equation 22}$$

- b. Calculate Average Carbon Stock for the Project Area:

The average carbon stock for the project area (reported in Mg C/ha) is calculated as the weighted average:

$$C_{Project\ Area}(Mg\ C/ha) = \frac{\sum [C_{Stratum}(Mg\ C/ha) \times Stratum\ Area\ (ha)]}{Total\ Project\ Area\ (ha)} \quad \text{Equation 23}$$

B5.4 Uncertainty in Carbon Stock Estimation

Accurate reporting of carbon stock estimates in seagrass projects requires not only the measurement of each carbon pool but also the quantification of uncertainty associated with those measurements.

B5.4.1 Error Propagation

a. Estimate Carbon Stock and Uncertainty for Each Carbon Pool

For each plot or core, calculate carbon stock for vegetative biomass (aboveground biomass, belowground biomass, and epiphytes) and soil organic carbon (SOC). For each pool, results should be expressed as:

$$\text{Carbon Stock}_{\text{pool}} = \text{Mean} \pm \text{Standard Error} \quad \text{Equation 24}$$

where SE (standard error) is computed from replicate plots or samples within the stratum.

- AGB, BGB, Epiphytes: Estimated from biomass samples collected using quadrats or cores, dried to constant weight, and converted to carbon stock using species-specific or default conversion factors.
- SOC: Estimated from soil bulk density, organic carbon concentration (%C_{org} from CHN analysis or LOI), and core depth.

b. Combine Uncertainty from All Carbon Pools (Error Propagation)

Once uncertainties are calculated for each pool, combine them using the root-sum-square (RSS) method:

$$SE_{\text{Total Pools}} = \sqrt{(SE_{\text{AGB}})^2 + (SE_{\text{BGB}})^2 + (SE_{\text{EPI}})^2 + (SE_{\text{SOC}})^2} \quad \text{Equation 25}$$

This provides the stand-level or plot-level total carbon stock uncertainty, typically expressed in Mg C/ha.

c. Account for Uncertainty in Area Estimates

Area estimates derived from GIS or remote sensing also carry uncertainty, which should be reported as:

$$Area_{pool} = Mean\ Area \pm Standard\ Error_{Area} \quad \text{Equation 26}$$

d. Propagating Stock and Area Uncertainty

To calculate total uncertainty for the project area, propagate both stock and area uncertainty as:

$$SE_{total} = Total\ Carbon\ Stock \times \sqrt{\left(\frac{SE_{Stock}}{Carbon\ Stock}\right)^2 + \left(\frac{SE_{Area}}{Area}\right)^2} \quad \text{Equation 27}$$

e. Report Total Carbon Stock with Uncertainty

The final result is presented as:

$$Total\ Carbon\ Stock = Mean \pm SE_{total} \quad \text{Equation 28}$$

Values should be presented with clear units (e.g., Mg C/ha), and where applicable, converted to Mg CO₂ using a 3.67 molar mass factor.

Root-sum-square propagation assumes independent errors. Where estimates share a model, carbon fraction, laboratory calibration or area estimate, account for that dependence. When combining strata, apply the corresponding area weights and include area uncertainty where material, following main Chapter 6.

B5.4.2 Monte Carlo Simulation (Optional Advanced Method)

Monte Carlo Simulation is a statistical approach used to account for uncertainty by running thousands of simulations that generate a distribution of possible outcomes. For seagrass carbon stock assessments, this method allows uncertainties in multiple parameters to be propagated simultaneously, providing a more robust estimate of overall carbon storage.

In seagrass projects, uncertain parameters typically include biomass estimates (aboveground, belowground, and epiphytes), soil bulk density, organic carbon concentration (%C_{org}), sediment depth, and mapped area. By sampling from probability distributions defined for these inputs, the simulation produces a range of possible total carbon stock values, from which both the mean and associated uncertainty (e.g., standard deviation, confidence intervals) can be reported.

Monte Carlo Simulation Steps Summarized:

a. Define Uncertain Inputs

Identify key variables with measurement or sampling uncertainty, such as aboveground biomass, belowground biomass, epiphyte biomass, soil bulk density, %C_{org}, sediment depth, and seagrass area. Assign appropriate probability distributions (e.g., normal, lognormal) to each parameter based on field data, literature, or expert judgment.

b. Run Random Simulations

Conduct thousands of iterations (typically 5,000–10,000). For each iteration, randomly sample from the distributions of each input variable and compute total carbon stock using the equations outlined in previous sections.

c. Analyze Output

Summarize the distribution of simulation results by calculating the mean, variance, standard deviation, and confidence intervals of total seagrass carbon stock.

d. Report Results

Present the outcomes as mean $\pm$ standard deviation or with 95% confidence intervals. Results can be expressed both per hectare (Mg C/ha) and as the total for the project area (Mg C).

e. Visualize

Graphical representations such as histograms or probability density plots can be used to illustrate the uncertainty range, the most likely values, and the overall distribution of carbon stock estimates.

B.6 Carbon Sink (Sequestration Rate) Estimation in Seagrass Ecosystems

Estimating carbon sequestration in seagrass ecosystems requires quantifying the net annual increase in stored carbon across different pools—most notably aboveground biomass, belowground biomass, epiphytes, and soil organic carbon. Depending on available data, monitoring design, and ecosystem characteristics, two principal methods are used: the stock-difference method and the gain–loss method.

The stock-difference method involves measuring carbon stocks in each relevant pool at two time points and calculating the net change over time. This approach is well-suited for monitoring carbon accumulation in seagrass restoration or protection projects where long-term plot-based measurements are feasible. It is particularly robust for aboveground biomass and soil organic carbon, as these pools tend to show measurable change over multi-year periods. When baseline and subsequent stock measurements are obtained through standardized field methods, this approach offers a direct and transparent estimate of sequestration.

In contrast, the gain–loss method estimates annual carbon fluxes by measuring gains—such as biomass production (leaf elongation, shoot growth, rhizome extension), and sediment deposition—and subtracting losses due to decomposition, grazing, or disturbance. In seagrass meadows, this method is commonly applied where high-resolution process data are available, including leaf production rates, shoot turnover, surface accretion (using marker horizons or sediment plates), and sediment carbon content. Isotope-based dating methods (e.g., ^{210}Pb) may also be used to estimate long-term soil carbon burial rates in seagrass sediments. The gain–loss method is especially useful for characterizing carbon dynamics at finer temporal and ecological scales, including sites experiencing rapid sedimentation or erosion.

Both methods are valid for estimating sequestration rates in seagrass ecosystems, and in many cases, a combination is applied—using stock-difference for vegetative biomass pools and gain–loss for soil carbon. The choice of method depends on project objectives, monitoring capacity, and the pools of interest. In all cases, the estimation should aim to meet minimum accuracy thresholds required for credible carbon accounting and potential carbon crediting.

B6.1 Stock-Difference Method for Seagrass

The stock-difference approach requires field-based measurements from the same sampling plots at two points in time (baseline and monitoring year). These values are then annualized and scaled to the project area.

Field-derived carbon stock values used here must come from consistent plot locations, methodologies, and stratification categories, as defined in the field data collection and stock estimation chapters (B.4 and B.5). Where relevant, the mean carbon density (e.g., Mg C/ha) per pool per stratum is used and scaled by the area represented by the sample plots.

B6.1.1 Carbon Stock Change in Aboveground Biomass

Aboveground biomass in seagrass ecosystems consists of leaves and shoots. To calculate stock change, biomass samples are collected using quadrats or cores at t_1 and t_2 , dried to constant weight, converted to carbon using a factor, and expressed as Mg C/ha.

Stock-difference formula:

$$\Delta C_{AGB} = \frac{(C_{t2,AGB} - C_{t1,AGB}) \times A}{t_2 - t_1} \quad \text{Equation 29}$$

Where:

- ΔC_{AGB} = Annual change in AGB carbon (Mg C/yr)
- $C_{t1,AGB}, C_{t2,AGB}$ = Mean AGB carbon stock at t_1 and t_2 (Mg C/ha)
- A = Area of ecosystem represented (ha)
- $t_2 - t_1$ = Time interval between measurements (years)

B6.1.2 Carbon Stock Change in Belowground Biomass

Belowground biomass consists of roots and rhizomes. It is measured directly through core samples. Measurements must be consistent across time intervals.

Formula:

$$\Delta C_{BGB} = \frac{(C_{t2,BGB} - C_{t1,BGB}) \times A}{t_2 - t_1} \quad \text{Equation 30}$$

The same spatial and temporal principles apply as with AGB.

B6.1.3 Carbon Stock Change in Epiphytes (if included)

Epiphytes growing on seagrass blades may be monitored if they contribute significantly to overall carbon storage. Their biomass is scraped, dried, and weighed at t_1 and t_2 .

$$\Delta C_{EPI} = \frac{(C_{t2,EPI} - C_{t1,EPI}) \times A}{t_2 - t_1} \quad \text{Equation 31}$$

B6.1.4 Carbon Stock Change in Soil/Sediment

SOC stock at t_1 and t_2 is estimated using bulk density, %Corg, and core depth, aggregated to Mg C/ha, and then compared across time intervals.

Formula:

$$\Delta C_{SOC} = \frac{(C_{t2,SOC} - C_{t1,SOC}) \times A}{t_2 - t_1} \quad \text{Equation 32}$$

Sampling depth should be consistent across time periods (e.g., 1 meter) or reported separately by depth intervals if different.

B6.1.5 Estimating Total and Annual Sequestration from Stock Change

Once each carbon pool's annual change has been estimated, the net annual sequestration is the sum across all included pools:

$$\Delta C_{total} = \Delta C_{AGB} + \Delta C_{BGB} + \Delta C_{EPI} + \Delta C_{SOC} \quad \text{Equation 33}$$

This estimate is expressed in Mg C /yr. To convert to CO₂-equivalent:

$$\Delta CO_2e = \Delta C_{total} \times 3.67 \quad \text{Equation 34}$$

Where 3.67 is the molecular weight ratio of CO₂ to C. This conversion expresses the carbon-stock change as a CO₂ quantity. A stock increase represents a removal, while a stock decrease represents an emission. Apply the emissions-positive/removals-negative convention when entering the result into the GHG balance. Calculate project net GHG benefits relative to the baseline, including applicable project emissions and leakage, in

accordance with main Section 9.6. The converted stock change alone does not establish an issuable credit quantity, and any gain or loss already represented in the stock change shall not be counted again.

The accuracy of this estimate is contingent on plot representativeness, measurement consistency, and time interval. Annualization should only be applied when the time difference ($t_2 - t_1$) is at least 1 year, preferably 2–5 years for greater reliability.

B6.2 Gain–Loss Method for Seagrass

The Gain–Loss method estimates net annual carbon sequestration by balancing measured carbon gains (e.g., seagrass biomass production, sediment carbon burial) against carbon losses (e.g., decomposition, disturbance, greenhouse gas emissions).

This method is especially useful when:

- Permanent plots provide growth or productivity data but long-term repeated stock measurements (stock-difference method) are not available;
- Sediment burial rates are known from marker horizons, sediment plates, or radiometric dating; or
- Estimating fluxes directly is more feasible than repeated total stock assessments.

The general formula applied is:

$$\Delta C = \Delta C_{Gain} - \Delta C_{Loss} \quad \text{Equation 35}$$

Where:

- ΔC = Net annual carbon sequestration (t C yr^{-1})
- ΔC_{Gain} = Annual carbon gains (t C yr^{-1})
- ΔC_{Loss} = Annual carbon losses (t C yr^{-1})

Inputs are derived from the field measurements and laboratory analyses described in Section B.4.

Calculate gains and losses separately for each stratum. Express every term in Mg C/yr , multiplying any per-hectare rate by the corresponding stratum area once. Sum the stratum totals to obtain the project total.

B6.2.1 Estimating Carbon Gains

a. Seagrass Biomass Increment (Above- and Belowground)

Biomass gains are estimated from leaf elongation rates, shoot turnover, and rhizome extension measured in permanent monitoring plots or from published productivity values. Harvested quadrat or core samples provide above- and belowground production estimates, which are then converted to carbon using a carbon fraction.

$$\Delta C_{biomass}(Mg\ C/yr) = \Delta G_{AGB} \times CF \times (1 + R) \times A \quad \text{Equation 36}$$

Where:

- ΔG_{AGB} = Annual growth rate of AGB (Mg biomass/ha/yr)
- CF = Carbon fraction (commonly 0.34) (Howard et al., 2014)
- R = Root-to-shoot ratio
- A = Area covered (ha)

A standing-stock root-to-shoot ratio shall be used to estimate belowground annual biomass gains only where its applicability to annual gains has been demonstrated.

Where epiphytes are included, estimate their annual carbon gain separately using measured or justified epiphyte production and an applicable carbon fraction, and add it once to the stratum gain subtotal. Do not include the same epiphyte material in seagrass biomass gains.

b. Soil Carbon Accumulation via Sediment Accretion

Seagrass soils accumulate carbon through vertical accretion and burial of autochthonous and allochthonous material. Accretion rates may be measured using marker horizons or subsurface sediment plates:

$$\Delta C_{soc}(Mg\ C/yr) = BD \times Z \times C_{org} \times 100 \times A \quad \text{Equation 37}$$

Where:

- BD = Bulk density (g/cm³)
- Z = Accretion rate (cm/yr)
- C_{org} = Organic carbon mass fraction (g C/g dry sediment), expressed as a decimal; e.g., 0.02 for 2%
- A = Area (ha)

The factor 100 converts g C/cm² to Mg C/ha.

Alternatively, a Carbon Accumulation Rate (CAR) derived from radiometric dating (e.g., ²¹⁰Pb) may be applied:

$$\Delta C_{soc}(\text{Mg C/yr}) = CAR \times A \quad \text{Equation 38}$$

Where:

- CAR = Carbon accumulation rate (Mg C/ha/yr)
- A = Stratum area (ha)

c. Radiometric Dating for Burial Rates

Radiometric dating (^{210}Pb) provides long-term estimates of sedimentation and carbon burial in seagrass soils. The Carbon Accumulation Rate for each depth interval k is calculated as:

$$CAR_k = BD_k \times C_{org,k} \times Z_k \quad \text{Equation 39}$$

Where:

- CAR_k = Carbon accumulation rate in interval k (g C/cm²/yr)
- BD_k = Bulk density of interval k (g/cm³)
- $C_{org,k}$ = Organic carbon fraction in interval k (g C/g dry mass)
- Z_k = Sedimentation rate for interval k (cm/yr)

Convert to Mg C /ha/ yr:

$$CAR_{ha} = CAR_k \times 100 \quad \text{Equation 40}$$

B6.2.2 Estimating Carbon Losses

Carbon losses represent the release of carbon from the ecosystem and must be subtracted from gains. Losses are categorized as follows:

a. Decomposition and Respiration

Losses from litter decomposition or root turnover can be estimated as:

$$C_{loss,resp} = M_{decomp} \times CF \times A \quad \text{Equation 41}$$

Where:

- $C_{loss,resp}$ = Annual carbon loss for the stratum (Mg C/yr)
- M_{decomp} = Annual dry biomass or litter mass decomposed per hectare (Mg dry biomass/ha/yr)

- CF = Carbon mass fraction, expressed as a decimal
- A = Stratum area (ha)

Decomposition rates may be sourced from site studies or literature.

Count decomposition as an ecosystem carbon loss only to the extent that carbon leaves the included accounting pools. Transfers from biomass or litter into an included soil carbon pool shall not be counted as ecosystem losses. Do not subtract a loss separately where it is already reflected in the stock-change or net burial estimate.

b. Disturbance and Physical Damage

Losses occur from storm events, dredging, anchoring, or coastal development. These can be estimated as the proportion of area or biomass lost and scaled by frequency of events.

c. Soil Disturbance and Oxidation

If sediments are disturbed (e.g., through dredging, reclamation, or aquaculture), oxidation of stored organic matter leads to CO₂ release. Default emission factors (e.g., IPCC 2014) may be applied where site-specific data are unavailable.

d. Methane (CH₄) and Nitrous Oxide (N₂O) Emissions

Assess CH₄ and N₂O emissions in the baseline and project scenarios. Quantify material emissions using measurements or justified applicable factors, particularly where eutrophication or disturbance may affect emissions. Document any exclusion under main Section 10.4.

Account for CH₄ and N₂O separately from the Mg C gain–loss subtotal. Convert these emissions to CO₂-equivalents using the applicable GWP values and include them in the GHG balance under main Section 9.6.

B6.2.3 Calculating and Reporting Sequestration Rate

Once annual carbon gains and losses have been quantified for each pool, the net annual carbon sequestration rate for the project is determined by subtracting total annual losses from total annual gains.

$$\Delta C_{net,i} = \Delta C_{Gain,i} - \Delta C_{Loss,i} \quad \text{Equation 42}$$

Where:

- i = Stratum index
- $\Delta C_{net,i}$ = Net annual carbon gain in stratum i (Mg C/yr)

- $\Delta C_{Gain,i}$ = Sum of annual carbon gains across included pools in stratum i (Mg C/yr)
- $\Delta C_{Loss,i}$ = Sum of annual carbon losses from included accounting pools in stratum i (Mg C/yr)

a. Aggregation Across Strata

Project net annual carbon gain is calculated by summing the stratum totals. Each stratum total already includes its area.

$$C_{net,project} = \sum_{i=1}^n \Delta C_{net,i} \quad \text{Equation 43}$$

Where:

- $C_{net,project}$ = Project net annual carbon gain (Mg C/yr).
- $\Delta C_{net,i}$ = Net annual carbon gain in stratum i (Mg C/yr)
- n = Number of strata

b. Conversion to CO₂-equivalent

To express results in terms of CO₂-equivalent for reporting and crediting purposes:

$$\Delta CO_2e_{net} = C_{net,project} \times 3.67 \quad \text{Equation 44}$$

Where 3.67 is the molecular weight ratio of CO₂ to C.

This conversion expresses net carbon gain as a CO₂ removal quantity; it does not by itself establish project net GHG benefits or an issuable credit quantity. Calculate project net GHG benefits relative to the baseline, including applicable project emissions and leakage, under main Section 9.6. Apply the emissions-positive/removals-negative convention when entering the removal into the GHG balance, and avoid counting any gain or loss twice.

c. Temporal Averaging and Reporting Period

- Results should be annualized over multi-year monitoring intervals (minimum 1 year, preferably 2–5 years). All reported values must be clearly linked to the field and laboratory methods (B.4), specify whether data are direct or literature-based, and present uncertainty estimates consistent with Section B.7.

d. Integration with Stock-Difference Estimates

If both methods are applied (e.g., stock-difference for biomass and gain–loss for soil burial), results should be combined at the pool level, avoiding double counting and following uncertainty combination procedures.

B.7 Uncertainty and QA/QC

Seagrass carbon accounting involves uncertainties from measurement errors, natural variability, and methodological limits. Setting clear precision targets, reporting results transparently, and applying strong QA/QC practices help reduce these errors and improve the credibility of findings.

B7.1 Sources of Uncertainty

Uncertainties in seagrass carbon assessments arise from four primary domains:

Measurement Uncertainty

Measurement uncertainty refers to errors in field measurements and laboratory analyses. In seagrass projects, sources include:

- Inconsistent placement of quadrats or corers, leading to variability in biomass estimates.
- Seasonal fluctuations in biomass, particularly leaf elongation and turnover, which can bias results if sampling time is inconsistent.
- Soil core compaction, incomplete recovery, or disturbance during extraction.
- Analytical issues such as inaccurate bulk density measurements, uncorrected presence of inorganic carbon, or reliance on Loss on Ignition without calibration against elemental analysis.
- Use of generic conversion factors for biomass (e.g., 0.34–0.40 C fraction) when site-specific values are unavailable.

Model Uncertainty

Model uncertainty arises from estimation methods and assumptions. For seagrass ecosystems:

- Cover-to-biomass relationships and published productivity rates vary widely among species and regions.
- Use of national or global average coefficients (e.g., absorption or productivity rates) may over- or under-estimate local conditions.
- Assumptions about the fate of eroded or exported carbon (whether redeposited or oxidized) remain uncertain in current science.

Sampling Uncertainty (Variability)

Sampling uncertainty reflects natural heterogeneity in seagrass meadows.

- Within a single meadow, carbon stocks can vary by depth, light availability, species composition, and sediment type.
- Spatial variability in soil organic carbon is often greater than in biomass pools, contributing substantially to overall uncertainty.
- Temporal variability is high, with seasonal and inter-annual changes in cover and biomass density.
- Because only a finite number of plots can be measured, sampling error is introduced when scaling up to the entire project area.

Area Estimation Uncertainty

Area estimation uncertainty arises from errors in delineating seagrass extent and project strata. Sources include spatial resolution, positional inaccuracies, classification errors, and differences in environmental conditions during image acquisition. Water depth, turbidity, sun glint, tidal conditions, and similarities between seagrass and other benthic habitats may affect the identification and mapping of seagrass meadows.

Where ecosystem extent is derived from remote sensing or GIS-based classification, area estimates shall be assessed using appropriate field reference data and validation procedures. Area uncertainty shall be quantified where relevant and incorporated into project-level carbon stock and GHG estimates in accordance with Chapter 6 of the Main Text.

B7.2 Reporting uncertainty in stocks and sinks

Transparent reporting is essential to ensure credibility. The standard convention include:

- Expressing uncertainty as a 95% confidence interval, either as a percentage of the mean, as an absolute range (e.g., $\pm$ Mg C/ha), or as minimum and maximum bounds.
- Reporting carbon stocks in Mg C/ha for a defined soil depth; conversion to Mg CO₂/ha can be made using the molar mass ratio ($\times 3.67$).
- State the adopted precision target, confidence level and whether the percentage represents confidence-interval half-width or full width. Assess and propagate uncertainty in accordance with main Chapter 6. Apply any uncertainty deduction only as required by the applicable certification framework.
- When high uncertainty is unavoidable, projects should apply the principle of conservatism by reporting values likely to understate, rather than overstate, GHG benefits.

B7.3 Recommended QA/QC Procedures

Quality Assurance (QA) and Quality Control (QC) are necessary to minimize errors, ensure transparency, and meet verification standards. The following practices are recommended for seagrass projects:

1. **Field Data Quality Checks:** Duplicate quadrat biomass or soil core measurements should be collected in at least 10% of plots. Differences beyond acceptable thresholds should trigger review or re-measurement. Sampling should be conducted at the same season in each monitoring cycle to reduce seasonal bias.
2. **Data Sheet and Entry QA:** Standardized underwater data sheets or waterproof digital tools should be reviewed daily. Typed data must be cross-checked with original records. An error log should be maintained.
3. **Instrument Calibration and Maintenance:** GPS, balances, and depth gauges must be calibrated regularly. Lab instruments (e.g., elemental analyzers, ovens) require calibration logs.
4. **Sample Handling and Lab QA/QC:** Chain-of-custody protocols should be followed. Include replicates and blanks in analyses. In soils, inorganic carbon must be removed or corrected before calculating %C_{org}.
5. **Spatial Data QA:** Seagrass maps derived from remote sensing should be validated with ground-truthing surveys. Multiple survey methods (e.g., drone, diver transects, quadrats) can be cross-checked to reduce misclassification error.
6. **Statistical QA and Sampling Review:** Assess sampling adequacy against the precision target documented in Section B3.5. Distinguish variability among plots, expressed by the coefficient of variation, from uncertainty in the estimated mean. Investigate outliers and document their treatment; do not discard valid observations solely because they are extreme. Reassess sample size using the observed variability.

By explicitly addressing uncertainties in biomass, soils, and mapped area—and by applying rigorous QA/QC protocols—seagrass blue carbon projects can ensure that their results are scientifically credible and suitable for inclusion in national GHG inventories or voluntary carbon markets.

B.8 Methodological Levels for Seagrass Carbon Accounting

The Methodological Level system provides guidance for selecting and documenting methods used to estimate seagrass ecosystem extent, carbon stocks, carbon stock changes, and relevant greenhouse gas emissions.

Three methodological levels—Basic, Standard, and Advanced—are distinguished by the specificity, representativeness, calibration, and validation of the underlying data and estimation methods.

The levels describe methodological approaches rather than mandatory sets of instruments. A higher methodological level does not automatically demonstrate greater accuracy or lower uncertainty. Uncertainty shall be assessed independently in accordance with Chapter 6 of the Main Text and Section B.7 of this Annex.

The tables below provide examples of methods corresponding to each level. They do not replace the minimum sampling, measurement, accounting, or monitoring requirements specified elsewhere in the methodology. Different methodological levels may be applied to different carbon pools and accounting components within the same project.

B8.1 Field Data Collection and Laboratory Methods by Measurement Category

The following table describes the general progression of field and laboratory methods used to obtain the inputs required for seagrass carbon accounting.

a. Aboveground and Belowground Biomass

Measurement category	Level 1 – Basic	Level 2 – Standard	Level 3 – Advanced
Seagrass cover and shoot density	Standardized quadrat-based cover observations, with shoot counts where required by the selected estimation method.	Representative, stratified field measurements of cover and shoot density, with appropriate replication and observer calibration.	Detailed site-specific measurements, potentially supported by calibrated and validated imaging methods or repeated observations where appropriate.
Species identification and	Field identification using appropriate guides, with	Verified species identification and	Detailed species and structural

Measurement category	Level 1 – Basic	Level 2 – Standard	Level 3 – Advanced
canopy characteristics	standardized observations of relevant canopy characteristics.	representative measurements of canopy or shoot characteristics across relevant strata.	characterization, supported by expert verification or validated analytical techniques where needed.
Biomass collection and processing	Standardized quadrat or core sampling, separation of relevant biomass components, and dry-mass determination.	Representative, replicated sampling across strata, with consistent sample separation, processing, and dry-mass measurement.	Detailed site-specific sampling and processing, potentially incorporating enhanced spatial or temporal replication where justified.
Biomass carbon content	Applicable published carbon fractions used with field-measured dry biomass where permitted.	Locally or regionally representative carbon fractions supported by appropriate laboratory analysis or calibration.	Replicated site-specific carbon-content measurements and validated analytical procedures where these improve the estimate.
SOC core collection and depth characterization	Soil coring to the required depth using equipment appropriate to the substrate, with depth intervals recorded in accordance with Section B.4.2.	Representative, stratified soil-core sampling with appropriate replication and depth characterization.	Enhanced spatial or vertical characterization, including additional replication or deeper cores where justified by sediment conditions and accounting objectives.
SOC bulk density and organic carbon concentration	Site-measured dry bulk density and organic carbon concentration determined through an applicable laboratory method, using justified conversion factors where appropriate.	Representative site measurements supported by locally or regionally calibrated analytical procedures and appropriate replication.	Detailed, replicated site-specific measurements, with enhanced depth resolution and validated laboratory analysis where warranted.
Epiphyte biomass, where included	Standardized collection and dry-mass	Representative epiphyte sampling	Detailed site-specific measurements,

Measurement category	Level 1 – Basic	Level 2 – Standard	Level 3 – Advanced
	determination of representative epiphyte samples, with applicable published carbon fractions.	across relevant strata, with locally appropriate carbon-content determination.	potentially including temporal replication and enhanced laboratory analysis where justified.
Sediment accretion and surface elevation, where relevant	Applicable published sediment-accumulation information or basic site observations, with justification of their relevance to the intended estimate.	Site-based measurements using suitable methods, such as marker horizons or subsurface sediment plates.	Replicated or longer-term measurements, potentially supplemented by radiometric dating or other validated techniques where appropriate.
GHG emissions, where relevant	Applicable published emission factors representative of relevant environmental conditions.	Locally or regionally representative emission factors, supported by site observations or targeted measurements.	Site-specific flux measurements or validated estimation methods, with temporal replication appropriate to the emission source.

The methods are illustrative. The use of advanced instruments or imaging techniques does not independently justify assignment to a higher methodological level. All measurements shall comply with the applicable sampling, sample-handling, laboratory, and QA/QC requirements specified elsewhere in this Annex, regardless of methodological level.

B8.2 Methodological Levels for Carbon Accounting Estimates

The following tables describe how data and measurement approaches may be used to estimate seagrass carbon stocks, carbon stock changes, and relevant GHG emissions.

Methodological levels shall be assigned to individual accounting components rather than treating the entire project as having a single level. The methodological level of an accounting estimate shall be determined from the collective suitability and quality of its underlying data and estimation procedures; the use of an individual measurement technique associated with a higher level does not automatically determine the level of the resulting estimate.

a. Carbon Stock Estimation

Accounting component	Level 1 – Basic	Level 2 – Standard	Level 3 – Advanced
Ecosystem Area / Extent	Existing seagrass distribution maps or available spatial datasets, supported by appropriate field confirmation and documented limitations.	Locally or regionally developed seagrass maps with systematic field validation, suitable spatial resolution, and consistent delineation of meadow extent.	Detailed site-specific maps supported by rigorous field validation, documented classification accuracy, and appropriate assessment of spatial uncertainty.
Aboveground Biomass (AGB)	Field-measured biomass combined with applicable published carbon fractions or conversion parameters.	Representative, stratified field measurements combined with locally or regionally appropriate carbon fractions and estimation parameters.	Detailed site-specific biomass estimation supported by validated measurements or locally calibrated estimation models, with enhanced spatial or temporal characterization where justified.
Belowground Biomass (BGB)	Field-derived biomass information or applicable published belowground-to-aboveground relationships, where permitted by the selected method.	Representative belowground biomass measurements or locally appropriate biomass relationships supported by field data.	Detailed site-specific belowground biomass measurements or locally calibrated and validated estimation models, where feasible and justified.
Soil Organic Carbon (SOC)	SOC estimation using site-measured soil depth and dry bulk density, with organic carbon concentration determined using an applicable laboratory method and justified conversion factors.	SOC estimation using representative, stratified soil measurements and locally appropriate or calibrated carbon-concentration estimates.	Detailed site-specific SOC estimation using replicated, vertically resolved measurements and validated analytical procedures.

Accounting component	Level 1 – Basic	Level 2 – Standard	Level 3 – Advanced
Epiphytes, where included	Representative epiphyte biomass measurements combined with applicable published carbon fractions.	Representative epiphyte measurements across relevant strata, using locally appropriate carbon-content estimates.	Detailed site-specific epiphyte measurements and validated carbon-content estimates, with temporal replication where warranted.

b. Carbon Stock Change and GHG Estimation

Accounting approach / component	Level 1 – Basic	Level 2 – Standard	Level 3 – Advanced
Stock-Difference Method	Comparison of carbon stock estimates at two comparable time points, supported by measured changes in the relevant accounting inputs and appropriately justified published parameters.	Repeated, representative measurements using locally appropriate parameters and consistent sampling and estimation methods.	Detailed repeated measurements, supported by site-specific calibration, validation, and enhanced characterization of spatial or temporal variability.
Gain–Loss Method	Estimation using applicable published rates or factors for carbon gains and losses, supported by relevant project activity and spatial data.	Estimation using locally representative measurements or parameters for the principal gains and losses, supplemented by published factors where justified.	Detailed site-specific estimation of relevant gains and losses, supported by direct measurements or locally calibrated and validated models.
GHG Emissions, where applicable	Estimation using appropriate published emission factors representative of relevant environmental conditions.	Estimation using locally representative emission factors, supported by site observations or targeted measurements.	Estimation using site-specific measurements or validated models, with appropriate characterization of temporal and environmental variability.

Sediment accretion, sediment carbon burial, and radiometric dating provide measurements or estimation inputs that may support the applicable carbon-accounting approach. They do not constitute separate ecosystem-level accounting approaches. Where GHG emissions are required for project accounting, their quantification shall not be omitted solely because a Basic methodological level is applied.

B.9 Monitoring

Monitoring is where project proponents systematically collect, manage, and analyze data to quantify changes in carbon stocks and greenhouse gas (GHG) emissions within the project boundary over time. For seagrass blue carbon projects, monitoring serves to:

- Ensure that emission reductions and carbon sequestration are real, measurable, and verifiable.
- Track the condition, extent, and bathymetric/stratification context of seagrass meadows and associated carbon pools.
- Detect deviations from the baseline scenario and confirm that project activities are implemented as planned.
- Provide the basis for ex-post crediting under relevant certification schemes.

B9.1 Monitoring Plan

The monitoring approach must be defined in the Project Monitoring Plan prepared at validation. This shall include:

- Parameters to be measured – Included pools (aboveground biomass, belowground biomass, epiphytes if applicable, soil organic carbon) and any applicable non-CO₂ GHG emissions required for project accounting (e.g., CH₄, N₂O in eutrophic/disturbed settings).
- Frequency of measurement – Follow the parameter-specific monitoring schedule in this annex. Verification is an independent review process and does not determine field measurement frequency.
- Field and remote sensing methods – Consistent with Sections B.3–B.7; methods should remain consistent between events (e.g., same quadrat size, core diameter, slicing scheme, LOI↔CHN calibration).
- Strata and plot management – Maintain permanent plots/transects where possible; relocate with GPS/RTK markers to reduce variance and improve temporal comparability.
- Data management – QA/QC procedures, uncertainty estimation, metadata standards, and archiving (raw sheets, photos, GNSS tracks, lab worksheets).

B9.2 Monitoring Schedule

Monitoring intervals are set by expected rates of change, feasibility, and each pool's contribution to total stock/sink.

Table 1: Minimum monitoring frequency by carbon pool

Carbon Pool / Parameter	Minimum Frequency	Notes
Aboveground biomass (AGB)	Every 3–5 years	Permanent quadrats/transects preferred; repeat measurements under comparable, site-appropriate seasonal conditions.
Belowground biomass (BGB)	Every 5 years	Measured directly (cores); keep core diameter/depth consistent.
Epiphytes (if included)	Every 3–5 years	Align with AGB campaigns; consider seasonal replication if material to accounting.
Soil organic carbon (SOC)	Every 10 years (every 5 years in early restoration)	Core to consistent depth (≥ 1 m where feasible); same slicing scheme; carbonate treatment rules unchanged.
Sediment accretion (MH/plates)	Annual (seasonal where feasible)	Use marker horizons in intertidal/shallow sites or subsurface sediment plates in submerged beds; combine with BD and % C _{org} for CAR.
Isotope geochronology (^{210}Pb)	One-off per stratum (repeat ~15–20 yrs or if regime shifts)	Validates long-term burial; reconcile with short-term accretion.
Areal extent / cover	Annual–biennial	RS update with ground-truth; track gains/losses and strata boundaries.
Oceanographic conditions (temp, salinity, PAR, turbidity)	Seasonal or continuous loggers	Supports assessment of growth, fluxes and the applicability of CH ₄ /N ₂ O estimation methods.
Non-CO ₂ GHG emissions (where applicable)	According to the applicable estimation method and monitoring plan	Quantify material emissions using measurements or justified applicable factors. Document exclusions under main Section 10.4.

Assess relevant GHG emissions in both the baseline and project scenarios. A source shall not be excluded merely because the project does not claim a flux benefit. Apply the exclusion provisions of main Section 10.4 and document their justification.

B9.3 Parameters and Data Collection

Monitoring should include biophysical, environmental, and geospatial parameters necessary for robust interpretation and RS cross-checks.

Table 2: Region-Specific Monitoring Parameters for Seagrass Ecosystems

Parameter	Carbon Pool / Component	Unit	Measurement / Analytical Method	Field / Lab Equipment	Notes
Species identification	Biomass pools	–	Field ID with guides; photo vouchers for verification	Field guide; GPS camera	Enables species/size-class stratification.
Cover (%)	AGB context	%	Visual classes or point-intercept in quadrats	Quadrat frame; slate	Calibrate observers with photo standards.
Shoot density	AGB	shoots m ⁻²	Count within 0.25–1 m ² quadrats	Quadrat; tally sheet	Pair with canopy height for AGB inference.
Canopy (shoot) height	AGB	cm	Ruler/meter stick from sediment to leaf tip	Meter stick	Record avg and max height.

Parameter	Carbon Pool / Component	Unit	Measurement / Analytical Method	Field / Lab Equipment	Notes
Epiphyte load/biomass	Epiphytes	g m^{-2}	Scrape subset blades; dry to constant mass	Scrapers; bags; oven; balance	If included as a pool, standardize scraping.
AGB/BGB dry mass	Biomass C	g m^{-2} ; Mg ha^{-1}	Destructive harvest (quadrat/core), oven-dry to constant weight	Core tubes (10–25 cm Ø); oven; balance	Keep core area and depth fixed for comparability.
Bulk density (BD)	SOC	g cm^{-3}	Dry to constant mass; volume by core geometry	Cores; calipers; oven; balance	Record penetration and recovered depths; assess and correct compaction where present. Recollect unsuitable cores.
%C _{org} (soil)	SOC	% dry mass	CHN (preferred) or LOI with local LOI→%C _{org} regression; carbonate removal where needed	Elemental analyzer; muffle furnace; HCl; desiccator	Acidify carbonate-rich samples consistently.
Soil depth to refusal	SOC context	cm	Probe/corer to refusal	Depth probe/auger	Needed for stock scaling.
Sediment accretion rate	SOC burial	mm yr^{-1}	Marker horizon (intertidal/shallow) or subsurface plate (subtidal)	Feldspar/sand; plates; pins; quadrat	Co-locate with BD/%C _{org} sampling.

Parameter	Carbon Pool / Component	Unit	Measurement / Analytical Method	Field / Lab Equipment	Notes
Long-term burial (CAR)	SOC burial	g C m ⁻² yr ⁻¹	²¹⁰ Pb/ ¹³⁷ Cs age–depth model (CF-CS/CRS)	Vibracorer; alpha/HPGe spectrometer	Report model choice and uncertainty.
Areal extent / strata area (A _{i,t})	All	ha	RS classification with ground-truth	Sentinel/Landsat/ UAV; GNSS	Provide accuracy/uncertainty (confusion matrix).
Oceanographic context	Supporting	various	Loggers or seasonal spot sampling (temp, salinity, PAR, turbidity, tides)	Multiparameter probe; PAR; turbidity; pressure sensor	Interprets biomass/flux variability.

B9.4 Tools and Methods

Field methods shall follow the applicable procedures in Sections B.3–B.6 of this Annex.

Remote sensing data may be used to:

- Detect seagrass cover change and verify boundary stability.
- Support bathymetry/depth-based stratification and species/cover classes where detectable.
- Identify disturbances (anchoring scars, dredging, storm impacts).
- Data sources may include Sentinel-2 and Landsat (with water-column/sunlight correction), commercial multispectral/hyperspectral imagery, UAV orthomosaics, and acoustic or video transects for submerged validation. Ground-truthing (transects, quadrats, drop-camera) is required to quantify map accuracy.

Data sources may include Sentinel-2, Landsat, UAV imagery, and other regionally available high-resolution datasets.

B9.5 Quality Assurance / Quality Control (QA/QC) and Uncertainty Assessment

Projects shall implement QA/QC procedures to minimize measurement error and bias:

- Crew training and calibration before each campaign; inter-observer tests for cover and shoot counts.
- Standardized underwater data sheets/metadata; daily review; photo logs of quadrats and cores.
- Cross-checks of $\geq 10\%$ of plots/cores by independent observers; plot relocation verified by GNSS/markers.
- Laboratory QA/QC: replicate analyses ($\geq 10\%$), blanks/standards; maintain LOI $\leftrightarrow$ CHN calibration curves; document carbonate removal steps.
 - RS QA: accuracy assessment (confusion matrix), report area uncertainty; reconcile RS change with field evidence.
 - Method change log: document and justify any deviations between events.

Estimate uncertainty for each included pool and the project total in accordance with Section B7 and main Chapter 6. Where sampling precision does not meet the adopted target, review the sampling design and increase sampling where necessary. Any uncertainty deduction shall follow the applicable certification requirements.

B9.6 Reporting templates

These templates support consistent reporting of seagrass monitoring results under this annex and the main methodology. Complete the applicable fields for each reporting period, retain the supporting field, laboratory, spatial and calculation records, and cross-reference them using unique identifiers. Expand rows as needed. Enter “not applicable” with a reason where a field does not apply; distinguish zero, not measured and missing data. The templates do not replace the project design document, approved monitoring plan or underlying calculation files.

Report quantities with their units, measurement dates and spatial and depth boundaries. Use carbon fractions as decimals (for example, 0.02), while organic-carbon percentages are entered as percentage values (for example, 2 for 2%). Keep carbon stocks, stock changes and GHG balances distinct. Report uncertainty under main Chapter 6 and use the applicable monitoring schedule; these templates introduce no new default factors or monitoring frequencies.

For projects covering more than one ecosystem, identify the contribution of each annex and consolidate shared sources, leakage and project-level results once. Annualized rates are not additional amounts to be added to reporting-period totals.

Template R1: Project and reporting period

Field	Entry
Project name and identifier	
Country, locality and proponent	
Report identifier, version and preparation date	
Reporting period start and end; duration and annualization basis	
Project start date and activity type; implementation completed during this period	
Applicable methodology and annex versions; monitoring-plan identifier	
Project boundary/map version and total area (ha)	
Baseline scenario and additionality documentation reference; changes since previous report	
Accounting approach by pool or component; methodological level and justification reference	
Previous report reference; changes in methods, boundaries or strata and comparability assessment	
Prepared by; internal reviewer; organization, roles and dates	

Template R2: Strata and spatial changes

One row per stratum. Areas must refer to dated, non-overlapping accounting units. Attach the boundary-change reconciliation where strata were split, merged, gained or lost; do not interpret an area change alone as a carbon-stock change.

Stratum ID	Habitat and management condition	Start area (ha)	End area (ha)	Map dates and versions	Area uncertainty reference	Change and evidence reference

Template R3: Monitoring implementation

One row per parameter or sampling component. Attach the sampling design and environmental observations required to interpret the measurements. Report the number of independent sampling units, distinguishing subsamples and repeat observations.

Parameter or pool and stratum	Method and SOP version	Planned frequency and dates	Actual dates and independent sample count	Responsible team	Deviation and corrective action	Data and QA record reference

Template R4: Biomass carbon stock summary

Repeat for each stratum, measurement date and scenario where biomass stocks are used. Supporting field records must preserve the original measurements and conversions; this table reports the resulting stratum estimates.

Field	Entry
Stratum ID; measurement date; scenario or observed inventory	
Independent plot/quadrat/core count and sampling-design reference	
Stratum area (ha) and spatial-record reference	

Pool	Mean dry biomass (Mg/ha)	Carbon fraction or parameter reference	Mean carbon stock (Mg C/ha)	Stratum carbon stock (Mg C)	Calculation and uncertainty reference
Aboveground living biomass					
Belowground living biomass					
Epiphytes, where included					

Retain the following in the linked field and laboratory records:

- Transect, quadrat and core identifiers; coordinates and reference system; measurement date, tidal state, water depth (m) and team.
- Species composition, vegetation cover (%), shoot density (shoots/m²), canopy height (cm) and representative photograph identifiers, where collected.
- Quadrat area (m²), biomass corer internal diameter (cm), core area (cm²), sampled depth (cm), and dry mass (g) separately for each measured biomass pool.
- Epiphyte inclusion decision, separation procedure and sample identifiers; distinguish sediment cores from biomass cores.

- Drying and weighing records, pool-specific carbon fractions and analytical/source references. Retain evidence for any relationship used to infer annual belowground production.

If carbon fractions differ among samples or species, calculate carbon before aggregation and cite the detailed calculation rather than imposing a single mean fraction. Do not add a subtotal to its component pools.

Template R5: Soil carbon and depth basis

Use one core record for each sampled core and one layer row for each analysed interval. The layer table may be supplied as a linked electronic schedule.

Field	Entry
Stratum, plot and core identifiers; date and coordinates	
Corer internal diameter (cm); penetration and recovered lengths (cm)	
Actual assessed depth (cm); refusal or other limitation	
Compression assessment and any correction; interval and volume basis	
Living-root/rhizome removal; retained dead material and pool allocation	
Laboratory/SOP reference; carbon analysis method; carbonate treatment and dry-mass basis	
Core photographs, chain-of-custody and sample register references	

Sample ID	Assessed depth interval (cm)	Dry bulk density (g/cm ³)	Organic carbon (%)	Layer stock (Mg C/ha)	Laboratory and calculation reference

Stratum ID	Common reporting depth (cm)	Independent core count	Mean SOC (Mg C/ha)	Stratum SOC (Mg C)	Uncertainty and calculation reference

Normally report measured stocks within 0–100 cm. Report shallower stocks at their actual depth and deeper stocks separately. Do not extrapolate a shallow stock to 1 m by a depth ratio. Identify any estimated unmeasured intervals separately and document their justification and uncertainty. Use a consistent depth basis for comparisons; document interval-boundary treatment and design-consistent averaging of cores.

Template R6: Carbon stock change

Complete the panel applicable to each pool and scenario. Where methods differ among pools, document their boundaries and demonstrate that the same change is not counted twice. These are carbon results, not project GHG benefits. For stock difference, change equals end stock minus start stock. For gain–loss, net change equals gains minus losses. Divide the interval change by elapsed years for the annualized result.

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Interval start and end: _____. Elapsed years: _____. Scenario: _____.
Area/depth comparability and boundary-change reconciliation: _____.

Stratum and pool	Start stock (Mg C)	End stock (Mg C)	Change (Mg C)	Annualized change (Mg C/yr)	Calculation reference

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Interval start and end: _____. Elapsed years: _____. Scenario: _____.
Rate integration and spatial scaling reference: _____.

Stratum and pool	Gains over interval (Mg C)	Losses over interval (Mg C)	Net change (Mg C)	Annualized net change (Mg C/yr)	Calculation and overlap check reference

Apply area once when converting per-hectare quantities to stratum totals. Preserve negative net changes. Where accretion or radiometric burial informs gains, record method, assessed dates/depths, rate units, carbon concentration convention, and whether the estimate is gross accumulation or net burial. Keep surface-elevation change distinct from accretion. Do not add historical interval rates as simultaneous annual gains or subtract losses already represented by a net burial estimate.

Template R7: GHG accounting summary

Complete the component ledger separately for baseline and project scenarios, using the same reporting period and compatible accounting boundaries. Link annual schedules where required. Include each term once, documenting overlap between stock changes, respiration and other measured fluxes.

Scenario and year/period	Source or removal and spatial scope	Gas or carbon basis	Input amount and unit	Conversion or GWP reference	Signed result (tCO ₂ e)	Calculation and overlap-check reference

Use positive values for emissions and negative values for removals in scenario balances. A stock increase converted to a removal therefore enters with the opposite sign to the positive stock-change result. Document use of 44/12 for carbon-to-CO₂ conversion and applicable 100-year GWPs for CH₄/N₂O. Identify whether measurements are expressed as gas mass or carbon/nitrogen mass and document any necessary conversion before applying GWP.

Accounting item	Reporting-period result (tCO ₂ e)	Annualized result (tCO ₂ e/yr)	Calculation reference
Net baseline GHG balance			
Net project GHG balance			
Baseline minus project before leakage			
Leakage deduction			
Net GHG benefit after leakage			

Calculate net GHG benefit as baseline balance minus project balance minus leakage under main Section 9.6. Leakage deductions shall not be negative. Do not add avoided emissions again where already represented in the baseline–project difference. For a multi-ecosystem project, provide the consolidated project table once and cross-reference each annex contribution.

Field	Entry
Applicable uncertainty/conservativeness adjustment, amount, unit and rule reference, or not applicable	
Non-permanence or reversal treatment and evidence reference, where applicable	
Relationship between reported benefit, any adjusted result and the consolidated project report	

Apply a deduction or buffer only where required by the applicable certification framework; identify its rule and calculation separately. Report any applicable adjustment separately and identify its rule and calculation. These reported estimates are not an issuance or certification decision.

Template R8: Uncertainty statement

Repeat this record for each material estimate and the consolidated result. Where an uncertainty budget is lengthy, attach it and retain its identifier here.

Field	Entry
Estimate identifier, scenario, period and spatial/pool/depth scope	
Point estimate and unit	
Standard error or other uncertainty measure; identify its type and unit	
Confidence level and interval limits, where applicable	
Relative precision measure and definition; adopted target and source, where applicable	
Sampling design, independent sample count and estimation/propagation method	
Included sources: sampling, model, carbon fraction, laboratory, area and other material sources	
Shared errors/covariance and area weighting; treatment of omitted sources	
Calculation reference, limitations and any additional sampling or corrective action	

Distinguish standard error, coefficient of variation and confidence-interval width. Do not treat them as interchangeable or combine incompatible uncertainty measures.

Template R9: Parameters and exclusions

Parameter register — one row per applied factor, model or conversion, including carbon fractions, allometry, LOI calibration, emission factors and GWP values as applicable.

Parameter ID and use	Value or equation and unit	Source with page/table/version	Site/species/depth applicability	Uncertainty or calibration evidence	Calculation reference

Exclusion register — record included, excluded and zero-valued terms distinctly. Assess project-level cumulative exclusions under main Section 10.4.

Pool, GHG or leakage term	Included/excluded/zero	Basis and applicable provision	Estimated omitted contribution (tCO ₂ e)	Evidence reference	Reassessment trigger

Field	Entry
Project-level cumulative de minimis assessment reference and period	
Combined contribution of terms tested (tCO ₂ e) and estimation basis	
Net GHG benefit before leakage used as denominator (tCO ₂ e)	
Calculated percentage, conclusion and treatment of non-positive/undefined denominator	
Evidence that exclusions do not overstate benefits; treatment of any adverse pool change	

Do not use a pool's share of standing carbon stock as the significance denominator. A non-positive or undefined denominator does not demonstrate that an exclusion passes. A source is not excluded merely because no flux benefit is claimed.

Template R10: QA review and risk follow-up

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Dataset or calculation ID	Check performed and coverage	Finding or limitation	Correction and status	Reviewer and date	Evidence/version reference

Cover field completeness, calibration, sample traceability, laboratory checks, data-entry review, units, formulas, spatial/depth comparability and double-counting checks as applicable. Retain original records and document corrections rather than silently replacing them.

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Risk/event ID and date	Affected stratum and extent (ha)	Observed or potential effect	Action, owner and due date	Accounting/reversal assessment reference	Follow-up status and evidence

Field	Entry
Raw field/laboratory records, photographs and spatial archive location	
Calculation files, SOP versions, parameter sources and uncertainty files	
Outstanding issues and their effect on the reported results	
Prepared by and date; internal technical review by and date	
Independent verification status and statement reference, if available	

Retain records under the main methodology's retention requirements. Internal review does not constitute independent verification.

B.10 Regional Parameter Tables

B10.1 Seagrass Species in the East and Southeast Asia Region

Table 3: Seagrass Species

Species	Family
<i>Enhalus acoroides</i>	Hydrocharitaceae
<i>Thalassia hemprichii</i>	Hydrocharitaceae
<i>Halophila ovalis</i>	Hydrocharitaceae
<i>Halophila beccarii</i>	Hydrocharitaceae
<i>Halophila decipiens</i>	Hydrocharitaceae
<i>Halophila minor</i>	Hydrocharitaceae
<i>Halophila spinulosa</i>	Hydrocharitaceae
<i>Halophila sulawesii</i>	Hydrocharitaceae
<i>Halophila nipponica</i>	Hydrocharitaceae
<i>Halophila okinawensis</i>	Hydrocharitaceae
<i>Cymodocea rotundata</i>	Cymodoceaceae
<i>Cymodocea serrulata</i>	Cymodoceaceae
<i>Halodule pinifolia</i>	Cymodoceaceae
<i>Halodule uninervis</i>	Cymodoceaceae
<i>Syringodium isoetifolium</i>	Cymodoceaceae
<i>Thalassodendron ciliatum</i>	Cymodoceaceae
<i>Ruppia maritima</i>	Ruppiaceae
<i>Zostera marina</i>	Zosteraceae
<i>Zostera japonica</i>	Zosteraceae
<i>Zostera caulescens</i>	Zosteraceae
<i>Zostera caespitosa</i>	Zosteraceae

Species	Family
<i>Phyllospadix japonicus</i>	Zosteraceae
<i>Phyllospadix iwatensis</i>	Zosteraceae

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