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Evaluating Leaf Area and Biomass Relationship in *Posidonia oceanica* (L.) Delile: A Tool for Non-Destructive Assessment

Francesco Pelizza [1,2,*], **Chiara Robello** [1,2], **Muriel Oddenino** [1,†], **Eva Germani** [3], **Francesca Capobianco** [1], **Alice Oprandi** [1] and **Monica Montefalcone** [1,3,4,†]

[1] Department of Earth, Environment and Life Sciences (DiSTAV), University of Genova, Corso Europa 26, 16132 Genoa, Italy; chiara.robello@edu.unige.it (C.R.); fracapobianco28@gmail.com (F.C.); alice.oprandi@edu.unige.it (A.O.)
[2] One Ocean Foundation (OOF), Via Gesù 10, 20121 Milan, Italy
[3] Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Piazzale Flaminio 9, 00196 Rome, Italy; evagermani1997@gmail.com
[4] National Biodiversity Future Center (NBFC), Piazza Marina 61, 90133 Palermo, Italy
* Correspondence: francesco.pelizza@edu.unige.it
† Deceased authors.

Abstract

Posidonia oceanica (L.) Delile is a seagrass endemic to the Mediterranean Sea and a significant contributor to human well-being through a variety of ecosystem functions, such as carbon cycling. Despite its ecological significance, most methods for estimating leaf biomass in this species are either destructive or expensive. In this study, 2500 individual leaves from 351 shoots of *Posidonia oceanica* were collected, across 16 sites in the Ligurian Sea, over two time periods (2016–2018 and 2024–2025), and analyzed for total leaf area and dry weight. An allometric equation following a power-law structure was derived using linear mixed-effects models and it was later validated via 10-fold cross-validation. Although some variations in the intercept term were observed, the allometric scaling structure remained consistent across space and time, providing the first robust species-specific allometric tool to estimate *P. oceanica* biomass, forming the basis of a proposed non-destructive protocol for meadow-scale biomass estimation.

Keywords: *Posidonia oceanica*; allometric relationship; leaf area; leaf biomass; carbon cycle

1. Introduction

Posidonia oceanica (L.) Delile is a seagrass endemic to the Mediterranean Sea and one of the most ecologically significant components of the basin's carbon cycle [1,2]. A defining feature of these meadows is their capacity for long-term carbon sequestration, achieved through the formation of the *matte*—a dense matrix of dead rhizomes, roots, and sediment that accumulates over centuries and stores substantial quantities of both organic and inorganic carbon [3–8]. The *matte* stocks vary between 40 and 237 kg C m^{-2} across the basin [2–9], with the total organic carbon stock trapped in *P. oceanica matte* estimated at 711–1067 million Mg C [9,10].

The second major contribution of *P. oceanica* to the carbon cycle lies in its exceptional primary productivity. The leaf blades are the most productive part of the plant, accounting for 79% of the carbon fixed per shoot, followed by sheaths (17%) and rhizomes (4%) [2]. Gross carbon fixation capacity ranges between 33.5 g C m^{-2} per year, in light-limited

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environments, to 426 g C m^{-2} per year, in shallow and well-illuminated areas [2,6]. This high productivity contributes directly to carbon storage carbon storage, since it is estimated that roughly between 10% and 36% of biomass produced in meadows is permanently buried in the *matte*, constituting a long-term carbon sink [8,11,12].

Given the importance of *P. oceanica* in Mediterranean carbon budgets, a range of monitoring techniques have been developed to quantify its productivity and carbon fixation. One of the most widely applied direct methods is the leaf marking technique originally described by Zieman (1974), in which divers pierce all leaves at a fixed reference point and, after an interval of 15–30 days, collect shoots and measure new tissue growth from the ligule to the mark, deriving biomass production over the elapsed period of time [13–16]. An alternative reconstructive technique is lepidochronology, which requires the sampling of cuttings and the analysis of each sheath's thickness to reconstruct decades of productivity [14,17,18]. More recently, non-destructive methods have been explored with the aim of reducing physical disturbance to the meadow. However, most of these techniques are expensive and require special materials, such as the aquatic eddy covariance, which measures oxygen flux above the canopy through an array of sensors [5].

Alongside these approaches, allometric relationships have been used as a means to derive *P. oceanica* leaf biomass and primary productivity without the need for destructive sampling [7,19]. Such techniques have been widespread for many years in the field of terrestrial botany [20–24]. Duarte (1991) made the first attempt at establishing an allometric relationship across seagrass species in the Mediterranean [25]. He demonstrated that a power-law relationship exists between morphological traits and biomass. Building on this work, subsequent studies have validated allometric power-law scaling of leaf biomass in terms of leaf area in species such as *Zostera marina* [26–29]. For *P. oceanica*, however, no reliable and validated allometric equation has been developed, with most existing productivity assessments still relying on destructive methods [30].

This gap persists because the biomass-to-area relationship is dependent on a range of site-specific factors, including depth, light availability, hydrodynamics, and nutrient availability, which introduce variability across meadows and seasons [30–32]. Establishing such a relationship would represent a significant step toward the development of non-destructive methods for estimating leaf biomass and, subsequently, primary productivity across *P. oceanica* meadows [33,34]. This prospect carries particular relevance in the current conservation context, where several restoration programs are being implemented across the Mediterranean Sea to prevent the decline of this seagrass [35–39].

The objective of this study is to evaluate whether a robust allometric relationship between *P. oceanica* leaf area and dry weight can be established and serve as the basis for a non-destructive biomass estimation equation. The derived equation is integrated with current seagrass monitoring techniques to outline a protocol for meadow-scale biomass assessment.

2. Materials and Methods

A dataset of roughly 2500 individual *Posidonia oceanica* leaves was assembled, collected from 351 shoots across 16 different sites, scattered around the region of Liguria, Italy (Figure 1). The Ligurian Sea was chosen as a case study because of its ecological heterogeneity, encompassing meadows at various depths, conservation states and subjected to several anthropic pressures [40–47]. These include physical damage from anchoring, fishing activities or worsening water quality from maritime and fluvial works, such as dredging and beach nourishment [30,40,41]. The sites selected for this study host *P. oceanica* meadows at various depths, with some located within Marine Protected Areas (MPA) (Table 1). While all sites are subject to differing degrees of anthropogenic pressure, site-specific measures of the nature and extent of these pressures fall outside the scope of this study, as comprehensive historical records are not readily available. Samples were always collected at the center of the meadow, in an area of high shoot density and cover. Shoots were collected across seasons in different proportions, with the highest amount in autumn (135), followed by summer (90), winter (63) and spring (63).

Figure 1. Map of the study area and location of the sampled meadows. On the bottom right, in red, the region of Liguria is highlighted. On the larger map, the black dots are all the meadows sampled between 2016 and 2018, in white are the meadows sampled between 2024 and 2025, and in yellow is Punta Pedale, the only site sampled in both periods.

Table 1. Sites selected for the study, with coordinates, sampling depth, and conservation status, indicating sites located within a Marine Protected Area (MPA). Anthropogenic pressures are classified following Holon et al. (2015) [42] and their references are indicated. TU = touristic facilities; A = boat anchoring; ANT = coastline anthropization (intensive urbanization and high usage of coastal areas); CE = coastal erosion; WQ = water quality degradation due to urbanization and coastal runoff; FI = recreational fishing; n.a. = data not available.

Site	Longitude	Latitude	Depth (m)	Protection	Pressures
Punta Pedale	44°19′15.4956″ N	9°12′56.5662″ E	12	No	TU, A, ANT, FI [43,44]
Paraggi	44°18′39.4164″ N	9°12′34.9164″ E	8	Yes	TU [43,44]
Niasca	44°18′30.6612″ N	9°12′37.9038″ E	7	Yes	TU [43]
Sanremo Porto Vecchio	43°48′45.4602″ N	7°46′37.6206″ E	13	No	A, ANT [43]
Bergeggi	44°14′15.9072″ N	8°26′39.3252″ E	12	Yes	TU, WQ [43]
Punta Manara	44°15′11.037″ N	9°24′32.2056″ E	15	No	WQ, ANT [45]
Monterosso	44°8′26.901″ N	9°38′38.6946″ E	18	Yes	WQ [46]
Prelo	44°20′25.3746″ N	9°13′32.0766″ E	14	No	ANT, TU [41,46]
Framura	44°12′15.1302″ N	9°32′51.954″ E	13	No	TU, A, ANT [47]
Camogli	44°20′42.6402″ N	9°9′10.1088″ E	15	No	WQ, ANT, TU [44,47]
Diano Marina	43°54′22.6188″ N	8°5′6.1758″ E	12	No	n.a.
Ventimiglia	43°47′11.7492″ N	7°35′25.998″ E	15	No	A, ANT, WQ [41]
Noli	44°12′25.128″ N	8°25′17.9646″ E	11	No	WQ, ANT [47]
Spotorno	44°13′35.5974″ N	8°25′28.3218″ E	16	No	WQ, ANT [47]
Vado Ligure	44°17′20.079″ N	8°27′34.3368″ E	15	No	WQ, ANT [47]
Ospedaletti	43°47′49.8624″ N	7°42′35.0598″ E	10	No	n.a.

2.1. Sampling Design

At each site, the number of replicates was chosen according to the guidelines given for biomass monitoring by the PREI index (*Posidonia Rapid Assessment Index*) [14,47]. Accordingly, a total of nine *P. oceanica* shoots were collected randomly from three different stations by SCUBA divers at each site.

All morphological analyses were performed in the laboratory following standardized procedures [14,17,30,48,49]. For each shoot, juvenile and severely damaged leaves were removed, the remaining leaves were counted, and their length and width were measured, to obtain the following descriptors: the number of leaves per shoot (n_leaves·shoot^{-1}), leaf width (cm) and leaf length (cm). Leaf length was defined as the longest extension of the leaf from apex to base and the leaf width as the longest extension of any two points on the blade parallel to each other and perpendicular to the length axis [50]. After measurements, all the epiphytes were scraped off from the leaves and the clean leaves were dried at 60 °C for 48 h to measure dry biomass (g *DW*).

For each shoot, we calculated the total leaf area (cm^2), mean leaf biomass per shoot (g *DW* shoot^{-1}), and the mean carbon content per shoot (gC shoot^{-1}). Because leaf area was estimated assuming a perfectly rectangular shape, a correction factor (CF) was calculated and applied using a subset of 160 randomly selected leaves, which were photographed using an OM System Tough TG-7 digital camera (OM Digital Solutions, Tokyo, Japan). The true leaf area was derived through the software Fiji (Version 1.54), National Institutes of Health, Bethesda, MD, USA, and divided by the corresponding rectangular estimate to obtain the CF [50–53]. Carbon content within shoots was then estimated using conversion coefficients gathered from the literature. The regional Mediterranean leaf tissue typically contains an average of between 37.8 ± 1.6% and 41.3 ± 0.4% carbon for leaf tissue [2,54], although earlier localized studies established lower values (27.4 ± 1.5%) [7]. Within this study, we used the values proposed by Pergent-Martini et al., (2021) [2], as they provide measurements of over a hundred leaves across various sites and depths, allowing the protocol the capacity to estimate carbon content across the whole Mediterranean basin. To finally derive an equation that estimates carbon content, without accounting for seasonal or depth-related variations, the Pergent-Martini et al. (2021) [2] conversion factor was inserted in the equation and the uncertainty of carbon content was calculated by combining the carbon fraction variability (CV = 4.23%) with the allometric absolute error, through propagation of relative errors in quadrature.

2.2. Data Analysis

To quantify the relationship between leaf dry weight (*DW*) and leaf area in *Posidonia oceanica*, a log–log allometric model (1) was created:

$$\log\left(DW_{ij}\right) = \alpha + \beta log\left(A_{ij}\right) + \varepsilon_{ij} \tag{1}$$

where DW_{ij} is the dry weight of leaves in a shoot i at site j, Aij is the corresponding leaf area, and εij represents the residual error. For comparison, the linear model of leaf area and dry weight (2) was also considered, expressed as follows:

$$DW_{ij} = \alpha + \beta A_{ij} + \varepsilon_{ij} \tag{2}$$

Diagnostics plots were subsequently performed to evaluate model adequacy, to verify assumptions of homoscedasticity and normality of residuals. To test differences in allometric scaling between the two sampling periods (i.e., 2016–2018 and 2024–2025), linear mixed-effect models (LMMs) with Gaussian error distribution were fitted on log-transformed data (Equations (3) and (4)).

$$log\left(DW_{ijk}\right) = \beta_0 + \beta_1 log\left(A_{ijk}\right) + \beta_2 Dataset_{ikj} + u_{0j} + u_{1j}\log(\mathrm{A_{ij}}) + \varepsilon_{ij} \tag{3}$$

$$log(DW_{ij}) = \text{Equation (3)} + \beta_3 log(A_{ij}) \cdot Dataset_{ikj} \tag{4}$$

where $Dataset_{ikj}$ represents the sampling period k at the site j, and u_{0j} and u_{1j} are site-specific random intercept and slope, respectively. In the first LMM (3), the sampling period ($Dataset_{ikj}$) and leaf area (A_{ij}) were included as fixed effects. In the second model (4), their interaction was also included. Because multiple leaves were sampled close by or within the same sites, to account for spatial non-independence, the term site was included as a random factor in both LMMs. The significance of the model terms was assessed using the Wald χ^2 test analysis of variance. Different combinations of fixed and random factors were compared using the Akaike Information Criterion (AIC) and likelihood ratio tests (LRTs) using maximum likelihood estimation. Fixed effects were evaluated using Kenward–Roger approximated F-tests. To quantify the proportion of variance explained by fixed and random components, marginal and conditional R^2 were computed following the Nakagawa and Schielzeth framework. Marginal R^2 describes the proportion of variance explained by fixed factors, and conditional R^2 includes fixed and random factors [55].

Finally, the predictive performance of the model was assessed using 10-fold cross-validation, as well as the Root of the Mean of Square of Errors (RMSE) and the Mean of Absolute value of Errors (MAE). In addition to predictive performance parameters, a 95% confidence interval was constructed using a parametric bootstrap procedure (999 iterations). For each iteration, the dataset was resampled and the LMM was refitted to extract allometric parameters from the fixed effects, combining them with residual noise drawn from the RMSE. The resulting distribution was used to estimate the 2.5th and the 97.5th percentile as lower and upper bounds of the confidence interval (CI). A last validation of the predictive power of the final equation was achieved through the Concordance Correlation Coefficient (CCC), assessing the agreement between observed and predicted biomass values.

All statistical analyses were conducted in R (version 4.5.0), using R-studio version 4.5.0 and Claude 3.5 Sonnet (Anthropic) as an assistant to generate and de-bug code to (i) compare LMMs using the lme4 and lmerTest packages; (ii) compute performance metrics (RMSE, MAE and CCC); and (iii) produce publication-quality figures using ggplot2. All code generated with AI assistance was reviewed, tested and validated. Claude was not used to interpret results or draw conclusions. All analytical decisions were made independently by the authors.

3. Results

3.1. Shoot Canopy Morphology

A total of 351 shoots were analyzed. Leaf dry weight per shoot ranged from 0.097 g to 2.158 g, with a mean value of 0.719 g (±SD = 0.4221 g). Total shoot leaf area varied between 28.21 cm^2 and 663.36 cm^2, with a mean of 196.75 cm^2 (±SD = 94.46 cm^2). The number of leaves per shoot ranged from 3 to 12, with an average of 5.69 leaves per shoot (±SD = 1.37 leaves). The site displaying the greatest total shoot area was Punta Pedale, with a mean area of 391 cm^2 (±SD = 155 cm^2) and a considerable variation in leaf size over time, going from 101 cm^2 to 663 cm^2. Diano Marina displayed the lowest mean area of 118 cm^2 (±SD = 34.1 cm^2), ranging from 54.2 cm^2 to 170 cm^2. When considering mean leaf area, Vado Ligure had the largest individual leaves, with a mean of 59.4 cm^2 per leaf (±SD = 12.8 cm^2) and Diano Marina resulted as having the smallest with a mean of 20.1 cm^2 (±SD = 4.52 cm^2). The correction factor for leaf area had a mean value of 0.996

(±SD = 0.086), indicating that manual measurements slightly underestimated the true leaf area on average.

Based on the measured shoot dry weights and carbon content values reported in the literature for *Posidonia oceanica*, leaf carbon stocks were estimated for each shoot. Using the most conservative estimate by Pergent et al. (1994), the mean carbon content of shoots was 0.197 ± 0.1162 g C shoot^{-1}, ranging between 0.0265 and 0.5913 g C shoot^{-1} [7]. Based on the values provided by Pergent-Martini et al. (2021), the mean carbon content of shoots is 0.2717 ± 0.16 g C shoot^{-1}, with a range varying between 0.0366 and 0.8157 g C shoot^{-1} [2]. As the latter values are derived from a comprehensive dataset compiled from various studies spanning the entire Mediterranean basin, they are considered more representative of regional variability and are therefore preferred for subsequent carbon stock estimates.

3.2. Leaf Area–Dry Weight Allometry

A strong positive relationship was observed between total shoot leaf area and total shoot leaf dry weight. A simple linear regression on raw-scale variables explained 74.4% of the variation in dry weight ($R^2 = 0.744$, $p < 0.001$). However, because plant size–biomass relationships typically follow power-law scaling [20,23–27,34,56], the relationship was further examined using a log–log transformation. Inspections of the marginal distributions of dry weight revealed a bimodal pattern, likely reflecting underlying a site- and season-specific structure in the data, which motivated the use of LMMs. Accounting for the hierarchical sampling structure, two random-effects structures were compared: a random-intercept model (Model 1, Table 2) and a random-intercept-plus-slope model (Model 2, Table 2).

Table 2. List of modeling equations employed in this study.

Model	Dependent Variable	Fixed Structure	Random Structure
1	Log(DW)	Log(Area)	1 | Site
2	Log(DW)	Log(Area)	1 + log(Area) | Site
3	Log(DW)	Log(Area) + Dataset	1 + log(Area) | Site
4	Log(DW)	Equation (3) + Log(Area) · Dataset	1 + log(Area) | Site

Within the first option, the factor site was inserted as a random intercept, allowing baseline dry weight to vary by site, while, in the second equation, it was inserted as a random slope, allowing the allometric scaling relationship to vary by site. The random-slope model provided a significantly better fit ($\chi^2 = 24.99$, $p < 0.001$) and a lower AIC (-160.2 vs. -139.2) compared with the first model, indicating that the allometric scaling relationship varies significantly among sites. To understand whether the origin of site variation was related to the temporal origin of the data (2016–2018 vs. 2024–2025), mixed-effect models were run including dataset as a fixed effect (Model 3, Table 2), as well as the interaction with log-transformed leaf area (Model 4, Table 2).

ANOVA comparison between Model 3 and Model 4 resulted as marginally non-significant ($\chi^2 = 3.752$, $p > 0.05$) with a very similar AIC (Table 3), leading to the retention of the simpler model without the interaction term. A final comparison, between Model 2 and Model 3, using LRT ($\chi^2 = 17.37$, $p < 0.001$) and Kenward–Roger approximated F-test ($F = 19.02$, $p < 0.001$), confirmed that the dataset variable significantly improved model fit, justifying its inclusion as a fixed effect in the final model. Based on these results, the final predictive equation relating leaf area to dry weight is

$$DW = 0.00248 \times \text{Area}^{1.122} \times e^{\epsilon};\ \text{with}\ \epsilon \sim N(0, 0.02867) \quad (5)$$

where 0.00248 (95% CI: 0.00141–0.00424) is the baseline coefficient and 1.122 (95% CI: 1.031–1.219) represents the estimated scaling exponent, indicating a slightly super-linear relationship between leaf area and dry weight and a clear deviation from isometric scaling (Figure 2).

Table 3. Comparison of model performances for LMM employed in this study, including Akaike Information Criterion (AIC), log-likelihood (LogLik), marginal R^2 and conditional R^2.

Model	AIC	LogLik	$R^2_{marginal}$	$R^2_{conditional}$
1	−139.21	73.606	0.727	0.919
2	−160.20	86.102	0.754	0.932
3	−171.82	92.909	0.835	0.921
4	−173.57	94.785	0.841	0.918

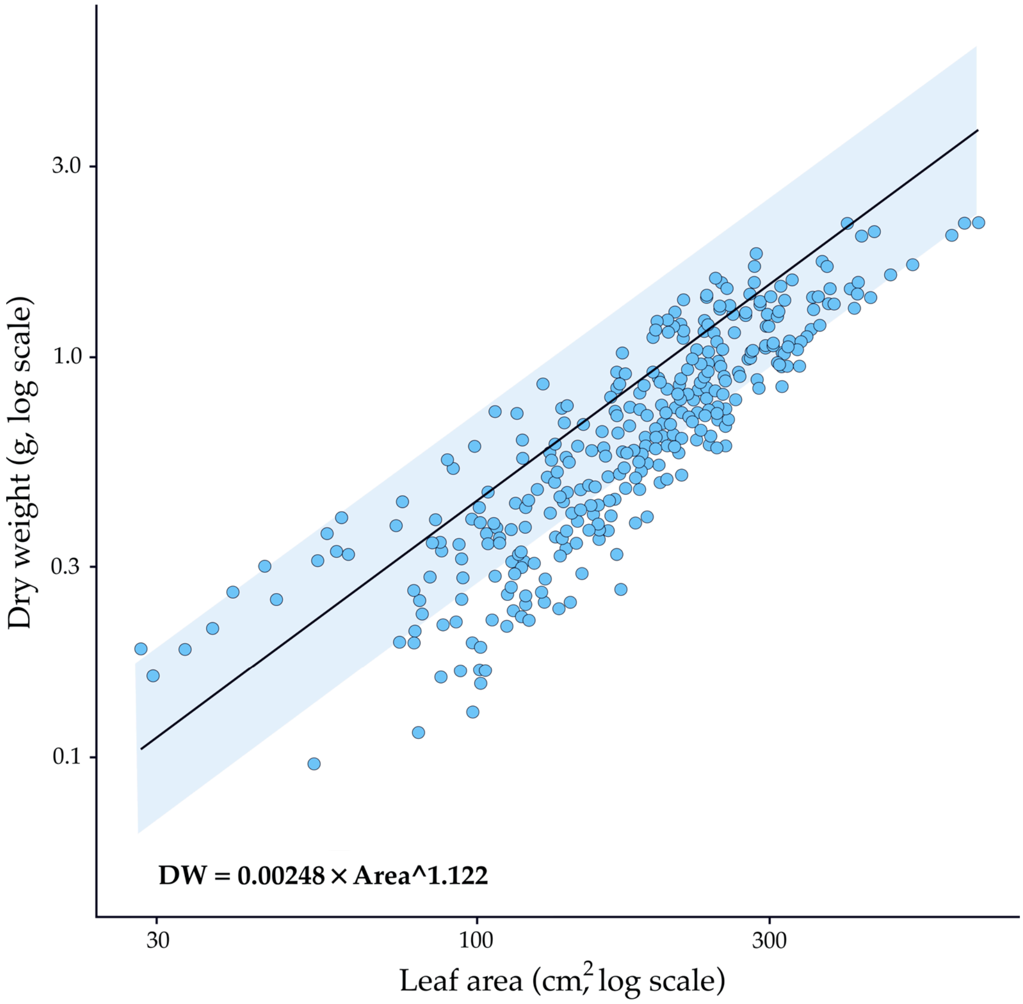

Figure 2. Relationship between log-transformed total shoot leaf area (cm^2) and log-transformed shoot dry weight (g), as defined by the power-law equation $DW = 0.00248 \times \text{Area}^{1.122}$, with the 95% CI as the blue band.

3.3. Model Validation

The predictive performance of the model was assessed using 10-fold cross-validation. Predictions were generated using fixed effects only, excluding random site effects to evaluate general predictive capacity. Folds were generated by random partitioning of observations. The model showed good predictive performance, with RMSE = 0.241 (log scale) and MAE = 0.189 (log scale). This corresponds to a multiplicative error of ×1.27, back-transformed from RMSE, and an absolute error of ±20.8% on the original scale, back-transformed from MAE. Model bias was negligible (bias = −0.0019). Model agreement was further evaluated using the CCC (0.915), indicating a strong reproducibility of biomass estimates derived from the allometric model (Figure 3). Residual diagnostics revealed no major deviations from normality or homoscedasticity, supporting model assumptions (Appendix A).

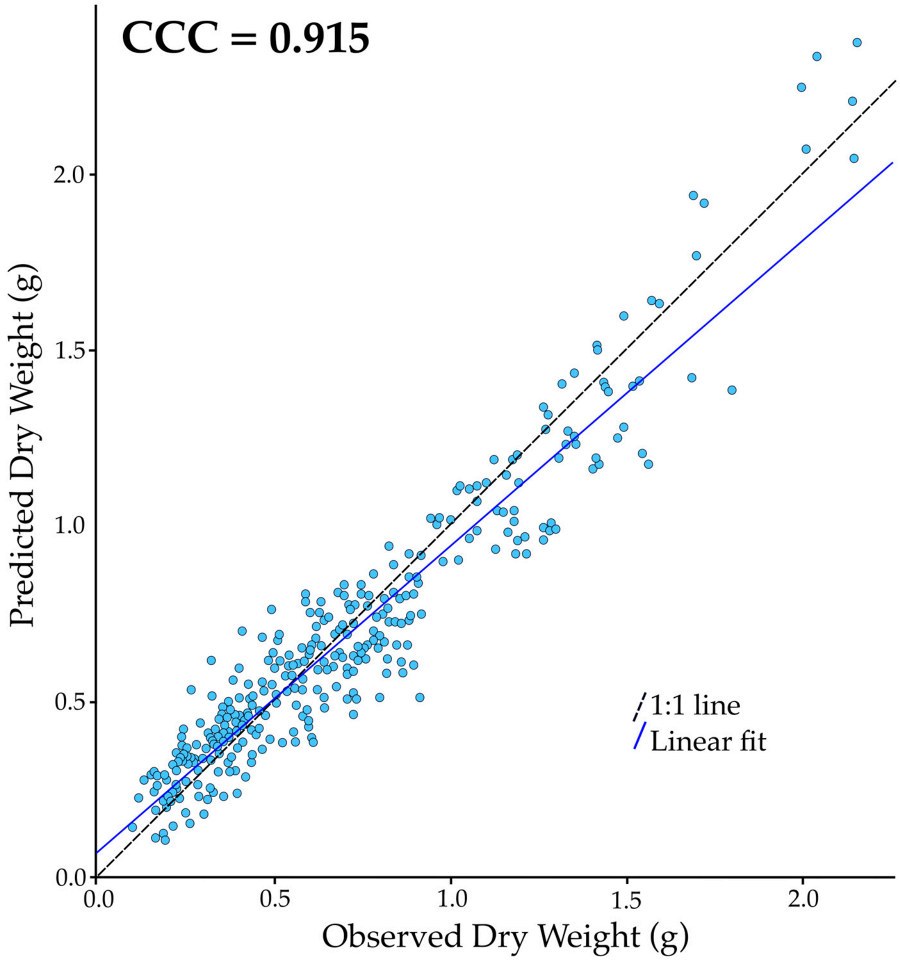

Figure 3. Predicted vs. observed leaf dry weight (g) from 10-fold cross-validation of the allometric model. The dashed 1:1 line represents perfect agreement between predicted and observed values, and the solid blue line that represents linear fit between predicted and observed values. The CCC indicates a strong agreement between predicted and observed values.

Combining the allometric equation for leaf biomass and area with the leaf carbon fraction reported by Pergent-Martini et al. (2021) [2] (37.8 ± 1.6%) as a fixed multiplier, an equation estimating carbon content (C) based on leaf area can be derived (Equation (6)). The combined prediction uncertainty of such equation is ±21.2%, propagating from the absolute error (±20.8%) and the variability of the carbon conversion factor (4.23%) [2].

$$C = 0.000952 \times \text{Area}^{1.122} \times e^{\epsilon};\ \text{with}\ \epsilon \sim N(0, 0.02867) \quad (6)$$

4. Discussion

This study provides one of the first robust allometric characterizations of the relationship between *P. oceanica* leaf area and dry weight. This relationship is described by an allometric power law equation, where the resulting exponent (β = 1.122) indicates a slightly superlinear relationship, meaning that leaf mass increases proportionally faster than area [31,56–59]. The validity of this scaling exponent across the two sampling periods was confirmed by the non-significant interaction between leaf area and dataset ($p > 0.05$), indicating that the departure from isometry is a consistent property of the species rather than a result of sampling period. The allometric factor's departure from perfect isometry suggests that larger leaves become progressively denser or thicker as they grow, likely reflecting progressive investment in structural tissue with increasing leaf size [25,60]. To account for the not-rectangular shape of *P. oceanica* leaves, a correction factor was applied (0.996 ± 0.086 SD), following Schrader et al. (2021) [50]. The correction factor indicates a slight underestimation of leaf area probably because *P. oceanica* leaves present natural irregularities along their blades or small herbivore bite marks, preventing the leaf outline from conforming perfectly to a rectangular approximation [4,14]. The robustness and uncertainty range of the allometric equation across 16 sites and two sampling periods confirms its potential as a reliable predictive tool for estimating *P. oceanica* leaf biomass from non-destructive area measurements.

The use of power-law allometric models to predict leaf biomass from morphological measurements has precedent across several terrestrial plant taxa [23,25,56,61–64]. Panayotidis and Simboura (1989) [65] were among the first to study the relationship between leaf surface and biomass in Mediterranean seagrasses, suggesting that the allometric relationship investigated has general value for many species [65]. Other studies, such as Echavarria-Heras et al. (2011), followed up on this approach, using the length ratio as a tool to estimate leaf biomass in *Zostera marina* through an allometric model [58]. Giovannetti et al. (2008) [30], whose work initially inspired this study, were among the first to test this on *P. oceanica* leaves and also found a significant relationship between leaf area and weight in the Ligurian Sea using a linear regression. They acknowledged the limits of a linear approach in its capacity to account for seasonal and environmentally influenced biomass peaks, suggesting further mathematical modeling was needed [30].

These limitations are addressed in the present study through the application of LMMs, which explicitly account for site-level variability as a random factor, enabling a more robust allometric characterization. Furthermore, the use of LMMs allowed for the comparison between sampling periods throughout this study. The absence of a significant interaction between the sampling period and the area indicates that the allometric relationship is not dependent on time. However, the significant effect of dataset as a fixed term indicates that for a given leaf area, biomass differs between sampling periods. This pattern suggests that environmental or temporal factors can affect the baseline biomass level between datasets without altering the underlying scaling mechanism. Considering the high concordance score (CCC = 0.915), the low prediction error observed during cross-validation and these results, the allometric model describes the relationship between *P. oceanica* leaf area and dry weight in a manner that accounts for spatial or temporal constraints, strengthening its value as a broadly applicable predictive tool.

The spatial and temporal independence of the allometric equation positions it as a practical foundation for non-destructive protocols to estimate *P. oceanica* biomass. Such protocols would involve the non-destructive measurement of leaf area in the field, using underwater measurements of leaf length and width to derive individual leaf area. A minimum of nine randomly selected shoots per station is recommended, consistent with standard *P. oceanica* monitoring guidelines [14]. The allometric equation derived here is then applied to each shoot to estimate shoot dry weight, which may be scaled to meadow level using shoot density and percentage cover to derive the leaf standing crop (LSC) [14]. Both shoot density and percentage cover can be measured with non-destructive protocols, using standard quadrat-based methods [14,66]. Within the application of allometric equation, the error term must be considered since the multiplicative error structure of the allometric model results in biomass predictions typically within ±20.8% of observed values, a margin that should be accounted for when interpreting meadow-scale estimates.

For large-scale or temporally repeated assessments, spatial heterogeneity in meadow structure must also be considered. Variations in shoot density and percentage cover—both of which influence the scaling from shoot-level to meadow-level biomass—should be monitored at pre-defined stations stratified according to the meadow's structural heterogeneity [2,14,41,66–68]. This stratified approach ensures that spatial gradients in meadow condition are adequately captured rather than averaged out.

Despite these limitations, the promising results observed within this study depict the allometric estimate as an attractive alternative to traditional sampling methods. Lepidochronology, for instance, requires the physical uprooting of shoots or the imposition of physical stress on the plant, potentially harming the meadow. In contrast, the allometric equation derived here provides a non-destructive method to estimate leaf biomass with predictive accuracy, requiring only the measure of leaf dimensions in the field. It must be acknowledged that the non-destructive nature of this approach carries inherent trade-offs. Physical sampling methods such as lepidochronology allow for the potential study of tissue carbon content and the reconstruction of growth patterns through the examination of rhizome scales, information that can be obtained only by physical sampling. The allometric approach is therefore best understood not as a wholesale replacement for destructive methods but as a tool that is especially valuable when the primary objective is biomass estimation and when meadow integrity must be preserved.

The need for such non-invasive monitoring is particularly relevant in restoration contexts. Pansini et al. (2024) and Bacci et al. (2024) both emphasize that restoration projects require monitoring metrics that do not damage transplanted patches, especially given the long timescales over which restoration must be assessed, often exceeding 10 years [16,69]. The allometric equation therefore opens up new possibilities for non-harmful monitoring of biomass, both at a local and seascape scale.

Estimating carbon content within leaves represents a further valuable application of this protocol in restoration and monitoring contexts. In this study, the Mediterranean average carbon content of 37.8% in *P. oceanica* leaves, reported by Pergent-Martini et al. (2021), is used to derive an equation estimating carbon content from leaf area [2]. It must be acknowledged that this is a general estimate that does not account for seasonal, geographical or ecological variations in leaf carbon content. Consequently, the carbon equation is most reliable when integrating data across multiple seasons and sites, rather than individual shoots sampled at a specific time and depth [32]. Furthermore, the conversion of dry weight to carbon content introduces a layer of uncertainty on top of the allometric confidence interval, as it combines the carbon ratio variability (±1.5–1.6 percentage points) with the equation's error term, resulting in a combined prediction uncertainty of ±21.2%. The difference in the conversion factors of Pergent-Martini et al. (2021) and Pergent et al. (1994) suggests that carbon content may vary with meadow ecological condition, season, and geographical location, which prompts caution when applying the fixed conversion factors across the dataset [2,7]. Nevertheless, the combination of literature data regarding carbon content and the allometric equation presented here represents a promising first step toward further methodological development of a reliable tool for estimating meadow carbon content in *P. oceanica* leaves. Such estimates could contribute to the growing body of work on Blue Carbon, providing a basis for carbon credit quantification to support funding of conservation and restoration projects [64], a factor that would greatly benefit restoration programs, in line with the objectives to achieve in the European Union Environmental Action Programme (EAP) [41,64–71]. However, such applications remain limited at the current stage of methodological development, as further applications of the allometric equation derived here are required for a standardized industrial scale of application.

Despite the promising applications of this allometric equation, several limitations must be acknowledged. The most significant one is the failure to account for environmental covariates such as depth or water quality, which can severely influence the morphology of *P. oceanica* leaves [5,11,30,31,72–74]. Instances of this can be found in Jiménez-Casero et al. (2023), where it is shown that the structure of *P. oceanica* leaves is affected by nutrient discharges through photo-physiological adjustments or the reduction in shoot architectural complexity [75]. Both these features severely affect the plant's capacity to allocate leaf tissue, thereby impacting the length and width of the leaf, which in turn can alter the allometric relation [76–79]. This limitation is further exacerbated by the constraints of the dataset, where the size range of leaves in shoots ranged between 28.21 cm^2 and 663.36 cm^2. This range excludes juvenile leaves or extremely large leaves, as most allometric models rely on mature leaves [2,14,34,64]. Future works should aim to expand the current work and cover a broader size range, including juvenile shoots, providing a greater relevance when applying the non-destructive protocol to restoration sites. Finally, although the Ligurian dataset is robust, further validation across meadows throughout the Mediterranean would help confirm the broader applicability of the derived equation, particularly for meadows subjected to a wider range of stressors that may alter leaf morphology and carbon content [16]. Such future operational field validation of this proposed protocol would include the study of meadow biomass through traditional sampling methods and the comparison of these results to the predictions of the allometric model. Once small-scale implementations have been tested and the protocol is fine-tuned, the large-scale implementation of biomass estimation could be tested. At larger scales, implementation would further require the use of advanced technologies such as remote sensing. For instance, the implementation of a side-scan sonar (SSS) or multibeam echosounder to measure a meadow's extent and delineate its boundaries provides key information in the creation of a sampling plan and in the accurate estimate of whole-meadow canopy biomass [9,80].

5. Conclusions

The allometric equation developed in this study represents a noteworthy tool for researchers and managers studying *Posidonia oceanica* leaf biomass from non-destructive measurements of leaf area. Validated across 16 sites and two sampling periods, the model demonstrates a stable allometric structure, with a consistent scaling relationship between leaf area and dry weight despite environmental and spatial variability. While differences in baseline biomass between datasets and sites were observed, the underlying allometric exponent remained constant, preserving a fundamental scaling relationship in *P. oceanica* leaves across conditions.

Thus, the allometric relationship described here represents an important step towards the development of a non-destructive approach for assessing seagrass biomass. By linking easily measurable morphological traits to dry weight, the model enables the estimation of shoot-level biomass and its subsequent scaling to meadow level using standardized measurements of shoot density and cover. Hence, this work provides one of the first frameworks for integrating individual-level measurements into seascape-scale biomass assessments. Importantly, this approach has not yet been tested in the field at full operational scale. However, this work initiates a body of research that establishes the basis for a non-destructive monitoring protocol, incorporating species-specific growth dynamics and extending this framework towards estimates of primary productivity.

Overall, the results highlight the strength of allometric scaling as a predictive tool in seagrass ecology and support the development of a standardized, low-impact method for long-term monitoring of *Posidonia oceanica* meadows.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author due to privacy.

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Abbreviations

The following abbreviations are used in this manuscript:

PREI	Posidonia Rapid Assessment Index
SCUBA	Self-Contained Underwater Breathing Apparatus
CF	Correction Factor
DW	Dry Weight
LMM	Linear Mixed-Effect Model
AIC	Akaike Information Criterion
LRT	Likelihood Ratio Test
RMSE	Root of the Mean of Square of Errors
MAE	Mean of Absolute value of Errors
CCC	Concordance Correlation Coefficient
CI	Confidence Interval
SD	Standard Deviation
EAP	European Union Environmental Action Programme

Appendix A

Appendix A.1. Distributional Diagnostics

Table A1. Per-site descriptive table for number of shoots analyzed and their resulting mean leaf area and dry weight.

Site	N of Shoots	μ Area (cm^2)	μ *DW* (g)
Bergeggi Isola	18	266	1.36
Niasca	27	135	0.685
Punta Pedale 2024–2025	27	147	0.717
Sanremo Porto Vecchio	27	164	0.831
Bergeggi	18	124	0.26
Camogli	18	190	0.551
Diano Marina	18	118	0.26
Framura	18	203	0.759
Monterosso	18	177	0.686
Noli	18	191	0.616
Ospedaletti	36	207	0.577
Prelo	27	206	0.831
Punta Manara	18	391	1.34
Punta Pedale 2016–2018	18	173	0.486
Sanremo	9	200	0.483
Vado Ligure	18	302	1.07
Ventimiglia	18	208	0.643

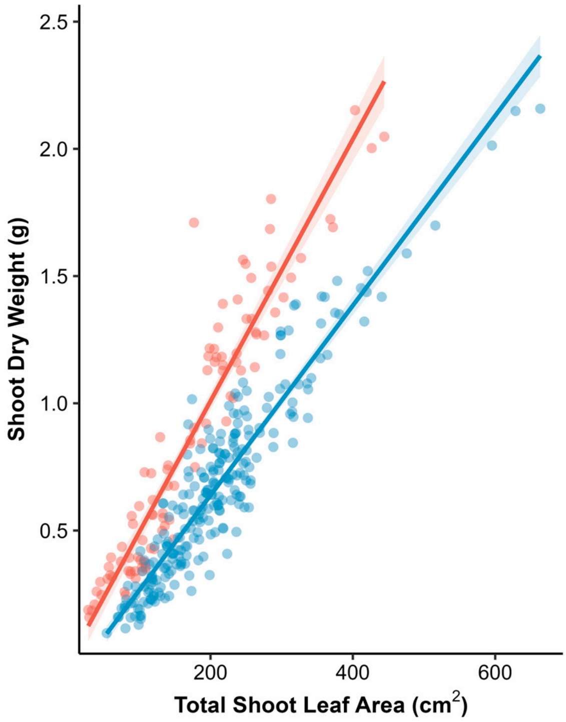

Figure A1. Regressions of datasets based on the time of sampling; in blue are samples collected between 2016 and 2018, and in red are those collected between 2024 and 2025.

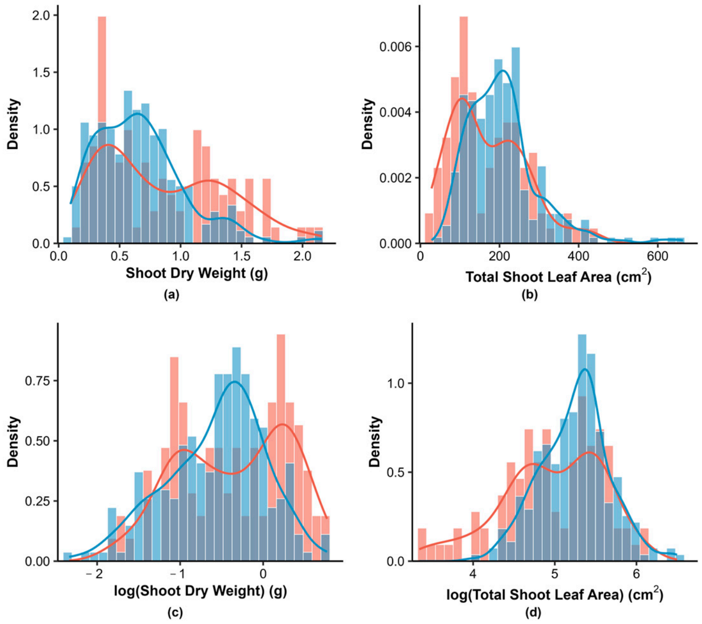

Figure A2. Histogram data distribution for dry weight and area; in blue are samples collected between 2016 and 2018, and in red are those collected between 2024 and 2025. (**a**) Distribution for shoot dry weight, (**b**) distribution for shoot leaf area, (**c**) distribution for log-transformed dry weight, (**d**) distribution for log-transformed shoot area.

Appendix A.2. Residual Diagnostics

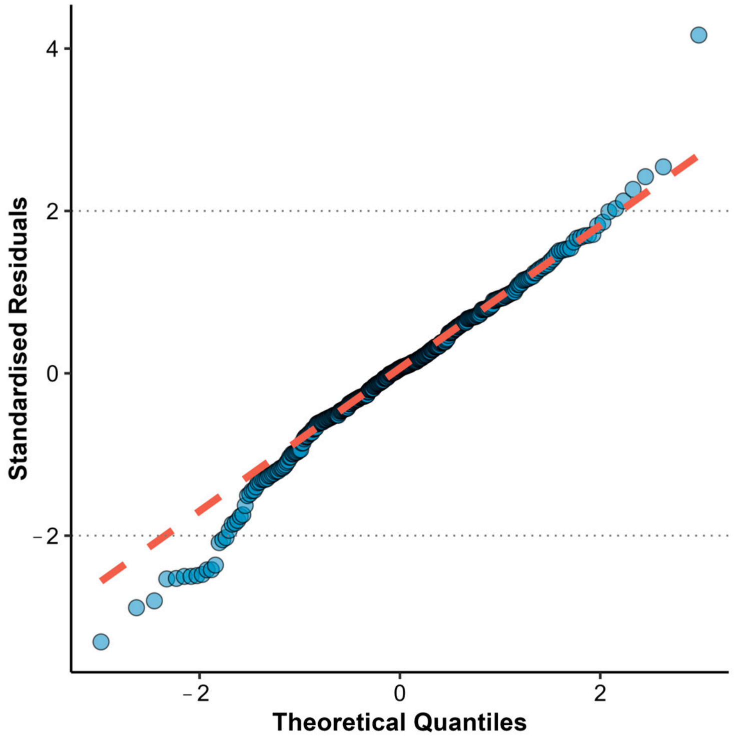

Figure A3. Q-Q plot of residuals for the final model.

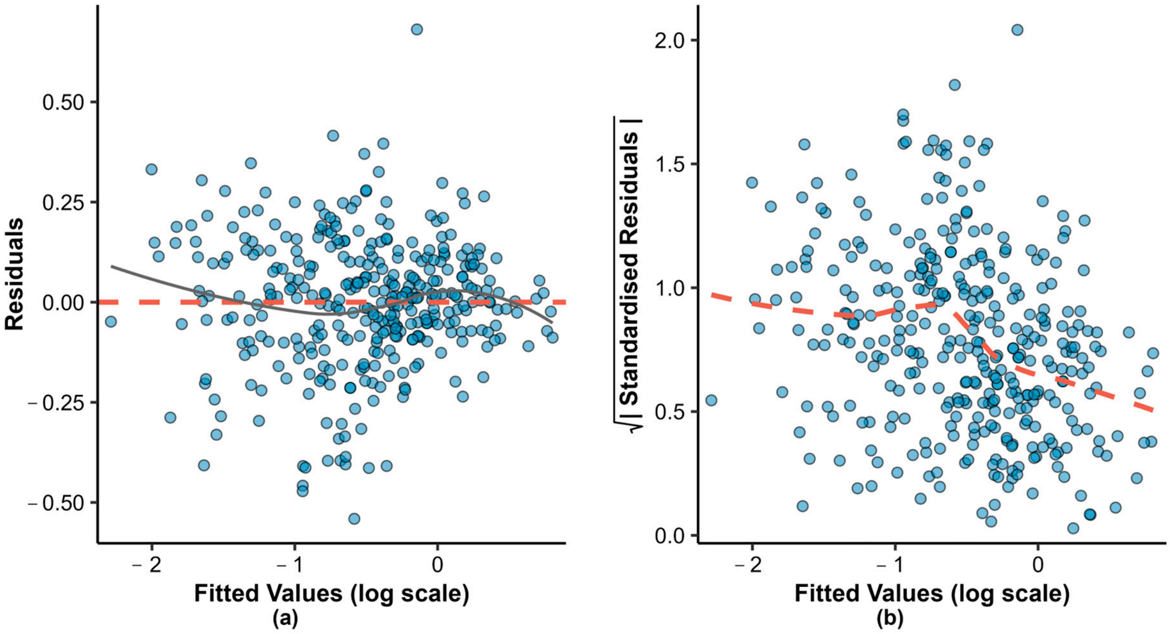

Figure A4. Linear Mixed Model diagnostic plots: (**a**) final LMM with residuals vs. fitted values, Equation (3), (**b**) scale–location plot.

Appendix A.3. Model Comparison

Table A2. Full LRT output (Model 1 vs. Model 2…), with degrees of freedom, X2 statistics and *p*-values.

Comparison	df	X^2	*p*-Value
Model 1–Model 2	2	24.993	<0.001
Model 2–Model 3	1	13.615	<0.001
Model 3–Model 4	1	3.752	0.053

Table A3. Kenward–Roger F-test output for final model, Equation (3).

Fixed Effect	Numerator df	Denominator df	F-Value	*p*-Value
Log(Area)	1	14.7	571.059	<0.001
Sampling Period	1	14.1	19.017	<0.001

Appendix A.4. Cross-Validation

Table A4. Fold-by-fold performance table (RMSE and MAE) for each of the 10 folds.

Fold	n	RMSE	MAE	Bias
1	36	0.2689	0.2070	0.0734
2	35	0.2309	0.1906	−0.0369
3	35	0.2498	0.1813	0.0472
4	35	0.2268	0.1630	−0.0657
5	35	0.2274	0.1892	0.0320
6	35	0.2131	0.1692	0.0172
7	35	0.2646	0.2135	0.0512
8	35	0.1969	0.1694	−0.0415
9	35	0.2405	0.1780	−0.0483
10	35	0.2795	0.2244	−0.0493
Overall	351	0.2412	0.1886	−0.0019

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