



## Short-term and long-term ocean acidification effects on seagrass performance: evidence from shallow CO<sub>2</sub> vents

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### 1. Introduction

Since the industrial revolution, the rapid increase of carbon dioxide (CO<sub>2</sub>) released into the atmosphere, has reached the concentration of 425 ppm in 2024 (Lan et al., 2024). These emissions are partially mitigated by the ocean which, absorbing this gas, induces a decrease in levels of ocean pH and changes to the chemistry of carbonates (Caldeira and Wickett, 2003; Quéré et al., 2018). This phenomenon is widely known as ocean acidification (OA), which has become one of the main effects of global climate change that concerns marine ecologists (Brewer, 2013; Sabine et al., 2004). According to the current predictions, the global ocean surface pH is likely to decrease by 0.3 units by 2100 (Representative Concentration Pathway 8.5 worst-case scenario, IPCC, 2022), causing extraordinary marine alterations from individual organisms to ecosystems (Connell and Leung, 2023; Fabry et al., 2008; Orr et al., 2005). This is particularly evident for Mediterranean Sea that, being a semi enclosed basin with limited water circulation, seems particularly affected by the effects of climate change (Chefaoui et al., 2018).

Although OA is expected to affect ecological dynamics, predicting its impact on marine biota, however, remains controversial, since the response of organisms is highly variable depending on their biological and ecological features (Connell and Leung, 2023; Doney et al., 2012; Kroeker et al., 2010; Ravaglioli et al., 2024a; Wernberg et al., 2024). Although calcified organisms could be negatively affected (Fabry et al., 2008; Kroeker et al., 2010), increased CO<sub>2</sub> may promote the performance of non-calcifying marine primary producers (Buapet et al., 2013; Koch et al., 2013), as in seagrasses.

Despite seagrass meadows are distributed over a limited portion of ocean surface (Short et al., 2007), they are among the most important shallow water ecosystems for the services they provide (Fourqurean et al., 2012; Hemminga and Duarte, 2000; Larkum et al., 2006;

Macreadie et al., 2014). These include the blue carbon sequestration in the coastal areas and a relevant contribution of carbon stored in deep oceans (Chang et al., 2024; Dahl et al., 2023; Lavery et al., 2013; Nordlund et al., 2018). Because of this, seagrasses are currently considered a potential nature-based solution in mitigating anthropogenic CO<sub>2</sub> emissions and climate change (Fakhraee et al., 2023; Forrester et al., 2024; Stankovic et al., 2021), although their use as a mitigation solution has yet to be adopted on a wide scale (Chausson et al., 2020; do Amaral Camara Lima et al., 2023; United Nations Environment Programme, 2020). Likely, this is likely due to several aspects: first, seagrass meadows are still highly threatened by anthropogenic local impacts (Bockelmann et al., 2012; Orth et al., 2006; Unsworth et al., 2019), and their conservation and management measures remain lacking (Griffiths et al., 2020) and, secondly, there are some gaps of knowledge regarding plant responses to both current and future climate conditions, highlighting the need from the scientific community to understand their acclimation and adaptation. Consequently, this claims for a collaborative effort from stakeholders towards well-designed conservation measures and seagrass restoration projects (Pansini et al., 2022, 2025; van Katwijk et al., 2016).

Predicting the effect of future OA on seagrasses is currently gaining momentum. As carbon-limited plants, higher levels of CO<sub>2</sub> may promote their performance in terms of photosynthetic activity, growth, biomass, and flowering event (Apostolaki et al., 2014; Koch et al., 2013; Kroeker et al., 2010). OA may also change the carbon balance of the seagrass community and alter their role as blue carbon ecosystems (Egea et al., 2018a; Yamuza-Magdaleno et al., 2025), as by promoting belowground biomass and therefore an increase in carbon sequestration (Palacios and Zimmerman, 2007; Russell et al., 2013). Moreover, seagrasses may act as a refuge for calcifying organisms against OA, although results may dependent upon species (Cossa et al., 2024; Ravaglioli et al., 2024b). However, the benefit received by the increased CO<sub>2</sub> on plants is not

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obvious and cannot be uniformly applicable to all seagrass species and environment (Borum et al., 2016; Guerrero-Meseguer et al., 2020; Hassoun et al., 2022; Ow et al., 2015; Pansini et al., 2023).

In this framework, the seagrass *Cymodocea nodosa* Ucria (Ascherson), widespread in the in the Mediterranean basin and occurring in the northeast Atlantic (Máñez-Crespo et al., 2020; Mascaró et al., 2009), serves as an excellent example for investigating seagrass acclimation and adaptation to OA. *C. nodosa* is particularly interesting because it is a fast-growing clonal species with a large phenotypic plasticity, occurring in several environmental conditions and for showing physiological and morphological adaptations to natural and anthropogenic stressors (Cancemi et al., 2002; Larkum et al., 2006), likely due to an efficient clonal integration among the apical and vertical shoots of the ramet (Tuya et al., 2013a, 2013b). Nevertheless, a well-documented regression in *C. nodosa* has been recorded due to anthropogenic pressure (Orth et al., 2006; Tuya et al., 2019) and effects of climate change (Chefaoui et al., 2016, 2018), so that this species has been listed in annexes of Barcelona and Bern conventions (82/72/EEC).

Scientific evidence suggests that low pH conditions may stimulate *C. nodosa* leaf abundance, photosynthetic activity, and carbon metabolism (Apostolaki et al., 2014; De los Santos et al., 2017; Yamuza-Magdaleno et al., 2025), but also determine the loss of phenolic protective compounds which may affect grazing and disease dynamics (Arnold et al., 2012; Jiménez-Ramos et al., 2017; Rodríguez et al., 2022). However, inconsistent results, probably due to a variety of experimental procedures, did not allow providing information on key ecological traits such as changes in seagrass above and below ground biomass, leaf area, and inorganic carbon accumulation (Apostolaki et al., 2014; De los Santos et al., 2017; Vizzini et al., 2019).

Most studies investigated the performance of *C. nodosa* using short-term manipulations in acidified mesocosms (Egea et al., 2018a; Rodríguez et al., 2022; Yamuza-Magdaleno et al., 2025) or field experiments that describe the long-term adaptations of the seagrass that naturally occur along natural low pH gradients (Apostolaki et al., 2014; Mishra et al., 2021; Vizzini et al., 2019). To our knowledge, only one field experiment has explored the short-term acclimation responses of transplanted control-pH plants in naturally acidified conditions. However, this study focused on changes in seagrass internal cell organisation which, after three weeks of low pH exposure, was negatively influenced, then interpreted as an apoptotic-like programmed cell death (Koutalianou et al., 2022).

This study assessed the performance of the seagrass *Cymodocea nodosa* to the short-term and long-term exposures to the projected OA scenario, with the hypothesis that seagrass productivity would increase under these conditions. To pursue this aim, a three-month field manipulative experiment was conducted in a natural sea acidified site (where shallow submarine CO<sub>2</sub> seeps occur) and other three control pH sites, where *C. nodosa* meadows are present. The experimental design allowed distinguishing between the effects of a short-term acidification exposure (by comparing transplanted plants from control pH sites to low pH site with translocated control pH plants) from those of a long-term acidification (by comparing untouched plants in control pH and low pH sites). As key ecological traits aboveground and belowground morphometry, biomass, and leaf physiology were investigated. Variability on both apical shoots (horizontal) and other shoots (vertical) of the cuttings were analysed, expecting that the response of the former (prostrate, which allows the meadow to expand) to OA would be enhanced respect to that of the latter. This integrative approach contributes to the knowledge of the consequences of the acidification conditions of the climate change scenarios on seagrasses, also disentangling the effects of plant manipulation (needed for the experiment) and origin.

## 2. Materials and methods

### 2.1. Study sites and environmental context

The experiment was done during summer 2024 in four sites across Lipari and Vulcano Islands, Italy (Fig. 1). In Levante Bay, Vulcano, a hydrothermal shallow water system (characterised by >90 % mol of CO<sub>2</sub> on and <2 % mol of H<sub>2</sub>S on dry gas basis) occurs and the strong pH gradient within less than 1 km allows simulating the effect of future OA on marine organisms (Aiuppa et al., 2021; Boatta et al., 2013; Duquette et al., 2017; Vizzini et al., 2013) by using sites along the gradient. One acidified site (LPH, mean pH 7.70) and three control pH sites, distancing about 0.3, 4.5 and 5 km from the LPH site, were selected (CPH, C1 and C2, mean pH 8.09, 8.09 and 8.10, respectively, Vizzini et al., 2013; Boatta et al., 2013). All the four sites exhibit similar temperature regimes, light intensity and hydrodynamics, as they are located inside bays and exposed to the east winds and currents, and in all of them *C. nodosa* meadows, with patch size diameter higher than 30 m, are located on volcanic sand at 4 ± 0.3 m of depth.

### 2.2. Experimental design and data collection

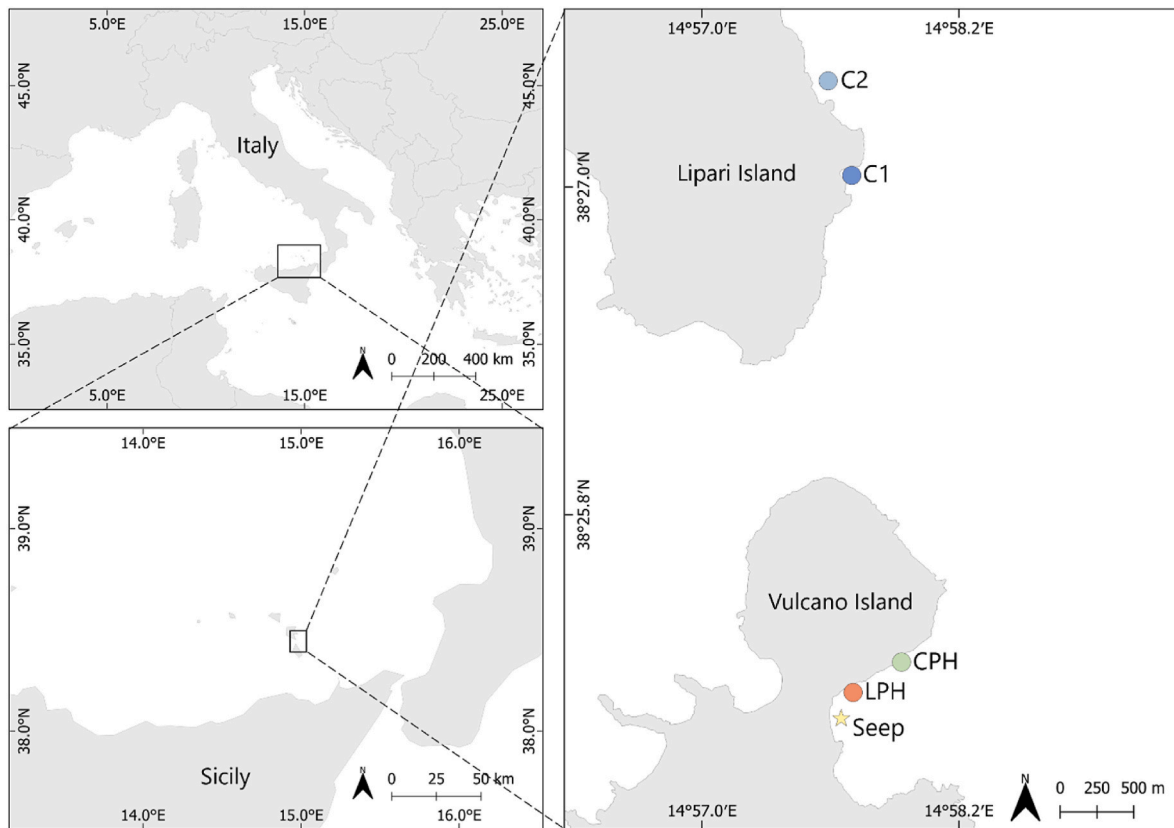
The manipulative experiment was set up on 5<sup>th</sup> July 2024 and lasted three months. *C. nodosa* cuttings, each bearing an apical and other four vertical shoots, coming from a terminal ramet, were randomly collected and manipulated as experimental units (EU). For each control site (CPH, C1 and C2), five EU were collected and translocated at the same depth within the same site (Chapman, 1986) while other five EU were transplanted in the acidified site (LPH), at the same depth. Conversely, in LPH five EU were translocated within the same site, to test the effect of plant manipulation and origin. Each EU was fixed to the substrate with the use of u-shaped pegs. On 5<sup>th</sup> October 2024, all the EU were removed, and, for each site, five additional *C. nodosa* untouched cuttings were also collected (Fig. 2 and S1). Overall, the EU consisted of a total of 55 cuttings (overall 625 shoots).

For the whole duration of the experiment, temperature and light intensity were measured every hour with a HOBO® Temp/Light UA-002-64 logger, while pH was measured at the beginning and at the end of the experiment with the use of a smarTROLL MP® probe recording every 2 s for 30 min (Fig. 3).

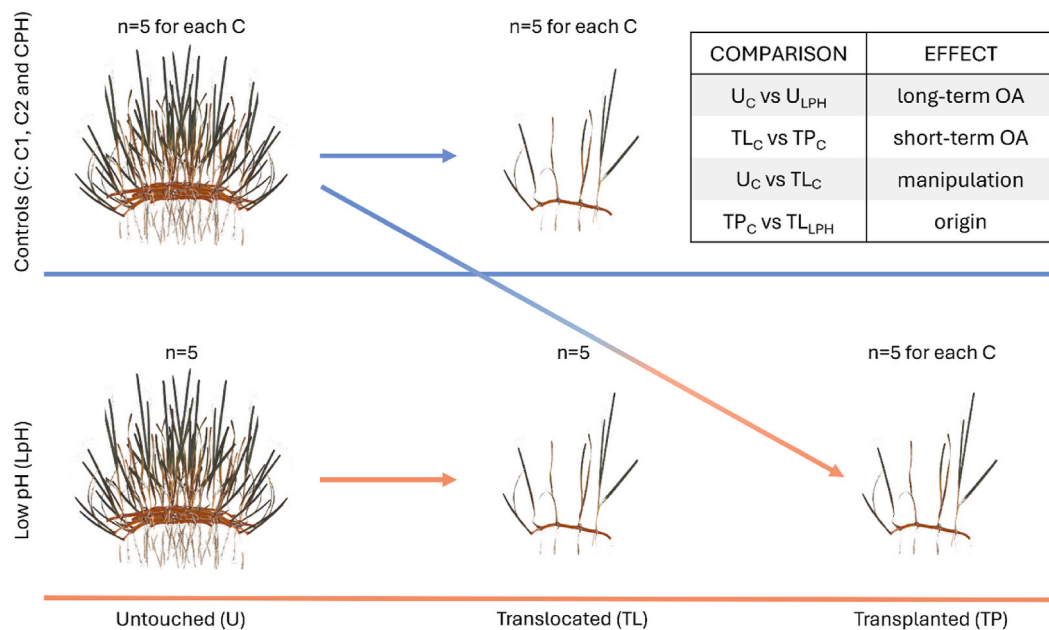
To disentangle any source of variability, several combinations of treatments between untouched, translocated, and transplanted cuttings of different sites were compared (Fig. 2). Overall, for each control site (C) the effects were evaluated as follows: Translocated<sub>C</sub> vs Transplanted<sub>C</sub> in LPH plants (short-term acidification); Untouched<sub>C</sub> vs Untouched<sub>LPH</sub> plants (long-term acidification); Untouched<sub>C</sub> vs Translocated<sub>C</sub> plants (manipulation); Transplanted<sub>C</sub> in LPH vs Translocated<sub>LPH</sub> plants (plant origin).

In the laboratory, *C. nodosa* cuttings were first cleaned from epiphytes and then each shoot per cutting was examined: shoot morphometry was measured in terms of number of leaves, leaf, root and internode length and leaf necrotic brown tissue length (considered when it covered the entire width and expressed in ratio on the total leaf length). Above and belowground shoot biomass was estimated on 24 h freeze-dried leaves and on 48 h–60 °C dried internodes and roots, respectively.

Shoot physiology was assessed by measuring the concentrations of total chlorophylls (a + b) and carotenoids (x + c) in the green portions of the leaves. These leaf tissues were ground into a fine powder using a Retsch® MM 400 mixer mill at 25 Hz for 3 min. Powdered samples (0.010 g) were then treated with ice-cold 100 % acetone until complete pigment extraction was achieved, followed by centrifugation at 4000 rpm for 10 min. The resulting supernatants were transferred into glass cuvettes for spectrophotometric analysis. Absorbance readings were taken at 662 nm, 645 nm, and 470 nm, corresponding to chlorophyll a, chlorophyll b, and total carotenoids, respectively. Pigment



**Fig. 1.** Location of experimental sites. In Vulcano Island two sites corresponding to the low and the control pH conditions were chosen (LPH and CPH). Two additional control sites were spanned among Lipari Island (C1 and C2).

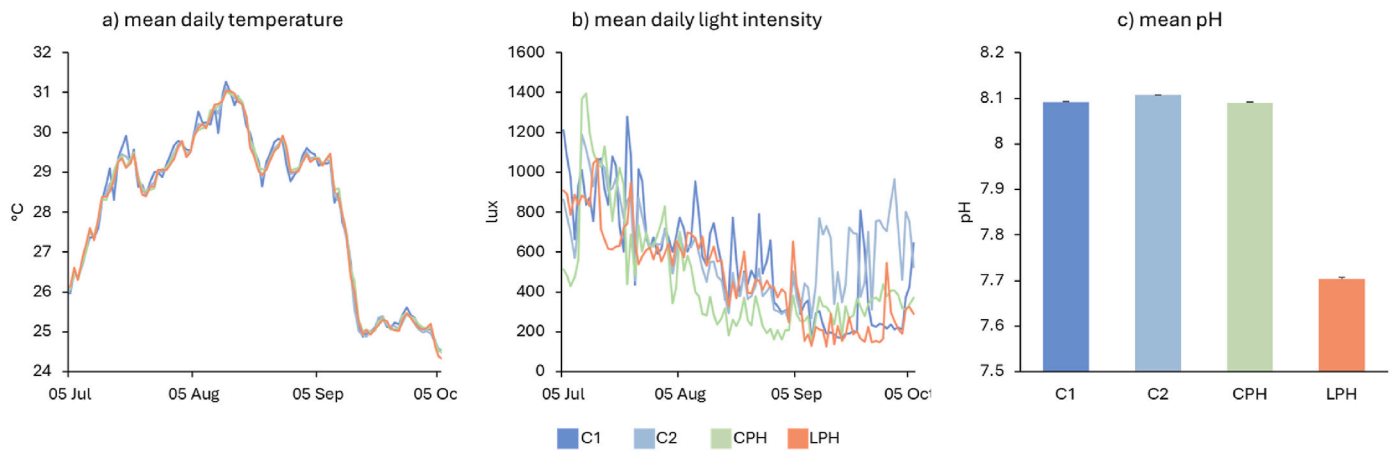


**Fig. 2.** Experimental set-up and effect type comparisons. For each Control site (C: C1, C2, and CPH), *C. nodosa* cuttings from an untouched (U) meadow were translocated (TL) and transplanted (TP) in the low pH site (LPH). In LPH, cuttings were only translocated. At the end of the study, all cuttings were removed and for each site untouched cuttings were collected. Comparison of treatments investigated the effects of long-term acidification ( $U_C$  vs  $U_{LPH}$ ), short-term acidification ( $TL_C$  vs  $TP_C$ ), manipulation ( $U_C$  vs  $TL_C$ ), and origin ( $TP_C$  vs  $TL_{LPH}$ ).

concentrations were calculated following the equations of Lichtenthaler (1987) and expressed as milligrams per gram of dry weight ( $mg\ g^{-1}$  DW).

### 2.3. Data analysis

To evaluate differences in *C. nodosa* morphology and physiology due

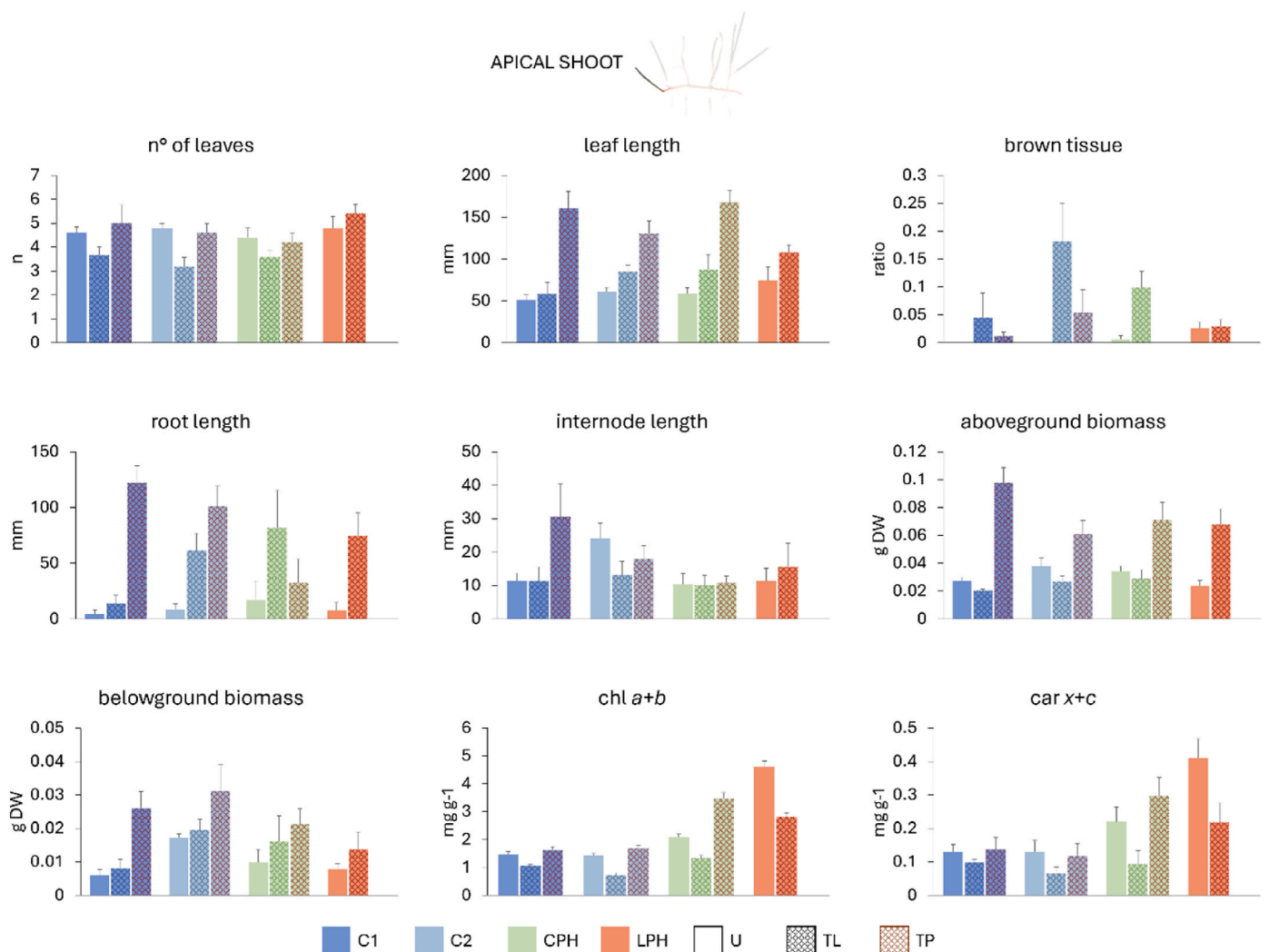


**Fig. 3.** a Mean daily temperature (°C) and light intensity (lux) at each site, from 5<sup>th</sup> July to 5<sup>th</sup> October 2024 measured with HOBO® Pendant Temp/Light UA-002-64 loggers. c) Mean (±SE) pH at each site at the beginning (5<sup>th</sup> July 2024) and at the end (5<sup>th</sup> October 2024) of the experiment, measured with a smarTROLL MP® probe recording every 2 s for 30 min.

to treatments, univariate statistics was used. Since clonal integration can affect seagrass performance (Liu et al., 2016; Ruocco et al., 2021), for each cutting data taken from the apical shoot were analysed separately from those of others shoots (from the 2<sup>nd</sup> to the 5<sup>th</sup> shoot), the latter

being pooled together, avoiding dependent samples.

Design was unbalanced due to some shoot mortality, so that for each control site a one-way permutational analysis of variance (PERMANOVA; Anderson, 2001) was run testing the effect of the treatment with



**Fig. 4.** Mean (±SE) number of leaves, leaf length, leaf brown tissue, root length, internode length, above and below ground biomass, chl a + b and car x + c of apical shoots in untouched (U, full colour bars), translocated (TL, gridded bars) and transplanted (TP, red-gridded bars) *C. nodosa* plants.

five levels: Untouched<sub>C</sub>, Translocated<sub>C</sub>, Transplanted<sub>C</sub>, Untouched<sub>LPH</sub> and Translocated<sub>LPH</sub>. Resemblance matrix built on Euclidean distance on untransformed data were estimated and each model used 9999 unrestricted permutations of raw data and a partial sum of squares. Differences in temperature, light and pH conditions between sites were evaluated with PERMANOVAs, since data did not meet parametric assumptions. Pair wise tests were run to investigate significant treatment comparisons of interest. Tests were run using Primer 6 v.6.1.13 & PERMANOVA + software (Clarke and Gorley, 2006).

### 3. Results

#### 3.1. Environmental features

Temperature and light regimes did not differ between the sites (mean temperature and lux 28.13 °C ± 0.02 SE and 530.86 lux ± 26 SE; Fig. 3a and b, Table S1), except for the LPH pH values were lower than in control sites (Fig. 3c–Table S1) with a mean difference of 0.37 (±0.02 SE).

#### 3.2. Plant traits

Overall, all *C. nodosa* traits responded quite consistently to treatments, showing a stronger response of the apical shoot rather than of the

other shoots of the cuttings, with the leaf physiology more affected than the leaf morphology (Figs. 4 and 5, Tables 1 and 2).

Short-term acidification (Translocated<sub>C</sub> vs Transplanted<sub>C</sub> plants) had a significant impact on the transplanted apical shoots in almost all the investigated traits, except for the number of leaves and the internode length, where no difference was detected. Specifically, under low pH conditions, the leaf length increased twofold ( $7.67 \pm 0.92$  vs  $15.3 \pm 1.13$  cm, hereafter mean ± SE) and the shoot biomass was four times higher ( $0.02 \pm 2^{-3}$  vs  $0.08 \pm 0.01$  g). Additionally, shoots originating from CPH did not exhibit any leaf brown tissue, contrary to the translocated ones ( $0.1 \pm 0.01$ ; Fig. 4, Tables 1 and 2). Moreover, at the low pH site, acidification promoted the apical root length ( $1.41 \pm 0.69$  vs  $12.21 \pm 1.52$  cm) and biomass ( $0.01 \pm 4^{-3}$  vs  $0.03 \pm 0.01$  g) of the C1 transplanted plants. Short-term OA also improved above ground production in the other shoots of the cuttings, including leaf length ( $5.1 \pm 1.1$  vs  $13.4 \pm 0.8$  cm) and biomass ( $0.01 \pm 3^{-3}$  vs  $0.03 \pm 9^{-3}$  g) The total concentrations of chl *a* + *b* and car *x* + *c* were also enhanced showing a strong positive upregulation in both apical ( $1.04 \pm 0.17$  vs  $2.26 \pm 0.6$  mg g<sup>-1</sup> DW and  $0.08 \pm 0.01$  vs  $0.18 \pm 0.05$  mg g<sup>-1</sup> DW, respectively) and other shoots ( $0.86 \pm 0.12$  vs  $1.74 \pm 0.2$  mg g<sup>-1</sup> DW and  $0.06 \pm 5^{-3}$  vs  $0.15 \pm 0.03$  mg g<sup>-1</sup> DW, respectively) across all C1, C2, and CPH plants, except for the other shoots in C2 plants (Figs. 4 and 5, Tables 1 and 2).

Considering the long-term acidification effect (Untouched<sub>C</sub> vs

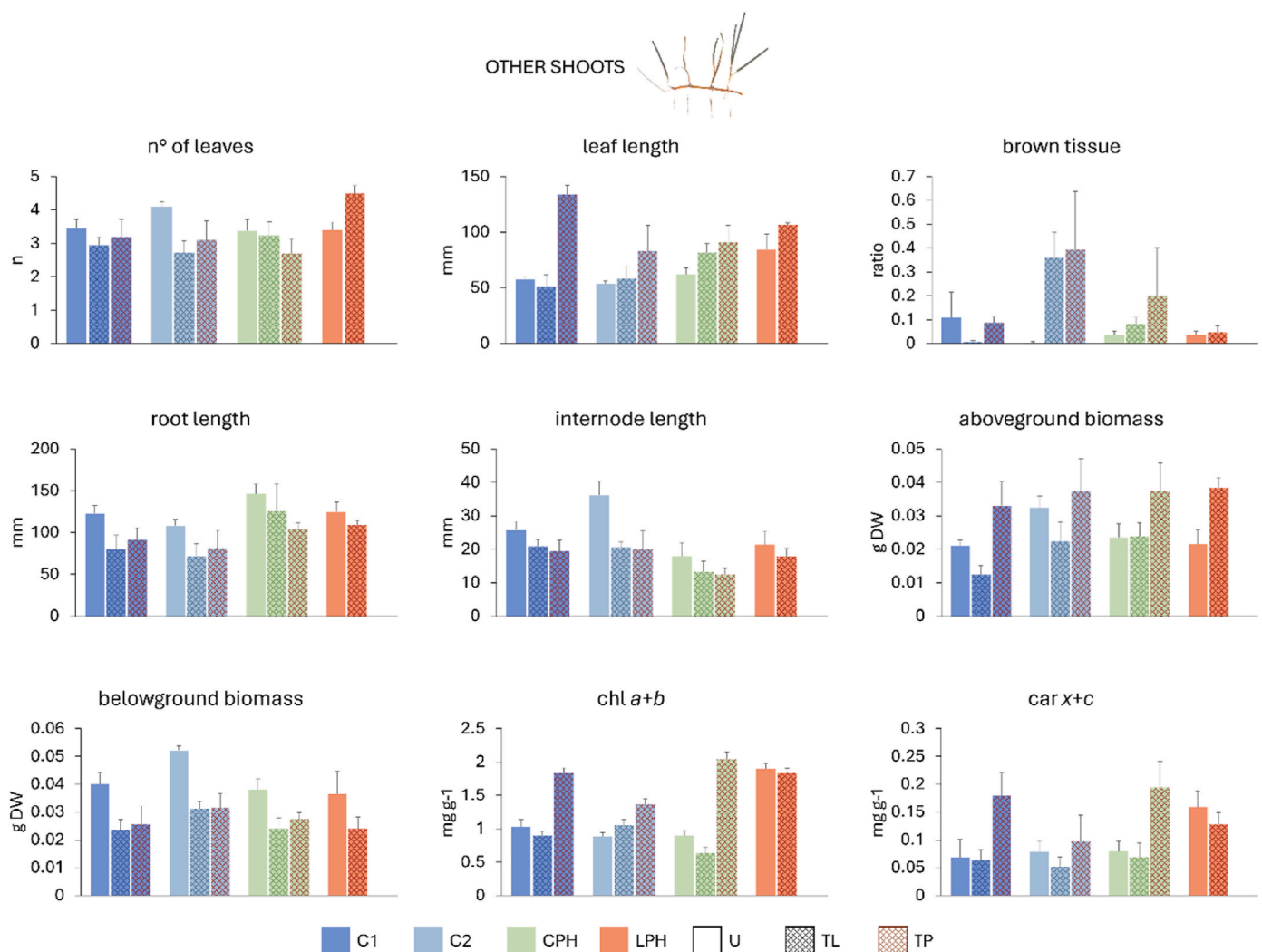


Fig. 5. Mean (±SE) number of leaves, leaf length, leaf brown tissue, root length, internode length, above and below ground biomass, chl *a* + *b* and car *x* + *c* of apical other in untouched (U, full colour bars), translocated (TL, gridded bars) and transplanted (TP, red-gridded bars) *C. nodosa* plants.

**Table 1**

PERMANOVA results on the effect of treatments (untouched = U, translocated = TL and transplanted shoots at low pH = TP) on *Cymodocea nodosa* morphology (number of leaves, leaf length, brown tissue, root length), biomass (above and below ground DW) and leaf pigments (chl *a* + *b* and car *x* + *c*) for apical and other shoots of plants from each control site (C1, C2, CPH). Significant values ( $P_{(perm)} < 0.05$ ) are highlighted in bold.

Site	C1					C2					CPH				
	shoot	apical		other		df	apical		other		df	apical		other	
		df	pseudo-F	$P_{(perm)}$	pseudo-F		$P_{(perm)}$	pseudo-F	$P_{(perm)}$	pseudo-F		$P_{(perm)}$	df	pseudo-F	$P_{(perm)}$
<i>n</i> <sup>o</sup> of leaves	4,22	1.24	0.335	3.09	<b>0.044</b>	4,24	4.51	<b>0.009</b>	4.53	<b>0.009</b>	4,24	2.97	<b>0.048</b>	3.69	<b>0.019</b>
leaf length	4,22	10.29	<b>&lt;0.001</b>	15.09	<b>&lt;0.001</b>	4,24	5.92	<b>0.002</b>	2.78	0.059	4,24	9.37	<b>&lt;0.001</b>	2.36	0.091
brown tissue	4,22	1.15	0.353	0.46	0.896	4,24	3.93	<b>0.012</b>	2.04	0.093	4,24	6.49	<b>0.002</b>	0.58	0.993
root length	4,22	15.79	<b>&lt;0.001</b>	2.59	0.072	4,24	7.91	<b>&lt;0.001</b>	2.65	0.063	4,24	2.42	0.083	0.99	0.442
internode length	4,22	1.77	0.155	0.99	0.433	4,24	1.07	0.398	3.77	<b>0.018</b>	4,24	0.31	0.911	1.46	0.262
above dw	4,22	17.66	<b>&lt;0.001</b>	6.88	<b>0.001</b>	4,24	7.05	<b>0.002</b>	1.9	0.149	4,24	6.96	<b>0.002</b>	2.55	0.061
below dw	4,22	5.28	<b>0.007</b>	1.68	0.195	4,24	3.69	<b>0.02</b>	4.57	<b>0.011</b>	4,24	1.12	0.372	1.87	0.159
chl <i>a</i> + <i>b</i>	4,24	34.8	<b>&lt;0.001</b>	37.91	<b>&lt;0.001</b>	4,24	39.92	<b>&lt;0.001</b>	42.45	<b>&lt;0.001</b>	4,24	15.1	<b>&lt;0.001</b>	59.39	<b>&lt;0.001</b>
car <i>x</i> + <i>c</i>	4,24	26.98	<b>&lt;0.001</b>	21.24	<b>&lt;0.001</b>	4,24	29.67	<b>&lt;0.001</b>	11.26	<b>&lt;0.001</b>	4,24	14.47	<b>&lt;0.001</b>	17.46	<b>&lt;0.001</b>

**Table 2**

Comparison of means (pair-wise tests) of *Cymodocea nodosa* variables testing the effects of short-term acidification (TL<sub>C</sub> vs TP<sub>C</sub>), long-term acidification (U<sub>C</sub> vs U<sub>LPH</sub>), manipulation (U<sub>C</sub> vs TL<sub>C</sub>), and origin (TP<sub>C</sub> vs TL<sub>LPH</sub>) on apical and other shoots of plants from each control site (C1, C2, CPH). Abbreviations: U = untouched; TL = translocated; TP = transplanted; ns = not significant. “-” symbol is shown when pair-wise test was not performed due to a lack of significant PERMANOVA results.

site	variable	comparison	effect type	C1		C2		CPH	
				apical	other	apical	other	apical	other
<i>n</i> <sup>o</sup> of leaves		TL <sub>C</sub> vs TP <sub>C</sub>	short-term OA	-	ns	ns	ns	ns	ns
		U <sub>C</sub> vs U <sub>LPH</sub>	long-term OA	-	ns	ns	ns	ns	ns
		U <sub>C</sub> vs TL <sub>C</sub>	manipulation	-	ns	U <sub>C</sub> > TL <sub>C</sub>	U <sub>C</sub> > TL <sub>C</sub>	ns	ns
		TP <sub>C</sub> vs TL <sub>LPH</sub>	origin	-	ns	ns	ns	ns	TP <sub>C</sub> < TL <sub>LPH</sub>
leaf length		TL <sub>C</sub> vs TP <sub>C</sub>	short-term OA	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>	-	TL <sub>C</sub> < TP <sub>C</sub>	-
		U <sub>C</sub> vs U <sub>LPH</sub>	long-term OA	ns	ns	ns	-	ns	-
		U <sub>C</sub> vs TL <sub>C</sub>	manipulation	ns	ns	U <sub>C</sub> < TL <sub>C</sub>	-	ns	-
		TP <sub>C</sub> vs TL <sub>LPH</sub>	origin	ns	TP <sub>C</sub> > TL <sub>LPH</sub>	ns	-	TP <sub>C</sub> < TL <sub>LPH</sub>	-
brown tissue		TL <sub>C</sub> vs TP <sub>C</sub>	short-term OA	-	-	ns	-	TL <sub>C</sub> > TP <sub>C</sub>	-
		U <sub>C</sub> vs U <sub>LPH</sub>	long-term OA	-	-	ns	-	ns	-
		U <sub>C</sub> vs TL <sub>C</sub>	manipulation	-	-	U <sub>C</sub> < TL <sub>C</sub>	-	U <sub>C</sub> < TL <sub>C</sub>	-
		TP <sub>C</sub> vs TL <sub>LPH</sub>	origin	-	-	ns	-	ns	-
root length		TL <sub>C</sub> vs TP <sub>C</sub>	short-term OA	TL <sub>C</sub> < TP <sub>C</sub>	-	ns	-	-	-
		U <sub>C</sub> vs U <sub>LPH</sub>	long-term OA	ns	-	ns	-	-	-
		U <sub>C</sub> vs TL <sub>C</sub>	manipulation	ns	-	ns	-	-	-
		TP <sub>C</sub> vs TL <sub>LPH</sub>	origin	ns	-	ns	-	-	-
internode length		TL <sub>C</sub> vs TP <sub>C</sub>	short-term OA	-	-	-	ns	-	-
		U <sub>C</sub> vs U <sub>LPH</sub>	long-term OA	-	-	-	U <sub>C</sub> > U <sub>LPH</sub>	-	-
		U <sub>C</sub> vs TL <sub>C</sub>	manipulation	-	-	-	U <sub>C</sub> > TL <sub>C</sub>	-	-
		TP <sub>C</sub> vs TL <sub>LPH</sub>	origin	-	-	-	ns	-	-
above DW		TL <sub>C</sub> vs TP <sub>C</sub>	short-term OA	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>	-	TL <sub>C</sub> < TP <sub>C</sub>	-
		U <sub>C</sub> vs U <sub>LPH</sub>	long-term OA	ns	U <sub>C</sub> > U <sub>LPH</sub>	ns	-	ns	-
		U <sub>C</sub> vs TL <sub>C</sub>	manipulation	ns	U <sub>C</sub> > TL <sub>C</sub>	ns	-	ns	-
		TP <sub>C</sub> vs TL <sub>LPH</sub>	origin	ns	ns	ns	-	ns	-
below DW		TL <sub>C</sub> vs TP <sub>C</sub>	short-term OA	TL <sub>C</sub> < TP <sub>C</sub>	-	ns	ns	-	-
		U <sub>C</sub> vs U <sub>LPH</sub>	long-term OA	ns	-	ns	ns	-	-
		U <sub>C</sub> vs TL <sub>C</sub>	manipulation	ns	-	ns	U <sub>C</sub> > TL <sub>C</sub>	-	-
		TP <sub>C</sub> vs TL <sub>LPH</sub>	origin	ns	-	ns	ns	-	-
chl <i>a</i> + <i>b</i>		TL <sub>C</sub> vs TP <sub>C</sub>	short-term OA	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>
		U <sub>C</sub> vs U <sub>LPH</sub>	long-term OA	U <sub>C</sub> < U <sub>LPH</sub>	U <sub>C</sub> < U <sub>LPH</sub>	U <sub>C</sub> < U <sub>LPH</sub>	U <sub>C</sub> < U <sub>LPH</sub>	U <sub>C</sub> < U <sub>LPH</sub>	U <sub>C</sub> < U <sub>LPH</sub>
		U <sub>C</sub> vs TL <sub>C</sub>	manipulation	U <sub>C</sub> > TL <sub>C</sub>	ns	U <sub>C</sub> > TL <sub>C</sub>	ns	U <sub>C</sub> > TL <sub>C</sub>	ns
		TP <sub>C</sub> vs TL <sub>LPH</sub>	origin	TP <sub>C</sub> < TL <sub>LPH</sub>	ns	TP <sub>C</sub> < TL <sub>LPH</sub>	TP <sub>C</sub> < TL <sub>LPH</sub>	ns	ns
car <i>x</i> + <i>c</i>		TL <sub>C</sub> vs TP <sub>C</sub>	short-term OA	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>	ns	TL <sub>C</sub> < TP <sub>C</sub>	TL <sub>C</sub> < TP <sub>C</sub>
		U <sub>C</sub> vs U <sub>LPH</sub>	long-term OA	U <sub>C</sub> < U <sub>LPH</sub>	U <sub>C</sub> < U <sub>LPH</sub>	U <sub>C</sub> < U <sub>LPH</sub>	U <sub>C</sub> < U <sub>LPH</sub>	U <sub>C</sub> < U <sub>LPH</sub>	U <sub>C</sub> < U <sub>LPH</sub>
		U <sub>C</sub> vs TL <sub>C</sub>	manipulation	U <sub>C</sub> > TL <sub>C</sub>	ns	U <sub>C</sub> > TL <sub>C</sub>	U <sub>C</sub> > TL <sub>C</sub>	U <sub>C</sub> > TL <sub>C</sub>	ns
		TP <sub>C</sub> vs TL <sub>LPH</sub>	origin	ns	TP <sub>C</sub> < TL <sub>LPH</sub>	TP <sub>C</sub> < TL <sub>LPH</sub>	ns	ns	TP <sub>C</sub> > TL <sub>LPH</sub>

Untouched<sub>LPH</sub> plants), morphological differences in untouched plants between control and low pH sites were irrelevant, except for the higher internode length of C2 apical shoots ( $1.13 \pm 0.42$  vs  $3.06 \pm 0.98$  cm). For the physiology, differences were detected in the pigment concentration in both apical ( $1.66 \pm 0.17$  vs  $4.61 \pm 0.2$  mg g<sup>-1</sup> DW and  $0.16 \pm 0.02$  vs  $0.41 \pm 0.05$  mg g<sup>-1</sup> DW, for chl *a* + *b* and car *x* + *c* respectively) and other shoots ( $0.97 \pm 0.04$  vs  $1.89 \pm 0.08$  mg g<sup>-1</sup> DW and  $0.07 \pm 3^{-3}$  vs  $0.15 \pm 0.02$  mg g<sup>-1</sup> DW, for chl *a* + *b* and car *x* + *c* respectively), with

a higher chl *a* + *b* and car *x* + *c* content in the acidified plants (Figs. 4 and 5, Tables 1 and 2).

The most significant changes due to the manipulation effect (Untouched<sub>C</sub> vs Translocated<sub>C</sub> plants) were found in the photosynthetic pigments. Translocated plants consistently displayed lower concentration of chl *a* + *b* and car *x* + *c* than the untouched plants across all sites, particularly in the apical shoot ( $1.66 \pm 0.17$  vs  $1.04 \pm 0.17$  mg g<sup>-1</sup> DW and  $0.16 \pm 0.02$  vs  $0.08 \pm 0.01$  mg g<sup>-1</sup> DW, respectively). The

manipulation of cuttings generally led to a reduction in morphological traits; however, this reduction was worthy of attention only in specific control sites and did not show a clear pattern between the apical and other shoots (Figs. 4 and 5, Tables 1 and 2).

In the low pH site, the plant origin (Transplanted<sub>C</sub> vs Translocated<sub>LPH</sub> plants) generally did not affect plant morphology ( $TP_C = TL_{LPH}$ ), with some exceptions depending on the plant site and response variable that, however, did not follow a consistent pattern. On the contrary, photosynthetic leaf pigments ( $2.26 \pm 0.6$  vs  $2.80 \pm 0.13$  mg g<sup>-1</sup> DW for chl *a* + *b* and  $0.18 \pm 0.05$  vs  $0.21 \pm 0.05$  for car *x* + *c*) responded to the treatment with a general decrease in the transplanted control plants rather than in the translocated low pH plants, except for the CPH plants for the car *x* + *c* concentration (Figs. 4 and 5, Tables 1 and 2).

## 4. Discussion

This three-month manipulative experiment, designed with multiple control sites, evidenced consistent patterns of plant performance, not only in locally adapted plants (LPH plants), but also in transplanted cuttings from control sites which allowed estimating the acclimation response. Results support the importance of conducting experiments in the field and providing fundamental information to address future hypotheses. Here, three general findings have been highlighted: first, *C. nodosa* cuttings exposed to low pH conditions exhibited a higher performance; second, changes in plant traits were considerably higher in apical shoots rather than in the other shoots; third, regardless the investigated effects, differences in plant physiological traits were more evident than those in the morphology.

### 4.1. Short-term acidification effect

Overall, the increase in morphological and physiological traits in plants that were exposed for 3 months at low pH with respect to those that were only translocated in the same control site highlighted and confirmed the hypothesis that OA may promote the seagrass performance, that are usually carbon-limited (De los Santos et al., 2017; Wang et al., 2024; Yamuza-Magdalenó et al., 2025).

The stronger increases observed in the aboveground photosynthetic tissues of the apical shoots (leaf length, biomass, and pigments) were attributed to a photoacclimation strategies (Cox et al., 2015) resulting in a higher vegetative development (Martínez-Crego et al., 2014; Palacios and Zimmerman, 2007; Russell et al., 2013). Moreover, a reduction in leaf brown tissue, although observed in a lesser extent, could be interpreted as the result of a positive compensatory response to the stress caused by the manipulation (this study; Minina et al., 2013). In the Mediterranean Sea, such changes may be particularly evident in *C. nodosa* which, as a pioneer seagrass species, may show rapid responses to environmental changes (Cancemi et al., 2002). Similar results were observed in other species such as *Cymodocea serrulata*, *Halodule uninervis*, *Zostera japonica* and *Thalassia hemprichii* (Ow et al., 2015; Wang et al., 2024).

Interestingly, belowground tissues were less affected by short-term OA, although increases in root length and biomass were observed, though not consistently across all control site populations. Similar to findings in terrestrial higher plants, CO<sub>2</sub> enrichment can stimulate shoot growth (as the source) and promote root growth (as the sink), thereby enhancing nutrient uptake and supporting a balanced source-sink relationship (Pan et al., 2020). In *C. nodosa*, this may also contribute to improved substrate anchorage likely assisting plants during storms, whose frequency is increasing with climate change. Our results suggest that *C. nodosa* allocates more resources to aboveground tissues under low pH conditions compared to belowground tissues, indicating a shift in tissue investment strategy as also observed by Piro et al. (2020), who reported significant modulation in leaf physiology with limited changes in root morphology and biomass under prolonged CO<sub>2</sub> exposure.

This hypothesis is also supported by the aboveground production

that was also enhanced in the vertical shoots of the cuttings, even if the effect of short-term OA was generally lower. A reduced development of vertical shoots in favour to the apical shoots, consistent with previous experiments (Mishra et al., 2021), may represent the result of a clonal integration, the ramet investing on the apical shoots destined to the exploration of new grounds (Ruocco et al., 2021; Tuya et al., 2013a, 2013b). Growing meristems may help the allocation of resources within the clone (Marbà et al., 2002; Terrados et al., 1997a, 1997b), increasing the performance of the apical shoots with respect of the others. The sharing of resources through clonal integration in *Cymodocea nodosa* has been demonstrated under adverse conditions (i.e., burial events), thus leading to an increased performance of the whole clone (Tuya et al., 2013a, 2013b; Song et al., 2013). However, only deeper analyses, as transcriptome profiling and global DNA methylation, could estimate to what extent the observed changes between the two shoot types could be ascribed to the clonal integration.

The overall improvement in the morphological performance is further supported by the lack of plant origin effect. Indeed, similar traits were observed between the cuttings transplanted in low pH and those translocated in low pH, indicating the ability of *C. nodosa* to acclimate to acidified environments, regardless its origin. This acclimation is likely mediated by interconnected physiological mechanisms that enhance photosynthetic efficiency and optimize resource allocation. These responses may be regulated by genetic mechanisms and/or species-specific adaptive strategies that reduce the influence of the plant's site of origin. Similar research has demonstrated, through genome-wide transcriptional analysis of *C. nodosa* under experimental ocean acidification, an upregulation of genes associated with photosynthesis and respiration, suggesting a coordinated metabolic response to elevated CO<sub>2</sub> levels (Olivé et al., 2017).

Regarding leaf physiology, this field three-month exposure resulted in an increased photosynthetic pigment content in the transplanted cuttings, observed both in the apical and other vertical shoots, confirming a high and rapid plastic response (Campbell and Fourqurean, 2013). Lowering pH levels stimulate the production of chlorophylls and carotenoids, which enhance the light-harvesting system and improve light use efficiency. This optimization boosts the photosynthetic electron transport system, leading to an increase in overall photosynthetic activity. The resulting energy surplus likely enabled primary producers to allocate more energy to biomass production, and consequently promoting the ecological success of the species under ocean acidification (Cheng et al., 1998; Egea et al., 2018b; Porzio et al., 2020; Rodríguez et al., 2022; Wang et al., 2024). Additionally, the effects of acidification are further supported by observations that plants transplanted to control sites exhibit reduced pigment content. This suggests a correlation between decreasing pH levels and pigment accumulation. These mechanisms could have also favoured further adaptive responses to the long-term exposures, as observed in the untouched cuttings.

### 4.2. Long-term acidification effect

The apical and other shoots in untouched plants were very similar in morphology (all morphological variables except only for the higher internode length of plants from one control site) regardless the site (controls or the low pH site), in agreement with some previous descriptive evidence (similar plant biomass both above and belowground) gained in plants of controls and low pH site (Apostolaki et al., 2014; Mishra et al., 2021). However, because of inconsistency in the literature, results are also in contrast with some other previous evidence which identified a higher (Mishra et al., 2021; Russell et al., 2013; Vizzini et al., 2019) or lower (Apostolaki et al., 2014; Vizzini et al., 2019) morphological and growth performance in the low pH adapted *Cymodocea* spp. plants with respect to control pH plants. This discrepancy could be ascribed to the different sampling procedures: while the latter studies sampled random shoots with cores, in our experiment the EU always consisted of cuttings coming from terminal ramets, consisting

of one apical shoot and four other vertical shoots (Máñez-Crespo et al., 2020). Although existing literature did not allow any generalisations for several plant ecological traits of naturally local acidified species, likely due to the differences among the various natural CO<sub>2</sub> vent systems (Vizzini et al., 2019), in our study the lack of differences observed between Untouched<sub>c</sub> and Untouched<sub>pH</sub> plants suggests that long-term OA exposition does not significantly impact on morphological seagrass traits. This finding provides evidence for a general adaptive compensatory strategy of *C. nodosa* in response to increased pCO<sub>2</sub> levels.

This result is supported by the consistently higher pigment concentrations observed under low pH conditions, suggesting enhanced photochemical efficiency. This pattern aligns with previous evidence reported for marine primary producers (Kelly and Hofmann, 2013; Porzio et al., 2017, 2020). However, it is important to note that the observed increase in photosynthetic activity does not necessarily result in greater plant biomass. This indicates the potential occurrence of compensatory mechanisms — possibly detectable through genetic analyses or metabolic profiling — that may be involved in physiological plasticity or local adaptation to acidified conditions over longer time scales (Porzio et al., 2020).

Concluding, the overall data evidenced that although some negative effects due to cutting manipulation were evidenced, especially on the physiology of the plant, they did not overshadow patterns due to acidification besides the specific location and plant origin. Therefore, *C. nodosa* may be considered a low pH tolerant seagrass, as the rapid response mechanisms would favour the initial acclimation to the short-term expected changes in the near future. Moreover, result gained by the long-term exposure suggests a possible stabilisation and adaptation in the distant future. Although in-situ manipulative studies may be limited by pseudo-replicated designs, this study stresses the importance of investigating the effects of climate change on marine biota in a natural environment analogue to future conditions, such as hydrothermal vents for OA. Given the growing interest in conservation and restoration worldwide, it is essential to continue researching on their performance in the face of climate change. One of the key challenges currently facing the ecologists is to understand how marine biota will cope with OA over the next future and this study claims for further efforts by scientific community in understanding how acclimation and adaptive mechanisms of the seagrass can influence the species interactions in future acidified marine ecosystems.

#### CRedit authorship contribution statement

**Arianna Pansini:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carmen Arena:** Writing – review & editing, Methodology, Investigation. **Alessia Crobu:** Writing – review & editing, Methodology, Investigation. **Ermenegilda Vitale:** Writing – review & editing, Resources, Methodology, Investigation. **Giulia Ceccherelli:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2025.122485>.

#### Data availability

Data will be made available on request.

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