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Managing biotic interactions during early seagrass life stages to improve seed-based restoration

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Abstract

1. Seagrasses are declining globally, and effective restoration actions to promote the recovery of degraded meadows are urgently needed. Harnessing positive plant interactions during early life stages is considered a valuable strategy to improve terrestrial and coastal habitat restoration. Yet, its application to seagrass restoration is still in infancy, and very little is known on the role played by biotic interactions in shaping newly established populations.
2. We assessed the feasibility of manipulating intraspecific and interspecific plant interactions to enhance seed-based restoration success using the seagrass *Posidonia oceanica* as a model. Specifically, we investigated in mesocosm whether seed germination increased with increasing seed density and whether increasing seedling density and planting a pioneer seagrass promoted facilitation among seedlings. To do this, seedlings were grown either as a single individual or in clumps of medium and high density, with and without *Cymodocea nodosa*, for 2 years encompassing their most critical growth stage.
3. Germination of *P. oceanica* seeds was not affected by seed density. *Posidonia oceanica* seedlings planted at medium and high density showed higher survival than those planted individually but only in the presence of *C. nodosa*. Seedlings planted at medium and at high density with *C. nodosa* performed better than those grown at low density or without *C. nodosa* due to a positive joint effect of intraspecific and interspecific interaction. *Cymodocea nodosa* plants grown with *P. oceanica* seedlings at high density were larger than those grown alone, indicating a mutualistic relationship.
4. *Synthesis and applications*. Our results show that in seagrasses positive interactions during early life stages can be promoted by planting seedlings in dense clumps in association with an early successional species. The incorporation of this novel nature-based approach in seed-based restoration could accelerate the recolonization of degraded seagrass habitats. In planning future seed-based restoration interventions, managers should assess both intraspecific and interspecific interactions established by seedlings of target species for identifying proper planting density/spatial configuration and potential benefactor species promoting facilitative mechanisms to maximize seedling planting success.

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KEYWORDS
competition, *Cymodocea nodosa*, facilitation, nature-based, plant–plant interaction, *Posidonia oceanica*, restoration, seedling

1 | INTRODUCTION

Seagrasses are foundation species delivering multiple key ecosystem services beneficial to human life and natural habitats, such as coastal protection, nutrient cycling and nursery habitat provisioning (Barbier et al., 2011; James et al., 2019). However, seagrass meadows are declining globally due to anthropogenic impacts and climate-change-related stressors (He & Silliman, 2019; Orth et al., 2006). The natural recovery of degraded meadows is unpredictable due to complex interactions among plant propagules and environmental conditions, and for some species, like the Mediterranean foundation seagrass *Posidonia oceanica* L. Delile, it can take decades or cannot occur at all (González-Correa et al., 2005; O'Brien et al., 2018). Seagrass restoration efforts performed worldwide over the last decades resulted in a variable success (van Katwijk et al., 2016; Tan et al., 2020). Thus, more effective conservation/restoration actions are necessary to counteract the decline of seagrass meadows and accelerate their recovery.

Seed-based restoration is currently considered as a promising, ecologically sustainable practice (Balestri & Lardicci, 2012; Balestri et al., 1998; Statton et al., 2013; Terrados et al., 2013). The use of sexual propagules has less impact on existing populations than traditional revegetation techniques involving the translocation of vegetative material (i.e. sod mats, rhizomes and cuttings) from donor populations to restoration sites (Balestri & Lardicci, 2006, 2012; Balestri et al., 1998; Statton et al., 2013; Tan et al., 2020; Terrados et al., 2013). It has also the potential advantage of mimicking natural ecological processes and ensuring genetic diversity which is critical for the long-term persistence and adaption of species to changing environmental conditions (Kendrick et al., 2016; Reynolds et al., 2012). However, seedling establishment is a particularly vulnerable phase, and most transplanting efforts conducted until now resulted in large seedling losses especially during the first months of transplanting (Balestri et al., 1998; van Katwijk et al., 2016; Suykerbuyk et al., 2016). Using seedlings previously grown in aquaculture tanks for the duration of their most critical growth stage could be a possible strategy to increase transplanting success (Balestri & Lardicci, 2012; Statton et al., 2013; Tanner & Parham, 2010). However, the lack of information on seedling growth requirements still prevents the application of this practice to many seagrasses. Moreover, for most species, there is a knowledge gap about the role of the interactions establishing among seedlings and heterospecific neighbours in controlling the dynamics of newly established populations (Statton et al., 2017; Vanderklift et al., 2020).

It is well known that plant–plant interaction outcome depends on the balance between negative and positive effects which may vary according to several factors such as environmental conditions and plant life stage (Callaway & Walker, 1997; Lortie et al., 2016). Theory and mounting experimental evidence indicate that under high stressful environmental conditions positive interactions can prevail over negative ones (Bertness & Callaway, 1994; Lortie & Callaway, 2006). The beneficial effects of these interactions on plant performance can be explained by a variety of mechanisms, including amelioration of physical conditions such as light and temperature, sediment stability, chemical stress reduction by detoxification of compounds (i.e. sulphide and ammonium) and protection from herbivores (Bertness & Callaway, 1994; Callaway, 1995; van der Heide et al., 2010; Maxwell et al., 2017). Environmental conditions at restoring sites might not be ideal for seedling growth. According to theoretical and empirical frameworks, if for a certain target species facilitation dominates over competition during the seed–seedling stage, then restoration efforts could benefit from planting seedlings in dense clusters or close to a benefactor plant. In contrast for those species in which competition dominates, then restoration could more likely benefit from planting seedlings in isolation. Numerous studies have shown the effectiveness of harnessing positive interactions to enhance seed-based restoration of terrestrial, and more recently of coastal habitats (e.g. salt marshes, wetlands and mangroves; Gómez-Aparicio, 2009; Renzi et al., 2019). For example, promoting intraspecific facilitation by increasing sowing density can increase seedling emergence, survival and growth rates of terrestrial plants (Barr et al., 2017; Burton et al., 2006). However, identifying the optimal seed/seedling density is often difficult since the relationship between positive effects and planting density can be not linear but rather hump-shaped, with a peak in the intensity of facilitation at intermediate densities (Chu et al., 2008; Zhang & Tielbörger, 2020). Harnessing the canopy effect of nurse plants or the proximity of a pioneer species is another valuable strategy to improve seed-based restoration. This is because these plants may act as benefactors by ameliorating local abiotic conditions via positive interspecific interactions (Bertness & Callaway, 1994; Brooker et al., 2008; Silliman et al., 2015), favouring the growth of seedlings planted close to them (Galindo et al., 2017; Padilla & Pugnaire, 2006; Yuan et al., 2019).

Only recently have seagrass ecologists recognized the potential benefits of incorporating facilitative interactions in restoration efforts (van Katwijk et al., 2016; Paulo et al., 2019; Valdez et al., 2020; Williams et al., 2017). However, no attempt has been made to manipulate intraspecific and interspecific interactions in seed-based restoration. The few studies that have addressed the effects of intraspecific interactions on seed germination and seedling establishment reported contrasting results. For example, for *Zostera marina* L. no effect of increasing seed density was found on germination (Orth et al., 2003), but positive effects were observed on seedling

survival (Bos & van Katwijk, 2007). Instead, for *Cymodocea nodosa* Ucria Ascherson negative effects of seed density both on germination and seedling survival were detected (Balestri et al., 2010).

We conducted two mesocosm experiments to examine the nature of intraspecific and interspecific interactions occurring at the seedling stage in the seagrass *P. oceanica* and assess the feasibility of manipulating these interactions for enhancing seed-based restoration. This slow-growing species forms extensive meadows which are currently under regression in many areas of the Mediterranean (Telesca et al., 2015). Both the species and habitat are protected by European legislation (Barcelona Convention, 1995; EEC, 1992), and there is growing interest in using pre-cultivated *P. oceanica* seedlings to recolonize degraded areas (Balestri et al., 1998; Guerrero-Meseguer et al., 2017; Terrados et al., 2013). The intensity of sexual reproductive events has increased in the last decades (Balestri & Vallerini, 2003; Diaz-Almela et al., 2004), and germinated seeds of *P. oceanica* have been found in sheltered, shallow (<3 m depth) sites on soft and hard substrates and within *C. nodosa* beds (Balestri et al., 2017). High seedling mortality rates have been observed in dense patches due to resource competition (Balestri & Lardicci, 2008; Balestri et al., 1998, 2017; Piazzi et al., 1999). Seedling mortality was lower on vegetated than on bare substrates possibly due to amelioration of physical and chemical stresses by macroalgae (Balestri et al., 2017; Pereda-Briones et al., 2020; Pereda-Briones et al., 2018). Seedlings cultivated in aquaculture systems can be maintained near to ideal growing conditions, but some stresses related, for example, to temperature are difficult to reduce. Here, we examined (a) the effects of increasing seed density on seed germination, (b) whether the nature and the intensity of interactions among seedlings would vary with increasing seedling density and (c) whether the presence of a pioneer species would modulate the effects of intraspecific interactions on seedling performance over 2 years encompassing the most critical life-history stage (8 months) of *P. oceanica* (Balestri et al., 1998). As pioneer species we selected the fast-growing seagrass *C. nodosa* (Buia & Mazzella, 1991). We hypothesized that the presence of this species would improve *P. oceanica* seedling performance and counteract negative effects of increasing seedling density via joint detoxification through aeration of the rhizosphere and shading, and thus reducing temperature and light stresses.

2 | MATERIALS AND METHODS

2.1 | Plant material collection and experimental system

Mature fruits of *P. oceanica* were collected along the shoreline of Livorno (Italy, Ligurian Sea; 43°28′54.97″N, 10°19′54.94″E) in early and in late April 2018. Apical plagiotropic rhizomes of *C. nodosa* were collected within a meadow (at 1.5 m depth) near Rosignano Solvay (Livorno, Italy) in late April 2018. Fruits and rhizomes were transported to the INVE Aquaculture Research Centre of Rosignano Solvay and placed in an outdoor aquaculture tank (7,000 L) appositely designed for growing seagrasses (Balestri & Lardicci, 2012). Mesocosms consisting of plastic pots (20 cm diameter × 20 cm depth) were filled with silica sand (0.5–1 mm sediment particle size, <0.01% organic content). A slow-releasing fertilizer, Pluscote Garden (16% N, 8% P, 16% K), was added at a rate of 1.5 g/L of substrate (Balestri et al., 2010). Mesocosms were left undisturbed for 2 days before running the experiments to allow sand consolidation. Seawater level in the tank was maintained at approximately 1 m by providing flowing seawater throughout the experiments. Seawater pH was 8.0–8.2, and salinity varied between 37.8 and 38.1. Seawater current speed was approximately 7 cm/s. Light intensity at the bottom of the tank was approximately 72% of the surface sunlight (Figure S1), and seawater temperature ranged from 9.2 to 28°C (Figure S2).

2.2 | Effect of seed density on seed germination

Mature fruits of *P. oceanica* collected in early April 2018 were opened by hand to remove seeds (Figure 1). Seeds were placed on the surface of the sediment in mesocosms to obtain three planting densities, 1 (low), 3 (medium) and 6 seeds (high), corresponding to approximately 32, 95 and 191 seeds per square meter, respectively. These densities were chosen to mimic seed densities observed in the field (Balestri & Lardicci, 2008). There were 12 replicates for each seed density level. The number of germinated seeds in each mesocosm was recorded for 15 days when no further germination events occurred. Final seed germination was calculated as percentage of the number of germinated seeds relative to the total number of seeds initially planted in mesocosms.

2.3 | Effects of seedling density and *C. nodosa* on seedling performance and intensity of interactions

The experiment was set up as a full factorial design with initial Seedling density (D: low vs. medium vs. high) and Cymodocea (C: *C. nodosa* presence vs. *C. nodosa* absence or bare sand) as independent factors. Each treatment combination was replicated four times. Before the start of the experiment, fruits of *P. oceanica* collected in late April 2018 were opened by hand, and the extracted seeds were sown in a circular pot (45 cm diameter, 5 cm deep) filled with natural sand and placed in a tank. Collected rhizomes of *C. nodosa* were cut into fragments of homogeneous size (6-cm long rhizomes with an apical meristem and three shoots) and individually planted in 12 mesocosms (one fragment per pot). An equal number of mesocosms was left unplanted to mimic bare substrate conditions. In May 2018, germinated seeds were removed from sand and randomly assigned to each treatment combination. Seedlings were planted at low density (1 seedling per mesocosm), medium density (2 seedlings per mesocosm) and high density (4 seedlings per mesocosm), corresponding to 32, 64 and 128 seedlings per square meter, respectively (Figure 1). The medium and high densities were chosen based on the

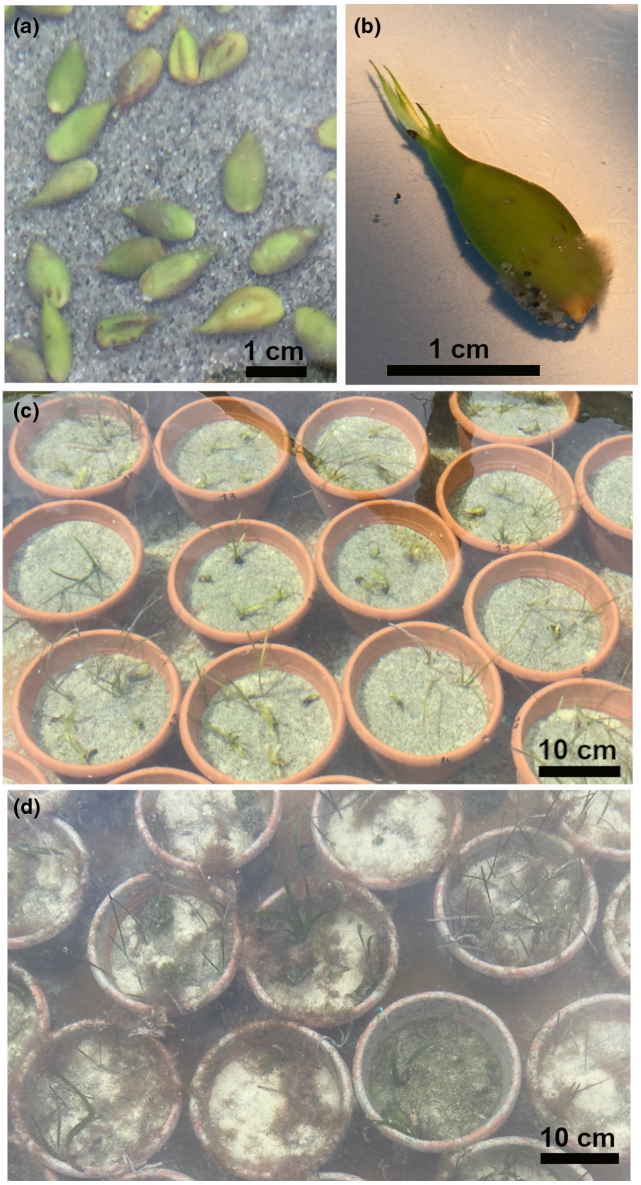

FIGURE 1 (a) Seeds and (b) germinated seeds of *Posidonia oceanica*. (c) Mesocosms containing *P. oceanica* seedlings at the start of the experiment (May 2018) and (d) after 22 months (March 2020)

minimum number of seeds that had germinated in the germination experiment described above. In these treatments, inter-seedling distance was approximately 2 cm. Four additional mesocosms were planted with one *C. nodosa* fragment alone to test for the effects of *P. oceanica* seedling density on its performance.

During the experimental period, mesocosms were periodically reallocated at random within the tank to minimize possible location effects on plant growth. Dead seedlings were not removed from mesocosms. The number of alive *P. oceanica* seedlings and the number of shoots produced by *C. nodosa* in each mesocosm were recorded monthly until the end of the experiment (June 2020). Final seedling survival was calculated as the percentage of initial number of seedlings planted in mesocosms survived at the end of experiment. All *P. oceanica* seedlings and *C. nodosa* plants were extracted from the sediment for morphometric measurements. For *P. oceanica* seedlings, the number of standing leaves per shoot and the length of rhizome were recorded. To determine total leaf area and total root length, leaves and roots of each seedling were individually scanned (black and white at 1,200 dpi of resolution; Epson Perfection, V550 Photo), and the images were processed using ImageJ software (Schindelin et al., 2012) and Rootnav software (Pound et al., 2013). Each seedling and *C. nodosa* plant was divided into shoot, rhizome and roots and dried at 70°C for 72 hr to obtain dry weights. Total plant biomass was calculated by summing leaves, rhizome and root weights. The final number of shoots and maximum leaf length of *C. nodosa* plants were also recorded.

2.4 | Statistical analyses

All statistical analyses were performed using R software (version 3.5.2; R Core Team, 2018).

To evaluate the effects of *P. oceanica* seed planting density on seed germination, data on final germination percentage were analysed with one-way univariate PERMANOVA (function adonis in VEGAN package; Oksanen et al., 2019) on untransformed data using Euclidean similarity matrix using unrestricted permutation of raw data (Clark & Gorley, 2015). Since there were not enough permutable units to get a reasonable test by permutation, *p*-values were obtained using a Monte Carlo random sample from the asymptotic permutation distribution (Anderson et al., 2008).

To assess the effects of initial seedling density and *C. nodosa* presence on seedling survival, final survival percentage data were analysed with two-way univariate PERMANOVA. To examine the effects of these factors on seedling performance, data of number of standing leaves, total leaf area, total root length, shoot biomass, root biomass, rhizome biomass and total biomass of seedlings were analysed using a two-way multivariate PERMANOVA followed by univariate PERMANOVAs on each variable. Due to a significant correlation among some variables, the multivariate analysis was restricted to the number of standing leaves, shoot biomass and root biomass. PERMANOVAs were performed on normalized untransformed data using Euclidean similarity matrix computed on 9,999 permutations of the residuals under a reduced model. Permutational analyses of multivariate dispersion (PERMDISP) were performed using the permutest function (VEGAN package, Oksanen et al., 2019) on statistically significant terms of each PERMANOVA analysis to check for differences in multivariate group dispersion. To examine how the intensity of intraspecific interaction changed with seedling densities and *C. nodosa* presence/absence, the relative interaction intensity index (RII; Armas et al., 2004) based on total biomass, shoot biomass, rhizome biomass and root biomass of seedlings was calculated separately for the medium and the high seedling density treatment with and without *C. nodosa* as follows:

$$\mathrm{RII} = \left(B_{\text{medium or high}} - B_{\text{low}}\right) / \left(B_{\text{medium or high}} + B_{\text{low}}\right),$$

where $B_{\text{medium or high}}$ is the average biomass of seedlings grown at medium or high density, and B_{low} is the biomass of the seedling grown alone on bare substrate in each mesocosm. The RII calculated in the absence of *C. nodosa* measured the intensity of intraspecific interactions while the RII calculated in the presence of *C. nodosa* quantified the intensity of both intraspecific and interspecific interactions on biomass variables. To quantify the intensity of the interspecific interaction alone, the RII based on each variable was calculated as follows:

$$\text{Interspecific RII} = \left(B_{\text{with } C.\,nodosa} - B_{\text{without } C.\,nodosa}\right) / \left(B_{\text{with } C.\,nodosa} + B_{\text{without } C.\,nodosa}\right),$$

where $B_{\text{with } C.\,nodosa}$ represents the biomass variable of a seedling grown with *C. nodosa* and $B_{\text{without } C.\,nodosa}$ represents the biomass of a seedling grown without *C. nodosa*. Mortality was included in all RIIs considering zero biomass for dead plants. RII ranges from −1 to 1 with negative values indicating competition and positive values net facilitative interactions (Armas et al., 2004). A one-sample mean *t*-test was used to test for significant departures from zero (i.e. neutral interaction) of each RII value. A nonparametric one-sample Wilcoxon signed-rank test was used when RII data did not meet normality assumption. Finally, separate one-way analyses of variance (ANOVA) were performed to test for the effects of *P. oceanica* seedling density on final shoot number, mean length of the longest leaf and total biomass of *C. nodosa* plants (GAD package; Sandrini-Neto & Camargo, 2020). Prior to ANOVAs, data were checked for normality and heteroscedasticity by Shapiro–Wilk test and Cochran' *C* test, respectively. Data of total biomass of *C. nodosa* plants were log (*x*) transformed to meet ANOVA assumption. Student–Newman–Keuls (SNK) test was used to establish the significance of differences among treatments when the ANOVA was significant at $\alpha < 0.05$.

3 | RESULTS

3.1 | Effect of seed density on seed germination

Seeds of *P. oceanica* started to germinate after few days in culture, and no germination event occurred after 15 days. Final percentage of seed germination was high in all treatments varying on average from 91.6% to 97.2% (Figure 2a,b). PERMANOVA did not detect any significant effect of seed planting density on final germination percentage (Pseudo-$F_{2,33} = 0.26$, $p = 0.764$).

3.2 | Effects of seedling density and *C. nodosa* on seedling performance and intensity of interactions

After the first year of the experiment (in June 2019), all *P. oceanica* seedlings planted with *C. nodosa* at medium density were alive while all seedlings planted at low density were dead (Figure S3). Most seedlings planted at medium and high density with *C. nodosa* (approximately 63% and 92%) survived to their first year. All these seedlings were still alive at the end of the experiment (in June 2020, Figure S3). Instead, a lower percentage of seedlings (approximately 25 and 45%) planted at these densities without *C. nodosa* were still alive in June 2020 (Figure S3). Seedlings grown with *C. nodosa* showed a higher percentage of survival than those grown without *C. nodosa* but only at high and medium seedling density (Table 1; Figure 2b). In the presence of *C. nodosa*, the survival of seedlings was lower at low density than at medium and high density (Table 1; Figure 2b).

Alive *P. oceanica* seedlings had a plagiotropic (horizontal) rhizome (length 1.5 ± 0.1 cm, mean ± *SE*) and a well-developed root system with a dichotomous pattern with a mean depth of approximately 6 cm (Supporting information Video S1; Figure S4). One seedling grown at

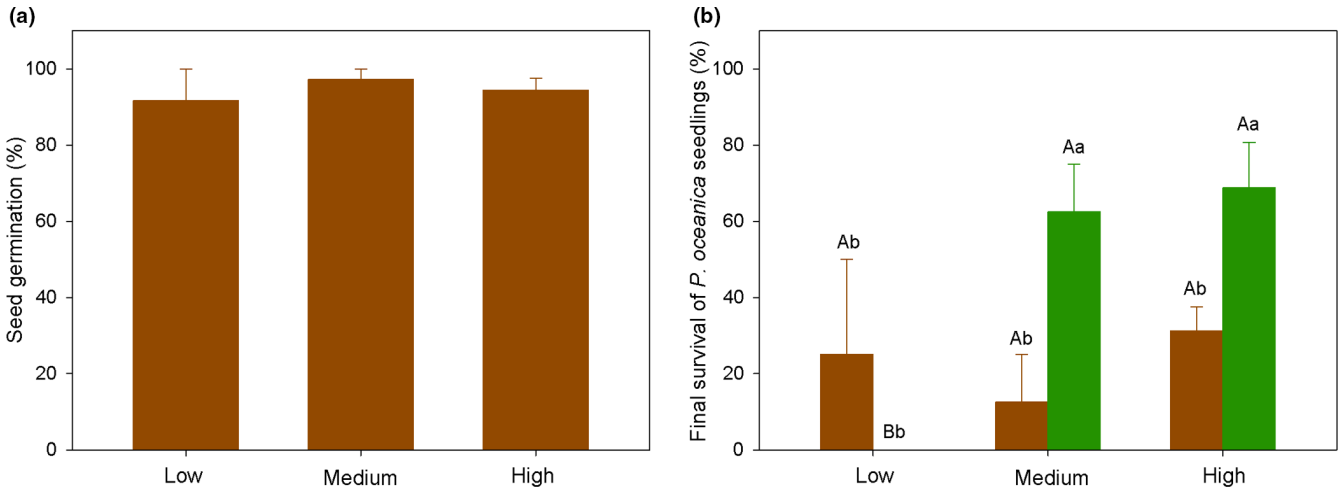

FIGURE 2 (a) Germination percentage of *Posidonia oceanica* seeds planted at low, medium, and high densities ($n = 12$). (b) Final survival percentage of *P. oceanica* seedlings planted at different densities, low, medium, and high seedling densities, without (brown bars) and with (green bars) *Cymodocea nodosa* ($n = 4$). Data are means ± *SE*. Uppercase letters denote significant differences ($p < 0.05$) among levels of the factor Seedling density and lowercase letters denote significant differences ($p < 0.05$) between levels of the factor Cymodocea

the high planting density on bare substrate had produced two shoots while the remaining ones had one shoot. Overall, seedlings grown at high density performed better than those grown at low density (Table S1). Seedlings planted at high density had a greater root and total biomass than those grown at low density, and they also showed a greater total leaf area compared to those grown at medium and low density (Table S1; Figure S5b,e,g). Moreover, seedlings grown with *C. nodosa* showed a greater number of standing leaves and total root length than those grown without *C. nodosa* but only at medium density (Table S1; Figure S5a,c). No significant effect of the investigated factors was detected on shoot and rhizome biomass (Table S1; Figure S5d,f).

TABLE 1 Summary of two-way univariate PERMANOVA testing the effects of *Cymodocea nodosa* (presence vs. absence) and seedling density (low vs. medium vs. high) on the percentage of final seedling survival of *Posidonia oceanica* seedlings

Source	*df*	Pseudo-*F*	*p*
Cymodocea *(C)*	1	3.48	0.076
Density *(D)*	2	3.90	**0.042**
C × D	2	4.32	**0.023**
Residual	18		
Pairwise test		C+: Low ≠ Medium = High	
		Medium, High: C+ ≠ C−	

Note: Results of pairwise comparisons are reported. Bold values indicate significance at $p < 0.05$. $n = 4$.

Abbreviations: C+, presence of *C. nodosa*; C−, absence of *C. nodosa* or bare substrate; Low, low density of *P. oceanica* seedlings; Medium, medium density of *P. oceanica* seedlings; High, high density of *P. oceanica* seedlings.

Intraspecific RII values, based on biomasses of seedlings grown without *C. nodosa* at medium density (Figure 3a–d), did not differ from zero (Table S2) indicating negligible interaction, except that the RII based on shoot biomass, which was negative indicating competition (Table S2). All RII values for intraspecific interaction for seedlings grown at high density did not differ from zero (Table S2; Figure 3a–d). In contrast, all RII values for intraspecific interactions of seedlings grown with *C. nodosa* at medium and high density (Figure 3a–d) were equal to +1 because none of the seedlings grown singly had survived, indicating a facilitative joint effect of intraspecific and interspecific interactions. For the same reason, all RIIs for interspecific interaction of seedlings grown at low density were equal to −1 indicating competitive exclusion by *C. nodosa*.

All *C. nodosa* fragments survived to the end of the experiment. The production of new *C. nodosa* shoots over the experimental period followed the typical seasonal growth pattern of the species in the Mediterranean with a peak of growth in summer. At the end of the experiment, plants grown with *P. oceanica* seedlings at high density had a greater number of shoots (ANOVA, $F_{3,12} = 9.56$, $p < 0.05$) and a larger total biomass ($F_{3,12} = 4.96$, $p < 0.05$) than those grown alone or with seedlings at medium and low densities (Figure S6). No difference in maximum leaf length was detected among treatments ($F_{3,12} = 1.30$, $p > 0.05$; Figure S6).

4 | DISCUSSION

Our study is the first to provide insights into the nature and intensity of intraspecific and interspecific interactions occurring in seagrass seedlings. It also demonstrates the usefulness of manipulating

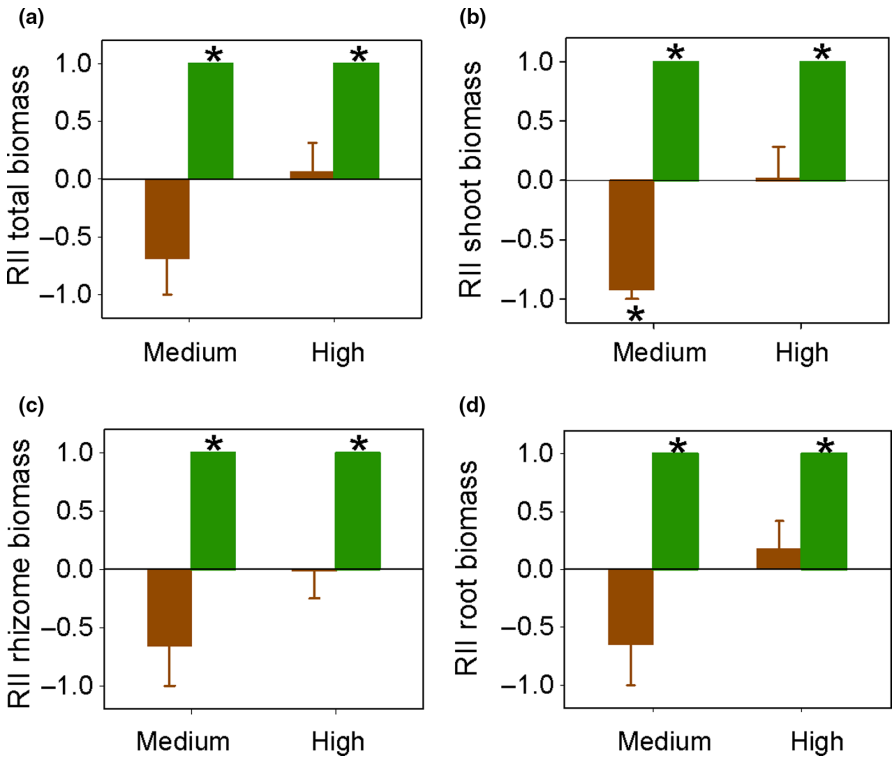

FIGURE 3 Relative interaction intensity index (RII) based (a) on mean total biomass, (b) mean shoot biomass, (c) mean rhizome biomass and (d) mean root biomass of *Posidonia oceanica* seedlings planted in each mesocosm at medium and high density without (brown bars) and with (green bars) *Cymodocea nodosa*. RII indices for seedlings grown without *C. nodosa* measured intraspecific interaction while RII indices for seedlings grown with *C. nodosa* measured the net joint effects of intraspecific and interspecific interactions on a certain variable. Data are means ± *SE* ($n = 4$). * denotes significant differences ($p < 0.05$) from zero (i.e. neutral interaction)

seedling density and surrounding vegetation to promote facilitative mechanisms and enhance seedling performance.

We found that seed germination was not affected by initial seed planting density, and the high percentages of germination (>90%) obtained here agree with the high germinability of *P. oceanica* seeds emerged from previous studies (Balestri et al., 1998; Fernández-Torquemada & Sánchez-Lizaso, 2013). This finding supports the hypothesis that *P. oceanica* seeds do not compete among them even when sown at high densities suggesting that the germination process is controlled by intrinsic factors. Initial seedling density did not affect the survival of seedlings grown without *C. nodosa*, and most seedlings died few months after germination. Seedling mortality might be attributed to high solar radiation (up to 1,449 μmol s^{-1} m^{-2}) and seawater temperature (up to 27.7°C) reached in summer. Studies have shown that *P. oceanica* plants growing in shallow sites (<5 m depth) are exposed to solar radiation up to 703 μmol s^{-1} m^{-2}, and to survive under these stressful conditions they must activate photoprotective and reparative processes to avoid chronic photo-damage and photoinhibition (Dattolo et al., 2014). Seawater temperatures above 27°C may limit the growth of seedlings by inhibiting their photosynthetic system (Guerrero-Meseguer et al., 2017) and leading to the formation of sulphide compounds toxic to plants (Holmer et al., 2003). We also observed that seedlings grown at high density showed more leaves and great total leaf area than those grown at low or medium density. Shoot biomass, however, showed some competitive effect at the medium density (negative RII) but this effect was not present at high density. We may speculate that the negative effects of increasing competition between low and medium density on seedling performance may have been overruled by self-facilitation at higher densities.

Contrary to our expectations, planting a single *P. oceanica* seedling close to *C. nodosa* resulted in competitive exclusion. *Cymodocea nodosa* has a guerrilla growth strategy showing widely distributed ramets connected by long spacers (Lovett-Doust, 1981), and it can quickly colonize bare substrates leading to changes of sediment characteristics (Larkum et al., 2006). Instead, *P. oceanica* has a phalanx growth strategy showing densely packed ramets connected by short spacers (Lovett-Doust, 1981). Interestingly, the reverse is true for the long-lived climax species *Thalassia testudinum* K.D. Koenig (guerilla species) and *Halodule wrightii* Ascherson (phalanx species) (Fourqurean et al., 1995; van Tussenbroek et al., 2016). Moreover, the rhizome of *P. oceanica* seedlings elongates horizontally very slowly, approximately 1 cm/year (in the present study). Thus, when grown with *C. nodosa*, a *P. oceanica* seedling could be outcompeted by this species likely due to substrate limitation and alteration of sediment characteristics. Our results also demonstrated that the addition of *C. nodosa* was beneficial for seedlings planted at medium and high density, leading to survival percentages up to five times higher than those observed at the same densities without *C. nodosa*. This positive effect could be related to an amelioration of physical stress likely due to high solar radiation via shading and/or chemical stress via detoxification from chemical compounds present in the sediment through increased aeration of rhizosphere by roots. Variations of endophytic and epiphytic microbial communities of plants could also have played a role. The presence of *C. nodosa* also caused a shift of interactions based on seedling biomasses from neutrality (for root biomass and total seedling biomass) or competition (for shoot biomass) to facilitation. This effect might be explained by the less damage to below-ground and above-ground seedling parts due to alleviation of physical/chemical stresses. It can also reflect the ability of *P. oceanica* to adjust its root architecture according to local biotic (i.e. presence of other macrophytes) and abiotic factors (i.e. substrate type) to maximize substrate exploration efficiency (Balestri et al., 2015; Guerrero-Meseguer et al., 2017; Pereda-Briones et al., 2018). These findings confirm our hypothesis that *C. nodosa* can modulate the interaction among *P. oceanica* seedlings, and they provide the first experimental evidence that this plant can act as a pioneer species facilitating substrate colonization by *P. oceanica*. Interestingly, even *C. nodosa* benefited from the presence of a higher *P. oceanica* seedling density, showing a greater shoot number and total biomass than when planted alone or with seedlings at medium density, suggesting mutual stress alleviation.

5 | CONCLUSIONS

Our results demonstrate the feasibility of cultivating *P. oceanica* seedlings in a nursery for an extended period before their transplantation in restoration sites. Importantly, they show that seed germination is not affected by sowing density, whereas the nature of the interactions that germinated seeds establish with conspecifics as they grow may depend on initial planting density and the presence/absence of *C. nodosa*. Therefore, manipulating simultaneously intraspecific interactions (i.e. by planting seedings at high density) and interspecific interactions (i.e. by adding *C. nodosa* close to seedlings) in culture could be effective in improving seedling survival. Here, we provide a proof-of-concept of the potential for incorporating facilitation mechanisms into seagrass seed-based restoration to enhance both the scale and success of interventions. This novel nature-based approach could enable restoration practitioners to maximize the number of seedlings available for restoration programs while reducing operational costs. For example, planting 100 seedlings at the high density level used here (4 seedlings per pot) with *C. nodosa* using our culture system requires a total of 25 pots and may lead to the production of up to 80 transplantable seedlings after 2 years. Instead, planting the same number of seedlings at the low density level (1 seedling per pot) without *C. nodosa* requires 100 pots leading to the production of up to 30 transplantable seedlings. Further investigations are needed to assess the effectiveness of our novel nature-based approach in the practice of transplanting *P. oceanica* seedlings in natural habitats. Indeed, the environmental conditions experienced by seedlings in culture differ from those occurring in a restoration site. For example, seedlings transplanted at a depth higher than that used here could be exposed to more favourable conditions, in terms of sunlight intensity and temperature. On the other hand, they could experience greater hydrodynamic stress in terms of wave exposure and sediment instability, and the positive effects of

intraspecific- and interspecific interactions could be probably more pronounced. Moreover, there is evidence that planting fast-growing pioneer species may accelerate the recovery of degraded sites by promoting substrate recolonization by late successional-climax species and temporarily replace their ecological services (Birch & Birch, 1994; Fourqurean et al., 1995; Gallegos et al., 1994). Previous seagrass restoration efforts have focused on planting a single climax species, but a recent study has shown the positive role that biodiversity could play in seagrass restoration (Williams et al., 2017). This study demonstrated that transplanting mixture of species with different morphologies and growth characteristics improved their survival and growth, and thus the trajectory towards successful restoration. Overall, our findings highlight the importance of carefully assessing the nature and strength of intraspecific and interspecific interactions occurring during the seedling stage of target species in planning future seagrass restoration and conservation interventions to identify proper seedling planting density/scheme and benefactor species that promote facilitation processes.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

E.B. and C.L. conceived the ideas and designed the methodology; E.B. and V.M. collected the data; E.B. and V.M. analysed the data; E.B., V.M. and C.L. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data available via the Dryad Digital Repository https://datadryad.org/stash/share/M9h0katm5VCYRhfTCUkYBUx_zRYNnnFM3BXgzV_fhMU (Balestri et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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