Harnessing spatial nutrient distribution and facilitative intraspecific interactions to enhance coastal dune restoration

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Abstract

Planting engineer species is widely used to restore coastal dune ecosystems. However, this approach needs to be improved to reduce plant mortality due to environmental stresses. Applying fertilizers and using designs promoting positive plant-plant interactions is increasingly advocated to enhance restoration of stressful coastal habitats. However, how the sign of plant interactions is affected by soil nutrient distribution pattern and varies depending on species architecture is poorly understood, and thus neglected in designing dune restoration interventions.

We conducted a one-year field-mesocosm experiment assessing the effects of homogeneous and heterogeneous nutrient distributions on functional traits and intensity of interactions in two common dune species with contrasting architecture. Clonal fragments of *Calamagrostis arenaria* (phalanx architecture) and *Sporobolus virginicus* (guerrilla architecture) were grown in monoculture either at low density or at high density with the same total supply of fertilizer applied either patchily or uniformly.

Calamagrostis arenaria allocated less biomass below- than aboveground under homogenous than heterogeneous nutrient conditions and generally produced more shoots when grown at high than at low density. Intraspecific interactions based on aboveground biomass, root biomass and total biomass shifted from positive to neutral under heterogeneous conditions while those on rhizome biomass switched from neutral to negative. *Sporobolus virginicus* produced shorter shoots under heterogeneous than homogeneous conditions, and intraspecific interactions on all biomass variables shifted from neutral to negative.

Our results demonstrate that nutrient distribution can affect functional traits and change the nature of intraspecific interactions differentially in phalanx and guerrilla species. Notably, they suggest that in nutrient homogenous substrates and high planting density could considerably increase the performance of phalanx species but reduce that of guerrilla species due to severe competition. Instead, in heterogenous substrates both species could perform similarly when planted at low and high density. These findings may significantly help restoration practitioners in choosing more effective planting designs and fertilization schemes for dune restoration.

Keywords: *Ammophila arenaria*, clonal plant, nature-based solution, plant-plant interaction, *Sporobolus virginicus*

Highlights

1. Revegetating degraded dune ecosystems is a critical step in coastal restoration.
2. Phalanx species grow better at high density in nutrient uniform substrate.
3. Guerrilla species grow better at low density in nutrient uniform substrate.
4. Plants performed similarly at low and high density under nutrient heterogeneity.
5. Promoting facilitation and manipulating nutrients enhance coastal restoration.

1. Introduction

The combination of climate change-driven factors, like sea level rise and flooding associated with intense storm events, poses a major threat to coastal ecosystems and services ( ). Naturally vegetated coastal dune systems act as a natural protective structure providing a physical barrier to flooding and wave attack (Defeo et al., 2009; Barbier et al., 2011). Dune vegetation plays a critical role in maintaining dune stability and promoting dune recovery after disturbances by facilitating incipient dune development, a role mediated by plant traits (Maun, 2009; Long et al., 2013; Feagin et al., 2015; De Battisti and Griffin, 2020; Edge et al., 2020). Severely damaged dune systems, however, may require re-establishment to provide an effective coastal defense. This process involves a series of interventions, including beach nourishment, installation of fences and planting of ecosystem engineers (i.e., species that fundamentally influence their community and ecosystem by creating or altering the physical structure of habitats, Jones et al. 1994, Wright and Jones 2006), or pioneer species that facilitate the colonization of later species through their beneficial effect on the environment (Bruno 2000; Calloway 1995; Bertness and Callaway 1994). The integration of this “soft” engineering nature-based practice and hard engineering structures may provide a realistic, cost-effective strategy to protect coastline and re-establish the multiple valuable services provided by coastal ecosystems (Hanley et al., 2020). However, the main problem in implementing the soft natural defense approach is the high rates of plant mortality caused by harsh dune habitat conditions (i.e., low soil moisture, sand burial, and sea spray) and improper planting (Wilson and Sykes 1999; Hesp 1991; Maun ….; Franks and Peterson, 2003; Martínez and García-Franco, 2008; Maun, 2009; Brown and Zinnert, 2018). Therefore, new planting strategies are needed to increase the effectiveness of coastal restoration.

Currently, the reconstruction of damaged or nourished dunes typically consists of planting dominant native species in monoculture, with plants normally spaced from 30-90 cm apart to minimize plant competition (Feagin et al., 2015; Silliman et al., 2015; Wootton et al., 2016; Miller et al., 2018). A fertilizer is usually added after planting to ensure satisfactory plant growth and accelerate restoration of dune communities, but this practice requires careful attention as it may damage dune communities favoring the growth of opportunistic grass species (Long et al., 2013;). However, planting designs and fertilization schemes are generally based more on practitioner experience rather than on ecological theories, and they neglect for example the nature (positive or negative) of the interactions occurring among individuals of the same species or of different species. A new method that has recently gained interest among coastal restoration ecologists, is the harnessing of positive (facilitative) plant-plant interactions by planting target species at high density or in an aggregated configuration (Halperm et al., 2007; Silliman et al., 2015; Renzi et al., 2019). This approach is inspired by ecological theories, which predict that in stressful environments positive interactions among plants are critical for plant survival (Bertness and Callaway, 1994; Franks and Peterson, 2003). Plants may facilitate each other through a variety of mechanisms, such as increasing water retention, protection from solar radiation or leaching of nutrients from decayed plant materials, ameliorating the harsh abiotic conditions ( ). However, recent attempts to restore coastal dunes have shown that the effectiveness of using facilitation-maximizing planting designs depends on abiotic conditions. Indeed, in more physically stressful dune zones, such as slopes, this planting design may increase transplant survival but fail to promote plant growth (Fischman et al., 2019). Instead, competition-minimizing designs may increase both transplant survival and growth in low-stress high dune areas, outperforming facilitation-maximizing designs (Fischman et al., 2019). However, the potential role of other factors such as the spatial distribution of soil nutrients in restoring sites in influencing the outcome of plant interactions and other measures of ecosystem functioning has been largely neglected.

Studies have demonstrated that in natural environments the availability of nutrients varies significantly, even at spatial scales detectable by individual plants (Ehrenfeld et al., 1997; Cain et al., 1999). Nutrients are usually scarce in dune systems, but a variety of factors such as the accumulation of beach wrack and litter and chronic atmospheric deposition of nitrogen can influence their spatial distribution (Maun, 2009;). Even the addition of fertilizers in restoring areas to relieve extreme nutrient limitation and allow the spread of dune plants (Long et al., 2013; Wootton et al., 2016; Miller et al., 2018) has the potential to influence nutrient distribution. Indeed, when dispersed uniformly across the whole planted area the fertilizer can lead to a uniform nutrient distribution while when added around the base of each plant can generate a patchy nutrient distribution. There is evidence that in nutrient heterogenous substrate some plants can concentrate their nutrient-acquiring organs in high nutrient areas relative to low nutrient areas (Birch and Hutchings, 1994; Day et al., 2003; McNickle et al., 2009). This foraging capacity enables these plants to benefit more, in terms of whole growth, from nutrient heterogeneous environments rather than homogeneous ones (Birch and Hutchings, 1994; Hutchings and Wijesinghe, 2008; Zhou et al., 2012). At the same time, the proliferation of nutrient acquiring organs in nutrient-rich patches may increase the intensity of competition among neighbor plants (Fransen et al., 2001; Day et al., 2003; Mommer et al., 2012). However, large variability in plant response exits among species.

In traditional restoration programs, one or two dune-building species are used, i.e., *Ammophila arenaria* and *Elymus farctus* in Europe; *Ammophila brevilugualta* and *Uniola paniculata* in North America; in Australia). Pioneer species such as *Sporobolus virginicus* are also used. These plants are clonal and spread vegetatively by rhizomes. Clonal species differ in their spatial architecture; phalanx plants produced densely packed ramets connected by short spacers (Lovett-Doust, 1981; Saiz et al., 2016), and thus they possess relatively poor ability to spread in a horizontal space across patches with different levels of nutrients. Guerrilla plants, by contrast, produced widely spaced ramets connected by long spacers (Lovett-Doust, 1981; Saiz et al., 2016), and therefore they may colonize quickly patches with different levels of nutrients. According to modelling and empirical studies, phalanx plants would monopolize locally abundant nutrients and thus would be favored in nutrient homogeneous environments whereas guerrilla plants would have an advantage in exploiting nutrients in heterogeneous environments by means of their foraging organs (Humphrey and Pyke, 1998; Ye et al., 2006; Saiz et al., 2016; Xue et al., 2018).

We conducted a one-year field-mesocosm experiment designed to develop more efficient planting methods to restore foredunes. Specifically, we investigated how the planting design (low and high plant density) and spatial nutrient distribution pattern (heterogeneous and homogeneous) affected plant traits and the intensity of intraspecific interactions in phalanx and guerrilla species widely used in dune restoration. To do this, clonal fragments of each of species were grown in monoculture either as single individual or with a conspecific individual with a fertilizer distributed patchily or homogenously in the substrate. For each species, we measured a suite of functional traits potentially related to ecosystem functioning and service provision ( ). These are plant height and number of shoots (both have been linked to wave attenuation potential), above-ground biomass (a proxy for productivity and related to wave attenuation potential, and below-ground biomass (linked to productivity and to sediment stability). The research hypothesis assumes that the spatial distribution of nutrients may influence the relationships among transplants in opposite direction in guerrilla and phalanx species. Planting at high density (i.e., using maximizing-facilitation designs) is expected to promote the growth of phalanx species but only in nutrient homogenous substrates and favor the growth of guerrilla species but only in heterogenous substrates. This assessment would improve coastal ecosystem restoration by assisting practitioners in determining under what conditions and for which type of species applying planting designs that maximize facilitation or alternatively that minimize competition.

2. Materials and methods

2.1 Study species and experimental design

Both selected species are perennial rhizomatous grasses. *Calamagrostis arenaria* subsp. *arundinacea* (Husn.) Banfi, Galasso & Bartolucci ex *Ammophila arenaria Calamagrostis arenaria* is a tall late successional species that generally colonizes embryonic dunes leading to the formation of high foredunes (van Puijenbroek et al., 2017; Reijers et al., 2019). *Sporobolus* *virginicus* (L.) Kunth is a pioneer species that rapidly colonizes beaches to form wide and low embryonic dunes (Hitchcock, 1971; Blits and Gallagher, 1991; Frosini et al., 2012; Balestri and Lardicci, 2013). Similar sized clonal fragments of the two species (two shoots and a rhizome portion approximately 2 cm long) were cut from local natural populations along the dune system of Rosignano Solvay (Italy, 43° 22′ 21.73″ N, 10° 26′ 24.32″ E) in early October. Before the start of the experiment, mesocosms consisting of plant growing containers (42 cm long, 18 cm wide, and 21 cm deep) were filled with pre-washed commercially available sand (silica sand 0.5 mm size, pH 8, organic matter < 0.01%).

For each of the two species as the target, the experimental design involved a factorial combination of two levels of spatial nutrient distribution (heterogeneous and homogenous) and two levels of planting density (low or 1 transplant per mesocosm corresponding to about 10 transplants m^2 and high or 2 transplants per mesocosm corresponding to about 20 transplants m^2). The low density was chosen as it has been used in previous restoration experiments (Gallego-Fernández et al., 2011;   ). Each treatment combination was replicated four times. In total, 32 mesocosms were established (2 species × 2 nutrient distributions × 2 planting density × 4 replicates). To generate the different nutrient distribution patterns, nutrients were supplied in the form of a slow-release fertilizer (Pluscote, 16%:8%:16% NPK, Orvital, Italy) and distributed either patchily or uniformly over the substrate. Total fertilizer supply (9 g) was the same in the two nutrient treatments to avoid confounding between the effects of nutrient concentration and spatial nutrient distribution. In the heterogenous treatments, the fertilizer was concentrated in three patches (6 cm diameter); one patch was in the middle of the mesocosm, and the other two patches were located laterally on opposite sides of the mesocosm (Fig. 1). Thus, each patch contained 3 g of fertilizer. In the homogeneous distribution treatments, the same total amount of fertilizer was distributed evenly into the surface of sand across the mesocosm (Fig. 1).

In all treatments, one rhizome fragment of the target species (either *C. arenaria* or *S. virginicus*) was planted on one side of the mesocosm in the space between the lateral nutrient-rich patch and the central nutrient-rich patch in the heterogenous treatments or in an equivalent position in the homogeneous treatments (Fig. 1). In the low planting density treatment, no other fragment was planted on the opposite side. This treatment mimicked a dispersed planting arrangement. In the high planting density treatment, a second fragment of the same target specie was planted on the opposite side of the mesocosm, in the space between the central nutrient-rich patch and the lateral nutrient-rich patch in the heterogenous treatments or in an equivalent position in the homogeneous treatments (Fig. 1). The distance between two transplants were about 15 cm, mimicking a clumped planting arrangement. In total, 48 rhizome fragments were planted. Mesocosms were randomly allocated in a foredune area (100 m^2) of Rosignano Solvay about 1 m each other and buried in the substrate. In order to protect plants against damage from herbivores and trampling the experimental area was fenced. Plants were watered once a day with tap water for the first week of the experiment and then left under natural weather conditions for one year. Total annual precipitation varied from 490.3 and 1324 mm, and mean annual temperature ranged from 15.1 to 16.3°C in this area during the experimental period.

2.2 Plant measurements

One year after planting, the survival of plants in each mesocosm was assessed. Aboveground parts of each plant in each mesocosm were cut where they emerged from the substrate. Shoot number and height were measured prior to plant collection. Then, belowground parts were carefully extracted by hand from sediment and separated into roots and rhizomes. All plant parts were cleaned from adherent sediment particles by using tap water, oven-dried at 70°C to constant weight and weighted. For each species grown at low density (i.e., alone), belowground biomass was calculated by summing root biomass and rhizome biomass. Total plant biomass was determined by summing the biomass of above- and belowground portions. Below- to aboveground biomass ratio was also calculated as changes in biomass allocation in dune plants can affect ecosystem functioning and service (Poorter et al., 2012). In the high planting density treatments, biomass measures were averaged between two plants.

Finally, the relative interaction index (RII, Armas et al., 2004) based on biomass variables of each species was calculated to quantify the intensity of intraspecific interaction:

$$RII = (P_1 – P_0) / (P_1 + P_0) \qquad (1)$$

where P_1 represents the average biomass of plants grown at high density, and P_0 is the biomass of the plant grown alone. RII values range from – 1 to 1, with positive values indicating facilitation, negative values indicating competition, and a zero-value indicating the absence of a significant interaction between plants. RIIs were calculated separately for each level of the factor nutrient distribution (heterogenous and homogeneous). This allowed us to assess how the effects of high planting density and nutrient distribution interacted in determining positive or negative interactions and plant cover.

2.3 Statistical analysis

All statistical analyses were conducted with R software (v. 3.5.1; R Core Team, 2018). Before performing the analyses, data were checked for normality and homoscedasticity using Shapiro–Wilk and Cochran *C* tests, respectively. For each species, the effects of treatments on plant response variables (number of shoots, shoot height, and biomasses) were analyzed separately using a two-way analysis of variance (ANOVA) with nutrient distribution, planting density, and their interactions as fixed factors (GAD package; Sandrini-Neto and Camargo, 2020). Data on shoot number of *C. arenaria* were square root transformed, and data on root biomass of *S. virginicus* were log transformed to meet the requirements for normality and homoscedasticity. Since no transformation improved normality of data for *C. arenaria* rhizome biomass, root biomass, below- to aboveground biomass ratio, and for *S. virginicus* shoot number, these variables were analyzed using a univariate two-way PERMANOVA based on the Euclidean distance of the data using 9999 permutations of the residuals under the reduced model (vegan package; Oksanen et al., 2019).

When in *posteriori* pairwise comparisons there were not enough permutable units to get a reasonable test by permutation, *P*-values were obtained by using a Monte Carlo random sample from the asymptotic permutation distribution (Anderson et al., 2008). Statistically significant terms were checked for differences in multivariate group dispersion with the permutational analysis of multivariate dispersion. Where appropriate, Student Newman-Keuls (SNK) test in ANOVAs and pairwise comparison test in PERMANOVAs were used to distinguish among levels of significant factors. Significant deviations of mean RII values from zero at the $P = 0.05$ level were assessed using a two-sided single mean t test. The effects of nutrient distribution treatment on RIIs were investigated using one-way univariate PERMANOVAs based on the Euclidean distance of the data since some RII data violated the assumption of normality. Significance levels were calculated from 9999 permutations of unrestricted permutation of raw data as this permutation method provides an exact test for one-way design (Anderson et al., 2008).

3. Results

One year after planting, all plants were still alive. *Calamagrostis arenaria* plants grown at low density had less shoots than those grown at high density regardless of nutrient distribution (Fig. 2; Table 1). Overall, the below- to aboveground biomass ratio was higher under heterogeneous than homogeneous conditions due to decreased root biomass (Fig. 2; Table 1). No difference among treatments was found for the remaining examined variables (Fig. 2; Table 1). Relative interaction intensity indices based on aboveground biomass, root biomass, and total biomass were positive and differed significantly from zero in the nutrient homogenous treatment indicating intraspecific facilitation while they were close to zero in the heterogenous treatment revealing no consistent plant-plant interaction (Fig. 3; Table 2). Instead, the RII index on rhizome biomass was negative and significantly different from zero in the heterogenous treatment suggesting a competitive interaction (Fig. 3; Table 2).

On average, *Sporobolus virginicus* plants had shorter shoots under nutrient heterogeneous than homogeneous conditions (Fig. 4; Table 1). There was a significant effect of the interaction between nutrient distribution and planting density for root biomass and total plant biomass (Fig. 3; Table 1). Plants grown at low density in the heterogeneous substrate had lower root biomass and total plant biomass than those grown in the homogenous substrate, and in this latter condition plants grown at low density had greater biomasses than those grown at high density (Fig. 4; Table 1). RIIs on all biomass variables were negative and significantly different from zero in the homogenous treatment indicating plant competition (Fig. 3; Table 2) while they did not significantly differ from zero in the heterogenous treatment suggesting no plant interaction (Fig. 3; Table 2). For both species, RIIs on root biomass and total biomass of plants grown under homogeneous and heterogeneous conditions differed significantly (Table 3). Significant differences between the two nutrient treatments were also observed for RII on aboveground biomass and rhizome biomass but only for *S. virginicus* (Table 3).

4. Discussion

The results of this study partially support the predictions that phalanx species would be favored under nutrient homogeneous conditions while guerrilla species would benefit from heterogeneous conditions (Humphrey and Pyke, 1998; Ye et al., 2006; Saiz et al., 2016; Xue et al., 2018). Our study indeed reveals that both phalanx and guerrilla species do not respond to nutrient heterogeneity by increasing their total plant biomass. There are several possible non-exclusive explanations for the lack of growth responses of clonal plants to nutrient heterogeneity. A first explanation is that in nutrient-poor habitats like dunes the costs of producing new nutrient-acquiring organs might be not compensated by benefits gained by a plant from those organs when it encounters nutrient-rich patches within heterogenous environments (Crick and Grime, 1987; Fransen and de Kroon, 2001). A second explanation is that plants respond to the establishment in a favourable patch by supporting ramet growth in bad patches at the expense of ramet growth in good patches (de Kroon et al., 1998; Wang et al., 2020). Here, we found that under nutrient heterogenous conditions, *C. arenaria* plants allocated comparatively more biomass in below- than aboveground organs compared to those grown under homogeneous conditions. This suggest that the species probably relies on the shift in biomass allocation to maximize nutrient acquisition and to cope with soil nutrient heterogeneity. Instead, *S. virginicus* did not show any shift in biomass allocation, even if produced shorter shoots in heterogeneous than homogenous substrate, suggesting that this species can rely on physiological integration to cope with heterogenous nutrient conditions.

Our study also confirms the hypothesis that nutrient distribution differently affected the intensity of interaction in phalanx and guerrilla species. Indeed, in nutrient heterogenous substrate *C. arenaria* exhibited less horizontal spread ability when planted at high than at low density likely due to increased competition for available space as suggested by the significant negative RII values observed for rhizome biomass. Instead, in nutrient homogeneous substrate intraspecific interactions were generally positive indicating that plants growing at high density produced more biomass and spread faster than those planted at low density. Many previous studies have documented the occurrence of negative interspecific plant interactions, while few studies have provided evidence of positive intraspecific interactions in highly stressful habitats like dunes (Harley and Bertness, 1996; Chu et al., 2008; Martínez and García-Franco, 2008; Fajardo and McIntire, 2011). Intraspecific facilitation can occur when the positive effects of conspecific neighbors, in terms of improved resources and microclimate conditions or reduced abiotic stresses, are greater than negative effects due to competition for resources (Harley and Bertness, 1996; Liancourt et al., 2005; Martínez and García-Franco, 2008). Here, the greater production of biomass observed for plants grown at high density than at low density could be attributed to self-facilitating mechanisms reducing abiotic stresses such as increasing water retention, protection from solar radiation or leaching of nutrients from decayed plant material. Instead, under homogeneous conditions *S. virginicus* plants performed less when grown at high than at low density due strong competition between conspecifics as suggesting by the negative RII values observed for intraspecific interactions. By contrast, the lack of relevant intraspecific interactions under heterogenous conditions suggests that this species may perform similarly when planted at high and low density.

The contrasting effects of heterogeneous and homogeneous nutrient distributions on functional traits and outcome of intraspecific interactions observed in this study can have important practical implications in coastal restoration. Notably, they indicate that planting rhizomes of *C. arenaria* at high density in nutrient homogenous areas could involve greater efforts, but it could result in enhancing sand trapping and accumulation efficiency and substrate binding. Planting *S. virginicus* at high density in nutrient homogenous substrate could lead to opposite effects. Instead, planting at high density in heterogenous substrates could not provide increased ecosystem benefits regardless of the architecture of the planted species.

5. Conclusions

This study reveals that both the performance and spread ability of dune plants varied significantly depending on their spatial architecture, planting density, and spatial distributions of soil nutrients. Notably, our results show that planting phalanx species such as *C. arenaria* in homogenous substrates at high density or clumped pattern rather than spaced apart or at low density resulted in total plant biomass up to four times greater. This suggests that in dune areas where nutrients are naturally distributed or supplied homogeneously in the form of fertilizers restoration efforts involving these species could benefit from using facilitation-maximizing planting designs, resulting in faster dune colonization and recovery of ecosystem services. However, planting guerrilla species like *S. virginicus* at high density rather than at low density resulted in decreasing plant biomass up to two times. Therefore, for these species a dispersed planting design that minimize plant competition could be a more efficient strategy. Instead, in dune areas where nutrients are distributed or supplied heterogeneously using low or high planting density could provide similar results in terms of total biomass regardless of species architecture. These findings are highly important for improving future coastal restoration based on soft natural defense interventions, enabling practitioners to select appropriate planting designs and fertilization scheme according to specific plants and environmental conditions.

Author contribution

Elena Balestri: Conceptualization, Validation, Investigation, Formal Analysis, Writing- Reviewing and Editing. Flavia Vallerini: Investigation, Visualization. Virginia Menicagli: Formal Analysis, Visualization. Claudio Lardicci: Conceptualization, Validation, Investigation, Supervision, Writing-Reviewing and Editing.

Declaration of competing interests

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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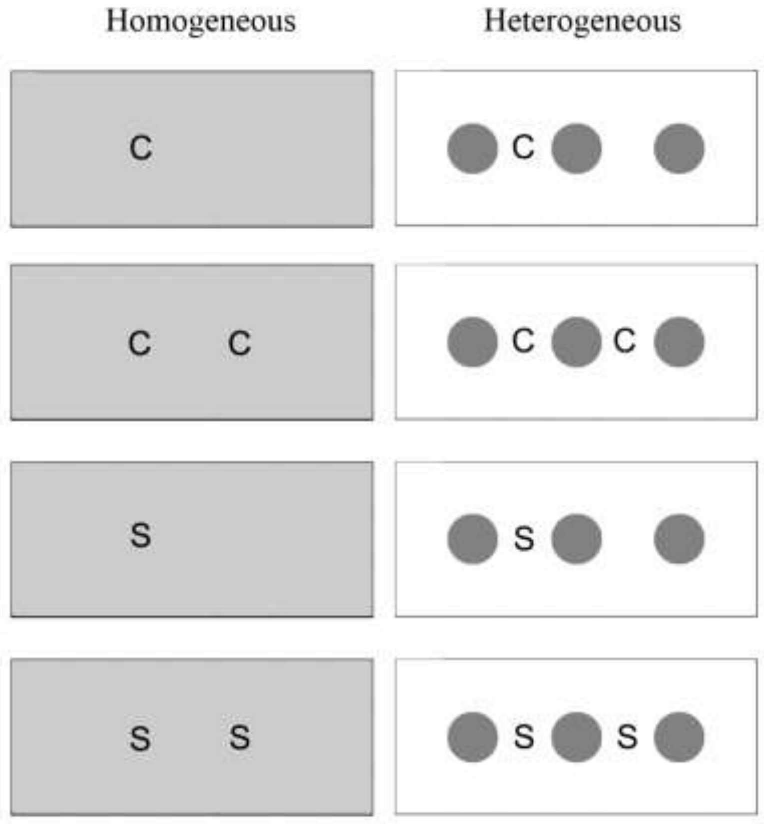

Fig. 1. Experimental layout showing the spatial distribution of nutrients (homogenous and heterogenous) and the initial position of transplants of *Calamagrostis arenaria* (C) and *Sporobolus virginicus* (S) in mesocosms. Transplants of each of the two species were grown either alone (low density) or with a conspecific neighbor (high density). Light grey indicates homogenous nutrient distribution in soil (i.e., all patches contained the same quantity of fertilizer). Black squares indicate nutrient-rich patches containing the fertilizer and white squares indicate cells without the fertilizer in the nutrient heterogeneous treatments. Total nutrient availability was the same in all treatments.

single column fitting image, color in online version only

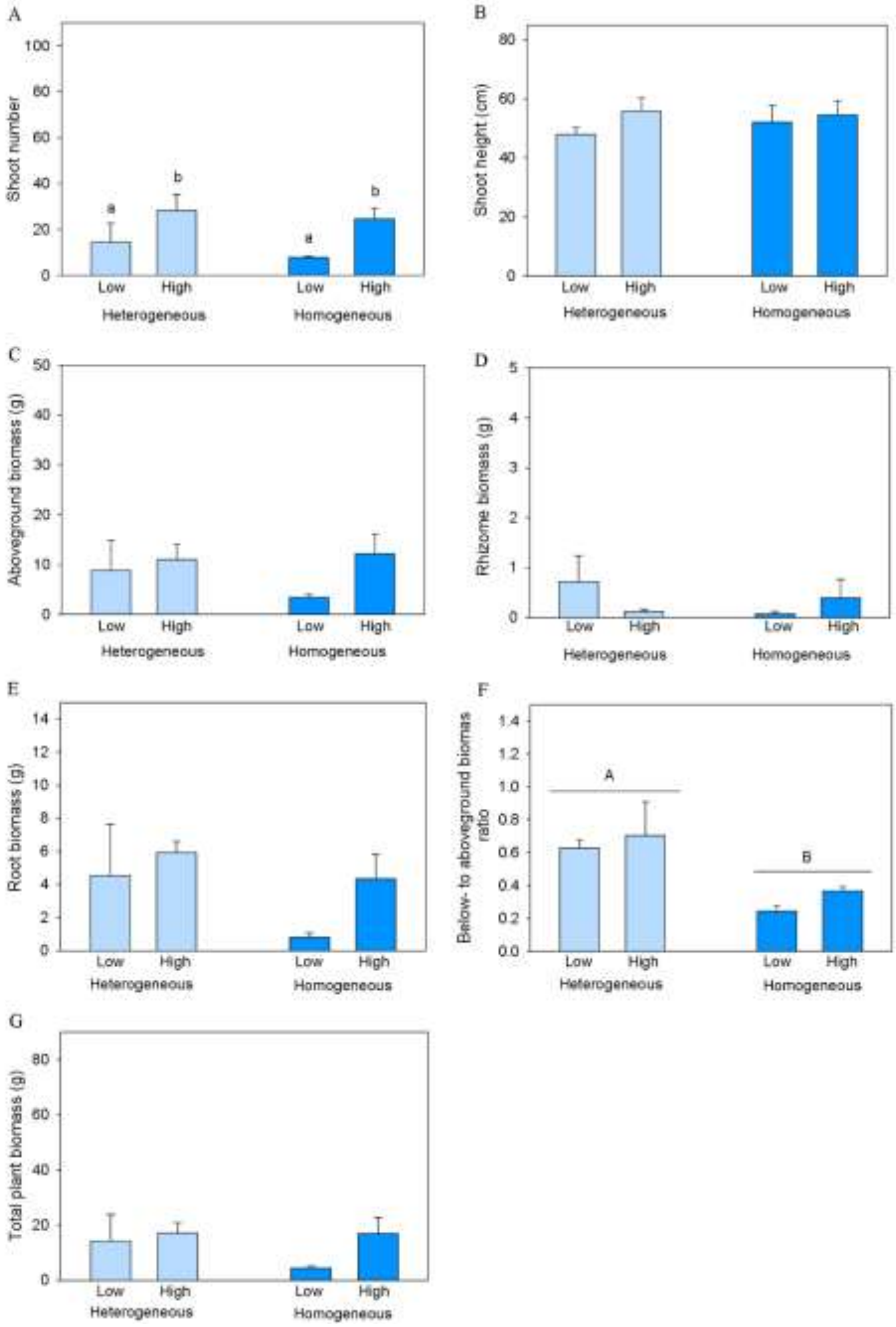

Fig. 2. *Calamagrostis arenaria* (phalanx species)**.** Effects of the nutrient distribution pattern (homogenous and heterogenous) and planting density (low density or single transplant, high density or with a conspecific transplant) on (A) shoot number, (B) shoot height, (C) aboveground biomass, (D) rhizome biomass, (E) root biomass, (F) below- to aboveground biomass ratio, and (G) total

biomass. Different letters above bars denote significant differences between treatments. Values are means ± SE. $n = 4$

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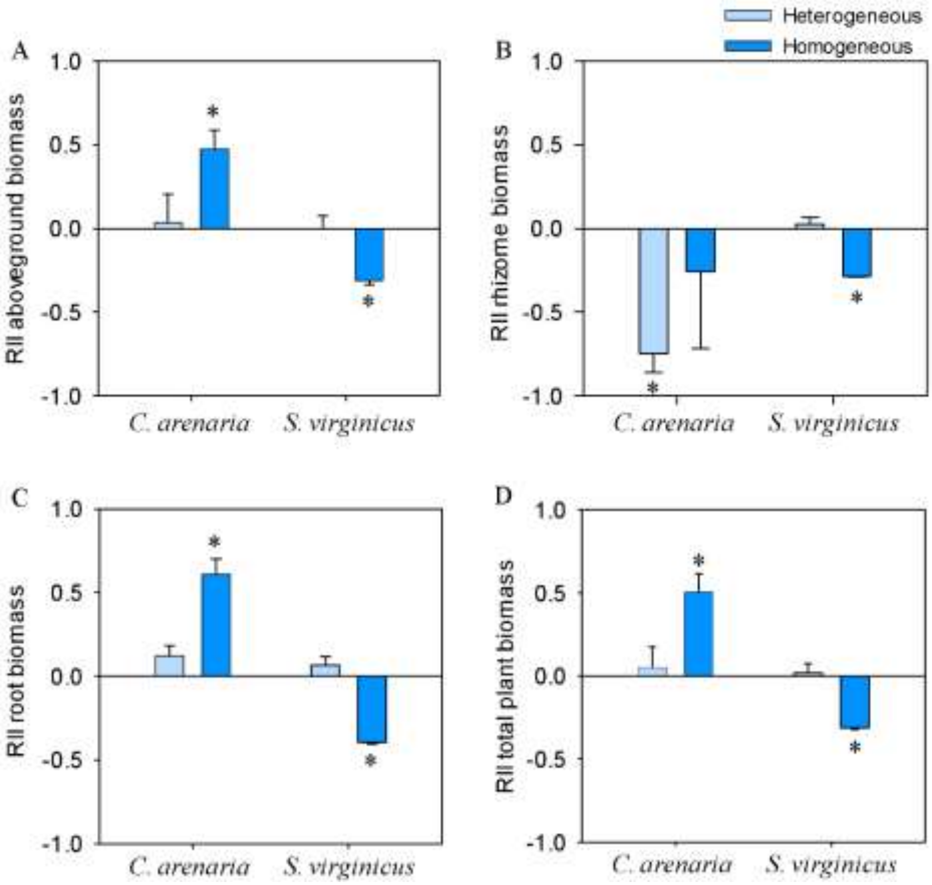

Fig. 3. Relative intraspecific interaction index (RII) based on plant biomass variables of the phalanx species *Calamagrostis arenaria* and the guerrilla species *Sporobolus virginicus* grown under different nutrient distribution conditions (heterogeneous and homogeneous). The symbol * on individual bars indicates values significant ($P < 0.05$). Values are means ± SE. $n = 4$

1.5 column-fitting image, color in online version only

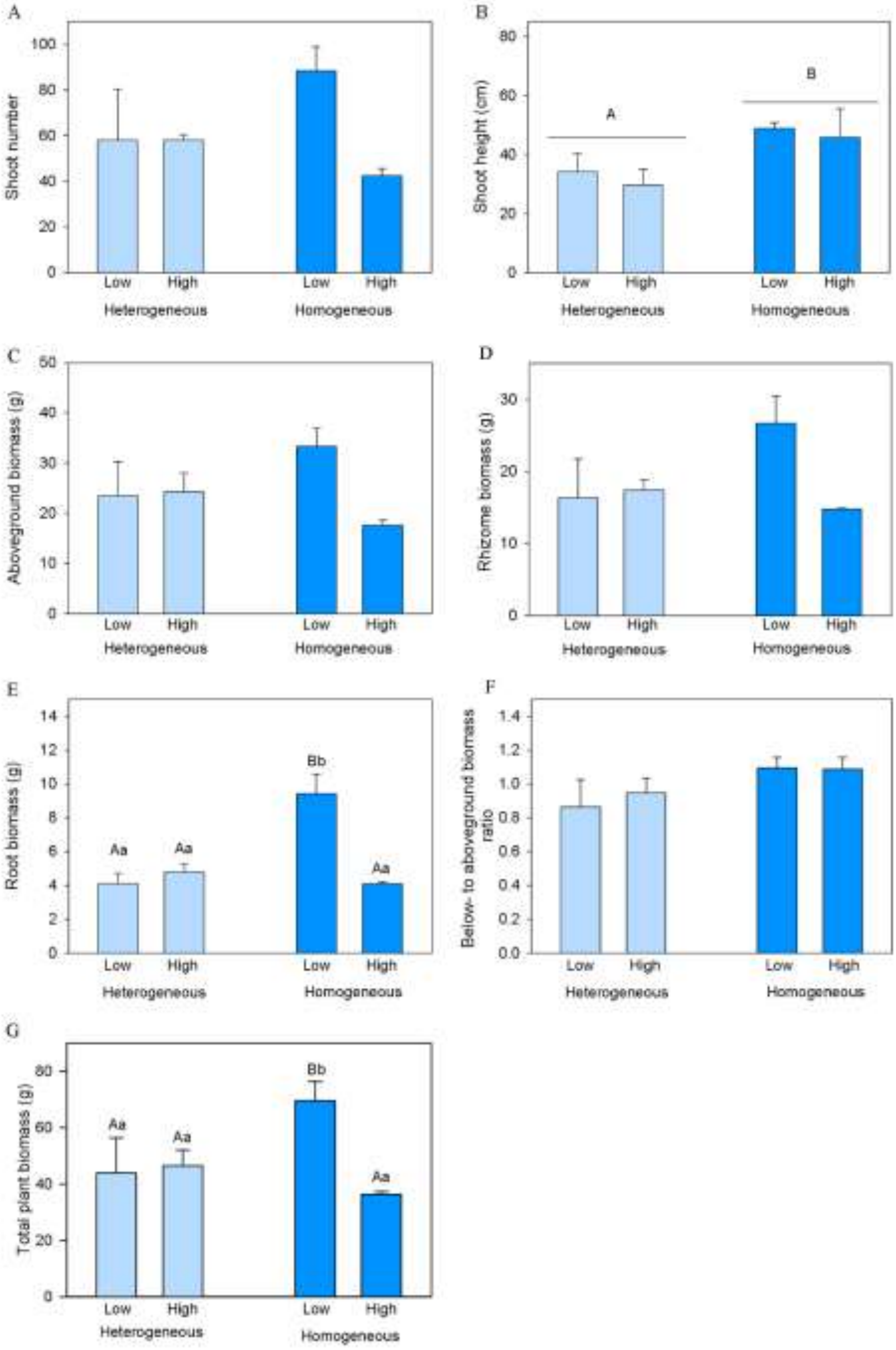

Fig. 4. *Sporobolus virginicus* (guerrilla species)**.** Effects of the nutrient distribution pattern (homogenous and heterogenous) and planting density (low density or single transplant, high density or with a conspecific transplant) on (A) shoot number, (B) shoot height, (C) aboveground biomass, (D) rhizome biomass, (E) root biomass, (F) below- to aboveground biomass ratio, and (G) total

biomass. Different letters above bars denote significant differences between treatments. Values are means ± SE. $n = 4$

2 column-fitting image, color in online version only

Table 1. Results of ANOVAs and PERMANOVAs on the effects of nutrient distribution pattern (homogeneous *vs.* heterogeneous) and planting density (low *vs.* high) on plant variables of *Calamagrostis arenaria* (phalanx species) and *Sporobolus virginicus* (guerrilla species). Values of P < 0.05 are in bold. Results of post-hoc comparison SNK and pairwise test are showed. LD: low density or alone; HD: high density or with a conspecific neighbor; Hom: homogeneous nutrient distribution; Het: heterogeneous nutrient distribution.

		Shoot number		**Shoot height**		**Aboveground biomass**		**Rhizome biomass**		**Root biomass**		**Below to above biomass ratio**		**Total plant biomass**	
Calamagrostis arenaria															
Source	**df**	**F**	***P***	**F**	***P***	**F**	***P***	**Pseudo-F**	***P***	**Pseudo-F**	***P***	**Pseudo-F**	***P***	**F**	***P***
Nutrient (Nu)	1	0.31	0.584	0.10	0.753	0.29	0.600	0.31	0.585	2.26	0.155	11.30	**0.001**	0.69	0.420
Density (D)	1	8.14	**0.014**	1.36	0.265	1.93	0.189	0.18	0.675	1.94	0.192	0.85	0.431	1.72	0.213
Nu x D	1	0.03	0.864	0.34	0.570	0.70	0.418	2.04	0.178	0.36	0.562	0.00	0.867	0.66	0.432
Residual	12														
Shapiro test		*P* = 0.361		*P* = 0.069		*P* = 0.052								*P* = 0.051	
Cochran's test		*P* = 0.112		*P* = 0.893		*P* = 0.156								*P* = 0.067	
Post-hoc test		LD < HD										Hom ≠ Het			
Sporobolus virginicus															
Source	**df**	**Pseudo-F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Nutrient (Nu)	1	0.35	0.561	5.80	**0.032**	0.13	0.717	1.32	0.272	10.70	**0.006**	3.22	0.097	1.02	0.332
Density (D)	1	3.34	0.092	0.35	0.563	2.95	0.111	2.63	0.130	8.87	**0.011**	0.13	0.719	3.90	0.071
Nu x D	1	3.38	0.090	0.01	0.898	3.67	0.079	3.72	0.077	20.90	**<0.001**	0.18	0.676	5.33	**0.039**
Residual	12														
Shapiro test				*P* = 0.220		*P* = 0.076		*P* = 0.105		*P* = 0.125		*P* = 0.126		*P* = 0.093	
Cochran's test				*P* = 0.178		*P* = 0.105		*P* = 0.084		*P* = 0.352		*P* = 0.114		*P* = 0.063	
Post-hoc test				Het < Hom						Hom: HD < LD LD: Het < Hom				Hom: HD < LD LD: Het < Hom	

Table 2. Results of one sample *t* test for significant difference from zero (i.e., no interaction) of the relative interaction index (RII) based on aboveground biomass, rhizome biomass, root biomass, and total biomass of the phalanx species *Calamagrostis arenaria* (a) and the guerrilla species *Sporobolus virginicus* (b) grown under different spatial nutrient distribution conditions, homogeneous and heterogenous. Values of $P < 0.05$ are in bold.

(a)		**RII Aboveground biomass**		**RII Rhizome biomass**		**RII Root biomass**		**RII Total plant biomass**	
	df	**t**	***P***	**t**	***P***	**t**	***P***	**t**	***P***
Homogeneous	3	4.27	**0.023**	-0.55	0.615	6.58	**0.007**	4.57	**0.019**
Heterogeneous	3	0.19	0.859	-6.56	**0.007**	1.97	0.143	0.42	0.698

(b)		**RII Aboveground biomass**		**RII Rhizome biomass**		**RII Root biomass**		**RII Total plant biomass**	
	df	**t**	***P***	**t**	***P***	**t**	***P***	**t**	***P***
Homogeneous	3	-11.97	**0.001**	-68.53	**<0.001**	-36.55	**<0.001**	-29.69	**<0.001**
Heterogeneous	3	0.009	0.992	0.64	0.565	1.42	0.250	0.32	0.768

Table 3. Results of one-way PERMANOVA testing the effects of nutrient distribution pattern (homogeneous *vs.* heterogeneous) on relative interaction index (RII) values for intraspecific interactions on biomass variables of *Calamagrostis arenaria* (phalanx species) and *Sporobolus virginicus* (guerrilla species). Values of $P < 0.05$ are in bold.

Variable		**RII**	
	df	**Pseudo-F**	***P* (MC)**
***Calamagrostis arenaria*:**			
Aboveground biomass	1,6	4.73	0.070
Rhizome biomass	1,6	1.05	0.342
Root biomass	1,6	19.27	**0.003**
Total plant biomass	1,6	7.50	**0.033**
***Sporobolus virginicus*:**			
Aboveground biomass	1,6	16.04	**0.007**
Rhizome biomass	1,6	61.25	**<0.001**
Root biomass	1,6	89.21	**<0.001**
Total plant biomass	1,6	33.48	**0.001**

df are degrees of freedom for the numerators and denominators of the PseudoF-statistic