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Seed photosynthesis enhances *Posidonia oceanica* seedling growth

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Abstract. *Posidonia oceanica* seeds demonstrate photosynthetic activity during germination as well as throughout seedling development, a fact which suggests that seed photosynthesis can influence seedling growth. This study examines the contribution of seed photosynthesis to changes in leaf and root biomass, the concentration of seed carbohydrate reserves (free sugars and starch) and elemental C, N and P content in seedlings exposed to different light/dark treatments. Green seeds attached to seedlings remained photosynthetically active for three months and photosynthetic activity in the seeds enhanced seedling growth in leaves and roots. It was estimated that ca. 29.26% of leaf biomass and ca. 42.31% of root biomass production was due to seed photosynthetic activity. Photosynthesis occurred at a higher rate in seeds than in leaves during early seedling growth, and extreme light limitation reduced the mobilization of carbon reserves stored in the seeds. These adaptations control leaf self-shading and plant competition in well-established *P. oceanica* beds while also promoting increased seedling survival rates in open, uncolonised areas where clonal growth is limited.

Key words: growth; photosynthesis; *Posidonica oceanica*; seagrass; seed.

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INTRODUCTION

Seagrasses play key ecological roles in coastal ecosystems where they form extensive meadows that support high biodiversity (Short et al. 2007). Seagrasses have developed unique ecological, physiological and morphological adaptations to a completely submerged existence, including internal gas transport, epidermal chloroplasts, submarine pollination, and marine dispersal (den Hartog 1970, Les et al. 1997). Seagrasses are clonal plants that have adapted to the marine environment and complete their entire life cycle in a saline medium, including flowering, pollen transport and seed germination (Phillips and Meñez 1988). The bulk of seagrass bed expansion occurs through clonal growth (Lewis and Phillips 1980, Phillips et al. 1981), although seeds are important for maintaining genetic variation within the population (Alberte et al. 1994, Williams and Orth 1998) and as agents of long-distance dispersal.

Posidonia oceanica (L.) Delile is a seagrass endemic to the Mediterranean Sea that forms beds considered one of the climax communities of the Mediterranean coastal area (Molinier and Picard 1952). *P. oceania* is a slow-growing K strategist that invests in multi-year vegetative growth which results in extensive meadows that dominate shallow, subtidal, Mediterranean coastal landscapes. This marine seagrass is long-lived and forms a deep network of root and rhizome material that can extend several meters deep and be thousands of years old. *P. oceanica* has the ability to reproduce sexually, through seeds, or asexually through the propagation of rhizome fragments (Molinier and Picard 1952). Flowering and fruit production is highly variable in space and in time, with intensive synchronised flowering episodes occurring at 8- to 10- year intervals (Balestri et al. 2003, Diaz-Almela et al. 2006). The mature fruit usually contains a single green non-dormant seed. The buoyant fruits are transported by currents away from parental shoots, enabling dehisced seeds to colonize sites outside the range of vegetative growth (Buia and Mazzella 1991). While the fruits of *P. oceanica* float, the seeds sink immediately upon release from the fruit, settle with the flat side down, and germinate within a few days (Caye and Meinesz 1984). The green seed remains attached to the young plant for one to two years after germination and supplies C, N and P to the developing seedling until about one year after germination (Balestri et al. 2009). It seems that during early seedling developmental phases the seeds supplement environmental supplies of N and P and display photosynthetic activity (Celdrán and Marín 2011), which suggests that they could contribute photosynthetically to the seedling. In that study, seed survival rates were higher in light rather than in dark conditions, which could have been due to seedling dependence on seed photosynthesis. Photosynthesis in non-dormant *P. oceanica* seeds could be an additional mechanism to supply C during early seedling growth. Little is known about the role that seed photosynthesis plays in seedling development and reserve mobilization yet this process is critical for understanding the factors influencing seedling survival. Although seedling recruitment is of great importance in the recolonization process after disturbances and the colonization of areas outside the range of vegetative growth (Balestri and Lardicci 2008), little is known about the environmental conditions that favor seedling growth and survival.

The aim of this study is to examine the contribution of seed photosynthesis to leaf and root growth and the mobilization of seed reserves and nutrients during the first three months of seedling development in the seagrass *P. oceanica*. To achieve these goals, changes in leaf and root biomass, the concentration of seed carbohydrate reserves (free sugar and starch) and elemental C, N and P content in seedling tissues were examined under varying light/dark conditions.

METHODS

Seed collection

P. oceanica fruits with no sign of dehiscence, herbivorism or mechanical damage were collected from the coastline of Murcia in southeastern Spain during May and June 2012. They were transported in a plastic container at a constant temperature to the laboratory, where they were placed in 200-L aquaria with similar conditions to those found in the field: 35 psu and 19°C. After extraction from the fruits, the seeds were individually placed on plastic mesh pots (8 cm high × 8 cm diameter) with a fiberglass substrate that favored high oxygen diffusion to the roots when plastic film was used as a covering in the light treatments. Because *P. oceanica* seeds have no dormancy period, germination occurred after maturation of the seed inside the fruit. Mature seeds extracted from the fruits were in the early stages of germination and without leaves when they were planted in the pots.

Experimental design

The effect of illumination on seedling growth was investigated by exposing the seeds and/or leaves from the seedlings to light or dark conditions for three months. The plastic control evaluated if the presence or absence of plastic film had any effect on seedling growth. Treatments ($n = 5$) were carried out with the following design (see Fig. 1):

1. Plastic control (PC): Seeds not covered by any plastic film. This control was under light conditions. Seeds and leaves were exposed to light.
2. Full light illumination (FL): Seeds covered by a transparent plastic film with a small hole in the apical side of the seed through which leaves exited towards the outside. This treatment was under light conditions. Seeds and leaves were exposed to light.
3. Partial light illumination (PL). Seeds covered by a black plastic film with a small hole in the apical side of the seed through which leaves exited toward the outside. This treatment was maintained under light conditions. Leaves were exposed to light, but

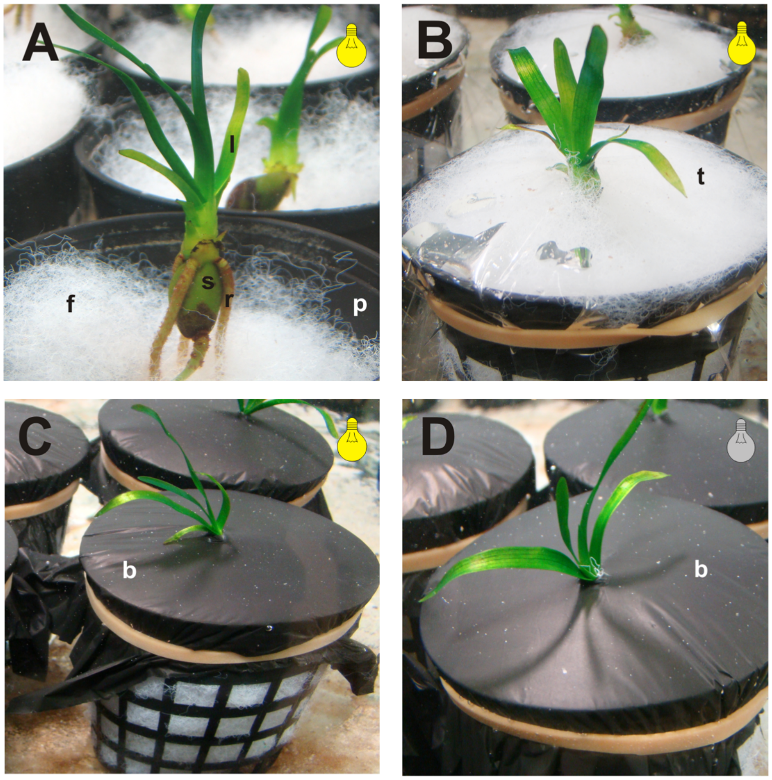

Fig. 1. (A) Plastic control. (B) Full light illumination treatment. (C) Partial light illumination treatment. (D) Full dark treatment. Abbreviations within panels are: r, roots; s, seed; l, leaf; p, plastic mesh pot; t, transparent plastic film; b, black plastic film; f, fiber glass.

the seeds were maintained in the dark with the black plastic film.

4. Full dark (FD). Seeds covered by a black plastic film with a small hole in the apical side of the seed through which leaves exited toward the outside. This treatment was maintained under dark conditions without light; both seeds and leaves were not exposed to any light.

Pots were placed inside five aquaria ($n = 5$) per treatment. Each (60-L) aquarium was filled with artificial seawater (35 psu) at the same temperature as the Mediterranean seawater during seed recollection (19°C). The water was not enriched with any nutrients. The aquaria were illuminated by halogen lamps with a photoperiod of 16:8 light:dark and delivering PAR light of 300 μmol photons m^{-2} s^{-1} at seed level. Water level and salinity within the aquaria were checked every second or third day. Seeds from FD were placed in an aquaria covered completely by aluminium foil to prevent light from entering. All aquaria were connected by a pump with a filter system that recirculated the water in order to maintain homogenous water conditions.

Mobilization of seed reserves and nutrients

To determine the contribution of seed reserves to growth in the different light treatments, free sugar and starch were assessed at the end of the experiment. Free sugars were extracted from the seeds in hot ethanol (80°C) (Zimmerman et al. 1989), evaporated with a heating block, dissolved in distilled water and analyzed spectrophotometrically using a 1% resorcinol assay standardized to sucrose (Huber and Israel 1982). Starch was extracted from ethanol-insoluble residues in 1N, KOH, and analyzed spectrophotometrically using the anthrone assay standardized to sucrose (Yemen and Willis 1954).

Seedlings were partitioned into three basic tissue components: leaves, roots and seeds (Fig. 1A) to examine the effect of light treatments on the nutritional status of each tissue component. For C, N and P analysis, tissue components were dried at 70°C and then ground into a homogeneous powder (MM 301 microgrinding device, Retch). Elemental analyses of C and N were performed on dry samples (2–6 mg) using a C:N:S analyzer (NA 1200, Carlo Erba). Elemental P analysis was performed using an inductively coupled plasma-mass spectrometer (Elan DCR II) in a 10-mg distilled water (DW) sample after digestion with HNO_3 and H_2O_2 in an Ethos-D microwave digester.

Seedling biomass

To examine the effect of light on seedling biomass, the dry weight of the three basic tissue components (leaves, roots and seeds) of the seedlings from the four treatments were dried at the end of the experiment at 60°C for 48 h. To calculate the contribution of the seed photosynthesis to seedling growth, the mean leaf and root biomass from PL was subtracted from the respective mean leaf and root biomass from FL. To calculate the contribution of only the leaves' photosynthesis, the mean leaf and root biomass of FD was subtracted from the respective mean leaf and root biomass. Finally, root and leaf biomass from FD represents the contribution of seed reserve mobilization.

Data analysis

Differences in leaf, root and seed biomass, seed reserves and nutrient concentrations among the seedlings from the four treatments were examined using a one-way ANOVA.

Seedling growth could be affected by light or dark exposition by generating more biomass as leaf or root tissue. However, the different growth investment of one of the two parts (leaves or roots) could also be due to changes in the expression level of light-regulated genes (Lifschitz et al. 1990, Batschauer et al. 1994, Terzaghi and Cashmore 1995) instead of seed or leaf photosynthesis. In that case, the separate statistical analysis of leaf or root biomass could be affected by this condition. To avoid that, "total biomass" (leaves and roots) was also analyzed.

Data were tested for homogeneity of variances using Levene's test ($\alpha = 0.05$) and normality using the Shapiro–Wilk test ($\alpha = 0.05$). After ANOVA, differences between specific treatments were determined with a post hoc Tukey HSD test ($\alpha = 0.05$).

RESULTS

Mobilization of seed reserves and nutrients

The one-way ANOVA revealed that starch concentration was significantly higher in FD than in treatments with total or partial seedling illumination (Tukey's HSD post hoc test, $P < 0.05$). Free sugars did not show significant differences between treatments (one-way ANOVA, $P > 0.05$; Fig. 2).

Elemental C, N and P concentrations and atomic C:N and N:P ratios and biomass in leaves, roots and seeds after 3 months of experimental treatment are shown in Figs. 3, 4 and 5, respectively. No significant differences were detected in elemental C and N concentrations or the C:N ratio between treatments (one-way ANOVA, $P > 0.05$). Leaves and roots tended to have higher P concentrations in treatments with shaded rather than illuminated seeds (Figs. 3 and 4); the differences were only statistically significant in roots (one-way ANOVA, $P < 0.05$). There were significant differences between the atomic N:P ratio of leaf and root tissues. In leaves, this ratio was higher in PC and FL than in treatments with shaded seeds (PL and FD) (Tukey's HSD post hoc test, $P < 0.05$). In roots, the post hoc test showed two N:P groups, but PL was not significantly different from PC–FL and FD.

Seedling biomass

Seedlings from the four treatments produced ca. seven leaves during the 12 weeks of the experiment, although leaf production depended on light exposure. There were significant differences in leaves, roots and leaf and root biomass among treatments (one-way ANOVA; $P < 0.05$) at 12 weeks after seed germination. Post hoc analysis confirmed that seedlings with seeds exposed to light (FL and PC) presented greater leaves, roots and leaf and root biomass than seedlings with seeds in darkness (PL and FD) (Tukey's HSD post hoc test, $P < 0.05$).

The effect of seed shading was as significant as keeping the entire seedling (leaves and seed) in darkness, as PL and FD did not present

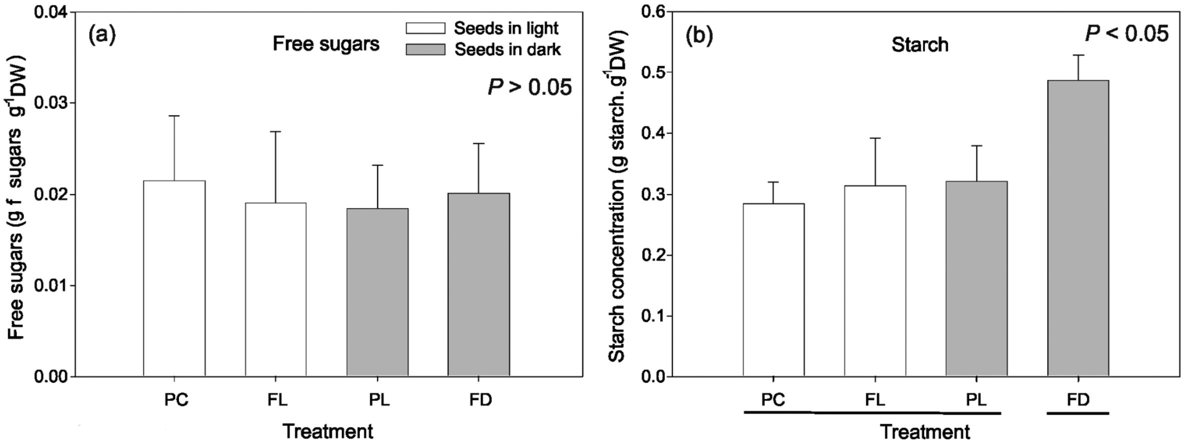

Fig. 2. Concentration of free sugars (a) and starch (b) in seeds of *P. oceanica* seedlings after 3 months of experimental treatment (mean ± SD). Horizontal bars represent significant differences (Tukey's HSD post hoc test, $P < 0.05$).

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Discussion

A major finding of the present study was that the high photosynthetic activity of *P. oceanica* seeds during early seedling growth enhances leaf and root growth, a response which reflects the adaptation of this endemic climax species to the oligotrophic conditions of the Mediterranean Sea.

Seagrass seedlings depend primarily on stored carbohydrate reserves complemented by autotrophic production until the photosynthetic apparatus of the seedling is capable of completely supporting the plant's carbon demands (Kaldy and Dunton 1999). The main carbohydrate stored in mature seagrass seeds (*P. australis*, *P. sinuosa*, *P. coriacea*, *Thalassia hemprichii*) is starch (Kuo and McComb 1989), which is presumably hydrolyzed to sugar and used to nourish early seedling growth (Hocking et al. 1981). The eelgrass *Zostera marina* L. responded to negative carbon balances during extreme light limitation by suppressing new root production, depleting carbohydrate reserves and gradually decreasing growth rates, although photosynthesis and respiration were unaffected (Alcoverro et al. 1999). This author also found that *Z. marina* died within 30 days under extremely limited light conditions, even though one-third of their carbon reserves remained immobilized in the rhizome, probably due to the effects of anoxia on translocation. Similarly to that study, the seeds maintained in FD contained more starch than partially or totally illuminated treatments, which suggests that extreme light limitation can prevent the full mobilization of carbon reserves in order to prevent a high respiration demand. Significant differences were observed in the biomass of leaves and roots as well as in the total biomass (leaves + roots biomass) (Fig. 6). This confirms that differences in seedling growth between treatments were due to seed photosynthesis and not any differential growth affected by light/dark conditions.

Extreme light limitation also imposes a dramatic reduction in growth rates and seedling survival. The ecological implications of this finding are very significant since light conditions become highly relevant in seedling growth. Seed burial during long periods of time or sequestration in depths where there is not enough light to activate seed photosynthesis implicates a low mobilization of the seed reserves that limits their growth rates and survival.

Another important consequence is that recruitment in a well-established *P. oceanica* meadow is not viable since this climax species displays very high LAI values (Olesen et al. 2002), which implies significant leaf self-shading within the canopy. The association between canopy morphology and the magnitude of leaf self-shading within the canopy has been described thoroughly

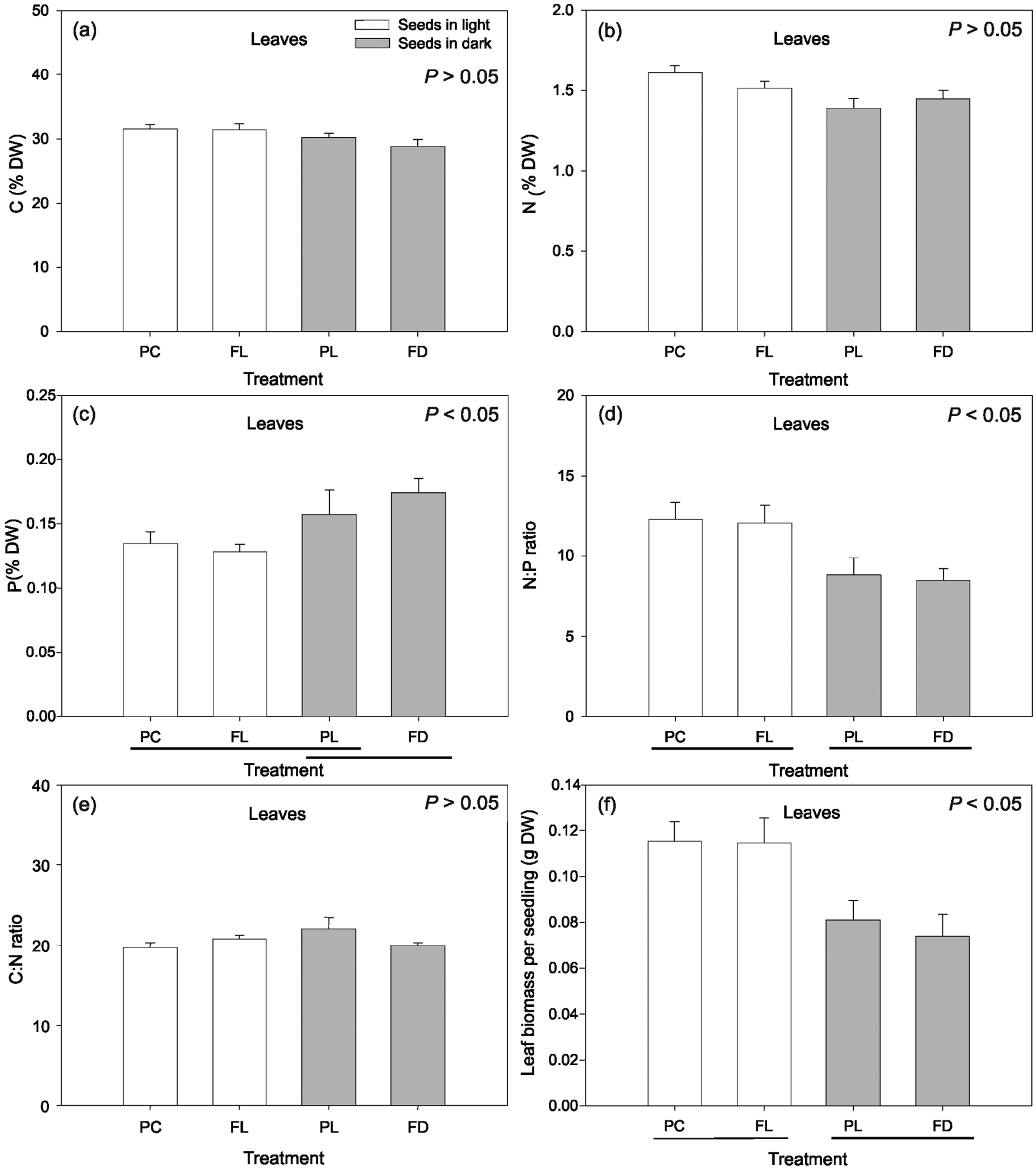

Fig. 3. Elemental C (a), N (b) and P (c) concentrations, atomic N:P (d) and C:N ratios (e) and biomass (f) in leaves of *P. oceanica* seedlings in PC, FL , PL and FD after 3 months of experimental treatment (mean ± SD). Horizontal bars represent significant differences (Tukey's HSD post hoc test, $P < 0.05$).

by Enríquez and Pantoja-Reyes (2005) and Hedley and Enríquez (2010). For seeds, light limitation imposed by mature meadows controls *P. oceanica* leaf self-shading and plant competition with its own recruits while also promoting increased seedling survival rates in open, uncolonized areas where clonal growth is limited. As stated by Terrados et al. (2013), seedlings

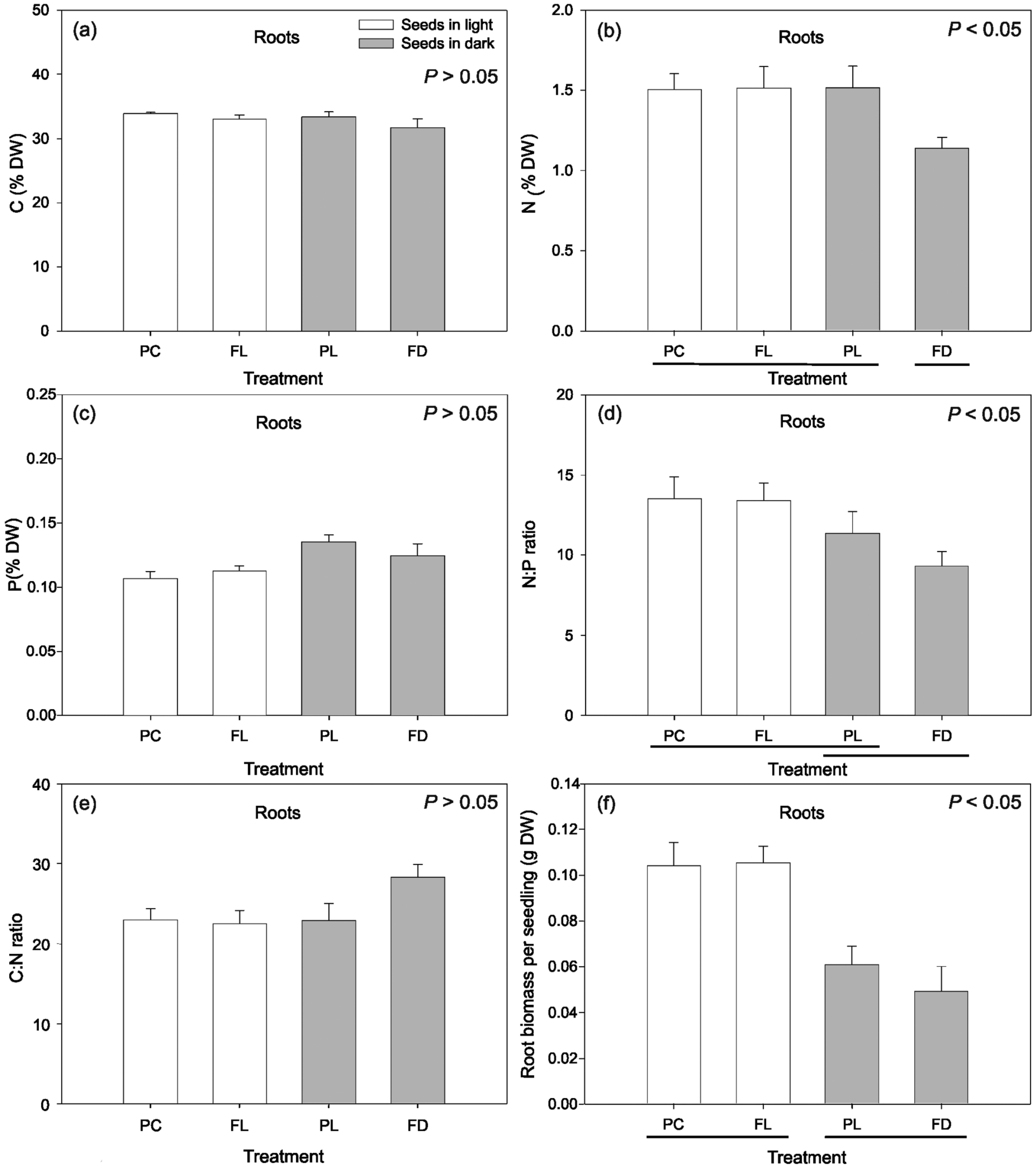

Fig. 4. Elemental C (a), N (b) and P (c) concentrations, atomic N:P (d) and C:N ratios (e) and biomass (f) in roots of *P. oceanica* seedlings in PC, FL , PL and FD after 3 months of experimental treatment (mean ± SD). Horizontal bars represent significant differences (Tukey's HSD post hoc test, $P < 0.05$).

planted inside a *P. oceanica* meadow led to complete mortality after 3 years, while seedlings planted in a dead meadow presented a survival rate of 44% during the same time frame. Shading was suggested as the process driving mortality, a conclusion which supports the results of the present study.

The establishment of a new seedling is the

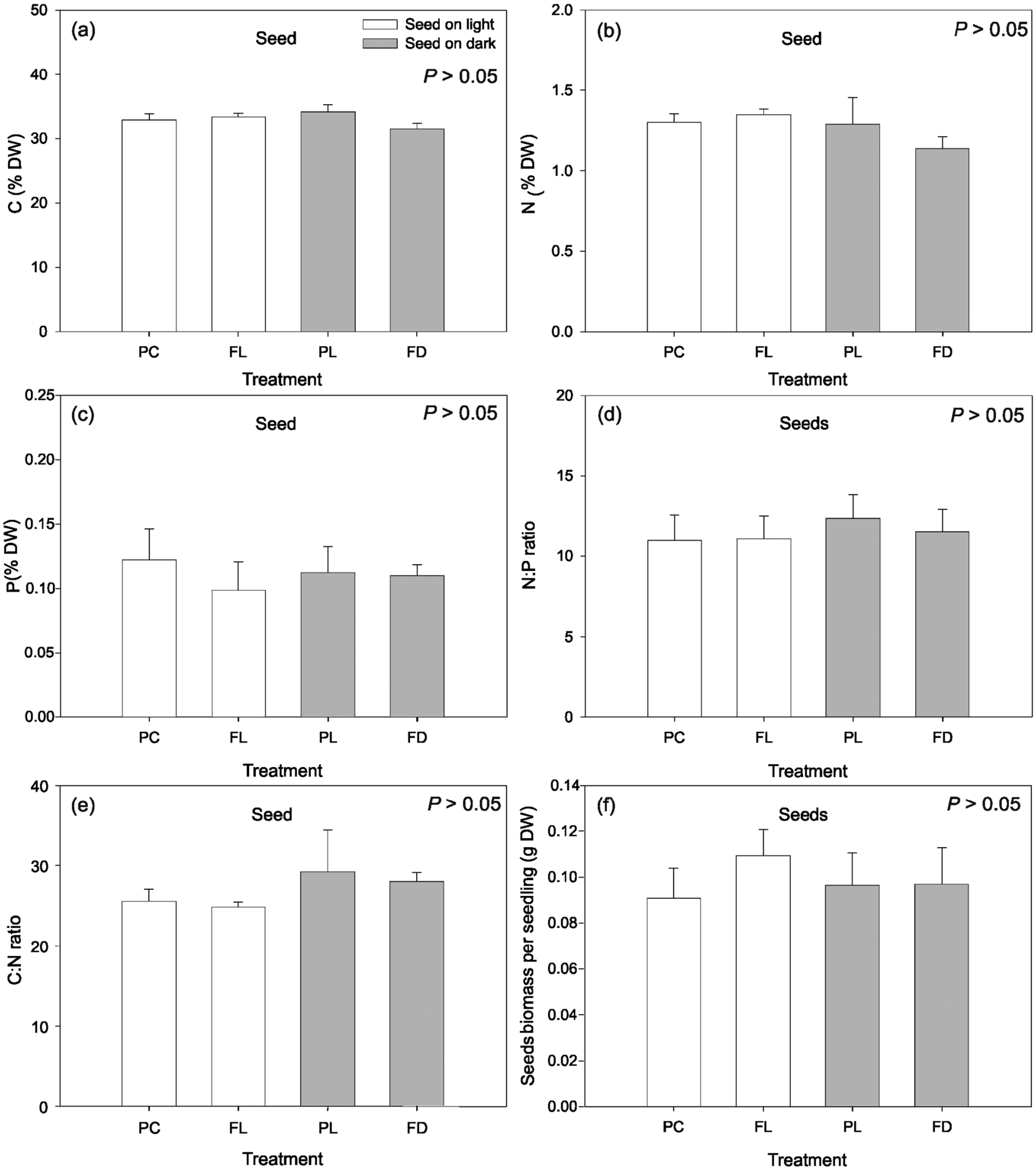

Fig. 5. Elemental C (a), N (b) and P (c) concentrations, atomic N:P (d) and C:N ratios (e) and biomass (f) in seeds of *P. oceanica* seedlings in PC, FL , PL and FD after 3 months of experimental treatment (mean ± SD). Horizontal bars represent significant differences (Tukey's HSD post hoc test, $P < 0.05$).

result of a seed being dispersed to an appropriate habitat and surviving complex biological interactions. The main constraints of seedling settlement could be classically summarized by sediment stability, nutrient limitation and light limitation. Duarte and Sand-Jensen (1996) found that P limitation may be a major cause of seedlings' failure to develop new patches in the

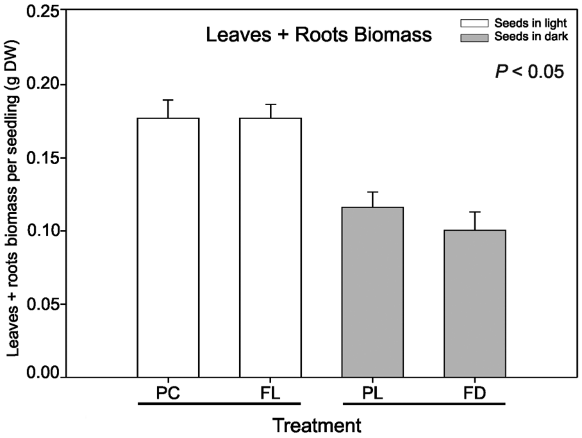

Fig. 6. Leaf and root biomass in *P. oceanica* seedlings in PC, FL, PL and FD after 3 months of experimental treatment (mean ± SD). Horizontal bars represent significant differences (Tukey's HSD post hoc test, $P < 0.05$).

Mediterranean seagrass *Cymodocea nodosa* (Ucria). On this basis, the authors supposed that severe nutrient deficiency is also the reason for the low reproductive success of *P. oceanica*. Balestri et al. (2009) studied nutritional constraints on the growth of *P. oceanica* seedlings in the northwestern Mediterranean Sea and suggests that P may play a major role in constraining the growth of *P. oceanica* seedlings. However, the results of the present study demonstrate that during the early development of a *P. oceanica* seedling, light is the limiting factor in its growth, not P. This finding reveals that light is a decisive factor for successful seedling growth.

In leaves, the N:P ratio was lower in PL and FD, while in roots, the N:P ratio was only lower in full dark treatments (FD), which could be related to lower nutrient demands due to the lower growth of seedlings treated in darkness . The lower N:P of PL and FD could indicate stress on seedling growth due to lack of photosynthesis rather than a nutrient limitation. Therefore, the generally low N:P ratio in both light and dark treatments in comparison with the atomic ratio observed in a field study using older seedlings with deflated reserves (Balestri et al. 2009) corroborates that, during early seedling development, P is not a limiting factor due to the high seed reserves. In FL and PC, the N:P ratio was near the stoichiometric ratio 16:1 described by Redfield (1934) for marine plankton, which is an indicator of suitable growth in light conditions with respect to treatments in darkness.

Balestri et al. (2009) found that the C, N and P content of *P. oceanica* seeds increased linearly with seed mass. Because seed size was homogeneously distributed between treatments, it was assumed that initial C, N and P reserves were identical between treatments.

The present study demonstrates that the seedlings with illuminated seeds (FL and PC) presented a significantly higher leaf and root biomass than treatments where seeds were not illuminated (PL and FD), which points to the clear influence of seed photosynthesis on seedling growth. Since there were not significant differences between FD and PL treatments, it was assumed that the photosynthetic contribution of leaves was practically nonexistent during early seedling growth. It can be estimated that ca. 29.26% of leaf biomass and ca. 42.31% of root biomass production was due to photosynthetic seed activity during early growth (3 months), as only 6.09% and 11.10% of leaf and root biomass, respectively, was contributed by leaf photosynthesis.

In addition, ca. 64.65% and 46.58% of leaf and root biomass, respectively, could be explained by mobilization of carbohydrate reserves from the seed, indicating that seed illumination did not act as a trigger mechanism for plant growth. This could be a survival mechanism for when seedlings are buried by wave action. *P. oceanica* seedlings have a relatively long shade tolerance; they are able to survive for three months following germination, albeit with significantly reduced foliar growth, which leaves the seedlings in unfavorable conditions during the light-limited winter period.

In conclusion, during the developmental phase of a seedling, photosynthetic activity and C, N and P reserves ensure the rapid growth of leaves and roots. The results of the present study demonstrate that seed photosynthetic activity enhances seedling growth of leaves and roots. This activity is functional and more active than that of leaves during early seedling growth. More studies are needed to increase existing knowledge about the reach of this strategy and the ecological implications of the same.

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