



Relationship between recruitment and mother plant vitality in the alien species *Acacia cyclops* A. Cunn. ex G. Don



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ABSTRACT

Acacia cyclops is a widespread invader in Mediterranean-climate regions. However, although its naturalization in the Mediterranean Basin dates back only a few years ago, and the invasion process has not been studied hitherto. We investigated seedlings recruitment strategy adopted by *A. cyclops* in a small island (Lampedusa, Italy) where its natural regeneration was strictly confined under mother plants canopy. Healthy plants (DCP), plants at incipient senescence (SCP) and dead plants (DP) were distinguished according to vitality and canopy status. Living plants were also characterized in relation to leaf C and N isotope composition. Regeneration pattern (seedlings and saplings abundance) was related to the microclimatic differences (soil temperature, air temperature and humidity, soil nutrients, light) observed between canopies and adjacent open areas, and among canopy types. Living canopies ensure milder conditions, reducing extreme values as well as fluctuations between night and day. However, beneath canopies (DP, SCP and DCP) seedlings may benefit from significantly higher soil nutrients content than in the outside, while light availability was much higher under DP. Saplings to seedlings ratio was found to be around 12 under DP, while under SCP it was slightly higher than 1, and just less than 0.5 under DCP. Moreover, saplings growth was significantly higher under SCP and DP, suggesting a prominent role of light in driving seedlings recruitment. Stable isotope analyses of C and N provided ecophysiological information in relation to changes in canopies structure. Thus, while seedling stage appears to be more nutrient-limited, subsequent sapling stage is much more light-limited. Although the species is not yet displaying an invasive spreading on the island, our study provides clear evidence that senescent canopies are better facilitators than healthy in preserving the invasive potential of *A. cyclops*. This finding suggests some best practices in order to gradually reduce the presence of the alien species within its pristine nuclei of introduction.

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1. Introduction

Species belonging to the genus *Acacia* have proven to be some of the most aggressive invading species, with vastly negative effects over terrestrial ecosystems. Australian Acacias (also termed ‘wattles’) are a group of leguminous woody plants that include some of the most important plant invaders globally (Richardson et al., 2011). The Italian peninsula has also been concerned; eight *Acacia* species are currently naturalized, and four of them already showed an invasive behavior (Celesti-Grapow et al., 2010; Pasta et al.,

2012a). Natural regeneration, establishment and recruitment of these species are key issues in understanding the spatial and temporally component of dynamic biological and chemical processes associated with plant invasions. Indeed, the study of both environmental factors and plant traits that modify the regeneration capability of *Acacia cyclops* is crucially important to understand its invasive potential at an early stage outside of its native area. *A. cyclops* natural regeneration in the semi-arid Mediterranean island of Lampedusa was dependent on the structure of covering canopy, leaf traits and vitality conditions.

Wattles are among the most widespread and prominent invaders within Mediterranean-type ecosystems, where they are able to produce major changes in species composition and ecosystem

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functioning, and thus threatening local biodiversity (Holmes and Cowling, 1997; Hellmann et al., 2010). For instance, many *Acacia* species establish almost pure stands in South African fynbos and greatly increase N soil content, especially due to symbiotic fixation. Actually, the slow decomposition rates of phyllodes may lead to a litter accumulation significantly higher in invaded than in uninvaded areas (Witkowski, 1991a; Yelenik et al., 2007), with strong and lasting ecological consequences (Marchante et al., 2011), especially where native species are adapted and evolved under low-resources conditions (Witkowski, 1991a).

A well known invader is *A. cyclops* A. Cunn. ex G. Don (Fam. Fabaceae), an evergreen shrub native of southwestern Australia (Cronk and Fuller, 1995). This alien plant belongs to the subgenus *Phyllodineae*, which includes 23 species with invasive behavior elsewhere (Richardson et al., 2011). *A. cyclops* has been widely used for stabilization of coastal areas and for afforestation purposes in semiarid regions, owing to its drought and salt resistance. The species is able to establish and survive in areas with low annual rainfall (250–300 mm; Gill, 1985; Witkowski, 1994). Like other *Acacia* species (Werner et al., 2010), *A. cyclops* is well adapted to Mediterranean climate and poor-nutrients environments, where it may regenerate and show a high competitive ability and a better acquisition of limiting resources, compared with that of native coexisting species (Morris et al., 2011). In particular, it is considered a transformer species in South Africa and in Portugal (Witkowski, 1994; Henderson, 2007; de Almeida and Freitas, 2006), where its invasiveness is mainly due to its large and persistent soil-stored seed bank (Milton and Hall, 1981; Richardson and Kluge, 2008). Seedlings fast growth rates (Witkowski, 1991b) and the long-distance seed dispersal provided by birds (Glyphis et al., 1981) exacerbate the invasive character of the species. Even if only 20–40% of seed population remains dormant in the soil after 1 year (Holmes and Moll, 1990), the annual seed production is very high (1977 seeds/m²; Milton and Hall, 1981) so that in time the seed bank can grow up to 7792 seeds/m² (Holmes, 1989). When established on a given area, the species causes deep alteration of N ecosystem cycling, mainly because of nitrogen fixation and litter accumulation (Stock et al., 1995; Marchante et al., 2011). Such an occurrence deeply affects structure and composition of invaded communities. Given all reasons, *A. cyclops* can be considered an ecosystem transformer (*sensu* Richardson et al., 2000) and, so, deserves to be included among the most aggressive alien plants.

Recently, *A. cyclops* started to spread also within coastal areas in the Mediterranean Basin, including Northern Africa and Sicily (Vilà et al., 1999; Badalamenti et al., 2013). Mediterranean insular ecosystems, known for their heritage of biological diversity and rate of endemism, could be seriously threatened by *A. cyclops* (La Mantia et al., 2011; Pasta and La Mantia, 2013; DAISIE database: <http://www.europe-aliens.org/speciesFactsheet.do?speciesId=12740#>). In Lampedusa, the largest island of the Pelagic Archipelago (Sicily), natural regeneration of *A. cyclops* attained a considerable abundance. Particularly, seedlings and saplings can be observed confined beneath the canopy of mother plants (Pasta et al., 2012a).

The present study aimed at understanding the patterns driving the early development steps of *A. cyclops* and thus to a potential invasion process, under extreme drought conditions. In particular, regeneration pattern was assessed according to mother plant vitality, and seedlings emergence to their definitive establishment. The main hypotheses are: (1) seed germination is favored by soil fertility (organic matter and N) as a consequence of consistent litter accumulation; (2) seedlings establishment and persistence is affected by light availability at soil level. Therefore, the canopy capability in affecting the microenvironment for seedlings was characterized underneath the canopy of both healthy, senescent and dead mother plants of *A. cyclops* in Lampedusa isle.

2. Materials and methods

2.1. Study site

The study was carried out in Lampedusa island (Sicily Region; Italy). *A. cyclops* regeneration was studied in Contrada Taccio Vecchio (35°31'02"N, 12°36'06"E, 46 m a.s.l.) on the North-Western side of the island, where about 5 ha have been afforested with *A. cyclops* together with *Acacia saligna* (Labil.) H.L. Wendl. and *Pinus halepensis* Mill. with the aim to promote the recovery of vegetation and to control soil erosion (Pasta et al., 2012b).

The soils, mainly originated by limestones and marls (Grasso and Pedley, 1988), are poorly evolved and generally shallow, showing an upper horizon only slightly differentiated, with abundant skeleton and low organic matter content (Fierotti, 1998). According to Rivas-Martínez (2008), the climate is classified as infra-Mediterranean upper-semiarid, with a mean yearly temperature of about 19 °C. Precipitations pattern follows a typical Mediterranean regime; rainfalls are mostly concentrated during the coldest months of the year. However, during the last 150 years, a significant decrease of annual rainfall from 500 to 300 mm has been reported, with a concurrent extension of the drought period up to 6 months (La Mantia et al., 2011). The windy character of the study area heavily affects the canopy structure and the overall evapotranspiration demand, thus affecting water availability at soil level. It is noteworthy that, along the last 30 years, uncontrolled fires did not affect the study area.

2.2. Plant material: mother plants selection and evaluation of regeneration patterns

The senescence process of *A. cyclops* individuals starts with leaves fall localized only in some main branches. Then, the canopy becomes gradually more and more sparse, and after about a year the plant eventually dies. Mother plants were chosen on the basis of different health conditions and canopy density. According to photosynthetic active radiation (PAR) extinction beneath individual canopies (expressed as % of full exposure), we distinguished healthy plants with dense canopy (DCP), plants at incipient senescence with sparse canopy (SCP) and dead plants (DP; Table 1). Three replicates per each canopy type were considered for the experiment. During March 2012, PAR measurements were hourly acquired by means of linear sensors (Apogee Inc., LQS 70–10 M). Regeneration patterns were assessed in terms of seedlings and saplings abundance under DCP, SCP and DP. Sample areas of 250 cm² were chosen proceeding northward from the trunk to the limit of the canopy projection, in steps of 50 cm. Seedlings, recognizable for compound leaves, were accounted separately from saplings, which display exclusively phyllodes, leaf-like modified petioles. The total number of seedlings and saplings, as well as the height of saplings, were assessed under each canopy type. The saplings/seedlings ratio was calculated. Expanded phyllodes, representative of the whole canopy, were sampled from both mother plants and saplings. Leaf area was measured by using a leaf area meter (Delta T Devices, USA), and leaf dry weight after stable weighing at 80 °C. Leaf mass area (LMA) and specific leaf area (SLA) were calculated. Total N content was measured by means of an elemental analyzer (NA 1500, Carlo Erba, Italy).

2.3. Stable isotopes analysis

Stable isotope ratios were determined using a continuous-flow, triple-collector, isotope ratio mass spectrometer (ISOPRIME, GV, Manchester, UK). Finely powdered sub-samples of dried leaves (≈0.5 mg for C; ≈2.5 mg for N) were quantitatively combusted in

Table 1
Plant characteristics and leaf isotopic composition.

Plant canopy	Canopy diameter (cm)	PAR (% extinction)	Leaf mass area (g m ⁻²)	Specific leaf area (cm ² g ⁻¹)	δ ¹³ C (‰)	δ ¹⁵ N (‰)	N * leaf mass area (g m ⁻²)
DP	600	39.58 ± 2.73	–	–	–	–	–
SCP	800	60.62 ± 3.57	240.64 ± 9.20	41.81 ± 1.64	–26.89 ± 0.29	–2.71 ± 0.13	3.26 ± 0.03
DCP	800	71.77 ± 2.89	209.08 ± 4.70	47.92 ± 1.05	–28.38 ± 0.37	–1.94 ± 0.29	2.85 ± 0.07

an elemental analyzer (Model NA 1500, Carlo Erba, Milan, Italy). Nitrogen oxides produced in the oxidation reactor were reduced to elemental N in a reactor filled with elemental copper and operated at 650 °C. Both CO₂ and N₂ were transferred in helium flow to the mass spectrometer for the determination of the isotopic ratios (R), ¹³C/¹²C and ¹⁵N/¹⁴N, respectively. The isotopic compositions of carbon and nitrogen (δ¹³C and δ¹⁵N, respectively) of the samples were determined according to the definitions described by Farquhar et al. (1989). In some detail, isotope ratios were used to calculate isotopic compositions, according to the following formula: $\delta = R_{\text{sample}}/R_{\text{standard}} - 1$, where the subscripts refer, respectively, to the isotopic ratio of the sample or to that of the international isotopic standards for carbon (VPDB) and for nitrogen (atmospheric N). It is worth noting that δ¹³C of plant structural carbon is the basic parameter for the calculation of carbon isotope discrimination (Δ¹³C). This latter isotopic notation is related to the expression of plant water-use efficiency (WUE) (for review see Brugnoli and Farquhar, 2000), an important physiological trait related to plant acclimation capability in drought-prone environments (Lauteri et al., 2004). Indeed, Δ¹³C in C₃ plants is the process descriptor of the ¹³C depletion in photosynthesis assimilated carbon, as compared to the ¹³C abundance in the atmospheric CO₂. Assumed homogeneity of δ¹³C in the atmospheric CO₂ of the study site, δ¹³C of leaf material is itself considered a reasonable proxy of assimilation weighed, plant water-use efficiency (Lauteri et al., 1993; Ripullone et al., 2004).

2.4. Microclimatic and radiation measurements

Air and soil temperature, and air humidity, were measured by means of sensors connected to data loggers (Onset® Hobo® data loggers), put under DP, SCP and DCP. The sensors were set in between the trunk and the edge of the canopy projection on the ground. The measurements were automatically recorded each 15 min on March 2012. All parameters have been expressed as a percentage respect to adjacent open areas, where the influence of *Acacia* canopy was considered negligible.

2.5. Soil and litter collection and analysis

Within each sample area delimited by the tree canopies, litter was carefully removed and weighed after 72 h oven-drying at 80 °C. Two soil samples were collected at 20 and 40 cm depth. Total N and C organic soil (SOC) content were determined with an elemental analyzer (NA 1500, Carlo Erba, Milan, Italy), on air-dried samples, after 2 mm sieving. Furthermore, three soil samples were taken in a fully exposed area in the neighborhood of each tested tree and analyzed as previously.

2.6. Statistical analysis

ANOVA was performed on overall plant type considering a randomized complete block, distance from trunk as main plot and depth as sub-plot. SAS procedure was used according to the adopted experimental design.

3. Results

3.1. Mother plant characteristics and recruitment patterns

The PAR measurements highlighted that under DCP the light extinction was higher than 70% of full exposure, under SCP the extinction percentage ranged between 40% and 60%, while under (DP) it was just less than 40% (Table 1). The canopy type of mother plants (DCP, SCP or DP) strongly affected the regeneration pattern. Indeed, saplings/seedlings ratio was around 12 under DP, while under living trees it was slightly higher than 1 for sparse canopies, and just less than 0.5 for dense canopies (Table 2). Moving away from the trunk, saplings number initially strongly decreases but then increases from 150 to 200 cm under living plants and from 100 to 150 cm under dead plants (Fig. 2). In addition to the higher total number, saplings growth was particularly enhanced under DP and SCP rather than under vigorous canopies (DCP). As a consequence, height was significantly lower under DCP, where saplings reached a mean height less than 16 cm, while heights of 24.8 and 20.6 cm were found under DP and SCP, respectively (Table 2).

LMA was significantly higher in SCP than in DCP (Table 1) and saplings (data not shown), mean values yielding, respectively, 240.8, 190.1 and 174.5 g m⁻². Despite of the large differences in LMA, no significant differences in leaf nitrogen content on leaf mass basis were observed, mean values being 1.36% of the leaf mass for both SCP and DCP and 1.34% for saplings (data not shown). However, owing to significant differences in LMA among the mother plant types, robust differences in nitrogen content on leaf area basis were found, SCP yielding significantly higher values than DCP (3.26 vs. 2.85 g m⁻²; Table 1).

3.2. C and N stable isotopes analysis

Leaf carbon isotope composition provided an ecophysiological explanation to the observed changes in mother plants canopy structure. Significant differences in δ¹³C and δ¹⁵N of leaves were observed comparing mother plants with sparse (SCP) and with dense canopy (DCP). It is noteworthy that SCP showed relatively enriched δ¹³C and depleted δ¹⁵N of leaf dry matter in respect to DCP. Particularly, δ¹³C mean values ranged from –26.9‰ to –28.4‰ and δ¹⁵N mean values from –2.7‰ to –1.9‰, in SCP and DCP, respectively (Table 1). Preliminary stable isotopes observations were achieved also on saplings, especially recruited under DP. Average values ± SE of δ¹³C and δ¹⁵N in leaves of saplings yielded, respectively, –30.0‰ ± 0.2‰ and –2.4‰ ± 0.3‰ (data not shown). Thus, the recruitment showed depleted values of δ¹³C in respect to both the living types of mother plants (DCP and SCP), whilst showing intermediate values of δ¹⁵N (Table 1).

3.3. Microclimatic and radiation measurements

Overall, variation in time of soil temperature under living and dead plants showed a very similar pattern, with lower values during the day, and higher during the night, compared with the outside (Fig. 1). By contrast, the canopy effect on air temperature and humidity was strongly different if the plant was living or dead. Under living plants, the canopy reduced consistently air

Table 2

Saplings/seedlings ratio, saplings height, as well as seedlings and saplings number under different *Acacia* canopy types. Different letters within the same column indicate significant differences after Tukey's HSD test ($p < 0.05$).

Canopy type	Saplings/seedlings ratio	Saplings height (cm)	Seedlings number (m ⁻²)	Saplings number (m ⁻²)
Dead (DP)	12.25 a	24.76 a	9.82 c	116.41 a
Sparse (SCP)	1.19 b	20.61 a	94.28 b	101.18 a
Dense (DCP)	0.40 b	15.88 b	195.31 a	77.28 b

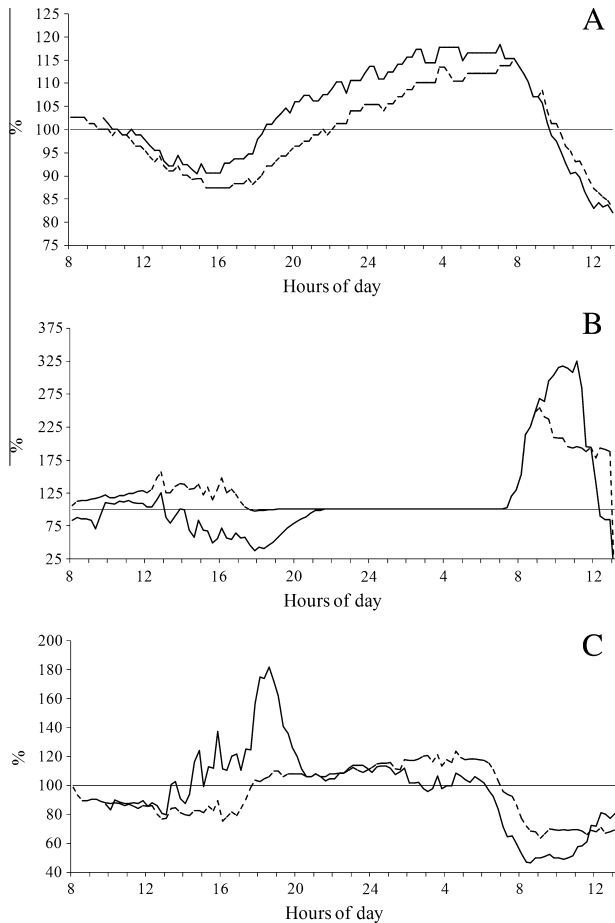


Fig. 1. (A) Soil temperature, (B) air humidity and (C) air temperature under living (dotted line) and dead plants (continuous line) (% respect to open air).

temperature and humidity fluctuations between night and day, while variations in air microclimatic parameters were far more pronounced under dead plants.

3.4. Soil and litter

SOC trend under the three different canopy types (DP, SCP or DCP) showed significant differences. Under DP, SOC sharply decreases from 50 to 300 cm far from the trunk (Fig. 3a). On the contrary, under living plants, both SCP, DCP and SOC decreases lightly up to 400 cm far from the trunk. In particular, SOC content is initially higher in DP and SCP, while beyond 200 cm is higher under living plants, both SCP and DCP.

Nitrogen trend was quite similar to SOC under SCP and DCP, whereas under DP the reduction with distance was much less marked (Fig. 3b). The highest values were always reached under SCP, irrespective of distance, whereas N content was higher under DP up to 200 cm, beyond becoming higher under DCP. However, according to canopy size, we found the highest SOC and N content

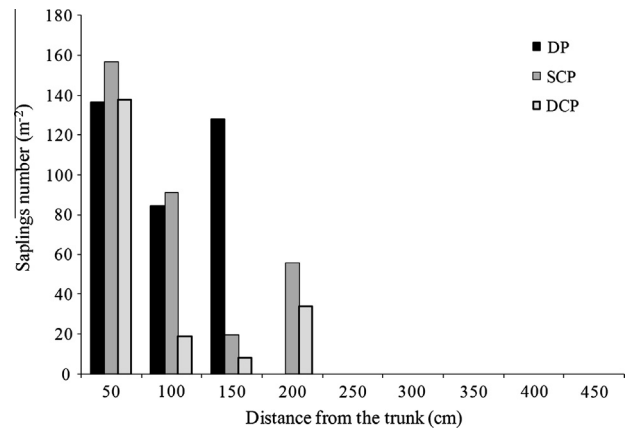


Fig. 2. Seedlings and saplings pattern in relation to distance from the trunk (DP = dead plants; SCP = plants at incipient senescence with sparse canopy; DCP = healthy plants with dense canopy).

associated with DCP, while the lowest values were found under DP (Table 3).

Overall, a highly significant difference between carbon and nitrogen soil content and distance from the trunk was found (Table 4). Just for nitrogen, a significant relationship with depth was also found. Both for carbon and nitrogen, no statistical significance was found for interaction depth × distance. As regards litter dry weight, all canopy types showed a similar decreasing pattern moving away from the trunk (Fig. 4). In DP, litter content sharply decreases to zero, while under SCP and DCP a more slowly reduction was found. In addition, litter dry weight was higher in DCP in the middle of the canopy while it was higher in SCP on the canopy edge and near to the trunk.

4. Discussion

For woody alien plants used for afforestation, seedlings recruitment and establishment patterns are strongly affected by how mother plants may alter the surrounding habitat and especially the understory conditions so as to trigger the naturalization process. As concerns *Acacia* species used in arid and semi-arid habitats such as *A. cyclops*, this is particularly true as open areas among adult plants are characterized by much more limited environmental conditions, both in terms of nutrient and water soil content.

Within our study site, the absence of natural regeneration outside of the canopies may be explained by a number of factors. First of all, the possible absence of dispersal vectors may have caused a spatial gradient in soil-seed density, and an abrupt reduction outward from the main stem (Milton and Hall, 1981). However a direct canopy effect may have played a major role by improving microclimatic and edaphic conditions underneath adult plants. Higher soil moisture and nutrients (N and C) content may considerably increase the chance for seedlings to survive and evolve into saplings. For instance, an overwhelming capability by *Acacia longifolia* to modify the environment, and especially nitrogen supply within plant communities, in driving the invasion processes has

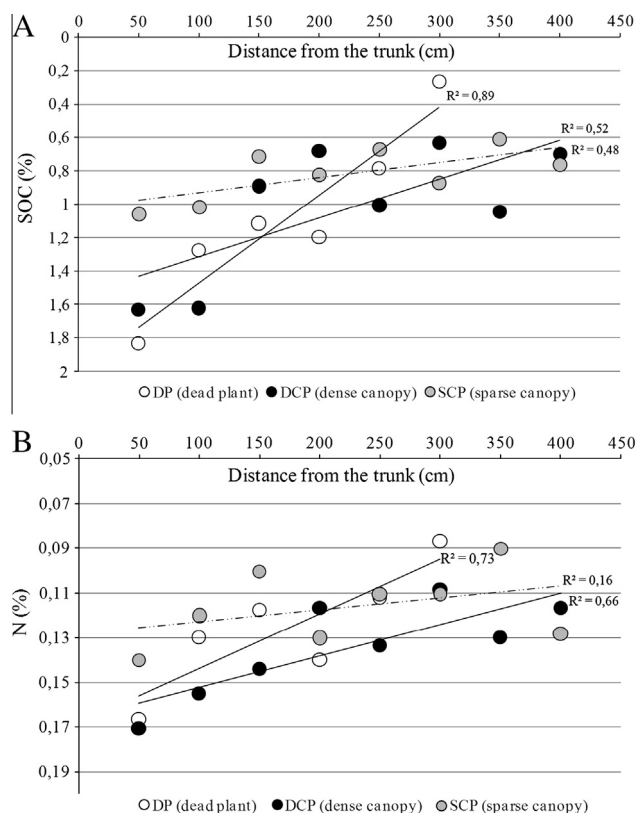


Fig. 3. (a) SOC (%) in relation to distance from the trunk; (b) N (%) in relation to distance from the trunk.

Table 3
SOC and N stock (0–15 cm depth) on area basis under different plant canopy types.

Plant canopy	SOC stock (kg m ⁻²)	N stock (kg m ⁻²)
DP	1.42 ± 0.18	0.34 ± 0.02
SCP	2.30 ± 0.16	0.34 ± 0.02
DCP	2.81 ± 0.32	0.31 ± 0.01

Table 4
ANOVA for soil carbon and nitrogen under different plant canopy types.

Source	DF	Carbon		Nitrogen	
		MS	F	MS	F
Rep	2	0.5297	0.51	0.0006	0.20
Depth	1	0.5022	4.86**	0.001	3.25**
Distance	6	0.1339	1.30	0.0013	3.69*
Depth * distance	6	0.0449	0.39	0.00005	0.16
Residual	24	0.1032	–	0.0003	–

* $p < 0.05$.

** $p < 0.001$.

been recently proved on a Portuguese coastal site, by means of innovative stable isotopes methodologies (Rascher et al., 2012). In general terms, the application of stable isotopes and geostatistical interpolation methods has been used at landscape, continental or global scales (isoscapes), to explicitly understand the spatial and temporal component of dynamic biological and chemical processes (West et al., 2008; Bowen et al., 2009a,b). In particular, the study based on combining native species foliar $\delta^{15}\text{N}$ with spatial information regarding plant location using geostatistical methods, demonstrated that it is possible not only to trace the N-focus point (*A. longifolia* individuals or small *Acacia* stands) as well as the effective area impacted by exotic N-addition into the surrounding

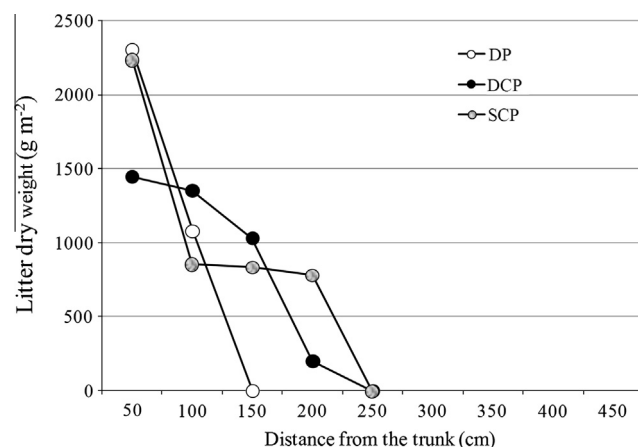


Fig. 4. Litter dry weight in relation to distance from the trunk (DP = dead plants; SCP = plants at incipient senescence with sparse canopy; DCP = healthy plants with dense canopy).

native vegetation. In our study, stable isotopes analyses on living mother plants (SCP and DCP) provided interesting physiological information on the mechanisms of recruitment adopted by *A. cyclops* within the Lampedusa landscape. Indeed, SCP showed significantly enriched values of $\delta^{13}\text{C}$ in leaf dry matter (Table 1) and, consequently, higher WUE than observed for vigorous mother plants (DCP). Such a finding, given the unhealthy conditions of SCP, has to be interpreted as a response to limiting environmental conditions (Farquhar et al., 1982; Brugnoli et al., 1988; Lauteri et al., 1997), whose abiotic or biotic determinants must be taken as unknown, at the moment. Although no measurements of plant-water relations have been acquired along the experiment, drought has to be regarded as one of the most limiting factors for growth and photosynthetic activity in Lampedusa. Thus, whatever the cause of the mother plants impairment, their relatively enriched $\delta^{13}\text{C}$ values indicate a decreased photosynthetic set-point (Farquhar et al., 1982), namely the internal to atmospheric CO_2 concentration ratio (C_i/C_a). A reduced CO_2 concentration in the leaf air spaces can be achieved by two main mechanisms: a reduced CO_2 diffusivity from the atmosphere to the carboxylation sites (either stomata or mesophyll resistances) or an increased biochemical drawdown due to increased carboxylation activity (extra-stomata effects). Moreover, $\delta^{13}\text{C}$ can be also a tracer of modifications in SLA and specific leaf weight (SLW) due to changes in leaf structure (Werner and Máguas, 2010). Thus, two considerations make more likely the effectiveness of differential diffusive constraints in explaining the observed variability of $\delta^{13}\text{C}$ in leaves of *A. cyclops*: thicker leaves in SCP rather than in DCP, and the senescent appearance together with a more depleted $\delta^{15}\text{N}$ of SCP, as compared with DCP. Leaf nitrogen isotope composition provides interesting insights in the plant nitrogen metabolism. Nitrogen fixation is expected to move $\delta^{15}\text{N}$ in leaves toward 0 (Shearer and Kohl, 1986). The more negative values of the impaired mother plants (SCP) indicate an unbalanced mutualism with fixing bacteria, owing likely to impaired root systems. This might explain a relatively high WUE, a reduced water uptake, a sparse canopy with thicker leaves and closer stomata in SCP as compared with DCP. However, due to thicker leaves, the leaf nitrogen content, on a leaf area basis (Table 1), resulted somewhat higher in SCP, and this could likely be ascribed to N recycling and translocation from senescent to surviving leaves in impaired mother plants.

Accordingly to our working hypothesis, adult *Acacia* canopies played a key role in the establishment of seedlings by protecting them under strong drought and nutrients-limited conditions. The observation that seedlings performance is facilitated underneath

adult plants is not new (Dunham, 1991; Isicheia and Muoghalua, 1992; Kahi et al., 2009). However, this study provides clear evidence that senescent canopies are better facilitators than healthy ones, as found for other invasive species (Rees and Paynter, 1997). The micro-environmental conditions under the canopies were found to significantly affect regeneration pattern, as shown by the wide differences detected in saplings/seedlings ratio, as well as in seedlings and saplings absolute number (Table 2).

In particular, we hypothesized that these differences may have been related to the incipient senescence process observed in many *Acacia* mother plants. Indeed, a significantly higher number of saplings were observed under dead plants than under living canopies, either dense or sparse (Fig. 2). Such an evidence may rely on litter accumulation and/or on the improvement of microclimatic conditions. Under dead plants seedlings experienced soil temperatures fluctuations as wide as under living plants (SCP and DCP). This suggests that litter layer is sufficient to mitigate extreme soil temperatures thus improving the overall soil water status. Conversely, living and dead canopies differently affected air humidity and temperature as well as light availability for seedlings (Fig. 1; Table 1). Indeed, living canopies reduced consistently air temperature and humidity fluctuations between night and day, ensuring milder microclimatic conditions, and protecting seedlings from direct sunlight, especially during the hottest hours of the day (Fig. 1). Variations in air parameters were by far more pronounced under DP, where seedlings are likely to experience much more stressful conditions. The higher light availability beneath DP than under DCP was associated to an increase of saplings/seedlings ratio from less than 1 up to more than 12 (Table 1). Such a trend reveals the prominent role of the combined effect of soil resources (water and nitrogen) and light in triggering seedlings growth and development, thus increasing or sustaining the invasive potential of *A. cyclops*. The reduced presence of seedlings under dead trees, at least partially, rely on the absence of seed production since several years after death. However, as already observed on *A. longifolia* in sand dunes (Máguas et al., unpublished) and on other *Acacia* species (Richardson and Kluge, 2008), *A. cyclops* is known to accumulate a robust soil seed bank (Holmes, 1989); thus, the observed regeneration patterns is more likely to depend on the lower resistance of seedlings to environmental stresses. In general saplings have higher survival rates than juvenile seedlings. Moreover, a faster development (Table 2) may ensure a better capability to cope with summer drought and to establish definitely within Mediterranean semi-arid environments. Our findings about the prominent role of light are consistent with other works concerning *A. cyclops*, which is confirmed to be a light-demanding species. Glyphis et al. (1981) found that only 6% of seedlings older than 3 months were capable to survive under the shade of a canopy, while in open areas the value for the same age class reaches up to 50%. Furthermore, a particularly negative effect of shading on root production has been found (Milton, 1982), so that seedlings growing under a dense canopy would be much more exposed to summer drought, as soon as light supply increases with canopy decaying.

In the present study, under *Acacia* canopies, seedlings may benefit from significantly higher N and C soil content than in the outside. In particular, SOC and N content were higher under DCP rather than under SCP and, especially, under DP (Table 3). This could be due to the combined effect of reduced protection from atmospheric exposure and of intense oxidative processes of organic material. However, dead plants are by far more effective on saplings recruitment than living ones, likely because of the increased light availability. Under living mother plants, litter layer allows germination and emergence of seedlings, but their development and definitive establishment is delayed or significantly slowed as long as a sufficient quantity of light reaches the ground. Thus, while the acacia's seedling stage appears to be particularly

nutrient-limited, the subsequent sapling stage is particularly sensitive to light limitations.

These results point out the importance of canopy and litter accumulation dynamics in *A. cyclops* invasion process, particularly referring to the latent and early phase. Such dynamics have striking environmental effects (Rascher et al., 2012) within the understory of the original mother plants, improving nutrient and water availability and reducing extreme temperatures and irradiances. In particular, the invasion process of *A. cyclops* appears to be favored by ageing or canopy decay. This greatly increases light availability for seedlings growth. However, further investigations are needed in order to define the ecophysiological bases and limits of the invasive potential of *A. cyclops*, as well as the light thresholds values that trigger the regeneration process by shifting saplings/seedlings ratio in favor of saplings.

5. Conclusions

Understanding the main factors allowing abundant regeneration of invasive species is of crucial importance for their management and control (Wilson et al., 2011); especially on Mediterranean islands, where plant invasions are an emergent issue (see Pretto et al., 2012 and references therein). The main limiting factors for seedlings emergence and survival in semi-arid environments are light and water availability, along with N soil content. In many water-limited ecosystems such as savannas, the presence of single trees with a scattered distribution is crucial for the improved soil conditions either in terms of fertility and water status, which determine favorable conditions for native species survival and thus preserve local biodiversity. Furthermore, Australian *Acacias* play a significant role in ecosystem structure and functioning in many parts of the world, from arid to wet ecosystems (e.g. Munzbergova and Ward, 2002). However, they successfully invade new habitats and deeply modify nutrients supply as well as biodiversity patterns (Hellmann et al., 2010).

This could be the case of Lampedusa, where the invasive behavior by *A. cyclops* (at an initial stage) has become apparent within afforested sites characterized by very shallow and nutrient-poor soils, where woody native species are poorly able to establish and reproduce. In particular, regeneration by self-sowing of *A. cyclops* was first observed in 1998, but just in the last few years the process has dramatically increased, so that now an impressive number of seedlings, saplings and young trees are actively growing and establishing. Within the study site, the regeneration process was strictly confined under *Acacia* canopies. We related the abundance of renewal with nitrogen, carbon and light availability, inside and outside of the tree canopies, considering healthy, senescent and dead mother plants. The spatial variability of C and N stable isotopes compositions among plants allowed us to clarify the main strategy of seedlings recruitment and establishment adopted by *A. cyclops* in Lampedusa. In particular, adult mother plants create safe conditions and may be beneficial also for the germination of some valuable woody native species such as *Pistacia lentiscus* L., as a consequence of consistent soil fertilization, but the overwhelming number of *Acacia* seedlings observed represents a serious threat for the insular biodiversity because *Acacia* will likely dominate at this site, at least in a long-term perspective (Pasta et al., 2012a).

Thus, apart from constantly monitoring the invaded area, a gradual removal of dead or senescent plants, under which most of saplings regeneration occurs, should be tested as a management practice for the control of the species. This could aid towards a progressive reduction of the invasive risk posed by *A. cyclops*, at least in those ecosystems where the invasiveness is still maintained at a latent stage. The invasive potential of *A. cyclops* in Lampedusa is not yet fully expressed but the species shows strong recruitment

ability, regenerating in mass new individuals in the pristine area of introduction.

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