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ORIGINAL ARTICLE

Seedling biochemical and ecophysiological traits improved under the patch-canopy microhabitats of medium-sized oak trees in a semi-arid forest

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Abstract

Key Message **Dimensions of mature oak trees influence seedling growth: excessive light and restricted soil moisture under the canopy of small trees and low light availability under large trees can limit growth whereas conditions are more favorable under trees of medium size.**

Abstract Nurse species play a key ecological role in the success of forest tree regeneration particularly in semi-arid ecosystems. However, the influence of nurse tree size on both microclimatic conditions and seedling development has been insufficiently explored. This study aimed to evaluate the effect of nurse oak trees dimensions (*Quercus brantii* Lindl.) on light and soil moisture availability as well as on the morphological, biochemical and ecophysiological characteristics of oak seedlings in oak forests in western Iran. Twenty-four oak trees were selected according to 3 DBH classes: <20 cm (small class), 20–50 cm (medium class) and ≥ 50 cm (large class). Three oak seedlings were randomly selected beneath the canopy of each tree. Seedling morphological traits, mesophyll conductance, leaf ion leakage, concentration in Ca, P, K, chlorophyll and carotenoids, as well as in several enzymes were measured. Beneath each tree, soil moisture and light availability were also measured. We found that the soil moisture, concentration in photosynthetic pigments and leaf area increased along the three DBH classes. The highest values for transpiration (5.15 mmol H_2O m^{-2}), photosynthesis rate (5.50 μmol CO_2 m^{-2} s^{-1}), mesophilic conductivity (0.042 mmol CO_2 $m^{-2}S^{-1}$), total seedling dry weight (1.67 g) and relative leaf moisture (75.58%) were observed in seedlings under the medium DBH class. The photosynthetically active radiation (PAR) decreased across the DBH classes, and the same trend was recorded in seedlings for leaf temperature, intercellular carbon dioxide concentration, leaf dry weight, calcium and potassium concentrations, proline concentration, malondialdehyde concentration, ion leakage, phenol content and activity of catalase and peroxidase enzymes. Using a principal component analysis (PCA) including all environmental factors and seedling characteristics, we showed that the three DBH classes offered contrasted conditions of microclimatic conditions and growth for the seedlings. We concluded that the microhabitat prevailing under the cover of tree oaks of the medium class was the most favorable to the early development stage of a natural or introduced oak regenerations in these water-limited areas.

Keywords Contrasted canopy conditions · Photosynthetic properties · Element concentration · Regeneration growth

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Introduction

Natural regeneration of forest systems is affected by a large set of biotic and abiotic factors and is often a slow and difficult process, particularly in sparse forests of semi-arid areas, (Heydari et al. 2017a; Alonso-Crespo et al. 2020). In such regions, canopies of woody plants play a key role in the establishment or non-establishment of associated species due to various effects on their environment (Peláez et al. 2019; O'Donnell et al. 2020a, b). The role of trees, due to their wide canopy and their major role in the formation of overstory canopy cover, is particularly significant in providing favorable microsite conditions to the establishment of a natural regeneration (Heydari et al. 2017a). For instance, in oak forests, previous studies have shown that the canopy of nurse trees facilitated the growth and development of oak seedlings through a variety of processes reducing environmental stresses, such as amelioration of the microclimatic factors, better nutrition and improvement of an ectomycorrhizal fungi network (Heydari et al. 2017b; O'Donnell et al. 2020a, b).

Among the environmental factors, light availability at the forest floor plays a crucial role for regeneration establishment (Pinchot et al. 2017; Helluy et al. 2021) and this resource is closely related to the structure, dimensions and density of the canopy of the overstory. In the understory, light availability is deeply variable from full light to full shade, which affects the initial growth and survival of oak seedlings (Guo et al. 2001; Sevillano et al. 2016). A low light level can reduce carbon dioxide uptake and carbohydrate production, leading to reduced growth (Barth et al. 2001). Although oaks grow slowly under heavy shade, low-to-moderate shade can be beneficial to seedling growth (Wagner et al. 2010). In fact, a high level can cause oxidative stress (Han et al. 2010) which can impair the growth and development of the seedlings for some species. In this process, a too large quantity of solar radiation is absorbed by the plant which limits the photosynthesis efficiency but increases the oxidative damage to the photosynthesis reaction center (Lichtenthaler and Rinderle 1998; Han et al. 2010). In these conditions, the rate of transpiration in plants is also usually increased, which indirectly reduces the water content in the leaves and slows down photosynthesis.

Plants have different defense systems to limit oxidative stress induced by light, involving antioxidant enzymes such as catalase, superoxide dismutase, peroxidase, ascorbate peroxidase and glutathione reductase (Shohael et al. 2006). Changes in light availability in the understory not only deeply influences seedling growth but also more generally their physiological and morphological attributes. Under shady conditions, seedlings usually invest more in their aboveground part than in their belowground system to increase their leaf area and, therefore, their photosynthetic activity and growth (Lambers et al. 2008). In contrast, at high light intensities, more leaves are produced, but due to low cell division, the size of the leaves is reduced and the direction and shape of the leaves are modified (Hatamian et al. 2014). For instance, Vera (2000) reported that seedlings of *Q. petraea* Matt and *Q. robur* L. grown under shade conditions have higher chlorophyll concentration, leaf area, height and relative leaf area, whereas root dry weight, shoot to root dry weight ratio, leaf thickness, growth rate and net uptake rate were higher under higher light conditions. Similarly, Pilehvar et al. (2012) examined the effect of 20, 40 and 100% of full sunlight on oak (*Quercus brantii*) seedling characteristics and found that under moderate-shade height growth, shoot/root ratio and mean leaf area increased, whereas collar diameter, root dry matter content, net uptake rate and relative growth rate decreased.

Light is of course not the only factor controlled by canopy cover and the amelioration of soil conditions usually recorded under tree canopy due to increased organic matter inputs can also influence seedling development. For instance, O'Donnell et al. (2020a, b) examining the effects of native oak (*Q. sinuata* var. breviloba) tree canopy cover on soil characteristics and growth of oak seedlings, noted that seedlings grown under the canopy of mature trees usually had smaller roots and shoots and had higher colonization rates by ectomycorrhizal fungi, higher concentrations of calcium, magnesium and manganese than seedlings grown in the open area (O'Donnell et al. 2020a, b).

The influence of mature oak trees on a large set of environmental factors is, therefore, of a major importance to understand regeneration dynamics. This is especially true in the harsh conditions of our semi-arid forests (the Zagros forests in western Iran) where natural oak regeneration is particularly scarce. In such conditions, the mature trees can play a key role by influencing access and availability to resources such as light which in turn can deeply influence growth and quality of the seedlings.

However, such resources are also largely controlled by the dimensions of the trees and we can hypothesize that small trees do not exert the same influence on seedling establishment than large trees. In fact, some previous works have shown that facilitation of nurse plants on beneficiary plants can increase with nurse plant size and this effect is especially noticeable at high biotic stress like arid or semi-arid environments (e.g. Peláez et al. 2019; Pugnaire et al. 1996). However, few studies have examined to what extent the responses of the beneficiary plants to this nurse effect is modulated by the dimensions of the nurse plant. In other words, does the amelioration of the environmental stress beneath the nurse plant canopy increase with the nurse size or is there a threshold size beyond which the positive effects are reduced?

The aim of this study is to investigate the influence of the nurse tree size on microclimatic conditions and ultimately on the establishment of a natural regeneration under the tree canopy. To achieve this goal, we have studied the morphological, physiological and chemical traits of 1-year-old oak seedlings naturally established under the canopy of nurse trees with contrasted dimensions. More specifically, our questions are the following:

- What is the influence of nurse trees of various dimensions (reflected by three diameter classes: small, medium and large) on the microclimatic factors in the understory?
- What are the main differences in physiological and chemical attributes of seedlings growing under nurse trees of variable dimensions and are they related to the microclimatic conditions?
- With these results, can we infer the most important trait-related strategies of the seedlings to cope with stressful conditions (i.e. excessive light and reduced soil moisture)?

Materials and methods

Study site description

Our study area is located in the Ilam province (western Iran) and is part of the Zagros Mountains in Iran which is a major hotspot of biodiversity (Salehi et al. 2013; Mirzaei et al. 2017). The Zagros forests are dominated by the native Brant's oak (*Quercus brantii*). This species covers more than 50% of this region from north to south due to its high adaptability and flexibility to various climatic and soil conditions (Sagheb-Talebi et al. 2014). In recent decades, the more frequent and intense droughts due to climate change and the worsening of the human disturbances (Moradizadeh et al. 2020; Heydari et al. 2020; Gheitury et al. 2020), have led to a significant mortality of these ancient oak forests and an alteration of their ecological value (Shiravand and Hosseini 2020). The study area was selected in a protected forest area in the north of Ilam city (latitude 46º20´–46º30´ N, longitude 33º40´–33º45´ E). The mean altitude of the study area is 1250 m a.s.l. and is characterized by a generally flat topography. The dominant species of this relatively sparse woody overstorey region is the Brant's oak (*Quercus brantii*) that associated with various woody species such as *Pistacia atlantica* Desf., *Acer monspessulanum* L. subsp. *Cinerascens* (Boiss. Yaltirik), *Cerasus microcarpa* Boiss., *Crataegus pontica* K. Koch. and *Daphne mucronata* Royle (see pictures in Supplementary Material 1). The climate is sub-Mediterranean, the average annual rainfall is 652.6 mm and the average annual temperature is 17° C. Soil is calcareous shallow with a clay loamy texture.

Sampling

We randomly selected 24 nurse trees evenly distributed in 3 DBH classes which also reflect a gradient of height and canopy dimensions: (1) small class, i.e. trees with a diameter at breast height (DBH) < 20 cm, mean canopy cover = 15.5 m^2 (2) medium class, (DBH = 20–50 cm, mean canopy cover = 36.0 m^2 (3) large class, (DBH ≥ 50 cm, mean canopy cover = 57.3 m^2). Trees were selected within a same area with very homogenous physiographic conditions to limit the spatial variability of the microsite conditions between the individuals. In autumn 2020, three 1-year-old oak seedlings were randomly selected beneath the canopy of each tree.

Measure of light availability and soil moisture

Photosynthetic active radiation (PAR) was measured in the field using a portable infrared gas analyzer (IRGA, ModLDC BiH model, UK). The measurements were taken beneath the canopy of each tree under sunny weather conditions from 12:00 to 14:00 at a constant height of 1.5 m. Beneath the canopy of each oak tree. Three soil samples were randomly taken from a depth of 20 cm and a composite sample was transferred to the soil laboratory. Soil moisture content was determined gravimetrically.

Seedling trait measurements

To better understand the photosynthetic performance and stress responses of oak seedlings, we measured a variety of morphological, physiological and chemical traits that are known to be correlated with seedling performance and stress (see Supplementary Material 2). The selected seedlings were carefully removed from soil to preserve the root system, placed in an icebox and immediately bring to the laboratory for measurements. For each seedling, the shoot (stem and leaves) was separated from the root system (at collar level): the height and the diameter at the base of the stem were measured. Leaf area was measured using a leaf area meter. Leaves and stems were then dried at 70 °C for 48 h and weighed. Specific leaf area was calculated as follows:

$$SLA = \frac{\mathrm{LA}}{\mathrm{DW}}, \tag{1}$$

where SLA is specific leaf area (g cm^{-2}), DW is the leaf dry weight and LA is the leaf area.

Before excavation, gas exchange (photosynthetic rate, transpiration rate, water use efficiency, intercellular CO_2 concentration, leaf temperature) was measured in the field using a portable infrared gas analyzer (IRGA, ModLDC BiH model, UK). Mesophyll conductance was calculated using Eq. 2 (Fischer et al. 1998).

$$\textit{Mesophyll conductance} = \frac{\text{Photosynthetic rate}}{\text{Intercellular CO}_2\text{ concentration}}. \tag{2}$$

The total chlorophyll and carotenoids of annual oak seedlings were measured according to Arnon (1975) method using 80% acetone and spectrophotometer at 470, 663, 645 nm.

We measured the relative water content according to the method of Diaz-Perz et al. (2006). An equal number of leaves were selected from each sample and immediately weighed (fresh weight) and then placed in distilled water for 24 h. After the elimination of the surplus of water, the leaves were weighed again (saturated weight). The leaves were then placed in the oven for 24 h at 70 °C and then weighed (dry weight). The relative leaf water content was calculated from the following equation (Diaz-Perz et al. 2006).

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100, \tag{3}$$

where RWC is the relative water content of the leaf in percentage, DW is the dry weight of the leaf, FW is the fresh weight of the leaf, TW is the saturated leaf weight.

To measure leaf ion leakage, some pieces were first prepared from the leaves of annual oak seedlings and the solutes extracted from them were analyzed using an electrical conductivity meter at a temperature of 25 °C. Finally, the percentage of ion leakage was calculated with Eq. 4 (Lutts et al. 1996).

$$\text{Leaf ion leakage} = \frac{\text{EC1}}{\text{EC2}} \times 100. \tag{4}$$

EC1 and EC2 are the electrical conductivity in the first and second readings with the electrical conductivity meter, respectively.

Proline content was measured based on Bates et al. (1973) method. The measurement of malondialdehyde (MDA) content, which has long been used as a lipid peroxidation marker, was measured according to the method of Heath and Parker (1968). Measurement of phenolic compounds was determined according to Follin–Ciocalteu reagent method (Singleton et al. 1965) and using a spectrophotometer with a wavelength of 765 nm based on the standard curve of gallic acid. To measure the activity of peroxidase and catalase enzymes, first, a solution containing the extracted enzymes was prepared. Leaf fresh was homogenized in in pre-chilled mortar and pestle with 50 mM potassium phosphate buffer (pH 7.5), 50 mM ethylenediamine-tetraacetic acid (EDTA), and 2% (w/v) insoluble polyvinylpyrrolidone (PVP). The homogenates were centrifuged at 14,000 rpm for 30 min at 4 ºC and the supernatants were processed for estimation antioxidant enzymes. Catalase activity was determined using the methods of Aebi (1984) and Luck (1974). The reaction mixture contained 50 mM phosphate buffer and 15 mM H_2O_2, and it was recorded spectrophotometrically at 240 nm for 1 min by decrease in absorbance. Enzyme activity was expressed as CAT (catalase) unit mg^{-1} protein. Peroxidase activity was measured by the H_2O_2-dependent oxidation of guaiacol at 470 nm (Chance and Maehly 1955).

The phosphorus concentration of shoots was calculated based on colorimetric method (yellow color of molybdovanadate) using a spectrophotometer (Olsen 1982). Potassium concentration was measured by the flame atomic diffusion method (flame photometry) and calcium and magnesium concentrations by the atomic absorption method (Waling et al. 1989).

Statistical analyses

Before analysis of data, the normality of data was tested using the Shapiro–Wilk and Kolmogorov–Smirnov methods in SAS 9.4. To analyze the effect of the three DBH classes (small, medium, large) on seedling traits we used an analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at 5% level of probability using SAS software ver. 9.4. To assess how multivariate seedling traits vary across the microhabitats defined by the different categories of nurse trees, a principal component analysis (PCA) was performed using PC-ORD 5 software. The PCA was applied on array composed of 24 nurse trees and 25 seedling trait values: each trait value was computed as the mean value of the 3 seedlings located under each nurse tree. The two environmental variables (light and soil moisture) were used as supplementary variables. Relationships between DBH with PAR and soil moisture were analyzed using linear regression analyses.

Results

Effect of DBH classes of the nurse trees on seedling traits

We detected significant differences between PAR (photosynthetic active radiation) and SM (soil moisture) across the DBH classes. Soil moisture was the highest under large and medium nurse trees (25.38 and 24.25%, respectively) and the lowest (19.63%) under small nurse trees (Table 1). In contrast, light availability in the PAR domain significantly decreased from trees belonging to the small class (1512 µmol m^{-2} s^{-1}) to trees of the medium class (733 µmol m^{-2} s^{-1}) and to trees of the large class (103 µmol m^{-2} s^{-1}).

Table 1 Effects of the DBH classes on abiotic factors and on seedling traits; data are mean ± standard error

	Traits	Small	Medium	Large	F
Abiotic factors	Soil moisture (%)	$19.62^{b} \pm 0.37$	$24.50^{a} \pm 0.86$	$23.87^{a} \pm 0.92$	25.47^{**}
	Photosynthetic active radiation (µmol m^{-2} s^{-1})	$1512.5^{a} \pm 106.4$	$733.5^{b} \pm 2.90$	$103.0^{c} \pm 14.80$	129.40^{**}
Ecophysiological traits	Photosynthetic rate (µmol CO_2 m^{-2} s^{-1})	$3.03^{c} \pm 0.3$	$5.5^{a} \pm 1.13$	$4.13^{b} \pm 0.11$	13.39^{**}
	Transpiration rate (mmol H_2O m^{-2} s^{-1})	$3.05^{b} \pm 0.34$	$5.15^{a} \pm 0.55$	$2.66^{b} \pm 0.28$	42.49^{**}
	Intercellular CO_2 concentration (mmol CO_2 m^{-2} s^{-1})	$525.6^{a} \pm 63.3$	$393.7^{b} \pm 47.3$	$472.1^{a} \pm 12.1$	8.26^{**}
	Leaf temperature (°C)	$35.25^{a} \pm 2.01$	$26.47^{b} \pm 0.78$	$23.46^{c} \pm 1.43$	69.32^{**}
	Mesophyll conductance (mmol CO_2 m^{-2} s^{-1})	$0.006^{b} \pm 0.0005$	$0.014^{a} \pm 0.0038$	$0.009^{b} \pm 0.0003$	14.40^{**}
	Water use efficiency (µmol CO_2 $mmol^{-1}H_2O$)	$0.9^{b} \pm 0.18$	$1.42^{ab} \pm 0.37$	$1.59^{a} \pm 0.46$	4.06^{*}
	Total chlorophyll (mg $g.fw^{-1}$)	$2.55^{b} \pm 0.37$	$4.50^{a} \pm 0.22$	$3.89^{a} \pm 0.51$	26.73^{**}
	Carotenoid (mg $g.fw^{-1}$)	$0.15^{b} \pm 0.028$	$0.30^{a} \pm 0.074$	$0.25^{a} \pm 0.016$	11.10^{**}
	Relative water content (%)	$26.86^{c} \pm 4.12$	$58.77^{a} \pm 5.93$	$44.18^{b} \pm 7.2$	29.36^{**}
Chemical traits	Leaf ion leakage (%)	$48.35^{a} \pm 3.43$	$37.95^{b} \pm 5.56$	$35.36^{b} \pm 3.92$	9.76^{**}
	Proline content (µmol $g.dw^{-1}$)	$12.24^{a} \pm 0.69$	$6.45^{c} \pm 1.89$	$8.69^{b} \pm 1.18$	18.79^{**}
	Malondialdehyde (nmol $g.dw^{-1}$)	$1.48^{a} \pm 0.058$	$0.48^{c} \pm 0.019$	$0.56^{b} \pm 0.055$	556.00^{**}
	Total phenol (mg Gallic acid $g.dw^{-1}$)	$0.34^{a} \pm 0.006$	$0.26^{b} \pm 0.006$	$0.08^{c} \pm 0.012$	1081.2^{**}
	Catalase activity (U $mg.protein^{-1}$)	$0.56^{a} \pm 0.082$	$0.43^{b} \pm 0.082$	$0.23^{c} \pm 0.024$	23.14^{**}
	Peroxidase activity (U $mg.protein.min^{-1}$)	$0.05^{a} \pm 0.006$	$0.04^{ab} \pm 0.008$	$0.03^{b} \pm 0.001$	5.55^{*}
Morphological traits	Stem length (cm)	$5.10^{c} \pm 0.68$	$17.58^{b} \pm 1.22$	$21.30^{a} \pm 1.41$	54.69^{**}
	Stem diameter (mm)	$2.15^{b} \pm 0.06$	$3.28^{a} \pm 0.23$	$3.23^{a} \pm 0.14$	16.07^{**}
	Leaf area (cm^2)	$48.90^{b} \pm 12.77$	$61.50^{a} \pm 4.43$	$66.90^{a} \pm 4.41$	2.31^{*}
	Specific leaf area (cm^2 g^{-1})	$86.6^{b} \pm 17.37$	$171.9^{a} \pm 16.95$	$205^{a} \pm 46.03$	16.52^{**}
Biomass and composition traits	Seedling dry weight (g)	$1.18^{b} \pm 0.02$	$1.67^{a} \pm 0.10$	$1.26^{b} \pm 0.08$	12.41^{**}
	Leaf dry weight (g)	$0.58^{a} \pm 0.141$	$0.36^{b} \pm 0.028$	$0.33^{b} \pm 0.045$	10.22^{**}
	Shoot Ca (g $kg.dw^{-1}$)	$12.67^{a} \pm 0.77$	$11.99^{ab} \pm 0.82$	$11.24^{b} \pm 0.2$	4.68^{*}
	Shoot P (g $kg.dw^{-1}$)	$1.06^{a} \pm 0.099$	$0.98^{a} \pm 0.023$	$0.95^{a} \pm 0.09$	2.09^{ns}
	Shoot K (g $kg.dw^{-1}$)	$4.82^{a} \pm 0.1$	$4.71^{ab} \pm 0.06$	$4.55^{b} \pm 0.17$	5.26^{*}
	Shoot Mg (g $kg.dw^{-1}$)	$2.64^{a} \pm 0.41$	$2.53^{a} \pm 0.09$	$2.30^{a} \pm 0.16$	1.77^{ns}

We found significant differences between photosynthetic rate, transpiration rate, intercellular CO_2 concentration, leaf temperature, mesophyll conductance, total chlorophyll concentration, leaf carotenoid concentration, relative water content, leaf ion leakage, proline content, malondialdehyde, phenol content, catalase activity, stem height, stem diameter, shoot dry weight, leaf area, leaf dry weight and specific leaf weight ($P < 1\%$) and on peroxidase activity, leaf area, shoots Ca and K concentration ($P < 0.05\%$) across the DBH classes, but water use efficiency, concentrations of the shoot in P and Mg were not significantly different (Table 1).

For the physiological traits, we recorded the highest transpiration (5.15 mmol H_2O $m^2.s^{-1}$) and photosynthesis rate (5.50 µmol CO_2 $m^{-2}.s^{-1}$) in seedlings under medium-sized nurse trees, while the lowest photosynthetic rate was observed in seedlings under small nurse trees (3.03 µmol CO_2 $m^{-2}.s^{-1}$), and the lowest transpiration (3.05 mmol H_2O $m^2.s^{-1}$) in seedlings under large nurse trees. Mesophyll conductance, contents in total chlorophyll and carotenoid, relative water content also peaked in seedlings under medium nurse trees although differences were not significant between seedlings under this class and the large class. In contrast, the lowest values were observed in seedlings under small nurse trees, which significantly differed from the two other classes. Intercellular CO_2 concentration and leaf temperature were the highest in seedlings under small nurse trees, while water use efficiency was maximal in seedlings under large trees. For the chemical traits, contents in proline, malondialdehyde and total phenol were the lowest in seedlings under medium trees and the highest in seedlings under small trees. In contrast, enzyme activity (catalase and peroxydase) was reduced in the large class, while leaf ion leakage was maximal in seedlings under small nurse trees. Variations in morphological traits indicated an increase of total length, leaf area and specific leaf area across the three classes (small, medium, large). However, stem diameter, which was minimal in seedlings under small nurse trees (2.15 mm), did not significantly differ between seedlings under medium and large nurse trees (respectively, 3.28 and 3.23 mm). For the biomass and composition traits, we noted that the aboveground biomass was the highest in seedlings under medium oak trees (1.67 g) and the leaf dry weight the highest in seedlings under small trees (0.58 g). The contents in Ca and Mg of the shoot were significantly higher in the small class than in seedlings under large nurse trees (12.67 vs 11.24 g kg.dw^{-1} and 2.64 vs 2.30 g kg.dw^{-1}) but did not differ between seedlings under the small and medium class (Table 1).

We found a negative linear relationship between the diameter at breast height of the nurse trees (DBH) and the photosynthetic active radiation measured below canopy while the relationship was positive between DBH and soil moisture (Fig. 1).

Results of the principal component analysis (PCA)

A PCA was achieved to more clearly identify the relationships between the different traits measured for seedlings and the microhabitats formed by the tree canopy. The two first components explained 74.69% of the total variance (Fig. 2). The first component (59.04% of the total variance) was positively and strongly related to soil moisture content, stem length and diameter, leaf area and specific leaf area, photosynthesis, mesophyll conductance, photosynthetic pigments and leaf relative water content (Table 2). In contrast, negative correlation coefficients were noted for photosynthetic active radiation, leaf temperature, leaf dry weight, intercellular CO_2 concentration, leaf ion leakage, contents in proline, malondialdehyde and phenol, contents in K and Ca in the shoot, as well as enzyme activities (catalase and peroxidase). Such traits characterized seedlings with active photosynthetic properties (leaf area and chlorophyll) but low osmotic regulators, antioxidant enzymes, and secondary compounds. Interestingly, the first component was positively related to soil moisture but negatively to the photosynthetic active radiation. Therefore, this first component reflected a gradient of environmental stress to seedling establishment and growth with no marked stress in its positive part and

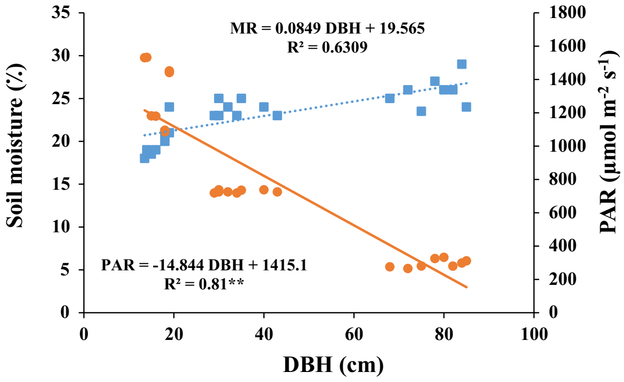

Fig. 1 Regressions between diameter at breast height (DBH) of the nurse trees with photosynthetic active radiation content (PAR, orange filled circle) and soil moisture (MR, blue filled square). Equations and coefficients of determination are indicated (**$P < 0.01$); ($n = 24$)

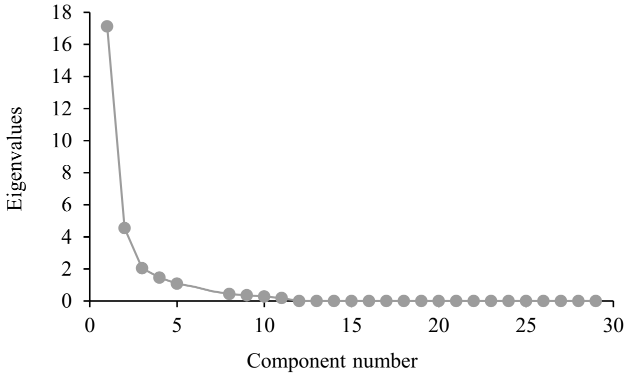

Fig. 2 Eigen values of each component of the PCA

Table 2 Principal component analysis for all traits measured in oak seedlings: the numbers in bold indicate significant correlation

Traits and abbreviations		Principal component	
		1	2
Stem length	SL	**0.947**	– 0.162
Stem diameter	SD	**0.887**	0.123
Leaf area	LA	**0.742**	– 0.144
Specific leaf area	SLA	**0.881**	– 0.182
Leaf dry weight	LW	**– 0.852**	0.063
Seedling dry weight	DW	0.529	**0.743**
Photosynthetic rate	Pn	**0.724**	**0.497**
Leaf temperature	LT	**– 0.934**	0.181
Mesophyll conductance	MC	**0.679**	**0.619**
Intercellular CO_2 concentration	ICC	**– 0.632**	**– 0.587**
Water use efficiency	WUE	**0.729**	– 0.023
Transpiration rate	T	0.299	**0.829**
Total chlorophyll	Chl	**0.868**	0.275
Carotenoid	Carotenoid	**0.750**	0.369
Relative water content	RWC	**0.771**	0.470
Leaf ion leakage	IL	**– 0.854**	0.105
Proline content	Proline	**– 0.830**	– 0.418
Malondialdehyde	MDA	**– 0.977**	– 0.121
Catalase activity	CAT	**– 0.755**	**0.548**
Peroxidase activity	POX	**– 0.663**	0.374
Total phenol	Phenol	**– 0.754**	**0.643**
Shoot K	K	– 0.617	0.461
Shoot P	P	– 0.555	0.206
Shoot Ca	Ca	– 0.646	0.408
Shoot Mg	Mg	– 0.402	0.371
Eigenvector value		17.121	4.541
Relative variance (%)		59.039	15.658
Cumulative variance (%)		59.039	74.697

favorable conditions in its negative part. The second component (15.46% of the total variance) was mainly related to seedling traits such as dry weight, transpiration rate, phenol content and mesophyll conductance.

The projection of the seedlings and traits on the two first components of the PCA according to the three DBH classes is shown in Fig. 3. It indicated that seedlings grown under medium trees had the most favorable photosynthetic properties such as photosynthetic rate, transpiration rate, photosynthetic pigments content, leaf relative water content and dry matter production. In contrast, seedlings grown under small or young native trees received a high level of photosynthetic active radiation resulting in higher oxidative stress, increased ion leakage and a higher content in malondialdehyde more susceptible to damage the cell membranes. In addition, due to this higher environmental stress, contents in phenols, osmotic regulators such as proline and antioxidant enzymes such as catalase and peroxidase enzymes, increased. Therefore, most of the seedlings growing under small nurse trees showed physiological characteristics indicating adaptations to light and oxidative stress. These seedlings usually produced a lower leaf area and smaller and thicker leaves in order to reduce transpiration. In contrast, seedlings grown under large trees exhibited opposite attributes (Table 1, Fig. 3).

Discussion

The microhabitat prevailing under the canopy of nurse oak trees provide favorable conditions for development of a natural oak regeneration in these water-limited areas. However, these conditions are mediated by nurse tree dimensions and opposite effects on light and soil moisture are recorded between small trees and large trees. Seedlings experience greater environmental stress under small trees and greater resource limitation under large trees and exhibit contrasted traits according to nurse tree size. We discuss below in more details the role of the two environmental variables included in this study (light and soil moisture) and the seedling responses according to the different types of traits.

Availability in soil moisture and light

Our results showed that soil moisture, which is one of the physical soil properties that plays a crucial role in water-limited forest systems like ours, was influenced by tree canopy. It was lower under trees of small dimensions (i.e. belonging to the small DBH class) than under trees of medium or large size (medium and large classes). Trees of the medium and large classes with large and thick canopy can provide a moderate shade reducing the loss of soil moisture due to direct sunlight and benefiting to seedling growth in the understory. In contrast, forest floor evaporation was considerably increased in the less shady conditions prevailing under the reduced canopy of trees of the small DBH class (Imani et al. 2016; Heydari et al. 2017a, b). For instance, in an open semi-arid pine forest, Yaseef et al. (2010) reported a soil evaporation representing 36% of the annual precipitation.

Soil moisture and light availability exhibited opposite patterns as the amount of PAR decreased with increasing DBH of mature trees, and seedlings of the small DBH class received more direct radiations than seedlings of the other two classes. Sarvade et al. (2014) found also that seedlings

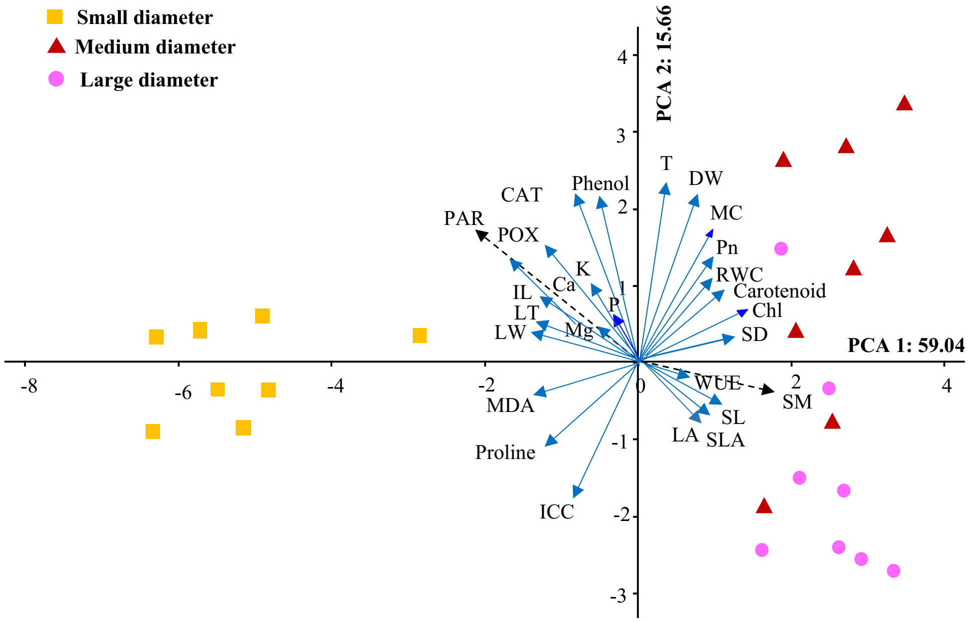

Fig. 3 PCA biplot of the explanatory variables (seedling trait values indicated by blue arrows) and individuals (tree nurses in the three DBH classes). The two environmental variables (PAR and SM: soil moisture) are indicated by black dotted arrows and the explained variance of each component is given. See Table 2 for abbreviations

under the canopy of larger trees were less exposed to direct radiations. In our semi-arid conditions, excessive sunlight, by increasing evaporation from the soil surface, can cause drought stress for 1-year-old oak seedlings and can reduce their growth (Shi et al. 2012).

Seedling ecophysiological traits

Oak seedlings of the small DBH class had higher leaf temperatures than seedlings of the large and medium DBH class. This increase in leaf temperature was probably explained by a decrease in cooling due to reduced evapotranspiration and higher air temperatures under trees of the small DBH class. We also found that the seedling transpiration rate decreased under trees of the large and small class. This result was consistent with the findings of several other studies. For instance, Cooper et al. (2004) reported for *Quercus ithaburensis,* a long-lived, deciduous oak, native to the eastern Mediterranean a decrease in leaf transpiration during the growth season of seedlings growing in shade compared to seedlings in sunnier conditions. Similarly, Valladares et al. (2005) found higher transpiration rates in sun than in shade phenotypes of two Mediterranean Quercus species (*Q. coccifera* and *Q. ilex*). In the small DBH class, the high amount of radiation contributed to decrease the transpiration rate of annual oak seedlings, probably due to the closing of the stomata and the reduction of the leaf mesophyll conductance. Seedlings reached the point of light saturation at a radiation intensity of 1512 µmol m^{-2} s^{-1}, and curiously the results showed that above this level both photosynthetic activity and aboveground growth were reduced. This reduction of photosynthesis in high radiation intensity conditions could be due, among other reasons, to the stomatal and non-stomatal limitations, which prevent the plant from using of the total amount of received radiation for its growth and dry matter production (Wilson et al. 2000). High light radiation leading to photoinhibition and soil moisture reduction were particularly unfavorable for plant growth (e.g. Valladares 2003). However, for seedlings established under the medium DBH class trees, levels of light and soil moisture were optimal, explaining a higher rate of leaf transpiration and a lower leaf temperature. In these conditions, the intensity of photosynthetic active radiation at the rate of 733 µmol m^{-2} s^{-1} increased the amount of effective light in photosynthesis and also increased seedling morphological traits such as stem length and stem diameter. Several previous studies have shown that a decrease of light intensity to certain levels reduced stomatal conductivity and photosynthesis in plants (Valladares, 2003; Miralles et al. 2011). For instance, Miralles et al. (2011) studying variations in traits of a Mediterranean shrub under shade levels of 0, 32, 48, 84 and 93%, showed that above 84% shading the concentration of stomatal carbon dioxide was increased and the photosynthesis rate was decreased. The authors also reported that by reducing leaf stomatal density under shade conditions of 84% and 93%, the yield of photosystem II (the second photosynthetic system, which includes light-dependent photosynthesis) and growth were reduced. This reduction was irreversible for 93% shade cover (Miralles et al. 2011). In our study, seedlings of the medium DBH class had the highest mesophilic conductivity and were more efficient in using sub-stomatal carbon dioxide.

The total chlorophyll content was significantly reduced under trees of the small DBH class most probably explained by photoinhibition due to intense light stress (Bertaminia et al. 2006). However, contents in photosynthetic pigments did not differ between seedlings of the large and medium class in relation to moderate light conditions. Similarly, Miralles et al. (2011) reported an increase of chlorophyll content under shade of 25%, 50% and 65% but a reduction after 80%.

Seedling chemical composition

Oak seedlings established under the canopy of trees of the large and medium DBH class trees had higher relative water content than seedlings of the small DBH class. The former also showed lower leaf temperatures and consequently, water loss through transpiration was reduced explaining the increase in the relative water content. In contrast, the relative water content of seedlings of the small DBH class decreased probably due to the lessening in soil moisture and increase in leaf temperature. In fact, this reduction in relative water content probably enabled seedlings to adapt to conditions of increased light stress and drought (Wang et al. 2012). Moreover, the light stress, by producing oxygen free radicals (oxidative stress), could have also caused the peroxidation of membrane lipids and increased the leaf ion leakage. The more stressful conditions under the canopy of trees of the small DBH class (i.e. excessive light and reduced soil moisture) could also explain the higher content of proline and malondialdehyde (MDA) in the leaves. In fact, production of proline is a mechanism to protect the cells, as proline is one of the amino acids that normally appears in response to stress and has various roles such as stabilizing membranes, proteins and cell structures and protecting cell functions against various reactive oxygen under environmental stress (Kaur & Asthir 2015). MDA is also an oxidative stress marker linked to cell membrane injury (Kocheva et al. 2009). Similarly, light stress also most probably explained the accumulation of secondary metabolites in particular phenols (André et al. 2009). The same process applied for catalase and peroxidase activities which were also higher in these conditions. The production of reactive oxygen molecules due to stressful conditions results in harmful effects on plant cells and defenses are activated by an array of antioxidant enzymes such as catalase and peroxidase (e.g. Gill et al. 2010).

Seedling growth and morphological traits

Seedling dimensions varied according to the DBH classes: stem diameter was the lowest in the small DBH class while stem length increased sharply from the small to the large DBH class (from 5.1 to 21.3 cm). In this latter class, seedlings were slenderer than in the medium and small DBH classes. The height to stem diameter ratios (H/D) increased from 2.37 cm/mm (small class) to 6.59 cm/mm (large class). These observations are typical of the "shade avoidance syndrome" (Franklin, 2008). The drastic reduction of light availability under the large canopies of trees of the large class as well as the potential modification of the light quality (reduction of the red to infrared ratio) favored seedling elongation as reported in many previous studies (e.g. Cole et al. 2011; Guerra-Santors et al. 2015). It was noteworthy that seedlings of the medium DBH class showed intermediate H/D values and that both stem diameter and seedling dry weight were the highest. These results indicate that microclimatic conditions under the canopy of medium and large-sized trees are the most favorable to seedling growth (see also Heydari et al. 2017a, b b). In contrast, environmental conditions are more adverse under trees of the small DBH class due to excessive light and reduced soil moisture (e.g. Miralles et al. 2011). Under the canopy of trees of the large DBH class, the dry aboveground biomass was also reduced due to insufficient light availability as previously observed (e.g. Sarvade et al. 2014).

Leaf area is one of the most important morphological indicators of oak seedlings in relation to the intensity of sunlight. The highest leaf area was recorded in oak seedlings of the medium and large DBH classes, suggesting a strategy to maximize light capture (see for instance Paiva et al. 2003). Oak seedlings located under trees of the small DBH class had the lowest leaf area in response to increased light radiation and reduced soil moisture. In such conditions, it was shown that mesophyll conductance was decreased as well as photosynthesis and growth, which finally reduced leaf area (Nonong & Nompo 2015). Despite the increase in leaf area across the DBH classes (from small to large), the leaf dry weight showed an opposite pattern which may be related to the decrease in leaf thickness (see for instance Aranda et al. 2005). Thicker leaves could be one of the ways in which leaves exposed to higher radiation intensities (such as seedlings under trees of the small class) achieved a higher photosynthetic rate under light saturation. On the other hand, seedlings had wider and thinner leaves under trees of the medium and large class due to lower light intensity and less evapotranspiration.

The highest concentrations of calcium and potassium were observed in seedlings under trees of the small DBH class while the lowest concentrations were found in seedlings of the large DBH class. In contrast, concentrations in phosphorus and magnesium did not significantly vary according to the three classes. These results could be explained by a concentration effect as the growth was also reduced in seedlings of the small DBH class (Sardans et al. 2013). Besides, they could also reflect different conditions of drought and light availability. For instance, Sardans et al. (2008) noted an accumulation of K in the stems of *Globularia alypum* submitted to water stress and concentrations of K were also the highest in leaves of *Quercus ilex* seedlings during the dry summer season (Sardans et al. 2013). Light could also have played a role as Odabas et al. (2009) observed reduced plant (*Laurocerasus officinalis* Roem) phosphorus and potassium concentrations under shade conditions although Villar-Salvador et al. (2004) did not find significant differences in the potassium, nitrogen and phosphorus concentrations in shoots of *Q. ilex* seedlings under 45% full light conditions.

Conclusion

Today, one of the most important steps in the protection of natural forest resources is natural regeneration which has always been challenging in semi-arid areas. In such areas nurse species, by providing favorable microhabitats, can provide a valuable help to overcome the ecological filters limiting a successful seedling establishment. Although *Q brantii* is supposed to be shade-intolerant, seedlings benefit the most of the shelter conditions of the medium-sized oak trees (medium DBH class: 20–50 cm). In these semi-shade conditions, soil moisture is higher and most of the growth and ecophysiological attributes are optimal as reflected by a greater photosynthetic pigment concentration, photosynthetic rate, transpiration level. In contrast, conditions are less favorable under trees of the small DBH class and of the large DBH class most probably due for the former to excessive light radiation and reduced soil moisture and for the latter. Therefore, medium-sized nurse trees seem the best option to enhance the early development stage of a natural or introduced oak regenerations in these water-limited areas. Our results also suggest that retention of existing oak forests is of a high importance to natural seedling establishment and that forest management operations such as thinning leading to an irregular tree distribution (i.e. with a large range of tree dimensions) has also to be promoted. Such an irregular structure is likely to create contrasted microhabitat conditions favorable to natural regeneration and more globally to other components of biodiversity. In treeless areas, shrubs can offer also suitable conditions for oak introduction as many studies have emphasized their role in improving soil nutrients, water infiltration, microclimatic conditions and protection against browsing (e.g. Gomez-Aparicio et al. 2004; Benayas et al. 2005; O'Donnell et al. 2020). Therefore, measures to protect or enhance growth of these nurse plants have to be encouraged to favor natural or artificial oak establishment in the future.

Author contribution statement HA-R: data curation, investigation, writing—original draft; JH: data curation, supervision, investigation, writing—review and editing; MH: conceptualization, methodology, resources, formal analysis, software, visualization, supervision, writing—original draft, writing—review and editing; SH: formal analysis, software, writing—review and editing; Isabel Miralles: writing—review and editing; BP: writing—review and editing.

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Data availability Not available to third parties.

Declarations

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

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