

## Study of a Natural Leguminous Plants/Cork Oak Association in Kroumirie: An Alternative Solution to Climate Change

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### Abstract

Despite the increase of the atmospheric CO<sub>2</sub>, cork oak (*Quercus suber* L.) forests don't stop degenerating. Deficits in water balance and in nutritional elements might be the main reasons. Standing as a potential regulator of the ecosystem nutrient dynamics, leguminous plants (Fabaceae, Leguminosae) are a good test case for individual species effects on Tunisian forests. They are the most diverse and widespread group of plants with the capacity of N<sub>2</sub> fixation, and are particularly abundant in Kroumirie forests. The use of natural legume species in Kroumirie, as soil fertility and grassland productivity enhancer, could be very interesting in the assessment of a new national land exploitation strategy aiming to increase carbon sequestration and climate change mitigation. Understanding the impact of these legume species on cork oak, using air nitrogen-fixation technique computation, would be crucial for future land management and policy decisions. A morphological and eco-physiological study of the *Cytisus triflorus* plant associated with Cork oak was carried out in Tabbouba, at Nefza region. Three sites were selected: CM1 (*Cytisus triflorus* alone), CM2 (Cork oak associated with *Cytisus triflorus*) and CM3 (Cork oak only). Morphological (height, diameter, density), physiological (stomatal conductance, water potential, transpiration, photosynthesis) and hydric parameters were measured for the two species. The morphological study results showed no significant difference sites for each species except for density parameter. On the other hand, physiological parameters measured for oaks trees clearly manifested significant differences in photosynthesis, transpiration and hydric conductance between CM2 and CM3 sites. The cork oak in association with the *Cytisus* are in better growth and productivity conditions than when they are alone.

**Keywords:** Association; legumes; cork oak; adaptation; climate change

### Introduction

The atmospheric CO<sub>2</sub> continue to increase by 2100 between 730-1200 μmol mol<sup>-1</sup> [1,2]. As a result and under adequate nutrients and water supplies, plant growth and biomass accumulation through CO<sub>2</sub> direct stimulation of photosynthesis are considerably enhanced [3-6]. The average enhancement of trees' photosynthesis due to higher CO<sub>2</sub> has been about 60% [7]. However, the responses vary considerably from one species to another [8]. This variation is tightly related to the position of the specie in the crown [9], nitrogen fertility level, season and co-occurring pollutant concentrations [10]. Nitrogen level decreases in the foliage of trees growing under elevated atmospheric CO<sub>2</sub> [11-13]. It is also decreased in the litter [14]. Unlikely, the quantity of litter increases 20-30% under elevated atmospheric CO<sub>2</sub> [15]. Whether or not nutrient mineralization rates will change because of soils high levels of CO<sub>2</sub> has been an unanswered question [16]. It is also uncertain whether decomposition rates will be significantly affected by elevated CO<sub>2</sub>, although the bulk of literature in this area suggests that the decrease in leaf litter N, coupled with an increase in lignin concentration, results in a slower decomposition rate [14].

The cork oak is the native hardwood forest species and is the most abundant in the Tunisian territory. It grows in the northwestern provinces starting from the Mediterranean coast and extending

southwards over the Kroumirie Mountains to the Mejerda Plain located about 50 km inland [17]. Climate scenarios for the 21<sup>st</sup> century show a tendency to decrease in summer rainfall and higher temperatures (IPCC 2001) suggesting that the forest could be affected in terms of productivity and mortality. The current climate changes aggravate the decline of Cork oak species. This situation has become worse because of the lack of natural regeneration and the technical operations such as protection and renewal.

Despite the increase of CO<sub>2</sub> atmospheric, cork oak forests keep degenerating and deficits in water balance and in nutritional elements are certainly behind this tendency. Elevated atmospheric CO<sub>2</sub> can substantially alter plant chemistry and leaf surface properties. Thus, it can alter host/pest interactions. For instance, levels of foliar N decline for trees growing under increased atmospheric CO<sub>2</sub> [18-20]. Leguminous plants, which are abundant in Kroumirie, could be a critical regulator of soil nutrient dynamics because of their high foliar nitrogen (N) and potential for symbiotic N fixation.

Legumes (Fabaceae, Leguminosae) are a good test case for individual species effects on Tunisian forest as they are potential regulators of ecosystem nutrient dynamics [5,21,22]. Legumes are the most diverse and widespread group of plants with the capacity of N<sub>2</sub> fixation [23,24] and are particularly abundant in Kroumirie forests [25-27]. Despite a general understanding of legumes as drivers of N dynamics, the impact magnitude of this plant group on our forest soils is poorly quantified. Studying the effects legumes have on soils and on

trees is crucial for understanding how community composition and functional group assemblages influence N dynamics.

Likewise, the natural legume species in Kroumirie are also highly altered; the soils are severely compacted and generally depleted in organic matter, nutrients and microbial life. A vast research program of introduction and selection of herbaceous and woody legume species has been developed in order to select the appropriate species to be used to rehabilitate the degraded cork oak forests. Part of this research is based on the speculation that selecting and increasing the appropriate legume species associated with the cork oak ecosystem should be crucial to increase soil fertility and primary productivity. This work aims to demonstrate the effect of legume species on Cork oak in terms of: water balance, gas-exchange and growth.

## Materials and Methods

### Associated legume

This study was focused in associating *Cytisus triflorus* with cork oak. *Cytisus* is one of the most characteristic genera of the Mediterranean flora. This species, native of lands surrounding the Mediterranean Sea, are present in scrubland and garigue on siliceous ground and often on acid soil. This legume is very peculiar. Actually, it

has shown a potential strength to resist summer drought and frequent disturbance events, such as fire and grazing. Furthermore, it can form both ectomycorrhizal and arbuscular mycorrhiza [28].

### Study area

Three different populations: CM1 (*Cytisus triflorus* alone), CM2 (cork oak associated with *Cytisus triflorus*), and CM3 (cork oak only) located in Tabouba (region of Nefza) were selected (Table 1).

They belong to the same bioclimatic stage of the Mediterranean wet floor with warm winter variant (sub-floor below) [29]. The sites chosen are circular and have a surface area of 500 m<sup>2</sup> (12.6 m radius). The circular form makes it possible to cut down the number of limit trees. Also, circular plots on the ground are easy and quick to materialize.

### Parameters measured

**Morphological parameters:** The sampling was carried out for all the individuals of sites. These parameters correspond to the density (number of individuals per unit area), total height of each species and diameter growth at breast height (dbh) for Cork oaks and at the base for *Cytisus*.

Forest	Sector	Soil type	Bioclimatic floor	Indicatif	Altitude (m)	Geographic coordinates	
						Width	Length
Tabouba	Nefza	Flys sch sandstone	Wet floor with warm winter variant	CM1	288	N36°54.261°	E009°06.175°
				CM2	280	N36°53.725	E009°03.927°
				CM3	280	N36°53.725°	E009°03.927°

**Table 1:** Main characteristics of sites studied.

**Functional and hydric parameters:** Measurements were made on 9 individuals per station except CM3 since it had only 4 cork oak individuals. For each individual, two samples of branches were taken. In the laboratory, branches were kept under ventilations and three water stress levels were applied. The first level corresponded to the initial state measured after sampling. The second one corresponded to a moderate water stress level of  $\Psi_b = -1.50 \pm 0.50$  MPa. The third and last one corresponded to several water stress levels of  $\Psi_b = -4.5 \pm 1.50$  MPa applied and measured after two to three days. For each stress level, leaf water potential was measured each time for three sheets belonging to the same sample using Scholander's chamber [30]. Functional parameters were measured too.

Gas exchanges were measured using a Li-Cor Li-6400XT Portable Photosynthesis System (Li-Cor, Lincoln, NE, USA) based on the IRGA principle (Infra Red Gas Analysis). The leaf stomatal conductance (g, in mol H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>), net carbon assimilation (A, in  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), and transpiration (T, in mmol H<sub>2</sub>O m<sup>-2</sup>s<sup>-1</sup>), were measured on the expended leaves of cork oak.. More than eight branches were taken from each site. Under appropriate conditions, they were cut and transported to the laboratory. The bases were kept merged in water in tubes. The experiments were led at a leaf temperature of 25°C and humidity of 50-60%. Leaves were placed under the clamp of the chamber assimilation (6 cm<sup>2</sup>) and acclimatized for 35 minutes. A program was then developed to vary the CO<sub>2</sub> concentration. For each

concentration applied, the stomatal conductance and the transpiration were measured.

Measurements of conductance were realized using HPMF methods (high pressure flow meter) using a Sperry conductimeter. The technique consisted in infusing degassed water at a positive pressure +P into the freshly cut branch and then measuring the flow at the entrance of this sample. The measured flow values (K<sub>i</sub> and K<sub>max</sub>, mmol s<sup>-1</sup>) were automatically recorded in a computer connected to the machine. For each sample, K<sub>i</sub> and K<sub>max</sub> were measured and the PLC was calculated:  $PLC = 100 \cdot (1 - K_i / K_{max})$ .

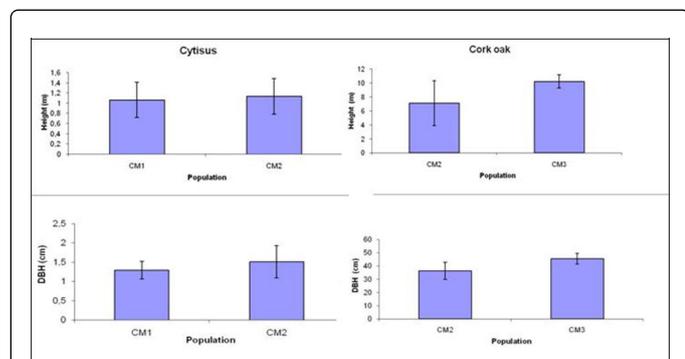
### Statistical analysis

Measurements were the object of a variance analyse to two factors (population and water stress). Significance levels were established at P<0.05. It was completed by a multiple comparison by Newman Keuls test (at 5%) according Dagnelie (1986).

## Results and Discussion

The results showed 140 plants /500 m<sup>2</sup> of *Cytisus triflorus* in CM1 and 30 plants/ 500 m<sup>2</sup> in CM3; 17 cork oak trees in CM1 and only 7 in CM3. Morphological parameters of all individuals located inside stations showed no significant differences between individuals of each species (Figure 1).

These results suggest that the *Cytisus* is beneficial for the oak growth. In fact, the root system of the oak is pivotal while that of *Cytisus* is superficial. Indeed, the two species are not in competition for water or nutriment. A deep and dense root development allows the tree to permanently balance water losses due to transpiration [31].



**Figure 1:** Mean ( $\pm$ SE) stand-level estimates of diameters at breast height (dbh) and height of cork oak and *Cytisus triflorus* in sites.

Measurements of leaf water potential show significant differences between sites and species (*Cork oak* and *Cytisus triflorus*) under severe water stress conditions (Figure 2).

For both species, the first level taken measurements varied between -5.31 and -8.28 bar. The second ones, under moderate stress conditions, varied from -15.9 to -19.61 bar. However, the last and third taken measurements revealed that the legume reached a maximum of

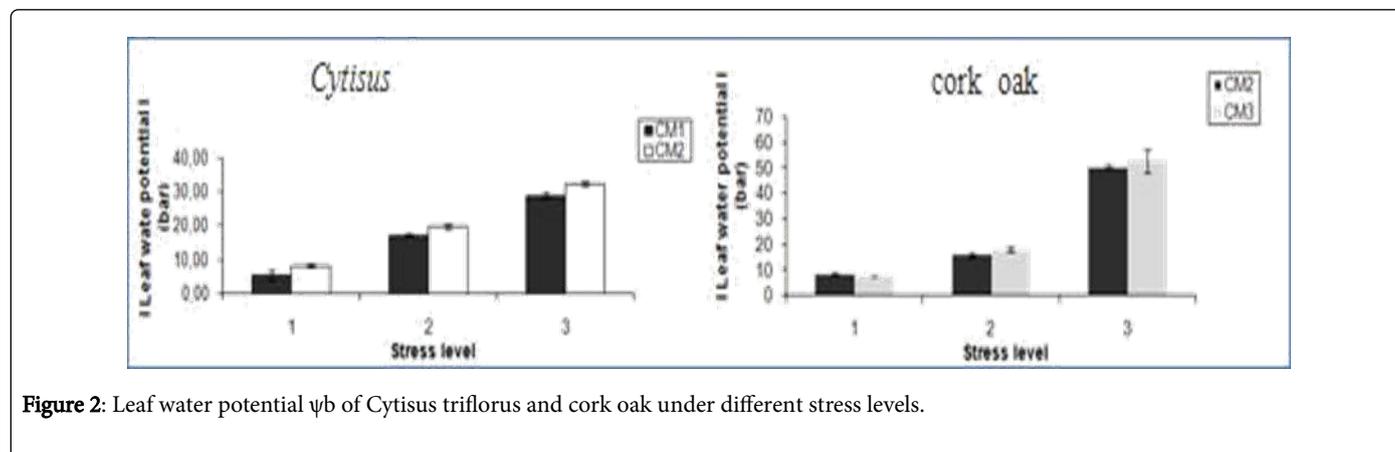
-32.30 bar ( $\pm$  0.57) at CM2 whereas the oak were able to withstand up to - 53 bar ( $\pm$  4.67) at CM3.

Concerning the eco-physiological parameters, results show significant differences in photosynthesis and transpiration between sites under different stress level conditions (Figure 3).

The cork oak behaves better in the presence of legume. With *Cytisus triflorus*, the photosynthesis and transpiration values of oaks in CM2 were higher than those found alone in CM3 for the three levels of potential. More the leaf water potential decreases, more the photosynthesis and the perspiration decreases. Under stress conditions, the reduction in photosynthetic activity occurs due to CO<sub>2</sub> availability decline caused by the restriction of CO<sub>2</sub> diffusion [32] and inhibition of ribulose-1,5-bisphosphate (RuBP) synthesis [33,34]. Even a small decrease in the water potential of a plant causes its stomata to close and, eventually, the intensity of photosynthetic assimilation of CO<sub>2</sub> to decrease. This decrease is less with legume (Figure 3).

*Cytisus triflorus* is a legume that presents intermediate characteristics between *Sclerophyllous* spp. It is considered as stress-tolerating, and summer deciduous species [35]. In fact, this species supplies the oak with required nitrogen to enhance its tolerance to water stress. Nitrogen treatment affects gas exchange and the photosynthetic capacity of the cork oak plants. Nitrogen fertilizer treatments could promote photosynthetic performance of *Quercus suber* by stimulating morphological and physiological responses [36]

Foliar and soil nitrogen content of cork in CM2 is higher than CM3. Legumes such as *Cytisus* are able to help cork oak reduce its vulnerability to stress and then fix foliar nitrogen. The association is beneficial to the soil fertility and therefore to the cork oak growth [37].



**Figure 2:** Leaf water potential  $\psi_b$  of *Cytisus triflorus* and cork oak under different stress levels.

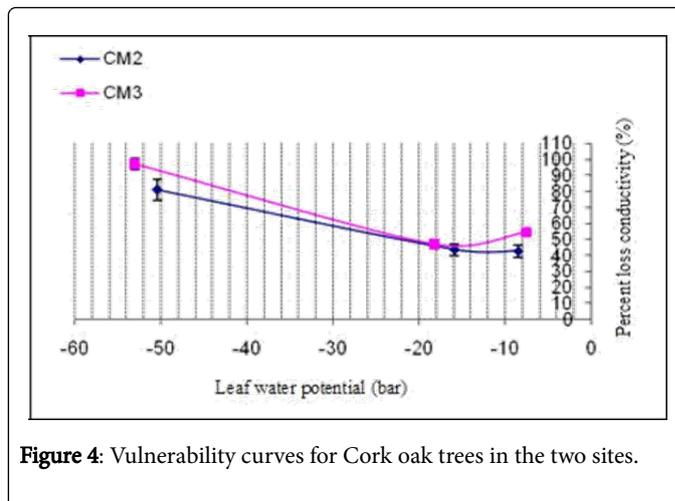
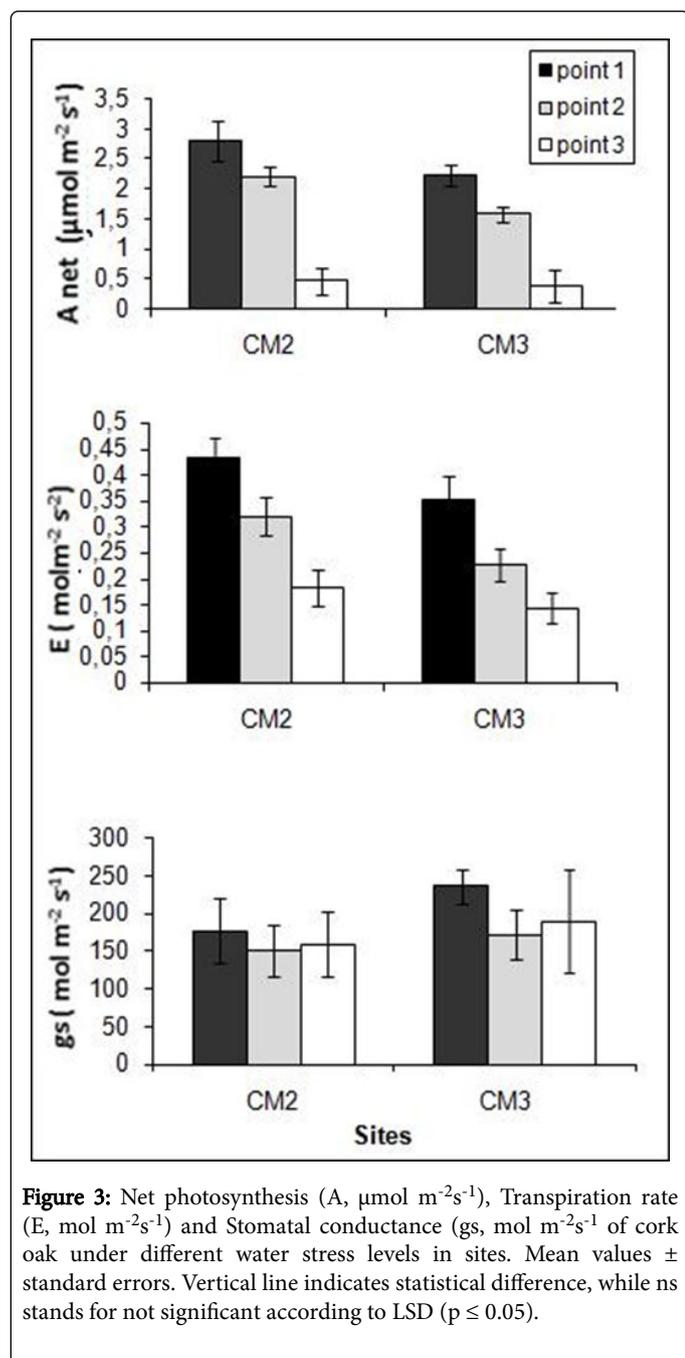
Differences in xylem vulnerability were observed through the vulnerability curves for cork oak trees in the two sites. The water potential that induces 50 PLC ( $\psi_{50}$ ) is a useful measurement of relative vulnerability of cork oak with and without legume.  $\psi_{50}$  can range between -11 bar in CM3 and -26 bars in CM2 (Figure 4).

These results show that oaks in CM3 are more vulnerable than those in CM2 where they are associated with *Cytisus*. Vulnerability to cavitation and stomatal physiology may co-evolve in cork oaks [38]. This is confirmed in CM3 where the cork oaks have the lowest photosynthesis and transpiration values at lowest density. They also have the highest leaf mass per area (LMA) values and the lowest foliar and soil nitrogen contents [37].

The association between species makes it possible to better valorize the environmental resources and exploit the complementarities between functional groups (species that can facilitate access to a resource by another species). Legumes produce almost all of the mineral nitrogen available for the associated plants. Though, the functioning of the association results in the facilitation and competition effects between species [39,40].

Nitrogen supplied by legumes is an essential plant macronutrient that influences plant growth, consequently biomass production and ontogenetic development, thus having a huge impact on plant performance, plant physiology and resource allocation constraints [41-43]. Nitrogen deficiency induces changes in many morphological and physiological parameters such as limitation of growth, leaf number and leaf area [44,45]. In various species, a significant decrease in CO<sub>2</sub>

assimilation and stomatal conductance has been reported [46,47]. Symbiotic nitrogen fixation by *Cytisus triflorus* is a major source of nitrogen in CM2 site. All these results suggest that the maintenance of cork oak species is improved by the association with legumes. Nitrogen (N) is an essential element for all living organisms as it is a component of vital molecules like proteins and nucleic acids [48,49]. N limits the productivity of terrestrial ecosystems in large areas in the world [50,51].



**Figure 4:** Vulnerability curves for Cork oak trees in the two sites.

Nitrogen is not present in most soil parent materials, but  $\text{N}_2$  is the major component of the atmosphere. Though, only a relatively small number of species like *Cytisus triflorus* have the ability to use atmospheric  $\text{N}_2$  for their own metabolism [52,53]. Associating cork oaks forest with legumes species will strongly affect the forest productivity and soil N content.

## Conclusion

This work shows that cork oaks associated with leguminous species exhibit a better eco-physiological behavior. Cork oak is one of the main species of Mediterranean ecosystem woodland and has high socioeconomic and environmental values. These last years, cork oak mortality rates increased. Also, a lack of natural regeneration has been perceived. For a future management of cork oak forests, we believe that fertilized trees with associated fixing  $\text{N}_2$  species will better resist to climate changes. Hence, *Cytisus triflorus* can help the cork oak reduce its vulnerability to stress. Though, additional studies should be carried out on proline, sugar analysis and composite organic volatile. The results elaborated in this work were very promising and could be further improved.

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