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Regeneration of pedunculate oak (*Quercus robur* L.) in a Mediterranean coastal forest: environmental constraints and plant traits variability

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INTRODUCTION

Regeneration challenges

Quercus robur L. is a species of high ecological and economic value yet shows poor natural regeneration in Mediterranean environments.

•**Limiting factors include**
- Competition with beech, hornbeam, and dense ground vegetation
- Browsing by ungulates
- Low acorn production and high seed predation
- Scarce light and soil moisture

Climate change
increases drought intensity, reducing seedling survival and pushing populations toward cooler, wetter areas.

Consequence: Reduced regeneration and risk of local population decline.

Adaptation and management strategies

Challenges in Mediterranean environments
Regeneration is hampered by aridity, summer droughts, and wildlife pressure.

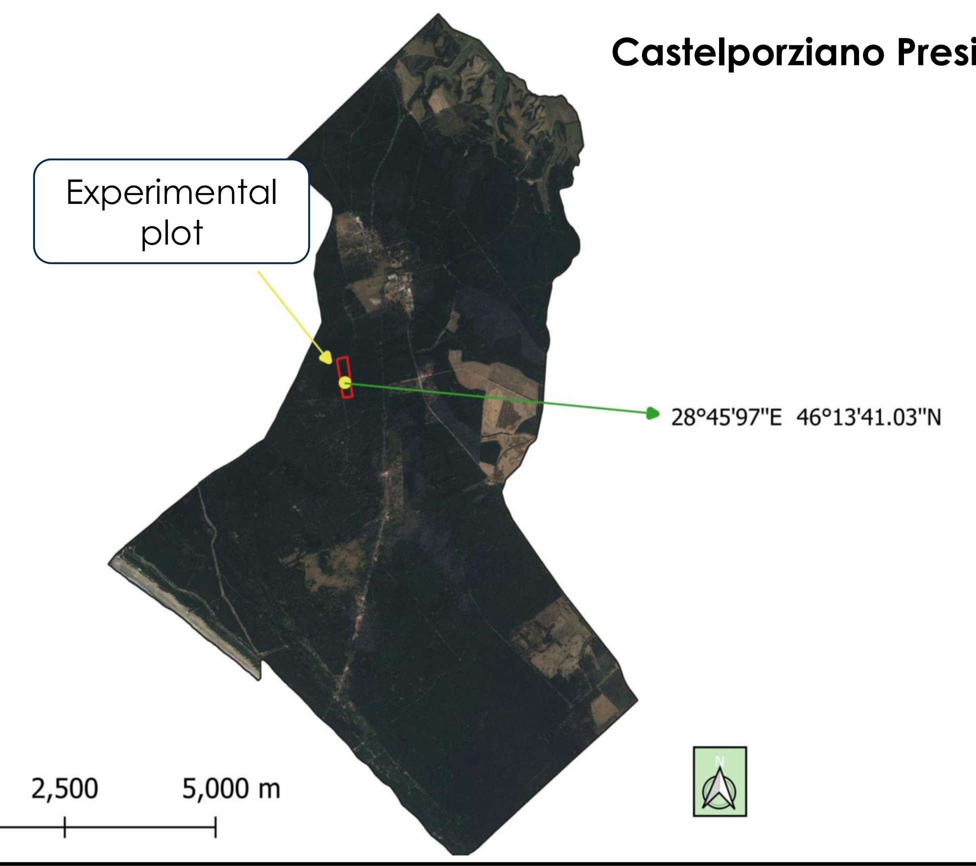
Management recommendations
- Maintain partial canopy cover to preserve a humid microclimate.
- Control competing vegetation and limit herbivory.
- Select drought-tolerant provenances/genotypes.
- Support regeneration using fencing and initial irrigation when needed.

Physiological adaptations to drought
- Development of deep root systems for water access.
- Stomatal regulation of photosynthesis to reduce water loss.
- Accumulation of osmoprotectants enhancing cellular drought tolerance.

OBJECTIVES

The aim of this study is to integratively assess how **summer aridity**, typical of Mediterranean climates, affects the **natural regeneration** processes of *Quercus robur* L., with a focus on the **physiological, adaptive, and genetic factors** influencing establishment, growth, and survival of young individuals. The research seeks to characterize the responses of **different size and age classes** of seedlings and saplings under both field (in situ) and controlled (ex situ) conditions, with particular attention **to post-stress recovery** capacity and potential **legacy effects**, in order to understand the resilience mechanisms enabling persistence under prolonged drought.
In parallel, the study aims to identify **key adaptive traits** promoting regeneration in Mediterranean environments, focusing on early germination and establishment phases, root development and deepening dynamics, the role of **abscisic acid** (ABA) in stomatal regulation, and the long-term survival determinants of oak seedlings.

Castelporziano Presidential Estate



STUDY AREA

- **Location:** Castelporziano Presidential Estate (Coastal Mediterranean forest south of Rome) (Italy).
- **Area:** ~6,000 ha (≈ 40 km²) with oak-dominated woodlands (*Q. robur* L., *Q. cerris* L., *Q. ilex* L., *Q. suber* L.)
- **Ecological role:** Mesophilic, temperate species at the **southern edge of its distribution**. Faces **recurrent droughts**, **heatwaves**, and **biotic stresses.**

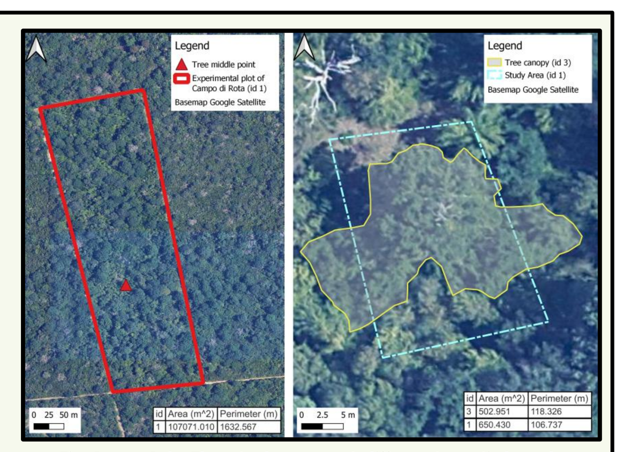
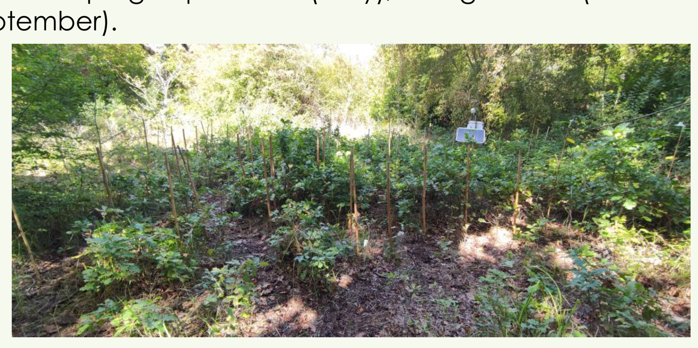


Fig.1-a e 1-b: The study area at different spatial scales

METHODS

In situ experiment

Sampling 45 plants of naturally regenerated seedlings under a **single mother tree.**
•Analysis along a **dimensional-generational gradient**
•Monitoring of **seasonal physiological responses**.
•Monitoring of **soil water content** (15, 30, 45, 60 cm)
•Four campaigns: pre-stress (May), drought stress (June-July), Recovery (September).



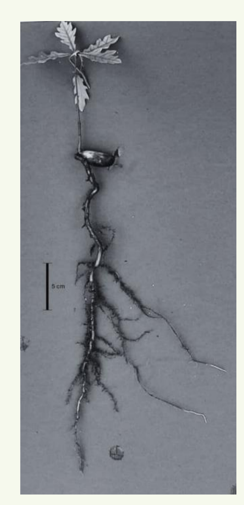
ABA Analysis

50 *Q. robur* L. seedlings (~2 years old)
Transplanted in 12x12x22 cm pots with peat + organic substrate

Acclimation phase: Grown in two experimental boxes (25 plants each) under initially saturated water conditions.
Drought stress:
- **Control box**: constant water supply.
- **Drought treatment**: water completely withheld to induce prolonged stress

Physiological monitoring: Measured stomatal conductance (gsw) with LI-600 (LI-COR Biosciences).
ABA quantification: ELISA kits for abscisic acid

Greenhouse experiment



Conditional growth:
•Seedlings (~ 6 months old)
• Grown pots (12 × 12 × 44 cm) filled with sand.
•Two water regimes:
➢ **High-water:** 4 cm from bottom
➢ **Low-water:** 12 cm from bottom

Roots analysis

Drought stress :
16 days: 11 drought + 5 recovery
•**35** plants tested
•**Gas exchange** measured with **LI-6800** (stomatal conductance, transpiration, CO_2 assimilation) before, during, and after drought.

RESULTS

In situ experiment

Seedling classification

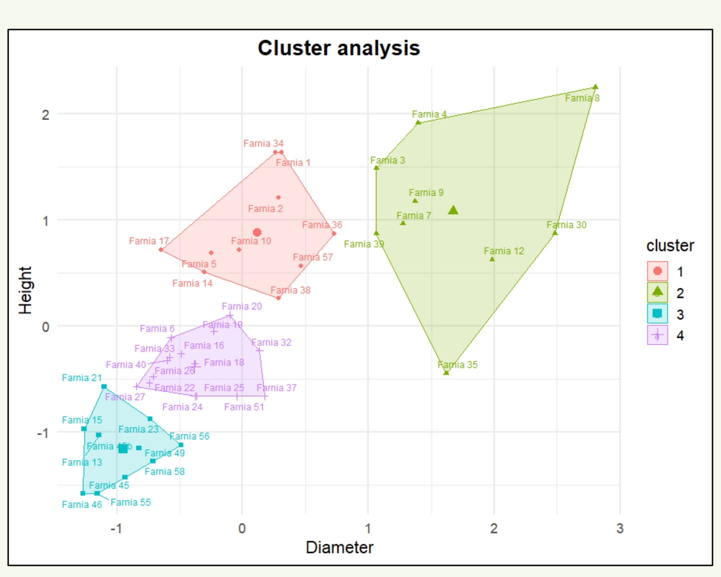


Fig. 2: Cluster analysis (K-means) on normalised values

Response to summer drought in different generations of seedlings

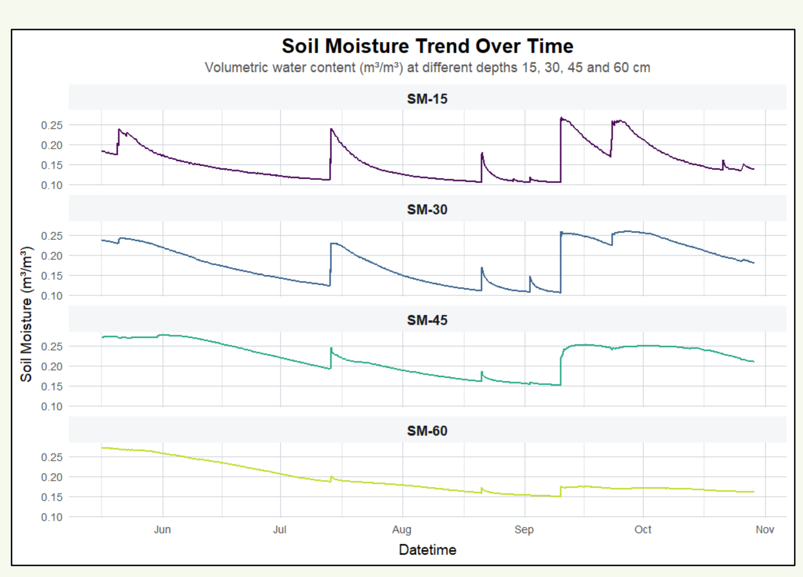


Fig. 3: Seasonal pattern of the soil water profile at different depths

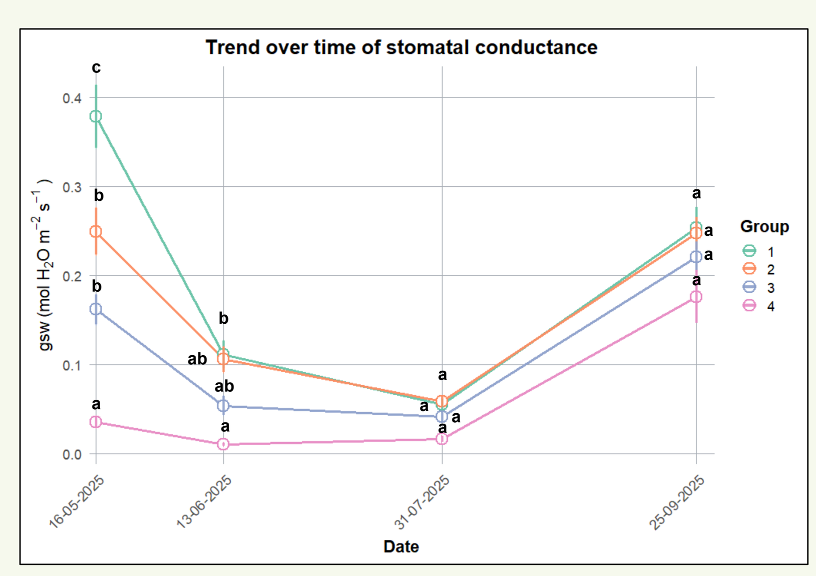


Fig. 4: Comparison of stomatal conductance (gsw) among groups for each sampling date (mean ± standard error). Different letters indicate statistically significant differences between groups ($p < 0.05$; ANOVA linear mixed model (LMM), Tukey post hoc test).

Stomatal conductance declined sharply from May to July across all groups. In September, groups 2–3 showed strong recovery, group 1 partial recovery, and group 4 exceeded initial values, reflecting surface moisture responsiveness (**Fig. 3**).

All soil profiles exhibited a pronounced decline in water content during summer, with a linear decrease at 60 cm and more precipitation responsive dynamics in shallower layers. By September, only partial recovery occurred in surface horizons, while deeper layers showed no evidence of recharge (**Fig. 4**).

Greenhouse experiment

Morfphological analysis

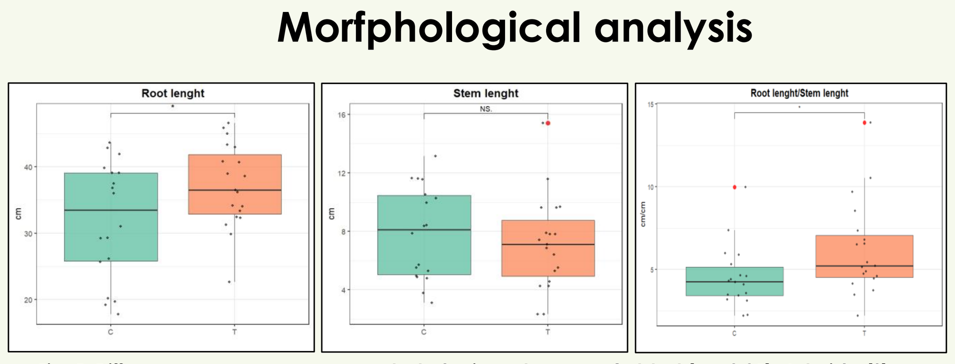


Fig. 5: Differences among groups were tested using a two-sample t-test ($p < 0.05$). Asterisks (*) denote statistically significant differences

Seedlings grown under lower water availability (T, 4 cm) developed significantly longer roots than those under optimal conditions (C, 12 cm), indicating strong morphological plasticity and adaptive capacity. This suggests that root deepening may represent a key functional trait in *Quercus robur* L., underpinning its drought tolerance strategy (**Fig. 5**).

All physiological parameters (gsw, A, WUE) declined under drought stress and partially recovered after rehydration, without returning to initial levels. No significant differences between treatments indicate an absence of measurable legacy effects on oak seedling physiology (**Fig. 6a,b,c,d.**).

Physiological response to water stress under controlled conditions

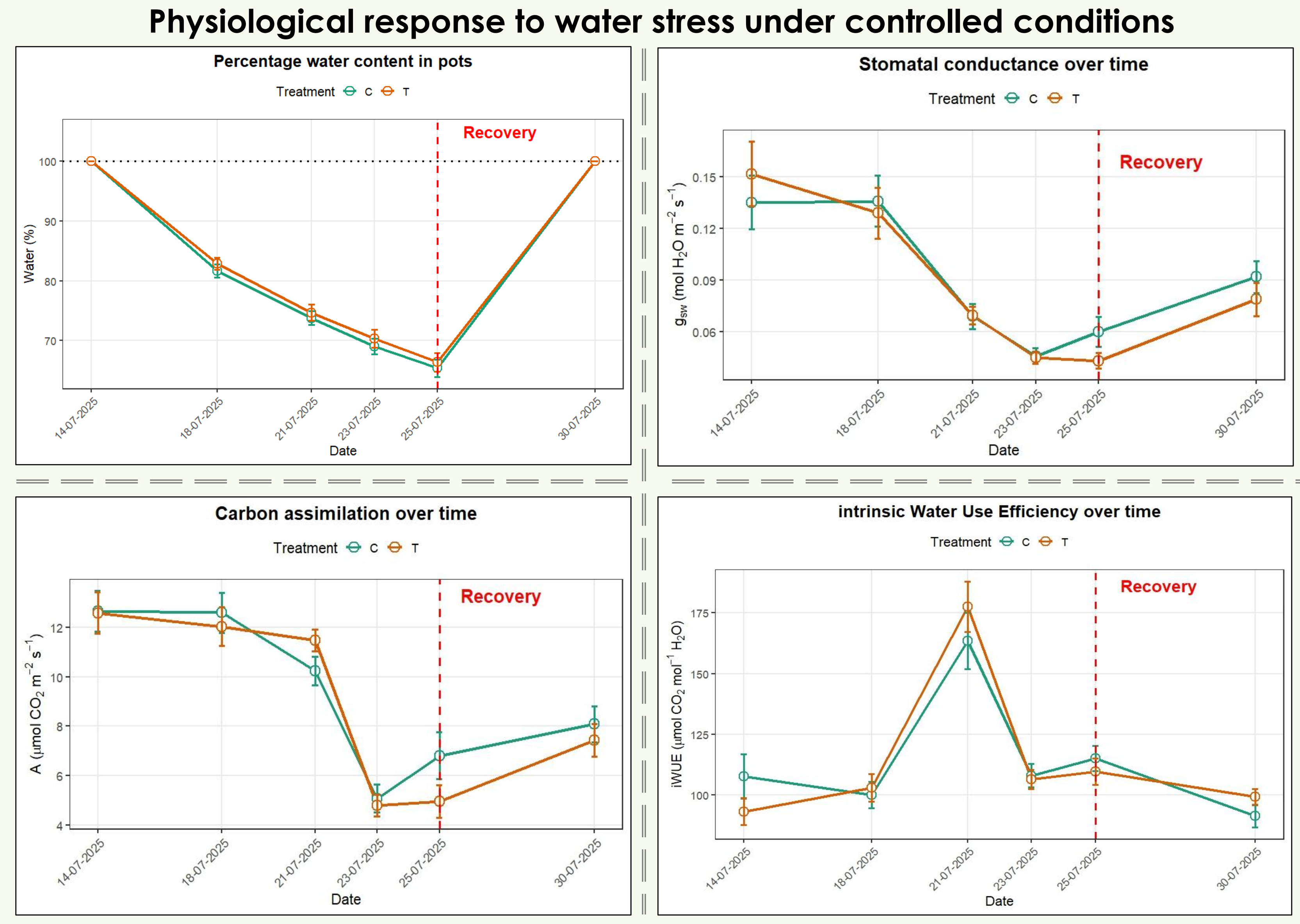


Fig. 6a-b-c-d. Mean ± standard error. No statistical significance between groups, Anova linear mixed model (LMM)

CONCLUSION

Summer **drought temporarily** homogenized physiological responses across size classes, while autumn **recovery** revealed distinct strategies related to root depth and access to soil water reserves.
All physiological parameters (gsw, A, WUE) showed a **marked decline** and only **partial recovery**, with no differences between treatments, suggesting the absence of legacy effects and a **strong influence of individual variability.**
Overall, the results suggest that root system architecture and abscisic acid (ABA) mediated regulation likely represent key functional traits in *Quercus robur* L., underpinning its adaptive capacity and drought tolerance, and potentially serving as reliable indicators of functional resilience under water-limited conditions..

2nd International Symposium on Biodiversity and Ecosystem Functions under Global Change
12 – 14 November 2025
Alghero (Sardinia – Italy)