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Widespread holm oak dieback in Mediterranean forests: the roles of carbon stress and hydraulic failure under recurrent drought events

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Abstract

In recent years, extensive tree dieback related to drought events have occurred in different Mediterranean forests. The aim of our research was to investigate the causes of widespread mortality of *Quercus ilex* L. observed in Tuscany.

Physiological traits and BOVCs were measured, during different seasons, in an experimental site established in the Maremma Natural Reserve, characterized by areas with high mortality rates of *Q. ilex*. To investigate specific physiological, biochemical and genetic mechanisms underlying *Q. ilex* dieback, we have also conducted a pot experiment on three-years old seedlings subjected to progressive water stress followed by re-watering, whereas control plants were maintained in well-watered conditions.

Results of both studies led us to hypothesize that *Q. ilex* dieback observed in the Maremma Natural Reserve may be attributed to both depletion in carbon reserves and hydraulic failure. Although holm oak is considered an isohydric species subjected to carbon starvation caused by fast stomatal closure in response to water deficit, the low water potential experienced by holm oak under recurrent droughts may have compromised xylem conductivity reducing its ability to recover from severe stress events. Considering the intrinsic high emission rates of monoterpenes of this species, variations in the production of these compounds may have implications for the atmospheric biochemistry in Mediterranean areas. In conclusion, our results contribute to elucidate possible physiological and molecular mechanisms underpinning recent holm oak forest mortality and provide guidance for understanding Mediterranean forest diebacks under climate changing conditions.

Keywords: drought-inducible genes; gas exchanges; Mediterranean ecosystems; *Quercus ilex*; secondary metabolites; water stress; xylem vessels.

Introduction

Mediterranean ecosystems are usually considered resilient to arid conditions, because of their capability to cope with and recover after severe stress events. However, Mediterranean region is a climate hotspot, where the effects of climate change are appearing more rapidly and impacting the area more harshly compared to other parts of the world (Gualdi *et al.*, 2013).

In recent years *Quercus ilex* (Holm Oak) forests have shown a widespread mortality in South Tuscany coastal areas

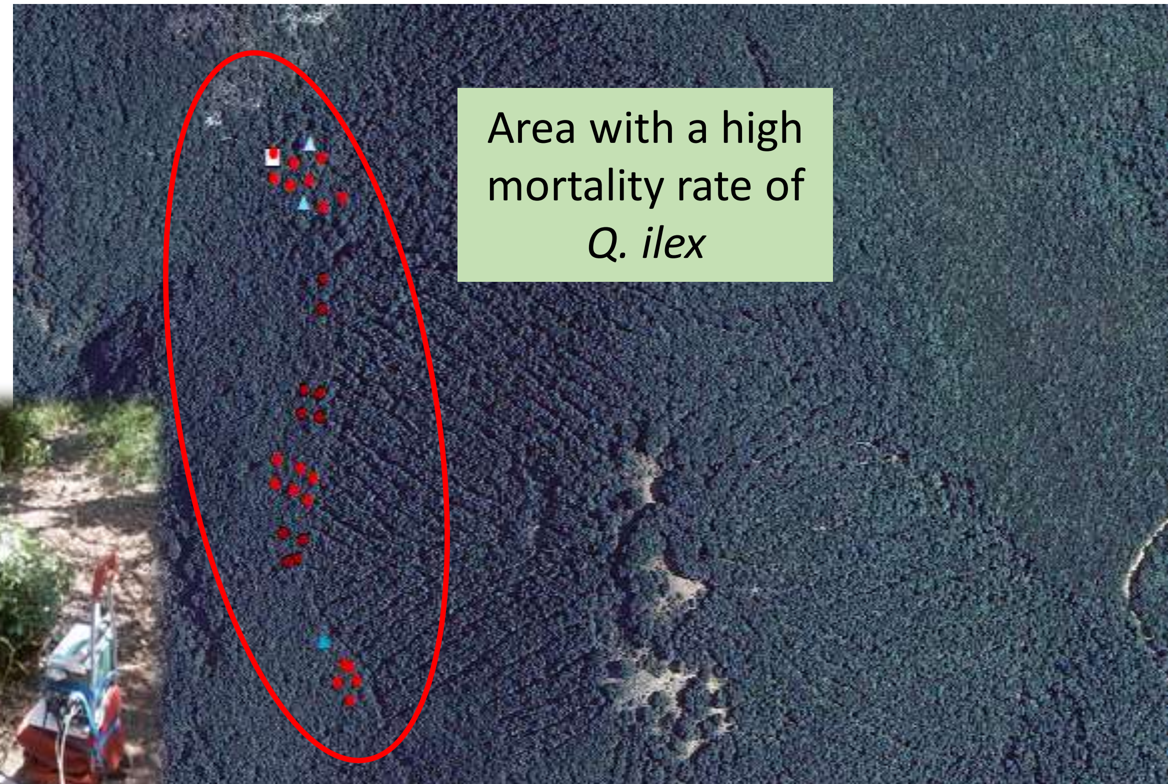
Aim of the study

Determining physiological and molecular mechanisms involved in climate-driven holm oak die-back in Mediterrranean area

Materials & Methods

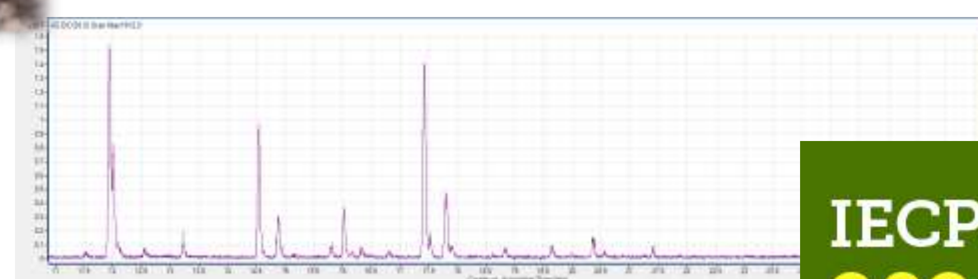
1) *In situ* monitoring of *Q. ilex* physiological traits

Three plots (each of 200 m^2) were selected in an area with a widespread tree mortality (80% of died holm hoaks)



Photosynthesis (P_n) and **stomatal conductance (g_s)** were measured using a LI-6400 system on a seasonal basis

Water potential (Ψw) was measured at midday and pre-dawn using a Schloander pressure bomb



BOVCs were collected at canopy level using SPME fibers desorbed in GC-MS

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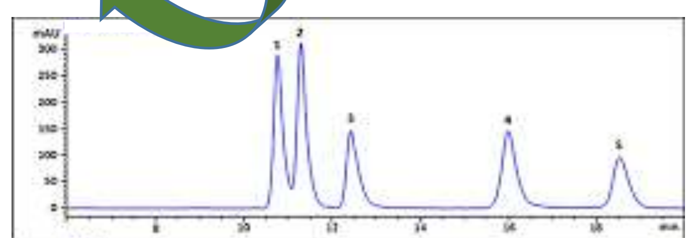
Materials & Methods

2) Induced-drought experiment on *Q. ilex* seedlings

10 three-year old seedlings of *Q. ilex* (~ 1m height) were subjected to a prolonged **water stress (WS)** followed by **re-watering (RW)**
10 seedlings were kept under **well-watered** conditions **(WW)**

chlorophyll fluorescence
gas exchanges
water-relations

Collection of wood samples for analysis of **non-structural carbohydrates (NSC)** by enzymatic digestion and HPCL quantification and gene expression



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Materials & Methods

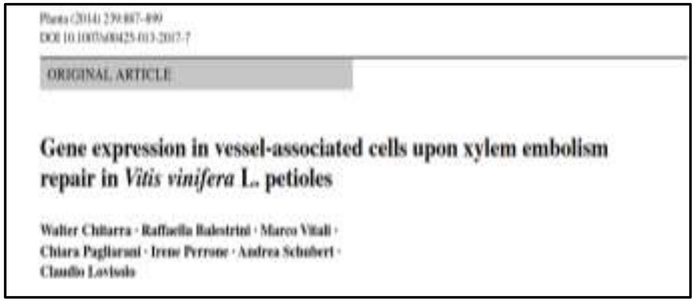
Gene expression: selection of *Q. ilex* genes and primer design

Gene ID	Putative function	Reference
AOS3	Jasmonic acid biosynthesis	Madritsch et al. (2019)
CDR1	Salicylic acid-dependent inducible resistance responses	Madritsch et al. (2019)
COMT	Plant cell wall remodeling; lignin biosynthesis	Madritsch et al. (2019)
CUL1	Plant cell wall remodeling	Madritsch et al. (2019)
CYP75B1	ROS scavenging	Madritsch et al. (2019)
EXPA	Plant cell wall remodeling	Madritsch et al. (2019)
NADK3	ROS scavenging	Madritsch et al. (2019)
PIP2	Aquaporin; water uptake and osmotic fluid transport	Chitarra et al. (2014)
SPS4	Sucrose-phosphate synthase	Chitarra et al. (2014)
WAK	Plant cell wall remodeling	Madritsch et al. (2019)
bHLH30	ABA-responsive gene expression	Madritsch et al. (2019)
detox	Detoxification of ROS	Madritsch et al. (2019)
NCED5 *	ABA synthesis	Chitarra et al. (2014)
NCED1 *	ABA synthesis	Chitarra et al. (2014)
GPT1 *	Glucose 6-phosphate/phosphate translocator	Chitarra et al. (2014)
SUC27 *	Sucrose transporter	Chitarra et al. (2014)
BAM3 *	Beta amylase	Chitarra et al. (2014)

Seventeen genes were selected on the basis of their putative involvment in **<u>foliar drought stress response of *Quercus* spp</u>**. (Madritsch *et al*., 2019). In addition, homologs of the well characterized **<u>*Vitis vinifera* water stress related genes</u>** (Chitarra et al., 2014) were searched in the available whole transcriptome of *Q. ilex* (Madritsch *et al*., 2019) and included in the assay

* WORK IN PROGRESS!


G3 Genes | Genomes | Genetics
INVESTIGATION
Elucidating Drought Stress Tolerance in European Oaks Through Cross-Species Transcriptomics


ORIGINAL ARTICLE
Gene expression in vessel-associated cells upon xylem embolism repair in *Vitis vinifera* L. petioles
Walter Chitarra · Raffaella Balestrini · Marco Vitali · Chiara Pagliarani · Irene Perrone · Andrea Schubert · Claudio Lovisolo

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Materials & Methods

Gene expression: RNA isolation and RT-qPCR

Four conditions:

In order to preserve RNA in samples, *Q. ilex* stems were dissected by using a **cryostat**. Total RNA was extracted by using a modified version of the extraction protocol by Carvalho *et al*., (2015) optimized for woody material

After DNAse treatment, RNA was converted into cDNA through SuperScript II RT and random primers

Diluted cDNA (1:2) was used in **RT-qPCR** to evaluate the relative gene expression of 12 genes linked to drought response in *Quercus* spp, in **three biological and two technical replicates for each of the four conditions**

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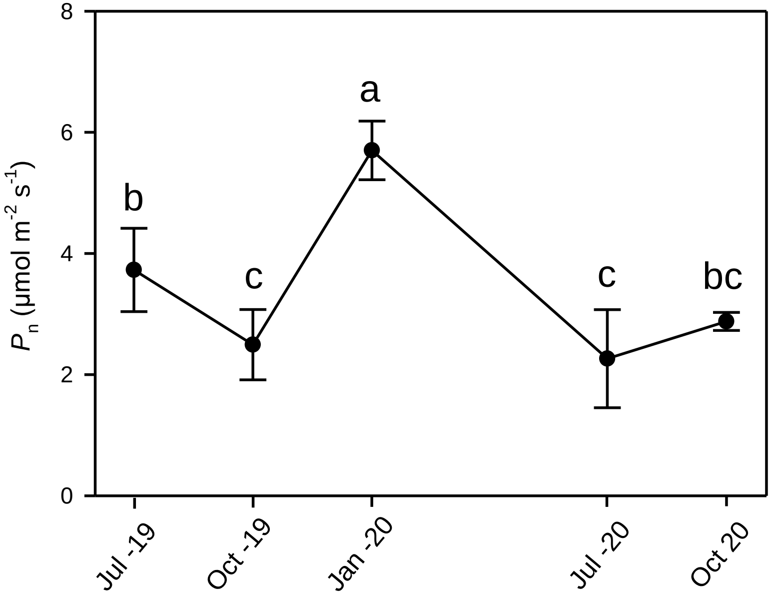
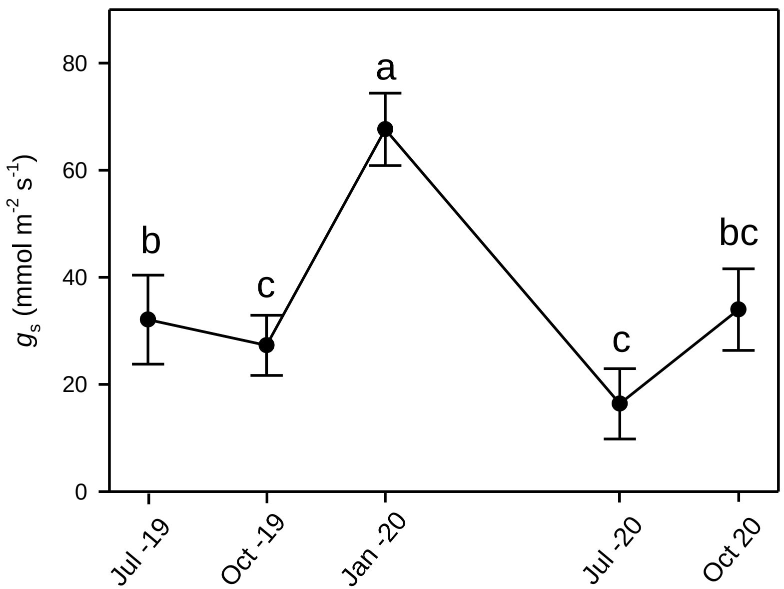
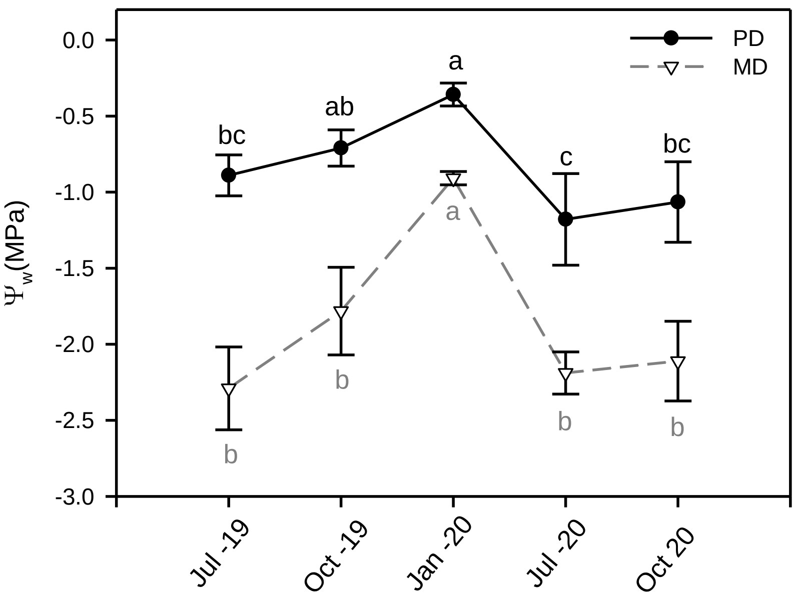
1) Results - Monitoring of *Q. ilex* physiology in Maremma Natural Reserve







Maximum photosynthetic activity was observed in January, whereas a **low stomatal conductance**, accompaigned by **low photosynthetic rates** were observed in **July and October**

The observed high difference between pre-dawn and midday Ψw in July and October suggests that the **xylem vessels were under tension** to sustain the photosynthetic activity

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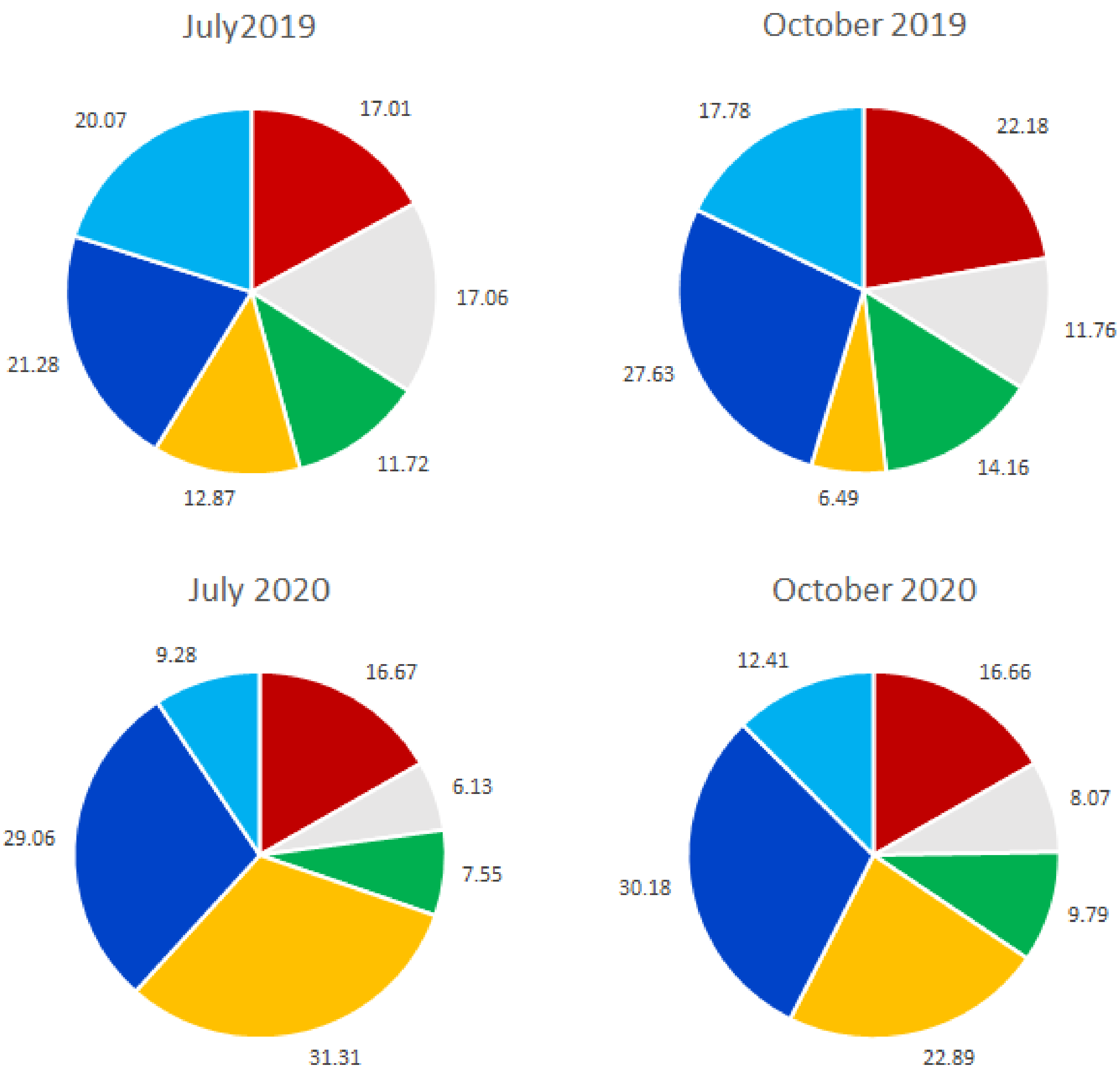
1) Results - Monitoring of atmospheric BVOCs in Maremma Natural Reserve



Changes in BVOC composition between years
and seasons may be related to environmental factors
and **<u>changes in vegetation composition and structure</u>**

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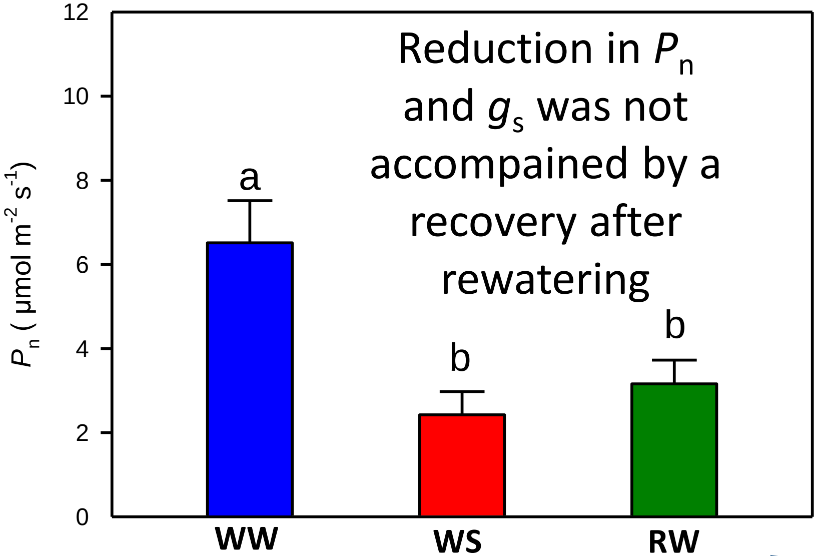
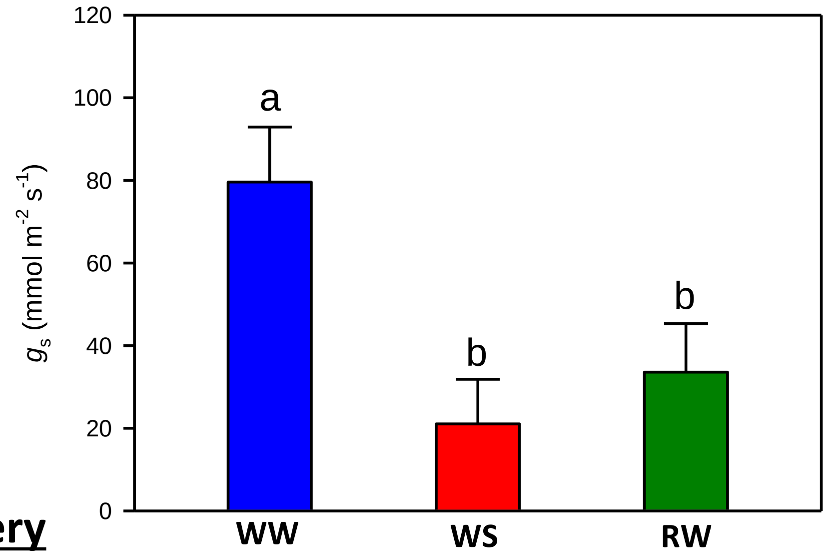
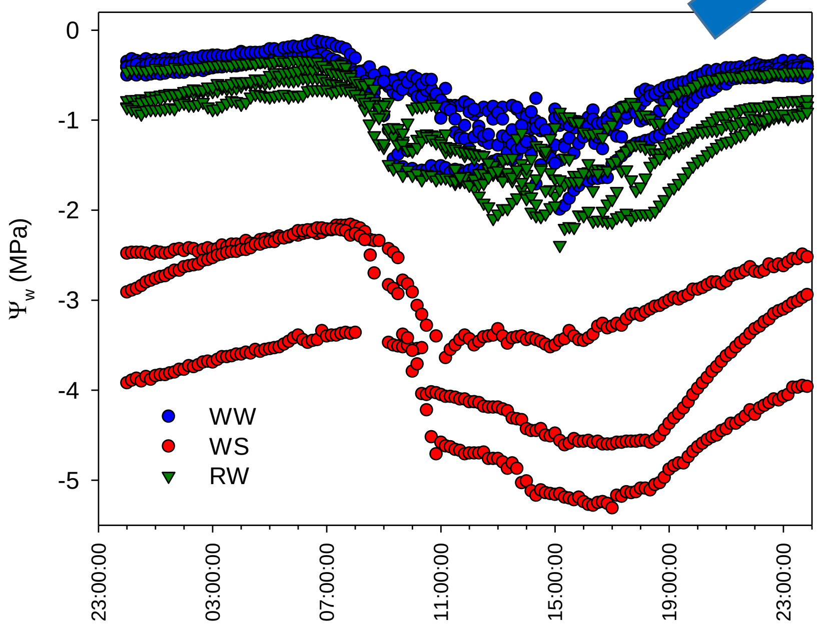
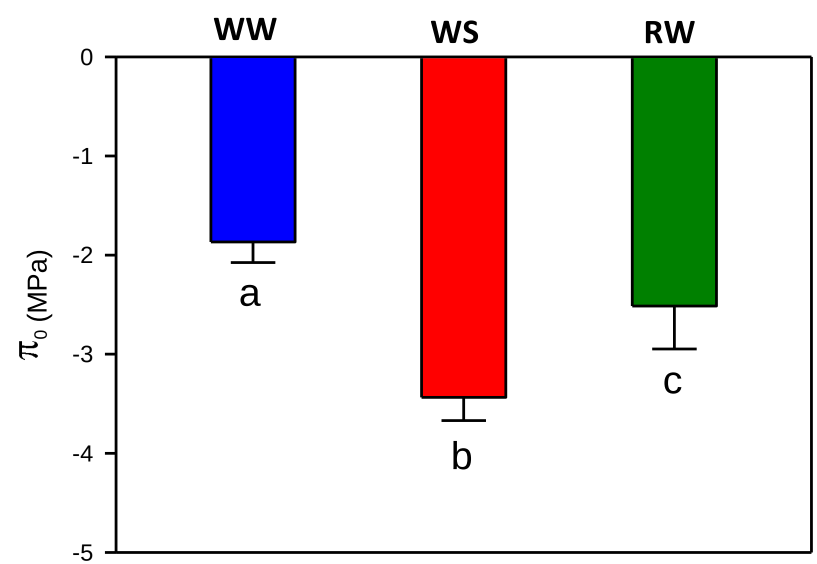
2) Results of *Q. ilex* pot experiment - Ecophysiology





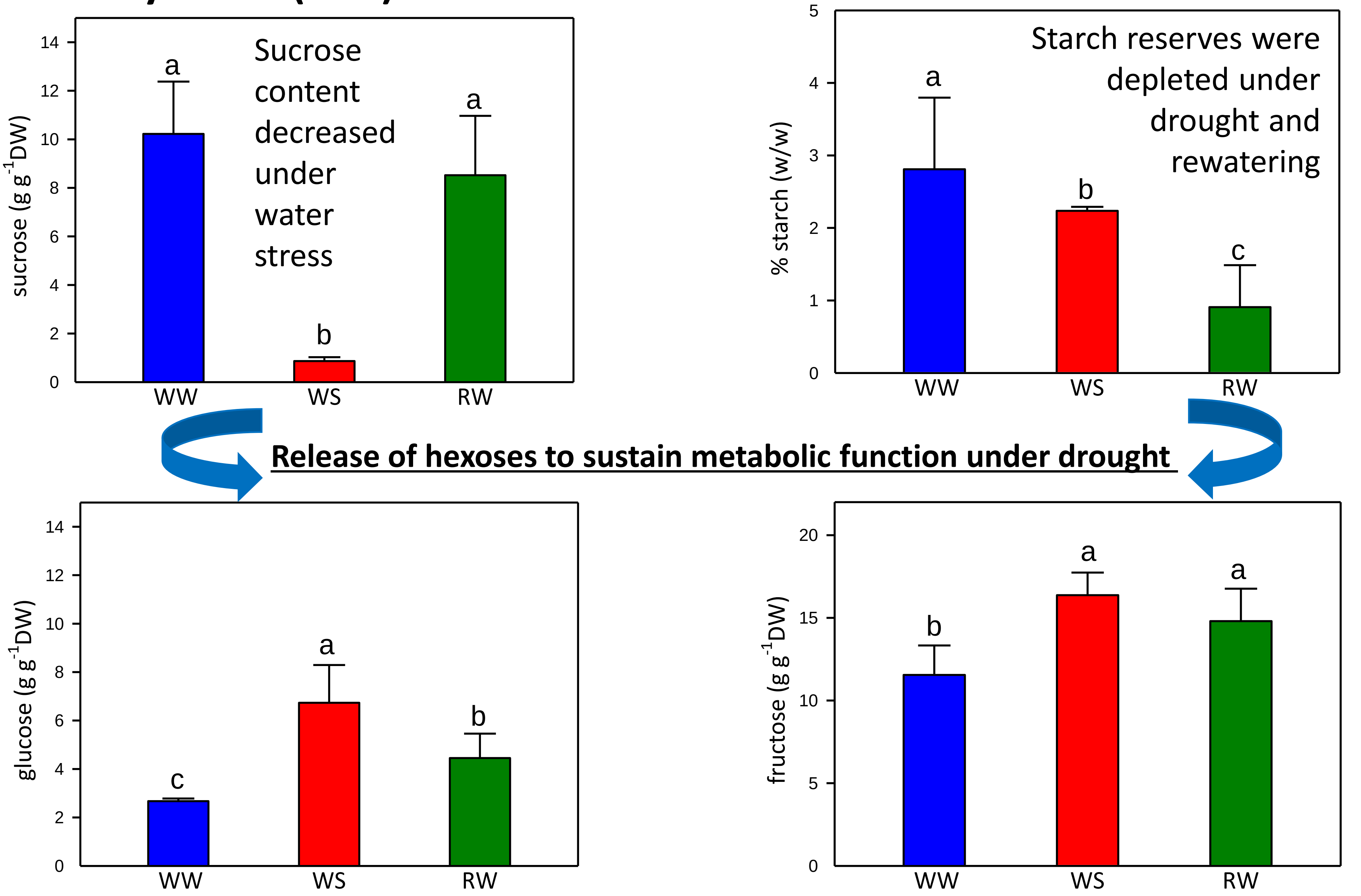


The **recovery in water potential** (Ψ_w) after rewatering

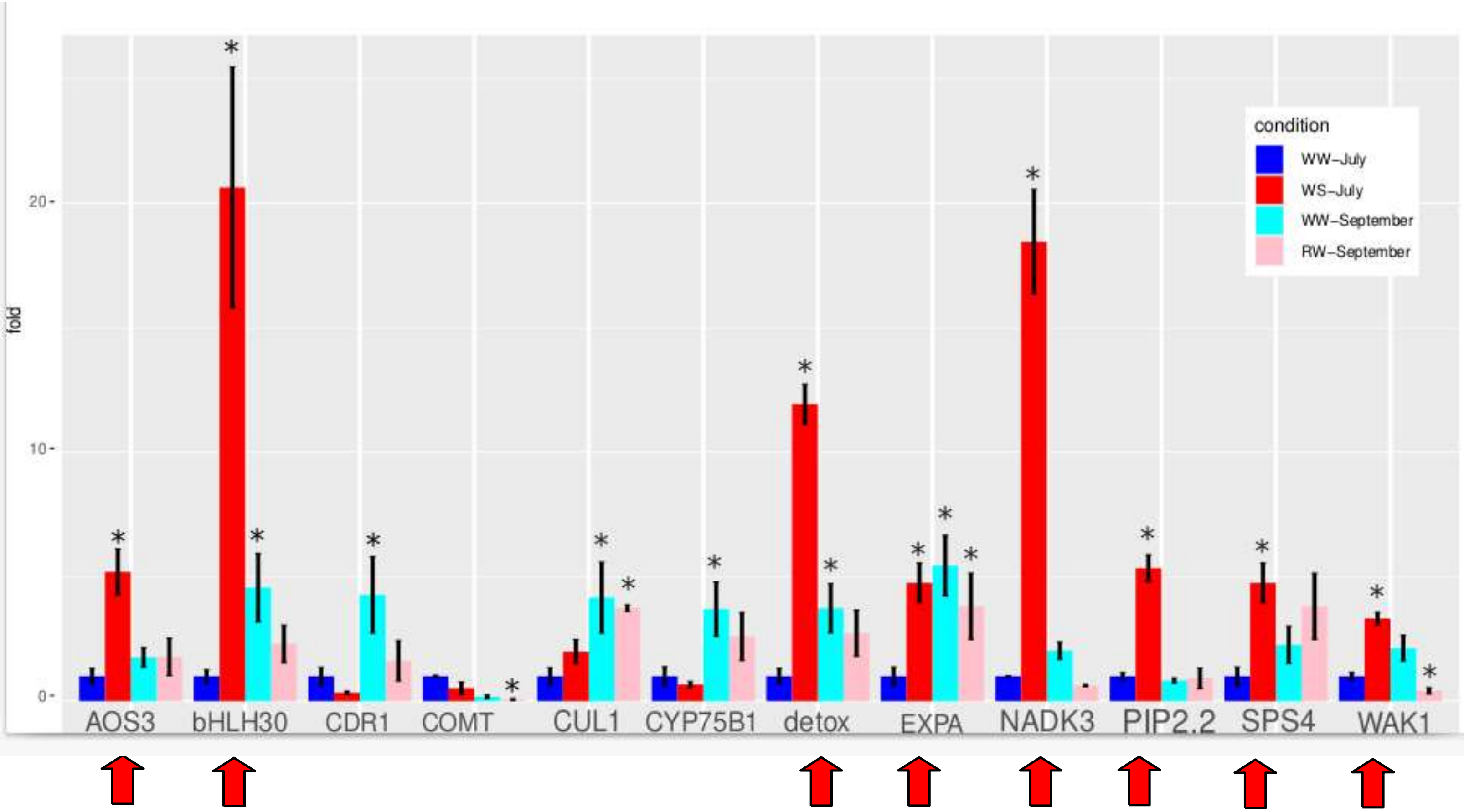


was accompained by a **partial reduction of osmotic potential at full turgor (π_o)**

2) Results of *Q. ilex* pot experiment – analysis of non-structural carbohydrates (NSC)



2) Results - Expression of *Q. ilex* genes putatively involved in drought response (by RT-qPCR)



Genes putatively related to **<u>ABA and jasmonic acid regulation, water uptake and osmotic fluid transport (aquaporin), sucrose synthesis, cell wall remodeling</u>** and **<u>ROS detoxification</u>** were significantly (*p-value* <0.05; permutation test integrated in REST2009) up-regulated in water stressed plants compared to the control (WW-July).

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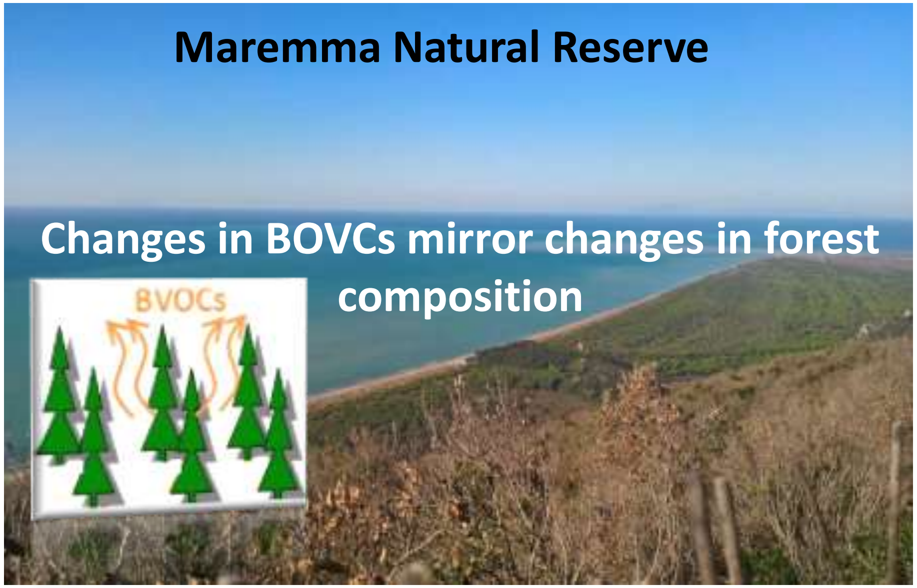
Discussion



The observed seasonal patterns in gas exchange showed a **<u>shift in the maximum of photosynthetic activity</u>** compared to the results reported for the same species in previous studies under field conditions (Corcuera *et al*. 2005).

In addition, the observed high differences between pre-dawn and midday water potentials indicated that holm oak trees are exposed to a **<u>prolonged water shortage not only in summer but also in autumn</u>**.

As a consequence, **<u>xylem vessels stayed under tension for a long period</u>** in order to maintain carbon assimilation.

The pot experiment allowed us to explore the biochemical and genetic mechanisms underlying **<u>the changes occurred in the wood parenchyma</u>** of *Q. ilex* during drought stress and rewatering

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Discussion

❖Our results highlighted the **key-role of carbohydrates** in the wood parenchyma during water stress

starch degradation and the activation of sucrose biosynthesis were important mechanisms to increase glucose levels in the cells of the wood parenchyma for **maintaining cellular metabolism** or to **preserve the efficiency of the xylem vessels** close to the cambium (Hammond *et al*. 2019).

❖ Changes in metabolic activities induced by drought in wood parenchyma are also suggested by the modification in the expression of genes involved abscisic acid and jasmonic acid metabolism as well as in ROS detoxification.

❖ The fine tuning of cellular metabolism uder drought allowed the recovery of hydraulic functionality after rewatering. However, the reduced stomatal conductance continued to limit photosynthesis. **This mechanism, while allows the protection the xylem vessels from embolism, exposes holm oak to the risk of carbon starvation**.

References

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