Microclimate, Soil Moisture, and Phenological Dynamics in an Eastern Mediterranean Evergreen Broadleaved Forest Stand

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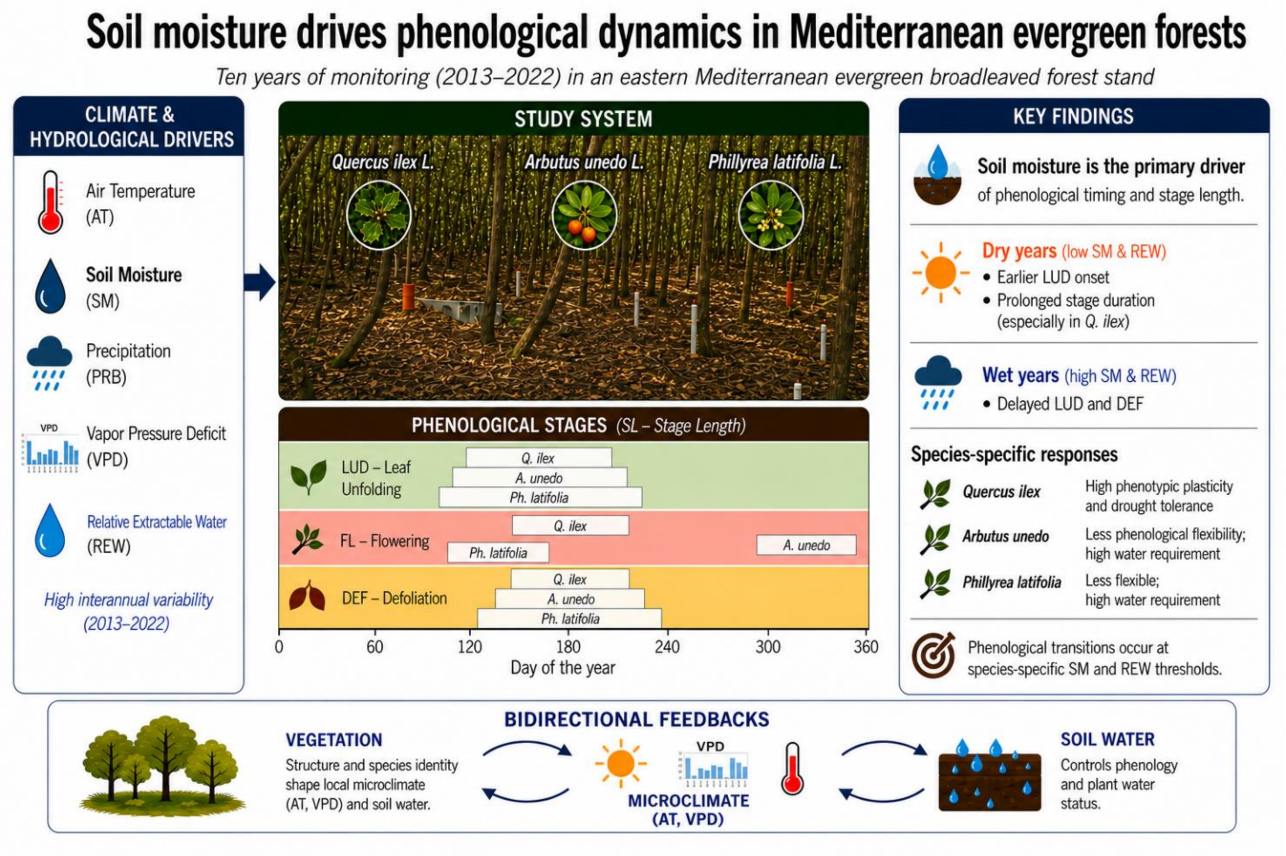

Abstract: This study investigates the phenological dynamics and microclimatic influences on three dominant evergreen species (*Quercus ilex, Arbutus unedo*, and *Phillyrea latifolia*) in an eastern Mediterranean forest stand over ten years (2013–2022). Phenological stages—leaf unfolding (LUD), defoliation (DEF), and flowering (FL)—were monitored alongside key microclimatic variables including air temperature (AT), vapor pressure deficit (VPD), precipitation (PRB), soil moisture (SM) and relative extractable water (REW). Results revealed strong interannual variability in phenological timing and stage length (SL), primarily driven by soil water availability rather than temperature alone. Reduced PRB and lower SM were associated with earlier LUD onset but prolonged stage duration, particularly in *Q. ilex*, highlighting its greater phenotypic plasticity and drought tolerance. In contrast, *A. unedo* and *P. latifolia* exhibited more limited phenological flexibility and higher water requirements for stage initiation. DEF was also delayed under wetter conditions, while FL responses varied among species, with significant delays observed in *A. unedo* during exceptionally dry years. Threshold analyses indicate that critical phenological transitions occur at species-specific SM and REW levels, suggesting differentiated ecohydrological strategies. Furthermore, vegetation structure and species identity influenced local microclimate, particularly AT and VPD, demonstrating bidirectional feedback between phenology and hydrometeorology. Findings suggest that while these ecosystems currently display resilience comparable to that of western Mediterranean counterparts, increasing drought intensity may alter competitive dynamics among species. Understanding these interactions is critical for forecasting ecosystem responses to climate change and informing forest management, guiding biodiversity conservation and restoration strategies. Promoting mixed species stands that enhance soil water retention and mitigate atmospheric dryness could improve forest resilience and support biodiversity conservation under future Mediterranean climate scenarios that predict increasing aridity and temperature extremes. Integrating field phenology with soil–plant–atmosphere interactions could lead to further research focusing on the functional diversity within eastern Mediterranean evergreen species and their role in shaping local microenvironments.

Keywords: Mixed species stands, Soil moisture, Phenology, Microclimate, Forest management, east Mediterranean

1. Introduction

The evergreen broadleaved species landscape constitutes very common forest ecosystems in the Mediterranean (MED). The Regional Climate Change Index (RCCI) analysis highlights the MED basin as a climate change (Giorgi 2006, Lazoglou et al. 2024) and biodiversity (Pausas & Millán 2019) key Hot-Spot. Climate projections consistently show a sharp reduction in average precipitation and greater variability during the dry (warm) season, which drives the region's high sensitivity to global change (Douville et al. 2021). If global warming reaches higher levels of intensity, then MED is expected to become progressively drier and drastically warmer (Lee et al. 2021).

Phenological stages are species-dependent (Wolkovich 2012, Spano et al. 2013) and directly linked to precipitation and air temperature (Richardson et al. 2013). Leaf unfolding, flowering and fruiting in Southern Europe showed an earlier onset (Vogel 2022). The role of phenotypic plasticity in the extended development period of forest species is relevant to the adaptive responses to climate variability and change (Franks et al. 2014, Bussoti & Pollastrini 2020).

The complex interactions of the phenological stages of evergreen broadleaved species (*maquis*), such as *Quercus ilex L.* (*Q. ilex*), *Arbutus unedo L.* (*A. unedo*) and *Phillyrea latifolia L.* (*P. latifolia*), with the environmental factors have been already investigated, focusing on their physiological, anatomical and morphological leaf traits (Ogaya & Penuelas 2004, Ogaya & Penuelas 2006, Gratani et al. 2006, Gratani et al. 2013, Barbeta & Penuelas 2016), resprout and plant shoot growth (Castell et al. 1994) and root functioning and ecohydrological adaptation strategies (Vico et al. 2015, Barbeta et al. 2015, Barbeta & Penuelas 2016). Drought tolerance (Bussoti & Pollastrini 2020), especially under drought-reduction experiment conditions (Ogaya & Penuelas 2004, Aguade et al. 2015, Barbeta et al. 2015, Penuelas et al. 2018, Ogaya & Penuelas 2021, Bogdziewicz et al. 2020, Campelo et al. 2023), water balance to promote management practices (Vicente et al. 2018) were also investigated against phenological stages such as growth, reproduction, plant seed regeneration and mortality. Additionally, phenological patterns such as fruit growth, maturation, and abscession (Ogaya & Penuelas 2004), defoliation to trees' growth (Camarero et al. 2016), and the advancement of the green season and flowering (Tuhami et al. 2023, Pareja-Bonilla et al. 2025) provided crucial insight into why plant phenology is changing in MED ecosystems and how these species exhibit varying responses. These insights are essential for developing adaptive management and conservation strategies under ongoing climate fluctuations, as they provide a mechanistic basis for anticipating species-specific vulnerabilities and effectively guiding sustainable forest management.

Most of the above research was conducted mainly in the western and central part of MED basin. To our knowledge, there are limited experiments in the eastern part of MED focusing on the responsive strategies of forest species to abiotic factors. Especially on *maquis* environments in their natural environment. Proutsos et al. (2022) estimated that Nestos's Delta (Greece) habitats were highly vulnerable and likely to be significantly affected by future climate variability because of decreased trends in annual precipitation. Other reported papers concerned monitoring for mainly deciduous species, such as *Fagus sylvatica L.* and *Castanea sative Mill.* in Greece and Italy (Doumkou et al. 2025), *Olea europaea L.* (Ulas & Gezerel 2004, Ozturk et al. 2021) or evergreen conifer species, such as *Pinus brutia* and *Cedrus libani* species in Mediterannean Turkey forests (Dogan Ciftci et al. 2024) and the endangered evergreen *Quercus alnifolia* in Cyprus (Anagiotos et al. 2012, Kougioumtzis et al. 2024).

The study of Penuelas et al. (2018) underscored the importance of integrating field experiments with long-term observations to accurately project and understand the ecological consequences of climate change in MED regions. Given climate change, our understanding of the contribution of different individual environmental parameters is of great importance and quite unknown to date (Wolkovich 2012), including their interactions with organisms (Parmesan and Hanley 2015, Ponce et al. 2022, Tuhami et al. 2023).

Having all the above information in mind from previous studies, we made the following hypotheses:

(i) Air Temperature (AT) is a primary driver of leaf unfolding (LUD) and flowering (FL), with earlier onsets under warmer conditions.

(ii) Soil moisture (SM) and Relative Extractable Water (REW) significantly affect defoliation and stage duration, especially under summer drought conditions.

(iii) High vapor pressure deficit (VPD) correlates with advanced or prolonged defoliation and may stress the flowering process.

(iv) Stage Length (SL) is modulated by the interplay of SM, REW and AT conditions.

These hypotheses lead to the main objective of our study, described as follows:

This 10-year study, conducted in a natural forest stand, investigates how dominant evergreen broadleaved species in the eastern Mediterranean respond phenologically to abiotic constraints, whether these responses mirror those observed in western Mediterranean ecosystems, and the SM and REW thresholds triggering critical phenological stages such as LUD, FL and defoliation (DEF).

2. Materials and Methods

2.1 Study area

The experimental plot (45 X 33 m) located near to Varetada village in western Greece (38° 50' 46.30"N, 21° 18' 15.29"E, alt: 340 m a.s.l.), represents a typical evergreen broadleaved forest ecosystem of the eastern Mediterranean (Greece) and forms part of the ICP Forests Level II plots network for intensive monitoring of European forest conditions. Vegetation structure shows significant heterogeneity in terms of dominance. Drawing on our empirical observations, most areas are dominated by *Q. ilex*, other evergreen broadleaf species, such as *A. unedo* and *P. latifolia*, also show a significant participation.

In the *Q. ilex* section, the tree layer shows a coverage greater than 90% (Figure 1) while the herbaceous layer is almost completely absent. The shrub layer shows signs of decline and a limited attempt to evolve into the tree layer. As a result, shrub species have thin trunks and elongated branches, in an effort to reach some light, since the dense canopy of *Q. ilex* blocks most of it. In the remaining section, where shrub species dominate, the vegetation appears particularly dense and erect. The vegetation height ranges from 6-15 m and the Leaf Area Index (LAI) measured 5.8 (Bourletsikas et al. 2023). It expresses intense dominance competition at a height of approximately 4 meters the ground. The result of this competition is the existence of many, small-diameter trunks and many dead branches. Despite the presence of more than thirty species, the herbaceous layer is relatively sparse under the shrub canopy. However, in openings between the shrubs, it becomes much denser, with ground cover sometimes exceeding 90%.

The site is characterized by hilly topography and is underlain by Eutric Cambisol fertile, well-drained soils (FAO, 1988), formed from flysch-derived parent material. In our knowledge, the forest has not been subjected to any silvicultural or managerial treatment by the local Forest Service for many decades. Thus, no land-use changes have occured, and the area has likely followed natural processes, such as ecological succession, biodiversity development, and structural changes without human disturbance.

The region's climate is classified as warm temperate with hot, dry summers, corresponding to the Csa type in the modified Koppen-Geiger climate classification system (Kottek et al. 2006). Regarding aridity, the broader area is classified as humid according to UNEP'S (1992) aridity classification system, based on Thornthwaite's (1948) and Thornthwaite's & Mather (1955) water balance approach (Tsiros et al. 2020, Proutsos et al. 2021).

2.2 Data collection and analysis

2.2.1 Meteorological data

The meteorological data were sourced from a meteorological station (MS) situated at coordinates 38° 50' 35.33"N, 21° 18' 25.40"E, located at a forest opening (alt. 320 m a.s.l.), approximately 420 meters from the experimental plot (Figure 1). All analyses were based on daily values of the measured or estimated variables.

Figure 1. Map of Varetada's study area location in Greece, along with vegetation composition within the experimental plot (top photo) and a view of the installed meteorological station (bottom photo).

AT (°C) and Relative Humidity – RH (%) values, including minimum and maximum values, were continuously monitored at 15-minute intervals, for the concerned 10-year period (2013-2022). Average daily values were calculated from the 96 daily 15-minute values. The analyzed dataset consisted of 3,652 days with measurements, and accounted for 96 days (2.63% of the dataset).

PRB (mm) was recorded on a weekly recording tape using a Belford rain gauge. It operates on a weighing principle, measuring the accumulated precipitation and converting it into mm, with simultaneous conversion of this measurement into millimeters (mm) of precipitation height. The maximum measurable precipitation depth of the recording tape is 300 mm, divided into two segments of 150 mm each. Daily PRB was calculated from the addition of the hourly resolution analysis based on tape readings.

VPD (kPa) was calculated by using the following equation (Allen et al. 1998):

$$\mathrm{VPD} = e_s - e_a \quad (1)$$

Where, e_s (kPa) stands for mean saturation vapor pressure and e_a (kPa) for actual vapor pressure. For the calculation of e_s and e_a, we used measured data from AT and RH, following the equations from Allen et al. (1998):

$$e_s = \frac{e^0(AT_{max}) + e^0(AT_{min})}{2} \quad (2)$$

$$e_a = \frac{RH}{100}\left[\frac{e^0(AT_{max}) + e^0(AT_{min})}{2}\right] \quad (3)$$

Where, $e^0(AT)$ (kPa) is the saturation vapor pressure at the measured daily AT (°C). This relationship is expressed by:

$$e^0 = 0.6108 \exp\left(\frac{17.27 * AT}{AT + 237.3}\right) \quad (4)$$

2.2.2 Soil Moisture and Relative extractable water

SM (m^3/m^3) was recorded hourly using ECH2O EC-5TM sensors (Decagon Devices Inc., Pullman, WA, USA) installed in three plot replicates (Bourletsikas et al. 2023). Sensors were positioned at three soil depths: –20, –40 and –70 cm. Daily SM values for each depth were derived by averaging the hourly measurements. Corresponding water storage (in mm) was calculated by multiplying the SM values by the thickness of each soil layer (in mm). The total soil profile was assumed to extend to 70 cm.

REW is the fraction of the available soil water (AWC) that the plants can use for their physiological needs. It constitutes a standardized measure for plant water stress studies, which ranges from 0 to 1 (from wilting point to field capacity, respectively) and it was calculated by using the following equation:

$$\mathrm{REW} = \frac{SM - \theta_{WP}}{\theta_{FC} - \theta_{WP}} \quad (5)$$

where, SM is the daily soil moisture values, $\theta_{WP} = 0.111$ m^3/m^3 is the SM value at the wilting point and $\theta_{FC} = 0.258$ m^3/m^3 is the SM value at field capacity (pF curves - Bourletsikas et al. 2023).

The dataset of soil-water related parameters used in this study consists of 262,944 values covering the period 2013-2022. The gaps in data generally are due to sensors' malfunctioning. In these cases, the missing data were completed by records from the soil sensors established at the same depths in the other replicates. In cases where at a specific depth all sensors were not operating (178 days – 4.9%), soil moisture across the whole profile was estimated by the measurements from the sensors in the remaining depths.

2.2.2 Phenological observations

Three phenological stages (LUD, DEF, and FL) were monitored for a period of ten years (2013 – 2022), for the three dominant species: *Q. ilex*, *A. unedo*, and *P. latifolia*. Observations and data collection inside the plot followed the guidelines outlined in the ICP Forests manual (Raspe et al. 2020). The phenological stages of the trees within the plot were scored based on observations of both the upper and middle parts of the crown (Table 1). A well-trained local observer conducted weekly assessments using binoculars. The observed information derived from the whole stand (45 X 33 m) as a percentage of each phenological stage and each tree species. For the FL stage, the reports included only absence or presence (Table 1). Different intensities (codes) of a phenological stage were repeated until the conclusion of the events (Raspe et al. 2020 – ICP Forests manual).

Table 1. Codes and Descriptions of the Quantitative and Qualitative information for the three phenological stages (Raspe et al. 2020 – ICP Forests manual).

Code	LUD and DEF		FL
Intensity	Description	Quantification	Description
1	not occuring	<1%	Absent
2	Infrequent	1 - 33%	Present

3	Common	>33 - 66%	Sparse
4	Abundant	>66 - 99%	Moderate
5	Complete	>99%	Abundant

3. Results and Discussion

3.1. Vegetation's phenological response to hydrometeorogical conditions

Figure 2 presents data on PRB, mean AT and SM for the first (_1) and second (_2) quarters of each observation year. In contrast, Figure 3 depicts phenological stages, their duration and intensity (different steps in each year).

Results show that AT remains relatively stable over the 10-year period, whereas PRB and SM showed considerable variability. By integrating the information from Figures 2 and 3, it becomes evident that SM is a key driver in triggering phenological stages. This is especially pronounced when comparing the wet year (2014) with the dry year (2017), where reduced PRB and SM corresponded with shifts in phenological timing and intensity. More specifically, in 2017, PRB was reduced by 29.3% in the first quarter (PRB_1) and by 79.5% in the second quarter (PRB_2) compared to the respective values in 2014. Correspondingly, SM_1 in 2017 showed decreased value (0.235 cm^3/cm^3) compared to 2014 (0.250 cm^3/cm^3) by 6.1%, whereas SM_2 also decreased but with a higher rate (26.8%). Therefore, the regional hydrological regime strongly influenced both the timing and duration of phenological stages.

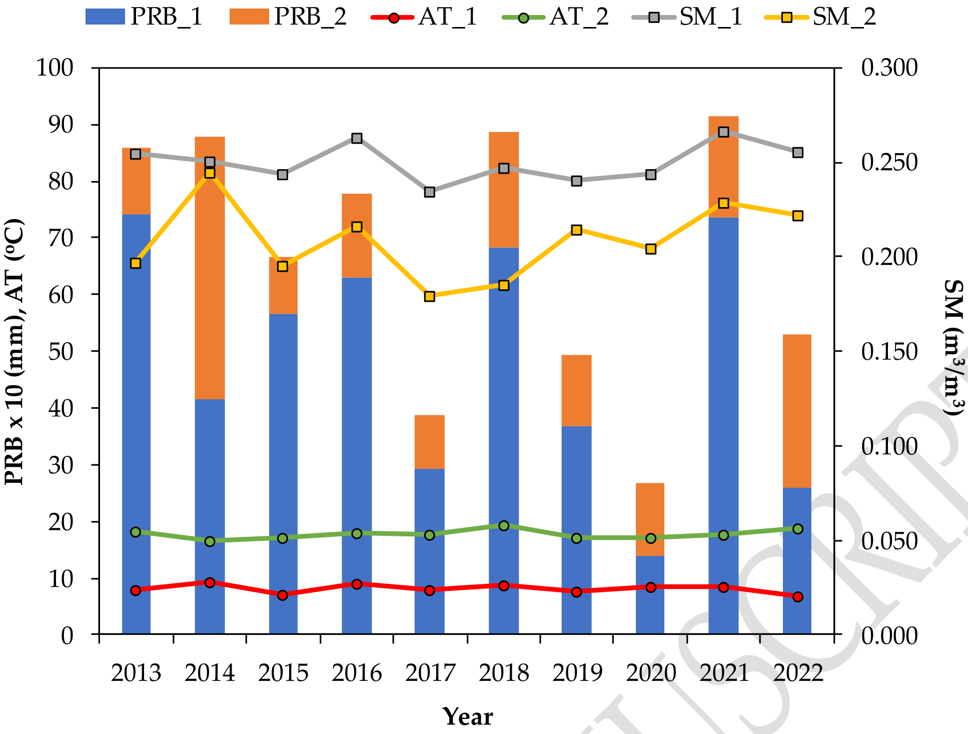

Figure 2. Precipitation (PRB × 10), mean air temperature (AT), and soil moisture (SM) recorded for the first (_1) and second (_2) quarters of each year.

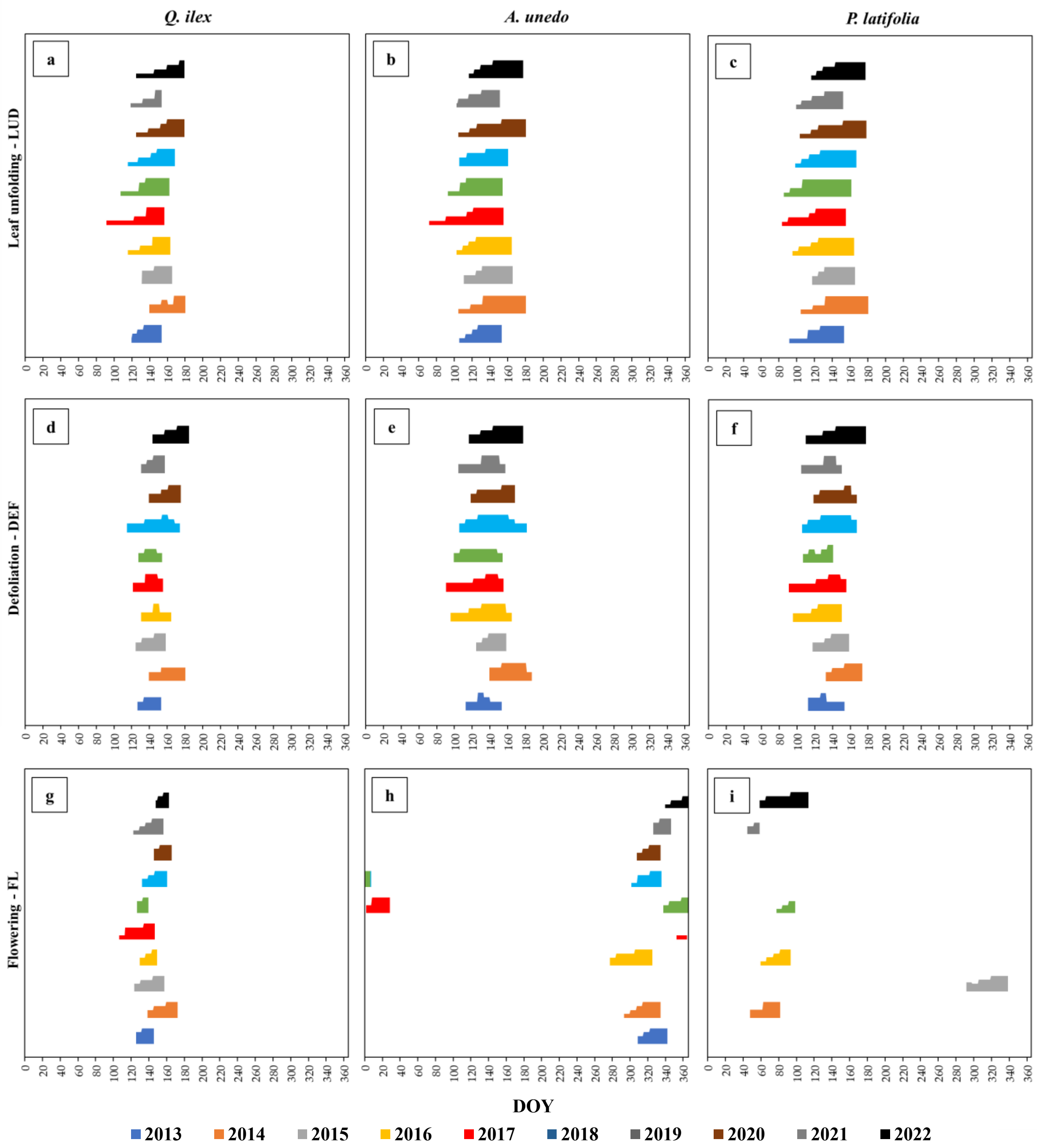

Figure 3. Stage length (SL) of phenological stages observed over a 10-year monitoring period in the study area: Leaf unfolding (LUD), defoliation (DEF) and flowering (FL) for the three dominant forest species *Q. ilex (a, d, g), A. unedo (b, e, h)* and *P. latifolia (c, f, i)*, respectively. The x-axis represents Day Of the Year (DOY). The stepwise patterns within each year's SL indicate the different intensity of each phenological stage (see Table 1).

Figure 3 shows that *Q. ilex* exhibited a notable 49-day delay in the onset of LUD in 2014 compared to 2017, while *A. unedo* and *P. latifolia* showed delays of 34 and 15 days, respectively. Though, it should be noted, that this pattern was not consistent in 2020, the driest year in terms of PRB. In 2020, LUD onset for *Q. ilex* occurred 35 days earlier compared to 2017. However, in this year SM_1 retained relatively high values (0.243 cm^3/cm^3), similar to 2014 (0.244 cm^3/cm^3), even if the PRB_1 was reduced, and this probably affected the beginning of the LUD stage, suggesting that increased water availability in the root-zone during the first quarter of the year may negatively affect the LUD stage onset. This relationship between SM and the onset of LUD is generally confirmed for all years of measurement, except for 2016 and 2021. During these years, SM_1 was high, exceeding 0.263 but the onset of LUD was delayed by 26 days compared to 2017, probably due to the higher air temperatures (AT_1) persisting at the site (9.2 ºC in 2016 and 8.5 ºC in 2021). These interactions indicate that both SM and AT jointly regulate phenological timing, with the plants adjusting stage duration, demonstrating remarkable adaptability.

DEF also exhibited a delayed onset during the wet year (2014). For *Q. ilex*, DEF began on 19 May, resulting in an 18-day delay compared to the start date of 1 May in 2017. Respectively, the delay for the beginning of the DEF stage for *A. unedo* was 49 days and for *P. latifolia* 42 days. The relatively shorter delay observed in *Q. ilex* under dry conditions may be linked to the greater plasticity of leaf morphological, anatomical, and physiological traits, as reported in mature Mediterranean maquis communities dominated by *Q. ilex*, *P. latifolia*, and *Pistacia lentiscus* in Italy (Gratani et al., 2006). Additionally, evidence of drought acclimatization in *Q. ilex* leaves was observed from litterfall traps installed inside the study area for LAI measurements (Bourletsikas 2024). Such acclimatization enhances tolerance to moderate drought conditions but does not confer resistance to severe water stress (Limousin et al. 2022), highlighting also vegetation adaptation mechanisms to confront drought (Barbeta & Penuelas 2016) and underscoring the need for further investigation.

Regarding FL, patterns also varied across the observation years, generally following LUD patterns, although dry conditions shortened FL duration. More specifically, in *Q. ilex,* FL started the same day with LUD in the wet year (2014), while it showed a delay of 17 days in the dry year (2017). In general, the FL stage for *Q. ilex* presents a 13-day delay compared with LUD stage, varying from 0 days (2014) to 21 days (2018). Especially, in 2018, the SL for FL was only 13 days. On the first day, the SM value recorded 0.124 m^3/m^3, corresponding to 8.5% REW, indicating that *Q. ilex* was subjected to intense water stress. Monthly SM was the lowest in September 2017 (0.116 m^3/m^3, close to θ_{WP}), followed by September 2018 and October 2017 (0.119 m^3/m^3), compared to all other months across all years. This indicates that natural vegetation experienced substantial water stress extending into late autumn. These conditions likely contributed to delayed FL of *A. unedo* by approximately 1.0 to 1.5 months (Figure 3), shifting it into winter months in some years (Bourletsikas et al., 2023).

3.2. Inversed Interaction of Phenology, Vegetation, and Hydrometeorology – Management context

Vegetation is a fundamental component of terrestrial ecosystems, closely interacting with hydrological and other environmental processes (Liu et al., 2023). Previous studies reported that an increase of the climate conditions intensity, may result in a prolongation of the growing season (Ogaya & Penuelas 2006, Gunderson et al., 2012). The latter, could lead to an increase of VPD (Yuan et al., 2019), evapotranspiration (Condon et al., 2020), variability in PRB (Sardans & Penuelas 2013, Proutsos et al. 2022, Douville et al. 2021), and SM imbalance (Deng et al. 2020, Zhang et al., 2021, Liu and Yang 2023). These remarkable changes can, on one hand, challenge vegetation resilience and on the other hand, redistribute vegetation community structure.

Our findings suggest that vegetation – having adapted to local hydrometeorological conditions – reveals an inverse influence on the selected parameters (SM, VPD and REW) related to water availability and the duration of phenological stages (SL). This bidirectional relationship is a key finding that requires further investigation, including consideration of additional biotic and/or abiotic factors, especially under future climatic scenarios (Lee et al. 2023).

Hydroecologically, in a management and future research context, these findings suggest incorporating species-level physiological traits, and seasonal phenological patterns, when assessing ecosystem resistance to drought and climatic variability (Menzel et al. 2006, Limousin et al., 2009). Selecting and assembling species based on co-occurring ecophysiological functions may enhance adaptive capacity, preserve stability in MED-type forest ecosystems, and support biodiversity conservation by maintaining functionally diverse communities and reducing the risk of drought-driven local extinctions. Additionally, long-term species-specific monitoring of phenological responses helps to better understand forest resilience and potential replacement in vegetation composition (Vico et al. 2015, Peñuelas et al. 2018), with direct implications for biodiversity conservation planning (e.g., identifying vulnerability species, prioritizing mixed stands and designing restoration strategies that sustain habitat heterogeneity. Future research should also integrate phenological observations with remote sensing techniques, including the use of Normalized Difference Vegetation Index (NDVI) thresholds, to effectively assess stress responses and changes in productivity (Maselli et al. 2014, Touhami et al. 2023, Benito-Verdugo 2024).

3.3. Hydrometeorological conditions driving the initiation of vegetation phenology

To identify threshold conditions, especially minimum ones, four microclimatic variables (SL, SM, VPD and REW) were investigated (Table 2). Among the studied species, *Q. ilex* demonstrated the highest resilience to limited soil moisture availability. This is evident from its lower water requirements for initiating phenological stages, compared to the other two species (Table 2). However, this implies a greater energy investment is required by *Q. ilex* to support these stages (Vicente et al. 2018). A prolonged drought period could erode this competitive advantage, making *Q. ilex* more vulnerable and potentially leading to its replacement by more drought-tolerant species (Ogaya & Peñuelas, 2006). This underscores the dynamic nature of competitive hierarchies in eastern MED forest ecosystems, driven by interannual variability in hydrological conditions. Fortunately, based on the available vegetative and climatic data, there is currently no clear evidence that such a shift is occurring—even though the first quarter of the century has already elapsed. Therefore, it is not evident how this natural forest will adjust to a potential prolonged drought.

Table 2. Mean and minimum values of the analyzed microclimatic variables for the three dominant forest species in the study area. Mean values correspond to the 10-year monitoring period (2013-2022), while the minimum values refer to conditions recorded on the onset dates of each phenological stage within the same period. Mean PRB refers to the total rainfall accumulated over the duration of each stage.

Phenological Stage	**Start (DOY)**		**PRB (mm)**	**SL (Days)**		**SM (m^3/m^3)**		**AT (ºC)**		**VPD (kPa)**		**REW**	
	Average	Min	Average	Average	Min	Average	Min	Average	Min	Average	Min	Average	Min
						Q. ilex							
LUD	115	90	99	49	34	0.219	0.195	16.0	12.5	0.769	0.090	0.752	0.591
DEF	129	114	68	36	27	0.201	0.124	19.2	15.1	1.083	0.089	0.710	0.483
FL	130	107	76	27	13	0.201	0.124	18.0	13.5	0.942	0.000	0.629	0.088
						A. unedo							
LUD	101	71	125	63	48	0.236	0.213	12.7	10.0	0.631	0.000	0.874	0.717
DEF	111	90	86	65	34	0.225	0.199	14.7	9.6	0.736	0.000	0.797	0.615
FL	315	277	205	34	21	0.202	0.142	12.6	4.9	0.429	0.000	0.640	0.215
						P. latifolia							
LUD	99	83	135	66	83	0.239	0.206	13.5	9.5	0.765	0.000	0.897	0.665
DEF	109	90	86	52	41	0.226	0.202	14.0	9.6	0.704	0.000	0.805	0.639
FL	97	45	155	34	14	0.253	0.197	10.1	1.3	0.296	0.123	0.994	0.602

The drought tolerance of *Q. ilex* was documented by several researchers through various phenological traits (Castell et al. 1994, Ogaya & Peñuelas 2006, Gratani et al. 2006, Aguadé et al. 2015, Camarero et al. 2016). According to Lobo et al. (2018), *Q. ilex* and *Q. suber* exhibited the lowest hydraulic conductivity and therefore the highest embolism resistance among Mediterranean oaks, with thresholds of -7.13 MPa and -5.52 MPa, respectively. Furthermore, any phenological response manifested as an extended development period may largely be attributed to the phenotypic plasticity of each forest species (Franks et al. 2014). This trait is further supported by the deeper rooting system of *Q. ilex* in comparison to *A. unedo* and *P. latifolia* (Castell et al. 1994, Vicente et al. 2018).

The difference observed in AT data between plant species, with *Q. ilex* consistently presenting higher microclimatic temperatures, probably reflects differences in canopy structure and albedo combined. Being a late-successional and well-established tree species (Bussoti & Pollastrini 2020), *Q. ilex* provides a denser canopy structure less efficient for radiation interception than the more diffuse crown of *P. latifolia* and *A. unedo* (Valladares et al. 2006, Moreno & Oechel 2012). This structural feature might favor local heating, as described for oak-dominated Mediterranean woodlands (Maestra & Cortina 2004). The higher water availability in *P. latifolia* plots could also be associated with its more conservative water use (Table 2) and a greater root-to-shoot ratio, which could affect water flux and storage in MED soils (Sardans & Penuelas 2013).

The higher relative extractable water (REW) observed under *P. latifolia* suggests that some sclerophyllous shrubs can enhance soil water storage capacity in the litter layer and exhibit lower transpiration demands (Gallardo & Merino 1993, D'Odorico et al. 2005).

Finally, it is worth noting that *A. unedo* and *P. latifolia* (compared with *Q. ilex*), appear to require lower VPD for phenological initiation, which could likely be attributed to differences in leaf conductance and stomatal sensitivity to atmospheric dryness (Flexas & Medrano 2002, Diaz-Guerra et al. 2019, Koutra et al. 2022).

3.4. Strengths and limitations of the research study

This study presents a valuable long-term investigation of the relationships among microclimate, soil moisture, and phenological dynamics in eastern MED forest ecosystems under conditions of climate variability. It provides important insights from relatively understudied eastern MED ecosystems, revealing regional ecological patterns that may differ from those reported in more extensively studied western and central MED areas. Comparative assessment of three dominant species enhances the ecological relevance of the work by highlighting species-specific adaptation mechanisms and responses to water limitation. Moreover, integrating long-term microclimatic, hydrological, and phenological data strengthens the study's contribution to understanding forest ecosystem responses to ongoing climate change and enriches current forest research in MED.

Despite the strengths of our long-term dataset and field-based methodology, some limitations should be considered. First, our reliance on a single experimental plot may constrain the generalizability of our findings to the broader eastern Mediterranean region, given the substantial variability in soil type, aspect, and forest structure. Second, although the 10-year study period exhibited significant interannual variation, it may not fully capture long-term climate trends or the occurrence of future extreme events. Furthermore, the relatively wet conditions during the study period may have resulted in an underestimation of drought impacts on phenological stages. Lastly, our exclusive focus on SM, AT, VPD, PRB and REW precluded the examination of other potentially influential drivers, such as nutrient availability, biotic interactions, and genetic variation, which may also affect phenological responses.

4. Conclusions

Our results highlight the importance of vegetation identity and phenology in shaping microclimatic and soil water conditions of eastern Mediterranean evergreen broadleaved forests. The differences observed in the case of the three sclerophyllous species considered (*Q. ilex*, *A. unedo*, and *P. latifolia*), indicate that functional leaf and stem traits related to canopy structure and water use strategy can be modulated by significant ecological variables such as air temperature, soil moisture, vapor pressure deficit, and relative extractable water.

Soil moisture (SM) and relative extractable water (REW) emerged as the primary controls on the timing and duration of phenological stages, exceeding the direct influence of air temperature alone. Early initiation and prolonged stage length were most evident in years with strong seasonal contrasts in water availability (e.g., 2014 vs. 2017), highlighting the importance of intra-annual hydrological variability. These findings confirm that phenological dynamics in eastern Mediterranean maquis ecosystems are closely link to root-zone water status rather than precipitation totals.

Among the studied species, *Q. ilex* showed that it can be better overcome water deficit periods, exhibiting competition against the other two species and maintaining earlier onset of leaf unfolding and a longer stage duration during dry years. This behavior aligns with its deep rooting system, low hydraulic conductivity, and high resistance to embolism, reinforcing its competitive advantage under moderate drought conditions. Explicit long-term water-stress studies in the eastern Mediterranean remain relatively limited, but existing evidence supports analogous vegetative adaptive dynamics to those in the western Mediterranean.

In contrast, *P. latifolia* and *A. unedo* showed less flexibility in phenological timing compared to *Q. ilex*, highlighting interspecific differences in adaptive strategies. Notably, flowering in *A. unedo* was substantially delayed in dry years (e.g. 2017), indicating that reproductive phenology is particularly vulnerable to late-summer and autumn water deficits.

Our results also reveal bidirectional feedback between vegetation and microclimate: As phenological stages progressed (especially during defoliation), local air temperature and atmospheric dryness (VPD) tended to increase, while soil water availability declined. These interactions indicate that vegetation not only responds to climate but actively modulates its immediate environment through canopy structure, transpiration, and litter dynamics.

Overall, the studied period was relatively wet compared to projected future conditions. These mature ecosystems are regionally adapted to the meteorological conditions of the broader area, demonstrating similar adaptive responses to these in western Mediterranean forests, concerning the delayed onset and the length of the phenological stages investigated. While the ecosystem currently exhibits such resilience, the consequences of more frequent and prolonged drought events remain uncertain. Long-term, species-specific monitoring (ideally combined with remote sensing approaches such as NDVI thresholds) will be essential to detect early warning signals of stress, potential species turnover, and probable shifts in community composition. From a management perspective, maintaining mixed stands with complementary hydraulic and phenological traits may enhance ecosystem stability under climate variability. Conservation and restoration strategies should prioritize functional diversity, safeguard deep-rooted species like *Q. ilex*, and preserve habitat heterogeneity to buffer against increasing climatic extremes.

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References

Aguadé, D., Poyatos, R., Rosas, T., & Martínez-Vilalta, J. (2015). Comparative drought responses of *Quercus ilex* L. and *Pinus sylvestris* L. in a montane forest undergoing a vegetation shift. Forests, 6(8), 2505-2529.

Allen, R. G., Pereira, L. S., Raes, D., & Smith, M. (1998). Crop evapotranspiration-Guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. Fao, Rome, 300(9), D05109.

Anagiotos, G., Tsakaldimi, M., & Ganatsas, P. (2012). Variation in acorn traits among natural populations of *Quercus alnifolia*, an endangered species in Cyprus. Dendrobiology, 68, 3-10.

Barbeta, A., & Peñuelas, J. (2016). Sequence of plant responses to droughts of different timescales: lessons from holm oak (*Quercus ilex*) forests. Plant Ecology & Diversity, 9(4), 321-338.

Barbeta, A., Mejía-Chang, M., Ogaya, R., Voltas, J., Dawson, T. E., & Peñuelas, J. (2015). The combined effects of a long-term experimental drought and an extreme drought on the use of plant-water sources in a Mediterranean forest. Global change biology, 21(3), 1213-1225.

Benito-Verdugo, P., González-Zamora, Á., & Martínez-Fernández, J. (2024). Recent Cereal Phenological Variations under Mediterranean Conditions. Remote Sensing, 16(11), 1879.

Bogdziewicz, M., Fernández-Martínez, M., Espelta, J. M., Ogaya, R., & Penuelas, J. (2020). Is forest fecundity resistant to drought? Results from an 18-yr rainfall-reduction experiment. New Phytologist, 227(4), 1073-1080.

Bourletsikas A. (2024). Hydrological regime of a natural evergreen broadleaved forest ecosystem in Western Greece with emphasis on soil water availability. PhD thesis, pp. 362 (in Greek with an extended summary in English).

Bourletsikas, A., Proutsos, N., Michopoulos, P., & Argyrokastritis, I. (2023). Temporal variations in temperature and moisture soil profiles in a Mediterranean Maquis Forest in Greece. Hydrology, 10(4), 93.

Bussotti, F., & Pollastrini, M. (2020). Opportunities and threats of Mediterranean evergreen sclerophyllous woody species subjected to extreme drought events. Applied Sciences, 10(23), 8458.

Camarero, J. J., Sangüesa-Barreda, G., & Vergarechea, M. (2016). Prior height, growth, and wood anatomy differently predispose to drought-induced dieback in two Mediterranean oak speciesk. Annals of Forest Science, 73, 341-351.

Campelo, F., Rubio-Cuadrado, Á., Montes, F., Colangelo, M., Valeriano, C., & Camarero, J. J. (2023). Growth phenology adjusts to seasonal changes in water availability in coexisting evergreen and deciduous mediterranean oaks. Forest Ecosystems, 10, 100134.

Castell, C., Terradas, J., & Tenhunen, J. D. (1994). Water relations, gas exchange, and growth of resprouts and mature plant shoots of *Arbutus unedo* L. and *Quercus ilex* L. Oecologia, 98, 201-211.

Condon, L. E., Atchley, A. L., & Maxwell, R. M. (2020). Evapotranspiration depletes groundwater under warming over the contiguous United States. Nature communications, 11(1), 873.

Deng, Y., Wang, S., Bai, X., Luo, G., Wu, L., Cao, Y., ... & Tian, S. (2020). Variation trend of global soil moisture and its cause analysis. Ecological Indicators, 110, 105939.

Dogan Ciftci, N., Şahin, A. D., Yousefpour, R., & Christen, A. (2024). Effects of climate trends and variability on tree health responses in the Black Sea and Mediterranean forests of Türkiye. Theoretical and Applied Climatology, 155(5), 3969-3991.

D'Odorico, P., Laio, F., & Ridolfi, L. (2005). Noise-induced stability in dryland plant ecosystems. Proceedings of the National Academy of Sciences, 102(31), 10819–10822.sciences.ucf.edu

Díaz-Guerra, L., Llorens, L., Bell, T. L., Font, J., González, J. A., & Verdaguer, D. (2019). Physiological, growth and root biochemical responses of *Arbutus unedo* and *Quercus suber* seedlings to UV radiation and water availability before and after aboveground biomass removal. Environmental and Experimental Botany, 168, 103861.

Doumkou, O., Markaki, M., Vanikiotis, T., & Kyparissis, A. (2025). Climate Effects on Phenology of Two Deciduous Forest Species Across Southern Europe. Forests, 16(4), 608. https://doi.org/10.3390/f16040608.

Douville, H., Raghavan, K., Renwick, J., Allan, R. P., Arias, P. A., Barlow, M., ... & Zolina, O. (2021). Water cycle changes. In Climate Change 2021: The Physical Science Basis, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1055–1210, doi:10.1017/9781009157896.010.

FAO-Unesco (1988), Soil map of the world. FAO-Unesco, Rome, Italy.

Flexas, J., & Medrano, H. (2002). Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. Annals of botany, 89(2), 183-189.

Franks, S. J., Weber, J. J., & Aitken, S. N. (2014). Evolutionary and plastic responses to climate change in terrestrial plant populations. Evolutionary applications, 7(1), 123-139.

Gallardo, A., & Merino, J. (1993). Leaf decomposition in two Mediterranean ecosystems of southwest Spain: influence of substrate quality. Ecology, 74(1), 152–161.

Giorgi, F. (2006). Climate change hot-spots. Geophysical research letters, 33(8).

Gratani, L., Catoni, R., & Varone, L. (2013). Morphological, anatomical and physiological leaf traits of *Q. ilex, P. latifolia, P. lentiscus*, and *M. communis* and their response to Mediterranean climate stress factors. Botanical Studies, 54, 1-12.

Gratani, L., Covone, F., & Larcher, W. (2006). Leaf plasticity in response to light of three evergreen species of the Mediterranean maquis. Trees, 20, 549-558.

Gunderson, C. A., Edwards, N. T., Walker, A. V., O'Hara, K. H., Campion, C. M., & Hanson, P. J. (2012). Forest phenology and a warmer climate–growing season extension in relation to climatic provenance. Global Change Biology, 18(6), 2008-2025.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. (2006). World map of the Köppen-Geiger climate classification updated.

Kougioumoutzis, K., Constantinou, I., & Panitsa, M. (2024). Rising temperatures, falling leaves: predicting the fate of Cyprus’s endemic oak under climate and land use change. Plants, 13(8), 1109.

Koutra, E., Chondrogiannis, C., & Grammatikopoulos, G. (2022). Variability of the photosynthetic machinery tolerance when imposed to rapidly or slowly imposed dehydration in native Mediterranean plants. Photosynthetica, 60(1), 88.

Lazoglou, G., Papadopoulos-Zachos, A., Georgiades, P., Zittis, G., Velikou, K., Manios, E. M., & Anagnostopoulou, C. (2024). Identification of climate change hotspots in the Mediterranean. Scientific Reports, 14(1), 29817.

Lee, J. Y., Marotzke, J., Bala, G., Cao, L., Corti, S., Dunne, J. P., ... & Christensen, H. M. (2021). Future global climate: scenario-based projections and near-term information.

Lee, H., Calvin, K., Dasgupta, D., Krinner, G., Mukherji, A., Thorne, P., ... & Park, Y. (2023). IPCC, 2023: Climate change 2023: Synthesis report, summary for policymakers. Contribution of working groups i, II and III to the sixth assessment report of the intergovernmental panel on climate change [core writing team, h. Lee and j. Romero (eds.)]. IPCC, geneva, Switzerland.

Limousin, J. M., Rambal, S., Ourcival, J. M., Rocheteau, A., Joffre, R., & Rodriguez-Cortina, R. (2009). Long-term transpiration change with rainfall decline in a Mediterranean Quercus ilex forest. Global Change Biology, 15(9), 2163-2175.

Limousin, J. M., Roussel, A., Rodríguez-Calcerrada, J., Torres-Ruiz, J. M., Moreno, M., Garcia de Jalon, L., ... & Martin-StPaul, N. (2022). Drought acclimation of Quercus ilex leaves improves tolerance to moderate drought but not resistance to severe water stress. Plant, Cell & Environment, 45(7), 1967-1984. https://doi.org/10.1111/pce.14326

Liu, Y., & Yang, Y. (2023). Spatial-temporal variability pattern of multi-depth soil moisture jointly driven by climatic and human factors in China. Journal of Hydrology, 619, 129313.

Lobo, A., Torres-Ruiz, J. M., Burlett, R., Lemaire, C., Parise, C., Francioni, C., ... & Delzon, S. (2018). Assessing inter-and intraspecific variability of xylem vulnerability to embolism in oaks. Forest Ecology and Management, 424, 53-61.

Maestre, F. T., & Cortina, J. (2004). Are Pinus halepensis plantations useful as a restoration tool in semiarid Mediterranean areas?. Forest ecology and management, 198(1-3), 303-317.

Maselli, F., Cherubini, P., Chiesi, M., Gilabert, M. A., Lombardi, F., Moreno, A., ... & Tognetti, R. (2014). Start of the dry season as a main determinant of inter-annual Mediterranean forest production variations. Agricultural and Forest Meteorology, 194, 197-206.

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aasa, A., Ahas, R., ... & Zust, A. N. A. (2006). European phenological response to climate change matches the warming pattern. Global change biology, 12(10), 1969-1976.

Moreno, J., & Oechel, W. C. (Eds.). (2012). Global change and Mediterranean-type ecosystems (Vol. 117). Springer Science & Business Media.

Ogaya, R., & Peñuelas, J. (2004). Phenological patterns of *Quercus ilex, Phillyrea latifolia,* and *Arbutus unedo* growing under a field experimental drought. Ecoscience, 11(3), 263-270.

Ogaya, R., & Penuelas, J. (2006). Contrasting foliar responses to drought in *Quercus ilex* and *Phillyrea latifolia.* Biologia plantarum, 50, 373-382.

Ogaya, R., & Peñuelas, J. (2021). Climate change effects in a Mediterranean forest following 21 consecutive years of experimental drought. Forests, 12(3), 306.

Ozturk, M., Altay, V., Gönenç, T. M., Unal, B. T., Efe, R., Akçiçek, E., & Bukhari, A. (2021). An overview of olive cultivation in Turkey: Botanical features, eco-physiology and phytochemical aspects. Agronomy, 11(2), 295.

Pareja-Bonilla, D., Arista, M., Morellato, L. P. C., & Ortiz, P. L. (2025). Better soon than never: climate change induces strong phenological reassembly in the flowering of a Mediterranean shrub community. Annals of Botany, 135(1-2), 239-254.

Parmesan, C., & Hanley, M. E. (2015). Plants and climate change: complexities and surprises. Annals of botany, 116(6), 849-864.

Pausas, J. G., & Millán, M. M. (2019). Greening and browning in a climate change hotspot: the Mediterranean Basin. BioScience, 69(2), 143-151.

Peñuelas, J., Sardans, J., Filella, I., Estiarte, M., Llusià, J., Ogaya, R., ... & Terradas, J. (2018). Assessment of the impacts of climate change on Mediterranean terrestrial ecosystems based on data from field experiments and long-term monitored field gradients in Catalonia. Environmental and Experimental Botany, 152, 49-59.

Ponce, Á., Alday, J. G., de Aragón, J. M., Collado, E., Morera, A., Bonet, J. A., & De-Miguel, S. (2022). Environmental drivers shaping the macrofungal sporocarp community in Mediterranean *Quercus ilex* stands. Forest Ecology and Management, 524, 120523.

Proutsos, N. D., Solomou, A. D., Koulelis, P. P., Bourletsikas, A., Chatzipavlis, N. E., & Tigkas, D. (2022, September). Detecting Changes in Annual Precipitation Trends During the Last Two Climatic Periods (1955-1984 and 1985-2018) in Nestos River Basin, N. Greece. In HAICTA (pp. 456-463).

Proutsos, N. D., Tsiros, I. X., Nastos, P., & Tsaousidis, A. (2021). A note on some uncertainties associated with Thornthwaite's aridity index introduced by using different potential evapotranspiration methods. Atmospheric Research, 260, 105727.

Raspe S, Fleck S, Beuker E, Bastrup-Birk A, Preuhsler T, 2020: Part VI: Phenological Observations. Version 2020-3. In: UNECE ICP Forests Programme Co-ordinating Centre (ed.): Manual on methods and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. Thünen Institute of Forest Ecosystems, Eberswalde, Germany, 14 p. + Annex [http://www.icp-forests.org/Manual.htm]

Richardson, A. D., Keenan, T. F., Migliavacca, M., Ryu, Y., Sonnentag, O., & Toomey, M. (2013). Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. Agricultural and Forest Meteorology, 169, 156-173.

Sardans, J., & Peñuelas, J. (2013). Plant-soil interactions in Mediterranean forest and shrublands: impacts of climatic change. Plant and soil, 365, 1-33.

Spano, D., Snyder, R. L., & Cesaraccio, C. (2013). Mediterranean Phenology. In Phenology: An Integrative Environmental Science (pp. 173-196). Dordrecht: Springer Netherlands.

Thornthwaite, C. W. (1948). An approach toward a rational classification of climate. Geographical review, 38(1), 55-94.

Thornthwaite, C. W. and Mather, J. R. 1955. The water balance. Publications in Climatology, Vol. 8, 1–104

Touhami, I., Rzigui, T., Zribi, L., Ennajah, A., Dhahri, S., Aouinti, H., ... & Khouja, M. L. (2023). Climate change-induced ecosystem disturbance: a review on sclerophyllous and semi-deciduous forests in Tunisia. Plant Biology, 25(4), 481-497.

Ulas, M., & Gezerel, O. (2004). Studies on the morphological, phenological and pomological characteristics of some table olive and olive oil cultivars in Cukurova region. In V International Symposium on Olive Growing 791 (pp. 103-107).

UNEP (1992). Middleton, N. J., & Thomas, D. S. (Eds.). World atlas of desertification (pp. ix+-69).

Tsiros, I. X., Nastos, P., Proutsos, N. D., & Tsaousidis, A. (2020). Variability of the aridity index and related drought parameters in Greece using climatological data over the last century (1900–1997). Atmospheric Research, 240, 104914. https://doi.org/10.1016/j.atmosres.2020.104914

Valladares, F., Guzmán, B., & Pearcy, R. W. (2006). Photoinhibition and drought in Mediterranean woody saplings: scaling effects and interactions in two co-occurring species. Tree Physiology, 26(4), 479–488.old.valladares.info

Vicente, E., Vilagrosa, A., Ruiz-Yanetti, S., Manrique-Alba, À., González-Sanchís, M., Moutahir, H., ... & Bellot, J. (2018). Water balance of Mediterranean *Quercus ilex* L. and *Pinus halepensis* Mill. forests in semiarid climates: a review in a climate change context. Forests, 9(7), 426.

Vico, G., Thompson, S. E., Manzoni, S., Molini, A., Albertson, J. D., Almeida-Cortez, J. S., ... & Porporato, A. (2015). Climatic, ecophysiological, and phenological controls on plant ecohydrological strategies in seasonally dry ecosystems. Ecohydrology, 8(4), 660-681.

Vogel, J. (2022). Drivers of phenological changes in southern Europe. International Journal of Biometeorology, 66(9), 1903-1914.

Wolkovich, E. M., Cook, B. I., Allen, J. M., Crimmins, T. M., Betancourt, J. L., Travers, S. E., ... & Cleland, E. E. (2012). Warming experiments underpredict plant phenological responses to climate change. Nature, 485(7399), 494-497.

Yuan, W., Zheng, Y., Piao, S., Ciais, P., Lombardozzi, D., Wang, Y., ... & Yang, S. (2019). Increased atmospheric vapor pressure deficit reduces global vegetation growth. Science advances, 5(8), eaax1396.

Zhang, H., Yuan, N., Ma, Z., & Huang, Y. (2021). Understanding the soil temperature variability at different depths: Effects of surface air temperature, snow cover, and the soil memory. Advances in Atmospheric Sciences, 38, 493-503.