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Environmental Controls on the Seasonal Variation in Gas Exchange and Water Balance in a Near-Coastal Mediterranean *Pinus halepensis* Forest

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Abstract: Aleppo pine (*Pinus halepensis* Mill.) is widespread in most countries of the Mediterranean area. In Greece, Aleppo pine forms natural stands of high economic and ecological importance. Understanding the species' ecophysiological traits is important in our efforts to predict its responses to ongoing climate variability and change. Therefore, the aim of this study was to assess the seasonal dynamic in Aleppo pine gas exchange and water balance on the leaf and canopy levels in response to the intra-annual variability in the abiotic environment. Specifically, we assessed needle gas exchange, water potential and $\delta^{13}C$ ratio, as well as tree sap flow and canopy conductance in adult trees of a mature near-coastal semi-arid Aleppo pine ecosystem, over two consecutive years differing in climatic conditions, the latter being less xerothermic. Maximum photosynthesis (A_{max}), stomatal conductance (g_s), sap flow per unit leaf area (Q_l), and canopy conductance (G_s) peaked in early spring, before the start of the summer season. During summer drought, the investigated parameters were negatively affected by the increasing potential evapotranspiration (PET) rate and vapor pressure deficit (VPD). Aleppo pine displayed a water-saving, drought avoidance (isohydric) strategy via stomatal control in response to drought. The species benefited from periods of high available soil water, during the autumn and winter months, when other environmental factors were not limiting. Then, on the leaf level, air temperature had a significant effect on A_{max}, while on the canopy level, VPD and net radiation affected Q_l. Our study demonstrates the plasticity of adult Aleppo pine in this forest ecosystem in response to the concurrent environmental conditions. These findings are important in our efforts to predict and forecast responses of the species to projected climate variability and change in the region.

Keywords: Aleppo pine; Greece; photosynthesis; water potential; $\delta^{13}C$; sap flow; canopy conductance; climate

1. Introduction

Aleppo pine (*Pinus halepensis* Mill.) is widespread in most countries of the Mediterranean area [1,2]. In Greece, Aleppo pine forms forests of economic importance (e.g., wood, resin, medicinal, and honey products) [1], comprising 26% of the coniferous forests in the country.

Aleppo pine can reach heights of 10–20 m, depending on the precipitation regime in the area, and has relatively shallow roots, usually not exceeding depths of 5 m [3]. The species is adapted to the xerothermic conditions (high temperatures and droughts) of the Mediterranean, due to its drought avoidance strategy of reducing stomatal conductance under water shortage [4]. This isohydric response allows Aleppo pine to limit the reduction of needle water potential and xylem cavitation, to which it is quite vulnerable [5,6]. Moreover, Aleppo pine has adapted its physiological activity to the seasonally changing climatic regime in the region. It actively grows during two periods of the growing season (spring and autumn) when temperatures are favorable and water is available. This behavior is more pronounced in the coastal regions than in continental forest sites [7]. On the other hand, extreme winter and summer temperatures and intensive summer droughts may cause growth activity to cease [8] and lead to extensive dieback and growth declines in Aleppo pine forests [9].

Improving our understanding of the driving factors that control Aleppo pine responses to climatic conditions is important for managing the species and forecasting its responses to climate variability, extremes, and change. Relationships between various physiological traits and the abiotic environment have been reported in the literature for Aleppo pine [10–12]. Studies have demonstrated a strong stomatal regulation in the species and coordination between foliage water potential and stomatal conductance to balance water loss [13]. However, needle and canopy stomatal responses to changes in evaporative demands, especially in combination with high temperatures remain unclear. Aleppo pine populations vary greatly in their response to extreme weather events across their distribution range [9,14]. Populations growing under the driest environments seem to be most impacted by extreme droughts and are prone to growth decline, but recover quickly. It is, however, unknown how the species will respond to the drier and hotter conditions forecasted for the Mediterranean basin under climate change [15], particularly in the eastern part [16]. Studying the seasonal dynamics of physiological traits in Aleppo pine in response to the concurrent climatic conditions will advance our understanding of the drivers that control growth and performance in the species and resilience of these forests. Few studies on the ecophysiological responses of Aleppo pine to drought regimes included Greek provenances and have focused on ecotypic variability assessed in plantations [17–20], not on adult trees of natural Aleppo pine forests. Thus, information on the ecophysiological responses of natural Aleppo pine forests in Greece is scarce.

In the present study, we assessed the seasonal dynamics of physiological traits of a mature near-coastal Aleppo pine ecosystem in Sani, Chalkidiki, northern Greece, over two consecutive years. We measured foliage gas exchange, water potential and stable carbon isotopic ratio, and tree sap flow rate and canopy conductance to characterize water balance dynamics of the species in response to climatic variability. Our specific aims were to (a) describe the seasonal variation in the physiological traits of Aleppo pine trees and (b) determine the climatic factors that control the observed seasonal trends. The combination of selected complementary techniques provides vital information from the needle to the stand level for assessing the performance of this dominant Mediterranean forest species under the prevailing climate change.

2. Materials and Methods

2.1. Site Description

The study was conducted at the peninsula of Kassandra, Chalkidiki, Greece. The experimental site is located at the Stavronikita forest (latitude: 40°06′22′′ N, longitude: 23°18′80′′ E, altitude 15 m.a.s.l., slope 1%, c. 300 m distance from the coast). The site is in a natural Aleppo pine (*Pinus halepensis*) stand with a mean tree height of 16 m, a mean diameter at breast height of 45 cm, a mean tree basal area of

0.19 m^2, and a stand basal area of 23.68 m^2ha^{-1}. The understorey consists of a maquis shrub vegetation, dominated with *Pistacia lentiscus* L., *Phyllirea media* L., and *Quercus coccifera* L. The soil has a high pH (7.5–8.2) and, according to European soil classification, it lies at the boundary between Calcari-chromic Vertisols and Chromic Luvisols [21,22].

2.2. Environmental Conditions

The climate on site is Mediterranean (Csa), according to Köppen-Geiger's classification, and is characterized by rainy winters and semi-arid growing seasons [23]. Micrometeorological data are available for the period 1978–1997 and from 2007 to present, from a fully automated weather station operating at a c. 50 m distance from the forest stand. Air temperature and air relative humidity (RHT2nl, Delta-T Devices Ltd., Cambridge, UK), photosynthetically active radiation (SKP215; Skye Instruments Ltd., Llandrindod Wells, UK), solar radiation (SKS1110, Skye Instruments, UK), wind speed (model 4.3515.30.000, THIES CLIMA, Göttingen, Germany), wind direction (WD4, Delta-T Devices Ltd., UK), precipitation (AR100 and RGB1, EM UK), and soil temperature at a depth of 15 cm (ST1, Delta-T Devices Ltd., UK) were continuously recorded. All parameters were data-logged on a 1-h basis (DL2e Delta-T Logger, Delta-T Devices Ltd., Cambridge, UK). Missing data due to a short-term malfunction of the meteorological station were completed after extrapolation from the respective data from the closest meteorological station of Loutra Thermis (latitude 40°30′ N, longitude 23°04′ E, 30 m.a.s.l.). The filling of the missing data gaps was performed by using the double-mass curve technique [24] followed by a t-test [25]. Moreover, vapor pressure deficit (VPD) was estimated using the RayMan model [26,27], while potential stand evapotranspiration (PET) and available soil water capacity (aSWC) of the study site were calculated with the water balance model WBS3. WBS3 is a forest–hydrological model that requires daily mean values of air temperature and daily total precipitation as meteorological inputs [28] and takes into account several forest stand parameters as input, as described in detail in a previous study [29].

An aridity index (AI) [30] was selected to estimate aridity conditions prevailing at the study area. A number of aridity indices have been proposed; these indicators serve to identify, locate, or delimit regions that suffer from a deficit of water availability [31]. The aridity index is estimated as follows (Equation (1)):

$$AI = P/PET, \quad (1)$$

where P is precipitation (mm), which in our study is equal to rainfall, and PET is potential evapotranspiration (mm). The boundaries that define the various degrees of aridity are shown in Table S1 [32].

2.3. Measurement Campaigns

Four dominant, non-neighboring Aleppo pine trees were selected for measurements and needle collection. Attention was paid to choosing healthy individuals, since infestation by the insect *Marhalina hellenica* (Genn.) is spread in *Pinus halepensis* forests of Chalkidiki. Three sun-exposed branches of the lower canopy (approximately three meters above ground) were marked and were thereafter used for measurements of gas exchange and midday water potential. After completion of each set of gas exchange measurements, the needles were sampled for carbon isotopic ratio analysis, as described below. Neighboring needles of the same branches were used for water potential measurements.

Measurements were conducted over two consecutive years on a monthly basis; gas exchange was measured from December 2007 to November 2009, while needle midday water potential was measured from January 2008 to October 2009. Needle $\delta^{13}C$ was determined from January 2008 to May 2009 due to technical limitations.

2.4. Gas Exchange and Needle Water Potential

For gas exchange measurement, we used the Li-6400 open path infra-red gas analyzer with a Li 6400-40 fluorescence chamber (Li-Cor, Lincoln, NE, USA). Maximum photosynthesis (A_{max}) and stomatal conductance (g_s) measurements were conducted on current year, fully expanded, and sun exposed needles between 10:00 and 13:00. The needles were carefully arranged in the 2-cm^2 cuvette in a way to exclude overlapping and to fully cover the area of the cuvette and they were acclimated for c. 10 min in the chamber at a CO_2 concentration of 400 ppm, under a photosynthetically active radiation (PAR) level of 1000 $\mu molm^{-2}s^{-1}$ from November to March and 1500 $\mu molm^{-2}s^{-1}$ from April to October. CO_2 flow rate was set to 300 $\mu mols^{-1}$ and temperature inside the chamber was controlled within the range of 17–28 °C, depending on the seasonal fluctuation of ambient air temperature.

Midday water potential (midday Ψ) measurements were conducted between 13:00 to 14:00 using a portable pressure chamber (model PMS 1003, PMS Instruments, Corvallis, OR, USA). The needles' water potential was measured after gas exchange measurements.

2.5. Needle $\delta^{13}C$ Signature

At the study site, new needles were fully expanded until the end of May each year. New needles (<1 year old), fully developed from the preceding May, were collected on a monthly basis from the same dominant Aleppo pine trees for the determination of the needle carbon isotopic ratio ($\delta^{13}C$). Samples were oven-dried at 65 °C until at a constant weight and then sent to the University of Nebraska Water Sciences Laboratory for analysis (https://watersciences.unl.edu/). Samples were finely ground and $\delta^{13}C$ was determined using mass spectrometry. The carbon isotope ratio ($\delta^{13}C$) of each sample was then determined as $\delta^{13}C$ (‰) = [(Rsample/Rstandard) − 1] × 1000, where Rsample is the $^{13}C/^{12}C$ of the sample and Rstandard is the $^{13}C/^{12}C$ ratio of the Vienna Pee Dee Belemnite (VPDB) standard.

2.6. Tree Sap Flow and Canopy Stomatal Conductance

Xylem sap flux was monitored using the thermal dissipation method [33,34]. In July 2008, 2-cm long Granier-type sensors and measurements were taken until early November 2009. Probe pairs were inserted radially into the stem of five dominant Aleppo pine trees averaging 43.5 cm diameter at breast height (DBH) with a vertical separation between the probes of approximately 12.0 cm. Probes were installed in the outer sapwood of the north-facing side of the stem and both probes and stems were insulated to minimize natural temperature gradients.

The temperature difference between the Granier-type probes was recorded at 10 second intervals and stored as 15 min averages on a data logger (CR10X Campbell Scientific, Logan, UT, USA) and used to obtain sap flux density by means of the equation derived empirically by Granier [33]. The daily maximum temperature difference was used as an estimate of the temperature difference under zero flow conditions. This variable was approximately constant over the study period (average coefficient of variation ± SE = 1.24% ± 0.04%).

Natural temperature gradients in the stem can interfere with sap flow measurements. These were measured over 60 days, but as values were consistently <5.2% of the sap flow signal, no corrections were applied [35].

The thickness of active sapwood was estimated using an allometric relationship obtained from a close by Aleppo pine site (in Peukochori, Chalkidiki; Radoglou K, unpublished data). For this purpose, 20 wood slices were used to estimate sapwood and heartwood areas. The equation best fitted to our data ($r^2 = 0.999$) was $A_s = 0.077 \times (DBH^{1.9905})$, where A_s stands for sapwood area (in m^2) and DBH for diameter at breast height (in m). Allometric relationships were also applied to estimate total tree leaf area [36] and used to calculate sap flow per unit leaf area (Q_l; $kgm^{-2}day^{-1}$).

Canopy stomatal conductance (G_s; mms^{-1}) was derived from sap flow measurements as described by [37].

Mean daily values of Q_l and G_s corresponding to days with mean daily values of VPD <0.1 kPa were excluded [38].

2.7. Statistical Analysis

Statistical analysis was performed with SPSS 23.0 (IBM Corp., SPSS for Windows, NY, USA) and OriginPro 8.0 (OriginLab Corp., Northampton, MA, USA). Relationships between physiological traits, as well as between physiological and single or combined environmental parameters, were examined using linear and non-linear regression analyses and coefficients of determination (adjusted R^2). The physiological traits tested were A_{max}, g_s, Ψ_{mid}, needle $\delta^{13}C$, Q_l, and G_s, while the respective environmental parameters were rainfall, air relative humidity, VPD, mean, maximum and minimum air temperature (T_{mean}, T_{max} and T_{min}, respectively), PET, net radiation, daytime net radiation, and aSWC of the actual day the physiological parameters were measured, or averaged over (a) the respective month, (b) the preceding month, (c) one week prior to measurements, and (d) two weeks prior to measurements. For the regression model between Q_l, VPD, and net radiation, mean hourly values for the wetter period (November to March of each study year, when data were available) were considered after excluding the ones corresponding to VPD <0.1 KPa. For all analyses, the environmental parameter(s) having an insignificant effect on each regression model ($p > 0.05$) were excluded from the model. When the combined effect of more than one environmental parameter on physiological traits was tested, only the independent environmental parameters were entered into the regression model. All tested significant regression models are presented in Table S2. The regression models with the highest adjusted R^2 and the highest significance level are presented in figures. The level of significance of each relationship ($p < 0.05$, $p < 0.01$, $p < 0.001$) is given in the respective plot.

3. Results

3.1. Climatic Conditions

The seasonal fluctuation of T_{mean} cumulative precipitation, mean aSWC, and mean VPD during the two-year study is presented in Figure 1. On average, 2009 was characterized by a combination of both higher average air temperatures and rainfall relative to 2008, resulting in lower VPD, higher aSWC, and higher aridity index (less xerothermic conditions) in 2009 compared to 2008 and to averages from the previous decade (Table 1).

Table 1. Annual cumulative rainfall and mean annual aridity index, aSWC and VPD during the study years and the previous decade.

Period	Rainfall (mm)	Aridity Index	aSWC (%)	VPD (KPa)
2008	542.1	0.77	36.9	0.55
2009	680.2	1.16	46.6	0.46
2008–2017	544.9	0.84	37.5	0.48

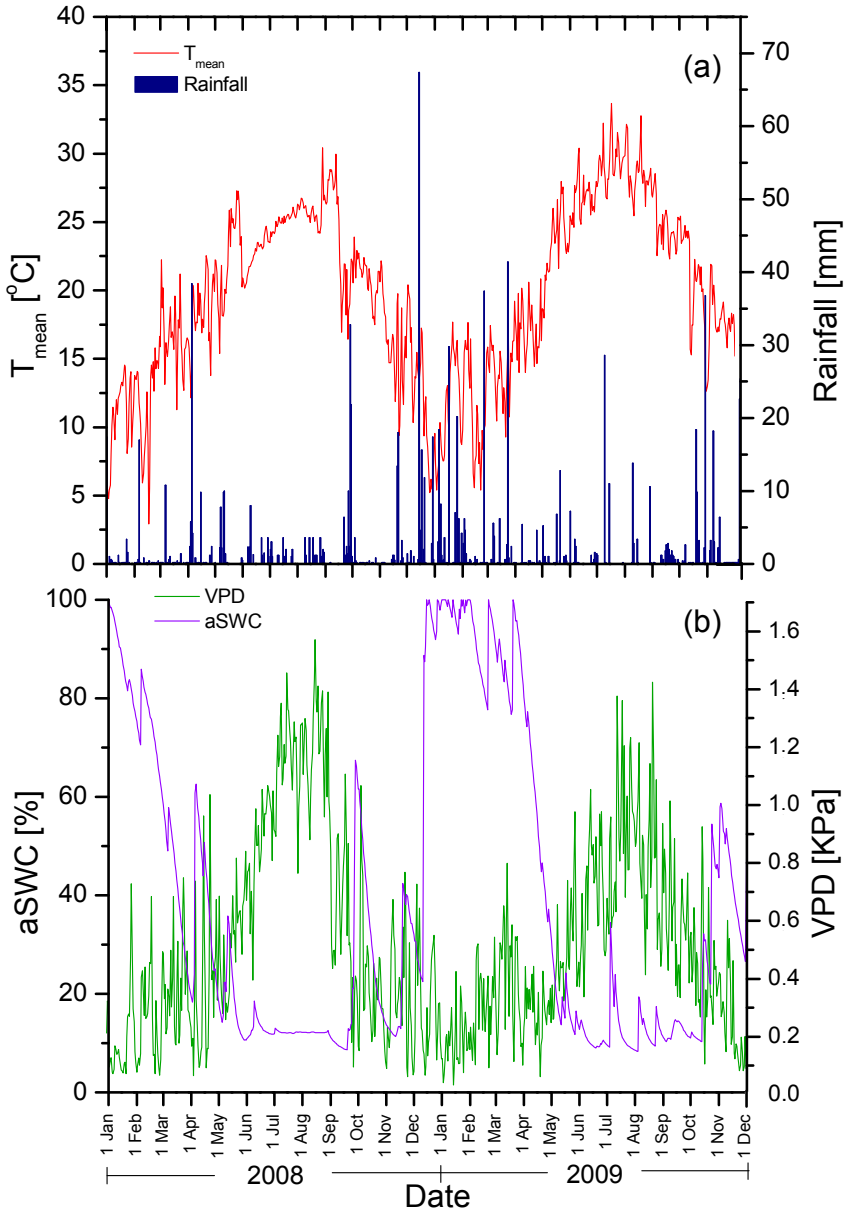


Figure 1. Daily values of: (**a**) Mean air temperature (T_{mean}) and rainfall and (**b**) available soil water capacity (aSWC) and vapor pressure deficit (VPD) during the study period.

3.2. Seasonal Patterns of Gas Exchange, Needle Water Potential, and $\delta^{13}C$ Composition

Gas exchange rates were high at the beginning of April in both years of measurements (Figure 2a,b). In 2008, a second pick in gas exchange was recorded in early July, before the summer drought was intensified, whereas in 2009 a second pick was evident in early October, after the completion of the drought season.

The combined effects of daytime net radiation and T_{mean} on sampling dates largely explains the variation in A_{max} between October and March ($R_{adj}^2 = 0.62$, $p < 0.05$; Figure 3). The particularly low A_{max} values observed in October and November 2008, compared to the same period in 2009, could be due to the substantially lower air temperatures of the former period compared to the latter. T_{mean} ranged from 8.5 to 15.5 °C in October–November measuring days of 2008 vs. 19.6 to 21.7 °C in 2009, while the T_{min} of the preceding nights was, similarly, lower in these measuring days of 2008 (5.4–11.2 °C) vs. 2009 (15.7–18.4 °C).On the other hand, the higher T_{mean}, increased aSWC, and decreased VPD during October–December of 2009, compared to the same period in 2008, resulted in

a substantial increase in gas exchange, which reached values comparable to those observed during the spring in Aleppo pine. The strong and significant relationship between A_{max} and g_s ($R_{adj}^2 = 0.59$, $p < 0.01$; Figure 4a) indicated a close stomatal regulation of photosynthesis during the study period.

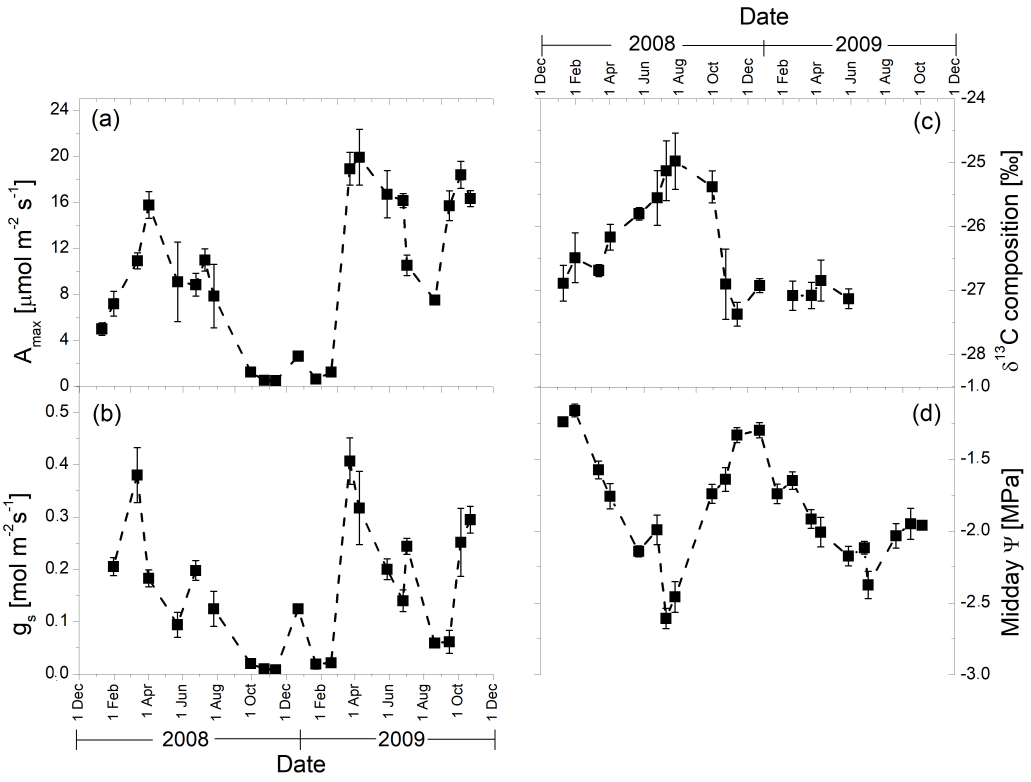


Figure 2. Seasonal pattern of monthly (**a**) maximum photosynthesis (A_{max}), (**b**) stomatal conductance (g_s), (**c**) $\delta^{13}C$ ratio, and (**d**) midday water potential (Ψ) during two consecutive years (2008 and 2009). $n = 4$ trees ± SE.

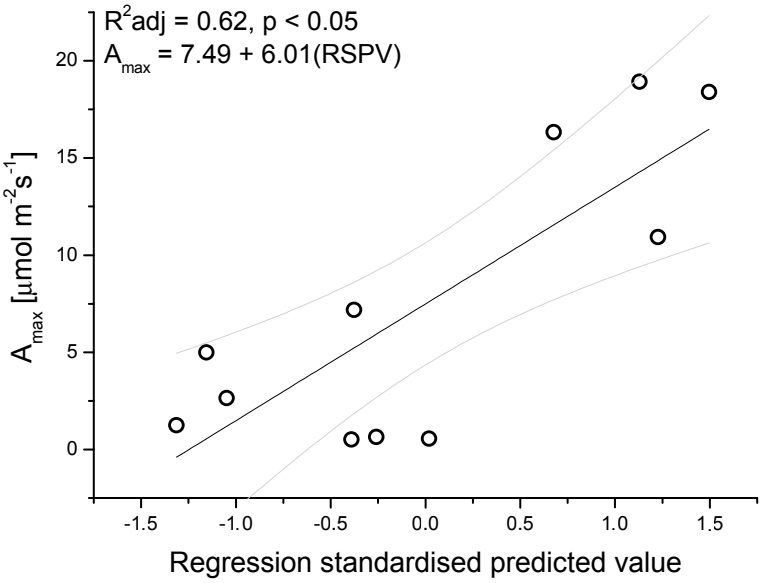


Figure 3. Regression model describing the combined effect of net radiation [kWm^{-2}] and mean air temperature [°C] on needle A_{max} during the period October–March. Mean daily values of net radiation and air temperature corresponding to the days of A_{max} measurements were used. For net radiation, only daytime values were used. RSPV stands for regression standardized predicted value. The confidence levels of the models are depicted by grey upper and lower bands.

The $\delta^{13}C$ ratio and midday Ψ of the current year needles displayed seasonal variability (Figure 2c,d) that was more pronounced in 2008 than 2009, consistent with the observed lower VPD and higher aSWC in 2009 vs. 2008 (Figure 1; Table 1). A significant negative linear relationship was recorded between $\delta^{13}C$ and Ψ ($R_{adj}^2 = 0.38$, $p < 0.05$; Figure 4b), with the highest $\delta^{13}C$ and the lowest midday Ψ values reported in August. Among all tested environmental parameters, the average VPD over two weeks period prior to sampling had the strongest effect on the $\delta^{13}C$ ratio of the needles ($R_{adj}^2 = 0.64$, $p < 0.001$; Figure 4a). The PET of the day of measurements was found to be the strongest predictor of midday Ψ ($R_{adj}^2 = 0.72$, $p < 0.001$; Figure 5b).

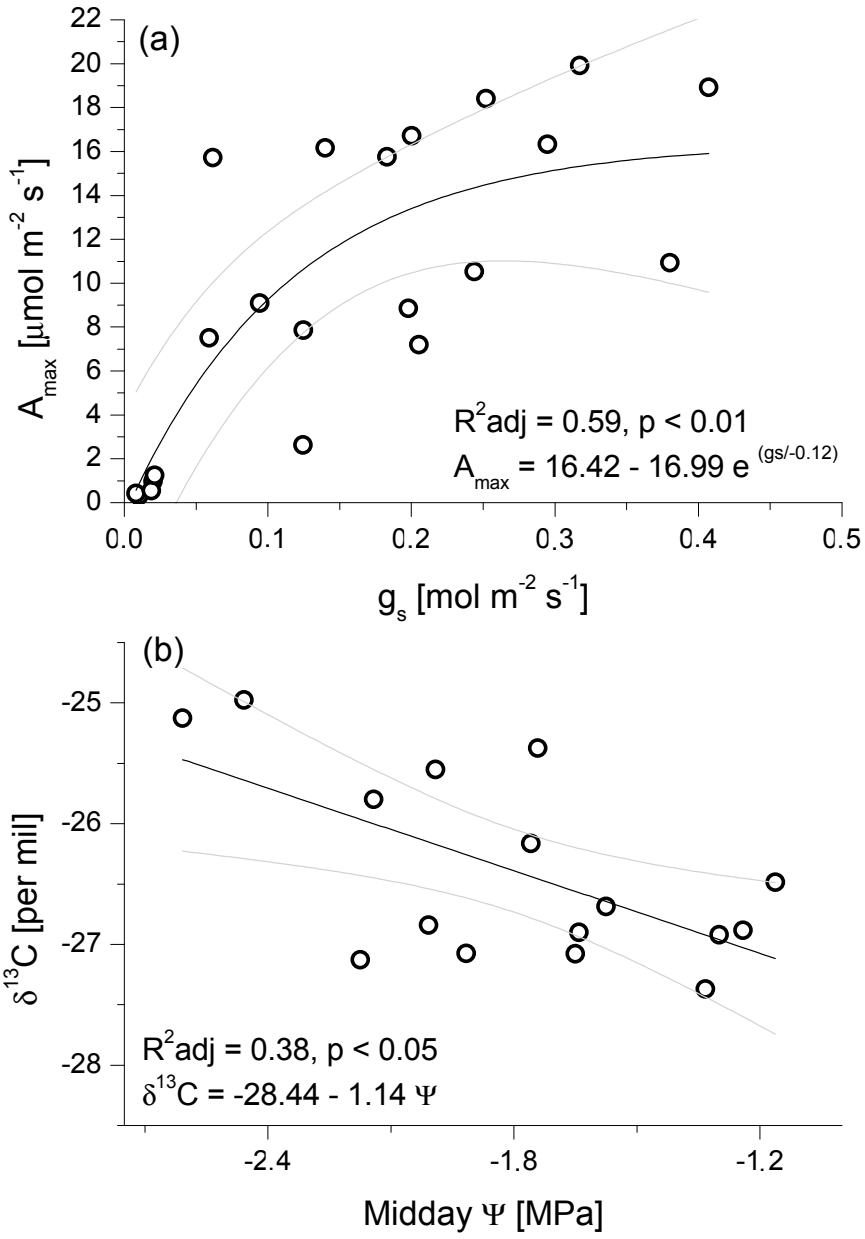


Figure 4. Regression models describing the relationship between needle (**a**) A_{max} and g_s and (**b**) $\delta^{13}C$ and midday Ψ. The confidence levels of the models are depicted by grey upper and lower bands.

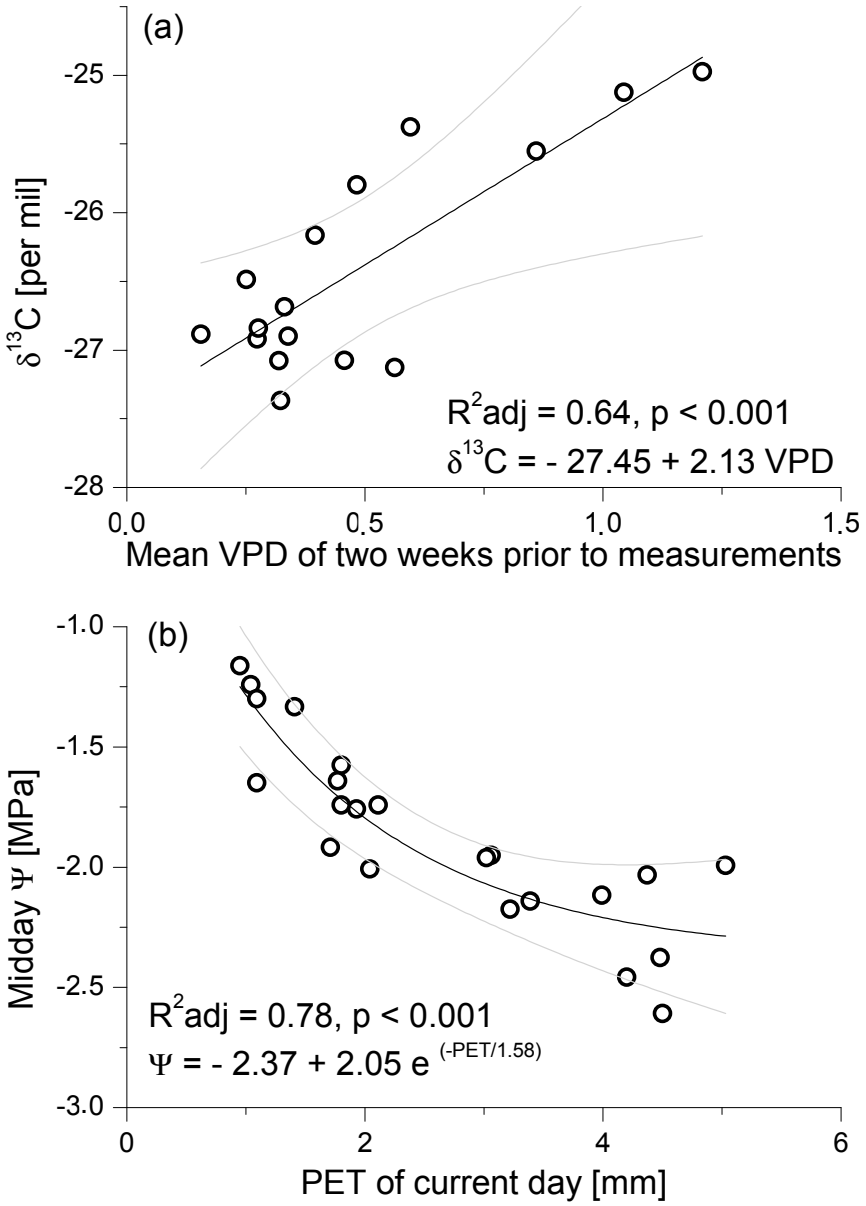


Figure 5. Regression models describing the relationship between (**a**) needle $\delta^{13}C$ and mean vapor pressure deficit (VPD) averaged over the preceding two weeks prior to measurements and (**b**) needle midday Ψ and potential stand evapotranspiration (PET) of the current day of measurements. The confidence levels of the models are depicted by grey upper and lower bands.

3.3. Seasonal Patterns of Sap Flow and Canopy Conductance

In 2009, maximum Q_l values were reached early in the growing season, when mean daily aSWC was still quite high (Figure 6a). Comparison with the same period in 2008 was not possible, since sap flow measurements were initiated in July 2008. In addition, datalogger malfunctioning resulted in missing values in April 2009, thus, not allowing comparison with the gas exchange maximum values in April. During the dry months, from July to September of both study years, similar declining trends were apparent in Q_l, with higher overall rates in less xerothermic 2009 compared to 2008 (Figure 6a). Q_l increased in October 2008, with the increase in aSWC, from 13% (in the second half of September) to 49% (first half of October), while mean daily values of VPD and net radiation were still not limiting. Q_l declined until December 2008, before increasing again to reach maximum values in March 2009 (Figure 6a), when VPD started to increase and aSWC was still very high (>85%). The combined effect of

net radiation and VPD during the wet season (November–March) of the study period largely explained the variation in Q_l ($R_{adj}^2 = 0.64$, $p < 0.001$; Figure 7). Thus, when water availability was not a limiting factor, Q_l was mainly controlled by VPD and net radiation.

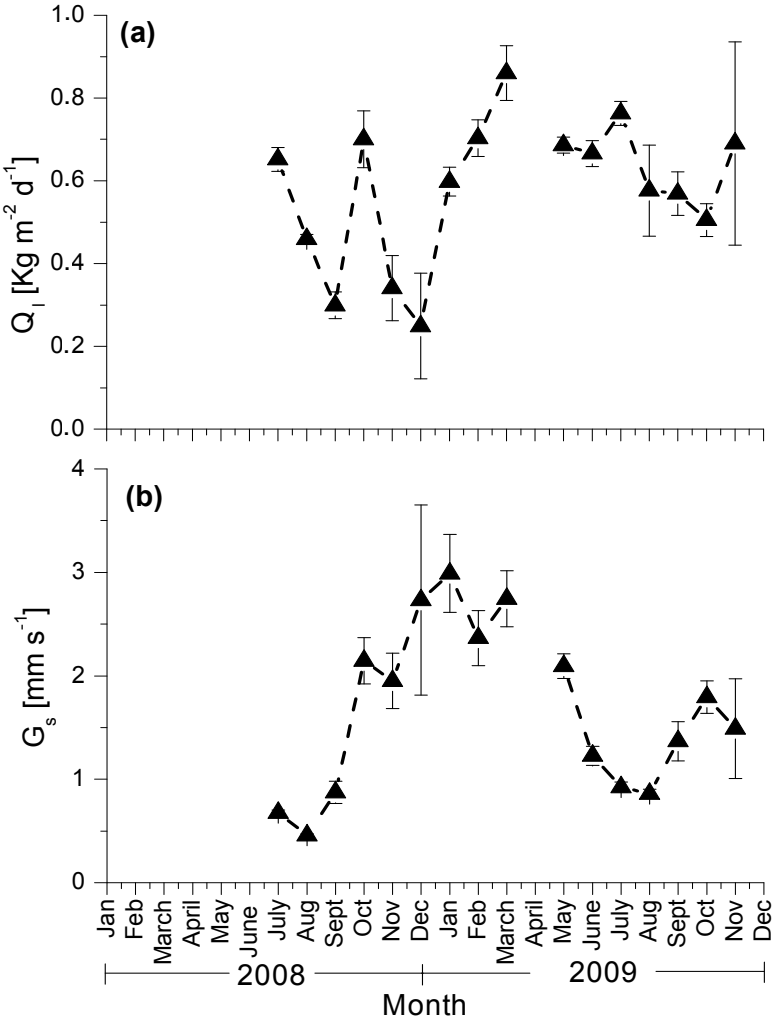


Figure 6. Seasonal patterns of mean monthly values of: (**a**) Sap flow per unit leaf area Q_l and (**b**) canopy stomatal conductance (G_s) measured over two consecutive years (2008 and 2009). $n = 5$ trees ± SE.

To investigate the causes of the drought-induced decrease in Q_l, canopy stomatal conductance (G_s) was derived from Q_l. The seasonal pattern of G_s was strongly controlled by PET ($R_{adj}^2 = 0.69$, $p < 0.001$; Figure 8a) and VPD ($R^2 = 0.62$, $p < 0.001$; Figure 8c) of the respective period, indicating increased stomatal control as drought progressed. G_s was also strongly related to aSWC ($R_{adj}^2 = 0.72$, $p < 0.001$; Figure 8b) and tracked its seasonal changes, which explains the high values in October and December 2008, as well as in January 2009 (Figure 6b).

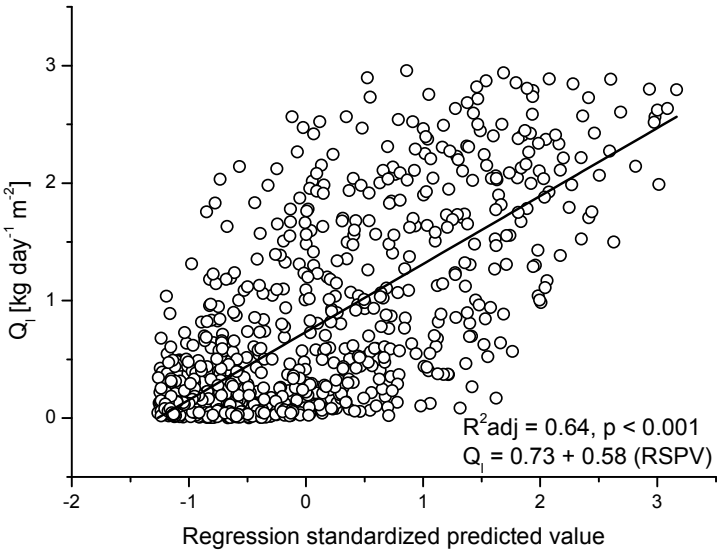


Figure 7. Regression model describing the combined effect of vapor pressure deficit (VPD) [kPa] and net radiation [kWm^{-2}] on Q_l. Mean hourly values of VPD and net radiation corresponding to the days' sap flow was monitored during the wet period (November to March) were used. RSPV stands for regression standardized predicted value.

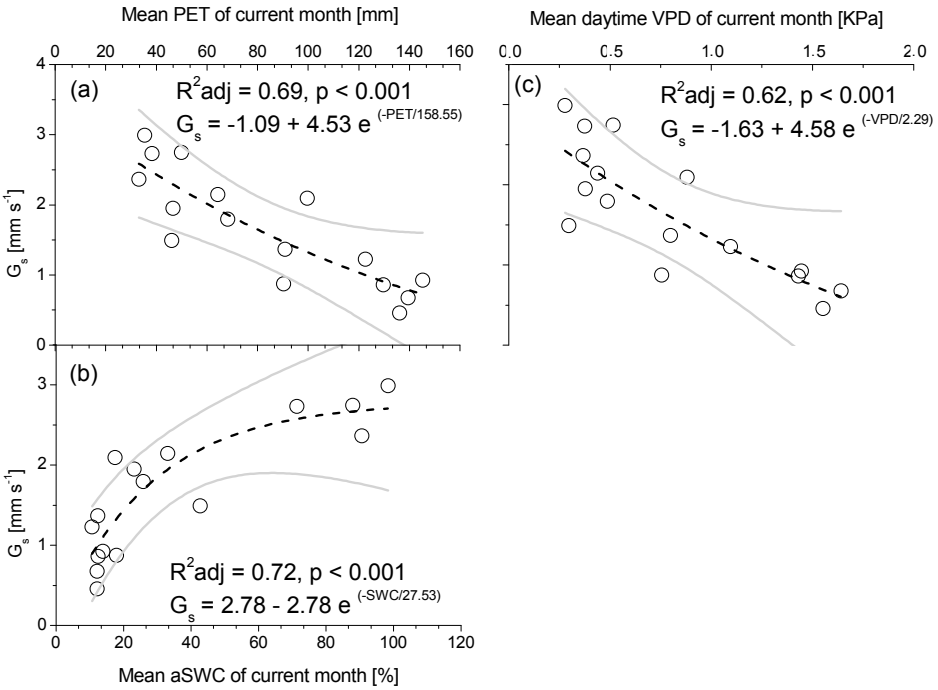


Figure 8. Regression models describing the relationship between mean monthly canopy stomatal conductance (G_s) and (**a**) potential stand evapotranspiration (PET), (**b**) available soil water capacity (aSWC), and (**c**) vapor pressure deficit (VPD). The confidence levels of the models are depicted by grey upper and lower bands.

4. Discussion

The seasonal variation in key physiological traits as impacted by the environmental conditions were investigated in a mature near-coastal Aleppo pine forest in Sani, Chalkidiki, northern Greece. The study site is characterized by semi-arid growing seasons (mean aridity index of April to October during the last decade was 0.38) and compared to other Mediterranean Aleppo pine forests, it falls

within the average rainfall range but has relatively high mean air temperatures (Table S3). Assessments were conducted during two consecutive years (2008 and 2009) differing in climatic conditions. The latter was characterized by considerably higher water availability, while the former was comparable or even drier than the last decade's average (Figure 1; Table 1). This enabled monitoring of the ecophysiological responses of Aleppo pine under a wider range of climatic conditions. Within this frame, the effects of key environmental parameters on the gas exchange and water balance of *Pinus halepensis* were tested.

Aleppo pine exhibited a bimodal pattern of A_{max} which peaked twice in each year, when conditions were favorable (Figure 2a), corresponding with the species growth activity in the spring and autumn [6]. Stomatal regulation over A_{max} was observed throughout the year (Figure 4a), which appeared to respond to the limiting environmental factors. During the xerothermic summer conditions, PET exceeded that of aSWC and caused a decline in midday Ψ to values comparable to those reported in adult Aleppo pine trees (c. −2.6 MPa) [6]. As a result, g_s also declined, thus limiting A_{max} (Figure 2a,b), in line with the isohydric water-saving strategy of Aleppo pine [4,8,39]. This reduction of g_s is probably a response to minimize conductivity loss. Similar midday Ψ levels caused a c. 30% loss of conductivity in Aleppo pine seedlings subjected to drought [4], indicating its relatively high vulnerability to xylem embolism [6].

Air temperature and net radiation appear to be key controllers of A_{max} during the period of October to March (Figure 3). High A_{max} rates were reached during the warm days of October–November 2009, but not during the substantially colder measuring days between October 2008 and March 2009 (Figure 2a). It has similarly been reported [40] that the decrease of night temperatures below 10 °C, accompanied by a photoperiod below 12 h, results in low photosynthetic rates in seedlings of *Pinus strobus* during autumn. The A_{max} of Mediterranean pines was also found to be controlled by the preceding night temperatures and internal factors during autumn and winter months [41], as well as by extreme preceding summer droughts [10]. Any photoinhibition effects on A_{max} during the colder months of 2008 can be excluded since Fv/Fm values remained high (above 0.84; data not shown) and the air temperature was not low enough to account for such a response [42].

The quite xerothermic summer of 2008 resulted in a low midday Ψ (Figure 2d) that was reflected in the $\delta^{13}C$ isotopic ratio (Figure 2c), which increased linearly with decreasing midday Ψ (Figure 4b). This has been observed in other forest species during water deficits [43], owing to decreased Rubisco discrimination against ^{13}C under stomatal closure due to abiotic stresses [44]. Midday Ψ was found to immediately respond to short-term changes in air evaporative demand, as similarly shown by [11], since current day evapotranspiration explains 72% of its variation ($p < 0.001$; Figure 5b). On the contrary, the air vapor pressure deficit over the last two weeks strongly affected the needle $\delta^{13}C$ (R^2 adj = 0.64, $p < 0.001$, Figure 5a), while this effect was less pronounced when shorter or longer time intervals were examined (Table S2). Foliar $\delta^{13}C$ being affected by recent environmental conditions has been previously reported [29,45] and may apply for Aleppo pine as well, as the isotopic signature of recently produced assimilates could be detected quicker in a conifer that maintains an active C metabolism through most of the year.

Sap flow per unit leaf area (Q_l) showed a seasonal water saving pattern, with maximum values in early spring and a gradual decline as summer drought progressed (Figure 6a) and stomatal control increased (Figure 2b) to prevent water loss. A second peak in Q_l occurred in autumn 2008 (Figure 6a) associated with aSWC increase (from 13% in the last half of September to 49% in the first half of October). It is also evident that, when water availability was not a limiting factor, Q_l was mainly controlled by VPD and net radiation (Figure 7). Our results indicate that, in accordance to the findings from other Mediterranean type ecosystems [37,46], interannual variability in sap flow of Aleppo pines can be substantial, to avoid periods of prolonged drought and high transpirational demands.

Consistent with the patterns of leaf-level responses and sap flow, canopy conductance (G_s) decreased during the summer drought months (Figure 6b) being strongly affected by the gradual increases in PET, VPD (Figure 8a,c) and air temperature (Table S2), as similarly reported in numerous other studies [37,47]. As PET increased, midday Ψ decreased (Figure 5b) and g_s followed the same

pattern to prevent conductivity losses [48]. On the other hand, g_s responded positively when water availability increased (Figure 8b), possibly due a gradual refill of previously cavitated tracheids [49].

The studied Aleppo pine stand exhibited plasticity to environmental conditions and showed the ability to recover from the effects induced by drought when climatic factors were improved. A similar response is reported for Aleppo pine growth when precipitation is increased [9]. However, during the period of increased water availability (October to March), varying patterns may be observed on the leaf and on the canopy level, responding to different parameters that seem to have a limiting effect. Thus, photosynthesis at the level of the lower canopy was greatly affected by air temperature and net radiation and increased when temperature was optimal (in October–November 2009), while sap flow and conductance at the canopy level responded positively to the favorable VPD, net radiation, and evapotranspiration during the same period of both years.

The results of the present study demonstrate the potential of this dominant Mediterranean forest tree species, to overcome the adverse conditions during summer droughts and to take advantage of more favorable water regimes occurring in early spring and occasionally also during autumn and winter, if other environmental parameters are not limiting, in a semi-arid ecosystem in Chalkidiki, Greece. Furthermore, some light is shed on the environmental controls over key physiological traits at the leaf and the canopy level.

5. Conclusions

By assessing the ecophysiological responses of the studied Aleppo pine forest in Northern Greece within a range of varying climatic conditions during a two year period, different but complementary patterns were revealed. During xerothermic periods, a typical isohydric behavior was exhibited by Aleppo pine; A_{max}, Q_l and g_s declined through stomatal control to limit Ψ reduction and loss of conductivity. However, in periods when water availability was not a limiting factor, the species was able to maximize its carbon gain if other controlling parameters, such as air temperature and net radiation, simultaneously ensured a favorable environmental regime. In conclusion, a high plasticity of the *Pinus halepensis* at the studied forest site to concurrent environmental conditions is indicated. Continuing studies are needed across Aleppo pine locations in the Mediterranean, particularly at its eastern part which is expected to be more prone to climate change, to improve our understanding of the species responses to ongoing climate variability.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/10/4/313/s1, Table S1: Classification of aridity index (AI) categories; Table S2: Significant regression models describing the relationship between physiological and single or combined environmental parameters over different time intervals. The adjusted R^2 values and the levels of significance are presented. The models in bold are the most significant ones with the highest adjusted R^2, explaining the variation in the respective physiological parameter, which are presented in figures; Table S3: Characterization of the study site, in comparison to other Aleppo pine sites of Mediterranean countries.

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References

1. Chambel, M.R.; Climent, J.; Pichot, C.; Ducci, F. Mediterranean Pines (*Pinus halepensis* Mill. and *brutia* Ten.). In *Forest Tree Breeding in Europe: Current State-of-the-Art and Perspectives*; Pâques, L.E., Ed.; Springer: Dordrecht, The Netherlands, 2013; Volume 25, pp. 229–265, ISBN 978-2-11128054-0.
2. Euro + Med PlantBase—The Information Resource for Euro-Mediterranean Plant Diversity. Available online: http://ww2.bgbm.org/EuroPlusMed/ (accessed on 12 February 2019).
3. Dafis, S.; Moulopoulos, C. Einwirkung der Bodenfeuchtigkeit auf das Wachstum und Wurzelbildung von Samlingen der Aleppo und Hartkiefer (*Pinus halepensis* Mill und *P. brutia* Ten). *Beih. Z. Schweiz. Forstver.* **1969**, *46*, 225–260.
4. Klein, T.; Cohen, S.; Yakir, D. Hydraulic adjustments underlying drought resistance of *Pinus halepensis*. *Tree Physiol.* **2011**, *31*, 637–648. [CrossRef] [PubMed]
5. Klein, T.; Cohen, S.; Paudel, I.; Preisler, Y.; Rotenberg, E.; Yakir, D. Diurnal dynamics of water transport, storage and hydraulic conductivity in pine trees under seasonal drought. *iForest* **2016**, *9*, e1–e10. [CrossRef]
6. Oliveras, I.; Martinez Vilalta, J.; Jimenez-Ortiz, T.; Lledo, J.; Escarre, A.; Piñol, J. Hydraulic properties of *Pinus halepensis, Pinus pinea* and *Tetraclinis articulata* in a dune ecosystem of Eastern Spain. *Plant Ecol.* **2003**, *169*, 131–141. [CrossRef]
7. Pacheco, A.; Camarero, J.J.; Ribas, M.; Gazol, A.; Gutierrez, E.; Carrer, M. Disentangling the climate-driven bimodal growth pattern in coastal and continental Mediterranean pine stands. *Sci. Total Environ.* **2017**, *615*, 1518–1526. [CrossRef]
8. Prislan, P.; Gričar, J.; de Luis, M.; Novak, K.; del Castillo, M.E.; Schmitt, U.; Koch, G.; Štrus, J.; Mrak, P.; Žnidarič, M.T.; et al. Annual cambial rhythm in *Pinus halepensis* and *Pinus sylvestris* as indicator for climate adaptation. *Front. Plant Sci.* **2016**, *7*, 1923. [CrossRef]
9. Gazol, A.; Ribas, M.; Gutiérrez, E.; Camarero, J.J. Aleppo pine forests from across Spain show drought-induced growth decline and partial recovery. *Agric. For. Meteorol.* **2017**, *232*, 186–194. [CrossRef]
10. Schiller, G.; Cohen, Y. Water regime of a pine forest under a Mediterranean climate. *Agric. For. Meteorol.* **1995**, *74*, 181–193. [CrossRef]
11. Klein, T.; Shpringer, I.; Fikler, B.; Elbaz, G.; Cohen, S.; Yakir, D. Relationships between stomatal regulation, water-use, and water-use efficiency of two coexisting key Mediterranean tree species. *For. Ecol. Manag.* **2013**, *302*, 34–42. [CrossRef]
12. Birami, B.; Gattmann, M.; Heyer, A.G.; Grote, R.; Arneth, A.; Ruehr, N.K. Heat Waves Alter Carbon Allocation and Increase Mortality of Aleppo Pine Under Dry Conditions. *Front. For. Glob. Chang.* **2018**, *1*, 8. [CrossRef]
13. Anderegg, W.R.L.; Wolf, A.; Arango-Velez, A.; Choat, B.; Chmura, D.J.; Jansen, S.; Kolb, T.; Li, S.; Meinzer, F.C.; Pita, P.; et al. Woody plants optimise stomatal behaviour relative to hydraulic risk. *Ecol. Lett.* **2018**, *21*, 968–977. [CrossRef] [PubMed]
14. Del Castillio, J.; Voltas, J.; Ferrio, J.P. Carbon isotope discrimination, radial growth, and NDVI share spatiotemporal responses to precipitation in Aleppo pine. *Trees Struct. Funct.* **2015**, *29*, 223–233. [CrossRef]
15. García-Ruiz, J.M.; López-Moreno, J.I.; Vicente-Serrano, S.M.; Lasanta-Martínez, T.; Beguería, S. Mediterranean water resources in a global change scenario. *Earth-Sci. Rev.* **2011**, *105*, 121–139. [CrossRef]
16. Sarris, D.; Christodoulakis, D.; Körner, C. Recent decline in precipitation and tree growth in the eastern Mediterranean. *Glob. Chang. Biol.* **2007**, *13*, 1187–1200. [CrossRef]
17. Grünwald, C.; Schiller, G. Needle xylem water potential and water saturation deficit in provenances of *Pinus halepensis* Mill. and *P. brutia* Ten. *Forêt Méditerranéenne* **1988**, *10*, 407–414.
18. Michelozzi, M.; Loreto, F.; Colom, R.; Rossi, F.; Calamassi, R. Drought responses in Aleppo pine seedlings from two wild provenances with different climatic features. *Photosynthetica* **2011**, *49*, 564–572. [CrossRef]
19. Klein, T.; Di Matteo, G.; Rotenberg, E.; Cohen, S.; Yakir, D. Differential ecophysiological response of a major Mediterranean pine species across a climatic gradient. *Tree Physiol.* **2012**, *33*, 26–36. [CrossRef]
20. David-Schwartz, R.; Paudel, I.; Mizrachi, M.; Delzon, S.; Cochard, H.; Lukyanov, V.; Badel, E.; Capdeville, G.; Shklar, G.; Cohen, S. Indirect Evidence for Genetic Differentiation in Vulnerability to Embolism in *Pinus halepensis*. *Front. Plant Sci.* **2016**, *7*, 768. [CrossRef]
21. European Soil Bureau Network—European Commission. *Soil Atlas of Europe*, 11th ed.; Office for Official Publications of the European Communities: Luxembourg, 2005; p. 128, ISBN 92-894-8120-X.
22. Orfanoudakis, M.; Democritus University of Thrace, Nea Orestiada, Greece. Personal communication, 2019.

23. Peel, M.C.; Finlayson, B.L.; McMahon, T.A. Updated world map of the Köppen-Geiger climate classification. *Hydrol. Earth Syst. Sci.* **2007**, *11*, 1633–1644. [CrossRef]
24. Dingman, S.L. *Physical Hydrology*, 2nd ed.; Prentice Hall: Upper Saddler River, NJ, USA, 2002.
25. Paparrizos, S. The Effect of Climate on the Hydrological Regime of Selected Greek Areas with Different Climate Conditions. Ph.D. Thesis, Faculty of Environment and Natural Resources, Albert-Ludwigs-University of Freiburg, Freiburg, Germany, 2016.
26. Matzarakis, A.; Rutz, F.; Mayer, H. Modelling radiation fluxes in simple and complex environments—Application of the RayMan model. *Int. J. Biometeorol.* **2007**, *51*, 323–334. [CrossRef]
27. Matzarakis, A.; Rutz, F.; Mayer, H. Modelling radiation fluxes in simple and complex environments: Basics of the RayMan model. *Int. J. Biometeorol.* **2010**, *54*, 131–139. [CrossRef] [PubMed]
28. Matzarakis, A.; Mayer, H.; Schindler, D.; Fritsch, J. Simulation des Wasserhaushaltes eines Buchenwaldes mit dem forstlichen Wasserhaushaltsmodell WBS3. *Bericht Des Meteorologischen Instituts der Universität Freiburg* **2000**, *5*, 137–146.
29. Fotelli, M.N.; Nahm, M.; Radoglou, K.; Rennenberg, H.; Matzarakis, A. Seasonal and interannual ecophysiological responses of beech (*Fagus sylvatica*) at its south-eastern distribution limit in Europe. *For. Ecol. Manag.* **2009**, *257*, 1157–1164. [CrossRef]
30. United Nations Environment Programme (UNEP). *World Atlas of Desertification*; Edward Arnold: London, UK, 1992; p. 69, ISBN 0 340 55512 2.
31. Paparrizos, S.; Maris, F.; Matzarakis, A. Integrated analysis and mapping of aridity over Greek areas with different climate conditions. *Glob. NEST J.* **2016**, *18*, 131–145.
32. Food and Agriculture Organization (FAO). *Forest Resources Assessment 1990: Tropical Countries*; FAO-UN: Rome, Italy, 1993; ISBN 92-5-103390-0.
33. Granier, A. A new method of sap flow measurement in tree stems. *Ann. Sci. For.* **1985**, *42*, 193–200. [CrossRef]
34. Granier, A. Evaluation of transpiration in a Douglas-fir stand by means of sap flow measurements. *Tree Physiol.* **1987**, *3*, 309–319. [CrossRef] [PubMed]
35. Do, F.; Rocheteau, A. Influence of natural temperature gradients on measurements of xylem sap flow with thermal dissipation probes. 1. Field observations and possible remedies. *Tree Physiol.* **2002**, *22*, 641–648. [CrossRef]
36. Mitsopoulos, I.D.; Dimitrakopoulos, A.P. Allometric equations for crown fuel biomass of Aleppo pine (*Pinus halepensis* Mill.) in Greece. *Int. J. Wildland Fire* **2007**, *16*, 642–647. [CrossRef]
37. Martínez-Vilalta, J.; Mangirón, M.; Ogaya, R.; Sauret, M.; Serrano, L.; Peñuelas, J.; Piñol, J. Sap flow of three co-occurring Mediterranean woody species under varying atmospheric and soil water conditions. *Tree Physiol.* **2003**, *23*, 747–758. [CrossRef]
38. Phillips, N.; Oren, R. A comparison of daily representations of canopy conductance based on two conditional time-averaging methods and dependence of daily conductance on environmental factors. *Ann. For. Sci.* **1998**, *55*, 217–235. [CrossRef]
39. Salazar-Tortosa, D.; Castro, J.; Rubio de Casas, R.; Viñegla, B.; Sánchez-Cañete, E.P.; Villar-Salvador, P. Gas exchange at whole plant level shows that a less conservative water use is linked to a higher performance in three ecologically distinct pine species. *Environ. Res. Lett.* **2018**, *13*, 045004. [CrossRef]
40. Chang, C.Y.; Unda, F.; Zubilewich, A.; Mansfield, S.D.; Ensminger, I. Sensitivity of cold acclimation to elevated autumn temperature in field-grown *Pinus strobus* seedlings. *Front. Plant Sci.* **2015**, *6*, 165. [CrossRef]
41. Awada, T.; Radoglou, K.; Fotelli, M.N.; Constantinidou, H.-I.A. Ecophysiology of three Mediterranean pine species under contrasting light regimes. *Tree Physiol.* **2002**, *23*, 33–41. [CrossRef]
42. Pflug, E.; Brüggemann, W. Frost-acclimation of photosynthesis in overwintering Mediterranean holm oak, grown in Central Europe. *Int. J. Plant Biol.* **2012**, *3*, e1. [CrossRef]
43. Fotelli, M.N.; Geβler, A.; Peuke, A.D.; Rennenberg, H. Drought affects the competition between *Fagus sylvatica* L. seedlings and an early successional species (*Rubus fruticosus*): Growth, water status and δ13C composition. *New Phytol.* **2001**, *151*, 427–435. [CrossRef]
44. Farquhar, G.D.; Ehleringer, J.R.; Hubick, K.T. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1989**, *40*, 503–537. [CrossRef]
45. Keitel, C.; Matzarakis, A.; Rennenberg, H.; Geßler, A. Carbon isotopic composition and oxygen isotopic enrichment in phloem and total leaf organic matter of European beech (*Fagus sylvatica* L.) along a climate gradient. *Plant Cell Environ.* **2006**, *29*, 1492–1507. [CrossRef] [PubMed]

46. Sánchez-Costa, E.; Poyatos, R.; Sabaté, S. Contrasting growth and water use strategies in four co-occurring Mediterranean tree species revealed by concurrent measurements of sap flow and stem diameter variations. *Agric. For. Meteorol.* **2015**, *207*, 24–37. [CrossRef]
47. Oren, R.; Phillips, N.; Ewers, B.E.; Pataki, D.E.; Megonigal, J.P. Sap- flux scaled transpiration response to light, vapor pressure deficit, and leaf area reduction in a flooded *Taxodium distichum* forest. *Tree Physiol.* **1999**, *19*, 337–347. [CrossRef]
48. Sperry, J.S. Hydraulic constraints on plant gas exchange. *Agric. For. Meteorol.* **2000**, *104*, 13–23. [CrossRef]
49. Tognetti, R.A.; Michelozzi, M.; Giovanelli, A. Geographical variation in water relations, hydraulic architecture and terpene composition of Aleppo pine seedlings from Italian provenances. *Tree Physiol.* **1997**, *17*, 241–250. [CrossRef]



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