

Esra Bayar

Influence of drought stress and N addition on the gas exchange, biochemical and growth traits in *Quercus ithaburensis*

Received: 28 April 2022; Accepted: 17 October 2022

Abstract: Forest tree species are likely to be affected by drought due to global climate change. Drought and nitrogen (N) affect plant growth. Therefore, we should understand the mechanisms underlying the N availability and species response to drought for plant development. This paper had two objectives. First, it determined the effects of N addition on water potential, gas exchange parameters, soluble sugar and photosynthetic pigment content, leaf N concentration, and growth parameters under drought stress and well-watered conditions. Second, it searched the drought response of *Quercus ithaburensis* Decne. subsp. *macrolepis* (Kotschy) Hedge&Yaltırık seedlings under N conditions with drought stress. In a greenhouse, one-year-old seedlings were exposed to two drought stress and nitrogen addition and three drought cycles. Drought stress significantly reduced stem water potential, relative water content, net photosynthetic rate, stomatal conductance, transpiration rate, leaf area, root collar diameter, seedling height, shoot and root dry weight. On the other hand, it increased water use efficiency and soluble sugar content. N addition is effective on soluble sugar content and photosynthetic pigment content, and promoted leaf N concentrations. Nitrogen addition under well-watered conditions stimulated the growth of *Q. ithaburensis* seedlings. Drought stress changed the physiological response of *Q. ithaburensis* seedlings (decreased water potential, net photosynthetic rate, stomatal conductance, and transpiration rate) in the short term, although nitrogen addition under drought stress has not changed the decreasing trend overall.

Keywords: Chlorophyll, drought stress, nitrogen, photosynthetic rate, *Quercus*

Addresses: E. Bayar, Isparta University of Applied Sciences, Faculty of Forestry, Forest Engineering Department, Isparta/Turkey;  <https://orcid.org/0000-0003-1137-297X/>, esrabayar@isparta.edu.tr

Introduction

Drought is the main abiotic factor limiting plant growth in arid and semi-arid regions with nutrient-deficient soils (Liu et al., 2013). Drought stress and nitrogen deficiency generally show up simultaneously in arid regions (Boussadia et al., 2010). Water and nutrient stress are limiting parameters for plant life (Graciano et al., 2005). The presence, intake, and use of water affect the presence, use, and solubility of nutrients (Alam, 1999). Plants develop

various adaptation strategies (e.g., stress avoidance and tolerance) to cope with water stress, including physiological and biochemical responses depending on the species. Some of these strategies are reductions in growth pattern, leaf water potential, relative water content, stomatal conductance, and transpiration rate, and increases in root length, shoot/root ratio, and accumulation of solutes (Seleiman et al., 2021). In response to water stress, plants can reduce leaf water content, and limit gas exchange parameters (Deligöz & Bayar, 2021; Koç, 2021; Koç et al.,

2021). Low water potential in soil and plants inhibits growth, and decreases cellular and textural development and uptake of essential nutrients (Dubey & Pessarakli, 2001). A decrease in soil moisture content has impacted WUE in high-nutrient oak seedlings (Welander & Ottosson, 2000). Also, long-term nutrient addition affects the leaf-water relationship (Bucci et al., 2006). Fertilizer type and amount, irrigation level can alter the results of the N response (Chen et al., 2018). Fertilization did not improve the reduction in net photosynthetic rates, stomatal conductance, or leaf water potential (Kleiner et al., 1992). There is a large body of research on plants' response to drought stress and N availability (Chen et al., 2016; Song et al., 2019; Wang et al., 2019; Zhang et al., 2021). Nutrient availability during drought stress can increase water use efficiency and promote faster recovery after drought (Gessler et al., 2017). Villar-Salvador et al. (2013) found that N fertilization did not affect the net photosynthetic rate but delayed the leaf senescence and shedding of *Q. faginea* L. N deficiency affects photosynthesis, sugar metabolism, and carbohydrate partitioning between tissues (Boussadia et al., 2010). Drought stress increased the soluble sugar content, decreased chlorophyll, and carotenoid concentrations of the seedlings (Jafarnia et al., 2018). Appropriate nitrogen supply can increase leaf N concentrations and chlorophyll content and promote physiological responses to drought (Xu et al., 2015). Moderate fertilization can improve chlorophyll content and photosynthetic performance under drought stress (Chen et al., 2016).

Oak species are distributed widely from humid to continental climate regions in Turkey (MÇBK, 2016). Turkey has a forest area of 22.93 million hectares (ha), 6.75 million ha of which consists of oak species (OGM, 2020). Of the broadleaved forests, oak species are the most ubiquitous. *Q. ithaburensis* is one of the 17 oak species in Turkey. It is mainly found in Western and South-western Anatolia but occasionally in Thrace and Central Anatolia (Öztürk, 2013). It is a drought-resistant species that can grow in shallow and poor soils (Siam et al., 2008). There is a growing body of research on the impacts of drought on oak species' physiological and biochemical properties [water potential (Deligöz & Bayar, 2018), gas exchange (Peguero Pina et al., 2009), carbohydrates, (Jafarnia et al., 2018) etc.]. However, only a small body of research investigates the effects of drought and N addition on physiological mechanisms on *Q.* species (Xu et al., 2015). Moreover, there is no consensus on the effect of the interaction between drought stress and N addition on physiological mechanisms. We do not know exactly how N addition changes drought tolerance in *Q. ithaburensis*. It is estimated that global climate change will cause drought and nitrogen accumulation (Wang et al., 2019). Therefore, we need to

understand the response of *Q. ithaburensis* to N addition and recurrent drought stress for effective afforestation in semi-arid, arid, semi-humid, and humid regions. This study focused on two questions: (1) Does N addition affect drought stress? and (2) How does N addition affect physiological, biochemical, and growth parameters in seedlings under drought and watered conditions? The research hypothesis was, "Although drought reduces growth and physiological responses, N addition under drought stress changes in stem water potential, net photosynthetic rate, water use efficiency, soluble sugar, chlorophyll content, and growth characteristics."

Materials and methods

Plant material and growth conditions

Q. ithaburensis seeds were collected from a seed stand (38°25'35"N, 30°02'36"E, Altitude: 1010 m) in Afyon-Sorkun in November 2018. They were soaked in water for 24 hours, and then, the empty and rotten ones were removed. The remaining seeds were stored in polyethylene bags in cold storage at +4 °C until sowing. Polyethylene plastic bags (18 × 30 cm) were filled with a peat perlite mixture (a ratio of 3:1). The seeds were sown in those bags at the Forest Nursery (37°53'N, 30°52'E) in February 2019. They were moved into a greenhouse in May 2020. They were watered regularly at field capacity to adapt them to the greenhouse until the trial. A data logger (Elitech RC-4HC) was placed inside the greenhouse to record the daily average temperature and relative humidity throughout the trial. The average air temperature was 23, 28, 27, and 24 °C in June, July, August, and September, respectively. The average relative moisture content was 51, 41, 42, and 47% in June, July, August, and September, respectively.

N addition and drought treatments

N fertilization was started in mid-May and continued once a month for four months. A total of 184 mg N (approximately 400 mg urea) was administered to each seedling. About 0.1 g of granular urea was dissolved in water and administered to the growing place of the seedlings each month. While applying nitrogen, it was made sure that there was no loss on the surface. The drought stress and the trial (N fertilization) started on 17 June 2020. The regime consisted of irrigation (WW: Well-watered, every 2–3 days and WS: Drought-stressed, every 30 days) and fertilization (N1: Nitrogen addition and N0: Non-nitrogen). The drought and fertilization were performed in triplicate (2 water treatments X 2 nitrogen treatments X 3 replicates X 27 seedlings

per replicate) according to a randomized trial design. It was continued to water the seedlings throughout the trial in control (well-watered) treatments. The seedlings had an initial diameter of 4.02 mm and a height of 13.05 cm. Drought stress was applied for 30 days and the seedlings were re-watered to close to field capacity after 30 days for recovery, and then the seedlings were exposed to drought stress again. This process continued for three months in three drought cycles (July, August, and September).

Water potential, relative water content and soil water content

Midday water potential (Ψ_{md}) was measured between 12:00 pm and 13:30 pm using a pressure chamber device (PMS Instruments, Corvallis, OR, USA; Scholander et al. (1965). Stem water potential was carried out at the end of each drought cycle and three seedlings from each iteration. Soil water content (SWC) (volumetric) was measured (20 cm) using a TDR 300 (FieldScout TDR 300 Soil Moisture Meter, Spectrum Technologies) soil moisture meter. Leaf relative water content (RWC) was calculated based on fresh weight (FW), turgid weight (TW), and dry weight (DW). Relative water content was calculated in three stages. First, leaf samples were weighed immediately. Second, they were saturated in a dark environment for about 24 hours and then reweighed. Third, oven-dry weight was determined at 105 °C for 24 h. Relative water content was calculated using the following equation (Ritchie, 1984):

$$RWC (\%) = 100 [(FW - DW) / (TW - DW)]$$

Gas exchanges

Net photosynthetic rate (A_{net}) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), transpiration rate (E) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and intercellular CO_2 concentrations (C_i) ($\mu\text{mol CO}_2 \text{ mol}^{-1}$) were measured using a portable photosynthesis (LI-COR LI-6400XT Lincoln, USA) device fitted with a chamber ($2 \times 3 \text{ cm}^2$) and a red/blue light source. The measurements were performed on three different seedlings (from each iteration) at the end of the drought cycles (every 30 days) between 09:00 am and 11:30 am and 13:30 pm and 15:00 pm. The CO_2 concentration, photosynthetically active radiation, and flow rate in gas exchange measurements were 400, 1250, and 500, respectively. Leaf temperature was adjusted depending on the temperature in the chamber of the greenhouse at the time of measurement. Afterward, the leaves were cut, and the leaf area was calculated using the Image J software program (Image J; Wayne

Rasband/NIH, Bethesda, MD, USA). Gas exchange parameters were recalculated based on the leaf area. Water use efficiency (WUE) was calculated using the following equation: A/E (Xu & Zhou, 2008).

Photosynthetic pigments, soluble sugars and N concentrations

At the end of each drought cycle, mature leaf samples were collected from the five seedlings at the iteration of each treatment. Chlorophyll pigment and total soluble sugar content were determined in each drought cycle. Leaf N concentration was examined at the end of the drought cycles. For chlorophyll pigment extraction, fresh leaf samples (0.1 g) were quickly crushed with acetone solution (80%) in a press until homogeneous. They were then placed in glass tubes (10 ml). They were mixed in a vortex at 3000 rpm for 10 minutes. They were then transferred to tubs (3 ml) and measured in a spectrophotometer at wavelengths of 450, 645, and 663 nm. Photosynthetic pigment contents based on absorbance values were calculated using the following equation (Arnon, 1949):

$$\text{Chlorophyll a} = 12.7(A_{663}) - 2.69(A_{645})$$

$$\text{Chlorophyll b} = 22.9(A_{645}) - 4.68(A_{663})$$

$$\text{Total chlorophyll} = 20.2(A_{645}) + 8.02(A_{663})$$

$$\text{Carotenoids} = 4.07(A_{450}) - [(0.0435 \times \text{Chlorophyll a}) + (0.367 \times \text{Chlorophyll b})]$$

For total soluble sugar and leaf N analysis, the samples were dried in an oven at 65 °C for 48 hours and then ground in a coffee grinder. Nitrogen was determined using the dry combustion method (LECO CN-2000). Total soluble sugar was calculated using the phenol sulfuric acid method proposed by Dubois et al. (1956). The dry sample (0.1 g) was homogenized in 10 mL of 80% ethanol for 24 hours and then centrifuged at 6000 rpm for 10 minutes. Afterward, a liquid sample (0.05 mL) and phenol solution (1 mL 5%) were added to each tube. The mixture was then vortexed. Afterward, H_2SO_4 (5 mL) was added to the mixture, which was vortexed again. It was kept at room temperature for one hour and then read at 490 wavelengths in a spectrophotometer in the tubs (3 mL).

Growth characteristics

The seedlings were removed after drought and N treatments. The seedling height (SH) (cm), root collar diameter (RCD) (mm), root (RDW) and shoot

(SDW) dry weight (g), and shoot/root ratio (S/R) of 45 seedlings (15 from each iteration) were measured. The seedlings were oven-dried at 65 °C for 48 hours to determine dry weight. Nine seedling leaves (three from each iteration) were used to determine specific leaf area (SLA) in July, August, and September. Specific leaf area was based on the leaf area/leaf mass equation. Leaf area (LA) was determined using the Image J software program.

Statistical analysis

A two-way analysis of variance of the General Linear Model was used to analyze the effects of recurrent drought stress and N treatment and their interaction. The Duncan test ($p < 0.05$) was used when there was a difference between water potential, SWC, RWC, gas exchange parameters, photosynthetic pigment, soluble sugar content, N concentration, and growth properties. Before ANOVA, arcsine transformation was performed for percentile (SWC, RWC and N concentration) data. Principal component analysis (PCA) was used to analyze the correlation between physiological and growth properties. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS 25.0).

Results

Cyclic drought stress and N addition impacts on SWC, RWC and Ψ_{md}

SWC, RWC, and Ψ_{md} responded significantly to drought stress, but N addition and their interaction did not affect SWC, RWC, and Ψ_{md} at the end of

drought cycles (Table 1). Drought stressed seedlings had lower SWC and Ψ_{md} (both N0 and N1) (Fig. 1). The midday stem water potential decreased to -3.9

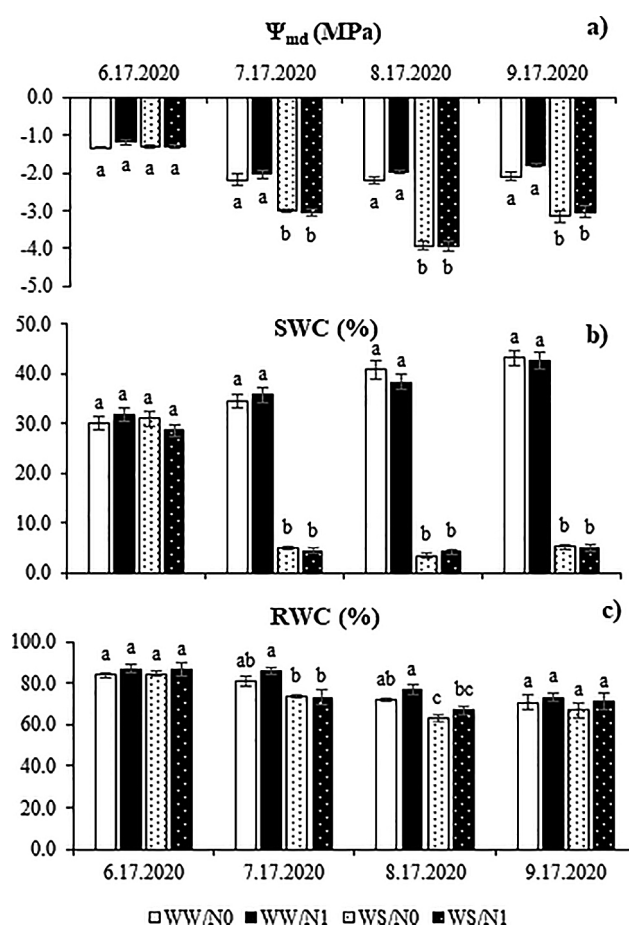


Fig. 1. Effects of drought stress and N addition on a) midday water potential (Ψ_{md}), b) soil water content (SWC), c) relative water content (RWC) in *Q. ithaburensis*

Table 1. The effects of drought stress and nitrogen addition on physiological and biochemical characteristics in *Q. ithaburensis* seedlings. S, drought stress effect; N, nitrogen effect; S×N, interactive effect of drought stress and nitrogen addition

| Parameters | S | | N | | S×N | |
|--|-----------|----|---------|----|-------|----|
| | F | P | F | P | F | P |
| Ψ_{md} (MPa) | 225.323 | ** | 1.970 | ns | 1.359 | ns |
| RWC (%) | 15.402 | ** | 3.533 | ns | 0.100 | ns |
| SWC (%) | 1.995.601 | ** | 0.040 | ns | 0.203 | ns |
| Anet ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) | 925.485 | ** | 0.312 | ns | 4.949 | * |
| gs ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) | 920.936 | ** | 0.001 | ns | 2.176 | ns |
| E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) | 1.084.275 | ** | 0.001 | ns | 3.881 | * |
| Ci ($\mu\text{mol CO}_2 \text{ mol}^{-1}$) | 73.083 | ** | 3.285 | ns | 4.546 | * |
| WUE ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) | 45.386 | ** | 3.763 | ns | 4.626 | * |
| TSC ($\text{mg g}^{-1} \text{ DW}$) | 18.979 | ** | 7.650 | * | 0.776 | ns |
| Chla (mg g^{-1}) | 9.477 | * | 67.062 | ** | 0.379 | ns |
| Chlb (mg g^{-1}) | 4.742 | * | 50.212 | ** | 0.586 | ns |
| Chla+b (mg g^{-1}) | 7.454 | * | 62.918 | ** | 0.006 | ns |
| Car. (mg g^{-1}) | 8.462 | * | 65.132 | ** | 0.002 | ns |
| Leaf N (%) | 11.482 | * | 144.980 | ** | 5.849 | ns |

ns: non-significant, * and ** significant at $P < 0.05$ and $P < 0.001$, respectively.

MPa in the seedlings subjected to drought stress in the second drought cycle. At the end of the second drought cycles, WS/N0 (63.1%) and WW/N1 (76.8%) had the lowest and highest RWC, respectively (Fig. 1).

Cyclic drought stress and N addition impacts on gas exchanges

Drought stress affected *Anet*, *gs*, *E*, *Ci*, and *WUE* (Table 1). Nitrogen addition had an insignificant effect on *Anet*, *E*, *gs*, *Ci*, and *WUE* ($P > 0.05$). However, the interaction between drought stress and N addition significantly affected *Anet*, *E*, *Ci*, and *WUE* ($P < 0.05$) (Table 1). At the end of the first drought cycle, WS/N1 had the lowest *Anet*, *gs*, *Ci*, and *E*. WS/N0 and WS/N1 had the lowest *Anet*, *gs*, and *E* at the end of the second and third drought cycle. Drought stressed seedlings (WS/N0 and WS/N1) had similar *Anet*, *gs*, and *E* but lower *Anet*, *gs*, and *E* than well-watered seedlings (WW/N0 and WW/N1). In the third drought cycle, WW/N1 had the highest *Anet*, *gs*, and *E*. Generally N addition under drought stress increased *WUE* (Fig. 2). The net photosynthetic rate was positively correlated with Ψ_{md} , SWC, *gs*, and *E*. Variables correlated with PC1 were Ψ_{md} , RWC, SWC, *Anet*, *gs*, *E*, and *Ci*. The water use efficiency was negatively correlated with Ψ_{md} , *gs*, *E*, and *Ci*. A decrease in SWC ($r: 0.918$, $p < 0.01$) leads to a decrease in Ψ_{md} ($r: 0.952$, $p < 0.01$), *Anet* ($r: 0.924$,

$p < 0.01$), *E* ($r: 0.941$, $p < 0.01$), *Ci* ($r: 0.660$, $p < 0.01$), *gs* ($r: 0.945$, $p < 0.01$) and an increase in *WUE* ($r: -0.560$, $p < 0.01$) (Fig. 3).

Cyclic drought stress and N addition impacts on soluble sugars, photosynthetic pigments and N concentrations

Both drought stress and N addition significantly affected the total soluble sugar content at the end of all drought cycles (Table 1). Drought stress generally increased the total soluble sugar content. Nitrogen addition under drought stress decreased the total soluble sugar content. Seedlings without N addition under drought stress had the highest total soluble sugar content (Fig. 4).

The interaction between drought stress and N addition significantly affected neither total soluble sugar nor photosynthetic pigment content (Table 1). At the end of the first and second drought cycles, seedlings with N addition (WW/N1 and WS/N1) had higher photosynthetic pigment content than those without N addition (WW/N0 and WS/N0) (Fig. 5). In general, well-watered seedlings with N addition (WW/N1) had higher Chl a, Chl b, Chl a+b, and carotenoid content than those without N addition (WW/N0). At the end of the drought cycles, N addition increased the N concentrations in the leaves. WS/N0 and WW/N0 had lower leaf N concentrations than

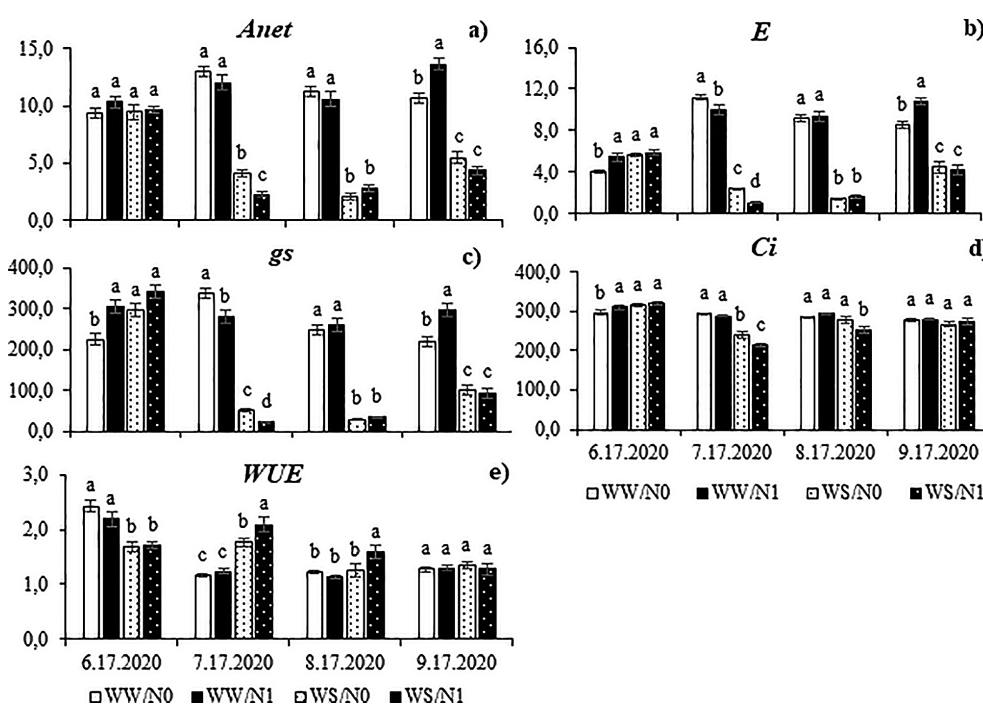


Fig. 2. Effects of drought stress and N addition on a) net photosynthetic rate (*Anet*, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), b) transpiration rate (*E*, $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), c) stomatal conductance (*gs*, $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), d) intercellular CO_2 concentrations (*Ci*, $\mu\text{mol CO}_2 \text{ mol}^{-1}$), e) water use efficiency (*WUE*, $(\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} / \text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1})$) in *Q. ithaburensis*

N addition treatments (Fig. 6). Variables with high correlation with PC1 are TSC, Chl a, Chl b, Car. and leaf N concentrations (Fig. 3).

Cyclic drought stress and N addition impacts on growth characteristics

Drought stress significantly reduced the RCD, SH, SDW, RDW, and LA of *Q. ithaburensis* seedlings. Seedlings without N addition under drought stress had lower SH. Nitrogen addition had a significant effect only on RCD. Seedlings under drought stress had lower RCD than others. WW/N1 had the highest

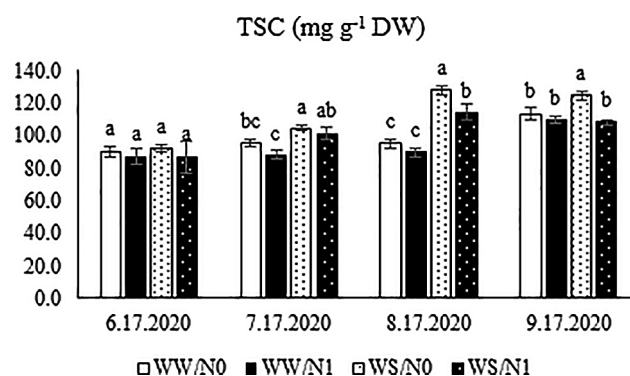


Fig. 4. Total soluble sugar content in *Q. ithaburensis* under drought (WW and WS) and N (N0 and N1) treatments

Table 2. The effects of drought stress and nitrogen addition on growth characteristics (RCD:Root collar diameter, SH:-Seedling height, SDW: Seedling dry weight, RDW: Root dry weight, S/R: Shoot/root dry weight ratio, SLA: Specific leaf area, LA: Leaf area) in *Q. ithaburensis* seedlings. Data are means \pm SE. The significance of the F-values from GLM are indicated as $P < 0.05$ (*); $P < 0.01$ (**)

| Treatment | RCD (mm) | SH (cm) | SDW (g) | RDW (g) | S/R | SLA (cm ² g ⁻¹) | LA (cm ²) |
|------------------------------------|-----------------|------------------|-----------------|-----------------|-----------------|--|-----------------------|
| WW/N0 | 5.71 \pm 0.13 | 16.24 \pm 0.63 | 1.40 \pm 0.09 | 6.52 \pm 0.41 | 0.22 \pm 0.01 | 132.71 \pm 2.70 | 7.99 \pm 0.43 |
| WW/N1 | 6.17 \pm 0.15 | 16.57 \pm 1.06 | 1.68 \pm 0.13 | 6.64 \pm 0.38 | 0.26 \pm 0.11 | 138.77 \pm 4.82 | 8.90 \pm 0.51 |
| WS/N0 | 4.48 \pm 0.16 | 13.36 \pm 0.79 | 1.05 \pm 0.08 | 4.56 \pm 0.34 | 0.56 \pm 0.34 | 140.05 \pm 3.08 | 7.36 \pm 0.47 |
| WS/N1 | 4.95 \pm 0.13 | 14.19 \pm 0.69 | 1.13 \pm 0.08 | 5.06 \pm 0.36 | 0.24 \pm 0.02 | 132.00 \pm 5.99 | 7.33 \pm 0.36 |
| Drought stress | 55.975** | 10.567* | 21.798** | 22.774** | 0.349 | 0.004 | 6.089* |
| N addition | 4.642* | 0.514 | 3.459 | 0.720 | 0.401 | 0.052 | 0.983 |
| Drought stress \times N addition | 0.992 | 0.09 | 1.043 | 0.27 | 0.284 | 2.622 | 1.123 |

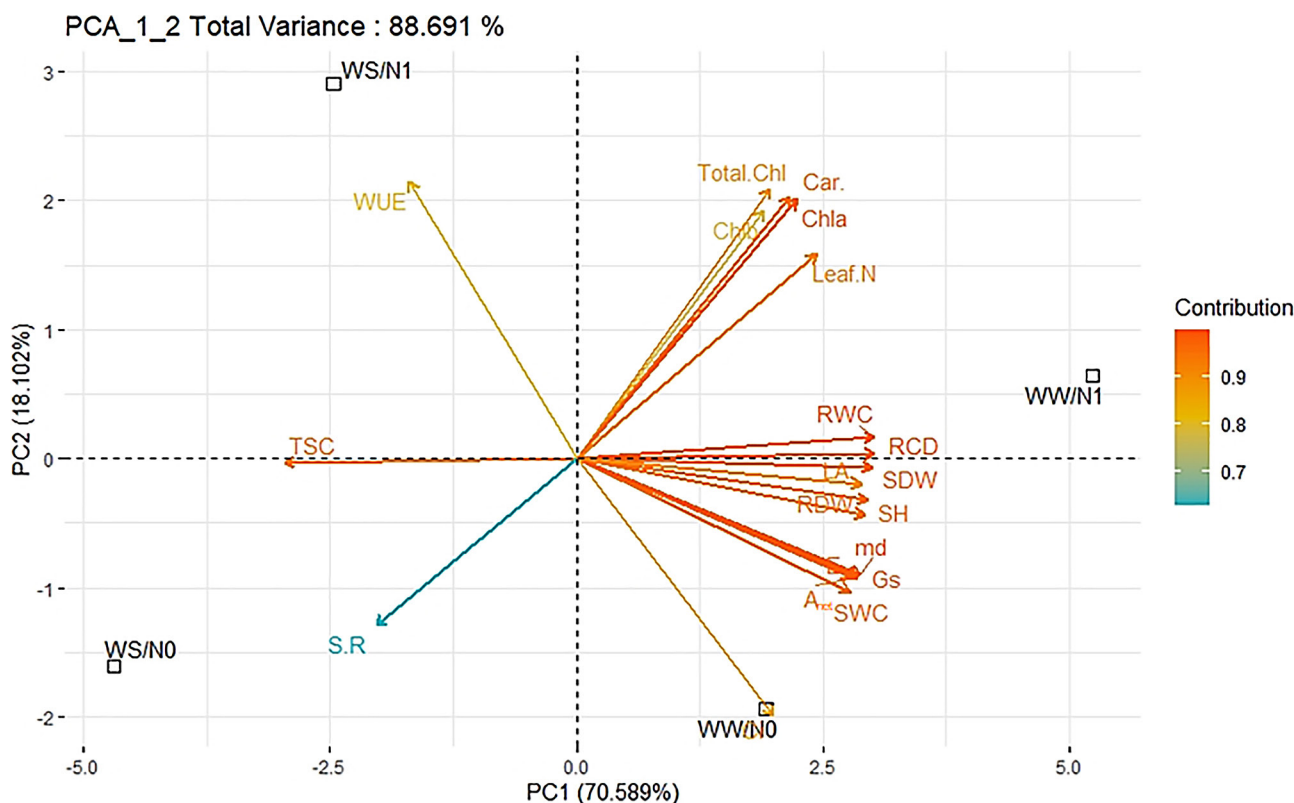


Fig. 3. Principal component analysis (PCA) of physiological, biochemical, and growth characteristics

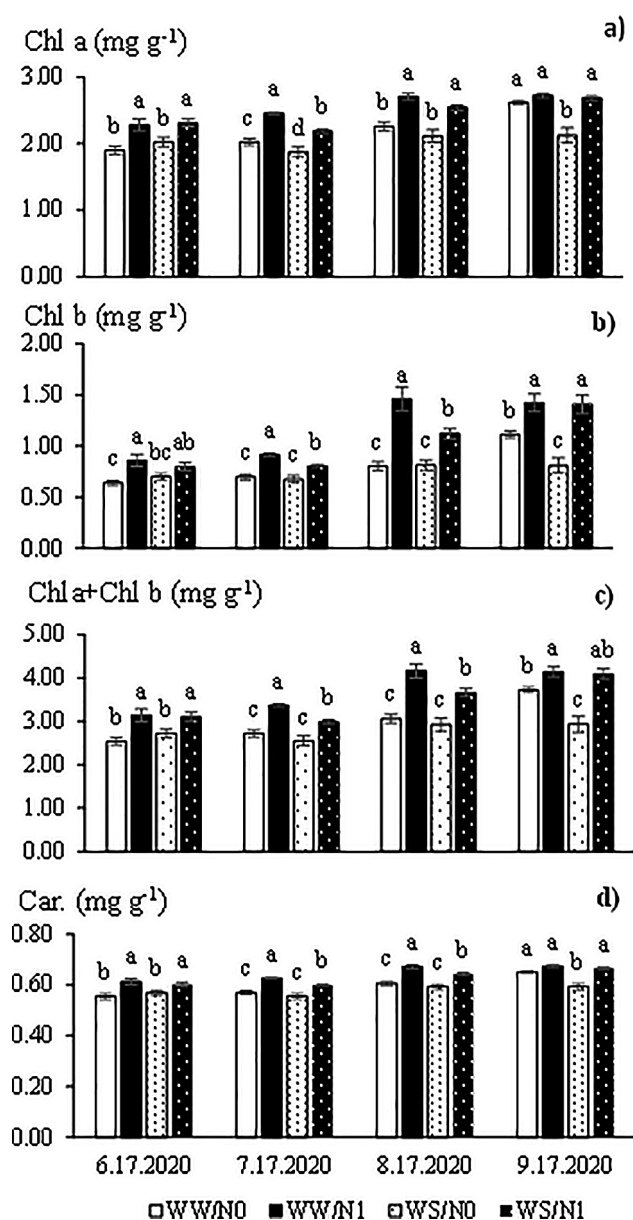


Fig. 5. a) Chlorophyll a (Chl a), b) chlorophyll b (Chl b), c) total chlorophyll (Chl a+b) and d) carotenoids (Car.) content of *Q. ithaburensis* under drought (WW and WS) and N (N0 and N1) treatments

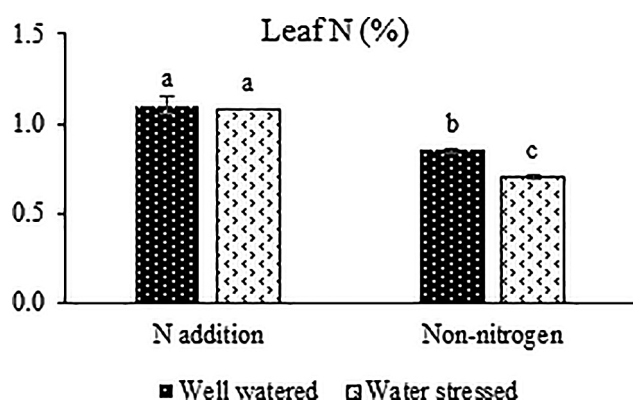


Fig. 6. Leaf N concentrations in *Q. ithaburensis* under drought and N treatments at the end of the drought cycles

RCD and LA. The interaction between drought stress and N addition did not affect RCD, SH, SDW, RDW, S/R, SLA, and LA (Table 2). RCD, SH, SDW, RDW, S/R, and LA are variables with high correlation in PC1 (Fig. 3).

Discussion

Drought stress and N addition effects on physiological and biochemical characteristics

This study investigated the effect of N addition and drought stress on stem water potential, net photosynthetic rate, stomatal conductance, transpiration rate, water use efficiency, total soluble sugar content, photosynthetic pigment content, and growth parameters on *Q. ithaburensis* seedlings. Seedlings under drought stress had lower SWC, Ψ_{md} , and RWC. Seedlings under drought stress had as low as -3.9 MPa midday leaf water potential. Nitrogen addition did not affect the water stress levels of *Q. ithaburensis* seedlings. Although the water relationship is not affected by the nutritional supplements, it depends on the presence of water (Correia et al., 1989). In the second drought cycle, seedlings without N addition under drought stress showed the lowest RWC and also well-watered *Q. ithaburensis* seedlings with N addition showed higher RWC. Wu et al. (2008) reported the same response in *Sophora davidii* (Franch.) Skeels. RWC increases when drought stress is accompanied by N addition (low N supply) and increase of N supply under well-watered conditions. Seedlings need nitrogen to support changes in all their cellular and physiological processes under drought conditions (Aliarab et al., 2020). Drought stress reduces SWC, Ψ (xylem water potential), and RWC (Deligöz & Bayar, 2021). The response of plants to drought plays a role in leaf morphology, leaf water potential, osmotic potential, photosynthesis, and stomatal conductance (Dickson & Tomlinson, 1996). Water stress causes a decrease in nutrient uptake, cell growth, leaf expansion and transpiration, and net CO_2 assimilation (Alam, 1999). Gas exchange parameters (e.g. *Anet*, *E*) decrease under water stress (Yin et al., 2006; Zhang et al., 2021). Based on the results, it can be reported that the reduction in SWC led to a reduction in *gs*, *E*, *Ci*, and *Anet* and an increase in *WUE* in drought-stressed seedlings. Net photosynthetic rate is highly correlated with *gs* and *E*. According to Chaves (1991), this correlation between *Anet* and *gs* is a common feature of drought-adapted species. In general, well-watered seedlings had similar *Anet*, *gs*, *Ci*, and *E* at the end of first drought cycle. Intercellular CO_2 concentrations generally depend on *gs* and the ability of mesophyll cells to assimilate intracellular CO_2 (Chen

et al., 2016). Stomatal closure reduces the C_i , which in turn changes the photosynthetic mechanisms. But the same photosynthetic mechanisms can also be affected independently of drought stress (Dickson & Tomlinson, 1996). The interaction between drought stress and N addition affected $Anet$, E , C_i , and WUE . Seedlings with N addition under drought stress had the highest WUE at the end of the first and second drought cycles. Seedlings under drought stress partially close their stomata, reducing C_i and photosynthesis, resulting in increased WUE at the end of the first drought cycles. Plants change to more efficient water use by partial closure of their stomata under drought stress (Esmaeilpour et al., 2016). A high WUE indicates that a plant has adapted to a lack of water for water conservation reasons (Chen et al., 2015). WUE in plants is greatly affected by nitrogen supply (da Silva et al., 2011). High nutrient supply due to a decrease in soil water content increases WUE (Welander & Ottosson, 2000). High nutrients may not improve drought tolerance (Kleiner et al., 1992). Low nitrogen decreases WUE , while high N increases it (Liu et al., 2013). N addition significantly increased net photosynthetic rate and stomatal conductance (Zhang et al., 2021). Yin et al. (2009) found that fertilization increased the net photosynthetic rate in poplars under well-watered conditions and its effect was negative under water-stressed conditions. Drought stress reduced the net photosynthetic rate in *Q. ithaburensis* seedlings. However, nitrogen addition increased the photosynthetic pigment content. Nitrogen addition under well-watered conditions increased the total N concentration of the leaves, resulting in an increase in the net photosynthetic rate at the end of third drought cycle. Both drought stress and N addition significantly affected Chla, Chl b, Chla+b, and Car. content and at the same time generally, well-watered seedlings with N addition showed higher Chla, Chl b, Chla+b, and Car. content. N addition may promote an increase in photosynthetic pigment content. Although seedlings with N addition (WS/N1 and WW/N1) had higher photosynthetic pigment content than without N addition (WS/N0 and WW/N0). N additions rise leaf N concentrations accompanied by higher net photosynthetic rate in Douglas-fir (Mitchell & Hinckley, 1993). There is a significant and positive relationship between photosynthetic capacity and leaf N content (Ripullone et al., 2003), which was consistent with our result. Total chlorophyll content (Chl a + b) is a good indicator of photosynthetic capacity. Research shows that photosynthetic pigments are reduced by drought stress (Wang et al., 2019) and increased by fertilization (Roca et al., 2018). Drought stress increases the activity of the chlorophyll-degrading enzyme (chlorophyll degradation) and decreases the chlorophyll content (Ajithkumar & Panneerselvam

2014). Fertilization promotes photosynthetic efficiency and increases photosynthetic pigment content, improving photosynthetic capacity under water deficiency (Chen et al., 2016). Nitrogen fertilization is recommended to increase the productivity of photosynthetic pigments under drought stress (Liu & Zhang, 2017). Reduced photosynthetic capacity is associated with N deficiency and negative feedback from the carbohydrate content in the leaf (Bousadia et al., 2010). Research also shows that drought stress increases the total soluble sugar content (Wu et al., 2013; Deligöz & Bayar, 2018). Drought stress increased soluble sugar content in needles of *Abies fabri* (Mast.) Craib. seedlings under unfertilized conditions, while it reduced under nitrogen supply (Guo et al., 2010). The results showed that the soluble sugar content was increased by drought stress and decreased by N addition. The total soluble sugar content decreased in N addition treatments under drought stress, which may be related to the fact that the seedlings use the total soluble sugar for growth. The reduction in the total soluble sugar content with N addition suggests that N addition modifies organic carbon allocation with more photosynthetic output for growth rather than improving stress tolerance (Zhou et al., 2011).

Drought stress and N addition effects on growth characteristics

The recurrent drought stress slowed down the plant growth. Drought stress significantly decreased RCD, SH, SDW, RDW, and LA. SLA was affected neither by drought stress nor by N accumulation. SLA, an indicator of leaf thickness, generally decreased under drought conditions. Thick leaves have a greater photosynthetic capacity than thin leaves because they generally contain higher chlorophyll concentrations and protein per unit leaf area (Liu & Stützel, 2004). Decreases in transpiration rate and water loss may lead to a decrease in LA under drought stress in *Q. ithaburensis*. Drought stress decreases LA, SDW, and RDW, resulting in significant differences in leaf areas between watered and N-treated seedlings (Dinh et al., 2017). The effect of N addition on SH, SDW, and RDW varies according to the irrigation regime and the amount of N (Rahimi et al., 2013; Song et al., 2019). The interaction between drought stress and N addition did not affect RCD and SH in *A. fabri* seedlings (Guo et al., 2010). In this study, nitrogen addition affected only RCD, and well-watered and N addition increased RCD in *Q. ithaburensis* seedlings. It is widely known that N fertilization is an essential determinant of RCD in seedlings (Pardos et al., 2005). In the third drought cycle, the well-watered with N addition treatments showed the highest $Anet$,

gs, and *E*, which may be one of the reasons for the increase in growth. Fertilization promoted plant growth in the well-watered conditions (Yin et al., 2009). Nitrogen addition affects plants' root collar diameter, height, and chlorophyll content (Razaq et al., 2017). Nitrogen deficiency affects plant productivity (decreasing net photosynthetic rate and leaf area) (Mu&Chen, 2021).

Conclusions

The results show that the presence of water is a limiting parameter in midday water potential and gas exchange. Under drought conditions, *Q. ithaburensis* seedlings demonstrated a drought adaptation mechanism that reduces *Anet*, *gs*, *E*, and *LA* and increases *WUE* and total soluble sugar content. In general, nitrogen addition under drought stress increased the *WUE* and also promoted the biochemical attributes (enhancing leaf N concentrations, and Chl content) of *Q. ithaburensis*. However, nitrogen addition did not affect the water stress level of the seedlings and did not change the declining tendency of the photosynthetic rate. This was a short-term study investigating the effect of a combination of drought stress and N addition.

Acknowledgments

The author is grateful to Prof. Dr. Serkan GÜLSOY, the coordinator of the Analytical Nature Clustering and Ordination Techniques Training (TÜBİTAK 2229-BİDEB) activity coordinator, and the study team for their contribution to the PCA analysis.

Conflict of interest

The author declares that they have no conflict of interest.

References

- Ajithkumar IP & Panneerselvam R (2014) ROS scavenging system, osmotic maintenance, pigment and growth status of *Panicum sumatrense* Roth. under drought stress. *Cell Biochemistry and Biophysics* 68: 587–595. doi:10.1007/s12013-013-9746-x.
- Alam SM (1999) Nutrient uptake by plants under stress conditions: Handbook of plant and crop stress (ed. by M Pessarakli) Marcel Dekker, New York, pp. 285–313.
- Aliarab A, Okati Vazifakhah E & Sadati SE (2020) Effect of soil moisture content and nitrogen fertilizer on survival, growth and some physiological characteristics of *Platycladus orientalis* seedlings. *Journal of Forest Science* 66: 511–523. doi:10.17221/34/2020-JFS.
- Arnon DI (1949) Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24: 1–15. doi:10.1104/pp.24.1.1.
- Boussadia O, Steppe K, Zgallai H, Ben El Hadj S, Braham M, Lemeur R & Van Labeke MC (2010) Effects of nitrogen deficiency on leaf photosynthesis, carbohydrate status and biomass production in two olive cultivars 'Meski' and 'Koroneiki'. *Scientia Horticulturae* 123: 336–342. doi:10.1016/j.scienta.2009.09.023.
- Bucci SJ, Scholz FG, Goldstein G, Meinzer FC, Franco AC, Campanello PI, Villalobos-Vega R, Bustamante M & Miralles-Wilhelm F (2006) Nutrient availability constrains the hydraulic architecture and water relations of savannah trees. *Plant, Cell and Environment* 29: 2153–2167. doi:10.1111/j.1365-3040.2006.01591.x.
- Chaves MM (1991) Effects of water deficits on carbon assimilation. *Journal of Experimental Botany* 42: 1–16. doi:10.1093/jxb/42.1.1.
- Chen L, Wang C, Dell B, Zhao Z, Guo J, Xu D & Zeng J (2018) Growth and nutrient dynamics of *Betula alnoides* seedlings under exponential fertilization. *Journal of Forest Research* 29:111–119. doi:10.1007/s11676-017-0427-2.
- Chen S, Bai Y, Zhang L & Han X (2015) Comparing physiological responses of two dominant grass species to nitrogen addition in Xilin River Basin of China. *Environmental and Experimental Botany* 53: 65–75. doi:10.1016/j.envexpbot.2004.03.002.
- Chen Y, Liu L, Guo Q, Zhu Z & Zhang L (2016) Effects of different water management options and fertilizer supply on photosynthesis, fluorescence parameters and water use efficiency of *Prunella vulgaris* seedlings. *Biological Research* 49: 12. doi:10.1186/s40659-016-0069-4.
- Correia MJ, Torres F & Pereira JS (1989) Water and nutrient supply regimes and the water relations of juvenile leaves of *Eucalyptus globus*. *Tree Physiology* 5: 459–471. doi:10.1093/treephys/5.4.459.
- Da Silva EC, Nogueira RJMC, da Silva MA & Albuquerque MB (2011) Drought stress and plant nutrition. *Plant Stress Global Science Books* 5: 32–41.
- Deligöz A & Bayar E (2018) Drought responses of seedlings of two oak species (*Quercus cerris* and *Quercus robur*). *Turkish Journal of Agriculture and Forestry* 42: 114–123. doi:10.3906/tar-1709-29.
- Deligöz A & Bayar E (2021) Impact of drought stress on water potential and gas exchange parameters in Macedonian oak (*Quercus trojana* P.B. Webb.) seedlings. *Turkish Journal of Forestry* 22: 366–370.

- Dickson RE & Tomlinson PT (1996) Oak growth, development and carbon metabolism in response to water stress. *Annals of Forest Science* 53: 181–196. doi:10.1051/forest:19960202.
- Dinh TH, Watanabe K, Takaragawa H, Nakabaru M & Kawamitsu Y (2017) Photosynthetic response and nitrogen use efficiency of sugarcane under drought stress conditions with different nitrogen application levels. *Plant Production Science* 20: 412–422. doi:10.1080/1343943X.2017.1371570.
- Dubey RS & Pessarakli M (2001) Physiological mechanisms of nitrogen absorption and assimilation in plants under stressful conditions: Handbook of Plant and Crop Physiology. 2nd ed. (ed. by M Pessarakli) Marcel Dekker Inc, New York, pp. 636–655.
- Dubois M, Gilles KA, Hamilton JK, Rebers PA & Smith F (1956) Calorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350–356. doi:10.1021/ac60111a017.
- Esmailpour A, Van Labeke M-C, Samson R, Boeckx P & Van Damme P (2016) Variation in biochemical characteristics, water status, stomata features, leaf carbon isotope composition and its relationship to water use efficiency in pistachio (*Pistacia vera* L.) cultivars under drought stress condition. *Scientia Horticulturae* 211: 158–166. doi:10.1016/j.scienta.2016.08.026.
- Gessler A, Schaub M & McDowell NG (2017) The role of nutrients in drought-induced tree mortality and recovery. *New Phytologist* 214: 513–520. doi:10.1111/nph.14340.
- Graciano C, Guamét JJ & Goya JF (2005) Impact of nitrogen and phosphorus fertilization on drought responses in *Eucalyptus grandis* seedlings. *Forest Ecology and Management* 212: 40–49. doi:10.1016/j.foreco.2005.02.057.
- Guo J, Yang Y, Wang G, Yang L & Sun X (2010) Ecophysiological responses of *Abies fabri* seedlings to drought stress and nitrogen supply. *Physiologia Plantarum* 139: 335–347. doi:10.1111/j.1399-3054.2010.01370.x.
- Jafarnia S, Akbarinia M, Hosseinpour B, Modarres Sanavi SAM & Salami SA (2018) Effect of drought stress on some growth, morphological, physiological, and biochemical parameters of two different populations of *Quercus brantii*. *iForest* 11: 212–220. doi:10.3832/for2496-010.
- Kleiner KW, Abrams MD & Schultz JC (1992) The impact of water and nutrient deficiencies on the growth, gas exchange and water relations of red oak and chestnut oak. *Tree Physiology* 11: 271–287. doi:10.1093/treephys/11.3.271.
- Koç İ (2021) Examining seed germination rate and seedlings gas exchange performances of some Turkish red pine provenances under water stress. *Duzce University Journal of Science and Technology* 9: 48–60. doi:10.29130/dubited.898820.
- Koç İ, Nzokou P & Cregg B (2021) Biomass allocation and nutrient use efficiency in response to water stress: insight from experimental manipulation of balsam fir, concolor fir and white pine transplants. *New Forests* 53: 1–19. doi:10.1007/s11056-021-09894-7.
- Liu F & Stützel H (2004) Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. *Scientia Horticulturae* 102: 15–27. doi:10.1016/j.scienta.2003.11.014.
- Liu J & Zhang J (2017) Effect of nitrogen on photosynthetic pigments of relay strip intercropping soybean under drought stress. *Advances in Engineering Research* 120: 892–895.
- Liu X, Fan Y, Long J, Wei R, Kjellgren R, Gong C & Zhao J (2013) Effects of soil water and nitrogen availability on photosynthesis and water use efficiency of *Robinia pseudoacacia* seedlings. *Journal of Environmental Sciences* 25: 585–595. doi:10.1016/S1001-0742(12)60081-3.
- MÇBK (2016) Oak Workshop Proceedings Book. https://marmaraarastirma.ogm.gov.tr/Documents/Mese_Calistayi_Bildiriler_Kitab%C4%B1.pdf.
- Mitchell AK & Hinckley TM (1993) Effects of foliar nitrogen concentration on photosynthesis and water use efficiency in Douglas-fir. *Tree Physiology* 12: 403–410. doi:10.1093/treephys/12.4.403.
- Mu X & Chen Y (2021) The physiological response of photosynthesis to nitrogen deficiency. *Plant Physiology and Biochemistry* 158: 76–82. doi:10.1016/j.plaphy.2020.11.019.
- OGM (2020) Forestry statistics 2020. General Directorate of Forestry, Ankara, <https://www.ogm.gov.tr/ekutuphane/Sayfalar/Istatistikler.aspx>.
- Öztürk S (2013) Türkiye meşeleri teşhis ve tanı kılavuzu. Orman ve Su İşleri Bakanlığı Orman Genel Müdürlüğü, Ankara.
- Pardos M, Royo A & Pardos JA (2005) Growth, nutrient, water relations, and gas exchange in a holm oak plantation in response to irrigation and fertilization. *New Forests* 30: 75–94. doi:10.1007/s11056-004-2553-x.
- Peguero Pina JJ, Sancho-Knapik D, Morales F, Flexas J & Gil-Pelegrín E (2009) Differential photosynthetic performance and photoprotection mechanisms of three Mediterranean evergreen oaks under severe drought stress. *Functional Plant Biology* 36: 453–462. doi:10.1071/FP08297.
- Rahimi A, Sayadi F, Dashti H & Tajabadi Pour A (2013) Effects of water and nitrogen supply on growth, water-use efficiency and mucilage yield of isabgol (*Plantago ovata* Forsk.). *Journal of*

- Soil Science and Plant Nutrition 13:341–354. doi:10.4067/S0718-95162013005000028.
- Razaq M, Zhang P, Shen HI & Salahuddin (2017) Influence of nitrogen and phosphorous on the growth and root morphology of *Acer mono*. PloS ONE 12: e0171321. doi:10.1371/journal.pone.0171321.
- Ripullone F, Grassi G, Lauteri M & Borghetti M (2003) Photosynthesis–nitrogen relationships: interpretation of different patterns between *Pseudotsuga menziesii* and *Populus* × *euroamericana* in a mini-stand experiment. Tree Physiology 23: 137–144. doi:10.1093/treephys/23.2.137.
- Ritchie GA (1984) Assessing seedling quality: Forest nursery manual: production of bareroot seedlings (ed. by ML Duryea & TD Landis) Martinus Nijhoff/Dr W. Junk Publishers, Oregon State University, Corvallis, pp. 243–259.
- Roca LF, Romero J, Bohórquez JM, Alcántara E, Fernández-Escobar R & Trapero A (2018) Nitrogen status affects growth, chlorophyll content and infection by *Fusicladium oleagineum* in olive. Crop Protection 109: 80–85. doi:10.1016/j.cropro.2017.08.016.
- Scholander PF, Hammel HT, Bradstreet ED & Hemmingsen EA (1965) Sap pressure in vascular plants: negative hydrostatic pressure can be measured in plants. Science 148: 339–346. doi:10.1126/science.148.3668.33.
- Seleiman ME, Al-Suhaibani N, Ali N, Akmal M, Alotaibi M, Refay Y, Dindaroglu T, Abdul-Wajid HH & Battaglia ML (2021) Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants 10: 259. doi:10.3390/plants10020259.
- Siam AMJ, Radoglou KM, Noitsakis B & Smiris P (2008) Physiological and growth responses of three Mediterranean oak species to different water availability regimes. Journal of Arid Environments 72: 583–592. doi:10.1016/j.jaridenv.2007.11.001.
- Song J, Wang Y, Pan Y, Pang J, Zhang X, Fan J & Zhang Y (2019) The influence of nitrogen availability on anatomical and physiological responses of *Populus alba* × *P. glandulosa* to drought stress. BMC Plant Biology 19: 63. doi:10.1186/s12870-019-1667-4.
- Villar-Salvador P, Peñuelas JL, Nicolás-Peragón JL, Benito LF & Domínguez-Lerena S (2013) Is nitrogen fertilization in the nursery a suitable tool for enhancing the performance of Mediterranean oak plantations? New Forests 44: 733–751. doi:10.1007/s11056-013-9374-8.
- Wang D, Huang G, Duan H, Lei X, Liu W, Wu J & Fan H (2019) Effects of drought and nitrogen addition on growth and leaf physiology of *Pinus massoniana* seedlings. Pakistan Journal of Botany 51: 1575–1585. doi:10.30848/PJB2019-5(30).
- Welander NT & Ottosson B (2000) The influence of low light, drought and fertilization on transpiration and growth in young seedlings of *Quercus robur* L. Forest Ecology and Management 127: 139–151. doi:10.1016/S0378-1127(99)00126-7.
- Wu F, Bao W, Li F & Wu N (2008) Effects of drought stress and N supply on the growth, biomass partitioning and water-use efficiency of *Sophora davidii* seedlings. Environmental and Experimental Botany 63: 248–255. doi:10.1016/j.envexpbot.2007.11.002.
- Wu M, Zhang WH, Ma C & Zhou JY (2013) Changes in morphological, physiological, and biochemical responses to different levels of drought stress in Chinese cork oak (*Quercus variabilis* Bl.) seedlings. Russian Journal of Plant Physiology 60: 681–692. doi:10.1134/S1021443713030151.
- Xu N, Guo W, Liu J, Du N & Wang R (2015) Increased nitrogen deposition alleviated the adverse effects of drought stress on *Quercus variabilis* and *Quercus mongolica* seedlings. Acta Physiologiae Plantarum 37: 107. doi:10.1007/s11738-015-1853-4.
- Xu Z & Zhou G (2008) Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. Journal of Experimental Botany 59: 3317–3325. doi:10.1093/jxb/ern185.
- Yin C, Pang X & Chen K (2009) The effects of water, nutrient availability and their interaction on the growth, morphology and physiology of two poplar species. Environmental and Experimental Botany 67: 196–203. doi:10.1016/j.envexpbot.2009.06.003.
- Yin CY, Berninger F & Li CY (2006) Photosynthetic responses of *Populus przewalski* subjected to drought stress. Photosynthetica 44: 62–68. doi:10.1007/s11099-005-0159-y.
- Zhang H, Li X, Guan D, Wang A, Yuan F & Wu J (2021) Nitrogen nutrition addition mitigated drought stress by improving carbon exchange and reserves among two temperate trees. Agricultural and Forest Meteorology 311: 108693. doi:10.1016/j.agrformet.2021.108693.
- Zhou X, Zhang Y, Ji X, Downing A & Serpe M (2011) Combined effects of nitrogen deposition and water stress on growth and physiological responses of two annual desert plants in northwestern China. Environmental and Experimental Botany 74: 1–8. doi:10.1016/j.envexpbot.2010.12.005.