Turkish Journal of Forestry | Türkiye Ormancılık Dergisi
2024, 25(1): 41-48 | Research article (Araştırma makalesi)

A comparison of photosynthetic gas exchange parameters measured under in situ and in vitro conditions in *Pinus nigra* subsp. *pallasiana* and *Pinus brutia* trees

Esra Bayar[a,*], Mehmet Said Özçelik[a]

Abstract: This study was conducted to compare the results of in situ and in vitro methods that can be used for measuring gas exchange parameters in two pine species. The study was carried out in a mixed *Pinus nigra* Arn. subsp. *pallasiana* (Lamb). Holmboe and *Pinus brutia* Ten. plantation in Kemer/Burdur, which has a semi-arid climate type located in the Western Mediterranean Region of Türkiye. In the first method, gas exchange parameters (net photosynthetic rate-A_{net}, stomatal conductance-g_s and transpiration rate-E) were measured directly on the tree using a mobile scaffold to reach to the tree canopy (in situ conditions). In the second method, branches were cut at approximately 50 cm and gas exchange parameters were determined in the same needles after the branch was immediately submerged in water (in vitro conditions). Measurements were taken between June and October 2022. Student's t-test was conducted to compare the results of in situ and in vitro photosynthesis measurement methods. No statistically significant differences were found between the results of the compared methods in terms of gas exchange parameters for both species. The results of the study showed that in vitro measurements of photosynthesis can be preferred to in situ measurements of photosynthesis in *P. nigra* and *P. brutia* under field conditions where access to the canopy of tall trees is not possible.
Keywords: Anatolian black pine, Branch cutting, Gas exchange, In situ measurement, Mediterranean forest, Turkish red pine

Pinus nigra subsp. *pallasiana* ve *Pinus brutia* ağaçlarında in vitro ve in situ koşullarda ölçülen fotosentetik gaz değişim parametrelerinin karşılaştırılması

Özet: Bu çalışma, iki çam türünde gaz değişim parametrelerini belirlemede kullanılabilecek in situ ve in vitro metotların sonuçlarının karşılaştırılması amacıyla yapılmıştır. Çalışma, Türkiye'nin Batı Akdeniz Bölgesi'nde yer alan yarı kurak iklim tipine sahip Kemer/Burdur orman alanındaki *Pinus nigra* Arn. subsp. *pallasiana* (Lamb). Holmboe ve *Pinus brutia* Ten. karışık plantasyon sahasında gerçekleştirilmiştir. Birinci yöntem olarak her iki türde gaz değişim parametreleri (net fotosentez hızı- A_{net}, stoma iletkenliği-g_s ve terleme oranı-E) kurulan bir iskele yardımıyla doğrudan ağaç üzerinde ölçülmüştür (in situ koşulları temsilen). İkinci yöntemde, ölçüm yapılan ibrelerin bulunduğu dallar yaklaşık 50 cm'den kesilip suya daldırılarak aynı ibreler üzerinde gaz değişim parametreleri belirlenmiştir (in vitro koşulları temsilen). Çalışma 2022 yılının Haziran-Ekim ayları arasında gerçekleştirilmiştir. In situ ve in vitro fotosentez ölçüm yöntemlerinin sonuçlarını karşılaştırmak için Student t testi uygulanmıştır. Örnekleme günlerinde, iki türde de gaz değişim parametreleri bakımından uygulanan metotların sonuçları arasında istatistiksel olarak anlamlı bir farklılık belirlenmemiştir. Çalışma sonuçları, boylu ağaçların dallarına erişimin mümkün olmadığı arazi koşullarında *P. nigra* ve *P. brutia* türlerinde in situ fotosentez ölçümleri yerine in vitro fotosentez ölçümlerinin tercih edilebileceğini göstermiştir.
Anahtar kelimeler: Anadolu karaçamı, Dal kesme, Gaz değişimi, Yerinde ölçüm, Akdeniz Ormanları, Kızılçam

1. Introduction

Photosynthesis is the main source of oxygen, food and energy on earth that provides sustainability of life; therefore, it is one of the most important bio-chemical processes (Flügge et al., 2016; Yin et al., 2022). During photosynthesis, higher plants convert sunlight energy to chemical energy via fixing CO_2 and releasing O_2 while coping with water loss (Millan-Almaraz et al., 2009). Due to its role in ecosystem functioning, research on photosynthesis has been of interest for more than 350 years (Huzisige and Ke, 1993). Up to date photosynthesis continues to be measured by researchers for various purposes such as plant growth, biomass allocation, species competition, ecosystem functioning and climate change mitigation (Field et al., 1989; Saxe, 1991; Hättenschwiler and Körner, 1997; Karnosky et al., 2003; Nunes et al., 2020; Sazeides et al., 2021; Schönbeck et al., 2022).

Several types of methods are available to measure or estimate gas exchange parameters at different plant levels, from leaves to entire stands (Hunt, 2003; Millan-Almaraz et al., 2009; Siebers et al., 2021). Measuring leaf gas exchange in situ conditions, where the petiole of the leaf is attached to the branch, is of crucial importance in the analysis of photosynthetic processes in plants among all methods (Gauthier and Jacobs, 2018). All commercially available

[a] Isparta Uygulamalı Bilimler Üniversitesi, Orman Fakültesi, Orman Mühendisliği Bölümü, Isparta, Türkiye
[*] **Corresponding author** (İletişim yazarı): esrabayar@isparta.edu.tr
Received (Geliş tarihi): 14.12.2023, **Accepted** (Kabul tarihi): 14.03.2023

Citation (Atıf)**:** Bayar, E., Özçelik, M.S., 2024. A comparison of photosynthetic gas exchange parameters measured under in situ and in vitro conditions in *Pinus nigra* subsp. *pallasiana* and *Pinus brutia* trees. Turkish Journal of Forestry, 25(1): 41-48.
DOI: 10.18182/tjf.1404940

portable photosynthesis measurement systems use a similar operating method, enclosing a part of the leaf or entire leaf in a chamber or cuvette (Haworth et al., 2018). Many studies have been conducted in which gas exchange parameters (net photosynthetic rate, stomatal conductance and transpiration rate) have been measured in seedlings under in situ conditions (Jafarnia et al., 2018; Deligöz and Bayar, 2021; Koç and Nzokou, 2023). Gas exchange measurements are relatively easy to obtain in seedlings because leaves are easily accessible compared to mature trees (Gauthier and Jacobs, 2018). However, for tall trees, in situ measurements usually require building up a permanent or a mobile elevated platform such as a ladder, a lift, or a scaffold to ensure that the portable photosynthesis measurement system remains stable during the measurement (Loewenstein and Pallardy, 1998; Gauthier and Jacobs, 2010; Gauthier and Jacobs, 2018; Bayar and Deligöz, 2020; Akalusi et al., 2021). It is difficult to carry an elevated mobile platform, especially in forest areas with steep slopes, rough or rocky terrain, or when branches that allow measurement exceed the platform's maximum height. In addition, such efforts may limit the sample size, can be time consuming and require more advanced security equipment for researchers (Gauthier and Jacobs, 2018; Akalusi et al., 2021). Therefore, researchers conducted different methods to eliminate the effect of unfavourable terrain conditions during photosynthesis measurements, i.e., branch beveling, leaf detaching, cracking, splitting, and girdling (Tang and Wang, 2011; Gauthier and Jacobs, 2018; Meng et al., 2019).

In this study, we chose an in vitro method that is currently used in field conditions and has been tested on various species in previous studies (Miyazawa et al., 2011; Tang and Wang, 2011; Meng et al., 2019; Verryckt et al., 2020; Akalusi et al., 2021). In this method, the piece of the branch containing the leaves to be measured is cut off from the tree with a sharp scissors, submerged into water without wasting any time, then it is once more cut under water to prevent cavitation (Koike, 1986; Pérez-Harguindeguy et al., 2013; Verryckt et al., 2020).There is still lack of information in the literature about whether in vitro measurements of photosynthesis can be conducted in pine trees. Therefore, comparing in situ and in vitro measurements of photosynthesis has become necessary for forested areas of the Mediterranean Region of Türkiye, where accessing the canopy of tall trees is a challenge and carrying a height adjustable platform is not reasonable due to unfavourable field conditions i.e. steep slopes, diverse topography, and rocky terrain.

The most common and ecologically important pine species of Türkiye, *Pinus nigra* Arn. subsp. *pallasiana* (Lamb). Holmboe (Anatolian black pine) and *Pinus brutia* Ten (Turkish pine) (Atalay, 2002) has been selected as research materials in the study. Moreover, these two species totally cover almost 45% of the forested areas of Türkiye (General Directorate of Forestry, 2022). The study aims (I) to compare the results of the gas exchange parameters measured under in situ and in vitro conditions in *P. nigra* and *P. brutia* trees and (II) to test if it is possible to use in vitro measurements especially in forests where carrying a platform is impractical. We hypothesized that there would be no statistically significant difference between the two methods in *P. nigra* and *P. brutia* growing under semiarid climate conditions.

2. Material and methods

2.1. Location of the study site and environmental conditions

The study site is located in Kemer/Burdur forest district in the western Mediterranean region of Türkiye (37° 21ˈ N, 30° 8ˈ E; Figure 1). The forest stand is a mixed *P. nigra*- *P. brutia* plantation established in 1991 and the seedlings of both species were obtained from Gölhisar Forest Nursery. The height of both *P. nigra* and *P. brutia* trees were approximately 10 m, and the diameter at breast height varied between 23.90 cm ± 1.44 cm and 26.20 cm ± 2.17 cm for *P. nigra* and *P. brutia* trees, respectively.

According to the nearest meteorology station (Tefenni) that provides 20 years of climate data, the study area has semi-arid characteristics in the Erinc climate index. Consistent with long-term climate data, the annual total precipitation in 2022 was recorded as 331.40 mm, and the average annual temperature and average annual relative humidity were recorded as 12.40 °C and 59.90%, respectively.

Soil water content was determined according to the gravimetric method monthly between June and October at fifteen locations randomly selected for homogeneous representation of the study site. Soil samples were taken from a depth of 0-20 cm and placed in sealed glass jars to prevent loss of soil moisture through evaporation. They were brought to the laboratory as soon as possible and their moist weight was determined on a 0.01 g precision balance. After that, the samples were placed in the oven and dried at 105°C for 24 hours. Soil water content was determined according to the ratio of the dry weight and wet weight records.

2.2. Experimental design

Ten individuals from each species were randomly selected. In selecting sample trees, care was taken to ensure that they were of similar diameter and height, had smooth trunks, were healthy, had no damage to their canopy, and had no visible disease. Measurements were always taken from the south-facing branches of the same sample trees approximately at the same height. Two different methods were applied to determine gas exchange parameters. In the first method: the gas exchange parameters were measured directly on the selected branch of each tree by using a mobile scaffold to reach to the canopy (Figure 2a). The measured needles were marked with a permanent ink pen after the measurement. In the second method, the piece of branch carrying the marked needles was cut to a length of approximately 50 cm from the apex with a sharp scissors. To prevent air insertion into the xylem vessels and damaging water absorption by causing cavitation, the cut branches were immediately immersed in water and their ends (approximately 5 cm) were cut again under water (Figure 2b) as recommended in previous studies (Miyazawa et al., 2011; Verryckt et al., 2020; Missik et al., 2021). Then, the same needles (marked) were placed in the chamber of a portable photosynthesis device and gas exchange parameters were measured again. The first method was referred as 'in situ' for the measurements made directly on the tree, and the second method, which was measured by cutting the branch, was referred as 'in vitro' in this study. The study period covered June to October 2022 and measurements were repeated at least once a month.

Figure 1. Location of the study site

Figure 2. Measurement of gas exchange parameters by a) in-situ method and b) in-vitro method

2.3. Gas-exchange measurements

Gas exchange measurements were carried out on clear days to minimise the effects of radiation. A portable photosynthesis measurement device, LI-6400XT (Lincoln, USA) was used for leaf-level gas exchange measurements. All measurements were completed between 09:00-16:00 o'clock with a 6 cm^2 chamber and an attached light source (6400-02B-red/blue/light). The calibration was performed as recommended by the producer for field conditions. Light curve was measured in the field and the photosynthesis photon flux density (PPFD) was determined. The PPFD, air flow rate, and reference CO_2 were set and held automatically at 1300 µmol m^{-2} s^{-1}, 500 µmol s^{-1} and 400 ppm, respectively. The leaf temperature is set depending on the air temperature during the measurement. From a south-facing branch of ten trees, sun exposed and fully developed five needles of one-year-old shoots were randomly selected and the needles were ordered in the cuvette in a flat plane without overlapping each other. Net photosynthetic rate (A_{net}, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}) and transpiration rate (E, mmol H_2O m^{-2} s^{-1}) were recorded on the device when they became stable (after 5 minutes on average) and they determined using the needle's total surface area (Svenson and Davies, 1992).

2.4. Statistical analyses

The statistical evaluation of the recorded data was performed with SPSS 25.0 Windows package programme. Initially, normality of error and homogeneity of variance were checked. Differences between the two applicated methods were analysed with Student's t test separately both for the two species (*P. nigra* and *P. brutia*) and the gas exchange parameters (*Anet*, *gs*, E). The data are presented as mean and standard error of the mean in two methods for both species.

3. Results

Total monthly precipitation during the sampling dates varied between 1.2 mm and 29.3 mm. Total precipitation was 58.40 mm during the summer period. Soil water content ranged from 8.70 % to 11.17 % (Figure 3). The average temperature values between 09:00 and 16:00 hours on the measurement days were recorded as 21.4 °C in June, 29.2 °C in July, 28.1 °C and 28.9 °C in August, 27.1 °C in September and 20.6 °C in October. Average relative humidity between measured hours was 62.4% in June, 19.8% in July, 52.0% and 28.6% in August, 19.7% in September and 41.6% in October.

As shown in Table 1 and 2, there was no statistically significant difference between in situ and in vitro measurements in the gas exchange parameters according to sampling dates in *P.nigra* trees at 95% confidence level. During all sampling dates, the results for A_{net}, g_s, and *E* were similar for both the in situ and in vitro measuments. According to the results gas exchange parameters were higher in early June, they decreased during the summer and slightly increased again in the fall (Figure 4a).

The in vitro A_{net}, g_s *and E* values of *P. brutia* samples were not significantly different from the in situ A_{net}, g_s, and E values (Table 1). A similar seasonal variation in the gas exchange parameters of *P. brutia* was determined as in *P. nigra* (Figure 4b).

Although A_{net} values were slightly higher in vitro measurements in general, they were not statistically significant. While the in situ net photosynthetic rate of *P. nigra* in June was 10.12 µmol CO_2 m^{-2} s^{-1} and in vitro *Anet* was 10.45 µmol CO_2 m^{-2} s^{-1}, these values decreased in August and recorded as 3.22 µmol CO_2 m^{-2} s^{-1} and 3.37 µmol CO_2 m^{-2} s^{-1}. Similarly, while the in situ net photosynthetic rate of *P. brutia* in June was 10.37 µmol CO_2 m^{-2} s^{-1} and in vitro *Anet* 9.70 µmol CO_2 m^{-2} s^{-1}, it was determined as 3.61 µmol CO_2 m^{-2} s^{-1} and 3.94 in August, respectively (Table 1 and Table 2).

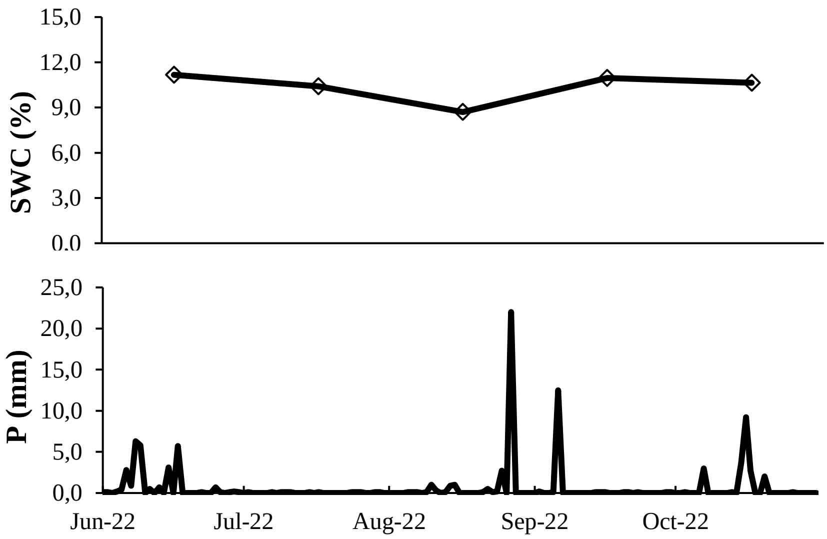

Figure 3. Temporal pattern in soil water content (SWC) and total precipitation (P) during the study period at the study site

Table 1. Comparison of gas exchange parameters in situ and in vitro methods (*Anet*: net photosynthetic rate, µmol CO_2 m^{-2} s^{-1}; *gs*: stomatal conductance, mol H_2O m^{-2} s^{-1}) and transpiration rates (E, mmol H_2O m^{-2} s^{-1}) in *P.nigra*

Gas exchange parameters	Methods	Sampling dates					
		07.06.2022	06.07.2022	09.08.2022	23.08.2022	15.09.2022	12.10.2022
A_{net}	In situ	10.12(0.25)[a]	4.94(0.63)[a]	3.87(0.22)[a]	3.22(0.25)[a]	3.31(0.51)[a]	4.91(0.38)[a]
	In vitro	10.45(0.14)[a]	5.69(0.48)[a]	4.35(0.29)[a]	3.37(0.24)[a]	3.79(0.44)[a]	5.33(0.34)[a]
g_s	In situ	0.10(0.00)[a]	0.04(0.00)[a]	0.03(0.00)[a]	0.02(0.00)[a]	0.02(0.00)[a]	0.04(0.00)[a]
	In vitro	0.11(0.00)[a]	0.04(0.00)[a]	0.03(0.00)[a]	0.02(0.00)[a]	0.03(0.00)[a]	0.05(0.00)[a]
E	In situ	2.03(0.06)[a]	1.55(0.19)[a]	1.05(0.05)[a]	1.02(0.06)[a]	0.80(0.10)[a]	0.92(0.06)[a]
	In vitro	2.07(0.05)[a]	1.78(0.19)[a]	1.15(0.06)[a]	1.17(0.06)[a]	1.08(0.10)[a]	1.04(0.07)[a]

The same superscript letters in the same line indicate there are not significant differences (p > 0.05). Mean and the standard error values of the measured gas exchange parameters in P. nigra samples (n=10).

Table 2. Comparison of gas exchange parameters measured in situ and in vitro methods (*Anet*: net photosynthetic rate, µmol CO_2 m^{-2} s^{-1}; *gs*: stomatal conductance, mol H_2O m^{-2} s^{-1}) and transpiration rates (E, mmol H_2O m^{-2} s^{-1}) in *P. brutia*

Gas exchange parameters	Methods	Sampling dates					
		07.06.2022	06.07.2022	09.08.2022	23.08.2022	15.09.2022	12.10.2022
A_{net}	In situ	10.37(0.42)[a]	6.59(0.57)[a]	4.53(0.55)[a]	3.61(0.17)[a]	4.12(0.44)[a]	4.84(0.24)[a]
	In vitro	9.70(0.38)[a]	6.77(0.30)[a]	5.09(0.51)[a]	3.94(0.16)[a]	4.40(0.51)[a]	5.20(0.19)[a]
g_s	In situ	0.12(0.01)[a]	0.05(0.00)[a]	0.04(0.00)[a]	0.03(0.03)[a]	0.02(0.00)[a]	0.04(0.00)[a]
	In vitro	0.12(0.01)[a]	0.06(0.00)[a]	0.04(0.00)[a]	0.02(0.03)[a]	0.03(0.00)[a]	0.04(0.00)[a]
E	In situ	2.39(0.12)[a]	2.16(0.13)[a]	1.46(0.13)[a]	1.22(0.04)[a]	0.96(0.10)[a]	1.08(0.04)[a]
	In vitro	2.44(0.12)[a]	2.33(0.20)[a]	1.55(0.26)[a]	1.17(0.04)[a]	1.06(0.13)[a]	1.17(0.05)[a]

The same superscript letters in the same line indicate there are not significant differences (p > 0.05). Mean and standard error values of the measured gas exchange parameters in P. brutia samples (n=10).

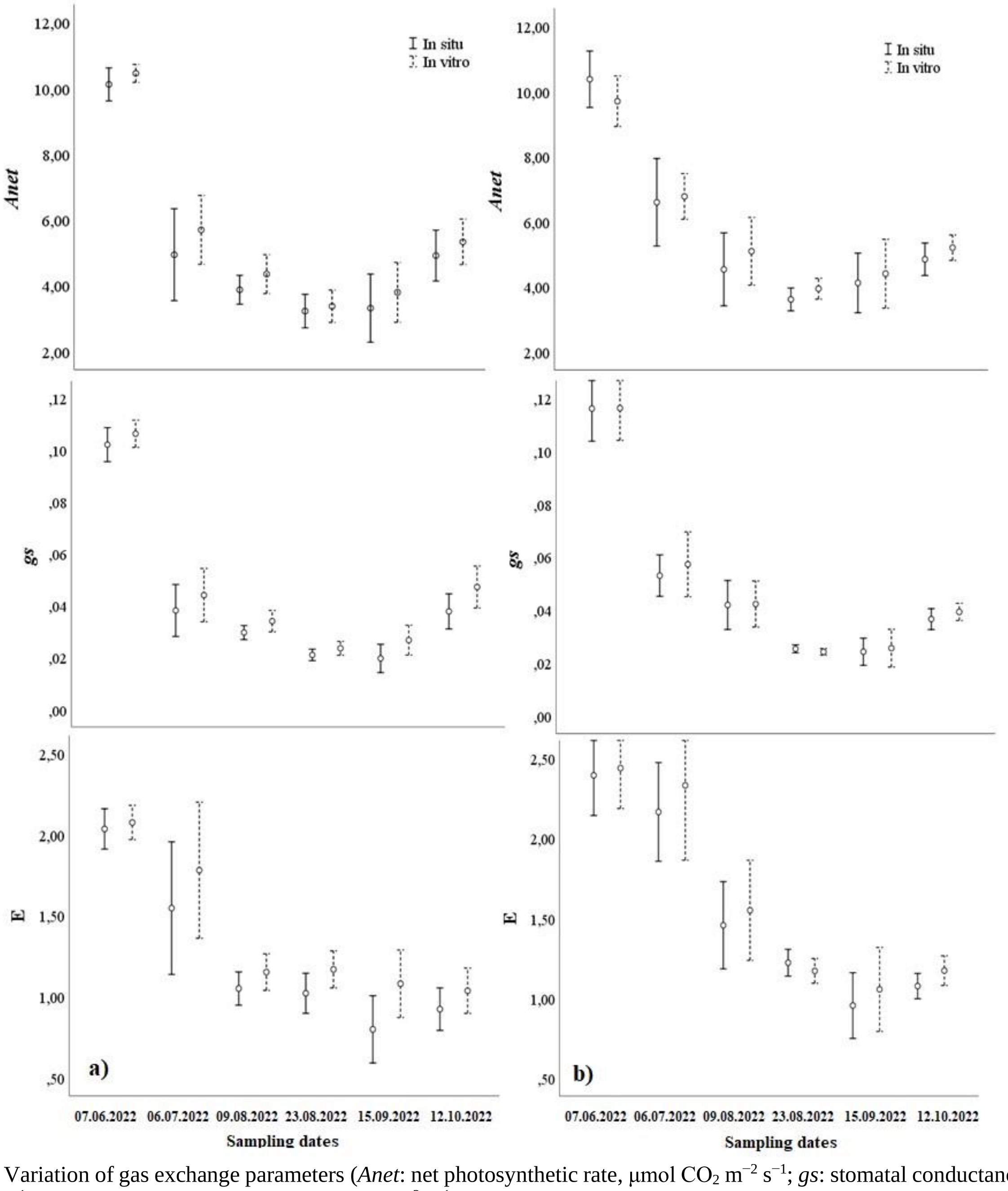

Figure 4. Variation of gas exchange parameters (*Anet*: net photosynthetic rate, µmol CO_2 m^{-2} s^{-1}; *gs*: stomatal conductance, mol H_2O m^{-2} s^{-1}) and transpiration rates (E, mmol H_2O m^{-2} s^{-1}) according to the method applied (solid line: in situ; dashed line: in vitro) during the study period in **a)** *P.nigra* **b)** *P. brutia*.

4. Discussions and conclusions

Studies on gas exchange measurements facilitate the understanding of the response of trees to changes in environmental conditions (Shinde et al., 2018; Gauthier and Jacobs, 2018). In this regard, it is also important to know the details of the measurement methods used to obtain the gas exchange parameters. In this study, we focused on comparing the results of in situ and in vitro photosynthesis measurements in *P.nigra* and *P. brutia* growing under semi-arid climate conditions in Burdur province of Türkiye.

According to the results, no statistically significant difference was found in net photosynthetic rate, stomatal conductance, and transpiration rate between in situ and in vitro measurements (Table 1 and 2) for both tree species. Seasonality, sampling dates and soil water content did not alter the results between in situ and in vitro methods on gas exchange parameters. The results of the study are consistent with the results of previous research where branch excision had no significant effect on A_{net} in various tree species (Miyazawa et al., 2011; Verryckt et al., 2020; Akalusi et al., 2021). After branch excision and re-cut under distilled water, Miyazawa et al. (2011) found that gas exchange rates and stomatal control of the excised leaves in response to environmental conditions did not change in *Lithocarpus edulis* Nakai trees. Akalusi et al. (2021) compared photosynthetic parameters and stomatal conductance in attached and detached foliage of *Abies balsamea* (L.) Mill. trees and they did not observe a significant effect of branch detachment on the results. Verryckt et al. (2020) measured leaf net photosynthetic rate on intact and cut branches in seven tree species from *Malvaceae, Burceraceae, Lecythidaceae* and *Fabaceae* families. They concluded that the branch cutting method was practical when the branch was re-cut under water and the leaves were given sufficient time to acclimate to the new environmental conditions. On the other hand, there are also reports indicating that the net photosynthetic rate significantly decreased after branch detachment (Santiago and Mulkey, 2003; Gauthier and Jacobs, 2018; Missik et al., 2021). Santiago and Mulkey (2003) reported a significant reduction in net CO_2 assimilation rates ranging from 14% - 87% for ten species from nine families. Similarly, Gauthier and Jacobs (2018) observed a sharp decrease in net photosynthesis (41% - 74%) within ten minutes after cutting the branches in *Quercus rubra, Q. alba* and *Juglans nigra* trees. Missik et al. (2021) also stated that branch excision immediately reduced photosynthesis and stomatal conductance by 27% to 62% in *Q. alba* L., *Acer saccharum* Marsh. and *Liriodendron tulipifera* L. trees.

One of the reasons that the results of photosynthesis measurements conducted on the intact branches and excised branches vary depending on the species could be the differences in the length of the vessels, therefore it is recommended cutting branches much longer than the species-specific vessel lengths (Missik et al., 2021). Another reason could be the isohydric or anisohydric behaviour of the species, since isohydric species tend to close their stomata in mild water stress and limiting their photosynthesis capacity whereas anisohydric species tend to maintain their photosynthetic activity under water stress conditions (Aguadé et al., 2015). Moreover, it is reported that wood anatomy is also a factor that can affect the results of in vitro measurements. Koike (1986) stated that the photosynthesis of diffuse porous trees can be easily measured by harvested shoots, whereas that of ring porous trees cannot be determined by the same method. Nevertheless, Tang and Wang (2011) found that in vitro photosynthesis measurements via cut shoots practicable for coniferous, diffuse porous and ring porous species.

Another possible factor affecting the results of in vitro photosynthesis measurements is the measurement time following the leaf detachment from the tree in case the leaves quickly lose their photosynthetic activity (Koike, 1986; Voronin and Fedoseeva, 2012; Gauthier and Jacobs, 2018; Akalusi et al., 2021). In this context, varying results from 3 minutes to 1 hour have been reported for various species by researchers. For example; Voronin and Fedoseeva (2012) observed that stomatal control of photosynthesis was retained within first 3–5 min after leaf excision in herbaceous and woody plants. Akalusi et al. (2021) reported that leaf gas exchange measurements can be obtained up to 30 minutes after leaf detachment in balsam fir. Koike (1986) found that leaf water supply and photosynthetic activity was effectively maintained for one hour following detachment of the leaves including both ring porous and diffuse porous tree species.

The main pursuance to pay attention to in all physical in vitro photosynthesis measurements is to ensure that water transport to the leaves continues at an adequate level after deteriorating in situ conditions. Thus, in order to eliminate the embolism effect that occurs when cutting a branch from a tree and to remove the embolized part of the branch, it is recommended to re-cut the cut branch under water before the leaf gas exchange measurements (Dang et al., 1997; Santiago and Mulkey, 2003; Verryckt et al., 2020; Missik et al., 2021). In this context, there are reports with bilateral results on whether re-cutting branches while the tension continues under water causes a significant decrease in hydraulic conductivity that can affect the gas exchange parameters or not. For instance, Wheeler et al. (2013) and Torres-Ruiz et al. (2015) emphasized that severing the branches even under water can still introduce embolism to the xylem that can affect the hydraulic conductivity. In response, Scoffoni and Sack (2015) and Venturas et al. (2015) reported that they could not determine an impact of such an artificial embolism on the xylem hydraulic conductance when the branches cut with native tension under the water. In our study, excising the branch and immediately re-cut with natural tension under water did not affect the net photosynthetic rate of the studied species. It is thought that the short length and narrow diameter of the tracheids, which provide water transmission in coniferous species, as well as the low water transmission rate, played an important role in obtaining this result (Akkemik, 2018).

Apart from the species-specific variations that affect the results of photosynthesis measurements in vitro conditions, differences in detail among the physical in vitro methods can also cause alteration in the results. For instance, Meng et al. (2019) compared several methods to find out the best method to measure photosynthesis in vitro conditions including "branch beveling" (the same method we applied in this study), cracking, splitting, girdling, and immersion in salicylic acid solution. According to the results of this research, it is proven that cracking has given the best results and the branch beveling underestimated net photosynthetic rate by values ranging from 9% to 65%. On the other hand, Tang and Wang (2011) indicated that girdling (instantly inserting the twigs into water after detaching, girdle phloem about 3 cm from the cut and remove all leaves except the target ones) is a more feasible method than branch beveling for a variety of tree species. In this study we found that the in vitro measurements (cutting the branch, immediately immersing to the water and re-cut under the water) gave slightly higher results than in situ measurements in *P. nigra* and *P. brutia* species, however the differences between the results were not statistically significant.

Carrying out photosynthesis measurements in conditions where the land and the plants to be measured are convenient for in situ measurements ensure the most reliable results (Gauthier and Jacobs, 2018). However, in vitro photosynthesis measurements are needed, especially in tall trees where it is difficult to reach the branches and in areas where setting up or transporting a platform is not possible. The results of the study supported the initially established hypothesis and provided a practical, time and cost-effective methodology to measure gas exchange parameters in the most widespread pine species (*P. nigra* and *P. brutia*) of Türkiye where in-situ measurements are not practical due to unfavourable terrain and stand conditions. There is a necessity for further in vitro photosynthesis measurement experiments, especially in Türkiye, which has a generous tree species diversity, heterogenous stand characteristics and variable terrain texture. Further experiments will help to picture if such methods are applicable for a variety of tree species with different physiological characteristics.

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*, 2505-2529.

Akalusi, M.E., Meng, F-R., Bourque, C.P.A., 2021. Photosynthetic parameters and stomatal conductance in attached and detached balsam fir foliage. Plant-Environment Interactions, 2(4):206-215.

Akkemik, Ü., 2018. Ağaç Fizyolojisi. İstanbul Üniversitesi-Cerrahpaşa Yayınları, İstanbul.

Atalay, İ., 2002. Türkiye'nin Ekolojik Bölgeleri. Orman Bakanlığı Yayınları, İzmir.

Bayar, E., Deligöz, A., 2020. Impacts of precommercial thinning on gas exchange, midday water potential, and chlorophyll content in *Pinus nigra* subsp. *pallasiana* stand from the semiarid region. Trees Structure and Function, 34(5): 1169-1181.

Dang, Q-L., Margolis, H.A., Coyea, M.R., Sy, M., Collatz, G.J., 1997. Regulation of branch-level gas exchange of boreal trees: roles of shoot water potential and vapor pressure difference. Tree Physiology, 17 (8-9): 521–535.

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(4): 366-370.

Field, C.B., Ball, J.T., Berry, J.A., 1989. Photosynthesis: principles and field techniques. In: Plant Physiological Ecology (Ed: Pearcy, R.W., Ehleringer, J.R., Mooney, H.A., Rundel, P.W.). Springer, New York, pp.209-253.

Flügge, U.-I., Westhoff, P., Leister, D., 2016. Recent advances in understanding photosynthesis. F1000Research, 5:2890.

General Directorate of Forestry (Türkiye), 2022. Official statistics. https://www.ogm.gov.tr/tr/e-kutuphane/resmi-istatistikler, Accessed: 10.12.2023.

Gauthier, M.M., Jacobs, D.F., 2010. Ecophysiological responses of black walnut (*Juglans nigra*) to plantation thinning along a vertical canopy gradient. Forest Ecology and Management, 259(5): 867–874.

Gauthier, M.M., Jacobs, D.F., 2018. Reductions in net photosynthesis and stomatal conductance vary with time since leaf detachment in three deciduous angiosperms. Trees, 32(5): 1247–1252.

Hättenschwiler, S., Körner, C., 1997. Biomass allocation and canopy development in spruce model ecosystems under elevated CO_2 and increased N deposition. Oecologia, 113(1): 104–114.

Haworth, M., Giovanni, M., Centritto, M., 2018. An introductory guide to gas exchange analysis of photosynthesis and its application to plant phenotyping and precision irrigation to enhance water use efficiency. Journal of Water and Climate Change, 9(4): 786–808.

Hunt, S., 2003. Measurements of photosynthesis and respiration in plants. Physiologia Plantarum, 117(3): 314–25.

Huzisige, H., Ke, B., 1993. Dynamics of the history of photosynthesis research. Photosynthesis Research, 38(2): 185–209.

Jafarnia, S., Akbarinia, M., Hosseinpor, B., Modares Sanavi, S.AM., Salami, S.A., 2018. Effects of drought stress on some growth, morphological, physiological, and biochemical parameters of two different populations of *Quercus brantii*. iForest Biogeosciences and Forestry, 11: 212-220.

Karnosky, D.F., Zak, D.R., Pregitzer, K.S., Awmack, C.S., Bockheim, J.G., Dickson, R.E., Hendrey, G. R., Host, G.E., King, J.S., Kopper, B.J., Kruger, E.L., Kubiske, M.E., Lindroth, R.L., Mattson, W.J., Mcdonald, E.P., Noormets, A., Oksanen, E., Parsons, W.F.J., Percy, K.E., Podila, G.K., Riemenschneider, D.E., Sharma, P., Thakur, R., Sôber, A., Sôber, J., Jones, W.S., Anttonen,S., Vapaavuori, E., Mankovska, B., Heilman, W., Isebrands, J.G., 2003. Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2 : A synthesis of molecular to ecosystem results from the aspen FACE project. Functional Ecology, 17(3): 289–304.

Koç, İ., Nzokou, P., 2023. Combined effects of water stress and fertilization on the morphology and gas exchange parameters of 3-year-old *Abies fraseri* (Pursh) Poir. Acta Physiologiae Plantarum, 45:49.

Koike, T., 1986. A method for measuring photosynthesis with detached parts of deciduous broad-leaved trees in Hokkaido. Journal of the Japanese Forestry of Society, 68(8): 423-428.

Loewenstein, N. J., Pallardy, S.G.,1998. Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: A comparison of canopy trees of three temperate deciduous angiosperms. Tree Physiology, 18(7): 431–439.

Meng, C., Liu, X., Chai, Y., Xu, J., Yue, M., 2019. Another choice for measuring tree photosynthesis *in vitro*. PeerJ, 7:e5933.

Millan-Almaraz, J.R., Guevara-Gonzalez, R.G., Romero-Troncoso, R., Osornio-Rios, R.A., Torres- Pacheco, I., 2009. Advantages and disadvantages on photosynthesis measurement techniques: A review. African Journal of Biotechnology, 8(25): 7340-7349.

Missik, J.E.C., Oishi, A.C., Benson, M.C., Meretsky, V.J., Phillips, R.P., Novick, K.A., 2021. Performing gas-exchange measurements on excised branches - evaluation and recommendations. Photosynthetica, 59(1): 61-73.

Miyazawa, Y., Tateishi, M., Komatsu, H., Kumagai, T., Otsuki, K., 2011. Are measurements from excised leaves suitable for modeling diurnal patterns of gas exchange of intact leaves?. Hydrological Processes, 25: 2924-2930.

Nunes, L.J.R., Meireles, C.I.R., Pinto Gomes, C.J., Almeida Ribeiro, N.M.C., 2020. Forest contribution to climate change mitigation: management oriented to carbon capture and storage. Climate, 8(2): 21.

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P. B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., de Vos, A.C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., ter Steege, H., van der Heijden, M.G.A., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M.V., Conti, G., Staver, A.C., Aquino, S., Cornelissen, J.H.C., 2013. New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of Botany, 61: 167–234.

Santiago, L., Mulkey, S. A., 2003. Test of gas exchange measurements on excised canopy branches of ten tropical tree species. Photosynthetica 41: 343–347.

Saxe, H., 1991. Photosynthesis and stomatal responses to polluted air, and the use of physiological and biochemical responses for early detection and diagnostic tools. Advances in Botanical Research, 18: 1-128.

Sazeides, C.I., Christopulou, A., Fyllas, N.M., 2021. Coupling Photosynthetic measurements with biometric data to estimate Gross Primary Productivity (GPP) in Mediterranean Pine Forests of different post-fire age. Forests, 12: 1256.

Schönbeck, L., Grossiord, C., Gessler, A., Gisler, J., Meusburger, K., D'Odorico, P., Rigling, A., Salmon, A., Stocker, B.D., Zweifel, R., Schaub, M., 2022. Photosynthetic acclimation and sensitivity to short- and long-term environmental changes in a drought-prone forest. Journal of Experimental Botany, 73(8): 2576–88.

Scoffoni, C., Sack, L., 2015. Are leaves 'freewheelin'? Testing for a Wheeler-type effect in leaf xylem hydraulic decline. Plant, Cell and Environment, (38): 534-543.

Siebers, M.H., Gomez-Casanovas, N., Fu, P., Meacham-Hensold, K., Moore, C.E., Bernacchi, C.R., 2021. Emerging approaches to measure photosynthesis from the leaf to the ecosystem. Emerging Topics in Life Sciences, 5(2): 261-274.

Shinde, S., Naik, D., Cumming, J.R., 2018. Carbon allocation and partitioning in *Populus tremuloides* are modulated by ectomycorrhizal fungi under phosphorus limitation. Tree Phyhsiology, 38(1): 52-65.

Svenson, S.E., Davies, F.T. Jr 1992. Comparison of methods for estimated surface area of water-stressed and fully hydrated pine needle segments for gas exchange analysis. Tree Physiology, 10: 417-421.

Tang, Y., Wang, C.K., 2011. A feasible method for measuring photosynthesis in vitro for major tree species in northeastern China. Chin J Plant Ecol, 35(4): 452-462.

Torres-Ruiz, J. M., Jansen, S., Choat, B., McElrone, A. J., Cochard, H., Brodribb, T. J., Badel, E., Burlett, R., Bouche, P. S., Brodersen, C. R., Li, S., Morris, H., Delzon, S., 2015. Direct x-ray microtomography observation confirms the induction of embolism upon xylem cutting under tension. Plant Physiology, 167(1): 40–43.

Venturas, M.D., Mackinnon, E.D., Jacobsen, A.L., Pratt, R.B., 2015. Excising stem samples underwater at native tension does not induce xylem cavitation. Plant, Cell and Environment, 38(6): 1060-1068.

Verryckt, L.T., Van Langenhove, L., Ciais, P., Courtois, E.A., Vicca, S., Peñuelas, J., Stahl, C., Coste, S., Ellsworth, D.S., Posada, J.M., Obersteiner, M., Chave, J., Janssens, I.A., 2020. Coping with branch excision when measuring leaf net photosynthetic rates in a lowland tropical forest. Biotropica, 52: 608–615.

Voronin, P.Y., Fedoseeva, G.P., 2012. Stomatal control of photosynthesis in detached leaves of woody and herbaceous plants. Russian Journal of Plant Physiology, 59(2): 281-286.

Wheeler, J.K., Huggett, B.A., Tofte, A.N., Rockwell, F.E. Holbrook, N.M., 2013. Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. Plant, Cell and Environment, 36: 1938-1949.

Yin, G., Verger, A., Descals, A., Filella, I., Peñuelas, J., 2022. Nonlinear thermal responses outweigh water limitation in the attenuated effect of climatic warming on photosynthesis in northern ecosystems. Geophysical Research Letters, 49(16):e2022GL100096.