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Influence of nitrogen and phosphorus additions on parameters of photosynthesis and chlorophyll fluorescence in *Cyclocarya paliurus* seedlings

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Abstract

Cyclocarya paliurus has been traditionally used as a functional food in China. A hydroponic experiment was conducted to determine the effects of N and P additions on photosynthesis and chlorophyll fluorescence (ChlF) of *C. paliurus* seedlings. N and P additions significantly altered photosynthesis and ChlF in the seedlings, but responses of these parameters to the N and P concentrations varied at different developmental stages. The greatest net photosynthetic rate (P_N) and actual photochemical efficiency of PSII (Φ_{PSII}) occurred in the treatment of 150.0 mg(N) L^{-1} and 25.0 mg(P) L^{-1} addition, whereas the highest maximum quantum yield of PSII (F_v/F_m) and water-use efficiency (WUE) were recorded with 150.0 mg(N) L^{-1} and 15.0 mg(P) L^{-1} on the 60th day after treatment. Significantly positive correlations of P_N with leaf relative chlorophyll content, transpiration rate, WUE, F_v/F_m, and Φ_{PSII}, as well as the Φ_{PSII} with the F_v/F_m, were found. Our results indicated that an optimal addition of N and P nutrients depends on their coupling effects on the photosynthetic capacity and PSII photochemistry.

Keywords: chlorophyll; net photosynthetic rate; nitrogen–phosphorus interaction; PSII photochemistry; water-use efficiency; wheel wingnut.

Introduction

Wheel wingnut [*Cyclocarya paliurus* (Batal.) Iljinskaja], a multi-function plant of the Juglandaceae family, is sporadically distributed in the subtropical mountain areas of China (Fang *et al.* 2006). The leaves of *C. paliurus* are commonly used in nutraceutical tea and Chinese medicine in China because of their health benefits (Fang *et al.* 2011), and have been approved as the new food raw materials by the National Health and Family Planning Commission of China in 2013 (Xie *et al.* 2016, Qin *et al.* 2021). Pharmacological studies have revealed that the health-promoting effects (such as antidiabetic, antihyperlipidemic, and anticancer) are attributed to abundant bioactive compounds in *C. paliurus* leaves, including polyphenols, flavonoids, triterpenoids, *etc.*

Highlights

- N and P addition notably affect photosynthesis and chlorophyll fluorescence in *Cyclocarya paliurus*
- The optimal addition quantity depends on the coupling effects of N and P nutrients
- Medium N and P concentrations promote net photosynthetic rate and maximum quantum yield

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Abbreviations: ChlF – chlorophyll fluorescence; DAT – day after treatment; E – transpiration rate; F_0 – minimum ChlF in the dark-adapted leaf; F_m – maximum ChlF in the dark-adapted leaf; F_v/F_m – maximum quantum yield of PSII; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; SPAD – leaf relative chlorophyll content; WUE – water-use efficiency; Φ_{PSII} – actual photochemical efficiency of PSII.

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Conflict of interest: The authors declare that they have no conflict of interest.

(Wu *et al*. 2017, Liu *et al*. 2018, Zhou *et al*. 2021, Qin *et al*. 2022), which results in the shortage of *C. paliurus* leaves for the nutraceutical tea production and medical use in the market. Thus, developing *C. paliurus* plantations and optimizing management practices of the plantations are the best choice for increasing leaf production yield and improving leaf contents of the above-specified bioactive compounds.

Nitrogen (N) and phosphorus (P) are essential macronutrients for plant growth and development, and they are also nutrients that most frequently limit primary productivity in all ecosystems in the biosphere (Aerts and Chapin 1999, Hall *et al*. 2005, Elser *et al*. 2007, Chen *et al*. 2022). Previous studies have shown that N and P deficiencies cause negative effects on plants (Zhang *et al*. 2014a, Wang *et al*. 2022a,b); photosynthesis always decreases under N and P deficiencies (Zhang *et al*. 2014a,b; Luo *et al*. 2016). As a vital constituent in proteins, nucleic acids, chlorophylls, and many secondary metabolites of plants (Luo *et al*. 2013), N plays an essential role in the enzymatic activities of photosynthetic processes (Güsewell, 2004). Overall, N deficiency stops leaf elongation (Marschner 2011), inhibits photosynthesis (Gregoriou *et al*. 2007), and reduces the size of chloroplasts in plants (Li *et al*. 2013). Similarly, as a main constituent of plasma membranes, P is also involved in nucleic acid synthesis, phosphorylation processes, and cellular energy transfer, while it is a component of numerous plant metabolites (Gan *et al*. 2016). P deficiency not only reduces the photosynthetic capacity of leaves (Neocleous and Savvas 2019) but also causes the premature development of plant reproductive organs and inhibition of stem and leaf growth (Sun *et al*. 2021). However, the responses of photosynthesis, chlorophyll (Chl) fluorescence, accumulation of secondary metabolites, and growth in plants to N and P applications are species- or genotype-specific (Zhang *et al*. 2014a, Deng *et al*. 2019, Zhao *et al*. 2021, Wang *et al*. 2022a).

Photosynthesis is a process by which green plants turn carbon dioxide and water into organic compounds (particularly carbohydrates) using light energy, whereas both chlorophyll (Chl) and carotenoids (Car) participate in photosynthesis (Bode *et al*. 2009). Chl content and photosynthetic traits, such as stomatal conductance (g_s), net photosynthetic rate (P_N), and water-use efficiency (WUE), are modulated by fertilizer application (Neocleous and Savvas 2019). Moreover, chlorophyll fluorescence (ChlF) is closely related to the processes in photosynthesis, and any effect of stress on photosynthesis can be detected using changes in the ChlF induction dynamics (Kong *et al*. 2016). ChlF is reported to be a more obvious and rapid indicator compared to other physiological indicators when plants are subjected to stress (Wang *et al*. 2015); it shows fast ChlF transients (OJIPs) and slow kinetics. Fast ChlF transients involve measuring the time-resolved fluorescence yield of PSII in response to a brief flash of light. The OJIP transient reflects the sequential redox changes that occur within PSII, such as the reduction of the primary quinone acceptor and the buildup of the secondary quinone acceptor (Strasser *et al.* 1995, Ebenhöh *et al.* 2014). Supplemented with the saturation pulse method (also called quenching analysis) is commonly used for studying slow kinetics in photosynthetic systems. This method involves the application of a strong light pulse that induces a state of saturation in the photosynthetic system, thereby allowing for the measurement of slow kinetics such as the rate of electron transport and the redox state of electron carriers (Hamerlynck and O'Connor 2022). Numerous studies have reported that N significantly affects both the photosynthesis and fluorescence characteristics of leaves (Wei *et al*. 2016, Lin *et al*. 2017). For example, Yu *et al*. (2014) reported that N deficiency significantly decreased the maximum efficiency of PSII photochemistry under dark adaption (F_v/F_m) and the photochemical quenching (q_P) of sugar beet leaves but increased the nonphotochemical quenching (q_N), and thus affected the photochemical activity of PSII and decreased photosynthesis.

Owing to its pharmaceutical values, many *C. paliurus* plantations are established in the subtropical region of China where N and P availability is most limited. Thus optimizing the application of N and P fertilization in the plantations is crucial to improve the plantation productivity and its leaf biomass production. Until now only some laboratory studies have been conducted to verify the effects of N forms and N availability on growth and secondary metabolite accumulation during the seedling stage of *C. paliurus* (Deng *et al*. 2019, Qin *et al*. 2020, 2022), while the responses of nitrogen metabolism, photosynthetic parameters and growth to N fertilization was investigated in the field (Qin *et al*. 2021). However, no studies have been conducted to reveal the coupling effects of N and P additions on photosynthesis and chlorophyll fluorescence as well as growth in *C. paliurus*. To understand the physiological response of *C. paliurus* to different addition regimes of N and P nutrients, the parameters of photosynthesis and chlorophyll fluorescence were investigated in this study. The objectives of the study were to (*1*) determine the response of photosynthetic parameters, chlorophyll fluorescence in particular, of *C. paliurus* seedlings to various N and P supply conditions, (*2*) investigate the connection between photosynthesis and chlorophyll fluorescence parameters, and (*3*) test the hypothesis that the parameters related both to primary (chlorophyll fluorescence) and secondary photosynthetic processes would be significantly affected by the addition of N and P nutrients, but the optimal addition quantity would depend on the coupling effects of N and P nutrients and vary with the seedling development stage. Findings from the present study would provide a theoretical basis for optimizing the fertilizer application in *C. paliurus* plantations growing on the N- and P-limiting regions.

Materials and methods

Plant materials: *C. paliurus* seeds were collected from Wufeng (30°1.44'N, 110°16.32'E) and Jinzhongshan (29°05'N, 102°16'E) in Hubei province and Guangxi province China in October 2021. To break seed dormancy and promote prompt germination, seeds were treated with exogenous GA3 (gibberellin A3; 2,000 mg L^{-1}) and stratification by the method of Fang *et al.* (2006). After the seeds germinated, the seedlings were transplanted into a non-woven bag container with an 8.0 cm inner diameter and 11.0 cm height for cultivation at Baima Teaching Experimental Base of Nanjing Forestry University. Substrate composition was soil: perlite: peat: organic fertilizer = 2:2:5:1 (v:v:v:v). In late June 2022, the *C. paliurus* seedlings with consistent growth were selected as research materials and moved to the greenhouse.

Growth conditions: During the experiment period, the air temperature, air humidity, and PPFD in a greenhouse were monitored using an *Environment detector* (*Xuzhou Fara Electronic Technology Co. Ltd.*, Hangzhou, China), and their detailed variations were showed in Fig. 1.

Hydroponic culture and treatment regimes: The 30-L polypropylene containers (50 cm length × 40 cm width × 15 cm height) were used in the hydroponic culture with the Hoagland nutrient solution, in which the concentrations of N and P were 1/2 full-strength Hoagland nutrient solution, while the concentrations of other elements were the full-strength Hoagland nutrient solution.

Two weeks after the hydroponic culture, the experiment was carried out on 29 June 2022. The experiment was arranged using a randomized design consisting of three N regimes [50.0 (N1), 150.0 (N2), and 250.0 (N3) mg L^{-1}] and three P treatments [5.0 (P1), 15.0 (P2), and 25.0 (P3) mg L^{-1}]. N was supplied in the form of $Ca(NO_3)_2 \cdot 4H_2O$, KNO_3, and $(NH_4)_2SO_4$, whereas P was supplied in the form of KH_2PO_4. For all treatments, other macro- and micronutrients were supplied based on full-strength Hoagland solution. Meanwhile, a nitrification inhibitor (dicyandiamide) with 7% of total nitrogen was added to eliminate the effect of NH_4^+-N nitrification on the experiment. Three replicates (12 plants per replicate) were set for each treatment, a total of nine treatments were designed and 108 plants were used. During the experimental periods, the pH of 6.0 ± 0.2 was initially adjusted for the solutions, and the nutrient solution was renewed every three days and aerated continuously.

Photosynthetic parameters: Photosynthesis measurements were conducted on the 30th and 60th days after the treatments (DAT, *e.g.*, on 29 July and 27 August 2022, respectively). The net photosynthetic rate (P_N), and transpiration rate (E) were measured using a portable photosynthesis system (*PP system CIRAS-3*, *Hansatech Instruments*, Germany) from 8:30 to 10:00 h in the morning. Water-use efficiency (WUE) was estimated at the level of the leaf instantaneous ratio of P_N to E (Chen *et al.* 2016). To ensure the consistency of light intensity, the red-blue light source configured by the instrument was used for the measurement, and the light quantum flux density was 1,200 μmol m^{-2} s^{-1}. Temperature sensor control type was used to track cuvette to ambient temperature. During the measurement, the healthy mature leaves with similar growth positions were selected in each treatment, and two plants were selected in each replicate for each treatment.

Chl *a* fluorescence parameters: The leaf Chl fluorescence parameters were measured using an *LI-600 Porometer/Fluorometer* (*Li-COR Instruments*, Lincoln, NE, USA) on 29 July and 27 August 2022, respectively, in parallel with the photosynthesis measurements. Measurements

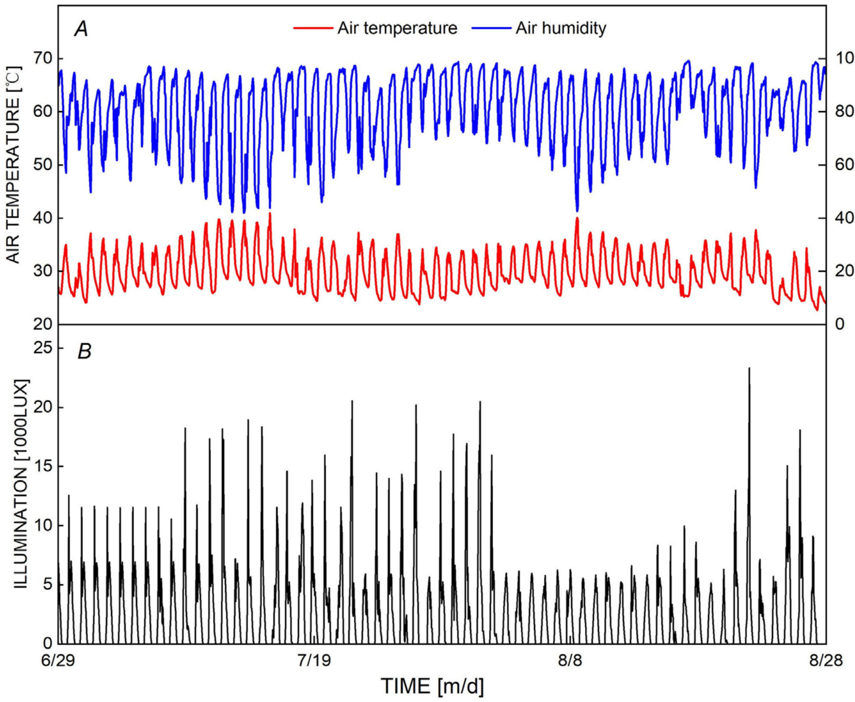

Fig. 1. Variations in air temperature and humidity (*A*) and illumination (*B*) during the experiment period.

were taken on 12 different leaves on the adaxial surface of the fully developed leaves for each treatment. Tissue was enclosed in a 0.75-cm^2 aperture clamp, held in its natural orientation to incident sunlight, with incident PPFD (about 1,000 µmol m^{-2} s^{-1}) serving as the actinic light source, with flux density measured with a Si-photodiode quantum sensor integrated into the *Li-600*. A modulated Chl fluorescence-measuring beam was provided by two LED-light sources focused on the cuvette aperture, filtered by a 750 + 40-nm bandpass filter, with fluorescence detected from 700 to 780 nm. The initial determination of steady-state fluorescence (F_s) used a sub-saturating modulated beam pulsed at 4–8 Hz over 2–5 s, followed by a saturating flash of 10,000 µmol(photon) m^{-2} s^{-1} intensity with a pulse width of 667 ns and 250–750 kHz frequency to determine light-adapted maximum fluorescence yield (F_m'). After dark adaptation (> 30 min), chlorophyll fluorescence measurements were repeated, and dark-adapted measurements provided baseline fluorescence (F_0; equivalent to F_s) and maximum fluorescence yield (F_m; equivalent to F_m') to determine optimal PSII yield (F_v/F_m) as $F_v/F_m = (F_m - F_0)/F_m$. Nonphotochemical quenching (NPQ) and photochemical quantum yield (Φ_{PSII}) were determined following the method of Maxwell and Johnson (2000) and Bilger *et al.* (2001) and were calculated using the equations: $\Phi_{PSII} = (F_m' - F_s)/F_m'$ and $NPQ = (F_m - F_m')/F_m'$, respectively.

Chlorophyll content estimation: As a reliable method for nondestructive chlorophyll detection (Akdemir *et al.* 2018), a *SPAD-502 Plus meter* (*Konica Minolta Optics*, Japan) was used to assess the leaf chlorophyll contents in the leaves. In parallel with the photosynthesis parameter measurements, the measurements were taken on the adaxial surface of 12 fully developed leaves for each treatment and were averaged to represent the leaf chlorophyll content of each treatment.

Statistical analysis: The experimental data were expressed as the mean ± standard error (SE). The main effects of N and P availability and their interactions were determined by two-way analysis of variance (*ANOVA*) using the statistical software *SPSS 19.0* (*SPSS*, Chicago, IL, USA), followed by *Duncan*'s multiple range test at $p<0.05$. If no significant interaction between N and P additions was detected for any measured parameters, then one-way *ANOVA* was conducted to compare parameter means across the treatments at $p<0.05$. Relationships among measured indexes were evaluated by *Pearson*'s correlation analysis.

Results

Leaf relative chlorophyll content: Two-way *ANOVA* results indicated that the leaf relative Chl content (SPAD) in the *C. paliurus* leaves was significantly affected by N and P additions, and their interactions (Table 1). On 30 DAT, the highest SPAD value was detected in the N2P3, followed by N2P2 (Fig. 2), which are 130 and 127% higher than that in the N1P1, respectively. However, on 60 DAT, the highest SPAD value was observed in N2P2, but no significant difference was observed between N2P2 and N2P3 (Fig. 2). Similarly, the lowest SPAD was also observed in N1P1 on 60 DAT. Overall, the SPAD values increased with prolonging treatment times and were significantly higher on 60 DAT than that on 30 DAT except N2P1 and N2P3 treatments (Fig. 2).

Photosynthesis and WUE: Two-way *ANOVA* showed that N and P additions affected P_N values, whereas their significant interaction was only detected on 60 DAT (Table 1). On the 30 DAT, P_N values in the N2 and N3 treatments were significantly higher than that in N1 treatment (Fig. 3*A*), and enhanced by 27.4 and 22.6%, respectively, when compared with N1. Similarly, more P additions also increased P_N values in leaves of *C. paliurus* seedlings (Fig. 3*B*). Compared to the P1 treatment, P_N values in P2 and P3 were enhanced by 11.9 and 13.4%, respectively. On 60 DAT, N and P additions had interactions on P_N (Table 1), and P_N in the leaves of plants ranged from 5.5 to 9.7 µmol(CO$_2$) m^{-2} s^{-1} (Fig. 3*C*). The greatest P_N occurred in N2P3, which is significantly higher than other treatments (except for N1P2), whereas the lowest P_N was observed in N1P1. Compared with P_N in the N1P1, the P_N in N2P3, N1P2, N2P2, N2P1, N3P3, N1P3, N3P1, and N3P2 increased by 77.4, 68.3, 62.2, 53.6, 41.4, 30.5, 19.5, and 11.0%, respectively.

Table 1. Two-way analysis of variance for the effects of nitrogen and phosphorus additions on parameters of photosynthesis and chlorophyll fluorescence in *Cyclocarya paliurus* seedlings. *, **, ***, and ns indicate the significances at $p<0.05$, $p<0.01$, $p<0.001$, and no significance, respectively. DAT – day after treatment; *E* – transpiration rate; F_0 – minimum ChlF in the dark-adapted leaf; F_v/F_m – maximum quantum yield of PSII; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; SPAD – leaf relative chlorophyll content; WUE – water-use efficiency; Φ_{PSII} – actual photochemical efficiency of PSII.

DAT	Factors	df	SPAD	P_N	*E*	WUE	F_0	F_v/F_m	NPQ	Φ_{PSII}
30	N	2	**	***	ns	***	***	***	*	***
	P	2	**	***	***	***	***	***	ns	***
	N × P	4	**	ns	***	***	**	***	***	***
60	N	2	***	***	*	***	***	***	***	***
	P	2	***	***	ns	***	***	***	ns	***
	N × P	4	***	***	***	***	***	*	***	**

Unlike P_N values, the *E* values were not significantly affected by N addition on 30 DAT and by P addition on 60 DAT (Table 1), whereas a significant interaction between N and P additions on *E* was observed on both 30 and 60 DAT. With an increasing amount of N and P, the *E* in leaves showed an irregular trend of variation (Fig. 4*A*). The highest *E* at 30 and 60 DAT were recorded in N2P2 and N1P2, respectively, while the lowest values were detected in N2P3 and N2P2.

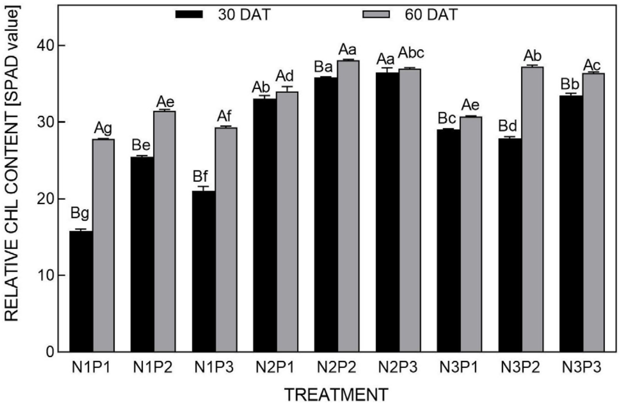

Fig. 2. Effect of nitrogen and phosphorus additions on the leaf relative chlorophyll content of *Cyclocarya paliurus* seedlings. Mean value ± SE ($n = 3$). *Different uppercase letters* indicate a significant difference under the same treatment at different times at $p<0.05$, *different lowercase letters* indicate a significant difference under the same time of different treatment at $p<0.05$.

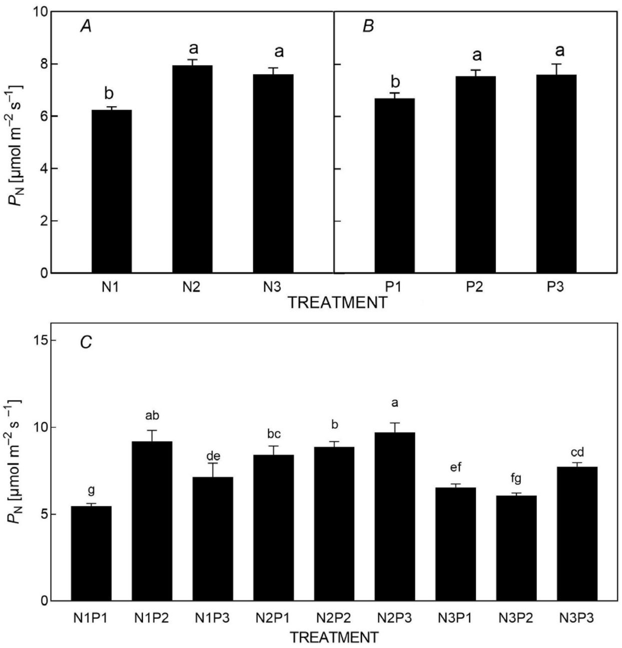

Fig. 3. Net photosynthetic rate (P_N) in leaves of *Cyclocarya paliurus* under different N (*A*) and P (*B*) additions measured on 30 DAT and 60 DAT (*C*), respectively. Mean value ± SE [$n = 9$ (*A*,*B*); $n = 3$ (*C*)]. *Different letters* indicate a significant difference between treatments at $p<0.05$.

In addition, WUEs in the leaves were significantly regulated by N and P additions as well as their interactions (Table 1). Similarly to P_N, the highest WUE was recorded in N2P3 at 30 DAT and in N2P2 at 60 DAT, respectively (Fig. 4*B*), which were significantly higher than other treatments. Compared to *E* in the N1P1 on 60 DAT, the *E* in N1P2, N2P3, N2P1, N3P1, N1P3, N3P2, N3P3, and N2P2 increased by 89.4, 54.6, 54.4, 50.2, 24.8, 14.7, 13.3, and 3.5% (Fig. 4*A*), respectively. In most cases, no significant difference in WUE was recorded between the two measurement times (Fig. 4*B*). However, the WUEs in N1P1 and N2P2 were significantly higher on 60 DAT than that on 30 DAT, but remarkably lower in N2P3 and N3P1 (Fig. 4*B*).

Chlorophyll fluorescence parameters: In most cases, the Chl fluorescence parameters (F_0, F_m, F_v/F_m, NPQ, F_m', and Φ_{PSII}) in *C. paliurus* seedlings were significantly affected by N and P addition as well as their interactions (Table 1). However, the Chl fluorescence parameters showed various responses to the treatments at the two measurement times. For example, on the 30 DAT, the highest F_0 appeared in N1P2, followed by N1P3, and the least value was found in N2P1, while on the 60 DAT, the highest F_0 was recorded in N3P2, followed by N3P1,

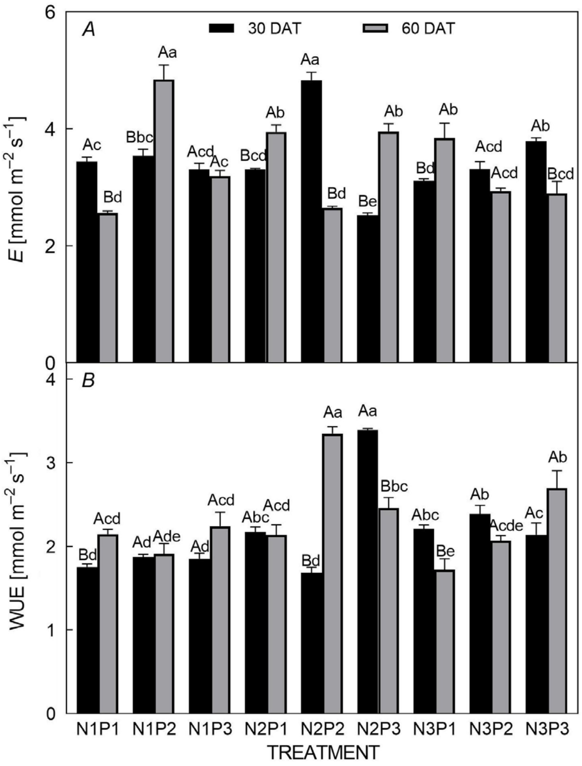

Fig. 4. Transpiration rate (*E*) (*A*) and water-use efficiency (WUE) (*B*) measured on 30 and 60 DAT in leaves of *Cyclocarya paliurus* under different nitrogen and phosphorus additions. Mean ± SE ($n = 3$). *Different uppercase letters* indicate a significant difference under the same treatment at different times at $p<0.05$, *different lowercase letters* indicate a significant difference under the same time of different treatment at $p<0.05$.

and the least was observed in N2P3 (Fig. 5*A*). Meanwhile, we noted that F_0 was significantly higher on 60 DAT than that on 30 DAT under N3 condition.

F_v/F_m represents the maximum photochemical efficiency or quantum yield and Φ_{PSII} represents the light-adapted PSII quantum yield. Our results showed that the values of F_v/F_m ranged from 0.776 to 0.818 among the treatments, but variation in the values was observed between the two DATs (Fig. 5*B*). The N1P3 showed the highest F_v/F_m, followed by N3P3, and the least in N1P1 on 30 DAT, whereas the highest F_v/F_m was found in N2P2, followed by N1P2, and the least in N3P3 on 60 DAT (Fig. 5*B*). The values of Φ_{PSII} ranged from 0.640 to 0.762 among the treatments, and their variation pattern was similar to the F_v/F_m (Fig. 5*C*). Contrary to the F_0, both F_v/F_m and Φ_{PSII} significantly decreased on 60 DAT when compared to the values on 30 DAT under N3 condition.

Our result also showed that the highest NPQ occurred in N2P3, followed by N3P1, and the least in N3P3 on 30 DAT. Similar to the F_0, the NPQ values were enhanced on 60 DAT when compared to the values on 30 DAT under the N3 condition (Fig. 5*D*).

Correlations among parameters of photosynthesis and chlorophyll fluorescence: Based on the indexes measured on the 30 and 60 DAT, Table 2 shows the relationship between photosynthesis and chlorophyll fluorescence. The results indicated that a significantly positive correlation of P_N with SPAD, *E*, WUE, F_v/F_m, and Φ_{PSII} was detected, whereas a negative relationship between P_N and F_0 was recorded. Meanwhile, WUE was significantly and positively correlated with SPAD and Φ_{PSII}, but negatively correlated with *E*. Some close correlations between dark-adapted chlorophyll fluorescence and light-adapted chlorophyll fluorescence were found (Table 2). *Pearson*'s correlation analysis indicated that F_v/F_m was positively related to Φ_{PSII} with a correlation coefficient of 0.736.

Discussion

As an important index to reflect photosynthetic capacity, P_N is defined as the CO_2 assimilation capacity per unit leaf area (Zhu *et al*. 2019). Our result found the photosynthesis response of *C. paliurus* seedlings to N and P additions was inconsistent at the two DATs (Table 1, Fig. 3). For instance, there was no significant interaction between N and P additions on P_N on 30 DAT, and P_N values enhanced with increasing addition amount of N and P (Fig. 3*A*,*B*), even if no significant differences in P_N were detected between N2 and N3 as well as between P2 and P3, suggesting that N2 and P2 are the optimum concentrations for P_N in this study. However, on 60 DAT, N and P additions showed interactions on P_N of *C. paliurus* seedlings, and both the highest P_N and WUE were achieved in N2P3 (Fig. 3*C*). The possible reasons are that the *C. paliurus* seedlings might have various requirement and utilization patterns of N and P at different growth stages, and there may exist a trade-off between N and P absorption in plants (Güsewell 2004). Furthermore, some studies indicated that N application would upregulate genes participating in photosynthesis and carbon fixation, and could improve photosynthetic ability (Midorikawa *et al*. 2014, Qin *et al*. 2022), whereas N deficiency inhibited the expression of photosynthesis-related genes, reduced metabolites

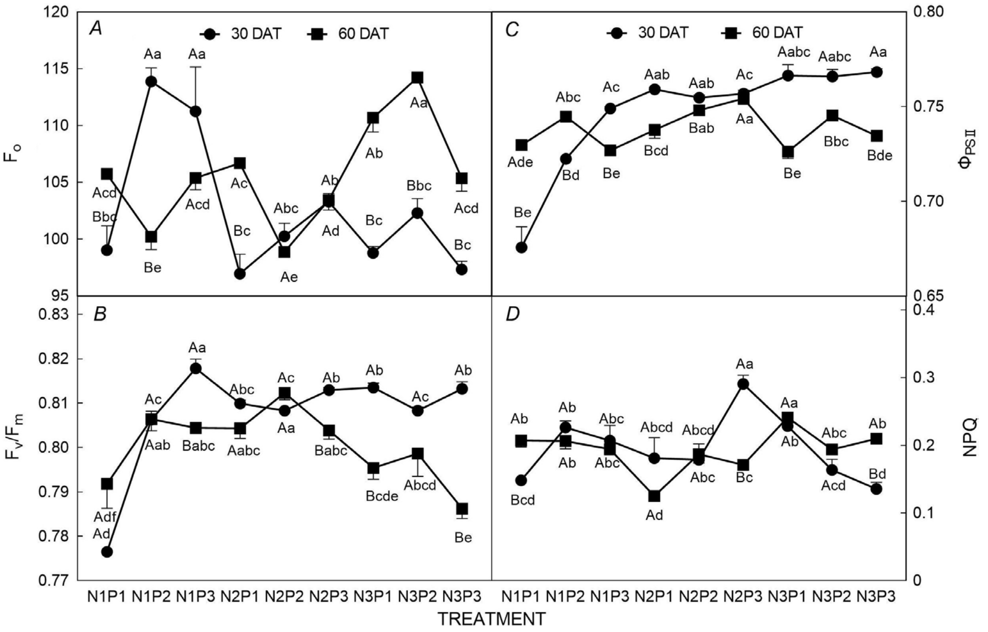

Fig. 5. Changes in chlorophyll fluorescence parameters measured on 30 and 60 DAT in *Cyclocarya paliurus* leaves under different nitrogen and phosphorus additions. F_0 – minimal fluorescence in dark-adapted leaf; F_v/F_m – maximum quantum yield of PSII; Φ_{PSII} – actual photochemical efficiency of PSII; NPQ – nonphotochemical fluorescence quenching. Values are means ± SE ($n = 3$). *Different uppercase letters* indicate a significant difference under the same treatment at different times at $p<0.05$, *different lowercase letters* indicate a significant difference under the same time of different treatments at $p<0.05$.

Table 2. Relationship between the leaf relative chlorophyll content (SPAD), net photosynthesis (P_N), transpiration rate (E), water-use efficiency (WUE), minimal fluorescence in the dark-adapted leaf (F_0), maximum quantum yield of PSII (F_v/F_m), nonphotochemical quenching (NPQ), and actual photochemical efficiency of PSII (Φ_{PSII}) in leaves of *Cyclocarya paliurus* seedlings ($n = 54$). *, ** indicate the significance at the 0.05, and 0.01 probability levels, respectively.

Index	Photosynthesis				Dark-adapted ChlF parameters		Light-adapted ChlF parameters	
	SPAD	P_N	E	WUE	F_0	F_v/F_m	NPQ	Φ_{PSII}
P_N	0.595^{**}	1						
E	0.013	0.353^{**}	1					
WUE	0.508^{**}	0.502^{**}	-0.611^{**}	1				
F_0	–0.110	-0.419^{**}	–0.141	–0.234	1			
F_v/F_m	0.085	0.399^{**}	0.161	0.222	-0.960^{**}	1		
NPQ	0.076	–0.149	-0.288^{*}	0.230	0.209	–0.140	1	
Φ_{PSII}	0.567^{**}	0.434^{**}	0.032	0.314^{*}	–0.242	0.155	0.057	1

Table 3. Multiple linear regression equations for predicting the relationships of photosynthetic capacities (Y) to nitrogen (N) and phosphorus (P) concentrations in the hydroponic cultures ($n = 54$). R_n, R_p, and R_{np} represent the partial correlation coefficients to the N, P, and the product of N and P concentrations (N × P), respectively.

Index	Fitted models	Multiple correlation coefficient	Partial correlation		
			R_n	R_p	R_{np}
P_N	$Y_{PN} = 2.801 + 0.580\ln(N) + 0.945\ln(P) - 0.041\ln(N) \times \ln(P)$	$R=0.484$, $p=0.004$	0.093	0.081	–0.017
F_v/F_m	$Y_{Fv/Fm} = 0.626 + 0.034\ln(N) + 0.067\ln(P) - 0.013\ln(N) \times \ln(P)$	$R=0.637$, $p<0.001$	0.578	0.594	–0.566
Φ_{PSII}	$Y_{\Phi PSII} = 0.479 + 0.049\ln(N) + 0.071\ln(P) - 0.013\ln(N) \times \ln(P)$	$R=0.676$, $p<0.001$	0.465	0.375	–0.330

in carbon metabolism, and upregulated the *CHL1* gene, which then led to Chl degradation in plants (Amiour *et al.* 2012, Curci *et al.* 2017). Our results showed that SPAD content and photosynthesis ability of *C. paliurus* were reduced as they grew under N limitation (N1; Figs. 2, 3*A*,*C*), following the report of Deng *et al.* (2019).

As another essential element, P is an important structural element in nucleic acids that regulate energy storage and transfer (Wang *et al.* 2013) and photosynthesis requires orthophosphate as a substrate. Thus, efficient use of N and P is believed to contribute to plant fitness (Aerts and Chapin 1999). However, N input is not paralleled by a similar increase in P inputs (Peñuelas *et al.* 2013). Our results showed that excess N availability may increase P limitation, thereby affecting the P_N (Fig. 3*C*), supporting the point that P additions alter plant N uptake (Ali *et al.* 2002, Reed *et al.* 2007), and increase the primary productivity for certain plant species (Seastedt and Suding 2007). Although both N and P enrichment increased the P_N of *C. paliurus* seedlings compared to N1P1 (*i.e.*, low N, low P treatment), P enrichment resulted in much greater P_N values in the low N (N1) compared to N enrichment (N3) at 60 DAT (Fig. 3*C*), demonstrating that P might be the more important limiting nutrient for *C. paliurus* growth in the present study. Indeed, both P and N simultaneously limited the photosynthetic capacity of *C. paliurus* seedlings (Fig. 3*C*), suggesting the dual nutrient limitation occurs in a physiological sense in this species under the hydroponic culture conditions of N1P1, as well as there is significant interaction between P and N addition on the photosynthetic capacity of *C. paliurus* seedlings.

Correlation analysis showed that WUE was significantly positive with P_N in the present study (Table 2). Usually, high N nutrients increase instantaneous WUE in wheat and coffee (Shangguan *et al.* 2000, DaMatta *et al.* 2002), however, the highest WUE was observed in N2P3 at 30 DAT and in N2P2 at 60 DAT in this study, respectively (Fig. 4*B*), indicating N2 is a suitable N concentration for the growth of *C. paliurus* seedlings whereas N3 is an excess N addition.

The F_0, F_m, F_v/F_m, NPQ, and Φ_{PSII} values are the most important Chl fluorescence parameters which are used in plant stress physiology studies (Thomas and Turner 2001, Baker and Rosenqvist 2004, Fu *et al.* 2012, Murchie and Lawson 2013). F_0 reflects mainly the emission from light-harvesting complexes (LHCs), while LHCs are mainly composed of various chlorophyll molecules that work to absorb photons and transfer them to the PSII reaction center for chemical reactions (Croce and van Amerongen 2013). Under normal photosynthetic conditions, absorbed photons by LHCs can pass efficiently to the PSII reaction center, resulting in low F_0 signals. However, under various stress conditions, such as low light, high temperature, and drought, the efficiency of the PSII reaction center decreases, LHCs produce auxiliary fluorescence, and the F_0 signal increases (Calatayud *et al.* 2006, Baker 2008, Tikkanen *et al.* 2011). It has been reported that F_0 would increase under stress conditions, but the F_v/F_m ratio would be reduced (Maxwell and Johnson 2000). In the current study, F_0 was significantly affected by N and P additions, and at 60 DAT, the F_0 values under high nitrogen treatment were higher than that under low nitrogen and medium nitrogen treatment (Fig. 5*A*), which may be a sign of the excessive N addition (Mauromicale *et al*. 2006).

The F_v/F_m value, the ratio of variable fluorescence to maximal fluorescence, can be used to estimate the potential efficiency of PSII in dark-adapted samples (Hura *et al*. 2007, Gorbe and Calatayud 2012, Murchie and Lawson 2013). An increase in F_v/F_m reflects higher light-use efficiency in plants (Baker 2008, Li *et al*. 2015) and less energy loss as heat (Jagtap *et al*. 1998, Jeon *et al*. 2006, Broetto *et al*. 2007). In general, F_v/F_m value increased with an increase of N or P concentrations, but exceeding a certain value of N or P concentration resulted in a decrease of F_v/F_m (Lapointe 1987, de Groot *et al*. 2003). Our results indicated that the F_v/F_m values increased with enhancing P concentration under low nitrogen conditions (N1), however, under the medium and high N conditions (N2 and N3), the F_v/F_m values decreased with the increase of P concentrations (Fig. 5*B*). The reduction in F_v/F_m may occur due to the decline of PSII maximum efficiency (Porcar-Castell *et al*. 2014). Moreover, NPQ represents an effective way how photosynthetic organisms can dissipate excessive irradiation into heat (Ralph and Gademann 2005, Pinnola *et al*. 2013), while the Φ_{PSII} value gives an estimation of the efficiency (Baker and Rosenqvist 2004) and represents the photochemistry at different photon flux density (Maxwell and Johnson 2000). Some studies indicated that there is an inverse relationship between Φ_{PSII} and NPQ (Maxwell and Johnson 2000, Massacci *et al*. 2008, Fu *et al*. 2012). However, the Φ_{PSII} was not significantly correlated with NPQ, but significantly correlated with the values of P_N and F_v/F_m in the present study (Table 2, $p<0.01$).

An increase in N availability at low N can be used for building extra machinery, whereas an increase in P availability at low P can be used to form structural compounds (*e.g*., nucleic acids and phospholipids) (Shi *et al*. 2020). Severe N and P deficiency or toxicity can lead to alterations in the photosynthetic apparatus and photosystems, thus causing photooxidative stress (Hernández and Munné-Bosch 2015, Shi *et al*. 2020). Our results found that the highest values of P_N, WUE, F_v/F_m, and Φ_{PSII} were achieved under different combinations of N and P concentrations at 60 DAT (Figs. 3*C*, 5). For example, the greatest P_N and Φ_{PSII} occurred in N2P3, whereas the highest F_v/F_m and WUE were recorded in N2P2, suggesting that P_N, WUE, maximal photochemistry efficiency, and actual quantum yield in PSII were significantly influenced by the N and P deficiency or toxicity due to photooxidative damage (Zhang *et al*. 2014a, Hernández and Munné-Bosch 2015, Shi *et al*. 2020).

Using N and P concentrations as variables, the multiple linear regression equations were well-fitted for predicting the photosynthetic capacities of *C. paliurus* seedlings (Table 3). These fitted models showed that P_N, F_v/F_m, and Φ_{PSII} were all positively correlated with N or P concentrations, but negatively related to the product of N and P concentrations, indicating that there exists an optimal combination between N and P availability, and a negative effect would occur when their concentrations exceed thresholds of plant requirement. The result partially confirmed the hypothesis that both photosynthesis and chlorophyll fluorescence are significantly affected by addition of N and P nutrients, but the optimal addition quantity would depend on the coupling effects of N and P nutrients.

Conclusions: Both photosynthesis and chlorophyll fluorescence parameters of *C. paliurus* seedlings were significantly affected by the addition of N and P nutrients, but responses of these measured parameters to various N and P concentrations varied at different development stages. The highest values of P_N, WUE, F_v/F_m, and Φ_{PSII} were achieved under different combinations of N and P concentrations, while the optimal addition quantity would depend on the coupling effects of N and P nutrients. The greatest P_N and Φ_{PSII} were observed in N2P3 [150.0 mg(N) L^{-1} and 25.0 mg(P) L^{-1}], whereas the highest F_v/F_m and WUE were recorded in N2P2 [150.0 mg(N) L^{-1} and 15.0 mg(P) L^{-1}] at 60 DAT. There existed a significantly positive correlation of P_N with SPAD, *E*, WUE, F_v/F_m, and Φ_{PSII}, whereas a negative relationship between P_N and F_0 was recorded. Meanwhile, the Φ_{PSII} was not significantly correlated with NPQ, but positively correlated with the F_v/F_m and P_N. Findings from the present study would provide a theoretical basis for optimizing the fertilizer application in *C. paliurus* seedling production as well as in its plantation management in the N- and P-limiting regions.

References

Aerts R., Chapin III F.S.: The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. – Adv. Ecol. Res. **30**: 1-67, 1999.

Akdemir B., Saglam C., Belliturk K. *et al*.: Effect of spatial variability on fertiliser requirement of olive orchard cultivated for oil production. – J. Environ. Prot. Ecol. **19**: 319-329, 2018.

Ali J., Bakht J., Shafi M. *et al*.: Uptake nitrogen as affected by various combinations of nitrogen and phosphorus. – Asian J. Plant Sci. **1**: 367-369, 2002.

Amiour N., Imbaud S., Clément G. *et al*.: The use of metabolomics integrated with transcriptomic and proteomic studies for identifying key steps involved in the control of nitrogen metabolism in crops such as maize. – J. Exp. Bot. **63**: 5017-5033, 2012.

Baker N.R.: Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. – Annu. Rev. Plant Biol. **59**: 89-113, 2008.

Baker N.R., Rosenqvist E.: Applications of chlorophyll fluorescence can improve crop production strategies: an examination of future possibilities. – J. Exp. Bot. **55**: 1607-1621, 2004.

Bilger W., Johnsen T., Schreiber U.: UV-excited chlorophyll fluorescence as a tool for the assessment of UV-protection by the epidermis of plants. – J. Exp. Bot. **52**: 2007-2014, 2001.

Bode S., Quentmeier C.C., Liao P.N. *et al.*: On the regulation of photosynthesis by excitonic interactions between carotenoids and chlorophylls. – PNAS **106**: 12311-12316, 2009.

Broetto F., Duarte H.M., Lüttge U.: Responses of chlorophyll fluorescence parameters of the facultative halophyte and C_3-CAM intermediate species *Mesembryanthemum crystallinum* to salinity and high irradiance stress. – J. Plant Physiol. **164**: 904-912, 2007.

Calatayud A., Roca D., Martínez P.F.: Spatial-temporal variations in rose leaves under water stress conditions studied by chlorophyll fluorescence imaging. – Plant Physiol. Biochem. **44**: 564-573, 2006.

Chen C.-C., Huang M.-Y., Lin K.-H. *et al.*: The effects of nitrogen application on the growth, photosynthesis, and antioxidant activity of *Amaranthus viridis*. – Photosynthetica **60**: 420-429, 2022.

Chen Y.H., Liu L., Guo Q.S. *et al.*: Effects of different water management options and fertilizer supply on photosynthesis, fluorescence parameters and water use efficiency of *Prunella vulgaris* seedlings. – Biol. Res. **49**:12, 2016.

Croce R., van Amerongen H.: Light-harvesting in photosystem I. – Photosynth. Res. **116**: 153-166, 2013.

Curci P.L., Cigliano R.A., Zuluaga D.L. *et al.*: Transcriptomic response of durum wheat to nitrogen starvation. – Sci. Rep.-UK **7**: 1176, 2017.

DaMatta F.M., Loos R.A., Silva E.A., Loureiro M.E.: Limitations to photosynthesis in *Coffea canephora* as a result of nitrogen and water availability. – J. Plant Physiol. **159**: 975-981, 2002.

de Groot C.C., Marcelis L.F.M., van den Boogaard R. *et al.*: Interaction of nitrogen and phosphorus nutrition in determining growth. – Plant Soil **248**: 257-268, 2003.

Deng B., Li Y., Xu D. *et al.*: Nitrogen availability alters flavonoid accumulation in *Cyclocarya paliurus* via the effects on the internal carbon/nitrogen balance. – Sci. Rep.-UK **9**: 2370, 2019.

Ebenhöh O., Fucile G., Finazzi G. *et al.*: Short-term acclimation of the photosynthetic electron transfer chain to changing light: a mathematical model. – Philos. T. Roy. Soc. B **369**: 20130223, 2014.

Elser J.J., Bracken M.E.S., Cleland E.E. *et al.*: Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. – Ecol. Lett. **10**: 1135-1142, 2007.

Fang S.Z., Wang J.Y., Wei Z.Y., Zhu Z.X.: Methods of break seed dormancy in *Cyclocarya paliurus* (Batal) Iljinskaja. – Sci. Hortic.-Amsterdam **110**: 305-309, 2006.

Fang S.Z., Yang W.X., Chu X.L. *et al.*: Provenance and temporal variation in selected flavonoids in leaves of *Cyclocarya paliurus*. – Food Chem. **124**: 1382-1386, 2011.

Fu W., Li P., Wu Y.: Effects of different light intensities on chlorophyll fluorescence characteristics and yield in lettuce. – Sci. Hortic.-Amsterdam **135**: 45-51, 2012.

Gan H., Jiao Y., Jia J. *et al.*: Phosphorus and nitrogen physiology of two contrasting poplar genotypes when exposed to phosphorus and/or nitrogen starvation. – Tree Physiol. **36**: 22-38, 2016.

Gorbe E., Calatayud A.: Applications of chlorophyll fluorescence imaging technique in horticultural research: a review. – Sci. Hortic.-Amsterdam **138**: 24-35, 2012.

Gregoriou K., Pontikis K., Vemmos S.: Effects of reduced irradiance on leaf morphology, photosynthetic capacity, and fruit yield in olive (*Olea europaea* L.). – Photosynthetica **45**: 172-181, 2007.

Güsewell S.: N:P ratios in terrestrial plants: variation and functional significance. – New Phytol. **164**: 243-266, 2004.

Hall S.R., Smith V.H., Lytle D.A., Leibold M.A.: Constraints on primary producer N:P stoichiometry along N:P supply ratio gradients. – Ecology **86**: 1894-1904, 2005.

Hamerlynck E.P., O'Connor R.C.: Photochemical performance of reproductive structures in Great Basin bunchgrasses in response to soil-water availability. – AoB Plants **14**: plab076, 2022.

Hernández I., Munné-Bosch S.: Linking phosphorus availability with photo-oxidative stress in plants. – J. Exp. Bot. **66**: 2889-2900, 2015.

Hura T., Grzesiak S., Hura K. *et al.*: Physiological and biochemical tools useful in drought-tolerance detection in genotypes of winter triticale: Accumulation of ferulic acid correlates with drought tolerance. – Ann. Bot.-London **100**: 767-775, 2007.

Jagtap V., Bhargava S., Streb P., Feierabend J.: Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) Moench. – J. Exp. Bot. **49**: 1715-1721, 1998.

Jeon M.-W., Ali M.B., Hahn E.-J., Paek K.-Y.: Photosynthetic pigments, morphology and leaf gas exchange during ex vitro acclimatization of micropropagated CAM *Doritaenopsis* plantlets under relative humidity and air temperature. – Environ. Exp. Bot. **55**: 183-194, 2006.

Kong D.-X., Li Y.-Q., Wang M.-L. *et al.*: Effects of light intensity on leaf photosynthetic characteristics, chloroplast structure, and alkaloid content of *Mahonia bodinieri* (Gagnep.) Laferr. – Acta Physiol. Plant. **38**: 120, 2016.

Lapointe B.E.: Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. – Mar. Biol. **93**: 561-568, 1987.

Li S., Yang W., Yang T. *et al.*: Effects of cadmium stress on leaf chlorophyll fluorescence and photosynthesis of *Elsholtzia argyi* – a cadmium accumulating plant. – Int. J. Phytoremediat. **17**: 85-92, 2015.

Li Y., Ren B., Ding L. *et al.*: Does chloroplast size influence photosynthetic nitrogen use efficiency? – PLoS ONE **8**: e62036, 2013.

Lin J., Wang Y., Sun S. *et al.*: Effects of arbuscular mycorrhizal fungi on the growth, photosynthesis and photosynthetic pigments of *Leymus chinensis* seedlings under salt-alkali stress and nitrogen deposition. – Sci. Total Environ. **576**: 234-241, 2017.

Liu Y., Cao Y.N., Fang S.Z. *et al.*: Antidiabetic effects of *Cyclocarya paliurus* leaves depends on the contents of antihyperglycemic flavonoids and antihyperlipidemic triterpenoids. – Molecules **23**: 1042, 2018.

Luo J., Qin J., He F. *et al.*: Net fluxes of ammonium and nitrate in association with H^+ fluxes in fine roots of *Populus popularis*. – Planta **237**: 919-931, 2013.

Luo J., Shi W., Li H. *et al.*: The conserved salt-responsive genes in the roots of *Populus* × *canescens* and *Arabidopsis thaliana*. – Environ. Exp. Bot. **129**: 48-56, 2016.

Marschner P.: Marschner's Mineral Nutrition of Higher Plants. 3rd Edition. Pp. 672. Academic Press, London 2011.

Massacci A., Nabiev S.M., Pietrosanti L. *et al.*: Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. – Plant Physiol. Biochem. **46**: 189-195, 2008.

Mauromicale G., Ierna A., Marchese M.: Chlorophyll fluorescence and chlorophyll content in field-grown potato as affected by nitrogen supply, genotype, and plant age. – Photosynthetica **44**: 76-82, 2006.

Maxwell K., Johnson G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Midorikawa K., Kuroda M., Terauchi K. *et al.*: Additional nitrogen fertilization at heading time of rice down-regulates cellulose synthesis in seed endosperm. – PLoS ONE **9**: e98738, 2014.

Murchie E.H., Lawson T.: Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. – J. Exp. Bot. **64**: 3983-3998, 2013.

Neocleous D., Savvas D.: The effects of phosphorus supply limitation on photosynthesis, biomass production, nutritional quality, and mineral nutrition in lettuce grown in a recirculating nutrient solution. – Sci. Hortic.-Amsterdam **252**: 379-387, 2019.

Peñuelas J., Poulter B., Sardans J. *et al.*: Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. – Nat. Commun. **4**: 2934, 2013.

Pinnola A., Dall'Osto L., Gerotto C. *et al.*: Zeaxanthin binds to light-harvesting complex stress-related protein to enhance nonphotochemical quenching in *Physcomitrella patens*. – Plant Cell **25**: 3519-3534, 2013.

Porcar-Castell A., Tyystjärvi E., Atherton J. *et al.*: Linking chlorophyll *a* fluorescence to photosynthesis for remote sensing applications: mechanisms and challenges. – J. Exp. Bot. **65**: 4065-4095, 2014.

Qin J., Yue X.L., Fang S.Z. *et al.*: Responses of nitrogen metabolism, photosynthetic parameter and growth to nitrogen fertilization in *Cyclocarya paliurus*. – Forest Ecol. Manag. **502**: 119715, 2021.

Qin J., Yue X.L., Fang S.Z. *et al.*: Nitrogen addition modifies the relative gene expression level and accumulation of carbon-based bioactive substances in *Cyclocarya paliurus*. – Plant Physiol. Biochem. **188**: 70-80, 2022.

Qin J., Yue X.L., Shang X.L., Fang S.Z.: Nitrogen forms alter triterpenoid accumulation and related gene expression in *Cyclocarya paliurus* (Batalin) Iljinsk. seedlings. – Forests **11**: 631, 2020.

Ralph P.J., Gademann R.: Rapid light curves: a powerful tool to assess photosynthetic activity. – Aquat. Bot. **82**: 222-237, 2005.

Reed S.C., Seastedt T.R., Mann C.M. *et al.*: Phosphorus fertilization stimulates nitrogen fixation and increases inorganic nitrogen concentrations in a restored prairie. – Appl. Soil Ecol. **36**: 238-242, 2007.

Seastedt T.R., Suding K.N.: Biotic constraints on the invasion of diffuse knapweed (*Centaurea diffusa*) in North American grasslands. – Oecologia **151**: 626-636, 2007.

Shangguan Z.P., Shao M.A., Dyckmans J.: Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. – Environ. Exp. Bot. **44**: 141-149, 2000.

Shi Q., Pang J., Yong J.W.H. *et al.*: Phosphorus-fertilisation has differential effects on leaf growth and photosynthetic capacity of *Arachis hypogaea* L. – Plant Soil **447**: 99-116, 2020.

Strasser R.J., Srivastava A., Govindjee: Polyphasic chlorophyll *a* fluorescence transient in plant and cyanobacteria. – Photochem. Photobiol. **61**: 32-42, 1995.

Sun T., Zhang J., Zhang Q. *et al.*: Transcriptome and metabolome analyses revealed the response mechanism of apple to different phosphorus stresses. – Plant Physiol. Biochem. **167**: 639-650, 2021.

Thomas D.S., Turner D.W.: Banana (*Musa* sp.) leaf gas exchange and chlorophyll fluorescence in response to soil drought, shading and lamina folding. – Sci. Hortic.-Amsterdam **90**: 93-108, 2001.

Tikkanen M., Grieco M., Aro E.-M.: Novel insights into plant light-harvesting complex II phosphorylation and 'state transitions'. – Trends Plant Sci. **16**: 126-131, 2011.

Wang J., Hui D., Ren H. *et al.*: Effects of understory vegetation and litter on plant nitrogen (N), phosphorus (P), N:P ratio and their relationships with growth rate of indigenous seedlings in subtropical plantations. – PLoS ONE **8**: e84130, 2013.

Wang L, Deng F., Ren W.-J.: Shading tolerance in rice is related to better light harvesting and use efficiency and grain filling rate during grain filling period. – Field Crop. Res. **180**: 54-62, 2015.

Wang T., Han H., Xie B. *et al.*: Comparative chlorophyll fluorescence and growth responses of two *Amaranthus* species to increased N supply variability. – Pol. J. Environ. Stud. **31**: 3867-3878, 2022a.

Wang Z.K., Chen Z.Y., Leite M.F.A. *et al.*: Effects of probiotic consortia on plant metabolites are associated with soil indigenous microbiota and fertilization regimes. – Ind. Crop. Prod. **185**: 115138, 2022b.

Wei S., Wang X., Shi D. *et al.*: The mechanisms of low nitrogen induced weakened photosynthesis in summer maize (*Zea mays* L.) under field conditions. – Plant Physiol. Biochem. **105**: 118-128, 2016.

Wu Z., Meng F., Cao L. *et al.*: Triterpenoids from *Cyclocarya paliurus* and their inhibitory effect on the secretion of apoliprotein B48 in Caco-2 cells. – Phytochemistry **142**: 76-84, 2017.

Xie J., Wang Z., Shen M. *et al.*: Sulfated modification, characterization and antioxidant activities of polysaccharide from *Cyclocarya paliurus*. – Food Hydrocoll. **53**: 7-15, 2016.

Yu X., Huang J.-X., Wang Y.-B. *et al.*: [Effect of nitrogen on chlorophyll fluorescence of blade of sugar beet.] – J. Nucl. Agric. Sci. **28**: 1918-1923, 2014. [In Chinese]

Zhang S., Jiang H., Zhao H. *et al.*: Sexually different physiological responses of *Populus cathayana* to nitrogen and phosphorus deficiencies. – Tree Physiol. **34**: 343-354, 2014a.

Zhang Z., Liao H., Lucas W.J.: Molecular mechanisms underlying phosphate sensing, signalling, and adaptation in plants. – J. Integr. Plant Biol. **56**: 192-220, 2014b.

Zhao C., Wang Z., Cui R. *et al.*: Effects of nitrogen application on phytochemical component levels and anticancer and antioxidant activities of *Allium fistulosum*. – PeerJ **9**: e11706, 2021.

Zhou M.M., Quek S.Y., Shang X.L., Fang S.Z.: Geographical variations of triterpenoid contents in *Cyclocarya paliurus* leaves and their inhibitory effects on Hela cells. – Ind. Crop. Prod. **162**: 113314, 2021.

Zhu Q., Kong L.J., Shan Y.Z. *et al.*: Effect of biochar on grain yield and leaf photosynthetic physiology of soybean cultivars with different phosphorus efficiencies. – J. Integr. Agr. **18**: 2242-2254, 2019.