

## Discrete nutrient utilizations in two *Bauhinia* species exposed to supplemental light-emitting diode spectra and exponential fertilization

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### Abstract

*Bauhinia* is a widely planted urban tree plant in tropical cities, which are frequently dwelling in a habitat exposed to inevitable streetlamp lighting. It is of practical meaning to figure out the lighting spectra that benefit nutrient utilization in corporation with a proper exponential fertilization regime. In this study, *Bauhinia brachycarpa* and *B. variegata* seedlings were cultured with exponential fertilization at the rate of 80 mg nitrogen (N) plant<sup>-1</sup> (N-phosphorus-potassium, 10-7-9) with an unfertilized control, and both were exposed to light-emitting diode spectra of R1BG5 (13.9% red, 77.0% green, 9.2% blue), R2BG3 (26.2% red, 70.2% green, 3.5% blue), and R3BG1 (42.3% red, 57.3% green, 0.4% blue). The R3BG1 spectrum resulted in smaller growing size and dry mass but higher nutrient concentration and root water content compared to the other two spectra. Exponential fertilization increased fresh mass for two *Bauhinia* species but only increased dry mass in *B. brachycarpa*. Compared to *B. variegata*, *B. brachycarpa* was verified to have a higher capacity to accommodate exogenous nutrient input through exponential fertilization. The R3BG1 spectrum was recommended for the illumination in streetlamps for *Bauhinia* because this spectrum can promote nutrient uptake without too fast rate of growth relative to other spectra with lower red-light proportions.

**Keywords:** fertilizer regime; LED illumination; light spectra; nutrient utilization; Yangtija

### Introduction

Urbanization is sprawling the area of built-up areas, where night light is being of increasing attention due to its remarkable contribution to urban economy and citizens' health (Xu and Gao, 2017; Xu *et al.*, 2019; Yang *et al.*, 2009). As lamps are usually constructed beside street trees, night light also means a supplemental illumination for growing organs that can change their growth and physiology (Sarala *et al.*, 2013; Tuharska *et al.*

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*al.*, 2014). Exposure to night light can shift biomass from roots to leaves and increased specific leaf area in woodland trees (Lockett *et al.*, 2022). Night light can also modify sap flow rate and water use efficiency (Lakatos and Buban, 1999). As a consequence, exposure to night light has been recognized as an environmental pollution, if tree phenology is modified without any control (French-Constant *et al.*, 2016). A better understanding of tree response to night light exposure is an essential precondition of mechanism for controlling tree phenology.

Trees evolve to acclimate to daytime sunlight and assimilate carbon dioxide through gas exchange used for photosynthesis. The photosynthetic rate is limited either by the capacity of ribulose biphosphate carboxylase (RuBPCase) or by that of Ribulose biphosphate (RuBP) regeneration (Wullschleger, 1993). Both of these, plus other enzymes involved in photosynthesis, are all reliable on nitrogen (N) utilization (Hikosaka and Hirose, 1998; Wullschleger, 1993). On the other hand, phosphorus (P) was not only the component for generating RuBPCase and RuBP, but also the most limited element in photosynthesis, stomatal conductance, and dry mass allocation (Fujita *et al.*, 2003; Zhou *et al.*, 2022). During night light exposure, it was found that utilizations in N and P were modified by extended photoperiod to contrasting states in varied organs (Wei *et al.*, 2013a). N utilization was also reported to vary in different species due to varied abilities of water uptake in response to different spectra (Liu *et al.*, 2021). Studies on tree seedlings revealed that nitrogen (N) and phosphorus (P) were absorbed at different application rates for specific growing stages, which resulted in their unsynchronized utilizations (Chu *et al.*, 2020; Wei *et al.*, 2020a; Zhao *et al.*, 2020). It is still insufficient to argue that nutrition utilization is a species-specific ability in response to night light spectra.

Nutrient deficiency is a key factor that stimulates plant functions with reliance on nutrient utilization (Fujita *et al.*, 2003; Wei *et al.*, 2013a). Low nutrient efficiency may also cause a risk in water deficit (Hardikar and Pandey, 2011; Xu *et al.*, 2019). Under streetlamps where plants are exposed to night light, it is a common practice to avoid nutrient deficit through fertilizer delivery. Soil environment under this condition, however, is highly heterogeneous that may impact N and P uptakes (He *et al.*, 2021; Wei *et al.*, 2017; Zhou *et al.*, 2022). As a strategy, it is necessary to fertilize plants and increase utilization and water uptake efficiency. As described in above mentioned parts, light-exposed plants use N and P in RuBP generation to make photosynthesis onward. The preference of a spectrum would upregulate needs for N and P uptakes, which further results in a joint effect across light spectra and nutrient availability on plant nutrient utilization (He *et al.*, 2021; Zhao *et al.*, 2019; Zhao *et al.*, 2020). This combined effect has implemental meanings for urban plants established under streetlamps, but, again, relevant tests are scarce on trials across different species.

*Bauhinia* is a genus of flowering plant in the legume family, Fabaceae. Many *Bauhinia* plants have a wide habitat range in subtropical and tropical climates; hence they are usually cultured as urban landscape plants (Daud *et al.*, 2007; Zheng *et al.*, 2020). *Bauhinia brachycarpa* is a widely planted legume in tropical cities where habitat is usually exposed to streetlamp illumination (Zhang *et al.*, 2019). *B. brachycarpa* usually distributes in soils enriched with rock fragments which generates a high heterogeneity with low nutrient availability (Hu *et al.*, 2021). *B. variegata* is another legume that is widely planted as a landscape ornamental species adjacent to streetlamps (Lemes *et al.*, 2019; Mazzini-Guedes and Pivetta, 2014). Extracts from *B. variegata* have been identified to have high medicinal values (Sharma *et al.*, 2011; Yadava and Reddy, 2003). Nutrient utilization in both *B. brachycarpa* and *B. variegata* are of high concentrations by departments of urban planning and landscape ecology.

In this study, both *B. brachycarpa* and *B. variegata* were cultured as seedlings in a controlled condition, where lighting was provided by light-emitting diode (LED) in different spectra for testing exposure effects. Seedlings were fertilized and examined for their N and P uptakes and water content. We aimed to disclose combined effects of night light exposure and fertilization on N and P utilizations in these two legume species. We also have objectives to reveal parameters that may take part in the outcomes of N and P utilizations and to exhibit the inner relationships between these parameters.

## Materials and Methods

### *Experiment layout*

This study was conducted in a greenhouse located at Changchun (43°47' N, 125°23' E), Northeast China. All growing conditions can be controlled in an indoor environment locally in this greenhouse. Indoor temperature was maintained by an air conditioner to a range of 26-42 °C (winter/summer temperatures, respectively). Air flow was controlled to  $\sim 1.3 \text{ m s}^{-1}$  which was in accordance with wind velocity in the city where *Bauhinia* plants are dwelled in (Liu, 2009). Relative humidity (RH) was controlled in a range of 61-83% which matched that in the urban habitat (Feng *et al.*, 2023; Liu *et al.*, 2009).

### *Plant materials*

Seeds of *B. brachycarpa* and *B. variegata* were obtained from an institution of forest science and development in Guangzhou. Seeds were collected, sterilized, dried and mailed to the greenhouse in late March, 2018. Seeds were sown in sands in an incubation chamber with a constant temperature of 44 °C in late April of 2019. Sands were sprayed to maintain the moisture of 90%. In mid-May, most seeds were germinant and over 70% grew to plantlets. Plantlets were transplanted to growing media made by mixed peat and perlite (75%:25%, v/v) (Zhiluntuowei A&F S&T Inc., Changchun, China). Transplanted plants were moved to growing plugs and trayed in an arrangement of 4 units  $\times$  8 units. Each plug had a height of 13 cm and diameter of 7 cm and a total of 32 plugs were placed in cavities embedded in one growing tray with 32 individuals per capita. Trays were placed in water of tanks which would also enable water uptake through pore pulling in growing substrates namely in a subirrigation system (Barreto *et al.*, 2015; Bumgarner *et al.*, 2015).

### *Fertilizer application*

Two weeks after transplant, plantlets grew to own stems that were strong enough to support aboveground growth. Exponential fertilization was employed for nutrient delivery to expect a steady state input of nutrients to growing seedlings. Ammonium nitrate, potassium phosphate, and potassium chloride were mixed and resolved in distilled water to a proportion of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O in 10%:7%:9% (w/w). Exponential fertilizer amounts are determined according to a model (Duan *et al.*, 2013):

$$N_T = N_S(e^{rt} - 1) \quad (1)$$

where,  $N_T$  is the final amount of N reserved in a seedling through exponential fertilization,  $N_S$  is the initial amount of this seedling. We employed  $N_S$  as the amount from another tree species that distributes in a similar ecological niche with *B.* which was suggested to be 1.4 mg N plant<sup>-1</sup> (Xu *et al.*, 2019).  $N_T$  was suggested to be 80 mg N plant<sup>-1</sup>, which fell in the range of application dose that can induce luxury nutrient uptake at a steady state of rate in tree seedlings (Salifu and Jacobs, 2006; Xu *et al.*, 2019). The coefficient  $t$  is the total time of nutrient delivery. Because juvenile *B.* seedlings need a cultivational period across a growing season for about four months, which required  $t$  to be 16 at a pace of applications once a week (Duan *et al.*, 2013). The specific rate of N delivery for every application ( $N_t$ ) was calculated as (Xu *et al.*, 2019):

$$N_t = N_S(e^{rt} - 1) - N_{t-1} \quad (2)$$

where,  $N_{t-1}$  is the total amount of N delivered to seedlings up to the latest time. Specific schedule of nutrient applications is listed in Table 1. Micro-elements were fed to seedlings through every fertilizer applications with nutritional solutions containing: 1 mM calcium chloride, 0.6 mM magnesium sulfate, 20  $\mu\text{M}$  ferric chloride, 6  $\mu\text{M}$  manganese chloride, 16  $\mu\text{M}$  boric acid, 0.3  $\mu\text{M}$  zinc chloride, 0.3  $\mu\text{M}$  cupric chloride, and 0.2  $\mu\text{M}$  sodium molybdate (Wei *et al.*, 2013b).

**Table 1.** Specific schedule of nutrient delivery through exponential fertilization over 16 applications

Application week	Nutrient delivery (mg plant <sup>-1</sup> )		
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
1	0.40	0.28	0.36
2	0.52	0.37	0.47
3	0.67	0.47	0.61
4	0.87	0.61	0.78
5	1.12	0.78	1.01
6	1.44	1.01	1.30
7	1.86	1.30	1.67
8	2.39	1.68	2.15
9	3.09	2.16	2.78
10	3.98	2.78	3.58
11	5.13	3.59	4.62
12	6.61	4.63	5.95
13	8.52	5.97	7.67
14	10.99	7.69	9.89
15	14.16	9.91	12.74
16	18.25	12.78	16.43
Total	80.00	56.00	72.00

#### *Illumination treatment*

Three types of light spectra were employed in this study. Panels embedded with light-emitting diodes (LEDs) emitted different lights in any of red, green, and blue lights. A spectrum was generated as the mixture of those three basic lights. Electric current of every panel was jointly controlled by two electric transformers. The red-light diodes were controlled by a 200-W electric transformer and diodes emitting green and blue lights were controlled by a 135-W electric transformer (Wei *et al.*, 2020c). Specific electric current output for three types of spectra are adjusted as follows:

- (i) The blue-light enriched spectrum was generated by controlling electric current for red light to be 10% of the full output and for combined green plus blue lights to be 50% (R1BG5).
- (ii) The green-light enriched spectrum was generated by controlling electric current for red light to be 20% of the full output and for combined green plus blue lights to be 30% (R2BG3).
- (iii) The red-light enriched spectrum was generated by controlling electric current for red light to be 30% of the full output and for combined green plus blue lights to be 10% (R3BG1).

Specific distributions of wavelengths and three-lights' components for three types of spectra can be seen in a previous study (Wei *et al.*, 2020c). The gross photosynthetic photon flux density (PPFD) was measured to be 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  by placing LED panels 28-36 cm over seedling shoot tips. This PPFD was suggested in another controlled-environment study that simulated streetlamp spectra on tree seedlings (Liu *et al.*, 2021). During the daytime (07:00 am - 05:00 pm) all seedlings received a same LED spectrum, i.e. the R1BG5, to maintain ordinary growth under an uniform lighting condition (Wei *et al.*, 2020c). LED lighting started since 06:00 pm and went on to 23:00 pm, which synchronized with the period when streetlamps were lighted on (Liu *et al.*, 2021).

#### *Sampling and determination*

Seedlings were fertilized for four months then both LED lighting and fertilization stopped. One week after cultivational practices stopped, ten individuals were randomly chosen and sampled with root plugs. All ten sampled seedlings were measured for height and root-collar diameter (RCD) and divided into shoot (leaves and stem) and root parts. Roots were rinsed with distilled water to fully remove adherent substrates. Cleaned

shoot and root parts were measured for fresh weight and oven-dried at 70 °C for 72 h to weight for dry mass. Tissue (shoot or root) water content ratio (*WCR*) was calculated as (Guo *et al.*, 2022):

$$WCR = \frac{(W_{fresh} - W_{dry})}{W_{fresh}} \times 100\% \quad (3)$$

where,  $W_{fresh}$  and  $W_{dry}$  are weight for fresh and dry masses, respectively. Dried samples were ground to pass 1-mm mesh and smashed powers were digested by sulfuric acid. Total N and P concentrations were only determined in shoot organs. Total N concentration was determined using the Kjeldahl method. Total P concentration was determined using the Molybdenum-anticolorimetry method (Zhao *et al.*, 2020). Nutrient (N or P) utilization (*NUI*) was assessed using models used by Li *et al.* (2017):

$$NUI = \frac{DWP}{SNC} \quad (4)$$

where, *BWP* is the dry mass accumulation in whole plant, and *SNC* is shoot nutrient concentration for N or P.

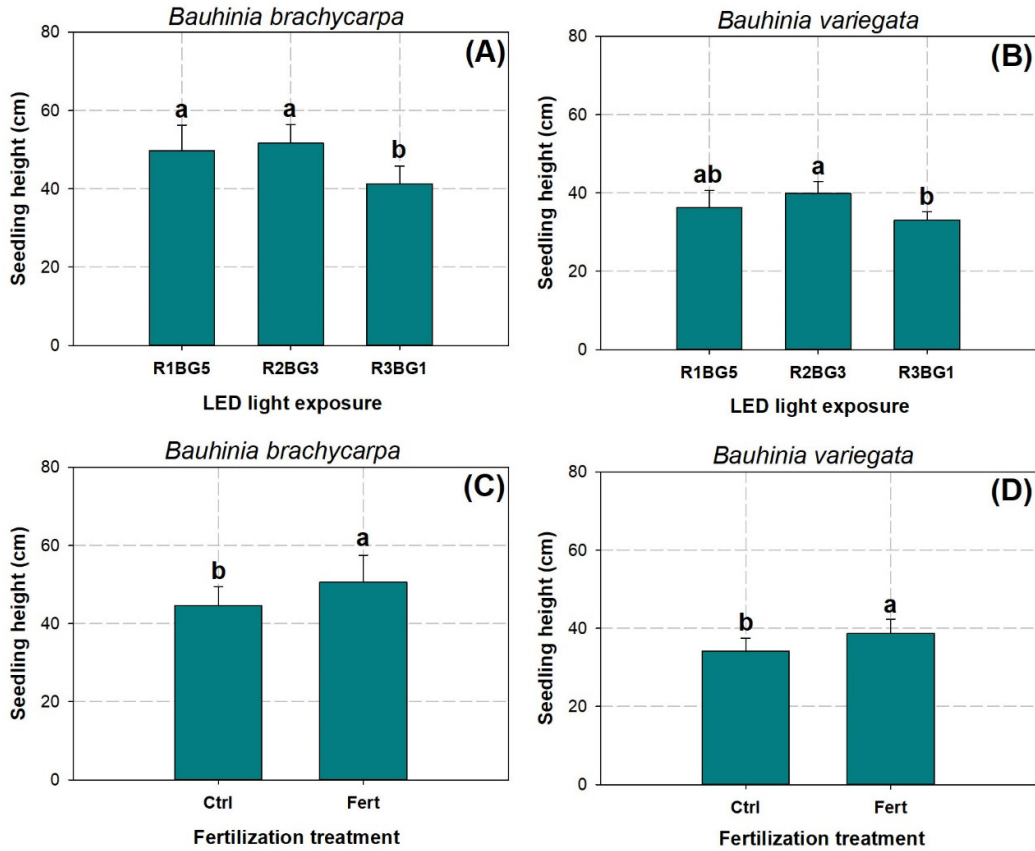
### *Statistical analysis*

Data passed tests for normality and variance homogeneity and no transformation was needed. All data were split to two groups by different species and all results were analysed both for two species. Analysis of variance (ANOVA) was used to detect interactive effects of lighting spectra ( $df=2$ ) and fertilization ( $df=1$ ) on plant parameters. When significant effect was indicated results were compared by Tukey tests ( $P<0.05$ ). Pearson correlation was used to detect relationship between *WCR* and *NUI*. Nutritional states of seedlings were diagnosed by synthesizing dry mass, nutrient content, and nutrient concentration in shoot part (Salifu and Jacobs, 2006). Vector analysis was used to plot relative difference of nutritional state between treatments.

## **Results**

### *Seedling growth*

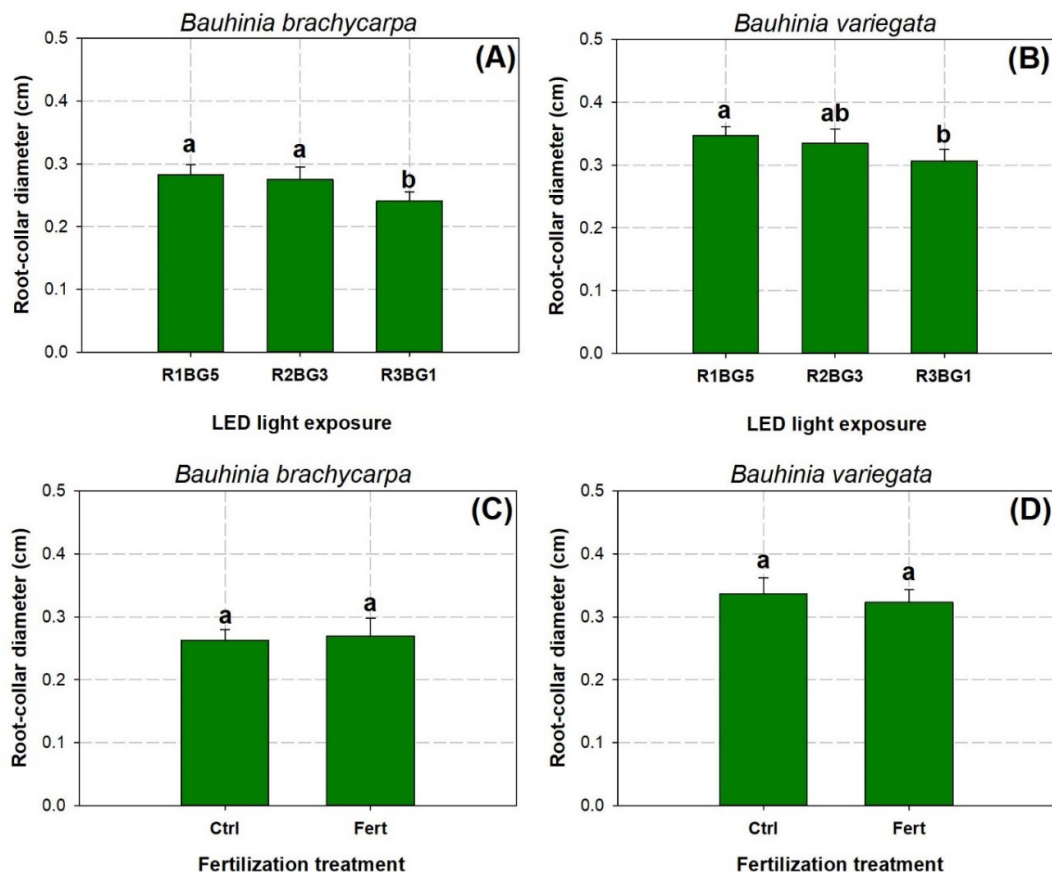
Lighting spectra and fertilization generated did not generate any interactive effects on seedlings height in either *B. brachycarpa* or *B. variegata* (Figure 1). The R3BG1 spectrum resulted in lower seedling height compared to the other two spectra for *B. brachycarpa* ( $P=0.0135$ ) (Figure 1A). In *B. variegata*, seedling height in the R3BG1 spectrum was lower than that in the R2BG3 spectrum, but the difference from that in the R1BG5 spectrum was not significant ( $P=0.0037$ ) (Figure 1B). Fertilization increased seedling height for both species (*B. brachycarpa*,  $P=0.0348$ ; *B. variegata*,  $P=0.0038$ ) (Figure 1C, D).



**Figure 1.** Treatments of Light-emitting diode (LED) light spectra (R1BG5, R2BG3, R3BG1) (A and B) and exponential fertilization (C and D) on seedling height in *Bauhinia brachycarpa* (A and C) and *B. variegata* (B and D)

Columns indicate means of each treatment and bars mark standard errors. Different letters mark significant differences according to Tukey test at 0.05 level.

Again, either lighting spectra or exponential fertilization had no interactive effects on RCD of two *B.* species (Figure 2). The R3BG1 spectrum reduced RCD compared to the other two spectra for *B. brachycarpa* ( $P=0.0049$ ) (Figure 2A). In *B. variegata*, however, the R3BG1 only resulted in a decline of RCD compared to the R1BG5 spectrum ( $P=0.0168$ ) (Figure 2B). Exponential fertilization failed to affect RCD in either species (Figure 2C, D).



**Figure 2.** Treatments of Light-emitting diode (LED) light spectra (R1BG5, R2BG3, R3BG1) (A and B) and exponential fertilization (C and D) on root-collar diameter in *B. brachycarpa* (A and C) and *B. variegata* (B and D)

Columns indicate means of each treatment and bars mark standard errors. Different letters mark significant differences according to Tukey test at 0.05 level.

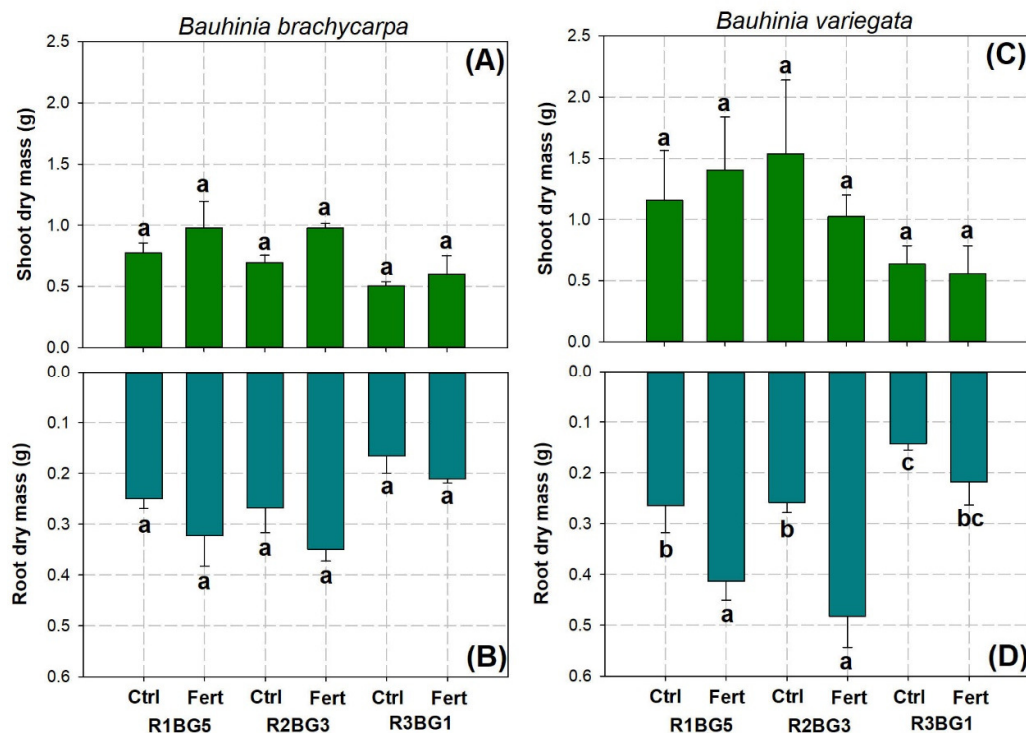
#### *Dry mass accumulation*

LED lighting spectra had a main effect on shoot dry mass in *B. brachycarpa* ( $P=0.0013$ ) (Figure 3A) and *B. variegata* ( $P=0.0142$ ) (Figure 3C). The R1BG5 and R2BG3 spectra resulted in shoot dry mass in  $0.88\pm 0.18$  g and  $0.84\pm 0.15$  g, respectively, both of which were higher than that in the R3BG1 spectrum ( $0.55\pm 0.11$  g) in *B. brachycarpa* (Figure 3A). Exponential fertilization increased shoot dry mass from  $0.66\pm 0.12$  g to  $0.85\pm 0.21$  g in *B. brachycarpa* ( $P=0.0059$ ). In *B. variegata*, the R1BG5 spectrum resulted in shoot dry mass in  $1.28\pm 0.38$  g and the R2BG3 resulted in  $1.28\pm 0.46$  g, which were both higher than that in the R3BG1 spectrum ( $0.60\pm 0.17$  g) (Figure 3C). Exponential fertilization did not change shoot dry mass in *B. variegata* which ranged between  $1.00\pm 0.41$  g and  $1.11\pm 0.50$  g ( $P=0.5449$ ).

Both LED lighting and exponential fertilization had a main effect on root dry mass in *B. brachycarpa* ( $P=0.0004$  and  $0.0032$ , respectively) (Figure 3B). The R3BG1 spectrum resulted in root dry mass in  $0.19\pm 0.03$  g, which was higher than that in the R1BG5 ( $0.29\pm 0.05$  g) and R2BG3 ( $0.31\pm 0.05$  g) spectra. Exponential fertilization increased root dry mass from  $0.23\pm 0.05$  g to  $0.29\pm 0.06$  g. However, LED lighting and exponential fertilization generated an interactive effect on root dry mass in *B. variegata* ( $P=0.0401$ ) (Figure 3D). In this species, exponential fertilization increased root dry mass in the R1BG5 and R2BG3 spectra but not in the

R3BG1 spectrum. Exponential fertilized seedlings exposed to the R1BG5 and R2BG3 spectra had greater root dry mass than other combined treatments in *B. variegata*.

Lighting spectra did not modify dry mass allocation between root and shoot in both species. In *B. variegata*, root to shoot dry mass ratio (RS) was decreased by exponential fertilization from  $0.42\pm 0.14$  to  $0.22\pm 0.07$ . In *B. brachycarpa*, fertilization did not cause any significant effect on RS in *B. brachycarpa*.



**Figure 3.** Treatments of Light-emitting diode (LED) light spectra (R1BG5, R2BG3, R3BG1) (A and B) and exponential fertilization (C and D) on dry mass in shoot (A and C) and root (B and D) of *B. brachycarpa* (A and B) and *B. variegata* (C and D)

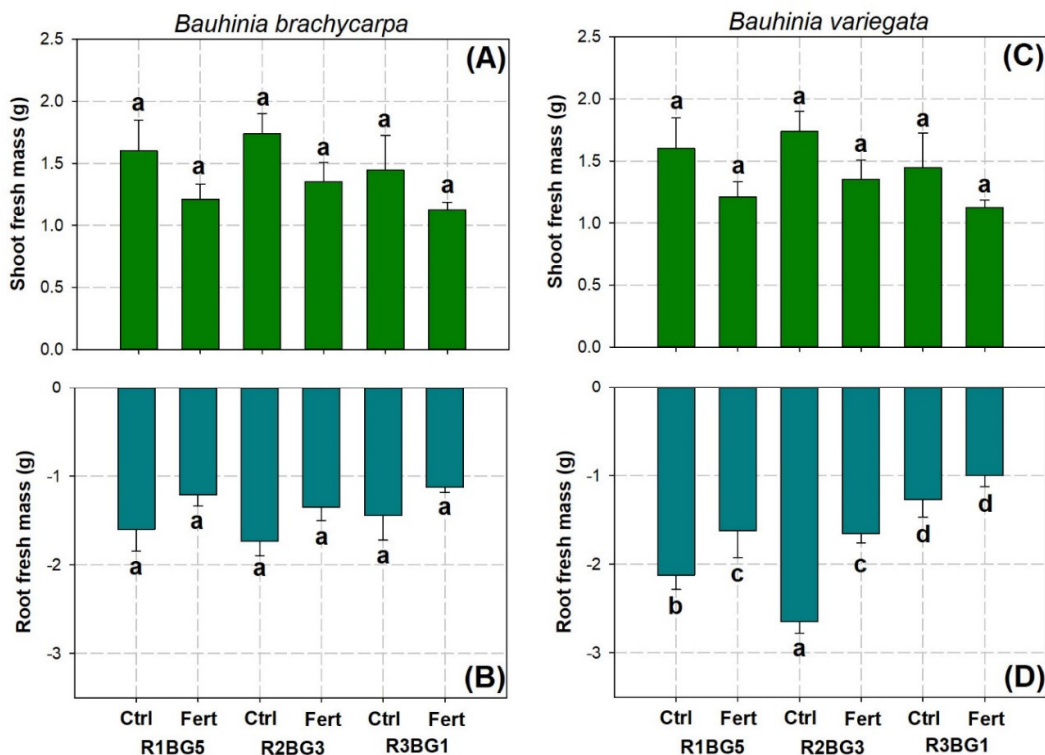
Columns indicate means of each treatment and bars mark standard errors. Different letters mark significant differences according to Tukey test at 0.05 level.

#### *Fresh mass accumulation*

LED lighting spectra resulted in significant effects on fresh mass in shoots of both *B. brachycarpa* ( $P=0.0074$ ) (Figure 4A) and *B. variegata* ( $P=0.0012$ ) (Figure 4C). In *B. brachycarpa*, the R1BG5 and R2BG3 spectra resulted in shoot fresh mass of  $3.06\pm 0.54$  g and  $3.04\pm 0.41$  g, respectively, both of which were higher than that in the R3BG1 spectrum ( $2.41\pm 0.19$  g) (Figure 4A). In *B. variegata*, the R1BG5 and R2BG3 also led to greater fresh mass in shoot ( $3.99\pm 0.60$  g and  $4.26\pm 0.52$  g, respectively) than that in the R3BG1 spectrum ( $2.97\pm 0.20$  g) (Figure 4C). Exponential fertilization increased shoot fresh mass by 21% in *B. brachycarpa* (Control,  $2.56\pm 0.20$  g; fertilization,  $3.11\pm 0.54$  g) and by 14% in *B. variegata* (Control,  $3.49\pm 0.55$  g; fertilization,  $3.99\pm 0.74$  g).

Lighting spectra did not cause any significant effect on root fresh mass in *B. brachycarpa* ( $P=0.1106$ ) (Figure 4B). However, lighting spectra imposed a significant effect on root fresh mass in *B. variegata* ( $P<0.0001$ ) (Figure 4D). The R1BG5 and R2BG3 spectra resulted in root fresh mass of  $1.87\pm 0.33$  g and  $2.15\pm 0.51$  g, respectively, while the R3BG1 spectrum only led to a lower level of root fresh mass as  $1.14\pm 0.20$  g. Exponential fertilization increased shoot fresh mass by 23% in *B. brachycarpa* (Control,  $1.59\pm 1.23$  g;

fertilization,  $1.23 \pm 0.13$  g) and by 29% in *B. variegata* (Control,  $2.02 \pm 0.56$  g; fertilization,  $1.43 \pm 0.33$  g). In addition, lighting spectra and fertilization also generated an interactive effect on root fresh mass in *B. variegata* (Figure 4D). Exponential fertilization decreased root fresh mass in the R1BG5 and R2BG3 spectra but not in the R3BG1 spectrum. Controlled seedlings exposed to the R2BG3 spectrum resulted in the highest level of root fresh mass.

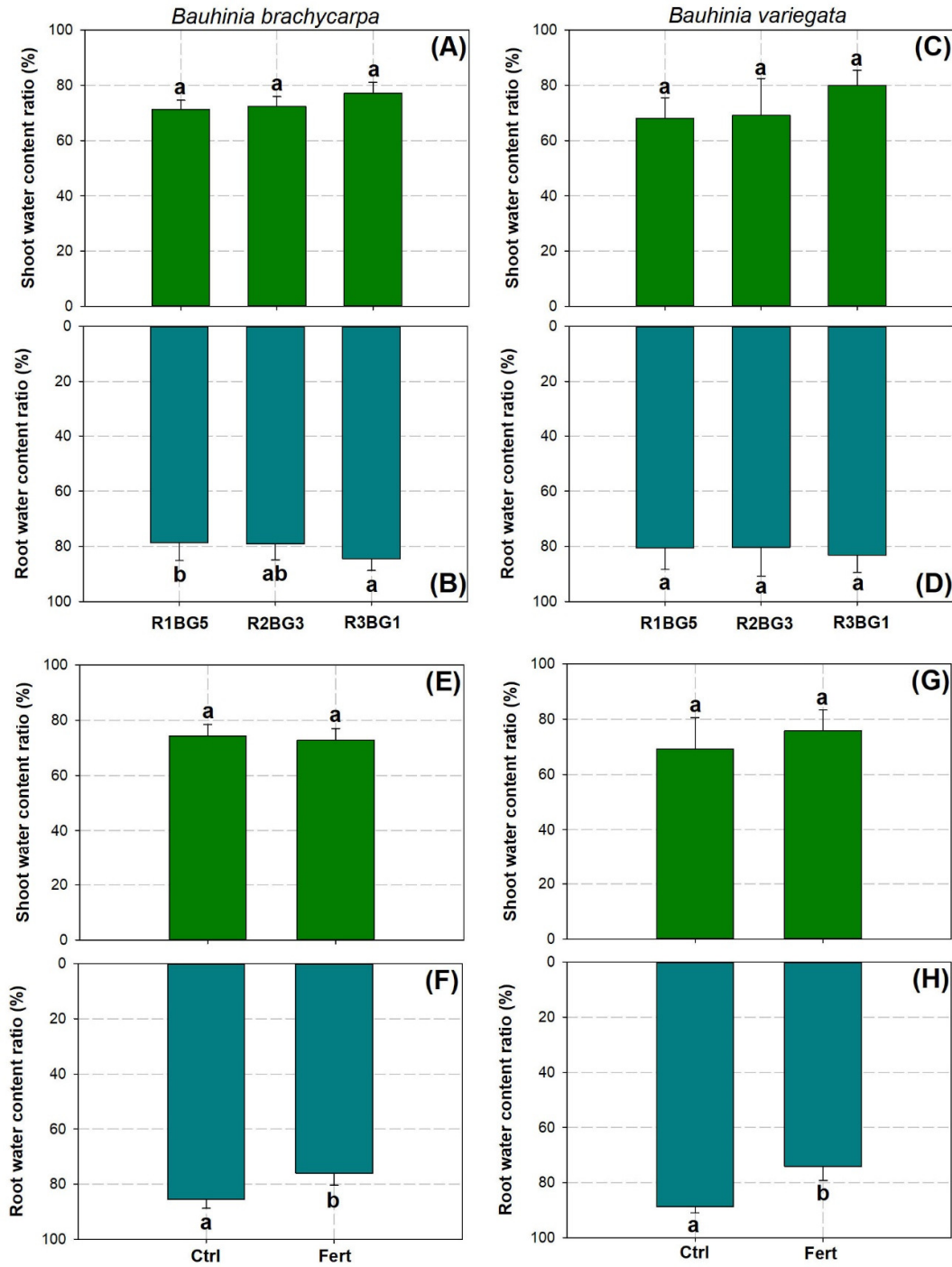


**Figure 4.** Treatments of Light-emitting diode (LED) light spectra (R1BG5, R2BG3, R3BG1) (A and B) and exponential fertilization (C and D) on fresh mass in shoot (A and C) and root (B and D) of *B. brachycarpa* (A and B) and *B. variegata* (C and D)

Columns indicate means of each treatment and bars mark standard errors. Different letters mark significant differences according to Tukey test at 0.05 level.

#### *Water content ratio (WCR)*

LED lighting spectra did not result in significant effects on *WCR* in shoots of *B. brachycarpa* ( $P=0.0741$ ) (Figure 5A) and *B. variegata* ( $P=0.1066$ ) (Figure 5C). Exponential fertilization also failed to affect shoot *WCR* in *B. brachycarpa* ( $P=0.1066$ ) (Figure 5C) and *B. variegata* ( $P=0.1725$ ) (Figure 5G). Root *WCR*, however, significantly responded to LED lighting spectra in *B. brachycarpa* ( $P=0.0001$ ) (Figure 5E). The R1BG5 spectrum resulted in root *WCR* of *B. brachycarpa* as  $78.66 \pm 6.53\%$ , which was lower by 7% than that in the R3BG1 spectrum (Figure 5B). LED lighting spectra lost its effect on root *WCR* in *B. variegata* (Figure 5D). Exponential fertilization reduced root *WCR* by 11% (control,  $85.46 \pm 3.36\%$ ; fertilization,  $76.10 \pm 4.32\%$ ) in *B. brachycarpa* ( $P=0.0001$ ) (Figure 5F) and by 16% (control,  $88.71 \pm 2.27\%$ ; fertilization,  $74.10 \pm 5.18\%$ ) in *B. variegata* (Figure 5H).

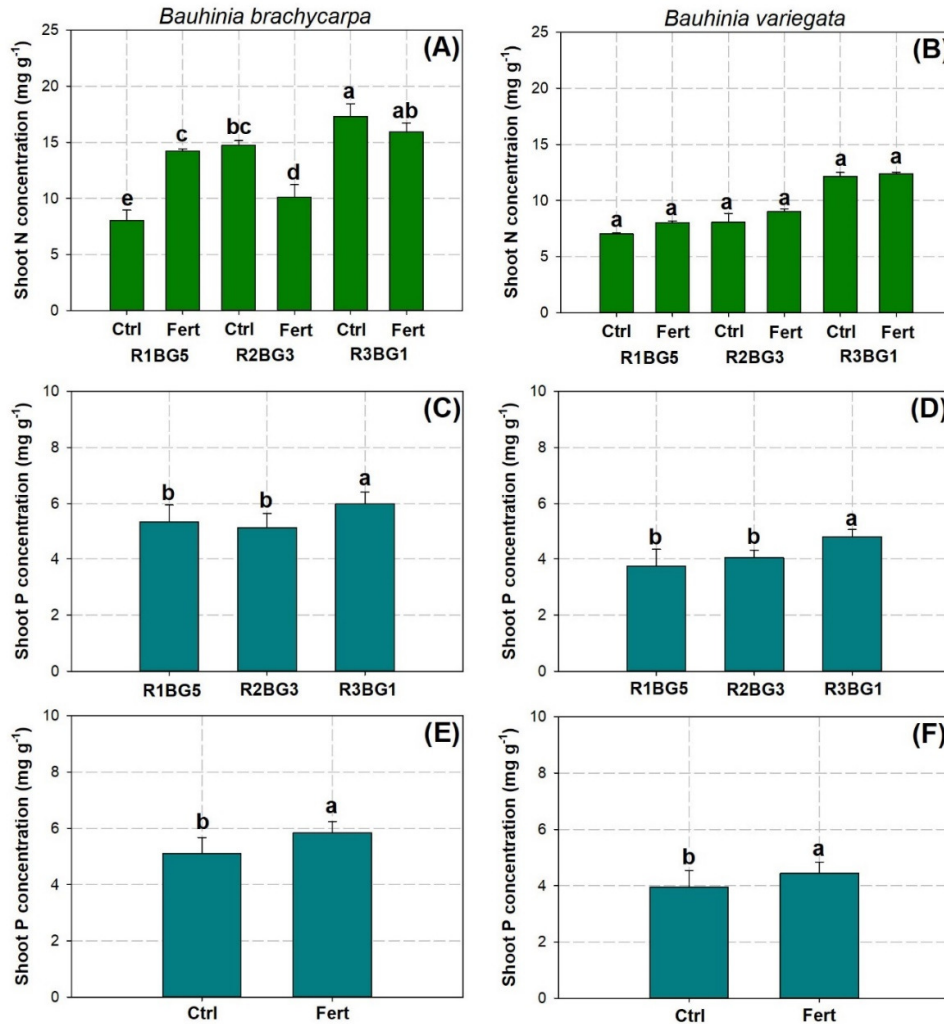


**Figure 5.** Main effects of Light-emitting diode (LED) light spectra (R1BG5, R2BG3, R3BG1) (A–D) and exponential fertilization (E–H) on water content ratio in shoot (A, C, E, G) and root (B, D, F, H) of *B. brachycarpa* (A, B, E, F) and *B. variegata* (C, D, G, H)

Columns indicate means of each treatment and bars mark standard errors. Different letters mark significant differences according to Tukey test at 0.05 level.

*N and P concentrations and contents*

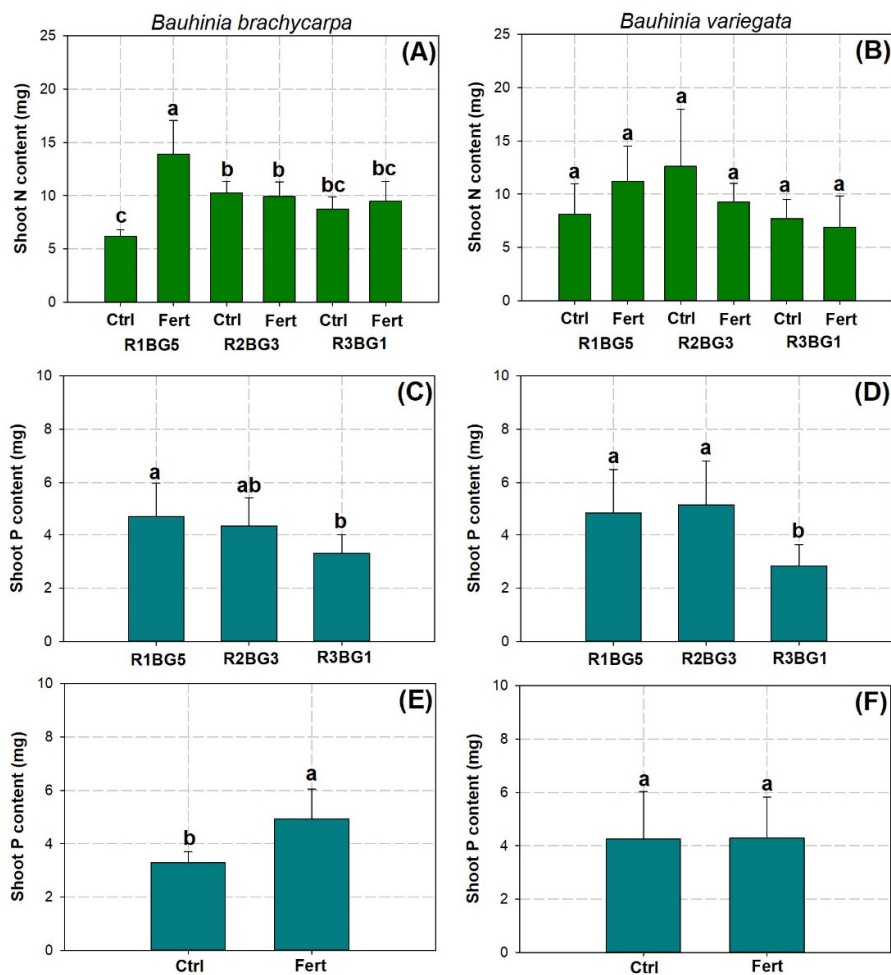
LED lighting spectra and exponential fertilization had an interactive effect on shoot N concentration in *B. brachycarpa* ( $P < 0.0001$ ) (Figure 6A). Exponential fertilization increased shoot N concentration in the R1BG5 spectrum, but decreased N concentration in the R2BG3 spectrum and generated no difference in the R3BG1 spectrum. Controlled seedlings in the R3BG1 spectrum had the highest level of shoot N concentration compared to that in the R1BG5 and R2BG3 spectra. In *B. variegata*, LED lighting spectra had a main effect on shoot N concentration which increased in an order following treatments of R1BG5 ( $7.51 \pm 0.52 \text{ mg g}^{-1}$ ) < R2BG3 ( $8.54 \pm 0.69 \text{ mg g}^{-1}$ ) < R3BG1 ( $12.27 \pm 0.26 \text{ mg g}^{-1}$ ) (Figure 6B). Exponential fertilization increased shoot N concentration in *B. variegata* from  $9.07 \pm 2.15 \text{ mg g}^{-1}$  to  $9.80 \pm 1.78 \text{ mg g}^{-1}$  ( $P = 0.0020$ ).



**Figure 6.** Combined effects of Light-emitting diode (LED) light spectra (R1BG5, R2BG3, R3BG1) and exponential fertilization on N concentration in shoot of *B. brachycarpa* (A) and *B. variegata* (B) and main effects of LED spectra (C and D) and fertilization (E and F) on shoot P concentration. Columns indicate means of each treatment and bars mark standard errors. Different letters mark significant differences according to Tukey test at 0.05 level.

Either LED lighting spectra or exponential fertilization had main effects on P concentration in shoots of *B. brachycarpa* ( $P=0.0094$  and  $0.0028$ , respectively) (Figure 6C, E) and *B. variegata* ( $P=0.0006$  and  $0.0107$ , respectively) (Figure 6D, F). In both species, shoot P concentration was higher in the R3BG1 spectrum than in the other two ones (Figure 6C, D). Exponential fertilization increased shoot P concentration for two species (Figure 6E, F).

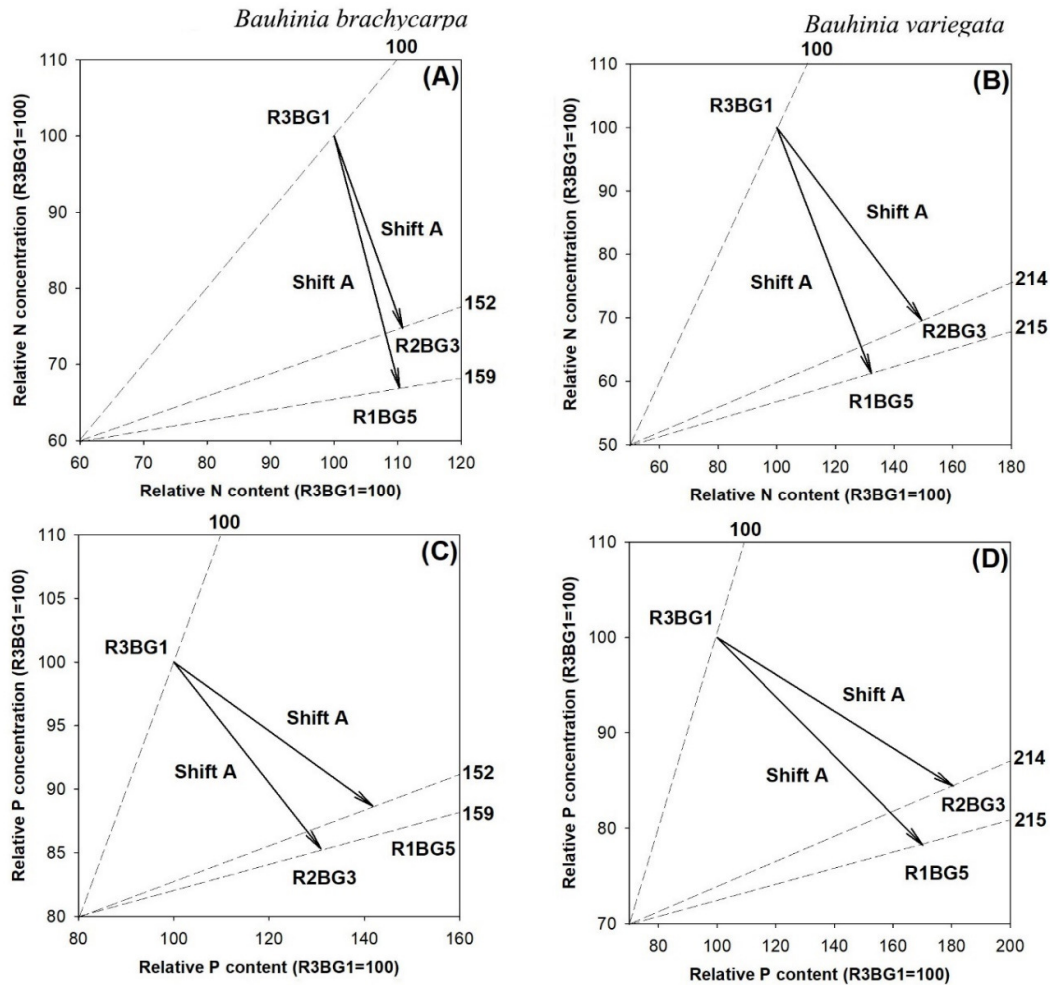
LED lighting spectra and exponential fertilization had an interactive effect on shoot N content in *B. brachycarpa* ( $P=0.0050$ ) (Figure 7A). Exponential fertilization increased shoot N content in the R1BG5 spectrum, but did not change N content in the R2BG3 and R3BG1 spectra. Fertilized seedlings in the R1BG5 spectrum had the highest level of shoot N content compared to that in the R1BG5 and R2BG3 spectra. In *B. variegata*, LED lighting spectra had no effect on shoot N content ( $P=0.2155$ ), neither did exponential fertilization ( $P=0.8331$ ) (Figure 7B).



**Figure 7.** Combined effects of Light-emitting diode (LED) light spectra (R1BG5, R2BG3, R3BG1) and exponential fertilization on N content in shoot of *B. brachycarpa* (A) and *B. variegata* (B) and main effects of LED spectra (C and D) and fertilization (E and F) on shoot P content. Columns indicate means of each treatment and bars mark standard errors. Different letters mark significant differences according to Tukey test at 0.05 level.

Both LED lighting spectra and exponential fertilization had main effects on P content in shoots of *B. brachycarpa* ( $P=0.0156$  and  $0.0004$ , respectively) (Figure 7C, E), but only LED spectra imposed a significant effect on shoot P content in *B. variegata* ( $P=0.0419$ ) (Figure 7D). In both species, shoot P content was higher in the R1BG5 spectrum than in the R3BG1 spectrum (Figure 7C, D). Exponential fertilization increased shoot P content in *B. brachycarpa* (Figure 7E) but not in *B. variegata* (Figure 7F).

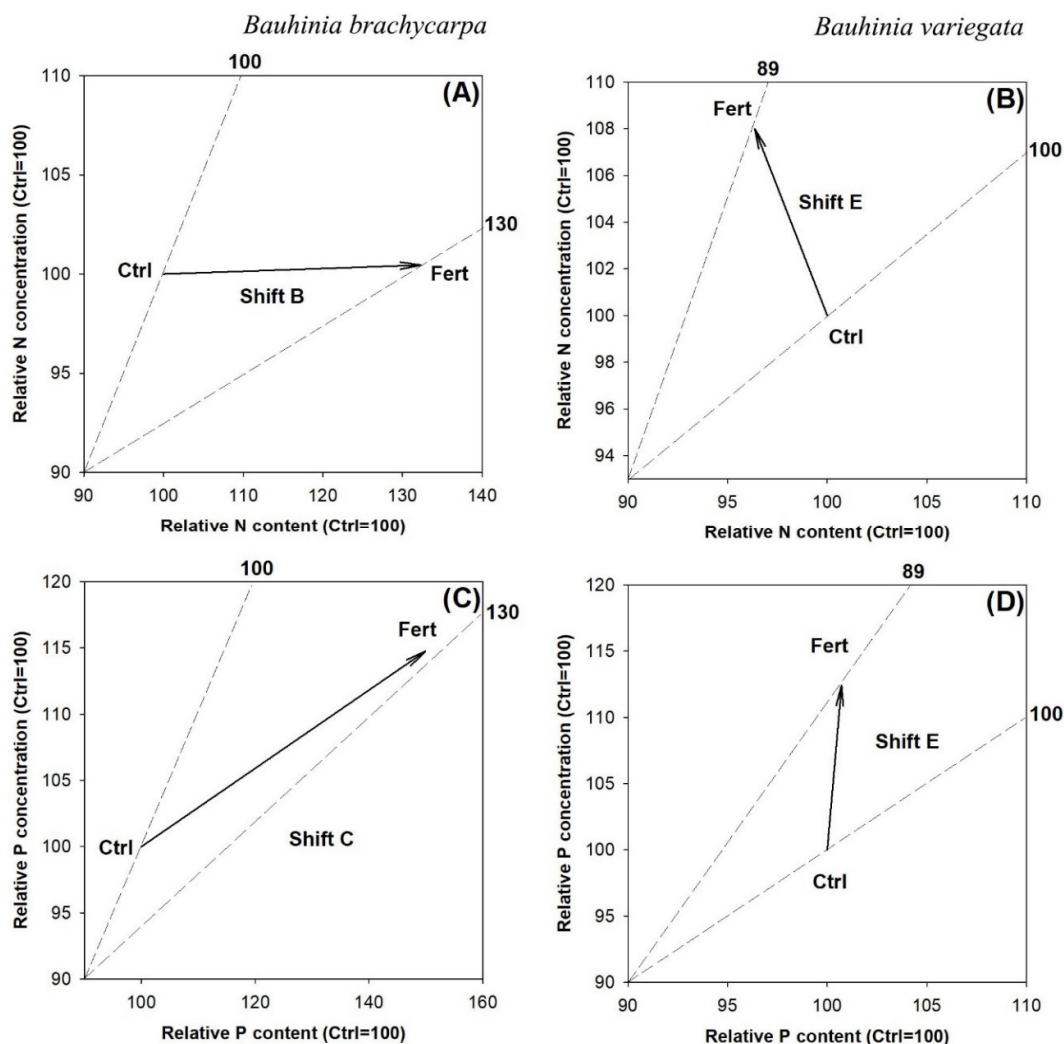
Relative to the R3BG1 spectrum, the R1BG5 and R2BG3 spectra resulted in relative increases in N content and dry mass but decreased N concentration; hence the latter two spectra were characterized to cause a status of N dilution in two species (Figure 8A, B). Similarly, the R1BG5 and R2BG3 spectra were characterized to cause P dilution relative to the R3BG1 spectrum (Figure 8C, D).



**Figure 8.** Vector analysis of N (A, B) and P states (C, D) in shoots of *B. brachycarpa* (A, C) and *B. variegata* (B, D) exposed to different LED light spectra. The spectrum R3BG1 is taken as the reference of 100 and relative values of Spectra R1BG5 and R2BG3 were estimated using vector nomographs. Shifts indicate nutritional states: Shift A, nutrient dilution caused by dry mass accumulation without sufficient nutrient supply.

Relative to the control, exponential fertilization increased dry mass accumulation without any changes of N concentration, which caused a steady state N delivery to *B. brachycarpa* (Figure 9A). However, exponential fertilization decreased dry mass but increased N concentration, which was characterized to be an excessively N supply to *B. variegata* (Figure 9B). Fertilized *B. brachycarpa* seedlings obtained increases in dry mass, P content,

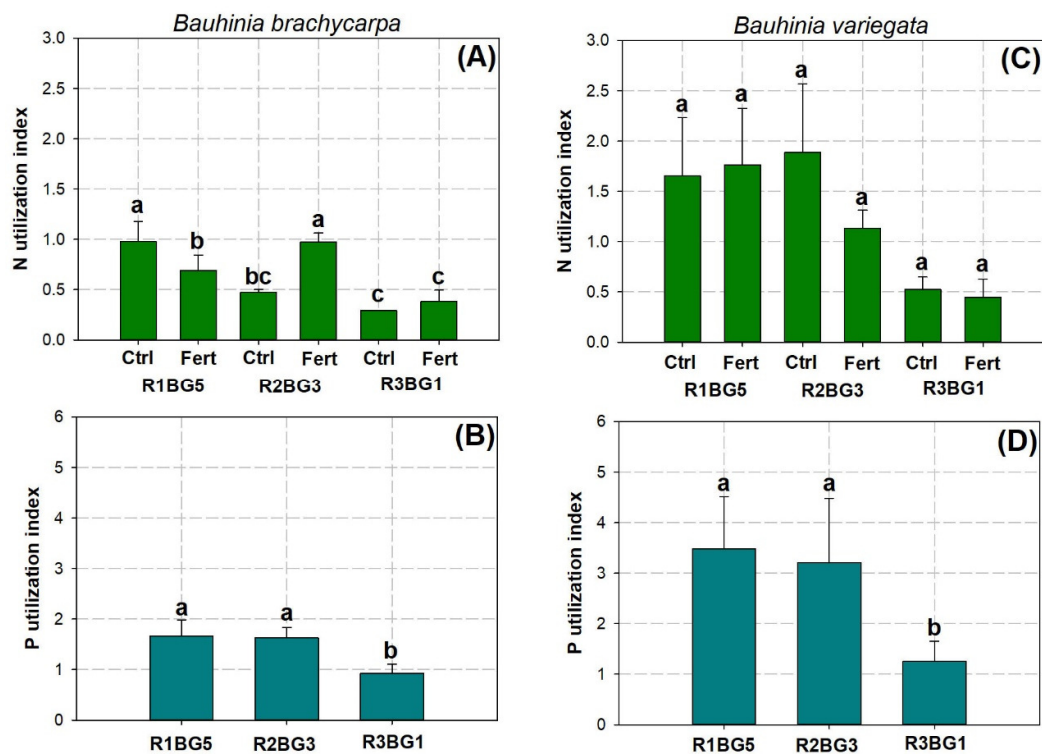
and P concentration, which was diagnosed to counter P deficiency in controlled seedlings (Figure 9C). In *B. variegata*, however, exponential fertilization decreased dry mass but increased P concentration, which was characterized to be a P supply excess (Figure 9D).



**Figure 9.** Vector analysis of N (A, B) and P states (C, D) in shoots of *B. brachycarpa* (A, C) and *B. variegata* (B, D) exposed to exponential fertilization (Fert) with unfertilized control (Ctrl) as the reference. Shifts indicate nutritional states: Shift B, steady-state delivery of nutrients to a sufficiency; Shift C, counter the nutrient deficiency caused by a limit; Shift E, nutrient supply excess that is caused by toxicity.

LED lighting spectra and exponential fertilization had an interactive effect on N utilization index in ( $P=0.0006$ ) *B. brachycarpa* (Figure 10A). Exponential fertilization decreased N utilization in the R1BG5 spectrum, but increased N utilization in the R2BG3 spectrum. In the R3BG1 spectrum, exponential fertilization did not cause any significant response of N utilization. In *B. variegata*, LED lighting spectra caused a significant effect on N utilization ( $P=0.0016$ ) (Figure 10C), which was lower in the R3BG1 spectrum ( $0.49\pm 0.14$ ) than in the R1BG5 ( $1.71\pm 0.50$ ) and R2BG3 spectra ( $1.51\pm 0.57$ ).

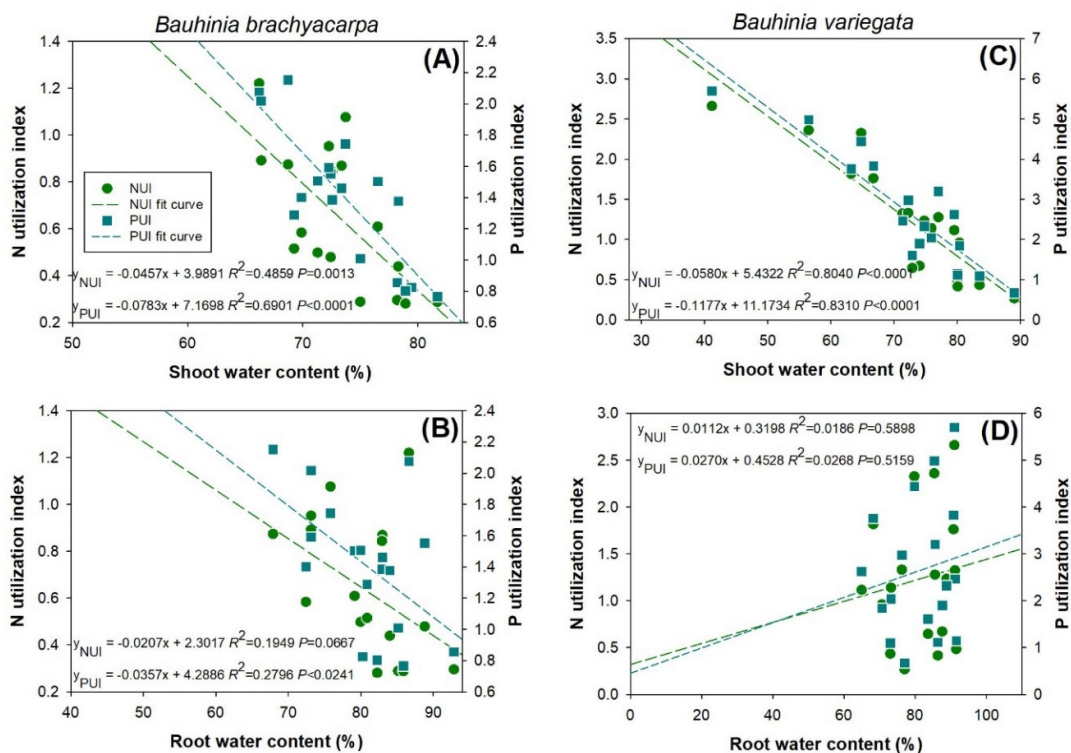
LED lighting spectra had significant effects on P utilization index in *B. brachycarpa* ( $P=0.0008$ ) (Figure 10B) and *B. variegata* ( $P=0.0068$ ) (Figure 10D). The R3BG1 spectrum resulted in lower P utilization compared to the R1BG5 and R2BG3 spectra in both species.



**Figure 10.** Combined treatments of Light-emitting diode (LED) light spectra (R1BG5, R2BG3, R3BG1) and exponential fertilization (Fert; unfertilized control, Ctrl) on N (A, C) and P utilizations (B, D) in *B. brachycarpa* (A and B) and *B. variegata* (C and D)

Columns indicate means of each treatment and bars mark standard errors. Different letters mark significant differences according to Tukey test at 0.05 level.

Shoot water content had negative relationships with N and P utilization indexes in *B. brachycarpa* (Figure 11A) and *B. variegata* (Figure 11C). Root water content had a negative relationship with P utilization (Figure 11B) in *B. brachycarpa*. In *B. variegata*, however, no relationship was found between root water content and N or P utilization indexes (Figure 11D).



**Figure 11.** Correlations between water content in shoots (A, C) and roots (B, D) of *B. brachycarpa* (A and B) and *B. variegata* (C and D)

## Discussion

### LED lighting spectra effect

In our study, the spectra of R1BG5 and R2BG3 resulted in a nutrient dilution status relative to the spectrum R3BG1 for both N and P statuses. This was caused by increased dry mass and nutrient content but decline in nutrient concentration. The R3BG1 spectrum had a higher proportion of red light in the wavelength (~42%) compared to the other two spectra (14-26%). Therefore, the decline in red light proportion in spectrum was verified to promote dry mass accumulation without sufficient nutrient uptake hence resulted in nutrient dilution. Our results concur with those found in *Aralia elata* (Wei *et al.*, 2020b) but contradict with findings on *Ficus hirta* and *Aplinia oxyphylla*. These together suggest that low-red-light spectrum may probably induce higher nutrient dilution in woody plants than in herbaceous plants.

We found that, the high-red-light spectrum (R3BG1) shaped *B.* seedlings with a smaller size, lower biomass accumulation, but higher N and P concentrations compared to another low-red-light spectrum (R1BG5). These changes can be identified in both species, suggesting that red light functioned to promote nutrient uptake at the cost of dry mass production. Low levels of N and P utilizations suggested that red-light induced high nutrient concentration, which, however, did not mean an efficient nutrient assimilation. Zhao *et al.* (2019) also found that the red-light spectrum can benefit N and P uptakes in well-fertilized *Larix principis-rupprechtii* seedlings. However, Gao *et al.* (2021) reported that, in *Quercus variabilis* seedlings, the green-light spectrum can promoted higher nutrient uptake than the red light. We are not sure whether the spectra variation on nutrient uptake is a species-specific effect. At least, we can conclude that red light spectrum did not benefit nutrient utilization in *B.* plants.

LED lighting spectrum did not cause response of water content in most organs except for *WCR* in roots which was higher in the spectrum with high red light in *B. brachycarpa*. Regarding root *WCR* was not changed by lighting spectra in *B. variegata*, we consider water content was a species-specific parameter. According to our correlation results, root *WCR* accounted for declines in N and P utilizations. It was also reported that a high red-light spectrum (77%) emitted by streetlamps increased whole-plant water content in maple but not in oak compared to a low red-light spectrum (46%) (Liu *et al.*, 2021). Red-light spectrum was also found to benefit water content in *Allium victorialis*.

#### *Exponential fertilization effect*

Although exponential fertilization was frequently used in nursery culture (Duan *et al.*, 2013; Li *et al.*, 2017), this can also be used as a simulating model to evaluate N loading through atmospheric deposition (Chen and Mulder, 2007; Seip *et al.*, 1999). In addition, controlled release fertilizer (CRF) is frequently used at transplant for newly planted trees (Iriño *et al.*, 2005; Jacobs *et al.*, 2005). Exponential fertilization can be used to test the steady state delivery of nutrients, which can also be a reference for the establishment of CRF formulation.

Exponential fertilization was developed to cope with the natural nutrient concentration decline in a dynamic of conventional fertilizer regime (Timmer, 1997). Its effect on dry mass accumulation depends on the nutritional status in which a seedling fall when receives fertilization (Salifu and Jacobs, 2006; Xu *et al.*, 2019). That is, when a seedling falls in a status of nutrient deficiency or lower, dry mass will increase with the growth of fertilizer application. When a seedling falls in a nutrient richness status, namely, luxury nutrient consumption or optimum nutrient state, additional fertilizer input will not cause any increment in dry mass accumulation (Salifu and Jacobs, 2006; Timmer, 1997).

In our study, exponential fertilization increased fresh mass for two *B.* species but only increased dry mass in *B. brachycarpa*. Vector analysis indicated that the two species responded to exponential fertilization as different nutritional statuses. Fertilization resulted in *B. brachycarpa* falling in the steady-state uptake of N, but meanwhile has caused N supply to be excessive in *B. variegata*. Exponential fertilization of P countered deficiency in *B. brachycarpa* while it also caused an excess in *B. variegata*. These meant that the two species had varied capacities to accommodate exogenous nutrient input, and *B. brachycarpa* had a higher nutrient accommodation capacity than *B. variegata*.

#### *Limits of this study*

Tests in this study have a rare relevance with those on other species. Although we established a complicated design testing two *B.* species, our rates of exponential fertilization still need to be tested to reveal the responses to a wider range of fertilizer spectrum. Secondly, our results about nutritional statuses in two *B.* species need more studies on physiological parameters to explain deep mechanisms accounting for species-specific difference. Thirdly, our study can be repeated under a field condition that employs all factors we used in a street. Finally, our results did not involve intervention of climate change, which will probably cause inevitable impacts on results and further works are suggested to incorporate this factor.

## **Conclusions**

Through a simulated study conducted at the controlled environment, we found nutrient utilization in two *B.* species are species-specific. Compared to *B. variegata*, *B. brachycarpa* had a higher capacity to accommodate exogenous nutrient input through exponential fertilization at 80 mg N plant<sup>-1</sup> through a total of 16 times of applications. *B. variegata* absorbed nutrients at the cost of water uptake but *B. brachycarpa*

assimilated nutrients in synchronization with root water uptake. A LED lighting spectrum with red-light proportion of ~42% was recommended for the illumination in streetlamps for *B.* because this spectrum can promote nutrient uptake without too fast rate of growth relative to other spectra with lower red-light proportions.

### Authors' Contributions

Conceptualization: Y.L.; Data curation: H.C., J.L., J.L.; Formal analysis: H.C., D.Z., L.B.; Funding acquisition: J.Y., Y.L.; Investigation: J.L., D.Z., L.B., Y.Y.; Methodology: J.Y.; Project administration: J.Y., Y.L.; Resources: J.L.; Software: J.L.; Supervision: H.C., J.Y.; Validation: Y.L.; Visualization: L.B., J.L.; Writing - original draft: H.C., J.L.; Writing - review and editing: Y.L. All authors read and approved the final manuscript.

### Ethical approval (for researches involving animals or humans)

Not applicable.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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