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ABSTRACT

All *Brassica alboglabra* plants were first grown aeroponically with full nutrients under full sunlight with average midday photosynthetic photon flux density (PPFD) of 1200 μmol m^{-2} s^{-1}. Thirty days after transplanting, plants were respectively, subjected to 10 days of average midday PPFD of 1200 (control, L1), 600 (L2) and 300 μmol m^{-2} s^{-1} (L3). Productivity, photosynthetic CO_2 assimilation and stomatal conductance were significantly lower in low-light (L2 and L3) plants than in high-light (L1) plants. Low light plants had the highest nitrate (NO_3^-) accumulation in the petioles. Low light also had an inverse effect total reduced N content. After different light treatments, all plants were re-exposed to another 10 days of full sunlight. Low-light plants demonstrated their ability to recover their photosynthetic rate, enhance productivity and reduce the NO_3^- concentration. These results have led to the recommendation of not harvesting this popular vegetable during or immediately after cloudy weather conditions.

Keywords: Aeroponic culture, Growth irradiance, Nitrate accumulation, Photosynthesis, Productivity

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INTRODUCTION

Since the edible parts such as young stems and leaves can accumulate plenty of NO_3^- thus, leafy vegetables are major sources of NO_3^- uptake by human (Boink and Speijers,

1999; Guadagnin et al., 2005). Investigations showed that about 80% of the total NO_3^- ingestion stems from daily diet of leafy vegetables (Dich et al., 1996). After ingestion, NO_3^- is reduced to nitrite (NO_2^-) by bacteria and some enzymes existing in the digestion system. NO_2^- reactions with various proteins in the digestive system to form nitroamines which are known to be mutagenic and carcinogenic (WHO, 1977). Due to the potential threats of NO_3^- accumulation in leafy vegetable, acceptable daily intake values of NO_3^- and NO_2^- recommended by the World Health Organization (WHO, 1977) is 3.6 mg kg^{-1}. Many countries have also set up NO_3^- concentration limits for vegetables (Santamaria et al. 1998; Zhou et al., 2000). For example, maximal level of NO_3^- in European Union defined lettuce and spinach are 2 to 4 and 2 to 3 g kg^{-1} fresh weight respectively depending on the time of harvest (Sušin et al., 2006).

Soilless culture such hydroponics or aeroponics is a system used for vegetable cultivation, which could produce homogenous and high-quality vegetables throughout the entire year. However, NO_3^- used in soilless culture as a sole nitrogen (N) source may lead to a higher NO_3^- content in certain vegetables (Lyons et al., 1994). For instance, in the study with aeroponically grown butter head lettuce (*Lactuca sativa* L. cv. Baby butter), it was found that shoot NO_3^- concentration was higher in lettuce plants grown under shade than under full sunlight (He et al., 2011). *Brassica alboglabra* (cv. Chinese broccoli), is one of the poplar vegetables consumed by the Chinese in Singapore and it is also one of the vegetables that are capable of accumulating large amounts of NO_3^- (Dich et al., 1996). NO_3^- content of *B. alboglabra* could also be depending on growth irradiances. For this popular vegetable, the leaf blade, petiole and stem are all edible parts. However, little is known about the NO_3^- concentration in their different edible parts.

The concentration of NO_3^- in plants depends on the species, the availability of NO_3^- to the plant, harvest period, the part of the plant as well as environmental factors such as light intensity (Cantliffe, 1973; Scaife and Stevens, 1983; Iversen et al., 1985; Kanaan and Economakis, 1992). Effects of growth irradiance on NO_3^- concentrations in different vegetable tissues have been well documented (Maynard et al., 1976; Lyons et al., 1994; McCall and Willumsen, 1998; Amr and Hadidi, 2001; Makus and Hettiarachchy, 2001). All these studies showed that NO_3^- concentrations of plant tissue are inversely related to growth irradiance. In Singapore, plants are exposed to intermittent days of clear and cloudy or haze weather. NO_3^- content of leafy vegetables grown in the greenhouse could be depending on growth irradiances (He et al., 2011). On the other hand, growth irradiance is one of the major factors affects both photosynthesis and productivity of vegetables grown in the tropical greenhouse (He, 2009; He et al., 2011).

N is the mineral nutrient required in the highest amounts by plants and is most frequently limiting growth and yield (Magaña et al., 2009). NO_3^- assimilation is one of the vital metabolic processes together with photosynthesis that affects the growth and development of plants. NO_3^- incorporation into biological molecules involves the reduction of NO_3^- to NO_2^- mainly via the enzyme nitrate reductase (NR) (Foyer et al. 1998; Forde, 2002; Macduff and Bakken, 2003; Cookson et al., 2005). NO_3^- content and the rate of NO_3^- assimilation in vegetables depends on the light intensity (Makus and Hettiarachchy, 2001; He et al., 2011) as in leaves, the regulation of NR is closely coupled to photosynthesis. Decrease in NR activity could also be linked to the decline in the rate of photosynthesis due to stomatal closure (Kaiser and Foster, 1989; Kaiser and Brendel-Benisch, 1991; Lawlor, 2002). Stomatal limitation has been reported to affect both

photosynthetic CO_2 assimilation rate (*A*) and internal CO_2 concentration, which inhibits metabolism (Lawlor, 2002). Kaiser and Förster (1989) concluded that NR activity was inhibited by lower internal CO_2 associated with *A*. Stitt et al. (2002) studied the links between carbon (C) and N metabolism and found that the latter is very sensitive to an inhibition of photosynthesis (Lillo et al., 2004). This research attempts to answer a few questions, i) does variation of growth irradiance affect the accumulation of NO_3^- in the different parts of *B. alboglabra* grown in soilless culture in Singapore? ii) what are the physiological bases that account for the different accumulation? iii) under what light condition should this plant be best harvested to reduce the NO_3^- concentration without substantially compromising productivity? Therefore, the main objectives of this study are to investigate the effects of growth irradiance on NO_3^- accumulation in different edible parts of *B. alboglabra*. The present study also addresses the physiological basis responsible for the final productivity and NO_3^- accumulation through the study of photosynthesis and the N metabolism by measuring *A*, stomatal conductance (g_s) and the total reduced N content under different growth irradiances.

MATERIALS AND METHODS

Cultivation of plants

A subtropical vegetable, *B. alboglabra* (*cv.* Chinese broccoli) was used. After germination, seedlings were inserted onto polyurethane cubes. The seedlings were then transferred to the greenhouse for 3 days for acclimatization before transplanting onto the aeroponic system as previously described by Lee (1993). All plants were exposed to fluctuating ambient temperature (26^oC – 36^oC) under 100% of prevailing solar radiation

with average maximal PPFD of 1200 µmol m^{-2} s^{-1}. They were supplied with full strength Netherlands Standard Composition. Nutrient solution conductivity and pH were maintained at 2.2 mS and pH 6.5 ± 0.5, respectively. The composition of full strength nutrient solution in mg l^{-1} was: K_2HPO_4, 187; $Ca(NO_3)_2.4H_2O$, 1237; $MgSO_4.7H_2O$, 609; K_2SO_4, 252; KNO_3, 293; FeEDTA, 20.52; $ZnSO_4.7H_2O$, 0.06; $CuSO_4.5H_2O$, 0.06; H_3BO_3, 0.59; $MnSO_4.H_2O$, 0.73; and $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.75. Thirty days after transplanting (DAT), plants were subjected to three different light treatments.

Light treatments

Thirty DAT, all plants were separated into three batches and were grown for further 10 days (40 DAT) under different light treatments. The levels of PPFD were recorded just above the top of the plants every day during midday. The three different light treatments were 1) 100% of prevailing solar radiation with average midday PPFD of 1200 µmol m^{-2} s^{-1} (control, L1), 2) covered with one layer of netting with average midday PPFD of 600 µmol m^{-2} s^{-1} (L2), and 3) covered with two layers of netting with average midday PPFD of 300 µmol m^{-2} s^{-1} (L3) over the 10-day period. Following the 10-day period of different light treatments, all plants were re-exposure to another 10-day (50 DAT) of 100% of prevailing solar radiation by removing the nettings with average midday PPFD of 1200 µmol m^{-2} s^{-1}.

Measurement of shoot and root fresh weight (FW) and dry weight (DW)

After removing the entire plant from the trough during harvesting time, the plants were separated into leaf blades, petioles, stems and roots, respectively. The FW of the different plant parts were weighed separately. After taking the FW, all the tissues were wrapped with aluminum foil and dried at 80^{o}C for 4 days to obtain DW.

Measurements of A and g_s

Ten days after different light treatments, for plants under each light treatment, A and g_s were first measured with a LI-COR Portable Photosynthesis System (LI-6400, Biosciences, U.S.) under a level of PPFD (from an internal LED light source) that was close to the average maximal growth irradiances. Immediately after the measurements of A and g_s, saturated photosynthetic CO_2 assimilation (A_{sat}) and saturated stomata conductance ($g_{s\ sat}$) were measured under a PPFD of 1200 μmol m^{-2} s^{-1} (also from an internal LED light source) on the same leaf. All measurements were carried out on the newly expanded leaves (the 6^{th} - 7^{th} leaves from the base) and were measured between 9:00 h (2 h after exposure to natural light) to 11:00 h in the greenhouse from the intact plants. Ten days after re-exposure to full sunlight, A and g_s, A_{sat} and $g_{s\ sat}$ were measured again via the same manner.

Measurement of NO_3^- concentration

It was determined using a Flow Injection Analyser (Model QuikChem 8000, Lachat Instruments Inc, Milwaukee, WI, USA) as described by Allen (1989). Dried plant tissue of 0.03 g was ground using a pestle and mortar with deionised water and then incubated at 37 °C for 2h. Sample turbidity was removed by filtration through a 0.45 μm pore diameter membrane filter prior to analysis. The NO_3^- was determined using a Flow Injection Analyser (Model QuikChem 8000, Lachat Instruments Inc, Milwaukee, USA). The principle of this method was to catalytically reduce NO_3^- to NO_2^- and measure the amount of NO_2^- present by a calorimetric reaction. NO_3^- is quantitatively reduced to NO_2^- by passage of the sample through a copperized cadmium column. The NO_2^- is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)

ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm.

Determination of total reduced N

Total reduced N content was determined by Kjeldahl digestion of dried samples in concentrated sulphuric acid. The samples were dried in an oven for 4 days set at 80 °C. After which, their dry weights were recorded before they were placed into a digestion tube with a Kjeldahl tablet and 5 ml of concentrated sulphuric acid. The mixture was then digested in a digestor until the mixture turned clear (about 90 minutes). After the digestion was completed, the mixture was allowed to cool for 30 minutes and the reduced nitrogen content was determined by a Kjeltec auto 1030 analyser. This result was later used to calculate the reduced nitrogen content (mg g^{-1}) present in the sample, and triplicate results were obtained for each treatment.

Statistical analysis

Differences between the control light (L1) and two other light treatments (L2 and L3), respectively were discriminated using Dunnett's procedure at $p < 0.05$.

RESULTS

Effects of growth irradiances on productivity

Both FW and DW of different plant parts were measured after the 20-day period of different light treatments and re-exposure to full sunlight. Responses of FW and DW to different light treatments were similar and only FW is shown in Figure 1. The FWs of the different plant parts before (30 DAT) and after (40 DAT) exposures to different growth irradiances are shown in Figures 1A and B, respectively. All plant parts continued to

grow during the 10-day of different light treatments. Leaf blade, petiole, stem and root (Figure 1B) from plants grown under deep shading (L3) for 10 days showed the lowest increases in FW compared to those obtained before light treatments (Figure 1A). Plants grown under full sunlight (L1) had the highest FWs of the different plant parts. Increases in the different plant parts under L2 were between those under L1 and L3, indicating that heavy shading (L3) had a greatest effect on plant growth. Although the FWs of different plant parts were still significant lower than the control L1 plants ($p < 0.05$), both L2 and L3 plants showed substantial growth after re-exposure them to full sunlight for 10 days (50 DAT) following different light treatments (Figure 1C).

Effects of growth irradiances on photosynthetic gas exchange and stomatal conductance

To study the photosynthetic performances and the maximal photosynthetic capacity after plants subjected to different light treatments, photosynthetic gas exchanges (A and g_s) were measured not only under the light intensities that were closed to the respective growth irradiance but also under the saturated light intensity (A_{sat} and $g_{s\ sat}$). After different light treatments for 10 days (40 DAT) both L2 and L3 plants showed significant decreases ($p < 0.05$) in their A and A_{sat} (Figure 2A) compared to the control L1 plants. The L2 and L3 plants also had much lower values of g_s than that of L1 plants when measured under the PPFD that was closed to their respective growth irradiances (Figure 2C). However, from the same leaves, there were no significant differences ($p > 0.05$) in $g_{s\ sat}$ among the different light treatments (Figure 2C). Upon re-exposure to full sunlight for 10 days, there were no significant differences in A_{sat} and $g_{s\ sat}$ between L1 and L2 plants (Figure 2B), indicating that L2 plants which were previously experienced shading had ability to fully

recover their photosynthetic capacities. After 10 days of re-exposure to full sunlight , however, A_{sat} was still significantly lower in L3 plants than in L1 and L2 plants ($p < 0.05$) although all plants had similar levels of $g_{s\,sat}$ (Figure 2 D).

Effects of growth irradiances on NO_3^- concentration

NO_3^- concentration of different plant parts were determined every 2 days over the 10-day period of different light treatments as well as the 10-day period of re-exposure of all plants to full sunlight. Both stem (Figure 3B) and petiole (Figure 3C) had much higher NO_3^- concentrations than that of leaf blade (Figure 3A) and root (Figure 3D) before the different light treatments. Over the 10-day of different light treatments, L1 plants had the constant lowest NO_3^- concentrations in all organs compared to those of L2 and L3 plants ($p < 0.05$). Although NO_3^- concentrations were slightly lower in all plant organs of L1 on day 10 (40 DAT) compared to day 0, statistically, the difference was not significant ($p > 0.05$). The slight lower NO_3^- concentrations could be due to the much bigger plant organs by 40 DAT (Fig. 1 B). NO_3^- concentrations increased markedly in leaf blade, petiole and stem of L2 and L3 plants with much greater increases found in L3 plants over the 10-day period of shading treatments (Figures 3A-C, $p < 0.05$). Compared to L1 plants, significant increases in root NO_3^- concentrations were only observed in L3 plants from day 4 of shading treatments (Figure 3D).

Over the 10-day of re-exposure to full sunlight, the control L1 plants had the lowest but constant NO_3^- concentration in all organs compared to those of L2 and L3 plants (Figures 3a-d). Over the same period, both L2 and L3 plants that were previously experienced 10 days of different shadings, exhibited significant and gradual decreases of

NO_3^- concentration in all organs with faster decreases found in L2 plants. By day 4, there were no significant differences in NO_3^- concentration in all plant parts between L1 and L2 plants. However, NO_3^- concentrations were significantly higher in leaf blade (except day 10) (Figure 3a), petiole (Figure 3b) and stem (Figure 3c) in L3 plants than in L1 and L2 plants over the entire period (Figure 3a-d).

Effects of growth irradiances on total reduced N concentration

After different light treatments for 10 days (40 DAT), except for the root, all the other plant parts of both L2 and L3 plants showed significant decreases ($p < 0.05$) in their total reduced N concentration (Figure 4A) compared to the L1 plants. After 10 days of re-exposure to full sunlight, however, except for the leaves of L3 plants, there were no significant differences in total reduced N concentration in all other plant organs among the plants that had previously exposed to different growth irradiances (Figure 4B).

DISCUSSION

Generally, the main source of dietary NO_3^- intake is considered to be the NO_3^- in vegetables (Santamaria et al., 1998). Previous researches suggested that the vegetables with high NO_3^- in the diet could put a human into the risk of gastrointestinal cancer and methemoglobinaemia (Bartsch et al., 1988). Hence, there is great concern about the NO_3^- content in the daily diet, especially in vegetables. *B. alboglabra* is a popular vegetable, mainly consumed by the Chinese population. The results of the present study show not only the productivity in terms of FW but also the NO_3^- concentrations of different edible parts fluctuating with growth irradiances. The productivities (Figure 1) of different *B.*

alboglabra parts were significantly decreased while the NO_3^- concentrations of different edible parts were significantly increased by shading (Figure 3). The reductions in productivity were more severe in deep shade plants (Figure 1).

In the present study, studies on photosynthetic gas exchange (Figure 2), total reduced N (Figure 4) of *B. alboglabra* provide certain physiological basis for the changes of productivity and accumulation of NO_3^- under different growth irradiances (Lillo et al., 2004; He, 2010; He et al., 2011). NO_3^- uptake and reduction were regulated by the supply of energy and C skeletons (Gastal and Saugier, 1989; Delhon, et al., 1996). Shading significantly decreased *A* of leaves (Figure 2) but increased NO_3^- concentrations of all plant tissues (Figure 3). Photosynthesis is directly and dramatically influenced by the amount of light striking leaves. It is well known that photosynthesis of single leaves immediately decline after subjecting to low growth irradiance (Lawlor, 1995; Logan, et al., 1998; Frantz, 2005). Light is also required for the development of leaves and biomass production of all plant tissues which is associate with both leaf area and photosynthetic rate (Taiz and Zeiger, 2002; Frantz, 2005). *B. alboglabra* is a sun loving plants. During the cloudy weather in Singapore, although it continues to grow, the growth of *B. alboglabra*, especially the biomass of leaf blade was much slower than during the sunny days. This was supported by the results of the present study that simulated the conditions of cloudy days (Figure 1A, 1B). After re-exposure to sunny conditions, except the roots, recoveries of growth were observed in different plant parts, especially the leaves (Figure 1C). During the shading period, low *A*, and g_s of shade plants were mainly due to the prevailing low light intensity which were required for photosynthesis and regulation of stomatal conductance (Bunce 2000). Lower rates of *A* could be partially

due to the low values of g_s (Figure 2C). However, the lower rates of A_{sat} (Figure 2A) in shaded plants than in unshaded control plants were not caused by the lower values of $g_{s\,sat}$ as all plant had similar values of $g_{s\,sat}$ ($p > 0.05$) (Figure 2B). Parallel analysis of leaf blade N concentration (Figure 4A) suggested that low A_{sat} could result from reduction of N metabolism especially the process of NO_3^- assimilation under low light. According to Solomonson and Barber (1990), as much as 25% of the energy of photosynthesis was consumed by the NO_3^- assimilation pathway. On the other hand, N determines the synthesis of amino acids and therefore of proteins and, ultimately, of all cellular components. The components of chloroplasts represent a large proportion of total leaf N (Lawlor et al., 2001). Therefore, reduction of *A* (Figure 2A) in *B. alboglabra* grown under suboptimal growth irradiance resulted in not only a marked decrease in its productivity (Figure 1B) but also accumulation of NO_3^- (Figures 3A-D) and inhibition of NO_3^- assimilation (decrease in total reduced N concentration) (Figure 4A). It has been reported that NO_3^- accumulation in plant tissues can occur when plants are shaded, photosynthesis becomes light-limited and NO_3^- reduction declines (Broadley et al., 2003). The ability of the plants to recover from its low photosynthetic rate after re-exposure to full prevailing solar radiation (Figure 2B) coupled with significant decreased NO_3^- accumulation (Figures a-d) and increased total reduced N concentration (Figure 4B). These results indicate that high growth irradiance, through the activation of photosynthesis and the production of sugars stimulates NO_3^- assimilation activity in *B. alboglabra* (Lillo et al., 2004). For *B. alboglabra* plants, NO_3^- uptake by the roots was transported to the stems (possibly as the strongest sink of NO_3^-) and via the routes of petioles to the leaves. Therefore, low sugar production induced under low light leads to

an inhibition of NO_3^- assimilation (Stitt et. al, 2002; Magaña et al., 2009). During low light treatment, accumulation of NO_3^- was higher in the petioles and stems since the supply of reducing equivalents (NADP or NADPH), C skeletons and energy (ATP) were limited for the reduction of NO_3^- to NO_2^- (Riens and Heldt, 1992). During low light treatment, NO_3^- concentration is lower in roots (Figure 3D) and this could be due to its transportation to stem and petiole where they were stored. This did not concur with the findings by Stöhr and Mäck (2001) on tobacco, also a C_3 plant whose roots accumulate NO_3^- in highest concentration during the light period. In the present study, NO_3^- accumulation under low light was highest in the petioles and stems but subsequent re-exposure to sunlight reduced much of its concentration (Figures 3b, c).

The present study simulated the local weather conditions by shading the *B. alboglabra* plants during the active vegetative growth stage for a period of 10 days. It was found that the edible parts of leaf blade, petiole and young stem accumulated large amount of NO_3^- especially under lower growth irradiance. After 10 days of deep shading both stems and petioles had much higher NO_3^- concentration of 20 to 22 mg g^{-1} DW (Figures 3B, C) or 200 to 250 mg Kg^{-1} FW, this level was between a maximum of 91 mg Kg^{-1} acceptable for fresh spinach and 791 to 1017 mg Kg^{-1} for fresh lettuce in Germany (Schwemmer, 1990), and, 700 mg Kg^{-1} present in the edible parts in China (Zhou et al., 2000). However, after re-exposure of shaded *B. alboglabra* plants to full solar radiation for 10 days, the highest NO_3^- concentration was about 15 mg g^{-1} DW in stems (Figure 3). Although those plants were previously subjected to shade did not gain the productivity as high as those plants which grown under full sunlight for the whole light cycle, they showed substantial growth after re-exposure them to full sunlight for 10 days (50 DAT)

following different light treatments (Figure 1 C). Based on these results, the harvesting of *B. alboglabra* is best carried out during sunny days or after some days of re-exposure to sunny days following cloudy or haze days. On the other hand, NO_3^- accumulation in the leaf blade was relatively lower compared to the petioles and stems, but consumers should bear in mind that the leaves constitute a much higher NO_3^- content by mass due to its highest biomass (Figure 1). Since it is not a cultural nor social health habit for consumer to inquire the growth history from the seller, a quick recommendation for consumers is to consume this vegetable lesser serving portion in the diet when they were purchased during the cloudy or haze days.

CONCLUSION

Severe light limitation has negative effects on productivity, photosynthetic CO_2 assimilation and NO_3^- assimilation of *B. alboglabra*. However, this effect is reversible upon re-exposure to full light condition. There is close relationship between NO_3^- concentration and total reduced N under different growth irradiances. High levels of NO_3^- accumulation were in stem and petiole of *B. alboglabra* after subjecting to lower growth irradiance, which has led to the recommendation of not harvesting this popular vegetable during or immediately after cloudy or haze weather conditions

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FIGURE CAPTIONS:

Figure 1. FW of different *B. alboglabra* organs before light treatment (A, 30 DAT), and after 10 days (40 DAT) of different light treatments (B), and re-exposure to 100% of prevailing solar radiation for 10 days (50 DAT) (C). Vertical bars represent standard error (n = 5). * denotes statistically significant difference between the control (L1) and low light treatments (L2 or L3) discriminated using Dunnett's procedure at $p < 0.05$.

Figure 2. *A and* A_{sat} (A) and g_s and $g_{s\,sat}$ (C) of *B. alboglabra* after 10 days (40 DAT) of different light treatments and, A_{sat} (B) and $g_{s\,sat}$ (D) of *B. alboglabra* after re-exposure to 100% of prevailing solar radiation for 10 days (50 DAT). Vertical bars represent standard error (n = 5). □ , *A* or g_s measured under different PPFDs similar to the growth irradiances, ■, A_{sat} and $g_{s\,sat}$, measured under saturated a PPFD of 1200 µmol m^{-2} s^{-1}. * denotes statistically significant difference compared to the control (L1) discriminated using Dunnett's procedure at $p < 0.05$.

Figure 3. Changes of NO_3^- concentration over the 10-day period of different light treatments and the 10-day period of re-exposure to 100% of prevailing solar radiation in leaves (A, a), petioles (B, b), stems (C, c) and roots (D, d) of *B. alboglabra*.). Vertical bars represent standard error (n = 5). * denotes statistically significant difference compared to the control (L1) discriminated using Dunnett's procedure at $p < 0.05$.

Figure 4. Total reduced N of different *B. alboglabra* organs after 10 days (40 DAT) of different light treatments (A), and re-exposure to 100% of prevailing solar radiation for 10 days (50 DAT) (B). Vertical bars represent standard error (n = 5). * denotes statistically significant difference compared to the control (L1) discriminated using Dunnett's procedure at $p < 0.05$.

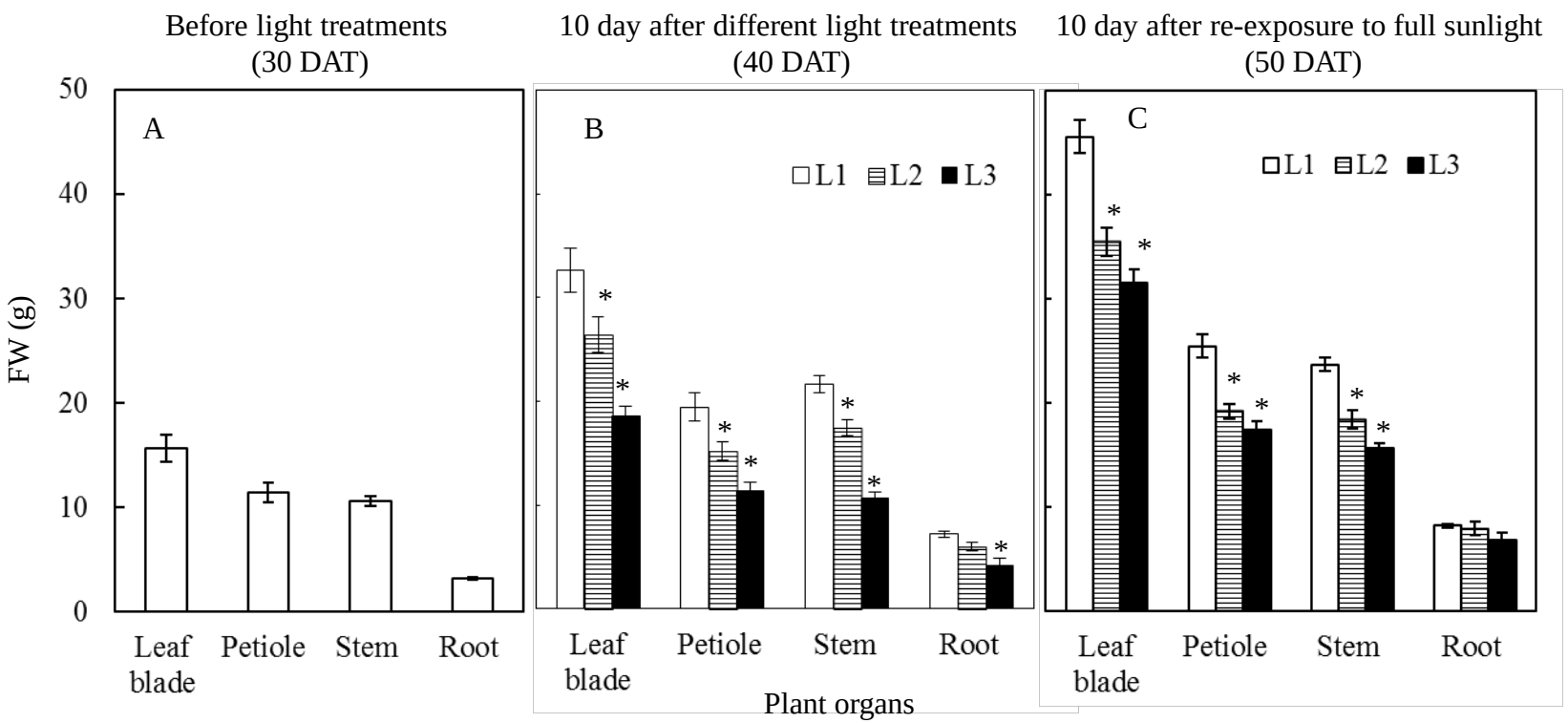
Before light treatments
(30 DAT)
10 day after different light treatments
(40 DAT)
10 day after re-exposure to full sunlight
(50 DAT)
A
B
C
L1
L2
L3
FW (g)
50
40
30
20
10
0
Leaf blade
Petiole
Stem
Root
Plant organs

Fig. 1

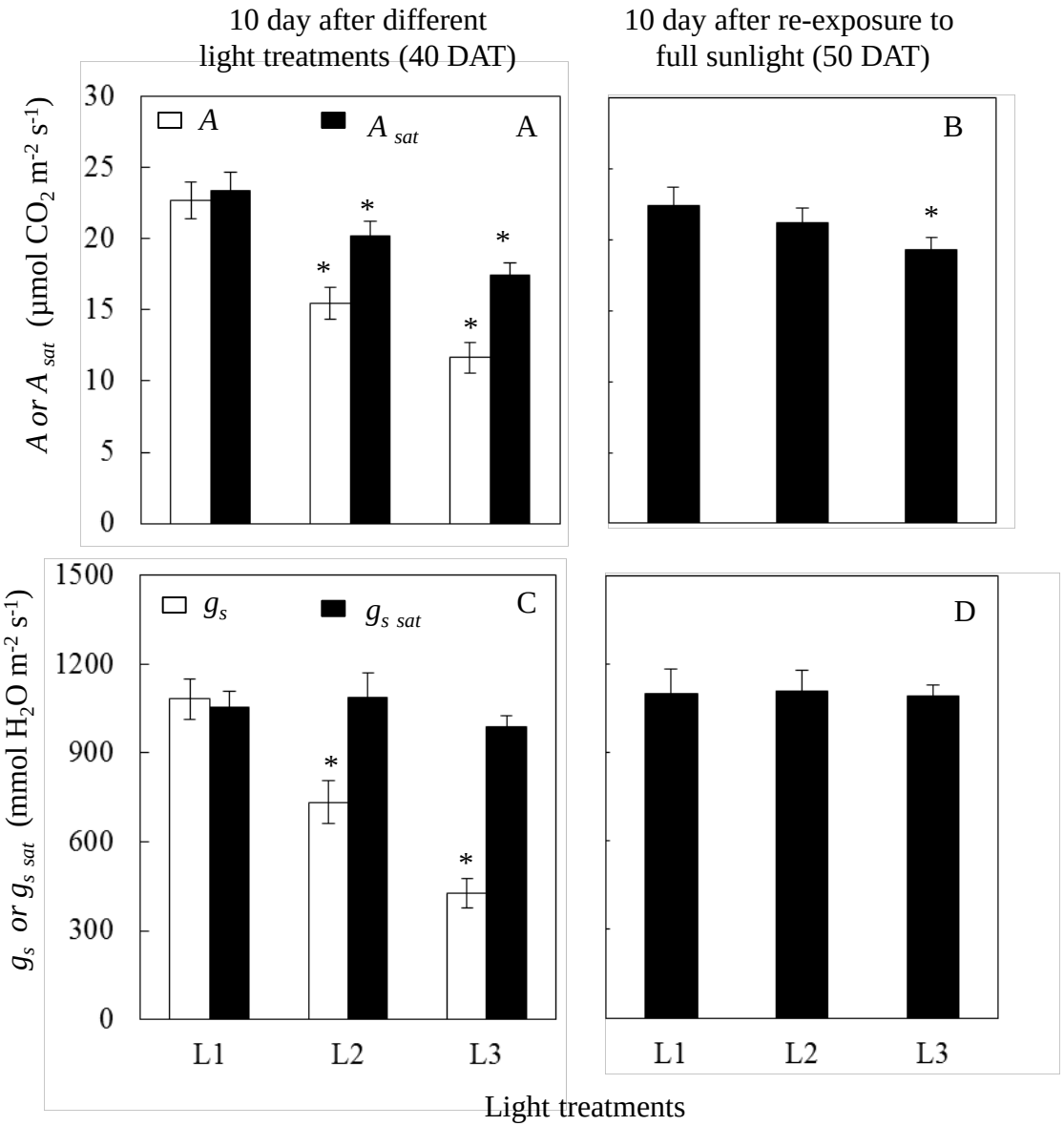
10 day after different light treatments (40 DAT)
10 day after re-exposure to full sunlight (50 DAT)
A or A sat (µmol CO2 m-2 s-1)
A
A sat
A
B
gs or gs sat (mmol H2O m-2 s-1)
gs
gs sat
C
D
L1
L2
L3
Light treatments

Fig. 2

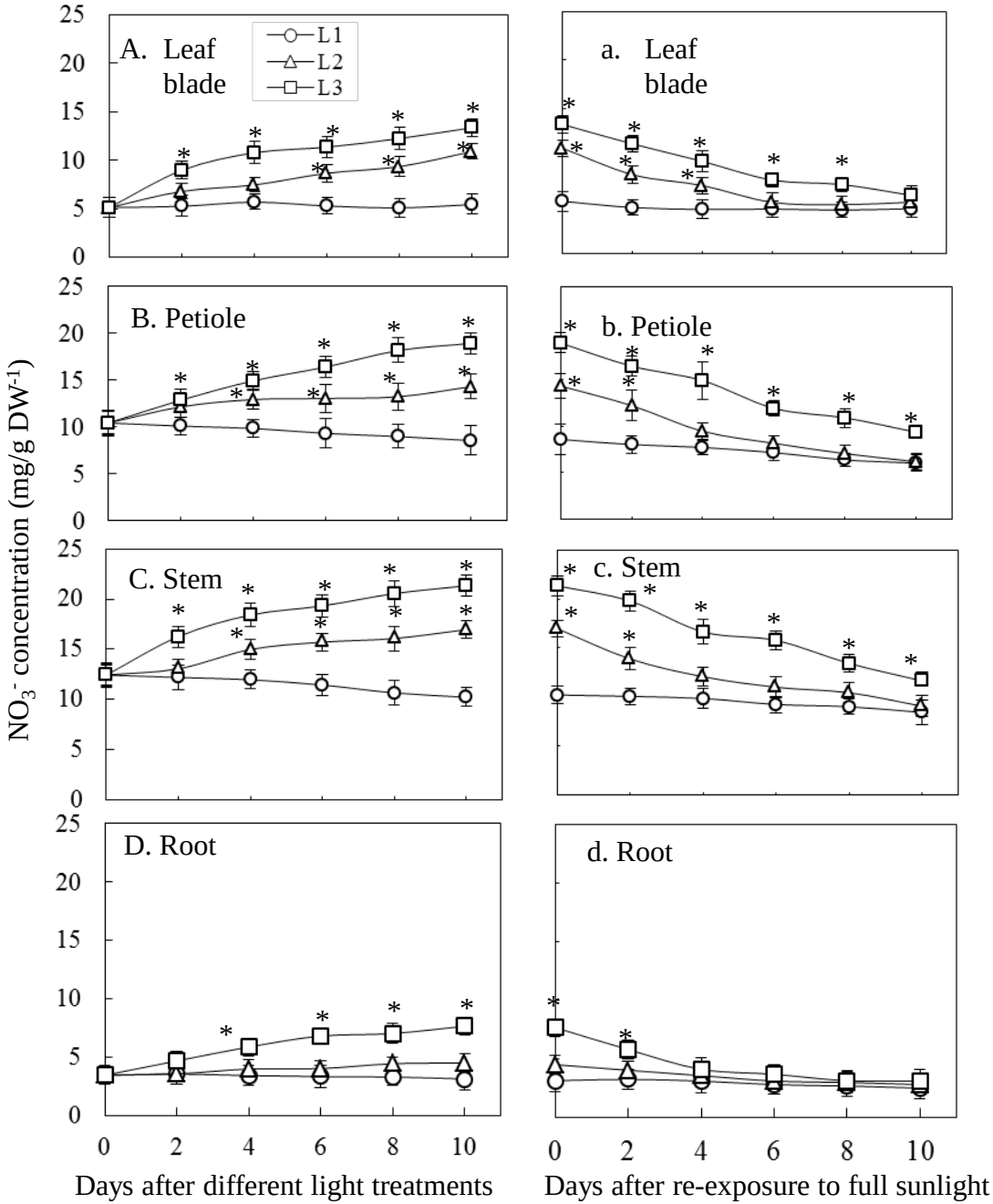
A. Leaf blade
L1
L2
L3
a. Leaf blade
B. Petiole
b. Petiole
C. Stem
c. Stem
D. Root
d. Root
NO3- concentration (mg/g DW-1)
Days after different light treatments
Days after re-exposure to full sunlight

Fig. 3

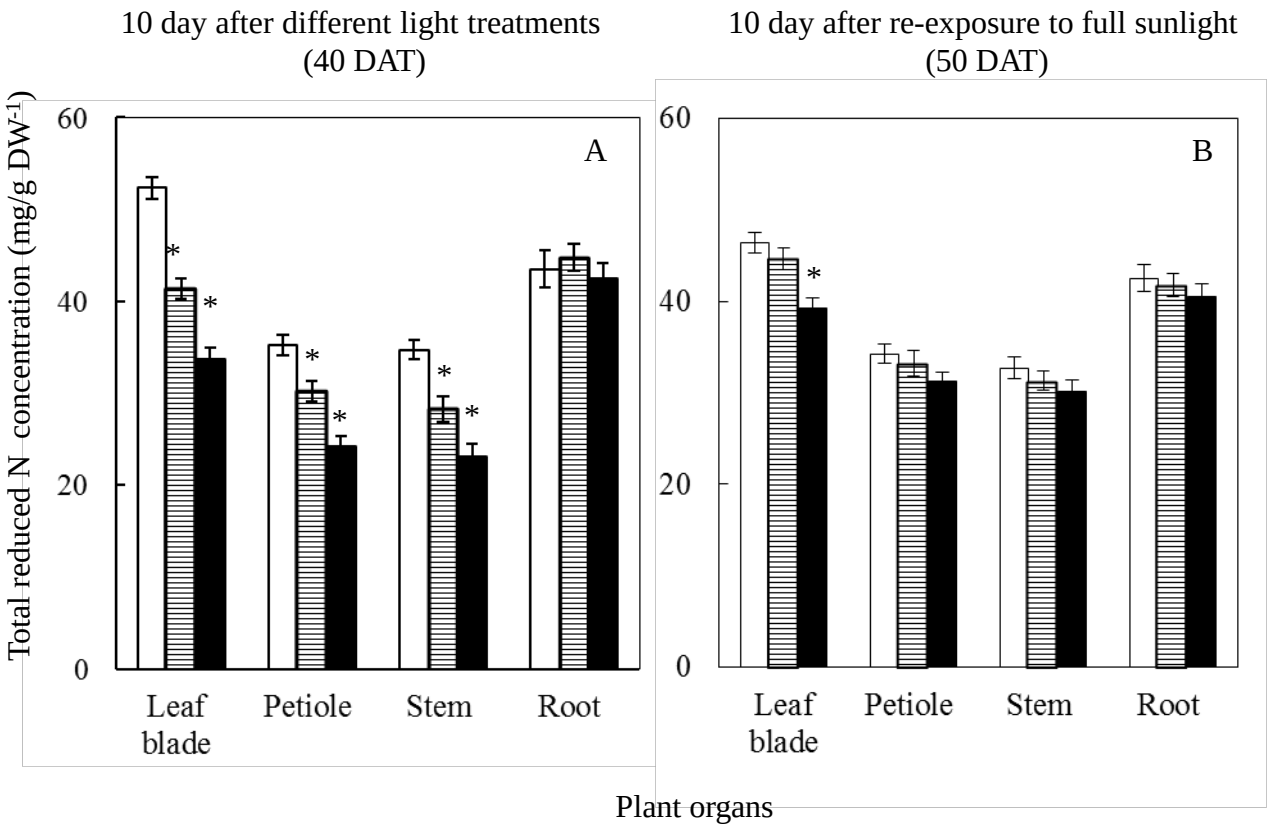
10 day after different light treatments
(40 DAT)
10 day after re-exposure to full sunlight
(50 DAT)
Total reduced N concentration (mg/g DW-1)
60
40
20
0
A
B
*
Leaf blade
Petiole
Stem
Root
Plant organs

Fig. 4