Nitrate Accumulation, Productivity and Photosynthesis of Temperate Butter Head Lettuce under Different Nitrate Availabilities and Growth Irradiances

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Abstract: Under low growth-irradiance and/or excessive nitrogen (N) fertilization, the roots of leafy vegetables could take up nitrate (NO_3^-) faster than the plant can convert it to organic nitrogen compounds. NO_3^- is suspected to have carcinogenic effect in human when eaten in high quantity. In this study, lettuce plants were first grown in an aeroponics system with full nutrients (full NO_3^-) under full sunlight. Six weeks after transplanting, plants were subjected to 7 days of full sunlight and shade with full, 1/2 and 0 NO_3^- respectively. Shoot NO_3^- concentration was higher under shade than under full sunlight regardless of NO_3^- availability after 7 days of treatments. The higher shoot NO_3^- concentration of shade plants was derived from the high NO_3^- accumulated in their roots during the 6 weeks of growth prior to treatments. There were no significant differences in NO_3^- concentrations of shoot and root after re-exposing all plants to full sunlight and full NO_3^- for another 7 days. Total shoot reduced N concentrations were similar among all plants regardless of treatments. These results indicate that reduction or withdrawing NO_3^- from nutrient solution did not affect N metabolism. Low productivity and photosynthesis under shade condition did not result from NO_3^- availability but they were directly caused by low growth irradiance. Thus, to prevent high accumulation of NO_3^- in the shoot, it may be a good practice to withdraw NO_3^- from nutrient solution during cloudy days or to extend the plant growth period a few more days under full sunlight before harvest.

Keywords: Growth irradiance, nitrate accumulation, photosynthesis, productivity.

INTRODUCTION

Vegetables that are capable of accumulating large amounts of NO_3^- include lettuce, spinach, cabbage, celery and Chinese broccoli [1]. NO_3^- is present in most food at low concentration. But green leafy vegetables contain higher concentration of NO_3^- than other foods. In general, NO_3^- is considered to be of low toxicity but when converted to NO_2^-, it interacts with haemoglobin and affects the oxygen transport, leading to a condition known as methaemoglobin [2].

Plants normally take up N from the soil in the form of NO_3^-, regardless of the form of N fertilizer applied. However, little NO_3^- accumulates in plants, when growth is normal, because the plant stems and leaves rapidly convert NO_3^- to amino acids and protein. Different environmental factors such as light intensity affect the concentration of NO_3^- in different vegetables. For instance, under low growth irradiance (cloudy days or haze), this balance can be disrupted so that the roots will take up NO_3^- faster than the plant can convert the NO_3^- to protein. NO_3^- accumulation is also dependent on the amount of N-fertilizer and time of application [2]. In countries such as those that experience the four seasons, NO_3^- levels in plants vary according to the seasons as the amount of NO_3^- accumulated in the plant tissues is closely related to the nitrate reductase (NR) activity that has been shown to be modulated by light intensity [3-5].

With regards to the NO_3^- content, the European Union established the maximum level for lettuce produced in open field as 2.5-4.0 mg g^{-1} fresh weight (FW) for the summer and winter seasons. For lettuce grown in the greenhouse, it is 3.5-4.5 mg g^{-1} FW [6]. Lettuce is the most popular amongst the salad vegetable crops. Both leafy and head types of lettuce are grown at the cool temperatures. We have successfully grown these two types of temperate lettuce in our tropical greenhouse by cooling their roots only [7-9]. He *et al*., [10] have shown that under natural tropical conditions in Singapore, aeroponically grown Chinese broccoli (*Brassica alboglabra)* subjected to low light had the highest NO_3^- accumulation in the petioles with lower accumulation in the leaves. However, there is little information on how growth irradiance and NO_3^- application influence the accumulation of NO_3^- in the shoot of lettuce grown in the tropics. This project aims to study shoot NO_3^- accumulation in lettuce by growing them under full sunlight and shade (simulating cloudy or haze weather) and they were supplied with different concentrations of NO_3^- under each growth irradiance at the later growth stage. Effects of growth irradiance and NO_3^- availability on the harvest yield and photosynthesis were used to evaluate whether manipulation of growth irradiance and NO_3^- affects the physiology and productivity.

MATERIALS AND METHODS

Plant Culture

A temperate vegetable, *L. sativa* cv. Nanda (butterhead lettuce) was used. Germinated seedlings were transplanted to

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an aeroponics system in the greenhouse. The roots of the plants were maintained at 25°C within sealed trough while aerial parts were exposed to the diurnal fluctuating ambient temperature (28°C – 40°C) under full sunlight. The PPFD of sunny days under full sunlight inside the greenhouse was about 800 µmol m^{-2} s^{-1}. The nutrient solution used to culture plants was based on full strength Netherlands Standard Composition. At full strength, the conductivity of the nutrient solution measured 2.2 mS and pH 6 ± 0.5. The composition of full strength nutrient solution in ppm 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; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.75. Six weeks after transplanting, plants were subjected to six different treatments of growth irradiance and supplied with NO_3^- described below.

Different NO_3^- Treatments under Different Growth Irradiances

Before the treatments, five plants were sampled. The shoot and root FW were recorded. The remaining plants were separated into 6 batches and were grown for further 7 days under two different levels of growth irradiances and supplied with three different concentrations of NO_3^-. The six different treatments were 1) full sunlight and full NO_3^- (188.75 ppm N), 2) full sunlight and ½ NO_3^- (94.38 ppm N), 3) full sunlight and 0 NO_3^- (0 ppm N), 4) shade (under two layers of black netting) and full NO_3^-, 5) shade and ½ NO_3^- and 6) shade and 0 NO_3^-.

The maximal PPFD were 800 µmol m^{-2} s^{-1} and 200 µmol m^{-2} s^{-1}, respectively, under full sunlight and shading on sunny days. Following the 7-day of different growth irradiance and NO_3^- availability treatments, all plants were re-exposed to another 7-day of full sunlight by removing the nettings of shade plants. Treatments with ½ NO_3^- and 0 NO_3^- were also changed to full NO_3^- concentration, that was, all plants were grown under full light and full NO_3^-.

Measurement of NO_3^-

Dried plant tissue was ground 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, WI, USA) by catalytically reducing NO_3^- to NO_2^- by passage of the sample through a copperized cadmium column. The NO_2^- was then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye had a magenta color which was read at 520 nm.

Measurement of Total Reduced N Concentration

Total reduced N content was determined by Kjeldahl digestion of dried samples in concentrated sulphuric acid [11]. The samples were dried in an oven (4 days set at 80 °C), their weights recorded then they were placed into a digestion tube with a Kjeldahl tablet and 5 ml of concentrated sulphuric acid. The mixture was then digested (about 90 minutes) until clear. After the digestion was completed, the mixture was allowed to cool for 30 minutes and the reduced N content was determined by a Kjeltec auto 1030 analyser. The reduced N content (mg g^{-1}) present in the sample was quantified through titration, and triplicate results were obtained for each treatment.

Measurement of Shoot and Root FW

After removing the entire plant from the trough during the harvesting time, the plant was separated into shoot and root respectively. The shoot and root FW were weighed separately.

Measurement of Photosynthetic CO_2 Assimilation, *A* and Stomatal Conductance, g_s

A and g_s were measured on newly expanded leaves (the 6th leaves from the base) between 1000 to 1130 h with LI-COR portable photosynthesis system (LI-6400, bio sciences, U.S.) in the greenhouse from the intact leaves. After the first 7-day of different growth irradiance and NO_3^- availability treatments, readings were taken with an LED light source which supplied 800 and 200 µmol m^{-2} s^{-1} of PPFD, which were close to the average maximal growth irradiance on the top of the leaves when plants were grown under full sunlight and shade, respectively, in the greenhouse. After re-exposing all plants to full sunlight and full NO_3^-, *A* and g_s of all plants were measured under 800 µmol m^{-2} s^{-1} of PPFD. The light source emitted in the wavelength ranging from 660 to 675 nm. Average ambient [CO_2] and relative humidity in the greenhouse were 380 ± 5 µmol mol^{-1} and 75% respectively. Leaf chamber temperature was set according to prevailing ambient conditions (28°C – 30°C).

Statistical Analysis

A two-way ANOVA was used to test for the effect of NO_3^- availability and growth irradiance on different parameters. A separate ANOVA was used to discriminate means across all treatments using Tukey's multiple comparison test. All statistical analyses were carried out using MINITAB software (MINITAB, Inc., United States, Release 15, 2007).

RESULTS

Effects of NO_3^- Availability and Growth Irradiances on Shoot and Root NO_3^- Concentration and Total Leaf Reduced N Content

NO_3^- concentrations were determined before the treatments. Their values were 12.45 ± 0.173 and 29.21 ± 0.212 mg g^{-1} DW for shoot and root, respectively. NO_3^- and total reduced N concentrations were measured again from both shoot and root after 7 days of different treatments (Fig. **1**). The interaction term "NO_3^- availability x growth irradiance" of two-way ANOVA was not significant for shoot and root NO_3^- concentration, and shoot and root total reduced N concentration, respectively (Table **1**, Fig. **1**). Separate ANOVA analysis shows that shoot NO_3^- concentration was much lower in plants under full sunlight than under shade regardless of NO_3^- availability. For plants grown under shade, they had the highest shoot NO_3^- concentration when they were supplied with full NO_3^- followed by those with ½ NO_3^- and the lowest shoot NO_3^- concentration was found in

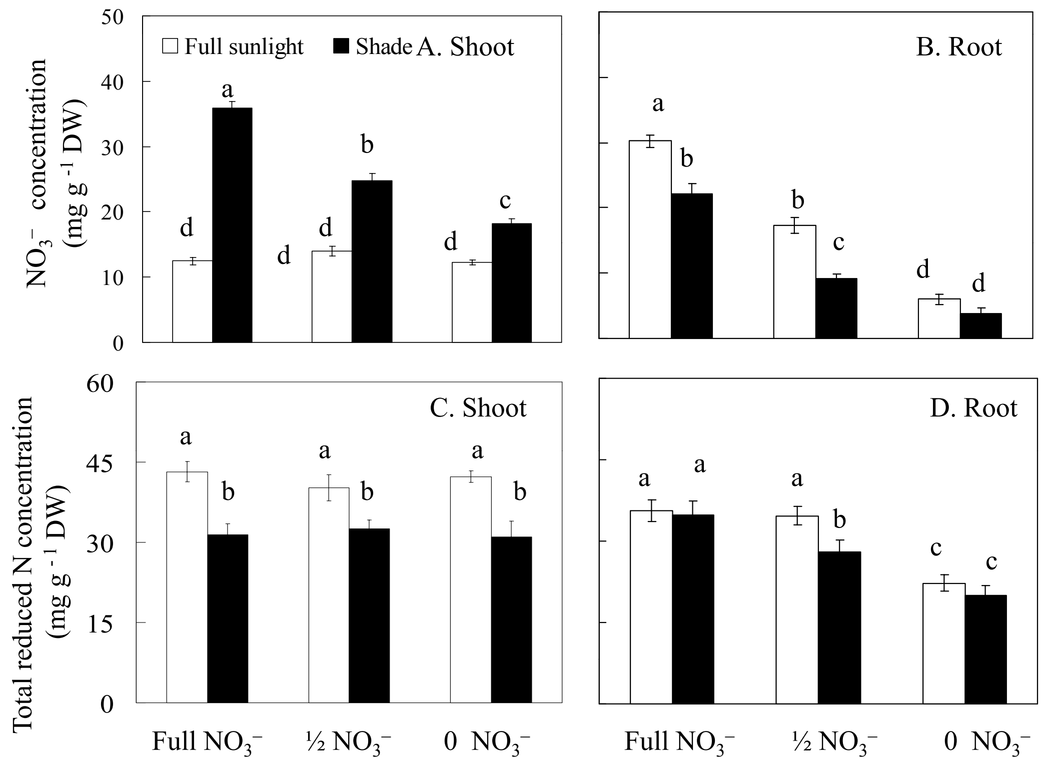

Fig. (1). NO_3^- (**A, B**) and total reduced N content (**C, D**) of *L. sativa* cv. Nanda after subjecting to two different growth irradiances and NO_3^- availabilities for 7 days. Each value is the mean of 5 different plants. Vertical bars represent the standard errors. Means with different letters above the bars are statistically different ($p < 0.001$) as determined by Tukey's multiple comparison test.

plants without NO_3^- for 7 days (Fig. **1A**, $p < 0.001$). However, there were no significant differences in shoot NO_3^- concentration when plants grown under full sunlight regardless of NO_3^- availability. Oppositely, root NO_3^- concentration was significantly higher in plants grown under full sunlight than under shade when full or ½ NO_3^- were supplied to them (Fig. **1B**, $p < 0.001$). Plants grown under both full sunlight and shade had significantly higher root NO_3^- concentration when supplied with full NO_3^- than with ½ NO_3^- and those with 0 NO_3^- (Fig. **1B**, $p < 0.001$). Total shoot reduced N concentrations were significantly higher in plants grown under full sunlight than under shade (Fig. **1C**, $p < 0.001$). However, there were no significant differences in shoot total reduced N among the different NO_3^- treatments for plants grown under both full sunlight and shade (Fig. **1C**, $p < 0.001$). Plants grown under full and ½ NO_3^- ($p > 0.05$) had similar level of root total reduced N and they were significantly higher than those of plants without NO_3^- for 7 days regardless of growth irradiances (Fig. **1D**, $p < 0.001$). After 7 days re-exposing to full sunlight and full NO_3^-, NO_3^- and total reduced N concentrations were also measured from both shoot and root (Fig. **2**). The interaction term "NO_3^- availability x growth irradiance" of two-way ANOVA for shoot and root NO_3^- concentrations, and shoot and root total reduced N concentrations were respectively, not significant (Table **1**, Fig. **2**). Separate ANOVA analysis shows that grown under full sunlight and full NO_3^-, all plants had similar levels of shoot or root NO_3^- concentrations (Figs. **2A, 2B**, $p > 0.05$), and shoot or root total reduced N concentrations (Figs. **2C, 2D**, $p > 0.05$) regardless of previous treatments.

Table 1. Two way Analysis of Variance of Physiological Variables, with P Values Presented for their Interaction

Figures	NO_3^- availability x Growth irradiance
Shoot NO_3^- (Fig. **1A**)	0.49
Root NO_3^- (Fig. **1B**)	0.32
Shoot Total Reduced N (Fig. **1C**)	0.66
Shoot Total Reduced N (Fig. **1D**)	0.45
Shoot NO_3^- (Fig. **2A**)	0.78
Root NO_3^- (Fig. **2B**)	0.45
Shoot Total Reduced N (Fig. **2C**)	0.53
Shoot Total Reduced N (Fig. **2D**)	0.72
Shoot FW (Fig. **3A**)	0.53
Root FW (Fig. **3B**)	0.47
Shoot FW (Fig. **4A**)	0.29
Root FW (Fig. **4B**)	0.68
A (Fig. **5A**)	0.51
g_s (Fig. **5B**)	0.33
A (Fig. **6A**)	0.46
g_s (Fig. **6B**)	0.27

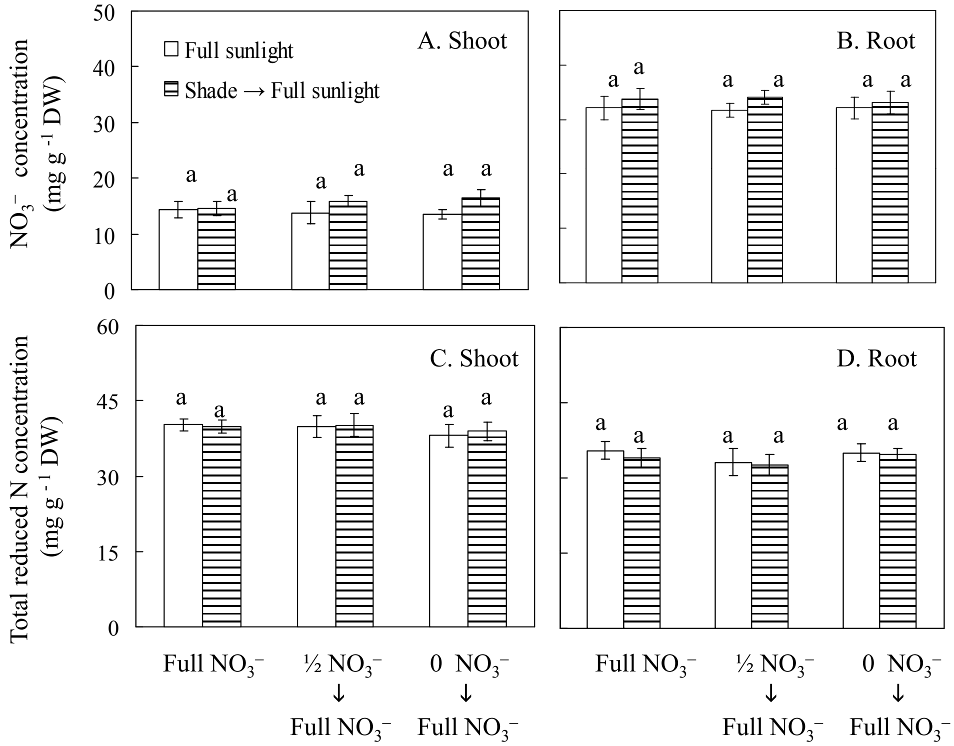

Fig. (2). NO_3^- (**A, B**) and total reduced N content (**C, D**) of *L. sativa* cv. Nanda after subjecting to two different growth irradiances and NO_3^- availabilities for 7 days and followed by re-exposure to full sunlight with full NO_3^- for another 7 days. Each value is the mean of 5 different plants. Vertical bars represent standard error. Means with different letters above the bars are statistically different ($p < 0.001$) as determined by Tukey's multiple comparison test.

Effects of NO_3^- Availability and Growth Irradiances on Productivity of Shoot and Root

Before the lettuce plants were subjected to different treatments, their FW of shoot and roots were 60.47 ± 3.65 g and 3.69 ± 0.28 g, respectively. All plants exhibited increased shoot FW (Fig. **3A**) and root FW (Fig. **3B**) after a further 7 days of growth under different condition. By the end of the treatments, the interaction term "NO_3^- availability x growth irradiance" of two-way ANOVA for shoot FW and root FW was respectively, not significant (Table **1**, Fig. **3**). Separate ANOVA analysis shows that shoot FW and root FW were significantly higher in plants under full sunlight than under shade regardless of NO_3^- availability (Figs. **3A, 3B**, $p < 0.001$). Compared to plants grown under full sunlight and full NO_3^-, shoot FW of all other plants were significant lower ($p < 0.001$) (Fig. **3A**). When grown under full sunlight with full NO_3^-, plants had the highest shoot FW followed by those with ½ NO_3^-, and the lowest shoot FW was recorded in plants grown without NO_3^- for 7 days. However, the higher root FW was found in plants grown under full sunlight with 0 NO_3^-. There were no significant differences in shoot FW or root FW among the different NO_3^- treatments when plants were grown under shade (Figs. **3A, 3B**). After re-exposing all plants to full sunlight and full NO_3^- for another 7 days, all plants continued to growth and had increased shoot FW (Fig. **4**). The interaction term "NO_3^- availability x growth irradiance" of two-way ANOVA for shoot FW and root FW was respectively, not significant (Table **1**, Fig. **4A**). Separate ANOVA analysis shows that

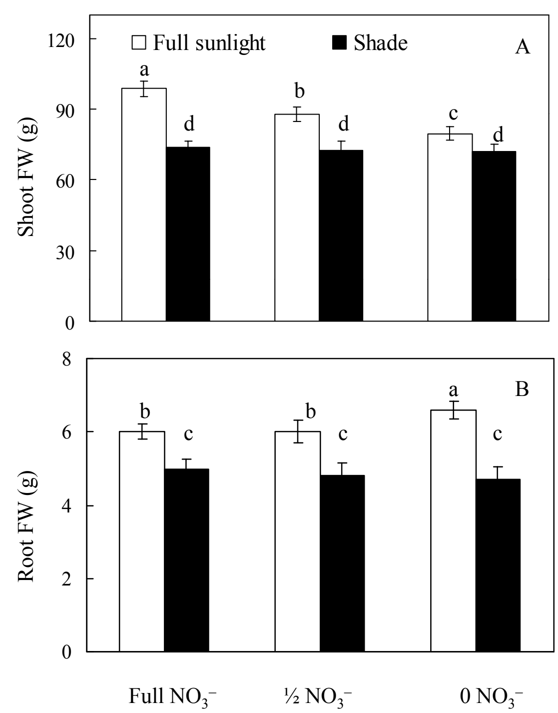

Fig. (3). Shoot FW (**A**) and root FW (**B**) of *L. sativa* cv. Nanda after subjecting to two different growth irradiances and NO_3^- availabilities for 7 days. Each value is the mean of 5 different plants. Vertical bars represent the standard errors. Means with different letters above the bars are statistically different ($p < 0.001$) as determined by Tukey's multiple comparison test.

those plants remained under full sunlight and full NO_3^- for the entire 14 days of treatment had the higher shoot FW than all other plants (Fig. **4A**, $p < 0.001$), which had no significant differences in their shoot FW regardless of their different previous treatments (Fig. **4A**, $p > 0.05$). After a further 7 days of growth under full sunlight and full NO_3^-, the average percentages of increment for shoot FW were 10% and 22% for plants grown previously under full sunlight and shade respectively. Not only shoot but also roots showed the increase in their growth after another 7 days of re-exposing to full sunlight and full NO_3^- (Fig. **4B**). Root FW was significantly higher in plants previously grown under full sunlight than in plants previously grown under shade (Fig. **4B**, $p < 0.001$). However, there was no significant difference in root FW among the plants previously treated with different NO_3^- availabilities after re-exposing to full NO_3^- for 7 day under the same growth irradiance (Fig. **4B**, $p > 0.05$). There were much lower increments in root FW for all plants compared to those shoot FW after 7 days of re-exposing to full sunlight and full NO_3^-. The average percentages of increment were 6% and 3% for plants grown under full sunlight and shade respectively.

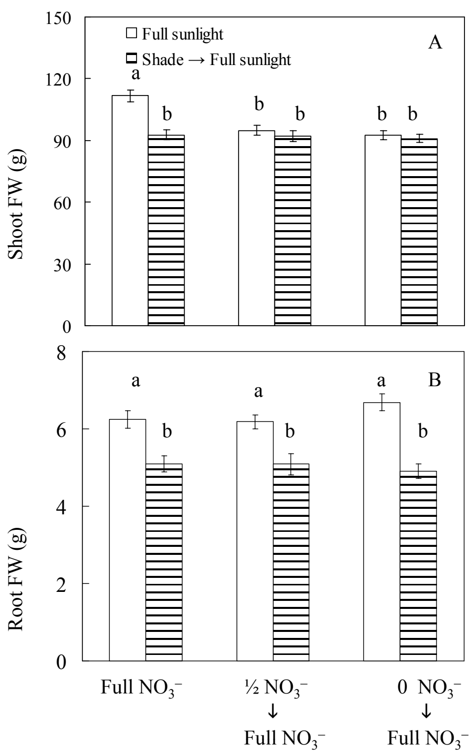

Fig. (4). Shoot FW (**A**) and root FW (**B**) of *L. sativa* cv. Nanda after subjecting to two different growth irradiances and NO_3^- availabilities for 7 days and followed by re-exposure to full sunlight with full NO_3^- for another 7 days. Each value is the mean of 5 different plants. Vertical bars represent standard error. Means with different letters above the bars are statistically different ($p < 0.001$) as determined by Tukey's multiple comparison test.

Effects of NO_3^- Availability and Growth Irradiances on *A* and g_s

A and g_s were measured under 800 and 200 µmol m^{-2} s^{-1} of PPFD, which were close to the average maximal growth irradiance on the top of the leaves when plants were grown under full sunlight and shade, respectively, in the greenhouse (Fig. **5**). After 7 days of different treatments, the interaction term "NO_3^- availability x growth irradiance" of two-way ANOVA was not significant for *A* and g_s, respectively (Table **1**, Fig. **5**). Separate ANOVA analysis shows that *A* and g_s were significantly higher in plants under full sunlight than under shade regardless of NO_3^- availability (Figs. **5A, 5B**, $p < 0.001$). When grown under full sunlight, plants supplied with full NO_3^- had the highest values of *A* and g_s followed by those with ½ NO_3^-, and the lowest values of *A* and g_s were recorded in plants with 0 NO_3^- (Figs. **5A, 5B**, $p < 0.001$). These results were similar to those of shoot FW (Fig. **3A**), indicating that both full sun light and full NO_3^- are required for achieving the maximal photosynthetic gas exchange, which was closely related to shoot productivity. Moreover, similar to shoot FW (Fig. **3A**), there were no significant differences in *A* and g_s among the different NO_3^- treatments when plants were grown under shade (Figs. **5A, 5B**, $p > 0.05$). After re-exposing all plants to full sunlight and full NO_3^- for another 7 days, *A* and g_s were measured under 800 µmol m^{-2} s^{-1} of PPFD, which were close to the average maximal growth irradiance on the top of the leaves. The interaction term "NO_3^- availability x growth irradiance" of two-way ANOVA for *A* and g_s was respectively, not significant (Table **1**, Figs. **6A, 6B**). It is surprising to see that all plants had similar levels of *A* and g_s (Figs. **6A, 6B**). Separate ANOVA analysis shows that there were no significant differences in *A* and g_s among all plants regardless of previous treatments under different NO_3^- availability and growth irradiances (Figs. **6A, 6B**, $p > 0.05$).

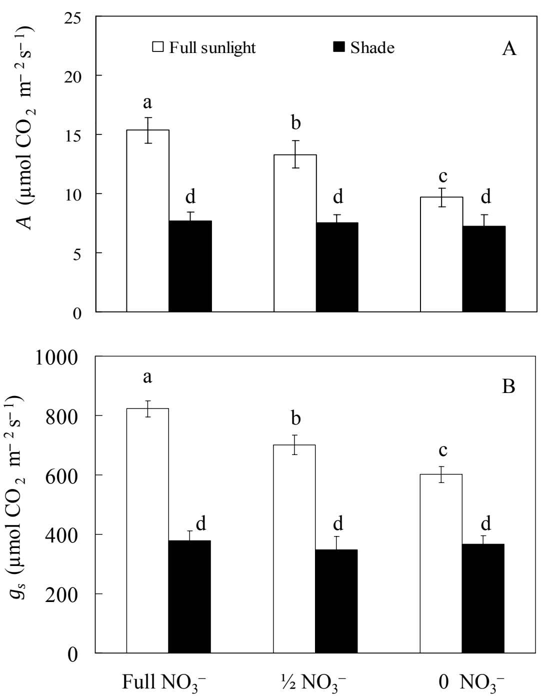

Fig. (5). *A* (**A**) and g_s (**B**) of *L. sativa* cv. Nanda after subjecting to two different growth irradiances and NO_3^- availabilities for 7 days. *A* and g_s were measured under 800 and 200 µmol m^{-2} s^{-1} of PPFD for plants were grown under full sunlight and shade, respectively, in the greenhouse. Each value is the mean of 5 different plants. Vertical bars represent the standard errors. Means with different letters above the bars are statistically different ($p < 0.001$) as determined by Tukey's multiple comparison test.

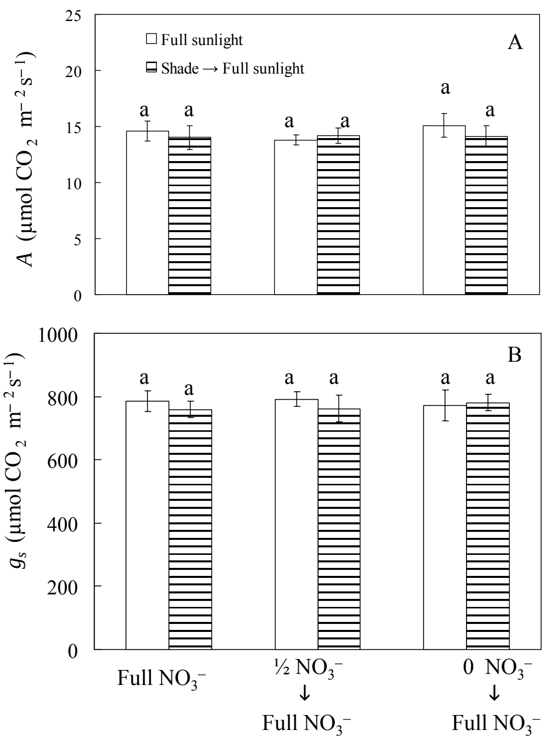

Fig. (6). *A* (**A**) and g_s (**B**) of *L. sativa* cv. Nanda after subjecting to different growth irradiances and NO_3^- availabilities for 7 days and followed by re-exposure to full sunlight with full NO_3^- for another 7 days. *A* and g_s were measured under 800 µmol m^{-2} s^{-1} of PPFD for all plants in the greenhouse each value is the mean of 5 different plants. Vertical bars represent standard error. Means with same letter above the bars are not statistically different ($p > 0.05$) as determined by Tukey's multiple comparison test.

DISCUSSION

It is well known that light influences the uptake of NO_3^- and its assimilation in plants [12-16]. In Singapore, plants are exposed to intermittent days of clear and cloudy (or haze) weather. NO_3^- concentration of leafy vegetable could be depending on growth irradiances [17, 18]. He and Lim [10] reported that when grown under low light in Singapore, aeroponically grown *B. alboglabra* (cv. Chinese broccoli) in the tropical greenhouse accumulated higher NO_3^- in their editable petioles and leaves than those of plants grown under high light. Reduced productivity, *A* and g_s were observed in low-light grown plants compared to those of plants grown under higher light. In this study, NO_3^- accumulation in both shoot and root, productivity and photosynthesis of lettuce plants were studied not only under different growth irradiances but also different levels of NO_3^- in the nutrient solution.

Aeroponics is a system used for the temperate lettuce cultivation in tropical Singapore by cooling the root-zone only, which could produce homogenous and high-quality vegetables throughout the entire year [7-9]. However, NO_3^- used in an aeroponic cultivation may lead to a high NO_3^- accumulation in leaves, especially during the cloudy or haze days. In this study, cloudy or haze weather was simulated by shading the lettuce plants for 7 days during the mature growth stage, i.e. 6 weeks after transplanting [19]. Shading caused higher NO_3^- accumulation in the shoot compared to plants grown under full sunlight. The higher shoot NO_3^- concentrations of shade plants were derived from the high NO_3^- accumulated in their roots during the 6 weeks of growth prior to treatments. NO_3^- uptake and transport are affected by growth irradiance [17,18]. Zhao and Oosterhuis [17] studied the effect of shade (63% light reduction) on the mineral nutrient status of *Gossypium hirsutum* plants. They found that an 8-day period of shade increased petiole NO_3^- by 145%. Similarly, in the present study, low light did not inhibit the uptake and transport of NO_3^-. Instead, it enhanced NO_3^- transport from root to shoot. The increases in shoot NO_3^- concentration under shade condition may result from several causes, namely, limitation by the availability of reducing power [20,21] or inactivation of **NR** [22-25]. However, it is interesting to note that plants grown under full sunlight had similar levels of low shoot NO_3^- concentration regardless of NO_3^- availability. All plants continued to grow during the 7 days of different treatments. Both full sunlight and full NO_3^- are required for achieving maximal shoot productivity. These results well agree with that in soilless culture, NO_3^- is required in the highest amounts by vegetable plants. When grown under full sunlight, NO_3^- deficiency limits growth and yield of vegetable crops [26,27]. Reducing or removing NO_3^- source from the nutrient solution under full sunlight may promote root growth and thus decreasing shoot FW as plants may spend more energy to uptake NO_3^- from the nutrient solution or to transport NO_3^- that accumulated excessively in the roots to the shoot [27]. However, NO_3^- availability had very little impact on shoot and root FW when grown under shade. Since removing NO_3^- from the nutrient solution after shading the plants did not cause further decreases in shoot FW, to avoid high accumulation of NO_3^- in the shoot, it would be a great benefit for both grower and consumer if NO_3^- would be totally withdrawn during the cloudy or haze days.

NO_3^- incorporation into biological molecules such as organic N compounds measured as total reduced N content in the present study involves the reduction of NO_3^- to NO_2^- mainly via **NR** [23-25]. Total reduced N concentration in vegetables depends on the light intensity [16, 28] as the regulation of NR is closely coupled to photosynthesis. In the present study, lower *A* and g_s of shade plants were mainly due to lower growth irradiance rather than the total reduced N as all plants have similar levels of shoot total reduced N. According to Solomonson and Barber [29], as much as 25% of photosynthetic energy was consumed by the NO_3^- assimilation pathway. Total reduced N content determines the synthesis of amino acids and therefore of proteins and, ultimately, of all cellular components. However, after shading the plants, reducing or withdrawing NO_3^- from nutrient solution had no impact on shoot and root total reduced N content, productivity and photosynthesis. These results indicate that low productivity and photosynthesis under shade condition for 7 days did not result from NO_3^- availability and NO_3^- assimilation (thus the total reduced N) but they were directly caused by low growth irradiance.

This study also simulated sunny weather following cloudy days by re-exposing all plants to full sunlight as well as full NO_3^-. All plants had similar low levels of shoot NO_3^- but higher level of total reduced N concentrations of shoot and root after 7 days of such treatments. These results further supported that high light stimulated NR activity and NO_3^- assimilation of shoot [22-25], which is closely related to its higher photosynthetic rate as all plants had similar *A* and g_s when measured under 800 µmol m^{-2} s^{-1}. It is interesting to see that increases in shoot FW were greater in plants previously grown under the shade than those which were remained under full sunlight after 7 days of full sunlight and full NO_3^- treatments. The higher shoot FW was due to higher biomass that was partitioned into the shoot rather than roots as the increment of root FW after exposing to full sunlight and full NO_3^- was much lower than that of shoot. In our previous studies, it was found that under favorable condition, the growth of lettuce aerial part was increased more than the growth of root, which leads to an increase in the shoot/root ratio [30]. This finding suggests that light is primary factor in partitioning the new assimilates that provide optimal proportions of shoot and root as plant production is driven by photosynthesis [31].

After 7 days of shading with full NO_3^-, shoot had the highest NO_3^- concentration about 39.25 mg g^{-1} DW or 0.36 mg g^{-1} FW. The acceptable level of NO_3^- for fresh lettuce was 0.791 to 1.017 mg g^{-1} FW in Germany and 0.7 mg g^{-1} FW in China [32]. However, after re-exposing the shaded lettuce plants to full sunlight and full NO_3^- for 7 days, the highest shoot NO_3^- concentration declined to about 12.12 mg g^{-1} DW or 0.11 mg g^{-1} FW. Although those plants were previously subjected to shade did not gain the productivity as high as those plants which were grown under full sunlight for the whole life cycle, however, they showed substantial and faster growth after re-exposing them to full sunlight for 7 days following shading. As mentioned earlier, totally withdrawing NO_3^- from the nutrient solution is a good practice to maintain low shoot NO_3^- concentration during the cloudy or haze weather. Based on the above calculation, the other good practice is not to harvest the lettuce vegetables immediately after cloudy or haze periods but to expose them for a few more days of full sunlight prior to harvest. This practice will also benefit both the lettuce grower and the consumer as there was more rapid shoot growth with low shoot NO_3^- concentration for those previously shaded plants after re-exposing to full sunlight and full NO_3^- for a few days.

CONCLUSION

Short-term shading the lettuce plants resulted in the highest level of shoot NO_3^- accumulation when supplied with full NO_3^-. Reduction of productivity and photosynthesis after shading the plants was caused by low light not NO_3^- availability. After re-exposing shaded plants to full sunlight and full NO_3^-, shoot NO_3^- concentration decreased to similar low level of those plants grown under full sunlight. Shaded plants showed substantial and faster growth after re-exposing them to full sunlight and full NO_3^- following shading. To avoid high NO_3^- accumulation in the editable shoot and at the same time benefiting the growers, recommendations include, 1) not to harvest the vegetable during or immediately after cloudy or haze weather, 2) totally removing NO_3^- from the nutrient solution during cloud or haze weather and then extend the growth period for another few more days under full sunlight supplied with NO_3^- before harvest.

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CONFLICT OF INTEREST

None declared.

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