Effects of elevated root zone CO2 and air temperature on photosynthetic gas exchange, nitrate uptake, and total reduced nitrogen content in aeroponically grown lettuce plants

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Abstract
Effects of elevated root zone (RZ) CO2 and air temperature on photosynthesis, productivity, nitrate (NO3–), and total reduced nitrogen (N) content in aeroponically grown lettuce plants were studied. Three weeks after transplanting, four different RZ [CO2] concentrations [ambient (360 ppm) and elevated concentrations of 2000, 10 000, and 50 000 ppm] were imposed on plants grown at two air temperature regimes of 28 °C/22 °C (day/night) and 36 °C/30 °C. Photosynthetic CO2 assimilation (A) and stomatal conductance (gS) increased with increasing photosynthetically active radiation (PAR). When grown at 28 °C/22 °C, all plants accumulated more biomass than at 36 °C/30 °C. When measured under a PAR > 600 μmol m−2 s−1, elevated RZ [CO2] resulted in significantly higher A, lower gS, and higher midday leaf relative water content in all plants. Under elevated RZ [CO2], the increase of biomass was greater in roots than in shoots, causing a lower shoot/root ratio. The percentage increase in growth under elevated RZ [CO2] was greater at 36 °C/30 °C although the total biomass was higher at 28 °C/22 °C. NO3– and total reduced N concentrations of shoot and root were significantly higher in all plants under elevated RZ [CO2] than under ambient RZ [CO2] of 360 ppm at both temperature regimes. At each RZ [CO2], NO3– and total reduced N concentration of shoots were greater at 28 °C/22 °C than at 36 °C/30 °C. At all RZ [CO2], roots of plants at 36 °C/30 °C had significantly higher NO3– and total reduced N concentrations than at 28 °C/22 °C. Since increased RZ [CO2] caused partial stomatal closure, maximal A and maximal gS were negatively correlated, with a unique relationship for each air temperature. However, across all RZ [CO2] and temperature treatments, there was a close correlation between maximal A and total shoot reduced N concentration of plants under different RZ [CO2], indicating that increased A under elevated RZ [CO2] could partially be due to the higher shoot total reduced N.

Key words: Elevated root zone [CO2], Lactuca sativa, NO3 uptake, photosynthesis, temperature, total reduced N.

Introduction
When grown in soil, plant roots normally are colonized by microorganisms and evolve more CO2 than sterile roots. It is not unusual for CO2 concentrations in the rhizosphere to be up to >10-fold higher than atmospheric CO2 concentrations (De Jong and Schappert, 1972; Norstadt and Porter, 1984). Below-ground CO2 efflux can be due to root and microbial respiration, and decomposition of the various carbon pools, including roots and litter (Pendall et al., 2004). Cramer and Richards (1999) reported that 14C taken up by tomato roots could be translocated to the shoots in...
an inorganic form. Teskey and McGuire (2002) have shown that dissolved CO$_2$ in the xylem sap could be carried upward in the stem when trees are transpiring, suggesting that the CO$_2$ in xylem sap is a mixture of CO$_2$ released by respiration of local tissues and CO$_2$ transported from soil, roots, and the lower portion of the stems. A direct correlation between CO$_2$ efflux from stems into the atmosphere and the CO$_2$ concentration in the xylem sap was observed, indicating that a portion of the dissolved CO$_2$ in xylem sap diffuses outward to the atmosphere (Teskey and McGuire, 2005). Hibberd and Quick (2002) provided $^{14}$C-labelled carbon to roots and xylem of two herbaceous species and subsequently found the assimilated label throughout the plants. Based on these findings, it was questioned whether the low CO$_2$ level in the RZ of aeroponically grown plants could regulate plant growth. By studying the responses of plant growth and photosynthesis to elevated RZ [CO$_2$], this not only contributes to the scientific literature but also helps growers to improve the production of aeroponically grown vegetable crops.

Elevated RZ [CO$_2$] has been reported to enhance plant growth (Viktor and Cramer, 2003; He et al., 2004, 2007; Cramer et al., 2005) although negative consequences for plant growth resulting from elevated RZ [CO$_2$] have also been reported (Cramer, 2002), especially in combination with O$_2$ deficiency (Boru et al., 2003). By using aeroponically grown lettuce plants in the greenhouse, previous results indicated that elevated RZ [CO$_2$] could alleviate midday depression of photosynthesis and hence increase leaf area, shoot, and root productivities (He et al., 2007). Recently, McGuire et al. (2009) confirmed that CO$_2$ transported in xylem sap can be fixed in photosynthetic cells in the leaves and branches of sycamore (Platanus occidentalis L.). The effect of elevated RZ [CO$_2$] on plant growth depended on a wide range of circumstances such as species considered, mineral nutrition, abiotic stress, the CO$_2$ concentration used (Cramer and Lips, 1995; Cramer and Richards, 1999; Cramer and Titus, 2001; Viktor and Cramer, 2003), and the RZ [O$_3$] (Boru et al., 2003; Cramer et al., 2005). Enriching the rhizosphere dissolved inorganic carbon (DIC) concentration increased the biomass of both control and salinized (50 mM NaCl) tomato (Solanum lycopersicum; formerly Lycopersicon esculentum) plants grown at high air temperature (daily maximum of 37 °C) and an irradiance of 1500 μmol m$^{-2}$ s$^{-1}$ (Cramer and Richards, 1999). When these plants were grown at irradiances <1000 μmol m$^{-2}$ s$^{-1}$, elevated rhizosphere DIC increased growth rates only of control plants grown at high shoot temperature (35 °C) or salinized plants at more moderate temperature (28 °C). In another study with tomato seedlings supplied with both NO$_3$ and NH$_4$$_3$, there was a significant increase in biomass accumulation in response to increased RZ [CO$_2$] (Viktor and Cramer, 2003). Cramer et al. (1993) suggested that DIC incorporation improved incorporation of N into amino acids in the roots as a consequence of greater supplies of anaplerotic carbon. The anaplerotic pathway makes use of inorganic carbon to build C$_4$ compounds, leading to amino acid and protein synthesis (Jeanneau et al., 2002; Giordano et al., 2003).

As the main organ involved in water and mineral uptake, roots may play an important role in adjusting nutrient uptake (especially N acquisition) to meet shoot demands caused by environmental changes (BassiriRad, 2000). Since >50% of leaf N is associated with the photosynthetic machinery (Evans, 1989), close relationships have been found for different species between photosynthetic capacity and leaf N content (Reich et al., 1994). Previously, the effects of elevated RZ [CO$_2$] on aeroponically grown lettuce were studied at two different air temperature regimes (28 °C/22 °C and 36 °C/30 °C day/night) in two controlled environment (CE) rooms. Elevated RZ [CO$_2$] stimulated photosynthetic CO$_2$ assimilation ($A$), with a greater increase at higher temperatures but decreased stomatal conductance ($g_s$) at both temperatures, reducing transpirational water loss (He et al., 2004). However, relationships between RZ [CO$_2$], root growth, N acquisition, and photosynthesis in aeroponically grown lettuce are poorly understood. To our knowledge, there is almost no information on the effects of interactions between elevated RZ [CO$_2$] and temperature on NO$_3$ uptake and assimilation rates and the relationship between N metabolism and photosynthesis under elevated RZ [CO$_2$]. It was hypothesized that the positive effects of elevated RZ [CO$_2$] on photosynthetic CO$_2$ assimilation might partly be due to increased NO$_3$ uptake and NO$_3$ assimilation rate and that these increases are strongly affected by growth temperature. To support this hypothesis, in the present study, the experimental design using aeroponically grown lettuce plants enables the investigation of the effects of elevated RZ [CO$_2$] and temperature not only on $A$ and $g_s$, measured under different PAR but also the plant growth especially the root growth associated with the uptake and accumulation of NO$_3$. The total product of N metabolism (i.e. the total reduced N) was also determined.

Materials and methods

Plant materials and cultural methods

Crisphead-type lettuce (Lactuca sativa L. cv. ‘Wintergreen’, South Pacific Seeds Ltd, New Zealand) were germinated on moist paper, and established on Grodan$^\text{®}$ rockwool in a CE room [28 °C/22 °C day/night, photosynthetically active radiation (PAR) $\sim$200 μmol m$^{-2}$ s$^{-1}$] at the New Zealand Controlled Environment Laboratory. Seedlings were then transplanted to 68-litre opaque plastic aeroponic bins (Stowers Ltd, New Zealand) in two CE rooms. Each bin lid held 24 plants, with roots initially contained in polyurethane cubes with sides of 19 mm. Each bin was supplied with temperature-controlled nutrient solution from a separate reservoir. Microsprinklers intermittently misted roots with recirculated full-strength Hoagland’s solution, at a frequency of one spray for 40 s per min. Solution pH was maintained at $\sim$6.5 and a conductivity of 2 mS. Air temperature was controlled in two CE rooms at 28 °C/22 °C and 36 °C/30 °C (day/night, ±0.5 °C) with relative humidity of 70%/65% (day/night, ±5%) in both. A 12 h photoperiod was provided by 1 kW high intensity discharge and tungsten halogen lamps, with a constant PAR of 650 μmol m$^{-2}$ s$^{-1}$ from 07:00 to 19:00 h. Fresh air was continuously circulated through the CE room with an average ambient [CO$_2$] of
360 ± 5 μmol mol⁻¹. Three weeks after transplanting, in each CE room, four different RZ [CO₂]: ~360 (ambient), 2000, 10 000, and 50 000 ppm were supplied to separate bins. In each CE room, there were two bins for each RZ [CO₂] treatment. Elevated RZ [CO₂] of 2000, 10 000, and 50 000 ppm was controlled, respectively, using pre-mixed CO₂–air mixtures (BOC Ltd, Auckland New Zealand), supplied from compressed air cylinders at ~0.5 l min⁻¹, with separate venting to the CE room exhaust air duct. [CO₂] at the shoot base was measured with a LI-COR 6400 photosynthesis system (LI-6400, Biosciences, Lincoln, NE, USA). No significant increase in atmospheric [CO₂] was found, indicating that the bin lids were gas-tight.

**Measurements of light response curves of A and gₛ in the CE rooms**

Two weeks after different elevated RZ [CO₂] treatments, light response curves of A and gₛ of the newly expanded leaves (the sixth leaves from the base) were measured between 08:00 h (1 h after exposure to light) to 11:00 h with an open infrared gas analysis system with a 6 cm² chamber (LI-COR) in the CE rooms using intact plants. Readings were taken with a light-emitting diode (LED) light source which supplied 300, 600, 900, and 1200 μmol m⁻² s⁻¹ of PAR. The light source emitted in the wavelength range 660–675 nm. Although a light source was used, a constant PAR of 650 μmol m⁻² s⁻¹ in the two CE rooms was maintained to minimize the time required for the leaves to adjust to the LED light source. Average ambient [CO₂] and relative humidity in the chamber were 360 ± 5 μmol mol⁻¹ and 70%, respectively. Leaf chamber temperature was set according to prevailing ambient conditions (28 °C or 36 °C). Photosynthesis measurements were recorded when both A and gₛ were stable (usually within 3–5 min) and then the PAR was increased to the next level. Due to the large number of samples, measurements were made on two different consecutive days in the two different CE rooms. For each treatment, four measurements were made from four different leaves. These measurements were repeated three times on the same leaves (total measurement period of 6 d).

**Measurements of leaf relative water content (RWC)**

RWC of the leaves was determined using leaf discs as (FW–DW)/(TW–DW)=100, where FW is the fresh weight, DW is the dry weight, and TW is the turgid weight of the leaf after floating on distilled water for 24 h.

**Measurement of FW and DW of shoot and root**

After plant removal from the bins (09:00 to 10:00 h), the polyurethane cube was carefully removed from individual root systems, and the plants were separated into shoot and root tissues. The shoots and roots were blotted carefully and weighed separately. All tissues were wrapped in aluminum foil, dried at 80 °C for 4 d, then reweighed.

**Determination of NO₃**

Dried plant tissue (0.03 g) was ground using a pestle and mortar with deionized water and then incubated at 37 °C for 2 h. Sample turbidity was removed by filtration through a 0.45 μm pore diameter membrane filter prior to analysis. The NO₃ was determined using a Flow Injection Analyser (Model QuikChem 8000, Lachat Instruments Inc., Milwaukee, WI, USA) by catalytically reducing NO₃ to NO₂ by passage of the sample through a copperized cadmium column. The NO₂ was then determined by diazotizing with sulphanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water-soluble dye had a magenta colour which was read at 520 nm.

**Determination of total reduced nitrogen (N)**

Total reduced N content was determined by Kjeldahl digestion of dried samples in concentrated sulphuric acid. The samples were dried in an oven (4 d set at 80 °C) and their weights were 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 (~90 min) until clear. After the digestion was completed, the mixture was allowed to cool for 30 min and the reduced N content was determined by a Kjeltac 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.

**Statistical analysis**

For light response curves of A and gₛ (Fig. 1) and changes in shoot and root DW and shoot/root DW ratio over a period of 3 weeks (Fig. 4), at each temperature regime, one-way analysis of variance (ANOVA) was used to test for significant differences between different RZ [CO₂], with means discriminated using Tukey’s multiple comparison test when main effect ANOVA tests were significant. For Figs 1 and 4, under each RZ [CO₂], a t-test was also used to test for differences of two air temperature regimes under the same RZ [CO₂]. For RWCC (Fig. 2), FW and DW of shoot and root and shoot/root ratio (Fig. 3), and NO₃ and total reduced N concentration of shoot and root (Fig. 5), after 3 weeks of different RZ [CO₂], treatments, a two-way ANOVA was used to test for the effect of RZ [CO₂] and air temperature on the these parameters (Table 1). A separate ANOVA was then used to discriminate means across all treatments. All statistical analyses were carried out using Minitab software (Minitab Inc., Release 15, 2007). Linear regressions between maximal A and either maximal gₛ or total reduced N concentration of the shoot were calculated with Microsoft Office Excel (2007).

**Results**

**Light response curve of A and gₛ**

After 2 weeks of different elevated RZ [CO₂] treatments, light response curves of A and gₛ were determined in attached leaves. Data obtained from the first 2 d are shown in Fig. 1. Similar data were observed over the next four consecutive days (data not shown). At 28 °C/22 °C, under a low PAR of 300 μmol m⁻² s⁻¹, no significant differences in A (F₃, 12=2.92, P >0.05) were observed among different RZ [CO₂] treatments. Instantaneous increases of A were observed when PAR was increased from 300 μmol m⁻² s⁻¹ to 1200 μmol m⁻² s⁻¹ (Fig. 1A). For instance, under the highest PAR of 1200 μmol m⁻² s⁻¹, A was significantly higher at each of the three different elevated RZ [CO₂] compared with those of plants grown at ambient RZ [CO₂] (Fig. 1A). A was similar at RZ [CO₂] of 10 000 ppm and 50 000 ppm, but it was much higher than ambient and RZ [CO₂] of 2000 ppm. (F₃, 12=60.95, P <0.05, 300 ppm <2000 ppm <10 000 ppm ≈50 000 ppm). The response of A to different RZ [CO₂] for plants grown at 36 ºC/30 ºC (Fig. 1B) was similar to that of plants grown at 28 ºC/ 22 ºC. However, compared with those plants grown at 28 ºC/22 ºC, under the same PAR, A was much lower in plants grown at 36 ºC/30 ºC (Fig. 1B) (P <0.05). Similar to A, under a low PAR of 300 μmol m⁻² s⁻¹, there were no significant differences in gₛ among different
RZ [CO\textsubscript{2}] treatments at both 28 °C/22 °C (Fig. 1C, $F_{3, 12}$=2.72, $P$ >0.05) and 36 °C/30 °C (Fig. 1D, $F_{3, 12}$=3.37, $P$ >0.05). Increases of $g_s$ were observed when PAR was increased from 300 µmol m\textsuperscript{-2} s\textsuperscript{-1} to 1200 µmol m\textsuperscript{-2} s\textsuperscript{-1} (Fig. 1C). However, $g_s$ was significantly lower at each of the three different elevated RZ [CO\textsubscript{2}] compared with that of plants grown at ambient RZ [CO\textsubscript{2}] at higher PARs at both temperature regimes (Figs. 1C, D). For example, under the highest PAR of 1200 µmol m\textsuperscript{-2} s\textsuperscript{-1}, $g_s$ values were significantly lower at elevated RZ [CO\textsubscript{2}] than at ambient RZ [CO\textsubscript{2}] at both 28 °C/22 °C (Fig. 1C, $F_{3, 12}$=54.08, $P$ <0.05) and 36 °C/30 °C (Fig. 1D, $F_{3, 12}$=53.71, $P$ <0.05). Tukey’s test showed that at both temperature regimes, $g_s$ was significantly lower in all elevated RZ [CO\textsubscript{2}] plants than in ambient RZ [CO\textsubscript{2}] plants, but $g_s$ values of 10 000 ppm and 50 000 ppm RZ [CO\textsubscript{2}] plants did not differ significantly (300 ppm >2000 ppm >10 000 ppm ≈ 50 000 ppm). At 36 °C/30 °C, although $g_s$ appeared to increase with increasing PAR, the magnitudes of these increases were significantly less than at 28 °C/22 °C ($P$ <0.05). It was interesting to note that at 300 µmol m\textsuperscript{-2} s\textsuperscript{-1}, the $g_s$ values of plants grown at 36 °C/30 °C (Fig. 1D) under all RZ [CO\textsubscript{2}] were significantly higher than at 28 °C/ 22 °C (Fig. 1C) ($P$ <0.05).

**RWC**

Leaves used for $A$ and $g_s$ measurements were harvested at 12:00 h on the same day for midday leaf RWC determination. The interaction term ‘RZ [CO\textsubscript{2}]×temperature’ of two-way ANOVA for midday leaf RWC was significant (Table 1, $P$ <0.001), indicating that RWC response to elevated RZ [CO\textsubscript{2}] was more sensitive at higher air temperatures. Midday leaf RWC was significantly higher in plants grown under all elevated RZ [CO\textsubscript{2}] than under ambient RZ [CO\textsubscript{2}] at both temperature regimes (Table 1, Fig. 2, $P$ <0.001). However, plants grown at 36 °C/30 °C had much lower midday leaf RWC than those grown at 28 °C/22 °C at each RZ [CO\textsubscript{2}] ($P$ <0.001). Midday leaf RWC was the lowest in plants grown under ambient RZ [CO\textsubscript{2}] of 360 ppm at 36 °C/30 °C and the highest in plants grown under elevated RZ [CO\textsubscript{2}] of 10 000 ppm and 50 000 ppm at 28 °C/22 °C (Fig. 2).

**Productivity of shoot and root**

Elevated RZ [CO\textsubscript{2}] treatments were begun 3 weeks after transplanting when lettuce plants were growing exponentially (He et al., 2009). By the end of the experiment, the interaction term ‘RZ [CO\textsubscript{2}]×temperature’ of two-way ANOVA for FW and DW of shoot and root, and shoot/root FW and DW ratios was not significant (Table 1). Separate ANOVAs show that FW and DW of shoot and root were significantly higher in plants at all elevated RZ [CO\textsubscript{2}] than at ambient RZ [CO\textsubscript{2}] at both temperature regimes (Figs. 3A–D, $P$ <0.001). Compared with those plants grown at ambient RZ [CO\textsubscript{2}], shoot FW of plants which were exposed to elevated RZ [CO\textsubscript{2}] showed 15–31% and 30–55% increases, respectively, at 28 °C/22 °C and 36 °C/30 °C. There were no significant differences in shoot FW and DW when roots were exposed to elevated [CO\textsubscript{2}] of 10 000 ppm and 50 000 ppm, but they were much higher than those of plants exposed to elevated RZ [CO\textsubscript{2}] of...
2200 ppm (Fig. 3A, B). However, root FW and DW of plants grown at elevated RZ [CO2] of 50 000 ppm were significantly greater than those at 10 000 ppm (Fig. 3C, D, P <0.001). Three weeks after elevated RZ [CO2] treatments, a greater biomass increase was found in roots than in shoots. For instance, shoot and root FW at 50 000 ppm RZ [CO2] were ~1.6-fold and 1.8-fold higher than at ambient RZ [CO2]. For the same plants, the DW of shoot and roots were, respectively, 1.8-fold and 2.5-fold higher than that of ambient RZ [CO2] plants. Higher FW of roots resulted in a significantly lower shoot/root ratio at elevated RZ [CO2] of 10 000 ppm and 50 000 ppm compared with that of ambient and 2000 ppm RZ [CO2] (Fig. 3E, P <0.001). However, higher root DW caused significantly lower shoot/root ratios at all elevated RZ [CO2] than at ambient RZ [CO2] (Fig. 3F, P <0.001).

As there were significant differences in shoot/root DW ratio among different RZ [CO2] (Fig. 3F), changes of shoot and root DW and shoot/root DW ratio were analysed over the 3 weeks of different RZ [CO2] treatments. Two days after elevated RZ [CO2] treatments, no significant differences in shoot and root DW were observed among different RZ [CO2] treatments at both temperature regimes (P >0.05). Increases of shoot and root DW were greater in all elevated RZ [CO2] plants than in ambient RZ [CO2] plants at both temperature regimes from day 4 of elevated RZ [CO2] treatment (Fig. 4A–D). For instance, 21 d after different RZ [CO2] treatments, shoot DW was significantly higher in all elevated RZ [CO2] plants than in ambient RZ [CO2] plants at both 28 °C/22 °C (Fig. 4A, F3, 16=96.72, P <0.05) and 36 °C/30 °C (Fig. 4B, F3, 16=111.62, P <0.05).

However, shoot DW of 10 000 ppm and 50 000 ppm RZ [CO2] plants did not differ significantly (300 ppm >2000 ppm >10 000 ppm ≈50 000 ppm). From 4 d after treatment, however, root DW was significantly greater in plants grown under RZ [CO2] of 10 000 ppm than of 50 000 ppm [CO2]. By day 21, plants grown under RZ [CO2] of 50 000 ppm had the highest shoot/root DW ratio among different RZ [CO2] at both temperature regimes (Fig. 4C, at 28 °C/22 °C, F3, 16=370.65, P <0.05; Fig. 4D, at 36 °C/30 °C, F3, 16=304.70, P <0.05; 300 ppm <2000 ppm <10 000 ppm <50 000 ppm). Under the same PAR, all plants had significantly higher shoot DW at 28 °C/22 °C that at 36 °C/30 °C (Fig. 4A–D, P <0.05).

For plants grown at 28 °C/22 °C under ambient RZ [CO2], the shoot/root DW ratio gradually increased over 3 weeks (Fig. 4E) while this ratio was constant for plants grown at 36 °C/30 °C under the same RZ [CO2] (Fig. 4F). The pattern of shoot/root DW ratio in response to elevated RZ [CO2] was similar under both temperature regimes. Shoot/root DW ratio of elevated RZ [CO2] plants first decreased and then increased during the 3 weeks of treatments. The initial decreases in shoot/root DW ratio as RZ [CO2] increased were much greater at 36 °C/30 °C than at 28 °C/22 °C, indicating that higher temperature enhanced the effects of elevated RZ [CO2] in partitioning more biomass to the roots. For example, 2 d after elevated [CO2] treatment, at 28 °C/22 °C plants grown under 50 000 ppm [CO2] had a significantly lower shoot/root DW ratio than under other RZ [CO2] (Fig. 4C, F3, 16=13.48, P <0.05, 300 ppm ≈2000 ppm >10 000 ppm >50 000 ppm). However, at 36 °C/30 °C, significant differences in shoot/root DW ratio were observed among different RZ [CO2] plants 2 d after treatments. Plants grown under RZ [CO2] of 300 ppm had the highest while those grown under RZ [CO2] of 50 000 ppm had the lowest shoot/root DW ratio (Fig. 4F, F3, 16=67.38, P <0.05, 300 ppm >2000 ppm >10 000 ppm >50 000 ppm). From day 8 after treatments, the shoot/root DW ratio gradually increased in all elevated RZ [CO2] treatments at both temperature regimes. By day 21, plants grown under RZ [CO2] of 50 000 ppm had the lowest shoot/root DW ratio among different RZ [CO2] (Fig. 4E, at 28 °C/22 °C, F3, 16=120.87, P <0.05; Fig. 4F, at 36 °C/30 °C,
NO₃⁻ uptake and total reduced N

Shoot and root NO₃⁻ and total reduced N concentrations were determined in the same plants used for measurements of plant productivity (Fig. 3). The interaction term ‘RZ [CO₂]×temperature’ of two-way ANOVA for NO₃⁻ and total reduced N concentration of shoot and root was not significant (Table 1). Shoot NO₃⁻ and total reduced N concentrations were significantly higher at all elevated RZ [CO₂] than in ambient RZ [CO₂] at both temperature regimes (Fig. 5A, B) \((P<0.001)\). There were no significant differences in NO₃⁻ and total reduced N concentrations of shoot when lettuce plants were exposed to elevated [CO₂] of 10 000 ppm and 50 000 ppm but they were significantly greater than those plants exposed to elevated RZ [CO₂] of 2000 ppm. At each RZ [CO₂], plants had significantly higher shoot NO₃⁻ and total reduced N concentrations at 28 °C/22 °C than at 36 °C/30 °C \((P<0.001)\). For roots, the differences in NO₃⁻ and total reduced N concentrations among the plants under different levels of RZ [CO₂] were similar to those of shoots at both temperature regimes. However, at each RZ [CO₂], plants grown at 36 °C/30 °C had significantly higher root NO₃⁻ and total reduced N concentrations than plants grown at 28 °C/22 °C (Fig. 5C, D, \(P<0.001\)).

Across all treatments, there was no correlation between maximal \(A\) and maximal \(g_s\) (Fig. 6A, \(P=0.14\)), but these variables were negatively correlated within each air temperature regime. However, a close correlation between maximal \(A\) and total reduced N concentration of the shoot was established in plants grown under different RZ [CO₂] and temperature treatments (Fig. 6B, \(P<0.001\)).
Discussion

There were substantial differences in \( A \) and \( g_s \) under a PAR \( \geq 600 \, \mu\text{mol m}^{-2} \text{s}^{-1} \) after the roots of aeroponically grown lettuce plants were exposed to different elevated [CO\(_2\)]. \( A \) increased with increasing RZ [CO\(_2\)] (Fig. 1) with an RZ [CO\(_2\)] of 10,000 ppm sufficient for maximizing productivity (Fig. 3). However, \( g_s \) was significantly lower at higher RZ [CO\(_2\)] than at ambient RZ [CO\(_2\)] (Fig. 1B, D) and, therefore, the enhancement of \( A \) under elevated RZ [CO\(_2\)] was not due to an increase in \( g_s \). Obviously, there was more internal CO\(_2\) available to plants grown under elevated RZ [CO\(_2\)] (He et al., 2004, 2007) as dissolved CO\(_2\) in the xylem sap could be carried upward in the stem when plants were transpiring (Teskey and McGuire, 2002, 2005). The xylem-transported CO\(_2\) could be fixed in green tissues (Hibberd and Quick 2002; McGuire et al., 2009).

When stomata are partially closed, leaf transpiration (E) decreases, thus plants become more water use efficient (WUE) if \( A \) is not decreased more than E. Growth of sunflower (Helianthus annuus) plants in elevated atmospheric [CO\(_2\)] decreased \( g_s \) and thereby transpiration, but increased net \( A \) and, therefore, WUE increased by 230% (control plants) and 380% (plants with severe water deficit) (Tezara et al., 2002). For plants with a normal water supply, pre-dawn and midday water potentials were less negative in high ambient [CO\(_2\)]. Consequently, photosynthetic limitation under low water supply may be less severe in high ambient [CO\(_2\)] (Clifford et al., 1993). In theory, the growth-limiting factors of aeroponically grown plants are the availability of light and CO\(_2\), as the roots are frequently misted with nutrient solution to minimize water and nutrient stress. However, similar to soil-grown plants, plant water availability may be limited by the volume of nutrient solution with which roots are in contact (determined by root surface area) and root hydraulic conductivity (Dodd et al., 2000). The former depends on root branching and the horizontal and vertical extent of root growth (Qin et al., 2007; He et al., 2009). Poor root development, as reflected
by lower root FW and DW (Fig. 3C, D) at 36 °C/30 °C, can limit water uptake by these plants, decreasing leaf RWC (He et al., 2001). High ambient RZ temperatures (>35 °C) inhibited root elongation and hair formation of aeroponically grown lettuce plants (Qin et al., 2002; Tan et al., 2002; He et al., 2009). When measured at a low PAR, gs at 36 °C/30 °C (Fig. 3D) under all RZ [CO2] was significantly higher than at 28 °C/22 °C (Fig. 1C), indicating that high temperature increased transpiration (He et al., 2004). Although gs appeared to increase with increasing PAR at 36 °C/30 °C, the magnitudes of these increases were much lower than at 28 °C/22 °C (Fig. 1C, D). Furthermore, this implies that under high PAR and high temperature, elevated RZ [CO2] increases midday leaf RWC (Fig. 2) while lower gs could reduce water loss and thus increase WUE (Farquhar et al., 1989).

Fig. 5. NO3– and total reduced N concentration of shoot (A, B) and root (C, D) of lettuce plants grown under different levels of elevated RZ [CO2] in two different CE rooms at 28 °C/22 °C and 36 °C/30 °C (day/night, ±0.5 °C) at a PAR of 650 μmol m−2 s−1 (12 h photoperiod) for 3 weeks. Each point is the mean of five measurements of five different plants from two different bins. Vertical bars represent the standard errors. Means with different letters above the columns are statistically different (P <0.001) as determined by Tukey’s multiple comparison test.

Fig. 6. Correlations between maximal A (A) and maximal gs (B) (data derived from Figs 1A and 5) of lettuce plants grown under different levels of elevated RZ [CO2] at 28 °C/22 °C and 36 °C/30 °C (day/night, ±0.5 °C) at a PAR of 650 μmol m−2 s−1 (12 h photoperiod) for 3 weeks.
Conflicting results of the effects of elevated RZ \( \text{CO}_2 \) on plant and root growth have been reported. Stolwijk and Thimmann (1957) showed that 2% RZ \( \text{CO}_2 \) inhibited pea (\textit{Pisum sativum} L.) root growth by 80% whereas 6.5% RZ \( \text{CO}_2 \) did not affect root growth of oat (\textit{Avena sativa} L.) or barley (\textit{Hordeum vulgare} L.). Geisler (1967) reported that 10% RZ \( \text{CO}_2 \) stimulated root development of pea. Bouma \textit{et al.} (1997) reported that 2% RZ \( \text{CO}_2 \) had no effect on either shoot or root growth of bean (\textit{Phaseolus vulgaris} L.). These contradictory results could be due to the differences in plant species, or in the concentration or duration of \( \text{CO}_2 \) treatment. In the present study, greater FW and DW biomass under elevated RZ \([\text{CO}_2]\) (Fig. 3) suggested that partial stomata closure causing higher midday RWC under elevated RZ \([\text{CO}_2]\) could alleviate water stress (especially at 36 \(^\circ\)C/30 \(^\circ\)C) and hence enhance productivity.

A major challenge to predicting whole-plant responses to elevated RZ \([\text{CO}_2]\) is understanding the complex relationships between elevated RZ \([\text{CO}_2]\) and other factors, especially temperature and nutrient availability, on different physiological processes. Acclimation of photosynthesis to growth at elevated \textit{ambient} \( \text{CO}_2 \) depends on the source/sink ratio within the plant and ‘sink capacity’ (Davey \textit{et al.}, 2006; Ainsworth and Rogers, 2007). Warmer temperature stimulates growth and respiration so that sink limitation would be less. Thus, \( \text{CO}_2 \) enrichment stimulates growth more at warmer than at cooler temperatures (Stitt 1991). The most likely explanation for sink limitation under elevated \textit{ambient} \( \text{CO}_2 \) is insufficient N supply (Rogers \textit{et al.}, 2006). In this study, a decreased shoot/root DW ratio during the first week of elevated RZ \([\text{CO}_2]\) treatments (Fig. 4E, F) implied that root sink strength immediately increased, thus increasing their capacity for utilizing photo-assimilate. Moreover, larger roots increased water and nutrient uptake, enhanced growth of shoots, and hence increased shoot/root ratios during the second and third weeks of RZ \([\text{CO}_2]\) treatment (Fig. 5).

The effect of elevated RZ \([\text{CO}_2]\) on plants could be the result of the C and N coordination that may regulate N uptake and assimilation. For whole plants, effects of elevated RZ \([\text{CO}_2]\) on C assimilation rate, especially under high light, could also be affected by N availability. Because elevated RZ \([\text{CO}_2]\) decreased stomatal conductance (and thus transpiration), it might be expected to have effects on mineral uptake. Most plants exposed to ambient elevated \([\text{CO}_2]\) exhibited enhanced growth rates, thus creating an increased demand for nutrients, especially N (Rogers \textit{et al.}, 2006; Sicher and Bunce, 2008). Decreased N concentration or content is usually interpreted as evidence that NO\textsubscript{3} uptake and assimilation have not kept pace with photosynthesis and growth in enhanced \([\text{CO}_2]\) (Pettersson and McDonald, 1994). Would limited N availability be likely to minimize \( \text{CO}_2 \) response below-ground as it did above-ground? RZ DIC increased NO\textsubscript{3} uptake compared with ambient RZ DIC (Cramer \textit{et al.}, 1996). Incorporation of exogenous DIC has been demonstrated through incorporation of \( \text{H}^{14}\text{CO}_3 \) into organic compounds by Cramer and Lewis (1993) although the assimilation of DIC is responsible for only a small contribution to the overall carbon budget (Cramer and Lips, 1995). Incorporation of DIC serves an anaplerotic function supplying carbon skeletons for amino and organic acid synthesis (Cramer \textit{et al.}, 1993). The anaplerotic pathway makes use of inorganic C to build the C\textsubscript{4} compounds leading to amino acid and protein synthesis (Jeanneau \textit{et al.}, 2002; Giordano \textit{et al.}, 2003). Although NO\textsubscript{3} uptake has been reported to be increased under elevated RZ \([\text{CO}_2]\) in the short term (Van der Merwe and Cramer, 2000), there was no change in the tissue total N with increased RZ \([\text{CO}_2]\) (Viktor and Cramer, 2003). However, Viktor and Cramer (2003) reported that there was a significant increase in the biomass of plants supplied with both NO\textsubscript{3} and NH\textsubscript{4} nutrition in response to increased RZ \([\text{CO}_2]\). In this study, growing plants aerophytically with ample nutrient supply by continually spraying the roots with nutrients, plus a larger root system under elevated RZ \([\text{CO}_2]\), increased not only leaf NO\textsubscript{3} but also total reduced N concentration (Fig. 5). Plants grown under elevated RZ \([\text{CO}_2]\) in the present study had lower \( g_s \) but higher \( A \), and higher productivity could also be partially due to DIC incorporation which allowed the improved incorporation of N into amino acids in the roots as a consequence of greater supplies of anaplerotic carbon for protein synthesis (Cramer and Lewis, 1993; Cramer \textit{et al.}, 1993; Viktor and Cramer, 2003). This was supported by the fact that greater differences in \( A \) were observed under higher PAR (Fig. 1) among the different elevated RZ \([\text{CO}_2]\) treatments, and the positive correlation between maximal \( A \) and accumulated total shoot reduced N (Fig. 6B). Numerous studies have demonstrated a positive linear relationship between the N concentration and amount of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in mature leaves. Decreases in the leaf N concentration and amount of Rubisco result in a reduced light-saturated photosynthetic rate (Li \textit{et al.}, 2009). The increased \( A \) under elevated RZ \([\text{CO}_2]\) could partially be due to the high shoot total reduced N not \( g_s \) (Fig. 6). Higher \( A \) at elevated RZ \([\text{CO}_2]\) increased the electron sink capacity and electron transport rate (unpublished data), and thus resulted in further increases of \( A \) under higher levels of PAR (Fig. 1).

Conclusions

Both elevated RZ \([\text{CO}_2]\) and higher temperature significantly affected the growth of lettuce plants. Although elevated RZ \([\text{CO}_2]\) had a greater effect (percentage growth increase) in the higher air temperature regime, it also had a considerable impact on photosynthetic gas exchange, water relations, and N metabolism at lower air temperatures. The benefits of elevated RZ \([\text{CO}_2]\) under both temperature regimes occurred within a few days: decreased \( g_s \), less water loss, higher midday leaf RWC, and also higher turnover of the Calvin cycle with higher supply of leaf NO\textsubscript{3} (thus higher levels of total reduced N and a higher amount of Rubisco). Over longer periods (days to weeks), acclimation of photosynthesis to elevated RZ \([\text{CO}_2]\) altered the source/sink ratio with increased ‘sink capacity’—the larger root system which in turn not only enhanced NO\textsubscript{3} uptake...
but also increased the capacity for utilizing photoassimilate. This research may have practical significance for aeroponic vegetable production by growing plants under elevated RZ [CO₂] to enhance productivity.

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