

Separating the effects of hypobaria and hypoxia on lettuce: growth and gas exchange

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The objectives of this research were to determine the influence of hypobaria (reduced atmospheric pressure) and reduced partial pressure of oxygen (pO₂) [hypoxia] on carbon dioxide (CO₂) assimilation (C_A), dark-period respiration (DPR) and growth of lettuce (*Lactuca sativa* L. cv. Buttercrunch). Lettuce plants were grown under variable total gas pressures [25 and 101 kPa (ambient)] at 6, 12 or 21 kPa pO₂ (approximately the partial pressure in air at normal pressure). Growth of lettuce was comparable between ambient and low total pressure but lower at 6 kPa pO₂ (hypoxic) than at 12 or 21 kPa pO₂. The specific leaf area of 6 kPa pO₂ plants was lower, indicating thicker leaves associated with hypoxia. Roots were most sensitive to hypoxia, with a 50–70% growth reduction. Leaf chlorophyll levels were greater at low than at ambient pressure. Hypobaria and hypoxia did not affect plant water relations. While hypobaria did not adversely affect plant growth or C_A, hypoxia did. There was comparable C_A and a lower DPR in low than in ambient total pressure plants under non-limiting CO₂ levels (100 Pa pCO₂, nearly three-fold that in normal air). The C_A/DPR ratio was higher at low than at ambient total pressure, particularly at 6 kPa pO₂ – indicating a greater efficiency of C_A/DPR in low-pressure plants. There was generally no significant interaction between hypoxia and hypobaria. We conclude that lettuce can be grown under subambient pressure (≈25% of normal earth ambient total pressure) without adverse effects on plant growth or gas exchange. Furthermore, hypobaric plants were more resistant to hypoxic conditions that reduced gas exchange and plant growth.

Introduction

The exploration of space requires the development of Advanced Life Support Systems (ALS) that have the capacity to recycle resources and produce food (Salisbury 1999, Wheeler et al. 2001). The biological component will include the use of higher plants for supplemental air and water purification, as well as providing food and

psychological benefits (NASA 1998). Plants grown in space environments will be subject not only to reduced gravity but also to the conditions designed mainly to fulfill requirements for the human environment (Wheeler et al. 2001). The International Space Station has CO₂ concentrations in its atmosphere that are many times in excess of those on Earth. The potential range of environmental

Abbreviations – ALS, Advanced Life Support Systems; C_A, carbon dioxide (CO₂) assimilation; DM, dry mass; DPR, dark-period respiration; LPPG, low-pressure plant growth; MCU, microcontroller units; pCO₂, partial pressure of CO₂; pO₂, partial pressure of O₂; RGR, relative growth rate; RH, relative humidity; RWC, relative water content; SLA, specific leaf area; VDC, voltage direct current signal.

conditions in an 'Advanced Life Support System' includes total gas pressures as low as 54 kPa (8 psi) and CO₂ concentrations as high as 700 Pa (7000 μmol mol⁻¹), some 19 times greater than on Earth (NASA 1998, 2004), and in great excess of the range that researchers are studying global change (Amthor 1991). Plants can tolerate wide variation in concentration of the essential gases, O₂ and CO₂, relative humidity (RH) and, possibly also, total gas pressure (He et al. 2003, 2006, Paul and Ferl 2006, Richards et al. 2006, Spanarkel and Drew 2002). Important environmental variables that have received limited research effort in relation to an ALS are reduced total atmospheric gas pressure at elevated CO₂ partial pressure.

There are several important advantages, from an engineering and cost-reduction viewpoint, associated with growing plants at hypobaric conditions in biomass production in a controlled lunar or Martian extraterrestrial base ALS environment. Utilization of low atmospheric pressure for crop production systems could lower the equivalent system mass, which is a metric used to compare diverse life support systems that account for all parameters that will contribute to the construction, launch, deployment and operation of the system (Drysdale et al. 1999). The reduced pressure differential between the plant growth facility and the external environment would reduce structural requirements, permit lighter materials to be used in its construction and improve safety issues by reducing external and internal pressure differentials (Corey et al. 2002, He et al. 2003, Paul and Ferl 2006). This would further reduce the payload volume and mass required for deployment. A hypobaric condition would reduce gas leakage from the controlled environment space to the external environment. For a lunar mission, the ambient pressure is near 0 kPa, whereas the Martian ambient pressure varies from 0.2 to 0.9 kPa. Pressure differences between the two environments drive leakage, with higher differences resulting in greater leakage. Furthermore, hypobaric conditions in the plant growth facility would require less buffer gas, typically N₂, to be transported or obtained in situ to supplement the physiologically active gases (CO₂ and O₂).

Previous studies have demonstrated that plant germination and seedling growth are possible at hypobaric conditions (Andre and Massimino 1992, Corey et al. 2002, Costes and Vartapetian 1978, Gale 1973, Goto et al. 1995, 1996, 2002, He et al. 2003, 2006, Musgrave and Strain 1988, Schwartzkopf and Mancinelli 1991, Spanarkel and Drew 2002). It is commonly known that plants grow at high altitudes, where pressures were well below 70 kPa (Davies et al. 2005, Gale 1972, Paul and Ferl 2006), although the invariable association between

increasing altitude and decreasing temperature confounds the issue of the effect of pressure alone. The question here is whether the rates of vegetative growth and morphogenesis compare closely with those at ambient pressure.

A potential limitation to plant growth under hypobaria is if the partial pressure of O₂ (pO₂) is reduced, oxidative phosphorylation can become limited (Drew 1997). Seedlings germinated and grew during a weeklong study at 6 kPa total gas pressure, provided the atmosphere was comprised predominately of oxygen (pO₂ = 5 kPa; ≈83% O₂); but at lower total pressures and therefore less O₂, seeds failed to germinate (Schwartzkopf and Mancinelli 1991). Low total pressure (21–24 kPa) did not inhibit seed germination and initial growth as long as pO₂ was 5 kPa or more (Musgrave and Strain 1988). Increasing pO₂ at low total pressure enhanced growth of rice and *Arabidopsis* (Goto et al. 2002).

Hypobaric environments are typically associated with hypoxia (low O₂) condition, particularly when total gas pressure is reduced below 50 kPa. Hence, there is a need to supply sufficient partial pressures of O₂ to avoid hypoxia under hypobaric conditions. However, the National Aeronautics and Space Administration (NASA) also has safety concerns because fire can be more easily propagated in enclosed environments with oxygen-enriched atmospheres above 23.5%. Nonetheless, *Arabidopsis* was grown at total pressure of 23 kPa with pO₂ of 21 kPa (≈91% O₂) in a hypobaric chamber equipped with a heater and other electronic devices without problems (Goto et al. 2002). There are conflicting reports in the literature about low-pressure effects on plant gas exchange (Corey et al. 1996, 2002, Daunicht and Brinkjans 1992, Goto et al. 1996, He et al. 2006, Iwabuchi and Kurata 2003, Richards et al. 2006, Spanarkel and Drew 2002); generally, studies suggest that carbon dioxide (CO₂) assimilation (C_A) and evapotranspiration increase under low pressure.

Hence, the objectives of this research were to determine the influence of hypobaria and the pO₂ on C_A, dark-period respiration (DPR) and growth of lettuce (*Lactuca sativa* L. cv. Buttercrunch).

Materials and methods

Low-pressure plant growth system

The low-pressure plant growth (LPPG) system is a fully automated system, capable of controlling pressure and gas concentrations in reduced pressure growth chambers. The LPPG system consisted of six growth chambers designed to operate at pressures as low as 5 kPa (Purswell 2002), as shown in Fig. 1. Total pressure and the pO₂ and

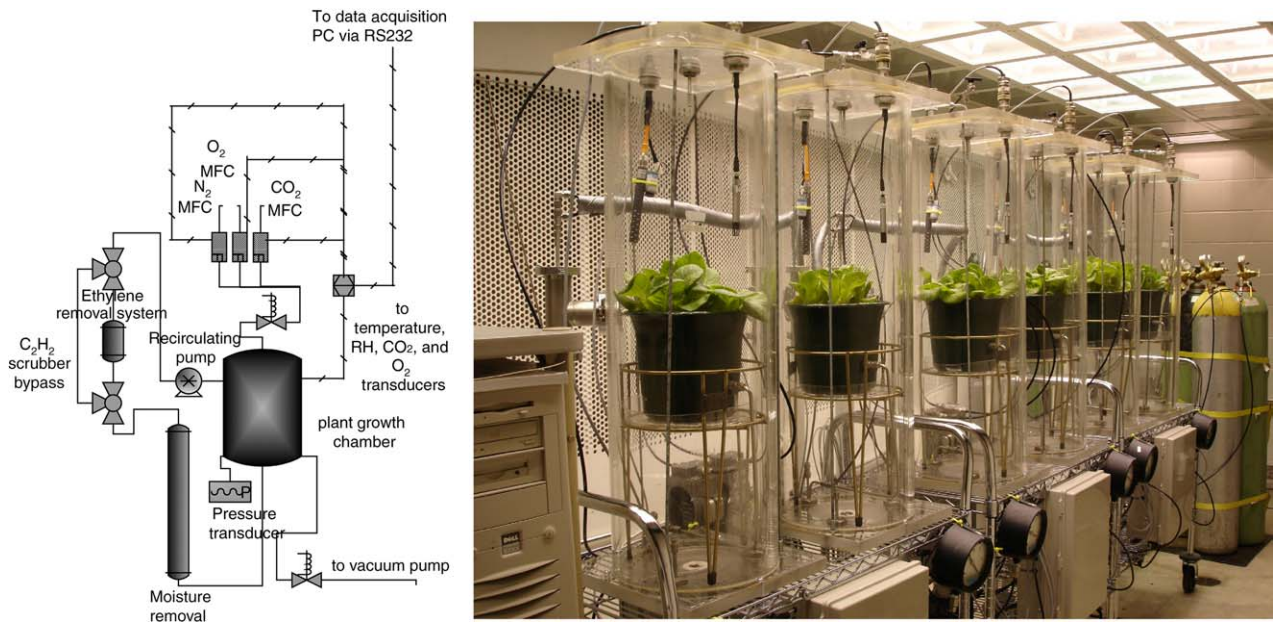


Fig. 1. Left: Schematic of LPPG. Right: Lettuce plants under different total pressure (101 or 25kPa)/pO₂ (12 or 21 kPa pO₂) in a 10-day study.

partial pressure of carbon dioxide (pCO₂) were controlled and monitored during experiments. The LPPG was a semi-closed system because O₂, CO₂ and N₂ are added and controlled (Fig. 1). Temperature was recorded, although not controlled directly by the LPPG system. Temperature control and lighting were provided by placing the LPPG system in an independent plant growth room. The chambers were independent so that conditions could be set to satisfy any statistical experimental design.

The six cylindrical LPPG chambers are constructed of clear acrylic to allow light into the chamber on all sides. They measured 0.31 m in diameter and 0.91 m in height and had a volume of 55.4 l. All fittings allowing gas, water and electrical connections into the chamber were vacuum rated. Metal racks holding two chambers apiece were situated in a growth room, which provided adequate lighting and temperature control. Each chamber was evacuated and maintained to the target reduced gaseous atmospheric pressure by a rotary vane vacuum pump (Bestech Model No. BD-20A; Bestech Industries, Inc., Kyunggi-Do, Korea), which was activated by signals from the control system.

The pressure in the chamber was measured using a pressure transducer (Ashcroft K2; Dresser Instruments, Addison, TX). Oxygen was measured by a motion stable, weak acid electrolyte oxygen sensor (MAX-250; Maxtec, Inc, Salt Lake City, UT) within each chamber. This sensor provided a stable signal over an oxygen concentration range of 0–100% (0–101 kPa pO₂). The CO₂ sensor selected used silicon-based non-dispersive infrared technology (model GMM222; Vaisala, Helsinki, Finland) to

optically determine the CO₂ concentration. The sensor response was converted to a voltage direct current (VDC) signal, which was proportional to the CO₂ concentration, and sent to another of the available analog-to-digital converter channels on the microcontroller units [(MCU), PIC16F877; Microchip Technology, Inc, Chandler, AZ]. Temperature and RH were measured with a HT-761 transmitter from Ohmic Instruments, Co (Easton, MD). This instrument provided two VDC outputs, proportional to temperature over the range 0–100°C and 0–100% RH, respectively. Temperature measurement was to a precision of ±0.3°C, and RH was measured to a precision of ±2%. The various sensors for pO₂, pCO₂ and RH were calibrated at the target pressures. Gas flow was controlled and measured using mass flow controllers (Model 1179; MKS Instruments, Andover, MA) (Fig. 1).

There was one MCU per growth chamber, which communicated with a central computer that acted as a switchboard between the user, the MCUs and the sensors. The control program on the computer was written in LABVIEW (National Instruments, Austin, TX). Set points for total pressure, pO₂ and pCO₂ in each chamber were inputs by the user to the LABVIEW program. The set points could be changed during experiments without stopping the control system.

Plant growth conditions

Lettuce (*Lactuca sativa* L. cv. Buttercrunch) was germinated in 20 × 14 cm plastic pots that were filled to within

1 cm of the top with fine-grade calcined clay [Profile Greens; Profile Products LLC, Buffalo Grove, IL (particle size < 1 mm, 74% porosity, 0.56 g cm⁻³ bulk density and 2.5 g cm⁻³ particle density)]. The inert, calcined clay was prewashed with deionized water and allowed to drain thoroughly before sowing. After the seeds were germinated, plants were supplied with a modified Hoagland's nutrient solution (pH 6.3) containing 4.0 mM Ca(NO₃)₂, 1.0 mM KH₂PO₄/K₂HPO₄, 2.0 mM KNO₃, 1.0 mM MgSO₄, 50 μM Fe as Fe-ethylenediaminetetraacetic acid, 1 mM NaCl and micronutrients: 50 μM B, 10.0 μM Mn, 1.0 μM Cu, 2.0 μM Zn and 0.3 μM Mo.

Ten days after imbibition, three seedlings were transplanted to 20-cm self-watering pots (S-series pots; Apollo Plastics, Ltd, Mississauga, Ontario, Canada) with a volume of (4 l) filled with prewashed calcined clay. The reservoir of the self-watering pots can hold 1 liter of nutrient solution. Lettuce seedlings were allowed to grow in normal atmospheric pressure in a controlled growth chamber for another 10 days. Only uniform, 20-day old plants were selected and transferred with their pots to the LPPG chambers for the treatments, so plants were 30-day old at the termination of 10-day studies. For the 10-day study, 1 liter of nutrient solution was in the reservoir at the beginning of the experiment, and an additional 1 liter was added during the study through an air-lock system.

Lighting was approximately 600 μmol m⁻² s⁻¹ at canopy level inside of the pressure chambers provided by Sylvania 400 watt metal halide (M400U) lamps with a 12-h/12-h light/dark phase, maximum/minimum temperature of 26.1 ± 0.6°C/20.0 ± 0.1°C and maximum/minimum RH of 91.2 ± 3.7% (dark period)/83.7 ± 2.7% (light period).

During the experiments, the gaseous total atmosphere in three chambers was reduced to 25 kPa, while three chambers were maintained at ambient pressure (101 kPa) as controls. To avoid chamber effects, low-pressure and ambient pressure chambers were rotated among replicated experiments. All gases (N₂, O₂ and CO₂) were controlled independently by computer-calculated data from the pressure, CO₂ and oxygen sensors. Each chamber utilized a counter flow heat exchanger to control humidity and condensation of excess water from the air. The heat exchanger had two 3-way valves, which is used to isolate it from the main system. Periodically, water was drained from the heat exchanger after first isolating it from the chamber. There was sufficient volume in the exchanger to hold condensate for the duration of the 10-day experiments. Air from each chamber was circulated at 25 l per minute by a diaphragm pump (Air Cadet; Cole Parmer, Vernon Hills, IL) through closed stainless steel chambers with steel coils for circulating chilled water at 13°C.

Gas exchange of lettuce plants during 10-day studies

Two experiments were designed to characterize the effects of varying pO₂ pressures on the C_A and DPR of lettuce under light conditions of 600 μmol m⁻² s⁻¹. The light/dark cycle was 12 h/12 h. Experiment 1 consisted of pO₂ at 101 (ambient total pressure)/21 kPa pO₂, 101/12 kPa pO₂ and 25 (low total pressure)/12 kPa pO₂ (so there would be an ambient and low-pressure treatments with equal pO₂). Experiment 2 consisted of pO₂ at 6, 12 or 21 kPa for both ambient (101 kPa) and low (25 kPa) total pressure. Supplementary CO₂ was added during the light cycle to maintain a minimum set point level of 100 Pa pCO₂. Without supplementary CO₂ during the light period, CO₂ levels would fall within several hours to a CO₂ compensation point of around 2–4 Pa CO₂ (20–40 μl L⁻¹ equivalent at 101 kPa) at 6 and 21 kPa pO₂, respectively (data not reported). The CO₂ was not controlled during the dark period. As previously mentioned, plants were 20-day old and were selected for uniform size at the time they were placed into the chambers.

CO₂ assimilation

In Experiments 1 and 2, C_A was measured by rate of CO₂ uptake (drawdown) during the light period cycle (Corey et al. 1996, 2002, Richards et al. 2006, Wheeler 1992). Measurements began 10 min after the lights were turned on during the 10-day study. The CO₂ accumulated in the chambers during the dark period cycle (without supplementary CO₂). To determine C_A, slopes of the regression lines of pCO₂ (Pa) over time (min) in each chamber were determined during the light cycle. The pCO₂ were measured by CO₂ sensors within each chamber independently at 1-min intervals during the light cycle. The change of pCO₂ in each chamber was related to C_A during the light period cycle. The minimum pCO₂ set point was 100 Pa pCO₂ for all the treatments in ambient and low-pressure chambers. When CO₂ levels were drawn down (assimilated by plants in the chamber) to 100 Pa pCO₂, which was equivalent to 1000 μmol mol⁻¹ at 101 kPa total pressure, the system added supplementary gas to maintain the set point level CO₂ in each chamber through the remaining duration of the light period cycle. The rates of C_A were obtained during the first 100–140 min (10 min after lights were turned on during the light cycle). Hence, at 1-min intervals, there were 100–140 measurements taken in determining the daily C_A in each replicated chamber per treatment. Each point of the regression lines was based on the daily slopes of C_A of lettuce plants from four replicate chambers (n = 4) during the 10-day study.

Because only six chambers in the growth room could be used during a given 10-day cycle, experimental runs required 12 chambers for gas exchange measurements in Experiment 1 (3 treatments \times 4 replications) and 24 chambers for Experiment 2 (6 treatments \times 4 replications) (see the *Statistical analysis*). For Experiment 2, final gas exchange measurements were made on day 10, plants harvested, leaf areas determined and C_A and DPR measurements converted to $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Dark-period respiration

The DPR was measured by CO_2 accumulation in chambers during the dark period (Richards et al. 2006, Wheeler 1992). The pCO_2 were measured by CO_2 sensors within each chamber independently at 1-min intervals during the dark period, as previously described. During the dark period cycle, no supplementary CO_2 was added to chambers and CO_2 was allowed to rise without maximum set points. To avoid any residual effects of the light cycle, data during the first 10 min of the dark cycle (720 min, 12 h) were not used for calculation. Hence, there were 710 1-min interval measurements taken nightly for DPR in each of the four replicated chambers per treatment. DPR was determined by the slopes of the regression equations of accumulated pCO_2 in each chamber during the dark period cycle. Each point of the regression line was based on the slopes of the DPR of lettuce plants from four replicate chambers ($n = 4$) during the 10-day studies.

Plant growth

In Experiment 2 at the termination of the 10-day study, plants were removed from chambers and leaf area, specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$), leaf and root dry mass (DM), leaf/root ratio (g g^{-1}) and chlorophyll content ($\mu\text{g cm}^{-2}$) were determined. Relative growth rate [(RGR) ($\ln \text{total plant DM}_2 - \ln \text{total plant DM}_1$) ($\text{time}_2 - \text{time}_1$) $^{-1}$] was also determined (Hunt 1982). For determining total plant DM_1 of RGR, five self-watering pots containing three uniform seedlings each were harvested at the same time the experiment was initiated for day 1. Leaf area was measured with an LI-COR LI-3000A leaf area meter (LI-COR, Lincoln, NE). Measurements of total leaf chlorophyll (Chl $a + b$) content were determined with a non-destructive method using a portable chlorophyll meter (SPAD-502; Minolta Camera, Co, Osaka, Japan). Ten measurements were recorded from the leaves of each chamber, and the contents of chlorophyll were calculated with a chlorophyll prediction equation (Carpio et al. 2005). Relative water content [(RWC) ($\text{fresh mass} - \text{DM}$) ($\text{saturated mass} - \text{DM}$) $^{-1}$] was also determined (Estrada-Luna and Davies 2003).

Statistical analysis

Replication was achieved by repeating treatments under the same conditions over time. Low-total-pressure and ambient total pressure chambers were run concurrently. Chambers were alternated during treatments between low and ambient total pressure to avoid any chamber effects. There were no chamber effects. All reported data were pooled from repeated, independent treatments. There were six chambers, so replications of treatments were run with combinations of total pressure and pO_2 and repeated as needed under the same environmental conditions. There were three plants per container and one container per chamber, with each container as a single replicate. In Experiment 1 (101/21, 101/12 and 25/12 kPa pO_2), only gas exchange was determined. For C_A and DPR, regression analysis was performed and slopes were determined and analyzed by analysis of variance (ANOVA) (SAS Institute, Inc, Cary, NC) to determine statistical differences among treatments, as previously described ($n = 4$).

In Experiment 2 (101/21, 101/12, 101/6, 25/21, 25/12 and 25/6 kPa pO_2), plant growth and gas exchange were determined, $n = 4$. As with Experiment 1 for C_A and DPR, slopes were determined and analyzed by ANOVA for statistical differences among treatments. ANOVA was also conducted to determine main effects and interactions, with mean separation by $\pm \text{SE}$ for both gas exchange and growth analysis. Coefficient of linear regression (r^2) was also determined for C_A and DPR with selected growth parameters of lettuce (*Lactuca sativa* L. cv. Buttercrunch) under ambient (101 kPa) pressure and hypobarica (25 kPa) with varying pO_2 .

Results

Patterns of DPR and C_A at 25/12, 101/21 and 101/12 kPa pO_2 during dark and light cycles

In Experiment 1, CO_2 accumulated each night period and increased because of higher DPR as the plants grew during the 10-day study (Figs 2 and 3). The pCO_2 was higher in ambient (101 kPa) than in low (25 kPa) total pressure chambers (Fig. 2), indicating higher DPR under ambient than under low total pressure. The maximum pCO_2 was around 200 and 180 Pa, respectively, for ambient pressure and low-pressure treatments at the end of 12-h dark period cycle of day 1 and increased to 400+ Pa and 330 Pa, respectively, for ambient and low pressure by the end of dark period cycle on day 10 (Fig. 2). From days 2 to 10, DPR increased 2.7-fold, 2.5-fold and 2.3-fold, respectively, for 101/21 kPa, 101/12 and 25/12 kPa pO_2 (Fig. 3). DPR was significantly higher under ambient

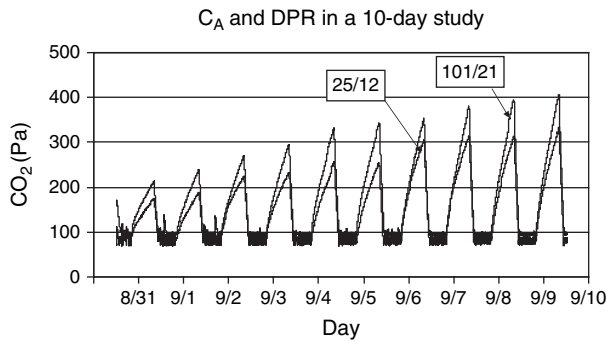


Fig. 2. Pattern of diurnal C_A and DPR via monitoring the pCO_2 in low (25/12 kPa pO_2) and ambient (101/21 kPa pO_2) pressure chambers during a 10-day study of lettuce. The pCO_2 was measured at 1-min intervals. CO_2 accumulated and peaked during DPR. Supplementary CO_2 was only added during the light period cycle when the pCO_2 reached a set point of 100 Pa.

than under low total pressure ($P < 0.05$), whereas there were no significant differences between 21 and 12 pO_2 (Fig. 3). It was interesting that the DPR rates at 25/12 were lower even after just 1 day of treatment (Figs 2 and 3), suggesting a direct, and almost immediate pressure, effect on DPR. The DPR of 101/12 kPa pO_2 was also higher than 25/12 kPa pO_2 , suggesting that pO_2 was not involved. DPR was lowest for low-pressure plants, and by day 10, DPR were 0.4, 0.4 and 0.3 Pa $CO_2 \text{ min}^{-1}$, respectively, for 101/21 kPa pO_2 , 101/12 kPa pO_2 and 25/12 kPa pO_2 (Fig. 3). At day 10, DPR of lettuce was 24.4 and 22.5% higher, respectively, in 101/21 and 101/12 kPa pO_2 chambers than in 25/12 kPa pO_2 chambers. During the 10-day study, the daily DPR increased 0.027, 0.027 and 0.022 $CO_2 \text{ Pa min}^{-1} \text{ day}^{-1}$, respectively, at 101/21, 101/12 and 25/12 kPa pO_2 (Fig. 3).

The C_A increased rapidly with lettuce plants as soon as lights were turned on during the light period in ambient and low-pressure chambers (Fig. 2). On day 2 within 110 min, lettuce plants in ambient and low-pressure cham-

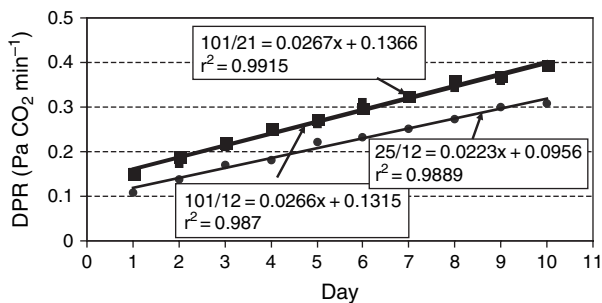


Fig. 3. DPR during a 10-day study of lettuce under different total pressure/ pO_2 at 101/21 kPa pO_2 , 101/12 kPa pO_2 , and 25/12 kPa pO_2 . Total pressure $P = < 0.05$, and oxygen (pO_2) $P = \text{NS}$; $n = 4$.

bers assimilated CO_2 produced during the 12-h dark period cycle down to the set point of 100 Pa pCO_2 ; the CO_2 drawdown period (for determining C_A) increased to around 140 min by day 10 (data not presented). From days 2 to 10, based on the slopes of the regression, C_A increased 1.9-fold, 1.9-fold and 2.2-fold, respectively, for 101/21, 101/12 and 25/12 kPa pO_2 (Fig. 4). However, total pressure and pO_2 had no significant effect on C_A . Looking at C_A on a daily basis over the 10-day study, the initial rate of C_A was about 0.8 Pa $CO_2 \text{ min}^{-1}$ for the first day among low-pressure treatment and ambient pressure treatment (Fig. 4). By day 10, C_A had increased over time, but was statistically the same among 101/21 101/12 and 25/12 kPa pO_2 , which were, respectively, 2.2, 2.0 and 1.9 Pa $CO_2 \text{ min}^{-1}$ (Fig. 4). During the 10-day study, C_A increased by 0.14, 0.13, and 0.13 Pa $CO_2 \text{ min}^{-1}$, respectively, for 101/21, 101/12 and 25/12 kPa pO_2 , which were statistically similar (Fig. 4).

Effect of hypoxia and hypobaria on DPR and C_A of lettuce

In Experiment 2 with pO_2 at 6, 12 or 21 kPa, DPR increased under low and ambient pressure as plants matured during the 10-day study (Fig. 5). The main effects of total pressure, pO_2 and time (days) on DPR were highly significant ($P < 0.001$) (Table 1); there was also a significant interaction of pressure \times time and \times time ($P < 0.001$). By day 5, DPR were higher in ambient than in low-pressure plants, and by day 10, low-pressure plants at 6 and 12 kPa pO_2 had lower DPR than ambient pressure plants (Fig. 5). With both ambient and low-total-pressure plants, DPR were lower at 6 kPa pO_2 than at 21 kPa pO_2 . At 6 kPa pO_2 , DPR was the same between day 5 and 10. On day 10, when plants were harvested and measurements could be expressed on a leaf area basis ($\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$), DPR of hypobaric plants was still significantly lower ($P < 0.01$) than ambient pressure, but

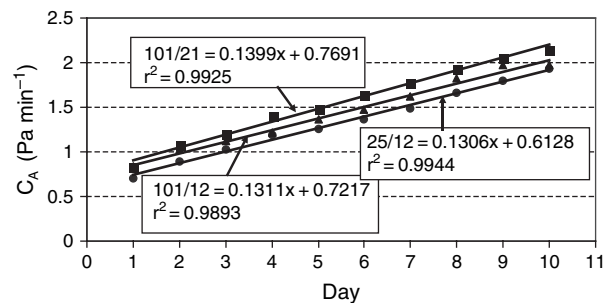


Fig. 4. C_A during a 10-day study of lettuce under different total pressure/ pO_2 at 101/21 kPa pO_2 , 101/12 kPa pO_2 , and 25/12 kPa pO_2 . Total Pressure (Pres) $P = \text{NS}$, oxygen (pO_2) $P = \text{NS}$; $n = 4$.

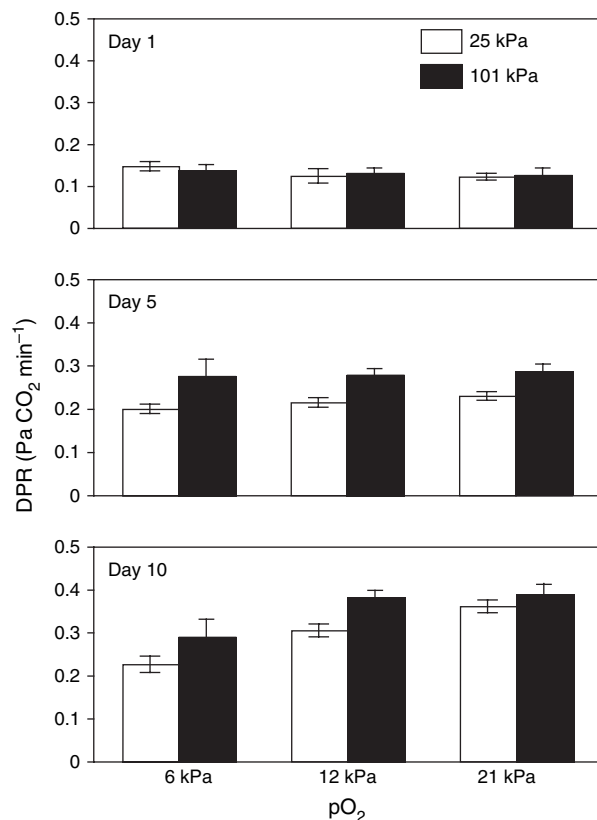


Fig. 5. DPR of lettuce at days 1, 5 and 10 under total pressure/pO₂ at 101/21 kPa pO₂, 101/12 kPa pO₂, 101/6 kPa pO₂, 25/21 kPa pO₂, 25/12 kPa pO₂ or 25/6 kPa pO₂. See Table 1 for significance; bars indicate \pm SE, n = 4.

the main treatment effect of pO₂ had no significant effect (Table 2, Fig. 6).

The C_A was significantly affected ($P < 0.001$) by the main effects of pO₂, time (days) and interaction of pO₂ × time, while total pressure had no significant effect

Table 1. Effect of total atmospheric pressure (Pres.), pO₂ and time on C_A (change CO₂ in chamber Pa min⁻¹), DPR (change CO₂ in chamber Pa min⁻¹) and C_A/DPR ratio. NS, non-significant or * ** * significant at $P < 0.05, 0.01, 0.001$ respectively; n = 4.

	Significance		
	C _A	DPR	C _A /DPR ratio
Pres	NS	***	***
pO ₂	***	***	***
Time	***	***	***
Pres × pO ₂	NS	NS	NS
Pres × time	NS	***	**
pO ₂ × time	***	***	***
pO ₂ × time × Pres	NS	NS	NS

Table 2. Day 10. Effect of total atmospheric pressure (Pres) and pO₂ on C_A ($\mu\text{mol m}^{-2} \text{s}^{-1}$), DPR ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$) and C_A/DPR ratio. NS, non-significant or * ** * significant at $P < 0.05, 0.01, 0.001$ respectively; n = 4.

	Significance		
	C _A	DPR	C _A /DPR ratio
Pres	NS	**	**
Oxygen (pO ₂)	***	NS	***
Pres × pO ₂	NS	NS	NS

(Table 1). These results concur with measurements taken on day 10 when C_A could be expressed in $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 2, Fig. 6). The C_A increased during the 10-day experiment at ambient and low total pressure (Fig. 7). At 12 and 21 kPa pO₂, there were no significant differences between low and ambient total pressure on C_A at day 1, 5 or 10 (Fig. 7, Table 1). However, by day 10, at 6 kPa pO₂ compared with 12 or 21 kPa pO₂, there was two-fold reduction in C_A under both low and ambient pressures (Figs 6 and 7). During day 10 at 6 kPa pO₂, there was a trend in greater C_A with low than with ambient pressure (Figs 6 and 7). The effect of 6 kPa pO₂ reducing C_A was associated with duration of the treatment; greatest reduction occurred at day 10, while there were no differences at day 1 (Fig. 7).

The C_A/DPR ratio was significantly affected ($P < 0.001$) by total pressure, pO₂, time (days) and the interactions of total pressure × time ($P < 0.01$) and pO₂ × time ($P < 0.001$) (Table 1). This concurred with day 10 measurements expressed as $\mu\text{mol m}^{-2} \text{s}^{-1}$, where main effects of total pressure ($P < 0.01$) and pO₂ ($P < 0.001$) significantly affected the ratio (Table 2). The C_A/DPR ratio was lowest at 6 kPa pO₂ at ambient and low total pressure (Figs 6 and 8). The C_A/DPR ratio was higher in low-total-pressure plants than in ambient total pressure plants, particularly at 6 kPa pO₂ (Figs 6 and 8). The higher C_A/DPR ratio suggests a greater efficiency of C_A to DPR with hypobaric plants. Neither total pressure nor time had a significant effect on the C_A/DPR ratio of 21 kPa pO₂ plants (Fig. 8).

Plant growth, RGR, leaf chlorophyll and RWC

Plant growth was comparable between lettuce plants at 101 and 25 kPa total pressures during the 10-day study (Table 3). However, plant growth (leaf area; SLA; leaf, root and total plant DM; and RGR) was lower at 6 kPa pO₂ than at 12 or 21 kPa pO₂ (Table 3). The main treatment effect of pO₂ was highly significant ($P < 0.001$) among all growth parameters, whereas total pressure or the interaction of pressure × pO₂ was not significant (Table 2). An

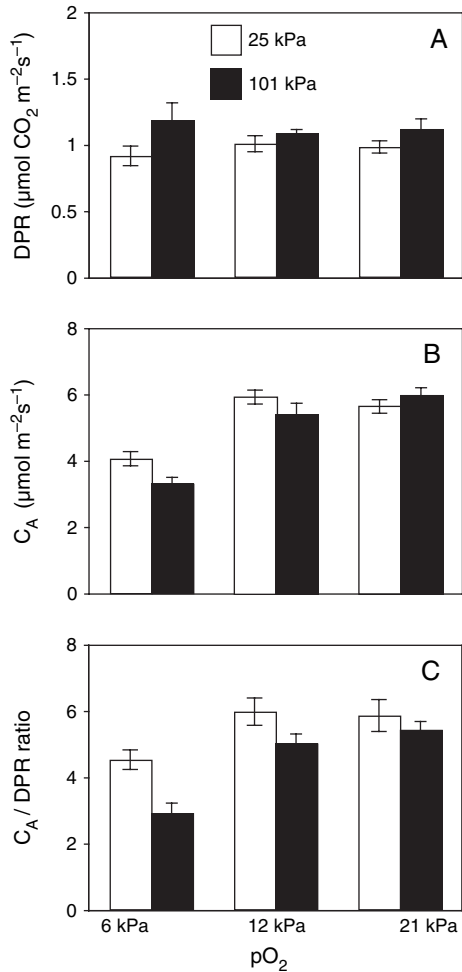


Fig. 6. Day 10. (A) DPR ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), (B) C_A ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) and (C) C_A/DPR ratio of lettuce under total pressure/ $p\text{O}_2$ at 101/21 kPa $p\text{O}_2$, 101/12 kPa $p\text{O}_2$, 101/6 kPa $p\text{O}_2$, 25/21 kPa $p\text{O}_2$, 25/12 kPa $p\text{O}_2$ or 25/6 kPa $p\text{O}_2$. See Table 2 for significance; bars indicate $\pm\text{SE}$, $n = 4$.

exception was RGR, which was significantly affected by $p\text{O}_2$ and the interaction of pressure $\times p\text{O}_2$, whereas the main treatment of total pressure was not significant (Table 3). RGR, which is a measure of net DM per unit DM per day [$\text{mg g}^{-1} \text{ day}^{-1}$], was lowest at 6 kPa $p\text{O}_2$ (compared with 12 or 21 kPa $p\text{O}_2$) and lower at 101/6 kPa $p\text{O}_2$ than at 25/6 kPa $p\text{O}_2$. Plants were 20-day old at the experiment initiation and 30-day old at harvest. Plants grew rapidly and had a 327–611% increase in total plant DM during the 10-day study (data not reported). The percent increase in leaf area and leaf, root and total plant DM was lowest at 6 kPa $p\text{O}_2$. However, the percent increase in root DM and total plant DM was greater at 25/6 kPa $p\text{O}_2$ than at 101/6 kPa $p\text{O}_2$, which is also reflected in the RGR.

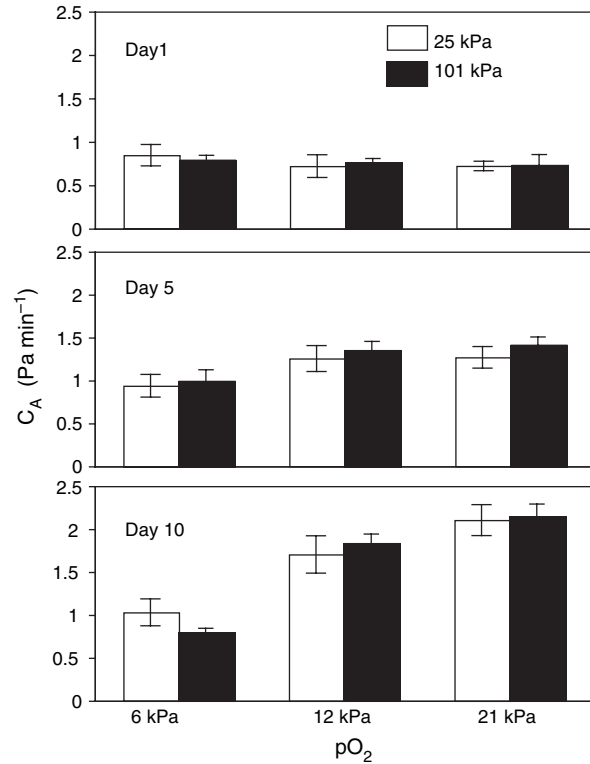


Fig. 7. C_A of lettuce at days 1, 5 and 10 under total pressure/ $p\text{O}_2$ at 101/21 kPa $p\text{O}_2$, 101/12 kPa $p\text{O}_2$, 101/6 kPa $p\text{O}_2$, 25/21 kPa $p\text{O}_2$, 25/12 kPa $p\text{O}_2$ and 25/6 kPa $p\text{O}_2$. See Table 1 for significance; bars indicate $\pm\text{SE}$, $n = 4$.

The SLA of 6 kPa $p\text{O}_2$ plants was lower than 12 or 21 kPa $p\text{O}_2$ lettuce, indicating thicker leaves in response to hypoxia. There was a non-significant trend (16% reduction) in lower SLA (thicker leaves) in 101/6 kPa than 25/6 kPa $p\text{O}_2$ plants.

Of all growth parameters, root growth was most sensitive to hypoxia with a 50–70% reduction in DM, and greater carbon partitioning in above-ground relative to below-ground DM (higher leaf/root ratio) occurred in 6 kPa $p\text{O}_2$ plants.

The coefficient of linear regression (r^2) was very high (0.91–0.96) between C_A and DPR with plant growth parameters (Table 4). Both C_A and DPR were highly correlated to leaf area; leaf, root and total plant DM; and RGR (Table 4).

RWC was not significantly different among low and ambient total pressures at 6, 12 and 21 kPa $p\text{O}_2$ and ranged from 94.4 to 94.9% (Table 3).

There were significant ($P < 0.001$) pressure and oxygen effects on leaf chlorophyll, with no interaction (Table 3). Leaf chlorophyll levels were lowest at 21 kPa $p\text{O}_2$ and greater at low total pressure (25 kPa) than at ambient total pressure.

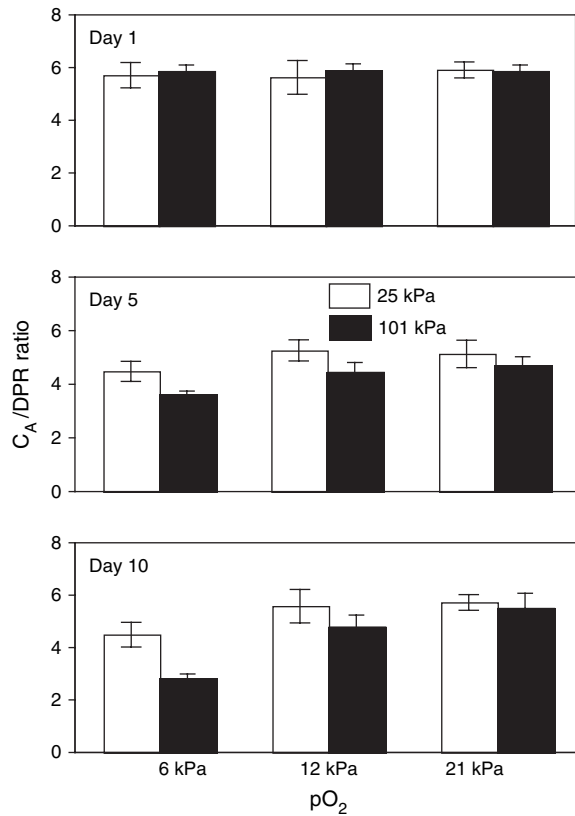


Fig. 8. C_A /DPR ratio of lettuce at days 1, 5 and 10 under total pressure/ pO_2 at 101/21 kPa pO_2 , 101/12 kPa pO_2 , 101/6 kPa pO_2 , 25/21 kPa pO_2 , 25/12 kPa pO_2 or 25/6 kPa pO_2 . See Table 1 for significance; bars indicate \pm SE, $n = 4$.

Discussion

This research demonstrates that lettuce can be successfully grown in a hypobaric environment. It is one of the first reports to clearly differentiate hypobaric from hypoxic responses in plant gas exchange and corresponding plant growth response. While hypobaria did not adversely affect C_A or plant growth, hypoxia did. There was no significant growth interaction between hypoxia and hypobaria, except with RGR. Hypobaric plants had similar C_A and around a 25% lower DPR (at 6 kPa pO_2) than ambient pressure plants. The C_A /DPR ratio was higher at low than at ambient total pressure, particularly at 6 kPa pO_2 – indicating a greater efficiency of C_A /DPR with low-pressure plants. Hypobaric plants were more resistant to hypoxic conditions (6 kPa pO_2), which reduced gas exchange, RGR and plant growth.

In a study of *Arabidopsis* gene expression at low atmospheric pressure, less than one half of the 200 genes dramatically up- or downregulated by hypobaria were similarly affected by hypoxia, suggesting that the response to hypobaria is unique and more complex than

Table 3. Effect of total gas pressure and pO_2 on leaf area, SLA, leaf, root and total plant dry matter (DM), leaf/root ratio, chlorophyll content, RGR [(In total plant $DM_2 - \ln$ total plant DM_1) ($time_2 - time_1$) $^{-1}$] and RWC [(fresh mass – DM)/(saturated mass – DM)] $^{-1}$] of lettuce (*Lactuca sativa* L. cv. Buttercrunch). ^aValues are means \pm SE, $n = 4$. NS, Non-significant or *, **, *** significant at $P < 0.05$, 0.01, 0.001, respectively.

Total pressure (Pres) (kPa)	pO_2 (kPa)	Leaf area (cm ²)	SLA (cm ² g ⁻¹)	Leaf DM (g)	Root DM (g)	Total plant DM (g)	Leaf/root ratio (g g ⁻¹)	Chl (µg cm ⁻²)	RWC (%)	RGR (mg g ⁻¹ day ⁻¹)
25	6	857 ± 18 ^a	44.5 ± 0.3	2.7 ± 0.1	0.4 ± 0.0	3.2 ± 0.1	6.4 ± 0.1	42.1 ± 1.7	94.6	0.18 ± 0.00
	12	1190 ± 69	50.1 ± 0.7	3.6 ± 0.1	0.8 ± 0.1	4.4 ± 0.2	4.3 ± 0.4	39.9 ± 0.5	94.4	0.22 ± 0.00
	21	1349 ± 39	54.5 ± 3.3	3.5 ± 0.2	0.8 ± 0.1	4.3 ± 0.3	4.5 ± 0.1	37.3 ± 0.5	94.6	0.21 ± 0.00
101	6	832 ± 25	37.6 ± 1.5	2.3 ± 0.1	0.3 ± 0.0	2.7 ± 0.1	7.0 ± 0.3	37.8 ± 1.4	94.9	0.16 ± 0.00
	12	1172 ± 96	49.9 ± 0.7	3.4 ± 0.4	0.8 ± 0.1	4.2 ± 0.5	4.2 ± 0.3	37.8 ± 0.7	94.5	0.21 ± 0.00
	21	1250 ± 57	54.2 ± 0.8	3.5 ± 0.2	1.0 ± 0.2	4.4 ± 0.3	4.2 ± 0.6	34.2 ± 0.7	94.4	0.22 ± 0.00
	Pres (significance)	NS	NS	NS	NS	NS	NS	***	NS	NS
	Oxygen (O_2) (significance)	***	***	***	**	***	***	***	NS	***
	Pres × O_2 (significance)	NS	NS	NS	NS	NS	NS	NS	NS	***

Table 4. Coefficient of linear regression (r^2) of C_A and DPR with selected growth parameters of lettuce (*Lactuca sativa* L. cv. Buttercrunch) under ambient pressure (101 kPa) and hypobaria (25 kPa) with varying pO_2 .

Total pressure (kPa)	pO_2 (kPa)	DPR	Leaf area	Leaf DM	Root DM	Total plant DM	RGR
C_A							
25	6	0.93	0.94	0.90	0.88	0.91	0.94
	12	0.95	0.92	0.91	0.91	0.94	0.98
	21	0.96	0.93	0.92	0.90	0.93	0.97
101	6	0.91	0.94	0.91	0.87	0.93	0.94
	12	0.94	0.92	0.91	0.90	0.92	0.99
	21	0.95	0.92	0.91	0.90	0.91	0.98
DPR							
25	6	—	0.81	0.83	0.91	0.89	0.90
	12	—	0.92	0.89	0.89	0.91	0.92
	21	—	0.93	0.90	0.91	0.92	0.92
101	6	—	0.86	0.79	0.89	0.87	0.90
	12	—	0.89	0.92	0.93	0.91	0.92
	21	—	0.91	0.90	0.92	0.91	0.94

an adaptation to reduced pO_2 (hypoxia) inherent to hypobaric environments, i.e. hypobaria does not equal hypoxia (Paul et al. 2004).

The pO_2 under which plant growth is reduced depends on the plant species, root zone aeration, plant age and plant growth rate. Given the specific conditions of this study, the 20-day-old lettuce plants had rapid growth and good root zone aeration (74% porosity) with the inert, calcined clay substrate, container system. Under hypoxic conditions of 6 kPa O_2 (\cong 29% of ambient sea-level pO_2), growth was reduced. At 12 kPa pO_2 , growth was close to adequate with some reduced leaf expansion (leaf area); however, plant DM, leaf/root ratio and RGR were comparable at 21 kPa pO_2 .

Roots were particularly sensitive to hypoxia with a 50% (25/6 kPa O_2) to 70% (101/6 kPa O_2) growth reduction, and greater carbon partitioning in above-ground relative to below-ground DM (higher leaf/root ratio). The respiratory enzymes, cytochrome *c* oxidase and succinate dehydrogenase, have a high affinity for O_2 , i.e. respiration is saturated with less than 0.1% O_2 . The metabolic activity of leaves is generally not reduced until atmospheric pO_2 levels are reduced to around 2 kPa, i.e. photorespiration studies typically reduce pO_2 to 2 kPa to minimize photorespiration and enhance net photosynthesis. Critical oxygen pressure for root respiration is around 10 kPa in a humid atmosphere (Saglio et al. 1984). Hence, O_2 supply to the roots likely became the problem, and not supply to the leaves, because O_2 diffusion is around 10 000-fold greater in air than in water. While root DM was significantly lower at 6 kPa pO_2 than at 12 or 21 kPa pO_2 , there was 25% greater root DM in 25/6 kPa pO_2 than in 101/6 kPa pO_2 plants. This may be explained in

part by the increased diffusion rates and reduced boundary layer resistance that occur with hypobaria, which may have led to greater O_2 diffusion and uptake in the 25/6 kPa pO_2 roots. Furthermore, under the conditions of this study, while root growth decreased under hypoxia, there was sufficient porosity and aeration with the inert, calcined clay substrate.

Leaves of all treatments were turgid and had similarly high RWC, so water stress was not an issue under hypobaria or hypoxia. In a number of preliminary studies under both greenhouse and growth chamber conditions, growth of lettuce plants with the container clay substrate system was highly satisfactory; the termination of the experiment after 10 days (30-day-old plants) was dictated in part because sufficiently high growth (of non-hypoxic plants) caused leaves to begin touching the inside chamber walls.

The SLA of 6 kPa pO_2 plants was also lower, indicating thicker leaves in response to hypoxia, which is often indicative of plant stress. SLA is a common variable in comparative plant ecology and is generally positively correlated to RGR (Shiple and Almeida-Cortez 2003). RGR is a measure of net DM per unit DM per day [$mg\ g^{-1}\ day^{-1}$] (Hunt 1982). A leaf with a high SLA will capture more light (photosynthetically active radiation) per unit mass than a leaf with low SLA (Poorter 2002). Under hypoxia, plant RGR and SLA of lettuce leaves decreased. Hypobaric plants had greater resistance to hypoxia: the RGR of 25/6 kPa pO_2 plants was greater than that of 101/6 kPa pO_2 plants. There was also a non-significant trend in higher (16%) SLA and total plant biomass; had the number of replications per treatment been larger than $n = 4$, statistical differences likely would have occurred.

Another advantage of hypobaric plants was greater leaf chlorophyll content than ambient pressure plants. Lettuce leaves phenotypically responded to hypoxia by producing higher chlorophyll levels, with highest leaf chlorophyll levels occurring in 25/6 kPa pO_2 plants. Low pressure has been reported to promote chlorophyll retention in a large number of plant species (Burg 2004). While C_A was not affected by total pressure, the C_A /DPR ratio was greater in hypobaric plants (Figs 6 and 8, Tables 1 and 2); the higher chlorophyll levels may partially account for the higher C_A /DPR ratio and the trend in higher C_A of hypobaric plants at 6 kPa pO_2 .

The C_A /DPR ratio is an indication of energy available for photosynthesis and growth, including how efficiently carbohydrates are utilized during DPR, and has been used to predict growth performance (Smith et al. 1995).

Both C_A and DPR were highly correlated to leaf area; leaf, root and total lettuce plant DM; and RGR (Table 4). Hypoxia (6 kPa pO_2) decreased C_A , DPR and the C_A /DPR ratio, which were tightly coupled to decreased plant biomass (Tables 3 and 4). While it is generally more

difficult to correlate C_A to growth rate, the reduction of C_A under hypoxia led to reduced growth under the environmental parameters of this 10-day study. While DPR has been used to predict plant growth rates of desert shrubs and crop plants, it requires close monitoring of other biological parameters (Smith et al. 1995). With other species, C_A and DPR have been positively correlated to RGR (Reich et al. 1998).

The C_A and DPR among all treatments were not statistically different on the first day of the second experiment; it was not until day 5 that treatment differences became apparent. Both C_A and DPR increased during the study, leading to an increase in leaf canopy and biomass of lettuce plants. However, hypoxia (6 kPa O_2) caused reductions in C_A , DPR and the C_A /DPR ratio. It was only feasible to convert the gas exchange rates from $\text{Pa CO}_2 \text{ min}^{-1}$ (perfect gas law: $PV = nRT$; see He et al. 2006) to $\mu\text{mol m}^{-2} \text{ s}^{-1}$ during the final day of harvest when leaf area could be accurately determined (Fig. 6). The values of C_A are in line with other studies of lettuce (Corey et al. 1996, He et al. 2003, 2006, Wheeler et al. 1994). The plants were 30-day old at harvest in the 10-day study, so some shading of leaves had occurred, which would have reduced C_A , compared with non-shaded leaves. The only statistical discrepancy occurred with DPR, which was statistically affected by total pressure, but the hypoxic effect was non-significant when units were converted to $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; otherwise all main treatment effects and the non-significant interaction of total pressure \times pO_2 were the same. We considered converting to leaf area based on the total canopy (ground) area basis of the chamber cylinders (rather than the actual leaf area, which we measured and report in this paper); but this would have been the least accurate and more variable method because leaf shading was occurring rapidly in some treatments; fast-growing lettuce leaves were starting to overlap and touch the inner chamber walls. Conversely, hypoxic plants had 34% lower leaf expansion and leaves did not fillup the circumference of the ground area, i.e. the values for C_A and the DPR would have been artificially higher. Utilization of total canopy (ground) area works well when the canopy cover is consistent among treatments (Richards et al. 2006, Wheeler 1992), but our hypoxic plants had smaller canopies and lower total light interception.

In a short-term study with lettuce at 70 kPa total pressure, DPR rates were higher under low pressure (Spanarkel and Drew 2002), which concurs with the higher DPR in a short-term (16 h) study with *Arabidopsis* (Richards et al. 2006). Conversely in another study with lettuce at 51 kPa, DPR decreased and C_A increased, which was attributed to greater inhibition of photorespiration at reduced pO_2 and lower pCO_2 . This concurs with

our longer term study with lettuce where hypobaric plants had a lower DPR and a trend in higher C_A under hypoxic conditions. It is important that longer term studies be conducted (i.e. seed to harvest), since plants acclimate, are highly plastic and conclusions may differ between short-term and long-term studies.

The reduction of C_A and DPR and during hypoxia (6 kPa O_2) was correlated to reduced RGR and lettuce biomass. By day 5, DPR were higher in ambient than in hypobaric plants, and by day 10, low-pressure plants at 6 and 12 kPa pO_2 had lower DPR than high-pressure plants. Lower pO_2 and hypobaria reduces respiration (Burg and Burg 1966). Storage of vegetable and fruits at very low pressure is known to decrease respiration and increase ethylene removal from tissue, possibly because of decreased ethylene biosynthesis and binding (Burg and Burg 1966). While ethylene production was not a focus of this study, high ethylene buildup is a problem with confined growth chamber systems, such as our LPPG system with extremely low leakage rates (less than 1% volume per day). We found that hypobaria reduced ethylene production and that hypobaric reduction of ethylene was greater than just that of hypoxia (low pO_2) (He et al. 2003).

It was surprising to see the reduced growth for a vegetative crop like lettuce at 6 kPa pO_2 , particularly at the ambient total pressure. Quebedeaux and Hardy (1973, 1976) reported good vegetative growth of soybean under reduced pO_2 levels and attributed this to the enhanced photosynthesis at low O_2 and ambient CO_2 . However, we maintained elevated CO_2 for all of our treatments ($100 \text{ Pa CO}_2 \cong 1000 \mu\text{l l}^{-1}$ at ambient total pressure), so there were no benefits to C_A by reducing the pO_2 to avoid photorespiration. Likely, pO_2 may have been important for leaf expansion or canopy spread (leaf area, SLA and leaf DM were reduced at 6 kPa pO_2), which in turn affected photosynthetic rates, the ratio of C_A /DPR and subsequent plant growth. As previously mentioned, roots had the greatest sensitivity to hypoxia; reduced root growth in turn affected leaf biomass.

Hypobaric environments are typically associated with hypoxic (low pO_2) conditions, particularly when total gas pressure is reduced below 50 kPa. Hence, there is a need to supply sufficient pO_2 to avoid hypoxia under hypobaric conditions. A major potential limitation to plant growth under hypobaric conditions is if the pO_2 is reduced, oxidative phosphorylation can become limited (Drew 1997). At 21 and 12 kPa pO_2 , C_A and plant growth were the same and highly correlated between hypobaric and ambient total pressure plants. Other studies reported that low total pressure (21–24 kPa) did not inhibit seed germination and initial growth as long as pO_2 was 5 kPa or more (Musgrave and Strain 1988). The increased

diffusivity of pO_2 under hypobaric conditions may maintain cells under more fully oxygenated conditions, thereby minimizing negative effects of low pO_2 . However, at 6 kPa pO_2 , hypoxia became a limiting factor, reducing C_A from 30 to 50% and total plant DM 26 to 39%, respectively, for hypobaric and ambient pressure plants. Greater tolerance to hypoxia occurred in 25/6 kPa pO_2 than in 101/6 kPa pO_2 plants, as indicated by a greater C_A /DPR ratio; lower DPR; higher RGR and leaf chlorophyll; and non-significant trends of higher C_A , higher SLA and total plant DM. In our study, plants at 25/6 kPa O_2 were sufficiently oxygenated to minimize hypoxic conditions. In a 31-day, low-pressure study with spinach, there were no differences in net C_A and transpiration rate under three different total pressures and pO_2 conditions [101/21, 25/21 and 25/10 kPa pO_2] (Iwabuchi et al. 1996); however, their lowest pO_2 was 10 kPa (48% of ambient sea-level pO_2) compared with 6 kPa (29% of ambient sea-level pO_2) for the current lettuce study.

An advantage of low pressure is that gaseous diffusion increases with the loss of boundary layer resistance (Rygalov et al. 2004). Changes in pressure affect gas diffusion rates and surface boundary layers and change convective transfer capabilities and water evaporation rates. A consistent observation from studies with plants at reduced pressures is increased evapotranspiration rates (Daunicht and Brinkjans 1992, Goto et al. 1996, Richards et al. 2006), even at constant vapor pressure deficits (Rygalov et al. 2004). While increased transpiration rates and potential desiccation losses under hypobaria have been reported (Iwabuchi and Kurata 2003) and increased water usage are concerns with hypobaria (Rygalov et al. 2004), we observed no differences in RWC or any wilting among 25/6 kPa pO_2 or any other treatments with lettuce. The biomass and surface area of lettuce are composed primarily of leaves, making it a prime indicator plant for drought; we observed no symptoms of wilting, and leaves were turgid at the termination of the experiments. With spinach plants, gas exchange rates were the same at 25 or 101 kPa; although there was an enhancement of gas diffusion at 25 kPa, leaves responded phenotypically by decreasing stomatal size and aperture, so that gas exchange rates were unaffected (Iwabuchi and Kurata 2003). In a short-term, 16-h study of *Arabidopsis*, C_A of suboptimal pCO_2 (40 Pa) plants was greater at low than at ambient pressure, but when pCO_2 was in a non-limiting range (70–100 Pa), C_A was insensitive to decreasing pressure (Richards et al. 2006). The latter observation of C_A under non-limiting pCO_2 concurs with our 10-day lettuce study.

As previously mentioned, hypobaria reduces respiration (Burg 2004). A reduction in pressure from 1.5 to 50 kPa opens an oat leaf's stomata in darkness and has the

same effect on other plants (Kirk et al. 1986). Dark stomatal opening at low pressure is most likely because of reduction in pCO_2 (Burg 2004). However, while hypobaric lettuce plants had lower DPR than ambient pressure plants, all plants were grown under elevated (non-limiting) CO_2 levels of 100 Pa $pCO_2 \cong 1000 \mu\text{l l}^{-1} CO_2$ at ambient total pressure. Under ambient total pressure and 100 Pa pCO_2 , soybean plants had a lower DPR and C_A and an increase in carbon gain/loss compared with 25 Pa pCO_2 plants (Griffin et al. 1999). A minor, 7% reduction in DPR occurred under ambient pressure with selected grassland species at 100 Pa pCO_2 (Tjoelker et al. 2001). Under ambient pressure, decreased DPR in elevated CO_2 is likely the result of direct inhibition of the activity of two mitochondrial enzymes: succinate dehydrogenase and cytochrome *c* oxidase; elevated CO_2 partial pressure leads to higher photosynthetic rates in a number of plant species, increasing the demand for reductant to be used in carbon fixation (Griffin et al. 2001). Griffin et al. (2001) reported that despite an increase in mitochondrial number in a number of plant species under elevated CO_2 , DPR decreased, suggesting the rate of ATP production via oxidative phosphorylation decreased. Supraoptimal CO_2 effects on soybean at ambient pressure were reported (Wheeler et al. 1993); stomatal conductance decreased at 100 or 200 Pa CO_2 but increased above 200 Pa CO_2 ; however, seed yield and total biomass were optimal at 100 Pa.

There are advantages of exposing lettuce to higher CO_2 conditions. During the light period cycle, the supplementary set point of pCO_2 was 100 Pa, which is much greater than earth ambient pCO_2 (36 Pa). The higher pCO_2 is in line with conditions most likely to occur under lunar and Martian controlled environment, agricultural systems (Corey et al. 2002, Paul and Ferl 2006). In our study, both C_A and DPR increased, leading to an increase in leaf canopy and biomass of the rapidly growing lettuce plants. The higher DPR is an indicator of the high growth rates of lettuce. Up to 50% of carbon fixed can be lost to respiration, with respiration being highly correlated to RGRs (Frantz et al. 2002, 2004). Most respiration in lettuce is the result of maintenance respiration, not growth respiration (Van Iersel 2003). While we are not suggesting that maintenance respiration per se is undesirable for plant growth, hypobaria reduced DPR at 6 and 12 kPa pO_2 . While the mechanism of reduced DPR under hypobaria is unknown, there may be growth advantages of low total pressure and higher pCO_2 to limit respiration losses, particularly under hypoxic conditions (Corey et al. 1996).

Low pO_2 also reduces the competition for CO_2 for RuBisCO, which lowers the CO_2 compensation point; however, high pCO_2 (100 Pa) substrate levels were used in this study, so photorespiration was not a limiting factor. In

a 24-h study with *Arabidopsis* seedlings under high pCO₂, hypobaria had no significant effect on the differential expression of five key photorespiration enzymes, including RuBisCO; there was less than two-fold changes in regulation of the targeted genes, suggesting no altered regulation within the photorespiratory pathway to hypobaria (Richards et al. 2006).

Photorespiration is nearly eliminated by elevating CO₂ above 100 Pa; hence, electron transport becomes the limiting factor for CO₂ fixation (Farquhar et al. 1980). Even under warm temperatures, photorespiration is reduced by raising pCO₂, with electron transport becoming the rate-limiting factor in CO₂ fixation (Frantz et al. 2002, 2004). Inhibition of photorespiration has been suggested as a reason for enhanced C_A of hypobaric plants (Corey et al. 1996, 2002, Goto et al. 1996, Iwabuchi et al. 1995, 1996). Richards et al. (2006) suggested that differences in photosynthetic drawdown rates with *Arabidopsis* was largely because of pO₂ effects on photorespiration and not because of hypobaria. Thus, it is important to explore the effect of total pressure (low compared with ambient) under varying pCO₂ and pO₂ and their subsequent effect on C_A, photorespiration and DPR

Concluding remarks

In summary, lettuce plants can be grown under hypobaric conditions (25 kPa) and were comparable to ambient pressure plants at 12 and 21 kPa pO₂. Under ambient total pressure, there was a higher DPR compared with low-pressure plants. Hypobaric plants were more resistant to hypoxic conditions (6 kPa pO₂), which reduced gas exchange and plant growth. There was a high correlation among DPR, C_A and plant growth. In a longer term study of 30+ days, hypobaric plants had greater biomass than ambient total pressure (101 kPa) plants (He et al. 2003). The considerably lower DPR (reduced consumption of metabolites) and higher C_A/DPR ratio could lead to greater plant growth (biomass production) under low pressure than under ambient conditions during longer crop production cycles.

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