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Soil CO_2 concentration does not affect growth or root respiration in bean or citrus

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ABSTRACT

Contrasting effects of soil CO_2 concentration on root respiration rates during short-term CO_2 exposure, and on plant growth during long-term CO_2 exposure, have been reported. Here we examine the effects of both short- and long-term exposure to soil CO_2 on the root respiration of intact plants and on plant growth for bean (*Phaseolus vulgaris* L.) and citrus (*Citrus volkameriana* Tan. & Pasq.). For rapidly growing bean plants, the growth and maintenance components of root respiration were separated to determine whether they differ in sensitivity to soil CO_2. Respiration rates of citrus roots were unaffected by the CO_2 concentration used during the respiration measurements (200 and 2000 μmol mol^{-1}), regardless of the soil CO_2 concentration during the previous month (600 and 20 000 μmol mol^{-1}). Bean plants were grown with their roots exposed to either a natural CO_2 diffusion gradient, or to an artificially maintained CO_2 concentration of 600 or 20 000 μmol mol^{-1}. These treatments had no effect on shoot and root growth. Growth respiration and maintenance respiration of bean roots were also unaffected by CO_2 pre-treatment and the CO_2 concentration used during the respiration measurements (200–2000 μmol mol^{-1}). We conclude that soil CO_2 concentrations in the range likely to be encountered in natural soils do not affect root respiration in citrus or bean.

Key-words: *Citrus volkameriana* L.; *Phaseolus vulgaris* L.; citrus; common bean; growth analysis; root respiration; soil CO_2 concentration.

INTRODUCTION

A large body of work describes the effects of elevated atmospheric CO_2 on shoot photosynthesis, shoot respiration and shoot growth. The amount of research being carried out on the effects of elevated atmospheric CO_2 on root growth and root respiration is increasing (e.g. review by Rogers, Runion & Krupa 1994). However, there is still little current research focusing on the effect of soil CO_2 concentrations on root processes, even though soil CO_2 concentrations generally greatly exceed that of the atmosphere. Soil CO_2 concentration is a function of CO_2-producing activity in the soil and soil diffusivity, resulting in concentrations that vary with depth (Johnson *et al.* 1994; Duenas *et al.* 1995), soil water content (500 for dry versus 50 000 μmol mol^{-1} for wet conditions; Bouma *et al.* 1997), soil type (4000–10 000 μmol mol^{-1} CO_2 at 50 cm depth; Duenas *et al.* 1995) and time of year (up to 14 000 μmol mol^{-1} at 15 cm; Johnson *et al.* 1994). The high but variable soil CO_2 concentrations may affect root physiology, as discussed in the next two paragraphs.

Reports on the short-term effects of soil CO_2 on root respiration have been contradictory. Root respiration of seedlings of Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] decreased by a factor of 4–5 when soil CO_2 concentrations were doubled (Qi, Marshall & Mattson 1994). The effect of CO_2 on root respiration was most prominent for concentrations between ≈200 and 2000 μmol mol^{-1}. However, the same CO_2 range had no effect on the root respiration rates of three desert species; their respiration rates decreased only for CO_2 concentrations of 3000 μmol mol^{-1} and higher (Nobel & Palta 1989; Palta & Nobel 1989). These contradictory results clearly indicate that the CO_2 concentrations used during respiration measurements may be critical. Qi *et al.* (1994) hypothesized that root respiration rates of Douglas fir showed a stronger CO_2 response than those of desert succulents, because, in their study, the total root respiration rate of Douglas fir could be ascribed to maintenance, as the seedlings were kept at the light compensation point. We considered this hypothesis unlikely, because slow-growing citrus exhibited no CO_2 response over a range of 400–25 000 μmol mol^{-1} (Bouma *et al.* 1997). However, none of the studies discussed above (Nobel & Palta 1989; Palta & Nobel 1989; Qi *et al.* 1994; Bouma *et al.* 1997) provides the quantitative data on root growth necessary for adequate testing of the hypothesis of Qi *et al.* (1994).

Long-term responses of root respiration to soil CO_2 concentration may differ from the short-term responses discussed above. Respiratory responses to soil CO_2 may be adapted to growth conditions in such a way that the respiratory enzymes are most sensitive to CO_2 concentrations outside the concentration range that is normally experienced by those enzymes (Amthor 1991). Long-term CO_2 effects on respiratory losses by the root may be assessed by growth analysis of plants with roots exposed to different soil CO_2 concentrations. Available reports are also contradictory on the effect of high soil CO_2 concentrations on

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plant growth. For example, increased growth was reported by Arteca, Poovaiah & Smith (1979), whereas reduced growth was reported by Stolwijk & Thimann (1957). Thus, the extent to which roots adapt to long-term exposure to high soil CO_2 concentrations is not clear.

In the present study we determined whether long-term exposure of roots to different soil CO_2 concentrations (600 versus 20 000 μmol mol^{-1}) caused differences in growth rates and affected the short-term respiratory response of roots from intact plants to soil CO_2. Moreover, by separating the growth and maintenance costs of a fast-growing species, we tested the hypothesis of Qi *et al.* (1994) that maintenance respiration is more sensitive to soil CO_2 concentrations than growth respiration. Bean (*Phaseolus vulgaris* L. genotype DOR 364) was used as a representative annual crop with a high relative growth rate. Although common bean has been studied previously by other investigators, results have been contradictory. Both growth stimulation (Bergquist 1964) and growth inhibition (Stolwijk & Thimann 1957) by high CO_2 have been reported. We included citrus (*Citrus volkameriana* Tan. & Pasq.) to allow comparison with our earlier work (Peng *et al.* 1993; Bouma *et al.* 1997) and to obtain measurements in the lower CO_2 range where Qi *et al.* (1994) observed the largest effect on root respiration. Moreover, citrus is valuable as a representative of woody perennials.

MATERIALS AND METHODS

Plant material

Citrus

Scarified seeds of the citrus rootstock Volkamer lemon (*Citrus volkameriana* Tan. & Pasq.) were germinated in flats filled with vermiculite (16 June 1995). Seedlings at the two-true-leaf stage (28 September 1995) were transplanted into respiration cuvettes (45 mm ID PVC tubing; 280 cm^3) with sterilized Candler fine sandy soil (Typic quartzipsamment) collected from the Citrus Research and Education Centre in Lake Alfred, FL, USA. Nutrients were supplied to be non-limiting, by increasing the frequency of addition of Hoagland's solution [5 mol m^{-3} KNO_3, 5 mol m^{-3} $Ca(NO_3)_2$, 5 mol m^{-3} KH_2PO_4, 2 mol m^{-3} $MgSO_4$, 1 mol m^{-3} Fe as FeEDTA and micronutrients; Hoagland & Arnon 1939] with plant size. Greenhouse temperature fluctuated between 20 and 35 °C depending on external weather conditions. Two months before the start of the experiment (start 14 May 1996), citrus seedlings were moved to a greenhouse with better temperature control, as described below for the bean experiments.

Bean

Seeds of common bean (*Phaseolus vulgaris* L. CIAT breeding line DOR 364) were obtained from CIAT in Cali, Colombia. Seeds were surface-sterilized in 7 mol m^{-3} NaOCl and 0·1% Triton X-100 (Sigma Chemical Co., St. Louis, MO) for 10 min, and germinated in 0·5 mol m^{-3} $CaSO_4$ for 36 h at 25 °C. Seedlings were then planted at a depth of 3 cm into respiration cuvettes (76 mm ID PVC tubing; 1400 cm^3). In our first experiment, planting, gas-exchange measurements and destructive harvests were staggered by 2 d, giving a total of six replicates of a single age. Plants were grown in solid-phase-buffered silica sand (Lynch *et al.* 1990) providing a constant availability of 10 mmol m^{-3} P. Twice daily (0700 and 1400 h), pots were irrigated with nutrient solution containing (in mol m^{-3}) 3·1 NO_3, 1·8 K, 1·2 Ca, 1·4 SO_4, 1·0 NH_4, 0·825 Mg, 0·05 Cl, 0·005 Fe-EDTA, 0·002 B, 0·0015 Mn, 0·0015 Zn, 0·000143 Mo and 0·0005 Cu. In the first experiment (22 March 1996–26 April 1996) P was added as 10 mmol m^{-3} KH_2PO_4. This P concentration appeared to be somewhat low, so a higher P concentration (50 mmol m^{-3}) was used in the second experiment (29 April 1996–26 May 1996). All plants were grown in a greenhouse in University Park, PA, USA (40° 85' N, 77° 83' W). Temperature was measured using copper-constantan thermocouples located between the pots (15 cm depth). Temperature ranged from a maximum of 30 °C (day) to a minimum of 20 °C (night). Light was supplemented from 0900 to 1100 h and from 1500 to 1700 h, with an average of 65 ± 15 μmol m^{-2} s^{-1}, and from 1100 to 1500 h, with an average of 110 ± 10 μmol m^{-2} s^{-1}, by 400 W metal-halide bulbs (General Electric Multivapor 400, USA). Maximum midday photosynthetically active photon flux densities reached 1400 μmol m^{-2} s^{-1} on clear days and 500 μmol m^{-2} s^{-1} on days with heavy cloud cover.

Experimental design

Citrus experiment

The respiration cuvettes in which the plants were grown had a removable lid with a small slot cut out for the stem, a drain at the bottom which could also be used as an air inlet, and an air outlet at the side just above the soil surface. One month before the respiration measurements, we closed the top of the respiration cuvettes and filled the slot plus the area around the stem with a flexible sealant (Terostat). Roots of six plants were exposed to air with a low concentration of CO_2 (≈600 μmol mol^{-1}) whereas the roots of the remaining five plants were exposed to air with ≈20 000 μmol mol^{-1} CO_2 (Fig. 1; technical details in legend). A soil CO_2 concentration of 20 000 μmol mol^{-1} is typical of potted citrus in this soil (Bouma *et al.* 1997). To monitor the effectiveness of our CO_2 treatments, gas samples were regularly pulled from the headspace of the respiration cuvettes and CO_2 concentrations determined by gas chromatography (5840 A, Hewlett-Packard, Palo Alto, CA). After 1 month of exposure to the two CO_2 treatments, root respiration was measured on roots exposed for 2 d at 200 μmol mol^{-1} CO_2, 2 d at 2000 μmol mol^{-1}, 2 d at 200 μmol mol^{-1} and 1 d at 2000 μmol mol^{-1}. Respiration rates were estimated with an infrared gas analyser (LI-COR 6252, Lincoln, NE) in differential mode, in an automated system that sampled between 11 respiration cuvettes, with

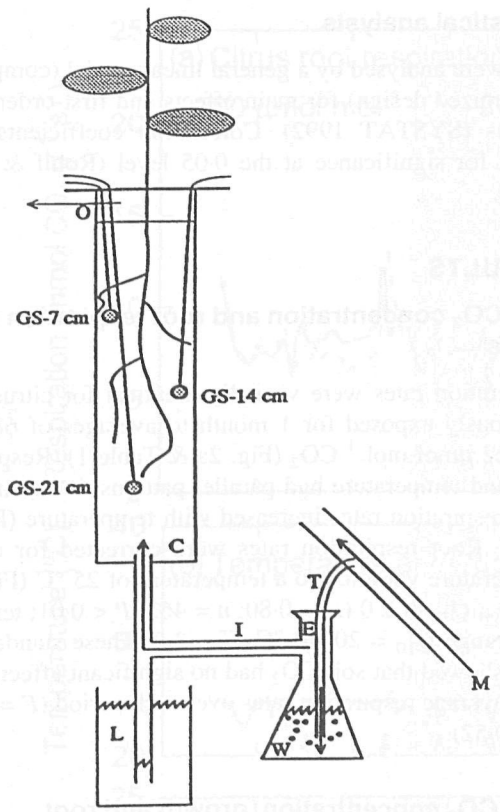

Figure 1. Apparatus to expose roots to different soil CO_2 concentrations. Ambient air mixed (high CO_2) or not mixed (low CO_2) with pure CO_2 was pumped into the bottom of a respiration cuvette (C) filled with sand. The incoming air was bubbled through water (W) to prevent desiccation and to check the flow. An equal flow per pot was obtained by using 1 mm ID tubing (T) from the main gas source (M) to the Erlenmeyer flask (E). After irrigation, the CO_2 perfusion was shut off for an hour to allow drainage through the water lock (L). Drainage could be enhanced by light suction on the air inlet tube (I). Shoots were kept at ambient CO_2 by sealing holes around the stem with a flexible sealant (Terostat) and by keeping the flow rates low (≈50 cm^3 min^{-1}). The air outlet (O) was on the side of the pots, above the soil surface. Soil CO_2 concentrations were measured in gas samples from the head space or from gas-sampling tubes (GS).

a 4 min time interval (Bouma *et al.* 1997). During the respiration measurements, irrigation water was equilibrated with the CO_2 concentration used for those respiration measurements by aerating the irrigation water with air from the 12th outlet of the gas-exchange system. The range of 200 to 2000 μmol mol^{-1} CO_2 was chosen to allow direct comparison to the study of Qi *et al.* (1994), where root respiration of Douglas fir was most affected by CO_2 shifts in this range, with only small responses at concentrations greater than 2000 μmol mol^{-1}. In addition, our infrared gas analyser (LI-COR 6252, Lincoln, NE) was calibrated for CO_2 concentrations only up to 3000 μmol mol^{-1}, preventing continuous respiration measurements at higher CO_2 concentrations without special instrumentation (e.g. Qi *et al.* 1994) or different cuvette designs (Bouma *et al.* 1997).

Bean experiment 1

In the first bean experiment we grew 72 plants with their roots in respiration cuvettes, which were essentially identical to those used for citrus. Two weeks after germination, the plants were divided into three groups of 24 plants each. Roots were exposed to a CO_2 concentration of ≈600 or 20 000 μmol mol^{-1} (Fig. 1; technical details in legend) or were allowed to establish a CO_2 diffusion gradient. Lids were placed on all 48 cuvettes with artificially maintained CO_2 concentrations. The 24 cuvettes that were given the CO_2 diffusion gradient treatment were kept open. The high-CO_2 treatment was preconditioned with 1 week of 5000 μmol mol^{-1}, before an increase to 20 000 μmol mol^{-1}. Soil CO_2 in all treatments was monitored in gas samples pulled from small chambers (top 3 cm^3 of a 10 cm^3 Nalgene syringe) inserted into the soil at 7, 14 and 21 cm depth. CO_2 concentrations were determined by gas chromatograph (5840 A, Hewlett-Packard, Palo Alto, CA; details described previously in Bouma *et al.* 1997). Six plants per treatment were harvested weekly, starting at week 2. Before harvesting at weeks 4 and 5, we measured root respiration at 500 μmol mol^{-1} CO_2, and shoot photosynthesis plus shoot respiration at ambient CO_2 (i.e. between 350 and 400 μmol mol^{-1}). Root respiration was measured as described for citrus (previous section). Shoot respiration (once a day) and photosynthesis (three times a day at the natural illumination level) were measured by briefly sealing the whole shoot in a custom-made 2·5 dm^3 cuvette, attached to an infrared gas analyser (LI-COR 6200, Lincoln, NE) in the closed mode.

Bean experiment 2

In the second experiment with bean we grew 36 plants as described for the first bean experiment. Low (*c.* 600 μmol mol^{-1}) and high (*c.* 20 000 μmol mol^{-1}) CO_2 treatments of the roots were started 14 d after germination, and were monitored using gas samples pulled from small chambers inserted at 14 cm depth. After 29 d we measured root respiration (methods as for citrus) on five high-CO_2-treated and six low-CO_2-treated plants. Respiration was measured for 2 d at 200 μmol mol^{-1}, followed by 2 d at 2000 μmol mol^{-1} and ending with 2 d at 200 μmol mol^{-1} CO_2. Irrigation water was equilibrated with the CO_2 concentration used for the respiration measurement. After 47 d, we repeated the root respiration measurements, but with the soil CO_2 concentrations in the opposite order (i.e. 2 d each at 2000, 200 and 2000 μmol mol^{-1} CO_2). Matched plants were harvested at the beginning ($n = 3$ per treatment) and end ($n = 6$ per treatment) of both sets of respiration measurements.

All respiration measurements were made on intact, undisturbed roots in soil. The inevitable contribution of microbial respiration to observed respiration rates was assumed to be negligible, as we used sterilized sandy soil which was sieved to remove organic matter (after Bouma *et al.* 1997 and references therein). All respiration rates were expressed per gram root dry weight. For bean, root

dry weight was corrected for changes over time when respiration measurements lasted several days (i.e. bean experiment 2). Actual dry weights were calculated by combining the relative growth rate based on all root weights with individual root dry weights at harvest. Such a correction was not necessary for citrus, because of its slow growth rate.

Our experiment was designed to maintain similar environmental conditions between CO_2 treatments. For example, frequent watering resulted in a similar pH in the leachate samples of all CO_2 treatments. The CO_2 concentration around shoots was regularly tested with a portable IRGA, and showed no effect of soil CO_2 treatments on the shoot environment.

Harvests and chemical analyses

Leaf area, root length, and biomass of leaves, stem, and roots were determined by destructive harvest. Roots were excavated by rinsing the sand with de-ionized water. For bean, the entire root system was cut into fragments up to 3 cm long. After vigorously mixing the root pieces, a random subsample of ≈100 segments was collected and exposed to 0·16 g dm^{-3} neutral red dye (Sigma Chemical Co., St. Louis, MO) for 1 h prior to scanning. For citrus, fine roots were separated from the woody tap root and subsampled as described for bean. Leaves and roots were scanned using a flat bed scanner (HP ScanJet II, resolution = 140 dots mm^{-2}, Hewlett Packard, USA). Leaf area, root length, and root diameter distribution were calculated using image analysis software (Delta-T SCAN, Delta-T Devices Ltd, Cambridge, UK). Plant material was freeze-dried at –60 °C for 72 h (bean experiment 1) or dried at 70 °C for 48 h (bean experiment 2) to 1 week (citrus). Drying periods were chosen to be long enough to obtain a constant dry weight. Root material harvested at day 28 (bean experiment 1) was analysed for C, H, N and O content (Fison Elemental Analyser EA1108, Fison Instruments, Italy).

Estimating maintenance respiration

Construction costs of roots [nmol CO_2 (g DW_{root})$^{-1}$] were calculated from elemental composition (after McDermitt & Loomis 1981). Respiratory costs of growth [nmol CO_2 (g DW_{root})$^{-1}$ s^{-1}] were derived by multiplying root construction costs with the relative growth rate of the root [g DW_{new} (g DW_{old})$^{-1}$ s^{-1}]. Net uptake rate of nitrate [mol N (g DW_{old})$^{-1}$ s^{-1}] was estimated as the product of the growth rate (g DW_{new} s^{-1}) and the N content of the plant [mol N (g DW)$^{-1}$], and divided by the root dry weight (g DW_{old}). Subsequently, costs for ion uptake were obtained by multiplying the net uptake rate of nitrate with the specific costs of nitrate uptake [1·2 mol CO_2 (mol N)$^{-1}$ s^{-1}, based on the range reviewed in tables 2, 3 and 4 of Bouma, Broekhuysen & Veen 1996, assuming a respiratory coefficient of 1·1 mol CO_2 (mol O_2)$^{-1}$]. Subtraction of respiratory costs of growth and ion uptake from overall respiration gave an estimate of respiratory costs for maintenance [nmol CO_2 (g DW_{root})$^{-1}$ s^{-1}] (after Peng *et al.* 1993).

Statistical analysis

Data were analysed by a general linear model (completely randomized design) for main effects and first-order interactions (SYSTAT 1992). Correlation coefficients were tested for significance at the 0·05 level (Rohlf & Sokal 1981).

RESULTS

Soil CO_2 concentration and root respiration of citrus

Respiration rates were virtually identical for citrus roots previously exposed for 1 month to averages of 686 and 21 322 μmol mol^{-1} CO_2 (Fig. 2a & Table 1). Respiration rate and temperature had parallel patterns, indicating that root respiration rates increased with temperature (Figs 2a & b). Root respiration rates were corrected for diurnal temperature variation to a temperature of 25 °C (Fig. 2c), using a Q_{10} of 2·0 (r^2 = 0·80; n = 462; $P < 0·01$; temperature range Q_{10} = 20–40 °C; Fig. 3a). These standardized data showed that soil CO_2 had no significant effect on the root average respiration rates over each period (F = 0·423; P = 0·52).

Soil CO_2 concentration, growth and root respiration of bean

Growth rates of roots and shoots were not affected by soil CO_2 concentration (i.e. no difference in the increase in dry weight development over time; Fig. 4; F_{leaf} = 0·178; P_{leaf} > 0·8; F_{stem} = 0·248; P_{stem} > 0·7; F_{root} = 0·312; P_{root} > 0·7). Soil CO_2 increased with depth for non-treated bean roots, presumably due to the diffusion gradient (Table 1). Depth did not affect soil CO_2 concentration for high- and low-CO_2 treatments, due to air-flow through the pot. Regardless of these different soil CO_2 concentrations, all beans grew with an equal exponential growth rate for the whole period studied (Fig. 4; note log scale for y-axis).

Growing beans at different soil CO_2 concentrations had no effect on root respiration rates determined at 500 μmol mol^{-1} CO_2 or net assimilation rate (NAR) determined at ambient CO_2 (bean experiment 1; Fig. 5; F_{resp} = 2·504; P_{resp} > 0·09; F_{NAR} = 0·066; P_{NAR} > 0·90). Construction costs of bean roots harvested at day 28 were also unaffected by CO_2 treatment (Table 2). The absence of an effect of soil CO_2 concentration on (a) the overall root respiration rate, (b) the root growth rate, (c) root construction costs and (d) the nitrogen content of the plant indicates that maintenance respiration was also not affected by soil CO_2 (Table 2). Although $R_{maintenance}$ for the low-CO_2-treated plants may appear to be higher, this apparent difference was only due to the non-significant variation in R_{total} that was carried over in the calculation of $R_{maintenance}$.

In the second bean experiment, root respiration rates were measured for several days on single plants, while alternating soil CO_2 concentration between 200 and 2000 μmol mol^{-1}. Plotting of respiration over temperature

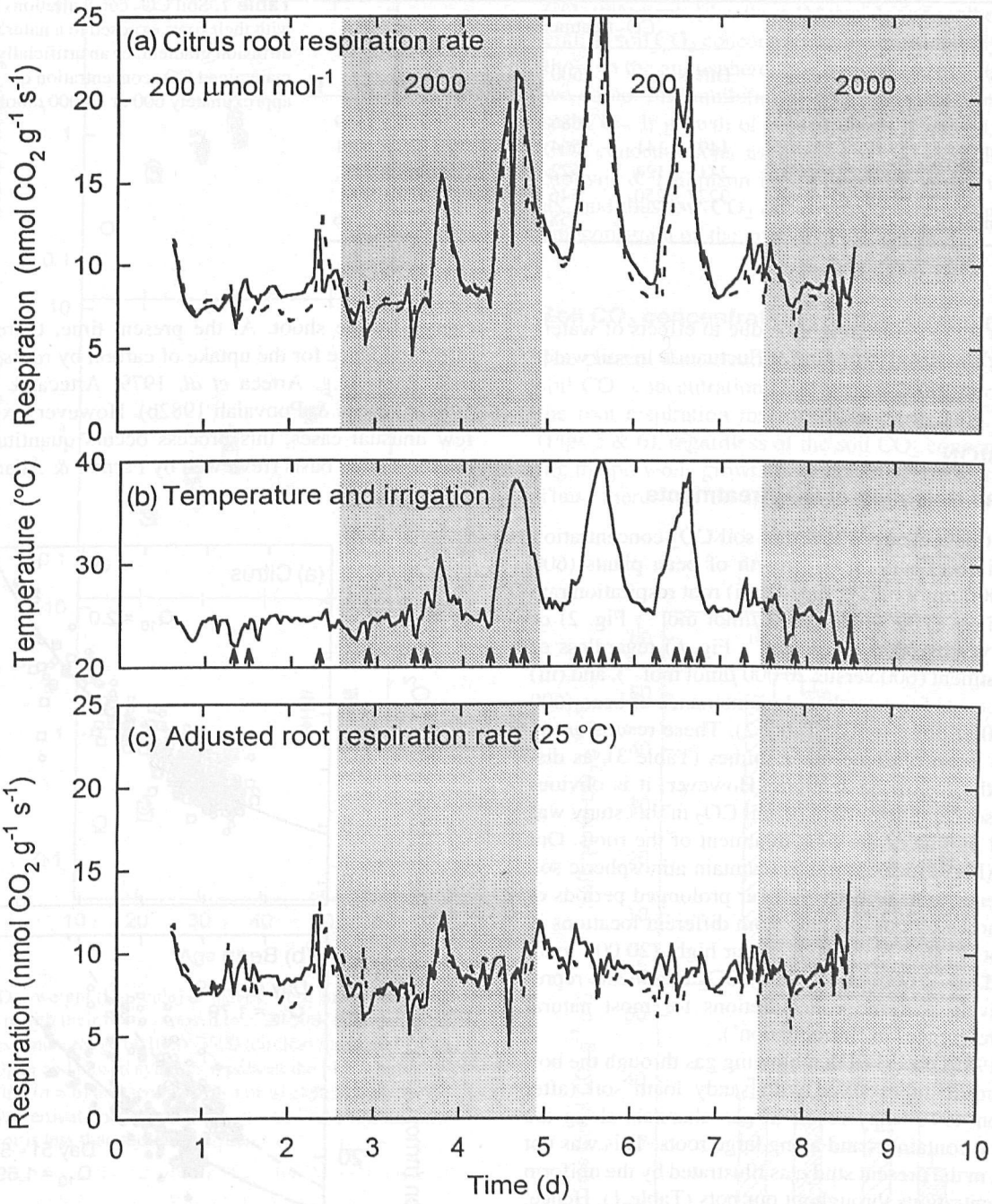

Figure 2. Respiration rates of citrus roots previously exposed to high (continuous line; $n = 5$) and low (dashed lines; $n = 6$) CO_2, measured at alternating 200 (light area) and 2000 (dark area) μmol mol^{-1} CO_2 (a & c). Root respiration was standardized to a temperature of 25 °C (c), to remove effects of temperature fluctuations (b, continuous line). Effects of watering (b, arrows) might explain remaining variation (c). Standard errors were not shown to enhance visibility, but averaged 1·19 (SD = 0·52; $n = 231$) and 1·26 (SD = 0·63; $n = 231$) for respiration measured on citrus roots previously exposed to high- and low-CO_2 concentrations, respectively.

yielded a Q_{10} of 1·79 ($r^2 = 0·79$; $n = 120$; $P < 0·01$) to 1·69 ($r^2 = 0·71$; $n = 177$; $P < 0·01$), depending on plant age (temperature range Q_{10} = 22–38 °C; Fig. 3b). The time dependence of the Q_{10} of root respiration was presumably due to a reduction of the root respiration rate per unit biomass with increasing root age (Fig. 6; $F_A = 27·9$; $P_A < 0·001$; $F_B = 6·02$; $P_B < 0·01$). This reduction of root respiration rates with increasing root age was independent of the soil CO_2 concentration at which the root respiration was determined. Thus, soil CO_2 concentration again did not affect root respiration rates of bean plants (Fig. 6; $F_A = 0·406$; $P_A > 0·52$; $F_B = 0·090$; $P_B > 0·76$), regardless of previous CO_2 treatment (Fig. 6; $F_A = 0·529$; $P_A > 0·47$; $F_B = 0·012$; $P_B > 0·91$). Short-term fluctuations of root respiration

Table 1. Soil CO_2 concentrations for plants with their roots exposed to a natural CO_2 diffusion gradient, or an artificially maintained CO_2 concentration of approximately 600 or 20 000 μmol mol^{-1}

Species	Depth (cm)	CO_2 treatments (μmol mol^{-1})		
		Diffusion	600	20 000
citrus	headspace		686 ± 30	21 322 ± 1369
bean (experiment 1)	7	1497 ± 141	564 ± 20	20 379 ± 1011
	14	2415 ± 124	622 ± 42	20 144 ± 938
	21	3227 ± 250	546 ± 34	18 956 ± 909
bean (experiment 2)	14		558 ± 25	25 013 ± 1276

adjusted for temperature might be due to effects of watering, although we tried to minimize fluctuation in soil water content.

DISCUSSION

Effects and reliability of CO_2 treatments

The present data clearly show that soil CO_2 concentration had negligible effects on (i) growth of bean plants (600 versus 20 000 μmol mol^{-1}; Fig. 4), (ii) root respiration rate of either citrus (200 versus 2000 μmol mol^{-1}; Fig. 2) or bean (200 versus 2000 μmol mol^{-1}; Fig. 6) regardless of the pre-treatment (600 versus 20 000 μmol mol^{-1}), and (iii) respiratory costs for growth and maintenance of bean (600 versus 20 000 μmol mol^{-1}; Table 2). These results are in contrast to those of some other studies (Table 3), as discussed in the following sections. However, it is obvious that the absence of any effect of soil CO_2 in this study was not caused by inaccurate CO_2 treatment of the roots. Our apparatus (Fig. 1) was found to maintain atmospheric soil CO_2 concentrations effectively over prolonged periods of time, as shown by gas samples from different locations in various pots (Table 1). Moreover, our high- (20 000 μmol mol^{-1}) and low- (600 μmol mol^{-1}) CO_2 treatments represent relatively extreme concentrations for most natural soils (see references in 'Introduction').

Good (1985) observed that blowing gas through the bottom of acrylic tubes filled with sandy loam soil (after Williamson 1970) may result in gas channels along the walls of the containers and along large roots. This was not a problem in the present study, as illustrated by the uniform CO_2 concentrations throughout our pots (Table 1). Hence, we did not use the method of Good (1985), in which initially all air is displaced with water, whereafter the water is displaced by the desired gas mixture.

Soil CO_2 concentrations and plant growth

Effects of high CO_2 concentrations in the soil on plant growth have been studied since the beginning of this century (for an early review see Livingston & Beall 1934). Soil CO_2 was expected to be utilized as a source of inorganic carbon for photosynthesis. However, studies of root and shoot growth as a function of soil CO_2 concentration yielded contradictory results (Table 3). Naturally, any CO_2 fixation in the roots will always depend on the acquisition of light energy by the shoot. At the present time, there is some direct evidence for the uptake of carbon by roots of terrestrial plants (e.g. Arteca *et al.* 1979; Arteca & Poovaiah 1982a; Arteca & Poovaiah 1982b). However, except for a few unusual cases, this process occurs quantitatively on only a limited basis (reviewed by Farmer & Adams 1996).

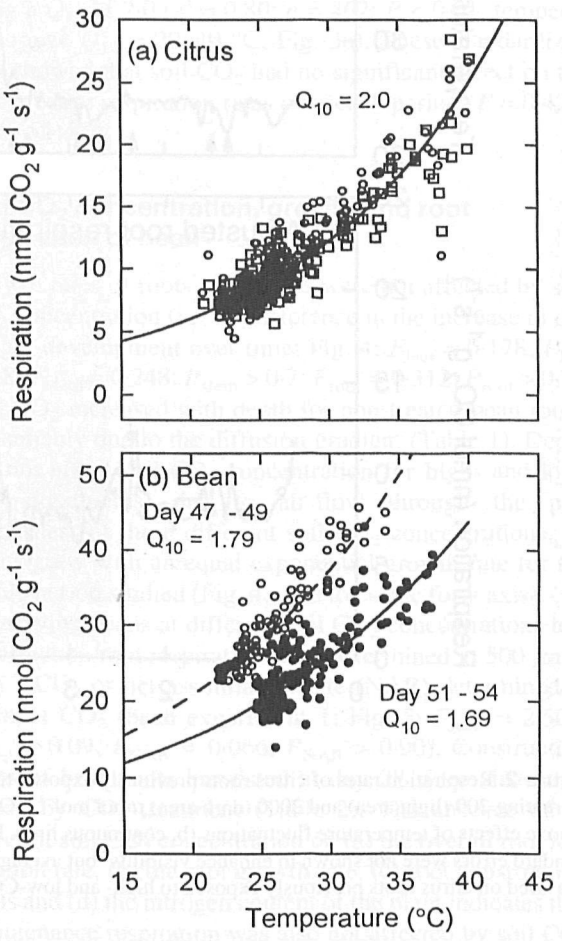

Figure 3. Relationship of root respiration rate (r; nmol CO_2 g^{-1} s^{-1}) with temperature (T; °C) for (a) citrus and (b) bean. For citrus, a Q_{10} value was obtained by exponential regression on all data, regardless of previous exposure to high (squares) or low (circles) CO_2 ($r = 1{\cdot}46^{(0.0708T)}$ with $r^2 = 0{\cdot}80$; $n = 462$; $P < 0{\cdot}01$). As respiration rate of bean roots decreased over time, Q_{10} values were determined separately for days 47–49 (open symbols; $r = 6{\cdot}48^{(0.058T)}$ with $r^2 = 0{\cdot}79$; $n = 120$; $P < 0{\cdot}01$) and days 51–54 (closed symbols; $r = 5{\cdot}39^{(0.052T)}$ with $r^2 = 0{\cdot}71$; $n = 177$; $P < 0{\cdot}01$).

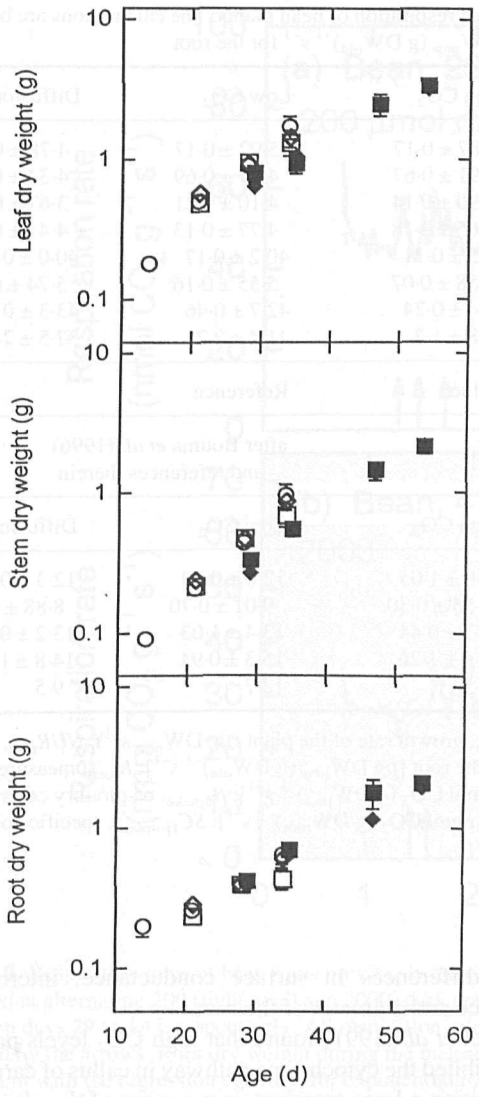

Figure 4. Dry weight (log scale) of leaves, stem and roots of bean plants grown with their roots exposed to *c.* 20 000 (diamonds), *c.* 600 (squares) and a range of 1000–3500 (circles) μmol mol^{-1} CO_2 (Table 1). Open and closed symbols represent the plants harvested during the first ($n = 6$) and second ($n = 3$ or 6) experiments, respectively. Vertical lines indicate the standard errors, unless the standard error is less than the symbol size.

Therefore, we are able to measure root respiration of intact plants by their CO_2 production; such measurements would be problematic if soil CO_2 was widely utilized as a source of inorganic carbon by the plant.

In the present study, soil CO_2 concentration had no effect on the growth of bean plants (Fig. 4), and no effect on the respiratory costs for growth and maintenance of bean (Table 2). The reason why these results and those of some of the earlier studies (Table 3) are contradictory is not clear. Perhaps some secondary effects occurred in some studies. Soil CO_2 concentration may affect bicarbonate formation and solution pH, which are known to affect many aspects of soil chemistry, notably nutrient availability. In the present study, we tried to maintain similar environmental conditions between CO_2 treatments (details in 'Materials and methods'). In general, as soil CO_2 concentrations are considerably higher than those in the atmosphere, it is not particularly surprising that we do not find inhibition of root respiration and growth by soil CO_2. If growth of certain plants is indeed inhibited by CO_2 concentrations as low as 10 000 μmol mol^{-1} (e.g. Stolwijk & Thinmann 1957), then soils with low soil porosity, and thus low CO_2 diffusivity, may represent an important constraint on the growth of such plants.

Soil CO_2 concentrations and root respiration

The present data clearly show that there was no effect of soil CO_2 concentration (200 versus 2000 μmol mol^{-1}) on the root respiration rate of either citrus (Fig. 2) or bean (Figs 5 & 6), regardless of the soil CO_2 concentration during the previous growth period (600–20 000 μmol mol^{-1}). Thus, there was no indication that root respiration was

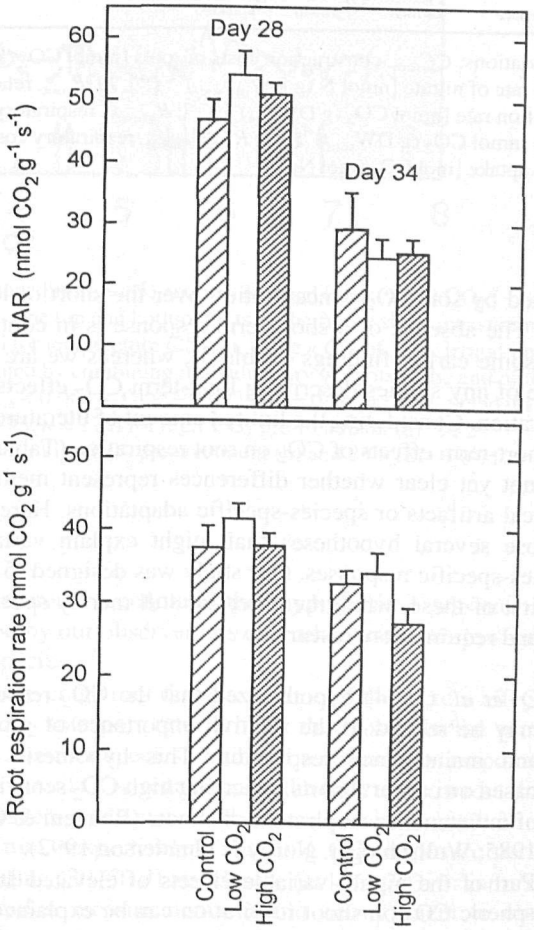

Figure 5. Shoot photosynthesis and root respiration rates of bean plants grown with their roots exposed to *c.* 20 000 (dark bar), *c.* 600 (white bar) and a range of 1000–3500 (intermediate bar) μmol mol^{-1} CO_2 (Table 1). Measurements were taken at plant ages of 28 and 34 d. The standard error is indicated at the top of each bar ($n = 6$). CO_2 treatments were not significantly different ($P = 0{\cdot}05$).

Table 2. Measured, literature and calculated parameters used to estimate maintenance respiration of bean plants. The calculations are based on a plant growth rate of 1·49 μg DW_{new} s^{-1} and a relative growth rate of 0·649 μg DW_{new} $(g\ DW_{old})^{-1}$ s^{-1} for the root

Observed parameters (units)		Tissue	High CO_2	Low CO_2	Diffusion
N content	(%)	leaves	4·87 ± 0·17	5·02 ± 0·17	4·78 ± 0·17
		stem	4·91 ± 0·67	4·91 ± 0·69	4·34 ± 0·38
		root	3·90 ± 0·14	4·10 ± 0·21	3·67 ± 0·16
		plant	4·65 ± 0·18	4·77 ± 0·13	4·41 ± 0·14
C content	(%)	root	39·5 ± 0·11	40·2 ± 0·17	40·0 ± 0·26
H content	(%)	root	5·58 ± 0·07	5·55 ± 0·16	5·74 ± 0·06
O content	(%)	root	43·4 ± 0·24	42·7 ± 0·46	43·3 ± 0·15
R_{total}	[nmol CO_2 $(g\ DW_{root})^{-1}$ s^{-1}]	root	37·8 ± 1·7	41·4 ± 2·2	37·5 ± 2·9

Literature parameter (units)		Value	Reference
$SC_{H\text{-uptake}}$	(mol CO_2 (mol N)$^{-1}$	1·2	after Bouma *et al.* (1996) and references therein

Calculated parameter (with equation)		High CO_2	Low CO_2	Diffusion
NUR_{root}	$[GR_{plant} \times [N]_{plant} / (1400 \times DW_{root})]$	13·0 ± 1·05	12·8 ± 0·79	12·3 ± 0·90
CC_{root}	(after McDermitt & Loomis 1981)	8·55 ± 0·30	9·01 ± 0·70	8·88 ± 0·27
R_{growth}	$(RGR_{root} \times CC_{root})$	12·7 ± 0·44	13·4 ± 1·03	13·2 ± 0·40
R_{uptake}	$(NUR_{root} \times SC_{H\text{-uptake}})$	15·6 ± 1·26	15·3 ± 0·94	14·8 ± 1·08
$R_{maintenance}$	$[R_{total} - (R_{growth} + R_{uptake})]$	9·5	12·7	9·5

Abbreviations: CC_{root}, construction costs of roots [mmol CO_2 $(g\ DW_{root})^{-1}$]; GR_{plant}, growth rate of the plant (μg DW_{new} s^{-1}); NUR_{root}, net uptake rate of nitrate [nmol N $(g\ DW_{old\ root})^{-1}$ s^{-1}]; RGR_{root}, relative growth rate of the root [μg DW_{new} $(g\ DW_{old})^{-1}$ s^{-1}]; R_{total}, measured root respiration rate [nmol CO_2 $(g\ DW_{root})^{-1}$ s^{-1}]; R_{growth}, respiratory costs of growth [nmol CO_2 $(g\ DW_{root})^{-1}$ s^{-1}]; R_{uptake}, respiratory costs of ion uptake [nmol CO_2 $(g\ DW_{root})^{-1}$ s^{-1}]; $R_{maintenance}$, respiratory costs for maintenance [nmol CO_2 $(g\ DW_{root})^{-1}$ s^{-1}]; $SC_{H\text{-uptake}}$, specific costs of nitrate uptake [mol CO_2 $(mol\ N)^{-1}$].

affected by soil CO_2 concentration over the short or long term. The absence of a short-term response is in contrast with some earlier findings (Table 3), whereas we are not aware of any studies describing long-term CO_2 effects on respiration. Considering the limited amount of literature on the short-term effects of CO_2 on root respiration (Table 3), it is not yet clear whether differences represent methodological artifacts or species-specific adaptations. Here we propose several hypotheses that might explain variable species-specific responses. Our study was designed to test the first of these, while the other are still merely speculative and require more research.

(1) Qi *et al.* (1994) hypothesized that the CO_2 response may be related to the relative importance of growth and maintenance respiration. This hypothesis was based on earlier reports indicating high CO_2 sensitivity of maintenance respiration of shoots (Reuveni & Gale 1985; Wullschleger, Norby & Gunderson 1992).

(2) Part of the highly variable effects of elevated atmospheric CO_2 on shoot respiration can be explained by separating the generally inhibiting direct effects from the variable indirect effects (Amthor 1991; Amthor, Koch & Bloom 1992). The occurrence of direct versus indirect effects depends on the CO_2 transport rate through the tissue (Amthor 1991). Hence, a species-specific CO_2 response of root respiration may be due to differences in surface conductance, internal air space and diameter of the root.

(3) Palet *et al.* (1991) found that high CO_2 levels partially inhibited the cytochrome pathway in callus of carnation, eliciting a large transient engagement of the alternative oxidase. Hence, the species-specific CO_2 response of root respiration may be due to species-specific variation in the relative contribution of cytochrome and the alternative non-phosphorylating pathway.

(4) Amthor (1991) describes several mechanisms that might enable tissue-specific regulation of the CO_2 sensitivity of enzymes. Such tissue specificity might vary among species to enable species to adapt to their native environments.

(5) The CO_2 sensitivity of respiration may only occur in acid soils, since, in soils that are buffered at a relative high pH, a relatively large portion of soil CO_2 may be transformed into HCO_3^- and CO_3^{2} (H. Lambers, University of Utrecht, the Netherlands, private communication). Hence, species-specific differences in rhizosphere pH modification may be an important factor in determining the sensitivity of a species to soil CO_2 concentrations.

(6) The CO_2 sensitivity of soil respiration may be proportional to the contribution of microbial activity, if plants are relatively insensitive to soil CO_2 concentrations (present data) and microorganisms are sensitive.

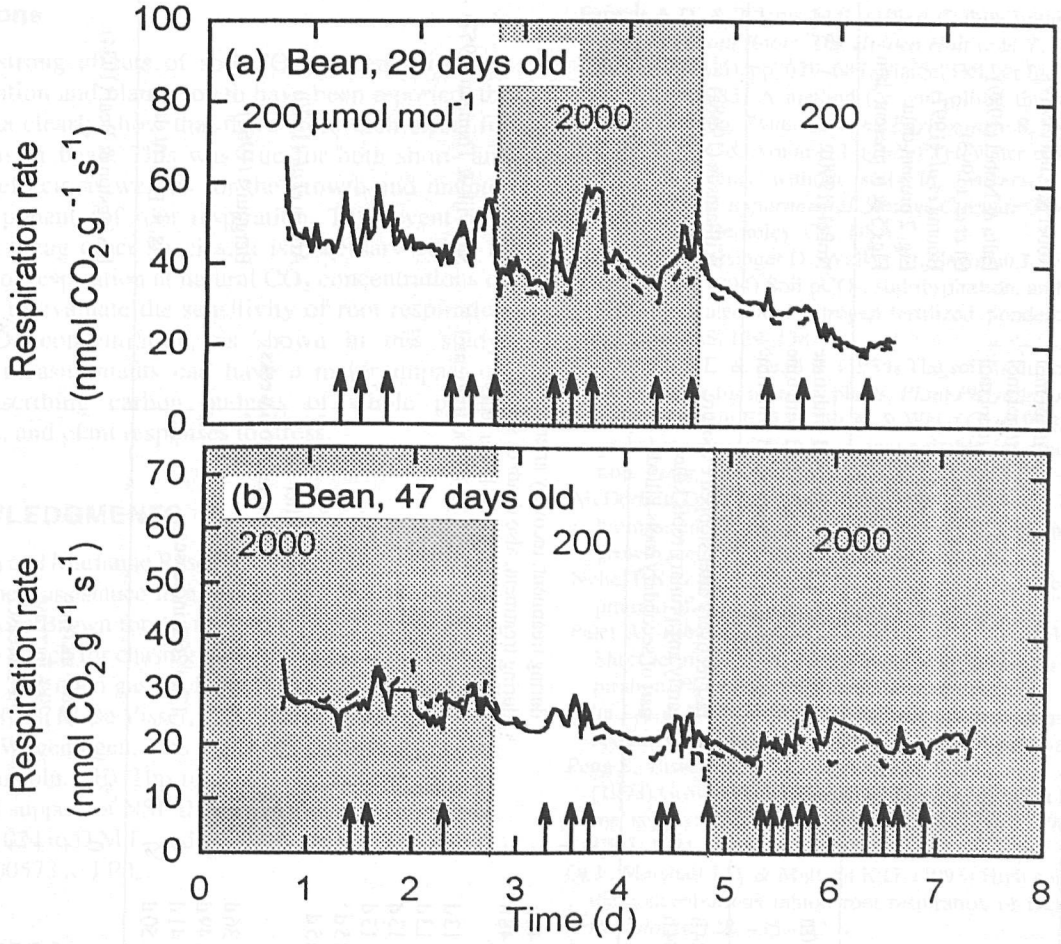

Figure 6. Respiration rates of bean roots previously exposed to high (continuous lines; $n = 5$) and low (dashed lines; $n = 6$) CO_2, measured at alternating 200 (light area) and 2000 (dark area) μmol mol^{-1} CO_2. The top and bottom parts of the figure show measurements started on days 29 and 47, respectively. All respiration rates were standardized for temperature (25 °C), using a Q_{10} of 1·74. Irrigations are indicated by the arrows. Root dry weight during the measurements was calculated by combining individual dry weights at the end of the experiment with the regression equation for exponential root growth ($\ln DW_{root} = 0{\cdot}062 \times \text{Time} - 2{\cdot}6978$; $r^2 = 0{\cdot}86$; $n = 95$). Standard errors were not shown to enhance visibility, but were on average 2·55 (SD = 0·95; $n = 175$) for high-CO_2 pre-treatment (a), 2·82 (SD = 1·16; $n = 174$) for low-CO_2 pre-treatment (a), 3·33 (SD = 0·98; $n = 180$) for high-CO_2 pre-treatment (b) and 2·68 (SD = 0·71; $n = 180$) for low-CO_2 pre-treatment (b).

Hypothesis 1 is not supported by the present data. Although we measured relatively large citrus seedlings with negligible growth compared to the amount of biomass to be maintained, we did not find a CO_2 response of root respiration. This finding is in agreement with our earlier work on citrus seedlings (Bouma *et al.* 1997), but this time for the CO_2 range (200 and 2000 μmol mol^{-1}) in which Qi *et al.* (1994) observed the most prominent CO_2 effect on root respiration of Douglas fir. The rapid growth of bean makes this species more suitable and interesting for testing hypothesis 1. Separation of the components of root respiration showed that neither growth respiration nor maintenance respiration was affected by soil CO_2 concentrations (Table 2). Although the pattern in Table 2 may seem to support hypothesis 1, this apparent pattern was merely due to the non-significant variation in R_{total} that was carried over in the calculation of $R_{maintenance}$. Therefore, we conclude that hypothesis 1 was not supported by our observations on both a fast- and slow-growing species.

The average root diameter is one of the factors affecting the CO_2 transport rate through the tissue. In the present study, neither species exhibited a respiratory response to shifts in soil CO_2, regardless of the difference in root diameter (average diameters of bean and citrus are 0·35 and 0·57 mm, respectively). The long CO_2 exposure periods preceding (30–50 d) and during (at least 20 h or more) the respiration measurements should be sufficient to obtain equilibrium between the CO_2 concentration in the soil and the root tissues, especially as Qi *et al.* (1994) observed a strong response after only 4 h exposure. This failure to observe differences between bean and citrus provides no support for (although cannot disprove) hypothesis 2 or most of the other hypotheses.

Table 3. The response of various root and plant parameters to exposure of the root to high CO_2 concentrations, for various species

Physiological trait	Species	CO_2 range (μmol mol^{-1})	Exposure (h or d)	Response + / 0 / –	Comments	References
Respiration	*Optunica ficus-indica*	300–20 000	4 h	0 / –	decrease only if $[CO_2] > 2500$ μmol mol^{-1}	Nobel & Palta (1989)
	Ferocactus acanthodes		4 h	0 / –	decrease only if $[CO_2] > 2500$ μmol mol^{-1}	
	Agave deserti	300–30 000	4 h	0 / –	decrease only if $[CO_2] > 2500$ μmol mol^{-1}	Palta & Nobel (1989)
	Douglas fir	100–7000	3 h	–	decrease over whole range	Qi *et al.* (1993)
	Citrus	400–25 000	46 h	0		Bouma *et al.* (1997)
	Citrus	400–2000	48 h	0		Fig. 2 present data
	Bean	400–2000	48 h	0		Figs 5 & 6, present data
Vitality	*Agave deserti*	5000, 20 000 and 100 000	0 to 10 h	–	uptake of neutral red decreased with time	Nobel (1990)
	Optunica ficus-indica	5000, 20 000 and 100 000		–	uptake of neutral red decreased with time	
	Ferocactus acanthodes	5000, 20 000 and 100 000		–	uptake of neutral red decreased with time	
Cell division	*Vicia faba* L.	0 (with 1 to 10% O_2)	4 to 24 h	–	fast recovery after stopped exposure	Williamson (1968a)
		110 000–200 000 (with 1 to 10% O_2)	4 to 24 h	0 / –	rarely any primary roots killed, but part of secondary roots killed	
Plant growth	*Nicotiana tabacum* L.	0 (with 2·5% O_2)		0		Williamson (1968b)
		185 000 (with 2·5% O_2)		0		
		0 (with 1% O_2)	24 to 48 h	–	during treatment; recovery afterwards	
		200 000 (with 1% O_2)	24 to 48 h	–	during treatment; also death of several root tips and chlorosis on lower leafs	
	Pisum sativum	0–70 000	10 to 13 d	0 / –	reduction only if $[CO_2] > 10\,000$ μmol mol^{-1}	Stolwijk & Thimann (1957)
	Phaseolus vulgarus	65 000	10 to 13 d	–		
	Vicia Faba	65 000	10 to 13 d	–		
	Helianthus annuus	65 000	10 to 13 d	–		
	Avena sativa	0–70 000	7 to 15 d	0		
	Hordeum vulgare	65 000	7 to 15 d	0		
	Solanum tuberosum	450 000	12 h	+	increased dry matter after 2 d	Arteca *et al.* (1979)
					increased tuberization after 3 to 6 weeks	
	Bean		14 to 36 d	+	8 to 41% increase	Bergquist (1964)
	Tomato		12 to 49 d	+	7 to 70% increase	
	White mustard		35 to 41 d	+	17 to 26% increase	
	Bean	400–25 000	up to 50 d	0		Fig. 4, present data
Shoot elongation	*Coleus blumei* (red)		45 d	0		Livingston & Beal (1934)
	Coleus blumei (green)		45 d	+	26% increase	
	Lysopersicum esculentum		24 d	+	29% increase	
	Cracaena sanderiana		45 d	+	9% increase	
	Lupinus albus		45 d	+	225% increase	
Metabolism	*Solanum tuberosum*	200 000	10 h	+	activity of PEP carboxylase in roots	Arteca & Poovaiah (1982b)

Conclusions

Although strong effects of soil CO_2 concentrations on root respiration and plant growth have been reported, the present data clearly show that there is no such effect for either citrus or bean. This was true for both short- and long-term effects as well as for the growth and maintenance components of root respiration. To prevent artifacts when using other species, it is necessary either to measure root respiration at natural CO_2 concentrations or thoroughly to evaluate the sensitivity of root respiration to soil CO_2 concentration, as shown in this study. Erroneous measurements can have a major impact on models describing carbon budgets of whole plants, ecosystems, and plant responses to stress.

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