Interactive effects of inorganic phosphate nutrition and carbon dioxide enrichment on assimilate partitioning in barley roots

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The combined effects of inorganic phosphate (Pi) insufficiency and CO_2 enrichment on metabolite levels and carbon partitioning were studied using roots of 9-, 13- and 17-day-old barley seedlings (*Hordeum vulgare* L. cv. Brant). Plants were grown from seed in controlled environment chambers providing 36 ± 1 Pa (ambient) or 100 ± 2 Pa (elevated) CO_2 and either 1.0 mM (Pi sufficient) or 0.05 mM (Pi insufficient) Pi. When values were combined for both Pi treatments, plants grown under enhanced CO_2 showed increased root dry matter, adenylates (ATP + ADP), glutamine and non-structural carbohydrates other than starch. In contrast with shoots, enhanced CO_2 partially reversed the inhibition of root dry matter formation imposed by Pi insufficiency. The Pi-insufficient treatment also increased sucrose, glucose and fructose levels in barley roots. The Pi and CO_2 treatments were additive, so that the highest soluble carbohydrate levels were observed in roots of Pi-insufficient plants from the elevated CO_2 treatment. Pi limitation decreased dry matter formation, acid-extractable Pi, nitrate, hexose-phosphates, glutamate, glutamine and acid invertase activity of barley roots in plants grown in both ambient and elevated CO_2. Adenylate levels in roots were unaffected by the moderate Pi insufficiency described here. Thus, the reduced hexose-phosphate levels of Pi-insufficient roots were not likely to be the result of low adenylate concentrations. The above results suggest that the capacity of barley roots to utilize carbohydrates from the shoot is inadequate under both Pi-insufficient and CO_2-enriched treatments. In addition, the Pi and CO_2 treatments used here alter the nitrogen metabolism of barley roots. These findings further emphasize the importance of avoiding nutrient stress during CO_2 enrichment experiments.

Introduction

Increasing atmospheric CO_2 levels are expected to increase the rates of carbohydrate synthesis and the biomass yields of plants within terrestrial ecosystems, primarily through the stimulation of net photosynthesis and the inhibition of photorespiration (Bowes 1993). This prediction is based on the observation that current atmospheric CO_2 levels do not saturate the photosynthesis rates of 95% or more of all terrestrial plant species (Stitt 1991). Over longer periods, the growth responses of plants to elevated atmospheric CO_2 are directly influenced by environmental factors, such as water and nutrient availability (Stitt and Krapp 1999). Nutritional status is particularly important because the increased growth rates of CO_2-enriched terrestrial plants create an enhanced demand for mineral nutrients (Campbell and Sage 2002). Plant growth rates under moderate nutrient insufficiency are usually enhanced by atmospheric CO_2 enrichment, but not to the same extent as under nutrient-sufficient conditions (Cure et al. 1988, Cardoso-Vilhena and Barnes 2001, Ziska 2003). A possible explanation for this observation is that plants utilize nutrients more efficiently under enhanced rather than ambient CO_2 (Stitt and Krapp 1999).

Phosphorus is a vital macronutrient for plant growth that functions in the formation of high-energy bonds, in the structure of certain biomolecules and membranes, and as an integral component of many metabolic reactions and signal transduction pathways (Duff et al. 1989,

Ticconi et al. 2001). The available supply of inorganic phosphate (Pi) can limit plant productivity in both agricultural and natural ecosystems. Consequently, plants have developed numerous morphological, biochemical and molecular mechanisms to cope with Pi insufficiency (Theodorou and Plaxton 1993). For example, Fredeen et al. (1989) have reported that Pi deficiency drastically impairs shoot and leaf development in comparison with root growth of hydroponically grown sugarbeet plants. In addition to favouring root growth, CO_2 enrichment increases the rates of Pi acquisition by stimulating organic acid exudation (Barrett and Gifford 1999). Pi limitation also severely inhibits leaf rates of photosynthesis, although this is reversed by petiolar feeding with Pi (Walker and Sivak 1986). Previous studies with intact leaves have shown that starch storage is enhanced at the expense of sucrose (Suc) synthesis when plants are deprived of Pi (Brooks 1986, Sicher and Kremer 1988, Fredeen et al. 1989). Other experiments have demonstrated that low concentrations of Pi modulate the activity of several key photosynthetic enzymes (Preiss 1984, Rao et al. 1989). In addition, acid phosphatase activity in white clover roots is induced by Pi insufficiency (Almeida et al. 1999). Because of the importance of Pi to photosynthesis, the majority of studies performed to date have focused on shoot tissue. In the current study, it was postulated that the effects of Pi insufficiency on roots should differ from those on leaves because photosynthesis dominates the metabolism of above-ground tissues.

Although much is known about the individual effects of CO_2 enrichment and Pi deficiency on photosynthate partitioning, very little is known about the interactive effects of these two environmental factors on plants. Sa and Israel (1995, 1998) previously reported that Pi deficiency severely impaired plant growth and nitrogen assimilation by nodulated soybean plants, and showed that this was not overcome by CO_2 enrichment. Cure et al. (1988) reported that plant growth under moderate Pi insufficiency was stimulated by CO_2 enrichment using soybean plants that were assimilating NO_3^-. Almeida et al. (1999), Barrett and Gifford (1999) and Campbell and Sage (2002) all showed that the growth responses of forage species to increased atmospheric CO_2 could be influenced by Pi availability. In the current study, the combined effects of CO_2 enrichment and Pi insufficiency on photosynthate partitioning were assessed within barley roots. The results indicated that Pi deficiency in combination with CO_2 enrichment had a pronounced effect on the carbon, nitrogen and phosphorus metabolism of barley roots.

Materials and methods

Plant materials

Single barley seeds (*Hordeum vulgare* L. cv. Brant) were sown in $1 \, dm^3$ rectangular pots filled with vermiculite (W. R. Grace & Co., Cambridge, MA). Seedlings were grown in matching controlled environment chambers (model M-2, EGC Corp., Chagrin Falls, OH) using the conditions and techniques described previously (Sicher 2001). Chamber air CO_2 partial pressures were controlled continuously at either 36 ± 1 or $100 \pm 2 \, Pa$ using infrared analysers equipped with set point controllers (model WMA-3, PP Systems, Haverhill, MA). These were the ambient and elevated CO_2 treatments, respectively. One-half of the pots were flushed daily with a complete mineral nutrient solution containing $1.0 \, mM$ Pi, which was provided as $NH_4H_2PO_4$ (Robinson 1984) The remaining plants were watered with $0.05 \, mM$ Pi, and appropriate adjustments were made to NH_4Cl and KCl concentrations to balance the counterion levels.

Seedlings were harvested 9, 13 and 17 days after sowing (DAS) and plants were quickly separated into shoots and roots. Freshly harvested roots were collected from plants between 4 and $5 \, h$ of the light period and were washed quickly in tap H_2O, carefully blotted dry on paper towels and then rapidly frozen in liquid N_2 to stop metabolism. No corrections have been applied for the possible loss of fine roots during the washing procedure. Where indicated, root and shoot tissue was dried in a forced-air oven at $80°C$ for 3 days prior to the quantification of dry matter. Roots were either extracted immediately or stored at $-80°C$ until analysis.

Metabolite analyses

Frozen roots initially were ground to a liquid N_2 powder using a mortar and pestle. Samples [approximately $0.25 \, g$ fresh weight (FW)] were extracted immediately in ground glass tissue homogenizers with $2 \, ml$ of 80% (v/v) ethanol at $4°C$. Homogenates were transferred to $15 \, ml$ centrifuge tubes and spun at $4000 \, g$ for $10 \, min$ in a Jouan model MR23i centrifuge (Jouan, Inc., Winchester, VA). The resultant pellets were washed with an additional $1 \, ml$ of 80% ethanol and centrifuged as above. A $1 \, ml$ aliquot of the combined supernatants was transferred to a sealed cryovial for storage at $-20°C$. The remainder of each extract was then evaporated to a minimum volume under a stream of N_2 at $45°C$ to remove excess solvent. The concentrates were reconstituted in a total volume of $1 \, ml$ with deionized H_2O and stored at $-20°C$. Root metabolites were analysed as described in detail elsewhere (Sicher 2001). The alcohol fraction was used for the determination of NO_3^-. The aqueous fraction was used for the determination of soluble sugars and amino acids. Pellets were gelatinized in $2 \, ml$ of boiling H_2O and starch was determined as glucose (Glc) liberated by the action of α-amylase and amyloglucosidase.

Hexose-phosphates (hexose-P) were extracted from frozen, powdered root tissue with $1 \, ml$ of 5% (v/v) $HClO_4$ with tissue grinders, as described above. The homogenates were transferred to $1.5 \, ml$ microfuge tubes and were spun for $4 \, min$ at $14 000 \, g$ in a microcentrifuge (model 5415C, Brinkmann Instr., Westbury, NY). The supernatants were neutralized with $4 \, M$ KOH and the resultant precipitates were removed by centrifugation, as

described above. Samples were stored at $-80°C$ until analysis. Both glucose-6-phosphate (Glc6-P) and fructose-6-phosphate (Fru6-P) were determined spectrophotometrically using coupled enzyme assays, as described previously (Sicher and Kremer 1990). Recoveries of standard hexose-P were about 85%. The neutralized extracts were also used to quantify Pi with a colorimetric procedure, as described previously (Sicher and Kremer 1988).

Adenylates (ATP + ADP) were measured in a recycling assay using KOH-neutralized samples extracted with $HClO_4$, as described above. Adenylates were detected spectrophotometrically in coupled enzyme assays at 340 nm and 25°C. Reaction solutions contained 50 mM HEPES-NaOH, pH 7.6, 1 mM Glc, 1 mM $MgCl_2$, 0.3 mM $NADP^+$, 2 units hexokinase, 1 unit Glc6-P dehydrogenase, 2.5 mM phosphocreatine, 2 units phosphocreatine kinase and 50 μl of sample in a total volume of 1 ml. Reactions were initiated with Glc and were linear for several minutes. The slope was directly proportional to the adenylate (ATP + ADP) concentration, and standard curves were prepared with known amounts of ATP or ADP. Standard recoveries were greater than 80%.

Enzyme measurements

Soluble acid invertase (β-fructosidase fructohydrolase, E.C.3.2.1.26) activity was determined as described by Pressey and Avants (1980). Frozen roots were ground to a liquid N_2 powder, as described above, and 0.25 g FW of each sample was transferred to ground glass tissue homogenizers. Samples were extracted at 4°C with 2.0 ml of extraction buffer containing 50 mM HEPES-NaOH, pH 7.4, 5 mM $MgCl_2$, 2 mM ε-aminocaproic acid, 1 mM benzamidine, 1 mM EDTA, 0.1% Triton-X-100, 5 mM DTT and 10% (v/v) glycerol. The homogenates were spun in a microcentrifuge at 4°C for 5 min, as described above, and the supernatants were collected in 15 ml centrifuge tubes on ice. The pellets were washed twice with 1 ml of extraction buffer and the supernatants were combined to yield the soluble enzyme fraction.

Acid invertase assays were performed at 37°C in a total volume of 1 ml containing 0.25 M acetate buffer, pH 5.0, 0.75 M Suc and 0.1–0.2 ml of extract from the soluble enzyme fraction. Assays were terminated at 0 and 60 min in a boiling H_2O bath. Soluble acid invertase activity was linear for at least 2 h and had a pH optimum between 4.5 and 5.5. The Glc liberated from Suc by the action of acid invertase was detected enzymatically (Bergmeyer et al. 1974). The activity of Suc synthase was determined at 30°C after subtracting invertase-dependent Suc cleavage according to Silvius and Snyder (1979).

Statistical methods

In general, five roots per treatment were analysed for each experiment, and results from two separate experiments were combined for analysis. A third experiment

was performed if measurement means from replicate experiments varied by more than 15%. Significant differences between means were determined by analysis of variance (Statview 5.0, SAS Inc., Raleigh, NC) with date or time, experiment and CO_2 treatment as nominal factors.

Results

Dry matter partitioning

Reducing the available exogenous Pi from 1.0 to 0.05 mM decreased the final shoot and root dry weight of barley plants grown at ambient CO_2 by 58% and 33%, respectively (Fig. 1). Increasing the ambient CO_2 partial pressure from 36 ± 1 to 100 ± 2 Pa significantly enhanced ($P \leq 0.01$) the shoot and root growth of barley seedlings in the Pi-sufficient treatment between 9 and 17 DAS. When combined over age and Pi treatments, shoot dry mass was greater ($P < 0.01$) in seedlings from the elevated compared with the ambient CO_2 treatment (Figs 1A and 1B). However, a CO_2–Pi interaction ($P \leq 0.01$) was detected for shoot dry mass and, in contrast with Pi-sufficient plants, CO_2 enrichment had no effects ($P > 0.05$) on the shoot growth of Pi-insufficient plants. On the final measurement date, the root dry mass was enhanced by 12% and 48% ($P \leq 0.01$) by CO_2 enrichment in the Pi-sufficient and Pi-insufficient treatments, respectively (Figs 1C and 1D). It should be noted that the CO_2–age–Pi treatment interaction was significant ($P \leq 0.01$) with respect to root dry mass. However, this was largely a result of a lack of definitive treatment
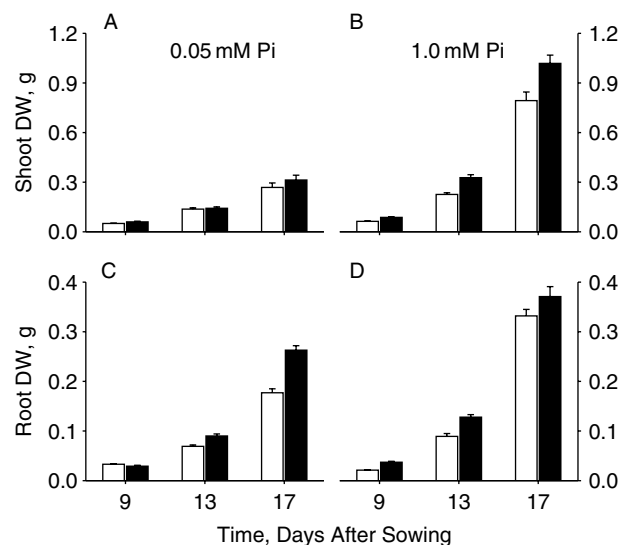

Fig. 1. Effects of CO_2 enrichment and inorganic phosphate (Pi) nutrition on biomass accumulation in roots and shoots. Barley plants were grown from seed in controlled environment chambers with either 0.05 mM (A) or 1.0 mM (B) Pi and with either 36 Pa (open bars) or 100 Pa (filled bars) CO_2. Plants were harvested 9, 13 and 17 days after sowing (DAS) as indicated, and separated into shoots (A, B) and roots (C, D). DW, dry weight.

effects on the 9-day sampling because the plants were small.

Non-structural carbohydrates

The effects of CO_2 enrichment and plant age on non-structural carbohydrate levels in the roots of Pi-sufficient and Pi-insufficient barley seedlings were determined 9, 13 and 17 DAS (Fig. 2). When means obtained for all harvest dates and CO_2 treatments were combined, root Suc concentrations were 3.1 ± 0.63 and $7.9 \pm 1.96\,\mu mol$ hexose g^{-1} FW ($P \le 0.01$) in the Pi-sufficient and Pi-insufficient roots, respectively (Figs 2A and 2B). Similar increases in Glc and Fru ($P \le 0.01$) occurred in barley roots when the Pi concentration of the nutrient solution was reduced from 1.0 to 0.05 mM (Figs 2C–F). The above findings were in contrast with root starch levels, which were unaffected ($P > 0.05$) by age, CO_2 or Pi (Figs 2G and 2H). Although the absolute effects were smaller than for the Pi treatment, soluble carbohydrate levels in barley roots were also increased by CO_2 enrichment. For instance, the mean concentrations of Glc, Fru and Suc were increased by 35%, 32% and 26%, respec-

tively, by CO_2 enrichment ($P \le 0.05$) when results for all three harvest dates in the Pi-sufficient treatment were combined. Similar increases in soluble carbohydrates in response to CO_2 enrichment were detected ($P \le 0.05$) in the Pi-insufficient treatment at 13 and 17 DAS.

In contrast with soluble sugars, both Glc6-P and Fru6-P concentrations in barley roots were decreased significantly ($P \le 0.01$) by Pi insufficiency (Fig. 3). Levels of Glc6-P were about one-third lower in Pi-insufficient than in Pi-sufficient samples. In contrast with soluble carbohydrates, neither Glc6-P nor Fru6-P levels in barley roots were affected by CO_2 enrichment ($P > 0.05$). The ratio of Glc6-P to Fru6-P in Pi-sufficient samples was about 3–4 : 1 when averaged over dates and CO_2 treatments. The CO_2–Pi interaction was significant ($P \le 0.01$) for Fru6-P levels in barley roots. This indicates that the mean difference between Fru6-P levels in the Pi-sufficient and Pi-insufficient treatments is greater at ambient than at elevated CO_2.

Inorganic anions

As expected, acid-extractable Pi in barley roots was decreased significantly ($P \le 0.01$) by Pi insufficiency (Fig. 4). Averaged over sampling dates and CO_2 treatments, Pi concentrations were 6.3 ± 0.65 and $0.6 \pm 0.13\,\mu mol\,g^{-1}$ FW in the Pi-sufficient and Pi-insufficient samples, respectively (Figs 4A and 4B). Regardless of Pi nutrition, Pi levels in barley roots were unaffected by CO_2 enrichment or by age ($P > 0.05$).

Root NO_3^- concentrations were greatest at 9 DAS, and then decreased with increasing age ($P \le 0.01$) under both Pi treatment conditions. However, the age-dependent
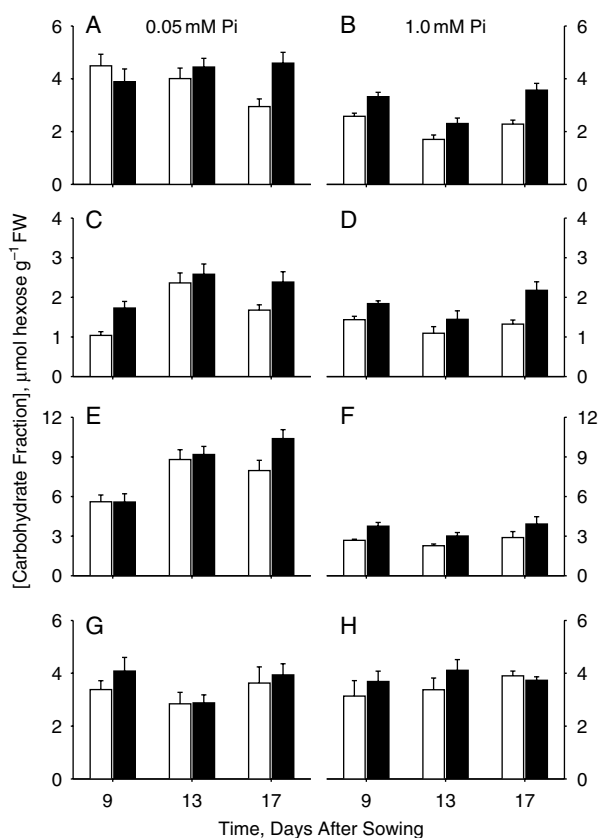

Fig. 2. Effects of CO_2 enrichment and inorganic phosphate (Pi) nutrition on root non-structural carbohydrate levels. Roots were harvested from 9-, 13- and 17-day-old barley seedlings between 4 and 5 h of the photoperiod, and glucose (A, B), fructose (C, D), sucrose (E, F) and starch (G, H) concentrations are shown. Other experimental details are as in Fig. 1. FW, fresh weight.
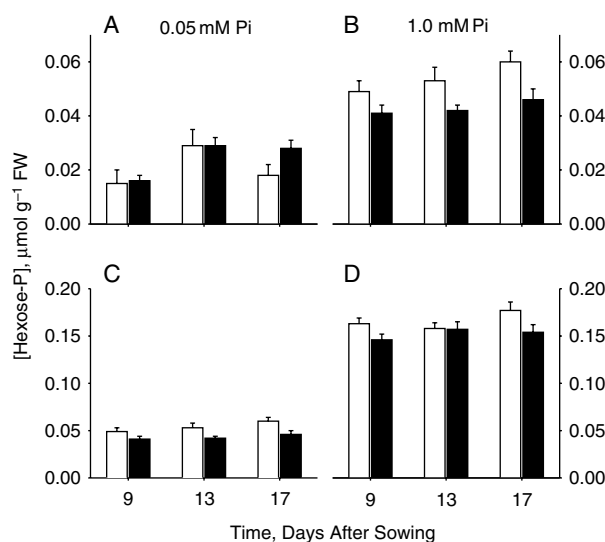

Fig. 3. Effects of CO_2 enrichment and inorganic phosphate (Pi) nutrition on root hexose-phosphate (hexose-P) levels. Barley roots were harvested from 9-, 13- and 17-day-old barley seedlings between 4 and 5 h of the photoperiod, and fructose-6-phosphate (Fru6-P) (A, B) and glucose-6-phosphate (Glc6-P) (C, D) concentrations are shown. Other experimental details are as in Fig. 1. FW, fresh weight.
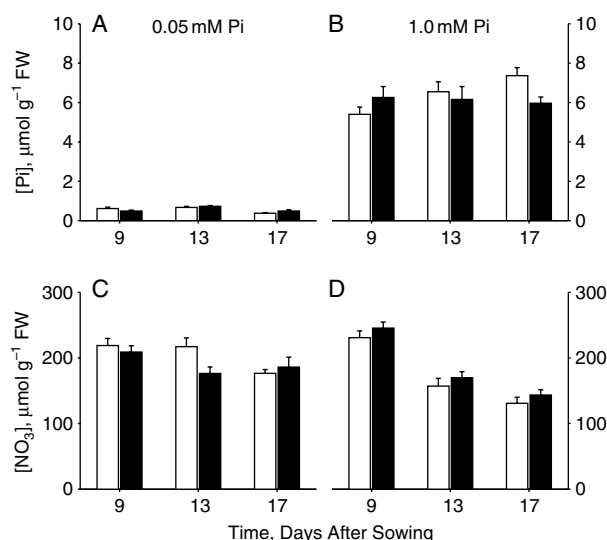
Fig. 4. Effects of CO$_2$ enrichment and inorganic phosphate (Pi) nutrition on root NO$_3^-$ and Pi levels. Roots were harvested from 9-, 13- and 17-day-old barley seedlings between 4 and 5 h of the photoperiod, and Pi (A, B) and NO$_3^-$ (C, D) concentrations are shown. Other experimental details are as in Fig. 1. FW, fresh weight.
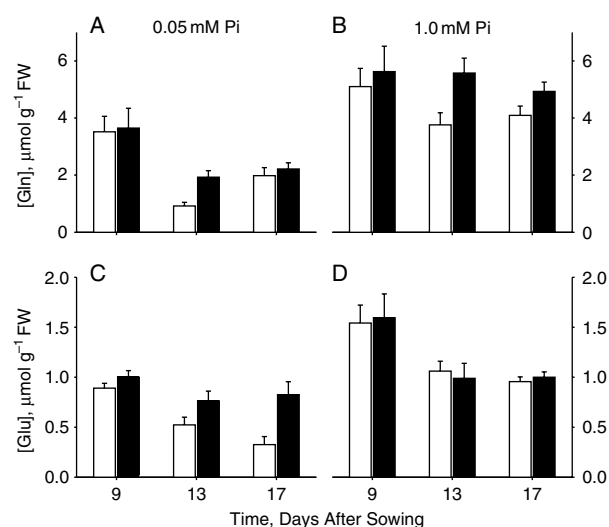

Fig. 5. Effects of CO$_2$ enrichment and inorganic phosphate (Pi) nutrition on root glutamine (Gln) and glutamate (Glu) levels. Roots were harvested from 9-, 13- and 17-day-old barley seedlings between 4 and 5 h of the photoperiod, and Gln (A, B) and Glu (C, D) concentrations are shown. Other experimental details are as in Fig. 1. FW, fresh weight.

decrease in NO$_3^-$ was greater in roots from the Pi-sufficient than from the Pi-insufficient treatment (Figs 4C and 4D). As a result, mean NO$_3^-$ levels were 9% lower ($P \leq 0.01$) in the Pi-sufficient compared with the Pi-insufficient treatment when results were combined over age and CO$_2$ treatment. Similar to Pi, root NO$_3^-$ levels were unaffected by CO$_2$ enrichment ($P > 0.05$).

Glutamate (Glu) and glutamine (Gln)

Both Glu and Gln levels in barley roots were decreased ($P \leq 0.01$) by Pi insufficiency (Fig. 5). In addition, maximal concentrations of Glu and Gln in barley roots were detected at 9 DAS, and levels of both amino acids decreased ($P = 0.01$) with increasing age. The mean concentrations of Gln were 4.9 ± 0.77 and $2.4 \pm 1.04 \, \mu mol \, g^{-1}$ FW ($P \leq 0.01$) in Pi-sufficient and Pi-insufficient barley roots, respectively, when results for both CO$_2$ treatments were combined (Figs 5A and 5B). Root Gln levels were also 23% greater in the enhanced compared with the ambient CO$_2$ treatment when means were averaged over both age and Pi treatments. Root Glu levels were 1.2 ± 0.29 and $0.9 \pm 0.14 \, \mu mol \, g^{-1}$ FW ($P \leq 0.01$) in the Pi-sufficient and Pi-insufficient treatments, respectively (Figs 5C and 5D). Combined over Pi treatments, Glu levels in barley roots did not differ ($P > 0.05$) between CO$_2$ treatments.

Adenylates

Adenylate (ATP + ADP) levels in barley roots were unaffected ($P > 0.05$) by Pi insufficiency (Fig. 6). The mean concentrations of adenylates in Pi-sufficient and Pi-insufficient barley roots were 2.3 ± 0.19 and

$2.2 \pm 0.17 \, \mu mol \, g^{-1}$ FW when averaged over age and CO$_2$ treatments. Adenylate levels in barley roots were increased by 32% ($P \leq 0.01$) by CO$_2$ enrichment. The mean concentrations of adenylates were 2.5 ± 0.18 and $1.9 \pm 0.17 \, \mu mol \, g^{-1}$ FW when averaged over age and Pi treatments. When tested separately, adenylate levels in the Pi-insufficient samples were unaffected ($P > 0.05$) by CO$_2$ enrichment. An age–Pi interaction was detected in this study as a result of differences in adenylate levels observed at 9 DAS.

Sucrose hydrolysis

Soluble acid invertase activity differed ($P \leq 0.01$) in the Pi-sufficient and Pi-insufficient root samples, but rates of Suc cleavage were unaffected ($P > 0.05$) by CO$_2$ enrichment (Fig. 7). The mean soluble acid invertase activities
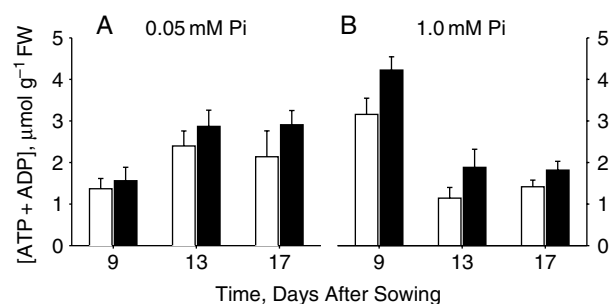

Fig. 6. Effects of CO$_2$ enrichment and inorganic phosphate (Pi) nutrition on root adenylate levels. Roots were harvested from 9-, 13- and 17-day-old barley seedlings between 4 and 5 h of the photoperiod, and adenylate [ATP + ADP] (A, B) concentrations are shown. Other experimental details are as in Fig. 1. FW, fresh weight.
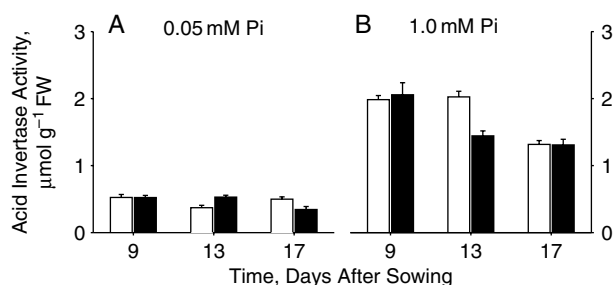
Fig. 7. Effects of CO_2 enrichment and inorganic phosphate (Pi) nutrition on root soluble acid invertase activity. Roots were harvested from 9-, 13- and 17-day-old barley seedlings between 4 and 5 h of the photoperiod, and soluble acid invertase activity (A, B) was measured in assays performed at 37°C and pH 5.5. Other experimental details are as in Fig. 1. FW, fresh weight.

were 1.7 ± 0.37 and $0.5 \pm 0.08 \, \mu\text{mol g}^{-1}$ FW ($P \leq 0.01$) when averaged over age and CO_2 treatments. Suc hydrolysis rates decreased with age in Pi-sufficient roots, but this was not observed in the Pi-insufficient root samples. The activity of Suc synthase in barley roots was unaffected by either CO_2 enrichment or Pi insufficiency (data not shown).

Discussion

Both CO_2 enrichment and Pi insufficiency affected root and shoot growth, partitioning and assimilate levels in barley roots. In comparison with the Pi-sufficient treatment, moderate Pi insufficiency reduced the root dry mass of barley seedlings by about one-third when plants were grown under ambient CO_2. The impact of Pi deficiency was more severe on shoots than on roots, so that total shoot growth was reduced by almost one-half when the Pi supply was decreased from 1.0 to 0.05 mM. Israel and coworkers (Cure et al. 1988, Israel et al. 1990) have shown that soybean plant growth responds positively to CO_2 enrichment under moderate Pi insufficiency. This was also true for barley roots examined in the present study. The root mass of CO_2-enriched barley seedlings from the Pi-insufficient treatment at 17 DAS was 80% of that of plants in the Pi-sufficient condition grown under ambient CO_2. In contrast with the results obtained with roots, barley shoot growth was only stimulated by CO_2 enrichment when the plants were Pi sufficient. These results confirmed the findings of Fredeen et al. (1989), who showed that root growth was favoured over shoot growth in response to Pi insufficiency. It should be noted that enhanced CO_2 also had a similar stimulatory effect on dry matter accumulation of moderately nitrogen-limited plants (Hocking and Meyer 1991, Ziska 2003). The above findings suggest that plants have the capacity to acclimate to moderate nutrient stress and to utilize the additional assimilates produced by CO_2 enrichment to manufacture dry matter, particularly below ground. Increased root growth in response to nutrient deficiency

is advantageous and allows plants to better obtain and utilize the available mineral supply.

Acid-extractable Pi of barley roots was decreased by about 95% on an FW basis in response to plant growth on Pi-insufficient nutrient solution. Much of the Pi in plant tissue is sequestered in the vacuole (Foyer et al. 1982, Woodrow et al. 1984). During extended periods of nutrient stress, Pi can be released from the vacuole. Clearly, Pi storage in the vacuole is an adaptive mechanism that provides Pi to the cytosol when the exogenous Pi supply is suboptimal.

Both Pi insufficiency and CO_2 enrichment increased non-structural carbohydrate levels in barley roots. However, the effects of CO_2 enrichment on soluble root carbohydrates, i.e. Suc, Glc and Fru, were small in comparison with the changes observed in response to Pi limitation. Non-structural carbohydrate amounts were lowest in roots of Pi-sufficient plants grown under ambient CO_2, and were highest in Pi-insufficient plants grown under elevated CO_2. Clearly, with both CO_2 enrichment and Pi insufficiency, the capacity of barley roots to utilize assimilates supplied by the shoot was restricted. Campbell and Sage (2002) speculated that elevated non-structural carbohydrate levels in plant tissues exposed to nutrient insufficiency would stimulate lateral root formation and thereby improve nutrient acquisition. Fredeen et al. (1989) have previously reported that Suc and starch levels are seven- and 11-fold greater in fibrous roots of soybean plants grown under Pi-insufficient than under Pi-sufficient conditions. These earlier starch results differed from the findings of the present study, which showed that Pi limitation did not alter starch levels in barley roots. In addition, only small increases in transitory starch in response to Pi limitation were detected in a previous study using barley primary leaves (Sicher and Kremer 1988). The differing root starch results between barley and soybean are probably the result of species differences. These contrasting results for root starch highlight the need for additional research on interactions between CO_2 enrichment and nutrient levels.

In contrast with soluble sugars, hexose-P levels in barley roots were reduced by Pi-insufficient relative to Pi-sufficient treatment. These results were in broad agreement with previous studies which showed marked decreases in phosphorylated photosynthetic intermediates in Pi-deficient leaves, primarily 3-phosphoglyceric acid and ribulose-1,5-bisphosphate (Brooks 1986, Dietz and Foyer 1986, Sicher and Kremer 1988, Rao et al. 1989). It is not surprising that leaf phosphorylated intermediates were decreased by Pi-limited growth conditions because photosynthesis was strongly inhibited by this treatment. Dietz and Foyer (1986) attributed the impact of Pi limitation on photosynthesis to inadequate rates of ATP-dependent ribulose-1,5-bisphosphate regeneration in the Calvin cycle.

Although the decrease in hexose-P levels in leaves can be explained readily, it is less clear why hexose-P levels in barley roots were reduced by moderate Pi insufficiency. It should be noted that, with the exception of 9-day-old

seedlings, adenylate levels (ATP + ADP) did not differ between Pi-sufficient and Pi-insufficient treatments in barley roots. This finding was in contrast with published adenylate levels for Pi-depleted sugarbeet leaves (Rao et al. 1989) and for *Brassica nigra* suspension cultures (Duff et al. 1989). In both of these previous studies, ATP and ADP levels were drastically reduced in the Pi-insufficient relative to the Pi-sufficient treatment. The impact of Pi insufficiency on leaves has been explained above. It should be noted that Duff et al. (1989) cultured suspension cells heterotrophically in the complete absence of Pi. The different adenylate results between this and the present study might be explained by the fact that the barley plants used here received a continual, moderately deficient supply of Pi as opposed to complete starvation. It was also interesting to note that CO_2 enrichment increased adenylate levels in barley roots from both Pi treatments. The author is unaware of previous CO_2 enrichment studies that measured adenylate levels in roots. Increased adenylates probably were associated with enhanced root dry matter formation that occurred in response to plant growth in elevated CO_2.

Evidence obtained in the current study suggests that Pi limitation affects the nitrogen nutrition of barley roots. First, Glu and Gln levels in barley roots were decreased by Pi insufficiency. This finding was significant because Glu and Gln are the predominant amino acids in roots (Geiger et al. 1999). These findings were in contrast with those of Duff et al. (1989) using *Brassica niger* suspension cells, who reported a five- to six-fold increase in free amino acid levels in association with Pi starvation. In addition, the age-dependent decrease in NO_3^- in barley roots between 9 and 17 DAS was greater in roots of Pi-sufficient than Pi-insufficient plants. One likely explanation for this observation is that the rates of NO_3^- utilization were impaired under Pi-limited growth conditions. In possible support of this conclusion, Israel et al. (1990) observed that the nitrogen utilization efficiency of non-nodulated soybean plants was decreased by 62% by Pi limitation. The nitrogen utilization efficiency is the ratio of total nitrogen to total biomass.

Suc produced by the shoot is transported by the phloem to the roots where it is metabolized for growth and development. Total non-structural carbohydrates were increased and hexose-P levels were decreased in barley roots by the combined effects of Pi limitation and CO_2 enrichment. This result suggests that the ability of root cells to utilize available Suc and hexoses provided by the shoot is inadequate under these treatment conditions. The finding that barley root adenylate levels were increased by CO_2 enrichment is in agreement with this assessment. Therefore, it was of interest to determine whether soluble acid invertase activity or Suc synthase were altered by either Pi insufficiency or CO_2 enrichment. Acid invertase and Suc synthase catalyse the initial steps in the utilization of Suc in sink tissues. The activity of Suc synthase in barley roots was unaffected by either CO_2 enrichment or Pi insufficiency (data not shown). However, soluble acid invertase activity was decreased by 70% on average in Pi-insufficient relative to Pi-sufficient barley roots. This finding was inconsistent with an earlier report (Fredeen et al. 1989) showing that acid invertase activity was almost doubled in soybean leaves when Pi was limiting. The activities of several enzymes and membrane transporters in higher plants have previously been shown to be increased by Pi deficiency as part of a starvation-induced rescue system (Theodorou and Plaxton 1993, Ticconi et al. 2001). In contrast with the results obtained with barley roots, Suc levels were decreased by Pi insufficiency in soybean leaves (Fredeen et al. 1989). Therefore, a negative correlation between changes in Suc levels and acid invertase activity was observed in both barley roots and soybean leaves in response to Pi limitation.

Taken together, the above results demonstrate that Pi insufficiency and CO_2 enrichment alter the carbon and nitrogen metabolism of barley roots. The Pi insufficiency imposed here decreased root growth by about half, but this was partially reversed by CO_2 enrichment. It is interesting to note that the effects of Pi insufficiency on starch, amino acids, adenylates and invertase activity differ between barley roots and the results of several earlier studies, as discussed above. This suggests that it is premature to generalize extensively about the specific effects of Pi insufficiency on metabolite levels in plants. Moreover, it is probable that the differences in metabolite levels between this and previous studies are largely attributable to the effects of Pi insufficiency on photosynthesis or to treatments involving complete Pi deprivation. Non-structural carbohydrates accumulated in roots in response to both Pi insufficiency and CO_2 enrichment. This indicates that the capacity of roots to utilize carbon imported from the shoot is inadequate under both of these treatments. As a final point, the above findings highlight the importance of avoiding nutrient stress during CO_2 enrichment studies.

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