

## Pseudoviviparous Reproduction of *Poa alpina* var. *vivipara* L. (Poaceae) during Long-term Exposure to Elevated Atmospheric CO<sub>2</sub>

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Pseudovivipary is an asexual reproductive strategy exhibited by some arctic/alpine grasses in which leafy plantlets are produced in place of seeds, with genetic conservation an advantage for stress tolerators in these nutrient-poor habitats. Photosynthetic metabolism and the development of this reproductive system were investigated under varying nutrient availability and predicted future CO<sub>2</sub> partial pressure (*p*CO<sub>2</sub>). *Poa alpina* var. *vivipara* L., grown at present ambient *p*CO<sub>2</sub> or ambient plus 340 µmol mol<sup>-1</sup> CO<sub>2</sub> (elevated *p*CO<sub>2</sub>), was supplied with either 0.05 mol m<sup>-3</sup> phosphorus and 0.2 mol m<sup>-3</sup> nitrogen, or 0.2 mol m<sup>-3</sup> phosphorus and 1.0 mol m<sup>-3</sup> nitrogen. Gas exchange measurements and determination of total non-structural carbohydrate (TNC), nitrogen and phosphorus contents revealed that parent plant leaf blade tissues experienced acclimatory loss of photosynthetic capacity after long-term growth at elevated *p*CO<sub>2</sub> (particularly so when nutrient availability was low); there were associated reductions in photosynthetic nitrogen and phosphorus use efficiencies (PNUE and PPUE). In addition, decreased PNUE and PPUE were exhibited by plantlets grown at elevated *p*CO<sub>2</sub> with low nutrient availability. Decreased reproductive dry matter in this treatment also resulted from a lack of reproductive initiation in daughter tillers, and altered phenology. Pseudoviviparous *P. alpina* is likely to be at a disadvantage in both vegetative and reproductive phases at predicted future elevated atmospheric CO<sub>2</sub> concentrations, particularly where nutrients are scarce and when in competition with species experiencing less acclimatory loss of photosynthetic capacity.

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**Key words:** Alpine meadow grass, elevated *p*CO<sub>2</sub>, nutrient availability, photosynthetic acclimation, photosynthetic nutrient use efficiency, *Poa alpina* var. *vivipara*, pseudovivipary, proliferation.

### INTRODUCTION

Habitually pseudoviviparous grasses reproduce asexually via the production of leafy spikelets. The upper portion of these spikelets, having reverted from sexual development back to vegetative development along the axis (a process termed proliferation; Bell, 1991), may be considered as a shoot system morphologically homologous with tillers. Characteristically, spikelets retain the dehiscence zone and often some mature floral organs typical of sexual spikelets (Fig. 1; cf. ephemeral proliferation—the inconsistent teratological development of leafy structures in response to abnormal temperature or disease; for a review, see Pierce, 1998). ‘Plantlets’ of habitually pseudoviviparous grasses are photosynthetically active (Lee and Harmer, 1980; Pierce *et al.*, 2000a) and, after dehiscing from the parent plant and being dispersed (primarily by the wind), may root and establish more rapidly in a short growing season than seeds from seminiferous varieties (Harmer and Lee, 1978). Pseudovivipary is an important reproductive strategy for grasses in nutrient-poor arctic/alpine environments, in which genetic conservation may confer selective advantage; it has been recorded in 41 species spanning 13 genera of

Poaceae (Pierce, 1998). These grasses are often locally abundant, may dominate nitrophilous communities in the Arctic (Summerhayes and Elton, 1928) and, aside from ecological importance, are also major fodder species in alpine regions, e.g. *Poa alpina* var. *vivipara* L. (viviparous alpine meadow grass) (Hegi, 1930; Steiner *et al.*, 1997).

In C<sub>3</sub> plants such as *P. alpina*, vegetative growth is limited not only by the availability of inorganic nutrients, but also by atmospheric carbon dioxide partial pressure (*p*CO<sub>2</sub>), which is predicted to double over the next century due to the burning of hydrocarbon fuels (Watson *et al.*, 1990). However, for many plants, including *P. alpina* (Baxter *et al.*, 1994a), an initially higher rate of growth in the seedling stage is not sustained at elevated *p*CO<sub>2</sub> (relative to plants grown at present day *p*CO<sub>2</sub>), with photosynthetic capacity and growth rates declining as photosynthetic metabolism becomes acclimated to the greater substrate availability.

During acclimation, extra photoassimilate, produced as a result of the initially higher photosynthetic rate, may cause a direct repression of transcription of genes controlling photosynthetic metabolism in flowering plants (van Oosten *et al.*, 1994, 1997; van Oosten and Besford, 1996). However, this does not appear to contribute to the initial acclimation of C<sub>3</sub> grasses, with source tissues exporting sufficient carbohydrates such that feedback inhibition is

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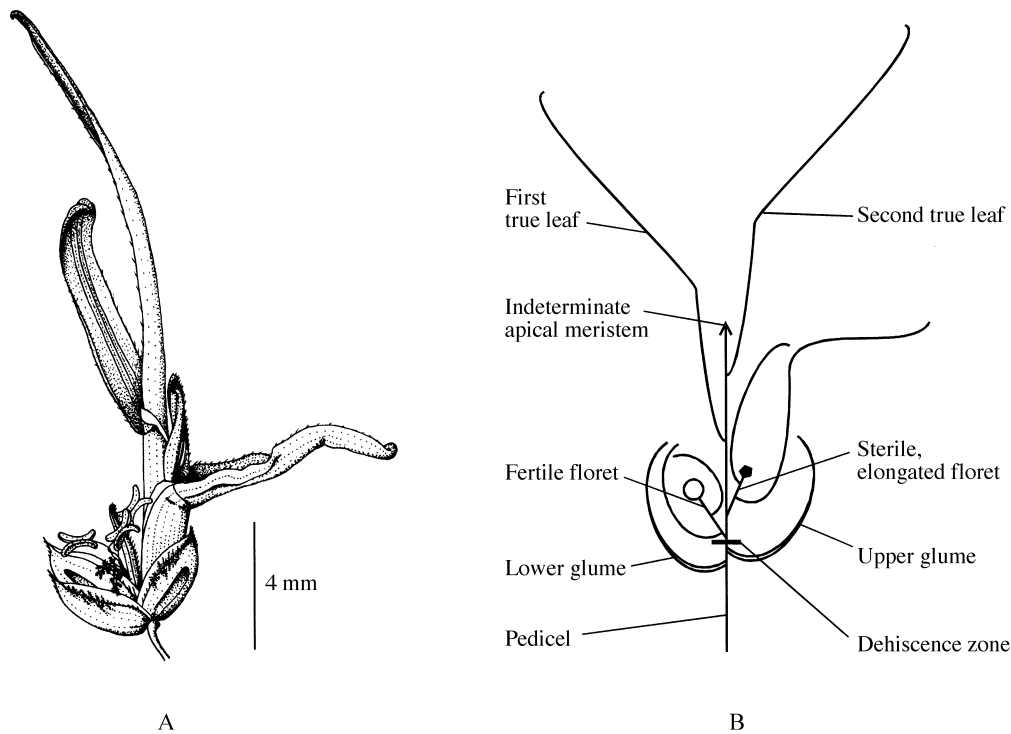


FIG. 1. Illustration (A) and diagrammatic representation (B) of a proliferated spikelet ('plantlet'), the propagule of the pseudoviviparous grass *Poa alpina* var. *vivipara*. Illustration by Simon Pierce.

prevented. For example, Harmens *et al.* (2000) have reported that acclimation is more dependent on leaf nitrogen content in *Dactylis glomerata* L. Indeed, regeneration of ribulose-1,5-bisphosphate (Ru1,5bisP) is a fundamental limitation to photosynthesis that is dependent on nitrogen and/or  $P_i$  availability (Farquhar *et al.*, 1980; Sharkey, 1985). Constrained by nutrient availability, longer-term exposure to elevated  $pCO_2$  promotes a decline in photosynthetic rates to a point where the regeneration of Ru1,5bisP is once again in equilibrium with its rate of use (Stitt, 1991). If excessive amounts of substrate cannot be processed rapidly enough, then  $P_i$  will also be locked up in the form of phosphorylated intermediate compounds, further limiting photophosphorylation (Stitt, 1991). A reduction in growth and limitation of sink size curbs sink strength, resulting in decreased flux between source and sink tissues, potentially inhibiting photosynthesis as a secondary effect. Thus, feedback inhibition of photosynthetic capacity in elevated  $pCO_2$  is potentially greatest in nutrient-limiting conditions (Arp, 1991), imposing greater constraints on growth than low nutrient availability alone. This has been reported for both vegetative *D. glomerata* (Harmens *et al.*, 2000) and *P. alpina* (Baxter *et al.*, 1997).

In the case of seminiferous plants, overall sink strength may increase with the production of fruit and seeds (Arp, 1991). However, the pseudoviviparous reproductive system of *P. alpina* is a source of carbohydrate (Pierce *et al.*, 2000a) and, therefore, unlikely to relieve feedback inhibition. As plantlets represent vegetative shoots with a photosynthetic efficiency equal to that of parent plant leaf blade tissues

(Pierce *et al.*, 2000a), it is possible that propagules of *P. alpina* exhibit similar physiological responses to elevated  $pCO_2$  and low nutrient availability to those of tillers.

Other factors may also affect the reproductive potential of these clonal plants. Increased tillering of *P. alpina* and other graminoids in elevated  $pCO_2$  (Tissue and Oechel, 1987; Baxter *et al.*, 1994a) may be especially pronounced under conditions of greater nutrient availability (Baxter *et al.*, 1997). Elevated  $pCO_2$  also affects the initiation, rate of development, number and longevity of flowers of many species (Garbutt and Bazzaz, 1984), responses that are potentially mediated by nutrient availability (Lang, 1965).

Reproduction via a pseudoviviparous system, being derived from a seminiferous system, must be initiated by environmental cues (i.e. chilling and shorter day lengths for these arctic/alpine species), with reproduction occurring late in the growth season (Pierce, 1998). Thus, elevated  $pCO_2$  and nutrient availability have the potential to limit pseudoviviparous reproduction in short arctic/alpine growing seasons. This study, which aims to investigate the effect of future substrate availability on pseudoviviparous reproduction, follows the synflorescence concept of reproductive architecture in grasses [i.e. that reproductive architecture is composed of repeated co-florescences ( $\approx$ spikelets) borne in groups termed 'paracladia', the inflorescence or 'main florescence' being the distal-most spikelet; *sensu* Vegetti and Anton, 1996; Vegetti and Weberling, 1996; for diagrammatic explanation, see Pierce *et al.*, 2000b]. The nomenclature of grasses follows Hubbard (1992).

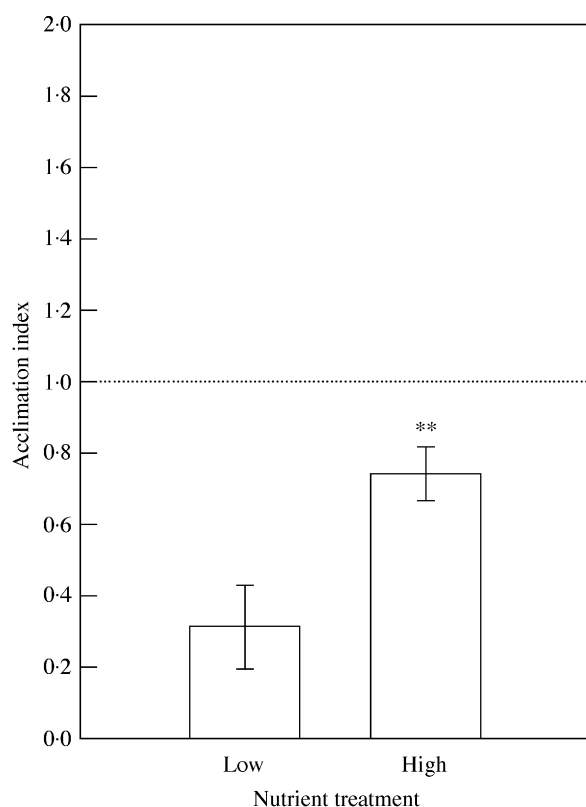


FIG. 2. Photosynthetic acclimation to elevated  $p\text{CO}_2$  of *P. alpina* var. *vivipara* plants grown at two nutrient regimes: one-fifth strength (low) or full-strength (high) Long Ashton nutrient solution. Data are means of six replicates  $\pm$  s.e. \*\* Significant difference between nutrient treatment means at the  $P < 0.01$  level determined by Student's  $t$ -test. An acclimation index  $< 1$  indicates a loss of photosynthetic capacity.

## MATERIALS AND METHODS

The plant material used was the same biotype of *Poa alpina* var. *vivipara* investigated by Pierce *et al.* (2000a, b), originating from the Hohe Mut ridge, Oetzal, Austria (45°50'12"N, 11°2'50"E) at an altitude of 2641 m a.s.l. Plantlets were initially raised in trays of sand using one-fifth strength Long Ashton nutrient solution (LA; Hewitt, 1966), until they possessed eight or nine fully expanded leaves, and were then transferred to the Institute of Terrestrial Ecology Solardome facility (Solardome Industries Ltd, Southampton, UK) at Abergwygregyn, Gwynedd, Wales, UK. This facility has been described by Stirling *et al.* (1997), and the environmental control and monitoring systems by Rafarel *et al.* (1995). Treatments consisted of ambient  $p\text{CO}_2$  (tracking local ambient of approx. 350  $\mu\text{mol mol}^{-1} \text{CO}_2$ ) and elevated  $p\text{CO}_2$  (ambient  $p\text{CO}_2$  with an additional 340  $\mu\text{mol mol}^{-1} \text{CO}_2$  at any point in time). Plants were cultivated in sand culture in 1-l square black plastic pots and were supplied with either one-fifth strength LA (modified with  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  to provide 0.05  $\text{mol m}^{-3}$  P and  $\text{NH}_4\text{NO}_3$  to provide 0.2  $\text{mol m}^{-3}$  N) or full-strength LA (0.2  $\text{mol m}^{-3}$  P and 1.0  $\text{mol m}^{-3}$  N) twice weekly, added to pots until saturated. Plants were watered twice daily with tap

water at 0600 and 1800 h using an automated sprinkler system.

Culms were tagged with the date of paracladial exertion (i.e. the date at which the main florescence became visible), and the length of the culm was measured every second day. Harvests of plant material were conducted every 10 d following the cessation of culm elongation growth. At all harvests six plantlets were removed from the distal half of the main axis paracladial zone, and six were removed from the proximal half (paracladial zones were apportioned into halves by spikelet number after Pierce *et al.*, 2000a). The remaining plant was divided into component organs, and these were further divided into green and non-green portions and dried to constant weight.

Ethanol-soluble and water-soluble carbohydrate contents of plant tissues were determined using the phenol-sulfuric acid method of Dubois *et al.* (1956). Tissue starch was determined using an enzymatic starch assay as described by Farrar (1993). Dry plant material (0.1 g sample) was digested using the Kjeldahl method (Allen, 1974; also detailed in Hind, 1993), and analysed for ammonium nitrogen and phosphate using a segmented flow autoanalyser (SAN<sup>PLUS</sup>; Skalar Analytical, Breda, The Netherlands). Photosynthetic nitrogen and phosphorus use efficiencies (PNUE and PPUE, respectively) of both parent plant leaf blade and paracladial zone tissues were calculated following the method of Baxter *et al.* (1994b):

$$E_{N/P} = \frac{A_N}{(N[P]/L_A)} \quad (1)$$

where  $E_{N/P}$  is photosynthetic nitrogen/phosphorus use efficiencies [in g (mean structural dry weight)  $\text{g}^{-1}$  (tissue nitrogen or phosphorus)  $\text{d}^{-1}$ ],  $A$  is net assimilation rate ( $\text{g m}^{-2} \text{d}^{-1}$ ),  $L_A$  is specific leaf area ( $\text{m}^2 \text{g}^{-1}$ ), and  $N$  or  $P$  is the leaf nitrogen or phosphorus content [ $\text{g N(P)} \text{g}^{-1}$  structural d. wt]. Net assimilation rate was calculated thus:

$$A_N = \frac{(w_2 - w_1)}{(t_2 - t_1)} \times \frac{(\log l_2 - \log l_1)}{(t_2 - t_1)} \quad (2)$$

where  $w_1$  and  $w_2$  are dry weights (g), and  $l_1$  and  $l_2$  are leaf blade or plantlet area ( $\text{m}^2$ ) at times  $t_1$  and  $t_2$  (d), respectively.

Infrared gas analysis (IRGA; Ciras 1, PP systems, Hitchin, UK) was used to quantify photosynthetic gas exchange of the youngest fully expanded leaf of the main axis 19 d prior to paracladial exertion. Measurements were taken in a saturating photosynthetic photon flux density (PPFD) of 1300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and at leaf temperature of 20 °C. In each nutrient treatment the photosynthetic capacity of plants grown at ambient and elevated  $p\text{CO}_2$  was compared at the same calculated intracellular  $p\text{CO}_2$  to produce an index of acclimation, after Stirling *et al.* (1997):

TABLE 1. Total non-structural carbohydrate (TNC) content and total nitrogen (N) and phosphorus (P) concentration of innovation zone leaf blade material at either 2 or 20 d after the cessation of culm elongation growth in *Poa alpina*

	Time (d)	Treatment			
		Low nutrient		High nutrient	
		Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>
TNC (mg glucose equivalent g <sup>-1</sup> total d. wt)	2	53.0 ± 3.2 <sup>a</sup>	64.0 ± 9.1 <sup>ab</sup>	56.0 ± 6.7 <sup>ab</sup>	65.0 ± 5.4 <sup>ab</sup>
	20	71.0 ± 15.6 <sup>b</sup>	64.0 ± 7.4 <sup>ab</sup>	62.0 ± 9.4 <sup>ab</sup>	59.0 ± 9.3 <sup>ab</sup>
N (mg g <sup>-1</sup> total d. wt)	2	24.3 ± 4.31 <sup>c</sup>	18.2 ± 0.85 <sup>ab</sup>	14.6 ± 2.86 <sup>abc</sup>	12.4 ± 2.11 <sup>a</sup>
	20	16.0 ± 1.38 <sup>ab</sup>	20.8 ± 3.37 <sup>bc</sup>	20.8 ± 1.16 <sup>bc</sup>	18.2 ± 1.91 <sup>abc</sup>
P (mg g <sup>-1</sup> total d. wt)	2	10.4 ± 0.42 <sup>c</sup>	6.9 ± 1.23 <sup>ab</sup>	8.5 ± 1.64 <sup>bc</sup>	4.3 ± 0.62 <sup>a</sup>
	20	9.8 ± 0.50 <sup>c</sup>	6.3 ± 0.19 <sup>ab</sup>	9.6 ± 0.25 <sup>c</sup>	6.3 ± 0.33 <sup>ab</sup>

Plants were grown in either one-fifth strength (low nutrient) or full-strength (high nutrient) Long Ashton nutrient solution, and either 350 μmol mol<sup>-1</sup> (ambient) or 690 μmol mol<sup>-1</sup> (elevated) CO<sub>2</sub>. Data are means of four replicates ± s.e. Different superscripts indicate significant differences at  $P < 0.05$  between all means for TNC data, or between treatment means and over time within each nutrient for N and P data, as determined by Tukey's multiple comparison procedure (ANOVA) (*sensu* Zar, 1999).

TABLE 2. Photosynthetic nitrogen and phosphorus use efficiencies (PNUE, PPUE; g structural d. wt g<sup>-1</sup> N(P) d<sup>-1</sup>) of parent plant leaf blade material over an 18-d period following cessation of culm elongation growth in *Poa alpina*

Nutrient	Treatment			
	Low nutrient		High nutrient	
	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>
PNUE	0.9 ± 0.32 <sup>b</sup>	0.1 ± 0.08 <sup>a</sup>	0.9 ± 0.51 <sup>ab</sup>	4.1 ± 0.87 <sup>c</sup>
PPUE	1.9 ± 0.64 <sup>a</sup>	0.3 ± 0.19 <sup>a</sup>	1.7 ± 0.92 <sup>a</sup>	11.9 ± 2.59 <sup>b</sup>

Plants were grown in either one-fifth strength (low nutrient) or full-strength (high nutrient) Long Ashton nutrient solution, and either 350 μmol mol<sup>-1</sup> (ambient) or 690 μmol mol<sup>-1</sup> (elevated) CO<sub>2</sub>. Data are means of four replicates ± s.e. Different superscripts indicate significant differences between treatment means for each nutrient use efficiency (PNUE or PPUE) at  $P < 0.05$  determined by Tukey's multiple comparison procedure (ANOVA) (*sensu* Zar, 1999).

$$I_A = \frac{(A'_e - A_a)}{(A_e - A_a)} \quad (3)$$

where  $I_A$  is the acclimation index,  $A'_e$  is net photosynthetic rate per unit leaf area obtained from plants grown in 690 μmol mol<sup>-1</sup> CO<sub>2</sub> (elevated) and measured at the same CO<sub>2</sub> concentration,  $A_a$  is the net photosynthetic rate obtained from plants grown and measured at 350 μmol mol<sup>-1</sup> (ambient), and  $A_e$  is the value obtained from plants grown at ambient  $pCO_2$  but measured at elevated  $pCO_2$ . An acclimation index equal to 1 indicates that no photosynthetic acclimation has occurred;  $I_A < 1$  indicates a loss of photosynthetic capacity, and  $I_A > 1$  indicates increased photosynthetic capacity.

## RESULTS

Ambient temperature was tracked by all solardomes; mean midday temperature was 16 ± 0.1 °C between May and August (26.5 and 5.2 °C maximum and minimum,

respectively), and mean midday PPFD inside the solar-domes was 304 ± 13.1 μmol m<sup>-2</sup> s<sup>-1</sup>.

### Acclimatory response of photosynthesis in parent plants

Elevated  $pCO_2$  resulted in acclimatory loss of net photosynthetic rate of fully expanded leaves of the parent plant in both nutrient treatments. However, at low nutrient availability, plants had a significantly lower photosynthetic acclimation index (i.e. had a greater acclimatory loss of photosynthetic capacity) compared with that of plants grown at 'high' nutrient status (0.3 ± 0.12 *cf.* 0.7 ± 0.08,  $P < 0.01$ ; Fig. 2).

### Parent plant growth and dry matter accumulation

Low nutrient availability alone resulted in lower whole plant dry weights at maturity (24–27 g at high nutrient availability compared with 7–11 g at low nutrient availability;  $P < 0.05$ ; data not shown), although CO<sub>2</sub> treatments did not affect whole plant dry weight in this study.

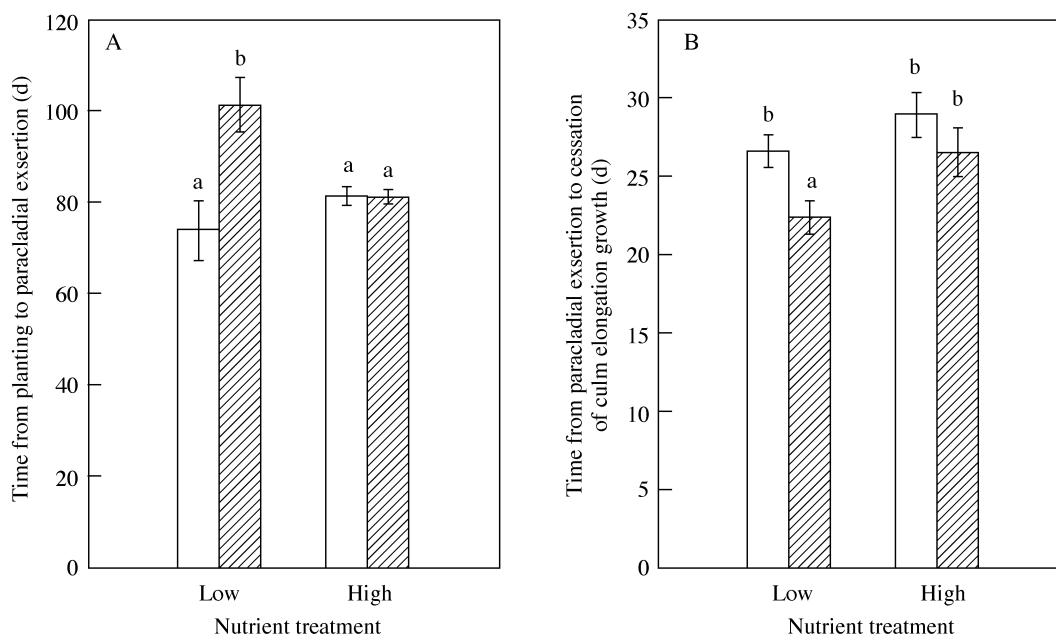


FIG. 3. Developmental timing of the culm of *Poa alpina*: time from planting to paracladial exertion (A) and time from paracladial exertion to cessation of culm elongation growth (B). Plants were grown at either 350  $\mu\text{mol mol}^{-1}$  (ambient; open bars) or 690  $\mu\text{mol mol}^{-1}$  (elevated; hatched bars) atmospheric CO<sub>2</sub>, and two nutrient regimes: one-fifth strength (low) or full-strength (high) Long Ashton nutrient solution. Data are means of twelve replicates  $\pm$  s.e. Different letters indicate significant differences at the  $P < 0.05$  level determined by Tukey's multiple comparison procedure (ANOVA) (Zar, 1999).

#### Leaf blade metabolite concentrations in parent plants

Plants grown at ambient  $p\text{CO}_2$  with lower nutrient availability showed an increase in total non-structural carbohydrate (TNC) content over time in parent plant leaf blades, a response not exhibited in other treatments, between which there were no differences ( $P < 0.05$ ; Table 1). Only the ambient  $p\text{CO}_2$  and low nutrient treatment resulted in decreases in tissue nitrogen concentration over time, and decreased tissue phosphorus concentrations were evident at elevated  $p\text{CO}_2$  ( $P < 0.001$ ; Table 1).

#### Photosynthetic nitrogen and phosphorus use efficiencies of parent plant leaves

Nutrient and CO<sub>2</sub> treatments had interactive effects on both photosynthetic nitrogen and phosphorus use efficiencies, with elevated  $p\text{CO}_2$  reducing PNUE in low nutrient conditions ( $0.1 \pm 0.08 \text{ g g}^{-1} \text{ d}^{-1}$ ), PPUE remaining low in low nutrient conditions ( $0.3 \pm 0.19 \text{ g g}^{-1} \text{ d}^{-1}$ ), and PNUE and PPUE increasing in high nutrient conditions ( $4.1 \pm 0.87$  and  $11.9 \pm 2.59 \text{ g g}^{-1} \text{ d}^{-1}$ , respectively;  $P < 0.001$ ; Table 2).

#### The flowering response

In low nutrient treatments, 27.3 and 28.6 % of plants flowered in ambient and elevated  $p\text{CO}_2$ , respectively, whereas in high nutrient treatments these figures were 62.5 and 70.2 %, respectively. In plants grown in elevated  $p\text{CO}_2$  and low nutrient conditions, the time prior to initiation of reproductive growth was increased (Fig. 3A) and the

period of culm elongation growth was reduced by approx. 5 d (Fig. 3B), whereas the other treatments did not affect developmental timing. Additional nutrients led independently to an increase in the number of daughter tillers in flower (Fig. 4A and B). Elevated  $p\text{CO}_2$  alone increased the number of flowering tillers in the high nutrient treatment, but interacted with low nutrient status to reduce the number of tillers in flower. This interaction was reinforced over time within all treatments except elevated  $p\text{CO}_2$  and low nutrient supply, resulting in the continued production of synflorescences (Fig. 4A and B). Total reproductive dry weight per plant was approx. 350 % higher in the high nutrient treatment compared with the low nutrient treatment at 20 d (Fig. 4C and D). There was no effect of, or interaction with, elevated  $p\text{CO}_2$ , other than an apparent increase in variability in total reproductive dry weight.

#### The main axis paracladial zone

The total number of spikelets of the main synflorescence was not affected by nutrient or CO<sub>2</sub> treatments (Table 3). Total dry weight of the paracladial zone was significantly increased in high nutrient conditions compared with low nutrient conditions, with further increases over time (e.g. a difference of 211 mg in plants grown at ambient  $p\text{CO}_2$  compared with a difference of 505 mg in plants grown at elevated  $p\text{CO}_2$  at 20 d; Fig. 5A and B). Elevated  $p\text{CO}_2$  and low nutrient availability interacted to reduce significantly the dry weight of the main axis paracladial zone ( $P < 0.001$ ; Table 6). The number of fully expanded leaves on plantlets

increased over time (Fig. 5C and D). The dry weight of distal plantlets was significantly greater than that of proximal plantlets in all treatments ( $P < 0.001$ ; Fig. 6; Table 7A). Distal plantlets had a greater leaf area ratio (LAR) than proximal plantlets (data not shown).

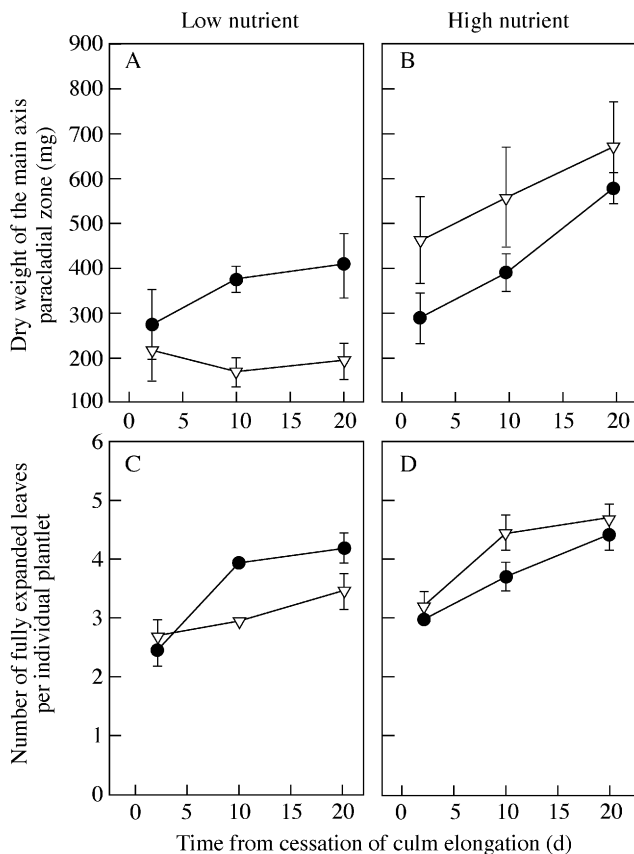


FIG. 4. A and B, Number of daughter tillers in flower at the time of cessation of culm elongation growth in *Poa alpina*. C and D, Total reproductive dry weight following the cessation of culm elongation growth. Plants were grown at either 350  $\mu\text{mol mol}^{-1}$  (ambient; closed symbols) or 690  $\mu\text{mol mol}^{-1}$  (elevated; open symbols) atmospheric CO<sub>2</sub>, and two nutrient regimes: one-fifth strength (low nutrient) or full-strength (high nutrient) Long Ashton nutrient solution. Data are means of four replicates  $\pm$  s.e.

TABLE 3. Mean number of spikelets in the main axis paracladial zone of *Poa alpina*

Nutrient treatment	CO <sub>2</sub> concentration ( $\mu\text{mol mol}^{-1}$ )	
	350	690
Low	58.8 $\pm$ 6.44	60.7 $\pm$ 6.08
High	64.5 $\pm$ 3.05	72.0 $\pm$ 3.35

Plants were grown in either one-fifth strength (low) or full-strength (high) Long Ashton nutrient solution, and either 350  $\mu\text{mol mol}^{-1}$  (ambient) or 690  $\mu\text{mol mol}^{-1}$  (elevated) CO<sub>2</sub>. Data are means of 12 replicates  $\pm$  s.e. Data were square-root transformed prior to statistical analysis. No statistical differences were detected at  $P < 0.05$  between treatments using analysis of variance (*sensu* Zar, 1999).

Plantlets possessed visibly senescent leaves from the time that culm elongation growth ceased, with the proportion of senescent material in the paracladial zone increasing over time ( $P < 0.001$ ; data not shown) until approx. 40 % of material in the paracladial zone was senescent at 20 d. High nutrient availability resulted in a greater proportion of senescent material ( $P < 0.05$ ), with no effect of, or interaction with, the elevated pCO<sub>2</sub> treatment (Table 6).

#### Leaf blade metabolite concentrations in plantlets

Total non-structural carbohydrate content of plantlets increased significantly over time in distal plantlets. Although TNC of distal plantlets did not differ among treatments, TNC of proximal plantlets was depressed at low nutrient availability and elevated pCO<sub>2</sub> 20 d after the cessation of culm elongation ( $P < 0.01$ ; Table 4). Phosphorus concentration of plantlet leaf tissue was significantly reduced in the elevated pCO<sub>2</sub> treatment, compared with ambient, at 2 d following paracladial exertion at both low and high nutrient supply ( $P < 0.05$ ; Table 5). Nutrient

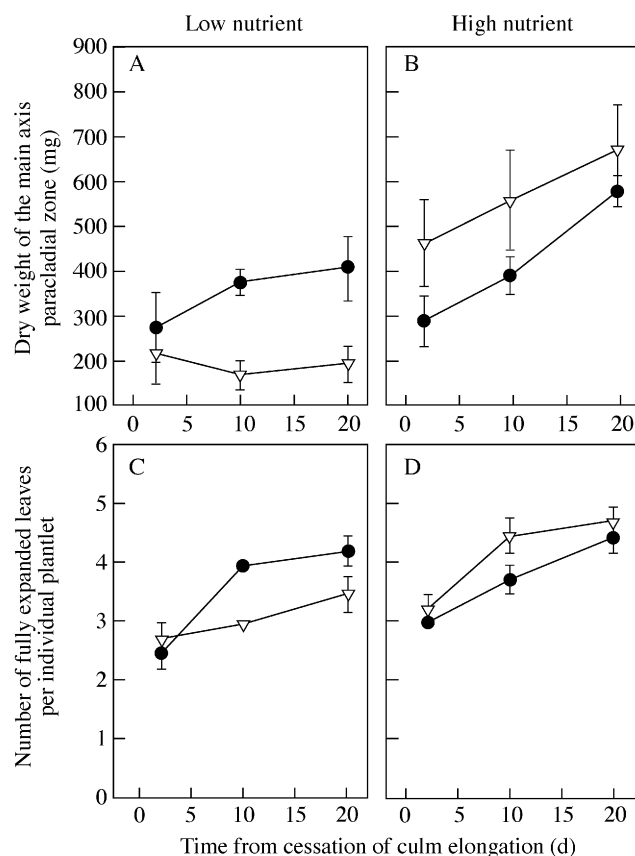


FIG. 5. A and B, Dry weight of the main axis paracladial zone after the cessation of elongation growth of the culm of *Poa alpina*. C and D, Number of fully expanded leaves of individual plantlets after the cessation of elongation growth of the culm (no distinction was made between distal and proximal plantlets). Plants were grown at either 350  $\mu\text{mol mol}^{-1}$  (ambient; closed symbols) or 690  $\mu\text{mol mol}^{-1}$  (elevated; open symbols) atmospheric CO<sub>2</sub>, and two nutrient regimes: one-fifth strength (low nutrient) or full-strength (high nutrient) Long Ashton nutrient solution. Data are means of four replicates  $\pm$  s.e.

and CO<sub>2</sub> treatments showed an interactive effect on both photosynthetic nitrogen and phosphorus use efficiencies, with elevated pCO<sub>2</sub> decreasing PNUE and PPUE in the low nutrient treatment ( $0.1 \pm 0.04$  and  $0.3 \pm 0.07$  g g<sup>-1</sup> d<sup>-1</sup>, respectively), and increasing PPUE in the high nutrient treatment ( $2.9 \pm 0.44$  g g<sup>-1</sup> d<sup>-1</sup>;  $P < 0.001$ ; Table 5).

## DISCUSSION

### Parent plant tissues

Parent plant leaf blade material of *P. alpina* var. *vivipara* exhibited acclimatory loss of photosynthetic capacity after

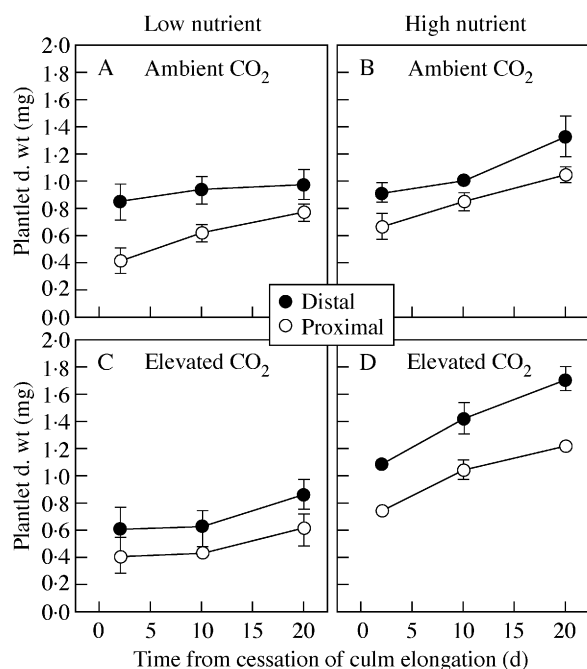


FIG. 6. Dry weight of plantlets from distal and proximal halves of the paracladial zone following the cessation of culm elongation growth in *Poa alpina*. Plants were grown at either  $350 \mu\text{mol mol}^{-1}$  (ambient) or  $690 \mu\text{mol mol}^{-1}$  (elevated) atmospheric CO<sub>2</sub>, and two nutrient regimes: one-fifth strength (low nutrient) or full-strength (high nutrient) Long Ashton nutrient solution. Data are means of four replicates  $\pm$  s.e.

long-term growth at elevated pCO<sub>2</sub>, an effect that was particularly pronounced at low nutrient availability. Harmens *et al.* (2000) reported a positive correlation between N content and acclimation for *Dactylis glomerata*. However, in the present study N, P and TNC contents of parent plant leaf blade material exhibited no difference among treatments that could account for acclimation directly, and they were not present in toxic concentrations (see Marschner, 1995). Lower photosynthetic nitrogen use efficiency indicates that N was utilized less efficiently in the production of dry matter at elevated pCO<sub>2</sub> when fewer nutrients were available. Also, photosynthetic phosphorus use efficiency remained low in this treatment, whereas both PNUE and PPUE were increased substantially by elevated pCO<sub>2</sub> when plants were grown at higher nutrient availability (as also observed by Baxter *et al.*, 1994b).

In addition, developmental differences were observed among treatments in the present study, indicating that CO<sub>2</sub> concentration and nutrient availability determine the developmental age of organs, and of the main shoot system via monocarpic senescence. Indeed, elevated pCO<sub>2</sub> may increase the developmental age of an organ by increasing the rate of cell division (e.g. *Dactylis glomerata*, Kinsman *et al.*, 1997). Changes in developmental age and senescence are associated with changes in protein turnover and the size of precursor pools (Peoples and Dalling, 1988), often resulting in a lack of correlation between leaf N and Rubisco concentrations (e.g. *Oryza sativa* L. 'Sasanishiki', Makino *et al.*, 1984). This suggests that the initial developmental response of *P. alpina* to elevated pCO<sub>2</sub> may determine the subsequent deployment of resources (expressed as PNUE and PPUE), thus governing photosynthetic capacity.

### Reproductive growth

Reduced reproductive dry weight observed in the low nutrient and elevated CO<sub>2</sub> treatment resulted from both a lack of flowering of daughter tillers and the subsequent suppressed growth response of the paracladial zone(s) produced. Daughter tillers may not have reached a threshold dry mass (developmental age) above which the apical meristem could be induced into reproductive development

TABLE 4. Total non-structural carbohydrate content (mg glucose equivalent g<sup>-1</sup> total d. wt) of plantlets from either distal or proximal positions in the paracladial zone at two time points from cessation of culm elongation in *Poa alpina*

Plantlet position	Time (d)	Treatment			
		Low nutrient		High nutrient	
		Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>
Distal	2	69 $\pm$ 15.1 <sup>a</sup>	50 $\pm$ 14.9 <sup>a</sup>	78 $\pm$ 3.9 <sup>a</sup>	90 $\pm$ 26.9 <sup>a</sup>
	20	171 $\pm$ 17.5 <sup>b</sup>	155 $\pm$ 19.9 <sup>b</sup>	170 $\pm$ 32.5 <sup>b</sup>	183 $\pm$ 16.3 <sup>b</sup>
Proximal	2	163 $\pm$ 36.9 <sup>ab</sup>	117 $\pm$ 34.7 <sup>a</sup>	201 $\pm$ 28.3 <sup>b</sup>	100 $\pm$ 22.4 <sup>a</sup>
	20	245 $\pm$ 12.9 <sup>c</sup>	141 $\pm$ 33.5 <sup>a</sup>	220 $\pm$ 19.6 <sup>bc</sup>	201 $\pm$ 2.9 <sup>bc</sup>

Plants were grown in either one-fifth strength (low nutrient) or full-strength (high nutrient) Long Ashton nutrient solution, and either  $350 \mu\text{mol mol}^{-1}$  (ambient) or  $690 \mu\text{mol mol}^{-1}$  (elevated) CO<sub>2</sub>. Data are means of four replicates  $\pm$  s.e. Different superscripts indicate significant differences between all means within each plantlet position (distal or proximal) at  $P < 0.05$  determined by Tukey's multiple comparison procedure (ANOVA) (*sensu* Zar, 1999).

TABLE 5. Total nitrogen (N) and phosphorus (P) concentrations of the paracladial zone of *Poa alpina* at two time points from paracladial exertion, and photosynthetic nitrogen and phosphorus use efficiencies (PNUE, PPUE) of the paracladial zone integrated over an 18-d period

Nutrient	Time (d)	Treatment			
		Low nutrient		High nutrient	
		Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>	Ambient CO <sub>2</sub>	Elevated CO <sub>2</sub>
N (mg g <sup>-1</sup> total d. wt)	2	16.1 ± 2.31 <sup>ab</sup>	14.4 ± 1.04 <sup>ab</sup>	11.4 ± 1.53 <sup>a</sup>	16.4 ± 0.75 <sup>ab</sup>
	20	11.9 ± 1.81 <sup>a</sup>	15.7 ± 1.49 <sup>ab</sup>	19.9 ± 2.27 <sup>bc</sup>	12.8 ± 2.79 <sup>a</sup>
P (mg g <sup>-1</sup> total d. wt)	2	5.4 ± 0.50 <sup>c</sup>	3.9 ± 0.21 <sup>b</sup>	5.1 ± 0.66 <sup>c</sup>	2.7 ± 0.01 <sup>a</sup>
	20	4.4 ± 0.08 <sup>bc</sup>	3.5 ± 0.16 <sup>ab</sup>	3.3 ± 0.54 <sup>a</sup>	2.8 ± 0.13 <sup>a</sup>
PNUE (g structural d. wt g <sup>-1</sup> N d <sup>-1</sup> )		0.6 ± 0.26 <sup>b</sup>	0.1 ± 0.04 <sup>a</sup>	0.4 ± 0.14 <sup>ab</sup>	0.5 ± 0.11 <sup>ab</sup>
PPUE (g structural d. wt g <sup>-1</sup> P d <sup>-1</sup> )		1.7 ± 0.57 <sup>bc</sup>	0.3 ± 0.07 <sup>a</sup>	1.0 ± 0.63 <sup>ab</sup>	2.9 ± 0.44 <sup>c</sup>

Plants were grown in either one-fifth strength (low nutrient) or full-strength (high nutrient) Long Ashton nutrient solution, and either 350 μmol mol<sup>-1</sup> (ambient) or 690 μmol mol<sup>-1</sup> (elevated) CO<sub>2</sub>. Data are means of four replicates ± s.e. Different superscripts indicate significant differences between all means within each nutrient for N and P contents, or between treatment means for each nutrient use efficiency, PNUE or PPUE, at *P* < 0.05 determined by Tukey's multiple comparison procedure (ANOVA) (*sensu* Zar, 1999).

TABLE 6. Summary of statistical significance of a balanced three-way ANOVA of data presented in Figs 4 and 5

Source	No. of daughter tillers in flower (Fig. 4)	Total reproductive dry weight (Fig. 4)	Dry weight of main axis paracladial zone (Fig. 5)	No. of leaves per plantlet (Fig. 5)	Senescence in paracladial zone
CO <sub>2</sub>	**	n.s.	n.s.	n.s.	n.s.
Nutrient	***	***	***	***	*
Time	***	***	*	***	***
CO <sub>2</sub> × Nutrient	***	n.s.	***	***	n.s.
CO <sub>2</sub> × Time	n.s.	n.s.	n.s.	n.s.	n.s.
Nutrient × Time	n.s.	***	n.s.	n.s.	n.s.
CO <sub>2</sub> × Nutrient × Time	*	n.s.	n.s.	n.s.	n.s.

Proportion data were arcsine-transformed and numerical count data were square-root transformed prior to analysis. \*\*\* *P* < 0.001; \*\* *P* < 0.01; \* *P* < 0.05; n.s., not significant (*P* > 0.05).

(Wareing and Phillips, 1990), which may also explain the general lack of flowering in low nutrient treatments. Spikelet number did not differ between treatments and thus differences in dry matter accumulation within the paracladial zone reflected the performance of plantlets. Although photosynthetic acclimation in plantlet tissues was not determined directly (as suitable cuvettes for gas exchange were not available), PNUE and PPUE of plantlet tissues showed a similar response to *p*CO<sub>2</sub> and nutrient availability to that of parent leaf blades, i.e. both were suppressed in low nutrient conditions with elevated *p*CO<sub>2</sub>, and PPUE increased in elevated *p*CO<sub>2</sub> with high nutrient availability. This was reflected in the lower dry matter accumulation in the low nutrient/elevated *p*CO<sub>2</sub> treatment relative to the other treatments imposed, providing further evidence (in addition to photosynthetic efficiency; Pierce *et al.*, 2000a) that plantlets have identical photosynthetic metabolism to parent plant shoot systems.

However, the growth response of plantlets was not entirely due to the direct physiological effects of resource availability. Elevated *p*CO<sub>2</sub> and low nutrient availability altered the development of the culm, with earlier senescence being promoted, and thus the physiological connection to

plantlets existed for a shorter period. Earlier senescence of the culm was also associated with the earlier senescence of plantlet material. The higher, and increasing, carbohydrate contents in the paracladial zone (compared with those of the parent leaf blades) indicate that carbohydrate export was inhibited by culm senescence, potentially leading to feedback inhibition of photosynthetic metabolism in plantlet tissues. Transport in the culm xylem is likely to have occurred at this point (Pierce *et al.*, 2000a), providing a route for the delivery of water and nutrients to photosynthetic tissues. The rapid senescence of plantlet material also indicates that plantlet health was dependent on the timing of culm senescence, an indirect effect of *p*CO<sub>2</sub> and nutrient availability. Additional developmental changes included a delay in floral induction at elevated *p*CO<sub>2</sub> with greater nutrient limitation. Such changes have been shown to be species- and biotype-specific, with nutrient-sufficient conditions usually resulting in either no change or earlier floral initiation (Garbutt and Bazzaz, 1984).

Plantlets produced towards the tip of the paracladial zone were consistently larger than proximal plantlets, irrespective of nutrient or CO<sub>2</sub> availability. Such inherent paracladial heterogeneity in other grass species results from a hierarchy



TABLE 7. Summary of statistical significance of a balanced four-way ANOVA of data presented in Fig. 6.

Source	Dry weight of distal and proximal plantlets (Fig. 6)
CO <sub>2</sub>	n.s.
Nutrient	***
Time	***
Position	***
CO <sub>2</sub> × Nutrient	***
CO <sub>2</sub> × Time	n.s.
CO <sub>2</sub> × Position	n.s.
Nutrient × Time	*
Nutrient × Position	n.s.
Nutrient × Time × Position	n.s.
CO <sub>2</sub> × Nutrient × Time	n.s.
CO <sub>2</sub> × Nutrient × Position	n.s.
CO <sub>2</sub> × Time × Position	n.s.
CO <sub>2</sub> × Nutrient × Position × Time	n.s.

'Position' relates to the location of plantlets in either the distal or proximal half of the paracladial zone. Proportion data were arcsine-transformed and numerical count data were square-root transformed prior to analysis. \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; n.s., not significant ( $P > 0.05$ ).

of phytohormonal dominance (e.g. rice, Patel and Mohapatra, 1992). The larger plantlets from distal paracladia have greater relative growth rates and are ten times more likely to establish than plantlets from proximal paracladia (Pierce *et al.*, 2000a).

The response of an individual plant to resource availability determines its competitive abilities and, ultimately, its survival and reproductive capacity. Clearly, reproductive potential of *P. alpina* var. *vivipara* is suppressed in conditions of elevated  $pCO_2$  and low nutrient availability, as is the vegetative phase (see also Baxter *et al.*, 1997). Therefore, where nutrients are limiting, pseudoviviparous *P. alpina* will be at a disadvantage compared with species that show less acclimatory loss or no change in photosynthetic capacity at predicted future  $pCO_2$ .

In conclusion, leafy plantlets of pseudoviviparous *P. alpina* have the same photosynthetic response to resource availability as parent plant leaf blades, with both showing decreased photosynthetic nutrient use efficiencies in elevated  $pCO_2$  when nutrients are scarce, and both are equally likely to experience acclimatory loss of photosynthetic capacity. In these conditions developmental changes such as a delay in paracladial exsertion, a general lack of paracladial exsertion, and exacerbated senescence in the culm and plantlets also decrease reproductive potential. Pseudoviviparous *P. alpina* growing in nutrient-poor habitats will be at both a vegetative and reproductive disadvantage compared with species that experience less loss of photosynthetic capacity during acclimation to future atmospheric CO<sub>2</sub> concentrations.

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