



## Phenotypic Plasticity of Grass Root Anatomy in Response to Light Intensity and Nutrient Supply

STEFAN WAHL, PETER RYSER\* and PETER J. EDWARDS

*Geobotanisches Institut ETH Zürich, Gladbachstr. 114, 8044 Zürich, Switzerland*

Received: 19 April 2001 Returned for revision: 3 July 2001 Accepted: 2 September 2001 Published electronically: 24 October 2001

The phenotypic plasticity of axial root anatomy was investigated in response to the availability of above- and below-ground resources using eight grass species of the genera *Bromus* and *Poa*. In a 7-week garden experiment nutrients were varied by a factor of five and light treatments of 100, 30 and 5.5% daylight were applied. Both nutrients and light influenced root structure. The effect of nutrients was largely explained by plant size, but this was not the case for light. Shading to 30% daylight led to a higher proportion of stele, larger stelar cells and larger xylem vessels. This can be understood either as an increased need for high transport capacity in the shade, where leaf area is larger but root mass lower than in full daylight, or as an increased resistance against desiccation, which is more of a hazard in open sites. Under 5.5% daylight, tissue mass density was reduced due to a lower proportion of stele, though xylem characteristics were not influenced when a correction for the effect of root cross-sectional area was applied. This response may be interpreted as a mechanism to maintain root function with a lower investment in biomass when growth is limited by low irradiance. The results show that the response of a plant to resource limitation is not restricted to those organs responsible for the acquisition of that resource. Furthermore, the qualitative response to shading depends on the absolute level of irradiance. For this reason, care is needed when comparing the results of shading experiments conducted under different irradiances.

© 2001 Annals of Botany Company

**Key words:** *Bromus*, hydraulic conductivity, light, nutrients, phenotypic plasticity, *Poa*, Poaceae, root anatomy, stele.

### INTRODUCTION

There is good reason to suppose that adjustments in anatomy and morphology of a plant in response to environmental conditions, i.e. phenotypic plasticity, are often adaptive, in the sense that they enhance the plant's fitness in that environment (Sultan, 2000). For example, plants tend to respond to resource limitation by allocating a larger proportion of their biomass to those organs involved in capturing the resource in shortest supply (Bloom *et al.*, 1985; Gedroc *et al.*, 1996). Thus, nutrient depletion commonly leads to increased biomass allocation to roots, while shading causes increased allocation to leaves and/or stems (Corrè 1983; Ryser and Lambers, 1995; Ryser and Eek, 2000).

The capacity of plants to acquire resources above- and below-ground is not determined directly by the biomass of roots and leaves but by leaf surface area (Lambers and Poorter, 1992) and root length (Ryser, 1998); it is therefore affected by the morphology and anatomy of these organs. A large leaf area per unit mass or a long root system per unit mass can be a result of either thin leaves or thin roots, respectively, or a result of low tissue density (Dijkstra and Lambers, 1989; Witkowski and Lamont, 1991; Ryser and Lambers, 1995). Among grasses, there is a relationship between tissue density in leaves and the proportions of cell wall and sclerenchyma in leaf cross-sections (Garnier and Laurent, 1994; van Arendonk and Poorter, 1994). Tissue density in roots has been related to the proportion of stele, the proportion of cell wall in the stele, and to characteristics of the tracheary system (Wahl and Ryser, 2000).

\* For correspondence. Fax +411 261 0595, e-mail [ryser@geobot.unmw.ethz.ch](mailto:ryser@geobot.unmw.ethz.ch)

We investigated phenotypic plasticity in the anatomical characteristics of roots in response to light and nutrient conditions. Such responses have been well studied in above-ground organs but less studied in roots. For example, it is known that under high light intensity many plants produce thicker leaves with more photosynthetically active tissue, while under shady conditions leaves have less sclerenchymatous tissue and more leaf area per unit dry matter (Björkman, 1981; Strauss-Debenedetti and Bazzaz, 1991; Thompson *et al.*, 1992). In contrast, leaves produced under low nutrient availability tend to contain a higher amount of sclerenchymatous tissue than those produced under high nutrient supply (van Arendonk *et al.*, 1997), which increases their robustness and reduces damage and loss.

The plastic responses of roots to drought and anaerobic conditions have been studied in some detail. A lack of oxygen can lead to the development of more aerenchyma tissue (He *et al.*, 1999), whereas drought causes increased deposition of suberin and lignin in the hypodermis and endodermis (Cruz *et al.*, 1992), and deposition of hydrophobic substances in epidermal cell walls (Watt *et al.*, 1996). Less is known about phenotypic plasticity of roots in response to light or nutrients. In poplar, a high nutrient supply led to the production of larger xylem vessels (Harvey and van den Driessche, 1999). The grasses *Dactylis glomerata* and *Brachypodium pinnatum* produced finer roots with a higher tissue mass density when the supply of nitrogen or phosphorus was low (Ryser and Lambers, 1995). A decrease in root diameter and an increase in root tissue mass density have contrasting effects on specific root length, which has been found to increase, decrease or remain unchanged in response to low nutrient supply (Ryser, 1998).

The level of irradiance also influences root characteristics, compensating for the reduced biomass allocation to roots in shade. Shade-grown (30 % daylight) *Dactylis glomerata* and *D. polygama* allocate less biomass to roots than those grown in full daylight but have a higher specific root length; as a result, the total root length per plant and nutrient uptake are the same as under full daylight (Ryser and Eek, 2000). This indicates that phenotypic plasticity in root diameter and tissue mass density can also be due to variation in above-ground resources; however, the anatomical basis of these changes is unknown.

Interspecific variation in root anatomy and root diameter is closely associated with the ecological requirements of the plant species (Wahl and Ryser, 2000). The aim of this study was to investigate intraspecific variation in root structure in response to different availabilities of above- and below-ground resources. Which anatomical characteristics respond to nutrients and light, and to what extent can such responses be regarded as adaptive? We studied phenotypic plasticity in root anatomy in eight ecologically contrasting grass species. Grasses are convenient for such work because they are ecologically diverse and yet have a comparatively uniform growth form.

## MATERIALS AND METHODS

### Experimental design and treatments

Eight grass species belonging to the genera *Bromus* and *Poa* were used (Table 1). The species selected, which included both annuals and perennials, varied widely in their habitat requirements with respect to nutrient and light conditions. Seeds were collected from populations in the Swiss Mittelland or were obtained commercially from Fenaco Sämereien, Winterthur, Switzerland (*P. trivialis* and *P. annua*). They were sown on perlite in a glasshouse. After germination, seedlings were transplanted into 1 l polyethylene bags (depth 0.32 m, width 0.064 m) containing quartz sand of 0.1–0.7 mm grain size. Small holes in the lower part of the bags ensured adequate drainage. There were six replicate plants per species and treatment combination.

TABLE 1. The eight grass species studied, their indicator values for nutrients and light and their characteristic habitat

	Ellenberg indicator values		
	Nutrients	Light	Habitat
<i>Bromus erectus</i> Huds.	3	8	Limestone grasslands
<i>Bromus sterilis</i> * L.	5	7	Road verges, arable land
<i>Bromus tectorum</i> * L.	4	8	Road verges, wasteland
<i>Poa angustifolia</i> L.	3	7	Dry grasslands
<i>Poa annua</i> * L.	8	7	Arable fields, wasteland
<i>Poa nemoralis</i> L.	4	5	Deciduous woodlands
<i>Poa pratensis</i> L.	6	6	Productive grasslands
<i>Poa trivialis</i> L.	7	6	Moist productive grasslands

The indicator values according to Ellenberg *et al.* (1992) express nutrient or light availability in the characteristic habitat on a scale from 1 (low availability) to 9 (high availability). Annual species are indicated with an asterisk.

Six treatment combinations with three photon flux densities (PFD; 100, 30, 5.5 % daylight) and two levels of nutrient supply were applied in a factorial design in an experimental garden at Zürich, Switzerland. The shade treatments were produced using frames (3 × 4 × 2.3 m) covered with an aluminium-coated shade cloth (OLS 60 and a combination of OLS 80 and black plastic net for 30 % PFD and 5.5 % PFD, respectively; Ludwig Svensson, Kinna, Sweden). Shading treatments were replicated in two blocks. The measured mean PFD values for the two levels of shading were  $30.4 \pm 0.4$  % and  $5.5 \pm 0.2$  % of the unshaded treatment. The influence of shading treatment on the microclimate was generally small. In the tents with 5.5 % PFD, afternoon maximum temperatures of 20–25°C outside were reduced by 2–3°C, whereas the nightly minima in the tents of approx. 5°C were about 1°C higher than outside. On a sunny day relative humidity was 50–60 % inside the shadiest tents, compared to 30–40 % outside. The influence of the 30 % PFD treatment on temperature and humidity was less than that of the 5.5 % PFD treatment.

The two nutrient treatments differed by a factor of five in the amount of nutrients applied. Nutrients were supplied weekly as 90 ml of diluted Hoagland solution containing (low/high): 18.4/91.9 µmol KH<sub>2</sub>PO<sub>4</sub>, 61.3/306 µmol KNO<sub>3</sub>, 29.4/147 µmol Ca(NO<sub>3</sub>)<sub>2</sub>, 16.6/83.1 µmol MgSO<sub>4</sub>, 0.009/0.044 µmol CuSO<sub>4</sub>, 0.0034/0.017 µmol ZnSO<sub>4</sub>, 0.074/0.370 µmol MnCl<sub>2</sub>, 0.19/0.95 µmol H<sub>3</sub>BO<sub>3</sub>, 0.002/0.010 µmol Na<sub>2</sub>MoO<sub>4</sub>, 0.009/0.044 µmol FeCl<sub>3</sub> and 0.01/0.05 µmol tartaric acid. For the first week all plants received the low nutrient treatment; the different nutrient treatments were initiated subsequently. To avoid any treatment effects of water availability, the polyethylene bags were placed in trays filled with 30 mm water. Capillary movement ensured a continuous water supply to the plants.

### Harvest and measurements

Plants were harvested on 25 Jun. 1997 after a growth period of 7 weeks, and were separated into above-ground biomass and roots. Fresh mass of above-ground parts was measured immediately after harvest, and dry mass after drying for at least 24 h at 70 °C.

To measure root tissue mass density, one nodal root per plant measuring 25–35 cm in length was selected. The root was rinsed in tap water to remove the sand and a section approx. 30 mm long was cut from the middle of the root. A sub-sample approx. 20 mm long was stored in 70 % ethanol for anatomical studies while the remainder was used to determine tissue mass density. For this purpose, root diameter was measured with a light microscope, and the volume was calculated by multiplying the cross-sectional area by the length, assuming a cylindrical form. Root samples were dried at 70°C for at least 24 h and the dry mass was determined to a precision of  $\pm 2$  µg using an analytical balance (MT 5, Mettler Toledo GmbH, Greifensee, Switzerland). Root tissue mass density was calculated as dry mass per volume (mg mm<sup>-3</sup>).

For anatomical measurements, the root pieces were embedded in 2-hydroxyethylmethacrylate (HEMA), also known as GMA (Igersheim and Cichocki, 1996). Slices

4  $\mu\text{m}$  thick were cut with a microtome. To distinguish cell walls from the background, the material was stained with Toluidine Blue, which stains lignin blue-green and cellulose and polycarboxylic acids red to reddish purple (O'Brien *et al.*, 1964; Gerlach, 1984). The sections were examined by light microscopy ( $\times 100$  and  $\times 200$ ) and digital images were obtained using a video camera (JVC TK 1280) connected to a computer.

We used the public domain NIH Image program version 1.62 (developed at the U.S. National Institute of Health and available on the Internet) on a Macintosh computer to measure various anatomical features. The cross-sectional areas (CSA) of the whole root, of the stele, and of large metaxylem vessels were determined by tracing their outlines with the cursor. Based on these measurements, two ratios were calculated: CSA stele:CSA root and CSA xylem:CSA root. The number of stelar cells and xylem vessels was counted. The proportion of cell wall in the stele CSA (excluding xylem elements which were measured separately) was determined using randomly chosen segments representing about 10% of the total CSA. The lumen of the cells was outlined and subtracted from the total area of the segment.

#### Statistical analysis

For the statistical analyses, SyStat version 5.2 (SyStat Inc., Evanston, IL 1992) was used. A logarithmic transformation was used for size data, an arcsine-square root transformation for quotients, and a square root transformation for count data (Sokal and Rohlf, 1995). A three-factorial ANOVA was used with species, nutrient treatment and light treatment as factors. The two plots for each light treatment were used as the split-plot factor. When analysing treatment effects on plant morphology and anatomy it is important to differentiate between allometric effects, i.e. treatment effects which are a result of differences in plant size between treatments, and genuine phenotypic plasticity (Coleman *et al.*, 1994). To correct for allometric effects, we used the log-transformed dry mass of above-ground plant parts as a covariate in the analyses of the above-ground dry mass to fresh mass ratio ( $\text{DM}/\text{FM}_a$ ) and root cross-sectional area. For analyses of other root parameters, the log-transformed root CSA was used as covariate to correct for any effects it might have on anatomical characteristics.

Relationships between covariant and transformed values of the tested parameters were found to be linear.

## RESULTS

#### Treatment effects on above-ground biomass and dry matter content

Both nutrient and light treatments had significant effects on the above-ground dry mass ( $\text{DM}_a$ ), and there was a significant interaction between these factors (Fig. 1A; Table 2). In full daylight and at 30% PFD, above-ground biomass was more than three-times greater under high compared to low nutrient supply. At 5.5% PFD there was no difference between the nutrient treatments. The two levels of shading had contrasting effects on growth. Under 30% daylight, above-ground biomass was slightly but significantly greater than under full daylight. In contrast, under 5.5% PFD, above-ground dry mass was only 14% of that in full daylight for the high nutrient treatment, and 42% of that in full daylight for the low nutrient treatment.

The nutrient and light treatments had contrasting effects on the dry mass to fresh mass ratio of the above-ground tissue ( $\text{DM}/\text{FM}_a$ ; Fig. 1B; Table 2). At 5.5% PFD,  $\text{DM}/\text{FM}_a$  was on average 41% lower than in full daylight. Even at 30% PFD there was a significant response in above-ground tissue density, although growth was not reduced compared to full daylight (Fig. 1A; Table 3). In contrast, a decrease in nutrient supply increased  $\text{DM}/\text{FM}_a$  by 10%, although the effect was only significant when size was included as a covariant in the analysis.

#### Root cross-sectional area and anatomy

The cross-sectional area of axile roots was significantly influenced both by nutrient and light treatments ( $P < 0.001$ ); under high levels of both resources roots tended to be thicker (Fig. 2A). However, root CSA was also closely associated with plant size: larger plants had thicker roots (Fig. 3). When the size effect was taken into account by including above-ground dry mass as a covariant in the analysis, the nutrient effect on root CSA was not significant, and the light effect was only weakly significant (Table 2).

All anatomical characteristics of the roots were significantly associated with root CSA (Table 2). Thus, as

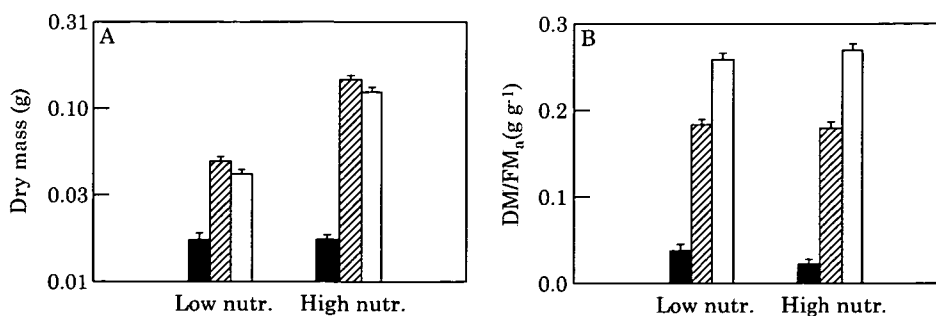


FIG. 1. Above-ground dry mass (A) and above-ground dry mass to fresh mass ratio ( $\text{DM}/\text{FM}_a$ ; B) as a function of two levels of nutrient supply and three levels of photon flux density (PFD). Mean values  $\pm$  1 s.e. without size correction. 5.5% PFD (■), 30% PFD (▨), 100% PFD (□).

TABLE 2. Results of ANOVA for above-ground biomass ( $DM_a$ ), dry mass to fresh mass ratio in above-ground organs ( $DM/FM_a$ ), cross-sectional area (CSA) of axile roots, tissue mass density of axile roots ( $TDM_r$ ) and the anatomical root traits: proportion of stele in root CSA, proportion of cell wall in stele CSA, number of stele cells, size of stele cells (CSA), number of xylem vessels, mean xylem vessel CSA and total xylem CSA

Parameters	$R^2$	Sp (d.f. = 7)	L (d.f. = 2)	N (d.f. = 1)	L x N (d.f. = 2)	Sp x L (d.f. = 14)	Sp x N (d.f. = 7)	Sp x L x N (d.f. = 14)	Log $DM_a$ (d.f. = 1)	Log CSA (d.f. = 1)
$DM_a^*$	0.90	115.8 <0.001	417.9 <0.001	232.0 <0.001	58.7 <0.001	6.9 <0.001	2.4 0.025	3.2 <0.001		
$DM/FM_a^\dagger$	0.92	41.5 <0.001	242.1 <0.001	26.9 <0.001	2.3 <0.108	3.5 <0.001	1.6 0.139	1.1 0.388	39.2 <0.001	
Root CSA*	0.64	12.8 <0.001	4.1 0.017	3.3 0.071	0.9 0.414	3.9 <0.001	0.9 0.504	1.2 0.260	7.7 0.006	
$TDM_r^\ddagger$	0.39	3.4 0.002	31.9 <0.001	0.0 0.914	0.6 0.560	0.9 0.608	0.2 0.976	1.6 0.073		5.8 0.017
Prop. stelet	0.67	44.0 <0.001	15.8 <0.001	0.0 0.927	3.7 0.026	2.1 0.012	1.9 0.068	1.4 0.163		11.0 0.001
Prop. cell wall st.†	0.36	3.4 <0.001	2.6 0.078	0.0 0.833	0.2 0.835	0.8 0.657	0.9 0.473	1.9 0.025	6.4 0.012	
Stele cell number‡	0.73	12.3 <0.001	10.4 <0.001	0.1 0.775	2.0 0.133	2.0 0.016	1.4 0.203	1.5 0.105		67.1 <0.001
Stele cell CSA*	0.64	10.7 <0.001	5.1 0.007	0.5 0.488	0.3 0.761	2.2 0.008	0.4 0.920	1.0 0.427		47.0 <0.001
Number vessels‡	0.74	24.0 <0.001	3.1 0.048	1.5 0.229	1.3 0.287	1.0 0.499	1.1 0.361	1.3 0.205		50.1 <0.001
Vessel CSA*	0.57	10.3 <0.001	12.4 <0.001	3.0 0.0833	5.8 0.0035	1.2 0.261	1.8 0.092	2.6 0.001		36.8 <0.001
Xylem CSA*	0.87	45.1 <0.001	16.4 <0.001	9.8 0.002	2.5 0.087	2.6 0.002	0.9 0.530	1.5 0.096		184.4 <0.001

The factors were species (Sp), light (L) and nutrients (N). The natural logarithm of dry mass above-ground and root CSA were used as covariates, respectively. The block effect with blocks nested within light was not significant and is not shown.  $R^2$  and degrees of freedom (d.f.) are given (total d.f. = 275). Transformations: \*natural logarithm, †arc sine, ‡square root.

TABLE 3. Contrasts between 100 % and 30 %, and between 30 % and 5.5 % photon flux density (PFD) for the measured traits (see Table 2)

Parameters	Contrasts			
	100%–30% PFD		30%–5.5% PFD	
	Pairwise mean differences	P	Pairwise mean differences	P
DM <sub>a</sub>	0.16	*	-1.57	***
DM/FM <sub>a</sub>	-0.056	***	-0.065	***
Root CSA	-0.041	—	-0.228	—
TDM <sub>r</sub>	-0.011	—	-0.042	***
Proportion stele	0.032	***	-0.043	***
Prop. cell wall stele	-0.022	—	-0.009	—
Stele cell number	0.656	—	-2.062	***
Stele cell CSA	0.088	*	0.015	—
Number vessels	0.117	—	-0.136	—
Xylem vessel CSA	0.175	**	0.137	—
Total xylem CSA	0.305	***	-0.041	—

Pairwise mean difference in least square means and the level of significance are given. \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$ .

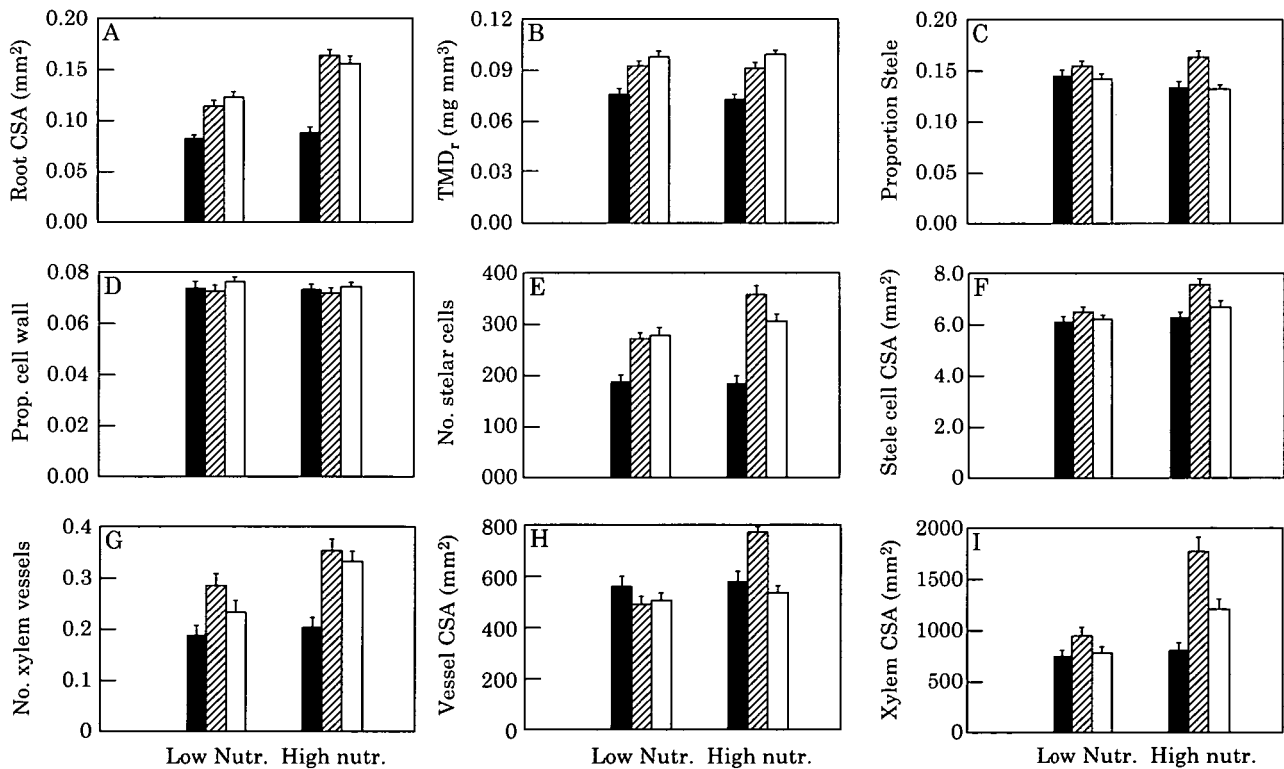


FIG. 2. Reaction of axile root anatomical traits to two levels of nutrient supply and three levels of photon flux density (PFD). Mean values  $\pm$  1 s.e. without size correction. A, Root cross-sectional area; B, root tissue mass density; C, proportion stele in root CSA; D, proportion of cell wall in stele CSA; E, number of stelar cells; F, stelar cell size (CSA); G, number of xylem vessels; H, mean xylem vessel CSA; and I, total xylem CSA. 5.5% PFD (■), 30% PFD (▨), 100% PFD (□).

nutrient supply influenced root CSA, it also had a strong influence on root anatomy in intermediate shade and in full daylight, but not at 5.5 % PFD (Fig. 2). Axile roots grown under high nutrient supply had more ( $P < 0.01$ ) and larger ( $P < 0.01$ ) stelar cells, and also more xylem vessels ( $P < 0.001$ ) with a larger CSA ( $P < 0.001$ ). Total xylem CSA was larger under high than under low nutrient supply ( $P < 0.01$ ). However, when root cross-sectional area was

included as a covariant in the analysis, nutrient effects on size and number of stelar cells and xylem vessels disappeared. Only the total xylem CSA remained significantly influenced by nutrient supply (Table 2); the  $P$ -value for the nutrient effect on mean xylem vessel size failed marginally to be significant ( $P = 0.051$ ).

Irradiance had a significant effect on all root anatomical parameters except on the proportion of cell walls in stele

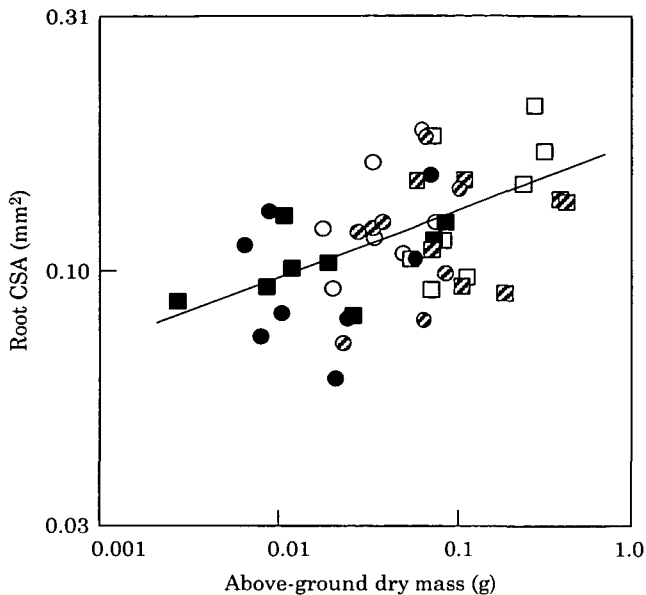


FIG. 3. Cross-sectional area of axile roots of the eight species studied in the six treatment combinations plotted against above-ground dry mass. Squares: high nutrient treatment. Circles: low nutrient treatment. Filled symbols: 5.5 % daylight. Hatched symbols: 30 % daylight. Open symbols: 100 % daylight. The line marks a significant regression ( $R^2 = 0.58$ ,  $P < 0.001$ ).

CSA ( $P = 0.078$ ), even when variation in root CSA was taken into account. The trends in response to increased shading were different for the progression from full daylight (100 % PFD) to 30 % PFD, compared with that from 30 % PFD to 5.5 % PFD (Fig. 2, Table 3). Thus, plants grown at 30 % PFD had a significantly higher proportion of stele than those grown under full daylight. This effect was due to the larger size of the xylem vessels and the parenchyma cells in the stele rather than to any difference in the numbers of xylem vessels and stelar cells. Total xylem CSA was significantly greater at 30 % PFD. The proportion of cell wall in the stele and the tissue mass density of axile roots ( $TMD_r$ ) were slightly lower in intermediate shade, but these differences were not significant. In contrast, plants grown at 5.5 % PFD had a significantly smaller proportion of stele compared to those grown at 30 % PFD. This effect was due to a smaller number of stelar cells, the size of the cells being unchanged. Root tissue mass density was significantly lower at 5.5 % PFD than at 30 % PFD. Plants grown at 5.5 % PFD were smaller than those grown at higher light levels, and had thinner roots ( $P < 0.001$ ) with smaller stelar cells ( $P < 0.01$ ), fewer xylem vessels ( $P < 0.001$ ), and a smaller area of total xylem ( $P < 0.001$ , Fig 2). However, none of these differences were significant if above-ground biomass or root CSA was included as a covariate in the ANOVA (Table 3).

There was a significant nutrient  $\times$  light interaction for xylem vessel CSA (Table 2). This was a result of the pronounced effect of nutrient supply on xylem vessel CSA at 30 % PFD, an effect that was not found either in full daylight or at 5.5 % PFD (Fig. 2).

Variation in root tissue mass density caused by the treatments was strongly associated with the variation in above-ground tissue density (General Linear Model with average  $TMD_r$  of each species in each treatment as dependent variables, species as categorical variables, and  $DM/FM_a$  as a continuous independent variable;  $P < 0.001$ ).

#### Interspecific variation in plastic response

There were no significant species  $\times$  nutrient interactions for any of the morphological or anatomical traits investigated (Table 2), although there was a weak interaction for above-ground dry mass. In contrast, several of the traits showed a significant species  $\times$  light interaction, indicating interspecific differences in light response. These traits included above-ground biomass, above-ground dry to fresh mass ratio, root CSA, proportion of stele in root CSA, stele cell size, stele cell number, and total xylem CSA (Table 2). In spite of this interspecific variation in light response, the rank order of species with respect to their parameter values across all six treatment combinations was remarkably stable. The Kendall Coefficient of Concordance (Zar, 1999) for above-ground biomass,  $DM/FM_a$  and all root parameters (corrected for the effects of above-ground biomass or root CSA, respectively) except  $TMD_r$  was over 0.57, indicating a highly significant concordance of species' values across all treatments (six treatment combinations and eight species;  $P < 0.001$ ).

## DISCUSSION

Both nutrient supply and irradiance had an effect on root anatomical characteristics. The results for nutrient supply could be completely explained as allometric effects related to differences in plant size and root thickness in different treatments. However, the influence of irradiance on root anatomy was not solely a size effect. The observed influence of above-ground resources on below-ground anatomy clearly demonstrates the importance of regarding a plant as a whole when considering its response to environmental conditions. It is evident that the organs directly associated with the acquisition of the limiting resource are not the only ones that are affected by changes in the supply of that resource.

The lack of a nutrient effect on root characteristics (other than an effect due to size) could be because our measurements were limited to axile roots. Other work using the grasses *Dactylis glomerata* and *Brachypodium pinnatum* has shown that when the whole root system is considered, root tissue mass density tends to be higher in plants grown with a low supply of nitrogen and phosphorus compared with those grown with high levels of these nutrients (Ryser and Lambers, 1995). Nutrient supply is also known to influence root topology in dicots, and thus root diameter distribution (Fitter and Stickland, 1991).

Shade had two major effects on the anatomy of grass roots: firstly, it led to modifications of the water conducting system; and secondly, tissue with lower construction costs per volume was produced. The first effect was evident at 30 % PFD, although growth was not

reduced compared to 100 % PFD. The second effect was pronounced at 5.5 % PFD, and was associated with reduced biomass production. The qualitatively different responses of plants growing under 30 % PFD and 5.5 % PFD demonstrate the importance of considering actual levels of resource availability, and whether or not they are growth limiting, when drawing conclusions about phenotypic plasticity.

The decrease in  $TMD_r$  was especially pronounced when irradiance became growth limiting, probably due to carbon limitation. The significance of this response may be that it allows plants to maintain root length and nutrient acquisition capacity, though at the expense of tissue robustness. A similar conclusion was reached for two *Dactylis* species which were able to maintain their total root length when shaded (30 % daylight) although the relative biomass allocation to roots was strongly reduced (Ryser and Eek, 2000).

A decrease in the investment in dry mass per root volume in the deepest shade was mainly a result of a smaller proportion of stele per root cross-sectional area, attained by a reduced number of stelar cells. However, the functional characteristics of stele were hardly influenced; although xylem is part of the stele, a reduction in the proportion of stele had no influence on xylem characteristics, suggesting that plants maintain the transport capacity of their roots even if investment of dry matter in dense stelar tissue is reduced. The percentage of cell wall in the stele cross-sectional area also remained unchanged.

An increase in irradiance compared to intermediate shade also led to a decrease in the proportion of stele, although in contrast to the response in deeper shade this was mainly a result of smaller stelar cells. Furthermore, full daylight led to significant changes in functional characteristics of the root: xylem vessels and the total xylem cross-sectional area were smaller in full daylight than under intermediate shade.

There are two possible explanations for these responses to light. Firstly, it is well known that under low light conditions plants often increase their leaf area ratio strongly while reducing their biomass allocation to roots (Corrè, 1983; Rice and Bazzaz, 1989). For example, Ryser and Eek (2000) found that leaf area ratio (LAR) of *Dactylis glomerata* almost doubled under 30 % PFD compared to full daylight, whereas the root mass ratio (RMR) was reduced by about 25 %. A higher relative transport capacity is required as the transpiring surface increases but the amount of roots decreases. According to the Hagen-Poiseuille law, conductance of a pipe increases with the fourth power of its diameter. The most cost-efficient way to increase the transport capacity of a root system is thus to increase the vessel diameter rather than producing more vessels in one root or by producing more roots (McCully and Canny, 1988). An increased xylem vessel size and total xylem cross-sectional area in shade also correspond well to the known relative constancy of the ratio between conducting cross-sectional area and leaf area (Huber value; Löscher, 2001).

Secondly, the difference in root anatomy between 30 % and 100 % daylight may also be regarded from the point of view of a potentially higher stress in full daylight.

Compared to plants growing in shade, those exposed to full daylight are often more prone to drought due to stronger winds, higher temperatures, and lower air humidities. In our experiment none of the plants were water-limited, but their response may still be adaptive to an increased risk of drought stress. Smaller cells in the stele and smaller xylem vessels indicate a higher resistance to low water potentials. It is known, for example, that local drought leads to smaller xylem vessels in sorghum (Cruz *et al.*, 1992). In above-ground parts of poplars and various species of Pinaceae, negative correlations have been demonstrated between hydraulic conductivity and both xylem vessel diameter and resistance to cavitation (Harvey and van den Driessche, 1999; Piñol and Sala, 2000). Furthermore, smaller root xylem vessels are non-functional for a shorter period after an embolism as they refill more quickly (McCully *et al.*, 1998). Small vessel size is associated with species found in habitats with a high probability of drought stress. For example, grasses of open and dry limestone grasslands, such as *Bromus erectus*, *Festuca ovina*, *Briza media* and *Poa angustifolia*, have smaller xylem vessels than species of more mesic open habitats, such as *Lolium perenne*, *Dactylis glomerata* or *Holcus lanatus*, or species of mesic habitats in shade or semi-shade, such as *Deschampsia caespitosa*, *Helictotrichon pubescens* or *Milium effusum* (Wahl and Ryser, 2000).

We hypothesize that the response of total xylem CSA to nutrient supply reflects the interplay between requirements for hydraulic conductance and for protection against drought. A greater xylem CSA under high nutrient supply, even when the effect of root CSA is taken into account, can be understood in terms of a need for an increased transport capacity. At high nutrient supply, plants have a relatively larger leaf area than at low nutrient supply, and invest a smaller proportion of biomass below ground. At 30 % PFD, the increase in total xylem area due to a higher nutrient supply was associated with a greater xylem vessel CSA. In full daylight the response may have been more 'cautious' with respect to a potential drought: mean xylem vessel size actually decreased, whereas the number of xylem vessels increased slightly.

Interspecific variation in plasticity has often been considered to reflect the ecological behaviour of the species (Crick and Grime, 1987; Grime *et al.*, 1991; Sultan, 2000). However, our data are not consistent with this conclusion. All eight grass species reacted in a similar way to variation in nutrient levels, and differences in light response were not easy to interpret. The rank order of species with respect to most of the characteristics studied remained similar under all treatment combinations. We conclude that even if plastic responses of root characteristics are an important aspect of the plant's performance when resources are limiting, interspecific variation in ecological behaviour may be better explained by the average characteristics than by the amount of plasticity in these characteristics.

#### ACKNOWLEDGEMENTS

We thank Anton Igersheim and Peter Endress from the University of Zürich for advice in anatomical work and for

facilities, Felix Wäckers for the use of equipment and Cathy Bayliss for comments on the manuscript. Stefan Wahl received financial support from the Swiss National Science Foundation (Grant 31-43392-95).

## LITERATURE CITED

- Björkman O. 1981. Response to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, eds. *Encyclopedia of Plant Physiology*. Berlin: Springer Verlag, 57–107.
- Bloom AJ, Chapin FS, Mooney HA. 1985. Resource limitation in plants—an economic analogy. *Annual Review of Ecology and Systematics* 16: 363–392.
- Coleman JS, McConnaughay KDM, Ackerly DD. 1994. Interpreting phenotypic variation in plants. *Trends in Ecology and Evolution* 9: 187–191.
- Corré WJ. 1983. Growth and morphogenesis of sun and shade plants. III. The combined effects of light intensity and nutrient supply. *Acta Botanica Neerlandica* 32: 277–294.
- Crick JC, Grime JP. 1987. Morphological plasticity and mineral nutrient capture in two herbaceous species of contrasting ecology. *New Phytologist* 107: 403–414.
- Cruz RT, Jordan WR, Drew MC. 1992. Structural changes and associated reduction of hydraulic conductance in roots of *Sorghum bicolor* L. following exposure to water deficit. *Plant Physiology* 99: 203–212.
- Dijkstra PD, Lambers H. 1989. Analysis of specific leaf area and photosynthesis of two inbred lines of *Plantago major* differing in relative growth rate. *New Phytologist* 113: 283–290.
- Fitter A, Stickland A. 1991. Architectural analysis of plant root systems. II. Influence of nutrient supply on architecture in contrasting plant species. *New Phytologist* 118: 383–389.
- Garnier E, Laurent G. 1994. Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. *New Phytologist* 128: 725–736.
- Gedroc JJ, McConnaughay KDM, Coleman JS. 1996. Plasticity in root/shoot partitioning: Optimal, ontogenetic, or both? *Functional Ecology* 10: 44–50.
- Gerlach D. 1984. *Botanische Mikrotechnik, 5th edn*. Stuttgart: Georg Thieme Verlag.
- Grime JP, Campbell BD, Mackey JML, Crick JC. 1991. Root plasticity, nitrogen capture and competitive ability. In: Atkinson D, ed. *Plant root growth. An ecological perspective*. Oxford: Blackwell Scientific Publications, 381–397. Special publication of the British Ecological Society 10.
- Harvey HP, van den Driessche R. 1999. Nitrogen and potassium effects on xylem cavitation and water-use efficiency in poplars. *Tree Physiology* 19: 943–950.
- He JB, Bögemann GM, van de Steeg HM, Rijnders JGHM, Voesebeck LACJ, Blom CWPM. 1999. Survival tactics of *Ranunculus* species in river floodplains. *Oecologia* 118: 1–8.
- Igersheim A, Cichocki O. 1996. A simple method for microtome sectioning of prehistoric charcoal specimens, embedded in 2-hydroxyethyl methacrylate (HEMA). *Review of Palaeobotany and Palynology* 92: 389–393.
- Lambers H, Poorter H. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research* 23: 187–261.
- Lösch R. 2001. *Wasserhaushalt der Pflanzen*. Wiebelsheim, Germany: Quelle und Meyer Verlag.
- McCully ME, Canny MJ. 1988. Pathways and processes of water and nutrient movement in roots. *Plant and Soil* 111: 159–170.
- McCully ME, Huang CX, Ling LEC. 1998. Daily embolism and refilling of xylem vessels in the roots of field-grown maize. *New Phytologist* 138: 327–342.
- O'Brien TP, Feder N, McCully ME. 1964. Polychromatic staining of plant cell walls by Toluidine Blue O. *Protoplasma* 59: 367–373.
- Piñol J, Sala A. 2000. Ecological implications of xylem cavitation for several *Pinaceae* in the Pacific Northern USA. *Functional Ecology* 14: 538–545.
- Rice SA, Bazzaz FA. 1989. Growth consequences of plasticity of plant traits in response to light conditions. *Oecologia* 78: 508–512.
- Ryser P. 1998. Intra- and interspecific variation in root length, root turnover and the underlying parameters. In: Lambers H, Poorter H, van Vuuren MMI, eds. *Inherent variation in plant growth. Physiological mechanisms and ecological consequences*. Leiden: Backhuys Publishers, 441–465.
- Ryser P, Eek L. 2000. Consequences of phenotypic plasticity vs. interspecific differences in leaf and root traits for acquisition of aboveground and belowground resources. *American Journal of Botany* 87: 402–411.
- Ryser P, Lambers H. 1995. Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. *Plant and Soil* 170: 251–265.
- Sokal RR, Rohlf FJ. 1995. *Biometry*. New York: W.H. Freeman and Co.
- Strauss-Debenedetti S, Bazzaz FA. 1991. Plasticity and acclimation to light in tropical Moraceae of different successional positions. *Oecologia* 87: 377–387.
- Sultan SE. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science* 5: 537–542.
- Thompson WA, Kriedemann PE, Craig IE. 1992. Photosynthetic response to light and nutrients in sun-tolerant and shade-tolerant rainforest trees: I. Growth, leaf anatomy and nutrient content. *Australian Journal of Plant Physiology* 19: 1–18.
- van Arendonk JJCM, Poorter H. 1994. The chemical composition and anatomical structure of leaves of grass species differing in relative growth rate. *Plant, Cell and Environment* 17: 963–970.
- van Arendonk JJCM, Niemann GJ, Boon JJ, Lambers H. 1997. Effects of nitrogen supply on the anatomy and chemical composition of leaves of four grass species belonging to the genus *Poa*, as determined by image-processing analysis and pyrolysis-mass spectrometry. *Plant Cell and Environment* 20: 881–897.
- Wahl S, Ryser P. 2000. Root tissue structure is linked to ecological strategies of grasses. *New Phytologist* 148: 459–471.
- Watt M, van der Weele CM, McCully ME, Canny MJ. 1996. Effects of local variations in soil moisture on hydrophobic deposits and dye diffusion in corn roots. *Botanica Acta* 109: 492–501.
- Witkowski ETF, Lamont BB. 1991. Leaf specific mass confounds leaf density and thickness. *Oecologia* 88: 486–493.
- Zar JF. 1999. *Biostatistical analysis, 4th edn*. Upper Saddle River, New Jersey, USA: Prentice Hall.