New Phytologist

Research

Assimilation and allocation of carbon and nitrogen of thermal and nonthermal *Agrostis* species in response to high soil temperature

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Received: *25 October 2005*
Accepted: *9 January 2006*

Summary

• We studied whether changes in the assimilation and allocation of carbon and nitrogen are associated with plant tolerance to high soil temperatures.

• Two *Agrostis* species, thermal *Agrostis scabra*, a species adapted to high-temperature soils in geothermal areas in Yellowstone National Park (USA), and two cultivars of a cool-season species, *Agrostis stolonifera*, L-93 and Penncross, were exposed to soil temperatures of 37 or 20°C, while shoots were exposed to 20°C.

• Net photosynthesis rate, photochemical efficiency, NO_3^--assimilation rate and root viability decreased with increasing soil temperatures in both species. However, the decreases were less pronounced for *A. scabra* than for both *A. stolonifera* cultivars. Carbon investment in growth of plants exposed to 37°C decreased more dramatically in both *A. stolonifera* cultivars than in *A. scabra*. Nitrogen allocation to shoots was greater in *A. scabra* than in both creeping bentgrass cultivars at 37°C soil temperature.

• Our results demonstrate that plant tolerance to high soil temperature is related to efficient expenditure and adjustment of C- and N-allocation patterns between growth and respiration.

Key words: *Agrostis*, carbon (C) allocation, heat tolerance, nitrogen (N) allocation.

New Phytologist (2006) **170**: 479–490

doi: 10.1111/j.1469-8137.2006.01684.x

Introduction

High temperature is a major environmental factor limiting growth, productivity and survival of cool-season plant species. Growth for cool-season perennial grasses often declines with increasing temperatures, and plant death occurs at temperatures above 30°C (DiPaola, 1992). High temperatures can have direct damaging effects associated with hot tissue temperatures, or indirect effects such as plant-water deficits caused by high evaporative demands. Plants can be damaged in different ways by either high air or high soil temperatures. Soil temperatures often reach injuriously high levels during summer, which strongly influences both shoot and root growth and survival of whole plants (Paulsen, 1994); however, different species and cultivars differ in their sensitivity to high temperature.

Studying plants adapted to extremely stressful environments has been an important tool used to unveil and understand mechanisms of plant tolerance to stresses. Several cool-season, C_3 grass species have recently been identified growing in geothermally heated areas in Yellowstone National Park (YNP), USA (Stout & Al-Niemi, 2002). Plants in geothermal areas in YNP are exposed to soil temperatures up to 50°C, but maximum high air temperatures below 25°C (Stout & Al-Niemi, 2002). Little is known about why and how thermal plants survive, and even thrive, in hot soils that are chronically permeated by hot steam. Stout & Al-Niemi (2002) showed that in heat-tolerant plants from heated soils in YNP, the levels of cytoplasmatic class I small heat-shock proteins (sHSPs) increased in a heat-correlated manner from rhizosphere temperatures between 40 and 48°C, confirming that

sHSPs may contribute to the adaptation of plants to geothermally heated environments. Delmer (1974) did not find specific heat-adaptation mechanisms in the above-ground parts of the thermal species *Mimulus guttatus*, collected from high-temperature soils (40 and 45°C) in YNP; however Stout *et al.* (1997) found that thermal grasses developed shorter and more highly branched roots at high soil temperatures than at low temperatures. *Agrostis scabra* ('thermal' rough bentgrass) is a predominant grass species in the thermal areas (Hitchcock, 1950; Tercek *et al.*, 2003). Rachmilevitch *et al.* (2006) found that the maintenance of root viability for thermal *A. scabra* under high soil temperatures was associated with downregulation of root respiration, particularly maintenance respiration. However, the exact nature of root morphological and physiological changes in plant adaptation to long-term heat stress from soils is still not well understood.

Carbon and nitrogen metabolism are basic processes, which are essential for plant growth and survival under environmental stress. Both are sensitive to changes in temperature. Carbon metabolism encompasses C assimilation through photosynthesis; consumption through respiration; and allocation to different metabolic pools (Lambers & Poorter, 1992). Temperature has a multitude of effects on C metabolism (Long & Woodward, 1988). Studies of plant exposure to relatively high air and soil temperatures have demonstrated that net photosynthetic CO_2-assimilation rate is generally inhibited by high temperatures (Long & Woodward, 1988; Hamerlynck *et al.*, 2000; Martin & Stabler, 2002; Haldimann & Feller, 2004), whereas respiration generally increases with increasing temperatures, at least in the short term, with a temperature coefficient (Q_{10}) *c.* 2 (Lambers, 1985; Criddle *et al.*, 1994). Therefore net C gain tends to be reduced at elevated temperature, and this may be aggravated by changes in allocation pattern favouring root growth at the expense of shoot growth. Consequently, growth is inhibited under heat stress (Amthor, 1989; Xu & Huang, 2000).

It is generally accepted that heat tolerance is positively related to C assimilation and negatively associated with C consumption (Lambers *et al.*, 1998). Carbon allocation to different metabolic processes depends on several internal factors, including plant growth and metabolic rates, and on environmental conditions (Larcher, 2003). Nitrogen uptake and metabolism are highly C-demanding processes (Noctor & Foyer, 1998), and are closely related to C metabolism (Foyer & Noctor, 2002). Rates of N assimilation usually decrease at extreme temperatures (Kenjebaeva & Rakova, 1995; Gao *et al.*, 2000; Epstein & Bloom, 2005). The maintenance of a positive C–N balance, and efficient allocation of C and N, may play a role in plant adaptation to both high air and soil temperatures (Epstein & Bloom, 2005; Gao *et al.*, 2000). However, how C and N allocation to different activities is related to root and whole-plant survival at high soil temperature is poorly understood. Understanding changes in C and N assimilation and allocation patterns may provide further insight into physiological mechanisms of plant tolerance to high soil temperatures.

The objective of this study was to investigate whether plant tolerance to the direct effect of heat in high soil temperature is associated with specific changes in C and N allocation and expenditure in general, and specifically to the unusual habitat of YNP. We compared two *Agrostis* species differing in heat tolerance: thermal *A. scabra* adapted to geothermally heated soils in YNP; and two cultivars of creeping bentgrass (*A. stolonifera*), L-93 and Penncross. Creeping bentgrass is a cool-season perennial grass, widely cultivated as turfgrass in cool-climate regions, and is sensitive to high temperatures (DiPaola, 1992). Relative growth rates (RGR), root viability, photochemical efficiency, rates of NO_3^- assimilation, and gas-exchange rates were determined to evaluate heat tolerance of the two *Agrostis* species. If C fixed during photosynthesis is predominantly allocated to respiration, growth and tissue construction of both shoots and roots, then the fraction of daily fixed C that is respired can be calculated, assuming no diurnal variation in shoot and root respiration and an average constant rate of net photosynthesis under constant day : night temperatures (Poorter *et al.*, 1990). Carbon-allocation patterns of two *Agrostis* species in response to high soil temperature were determined, taking into account C content, RGR, and daily C respired in shoot dark respiration and in roots. Similarly, N allocation to shoots and roots was calculated by determining total N content in shoots and roots.

Materials and Methods

Plant materials

Plants of thermal *Agrostis scabra* Willd., and two cultivars of creeping bentgrass (*Agrostis stolonifera* L.), L-93 and Penncross, were established in sand in polyethylene bags with holes at the bottom for drainage (5 cm diameter, 40 cm length) in a glasshouse. The tubes were designed to enable plant growth to occur in well drained sand in polyethylene bags while root-zone temperature was constant. A detailed description of the water bath that controlled air and soil temperature separately is given by Xu & Huang (2001).

Plants were watered daily and fertilized twice a week with a nutrient solution (60 ml) containing 1 mM KNO_3, 0.2 mM $CaSO_4$, 0.35 mM KH_2PO_4, 2 mM $MgSO_4$, 0.2 g l^{-1} Fe-NaEDTA, and micronutrients (Epstein, 1972). After establishment for 6 wk in the glasshouse, plants were transferred to a controlled-environment walk-in growth chamber (Conviron, Winnipeg, Canada) with a 14-h photoperiod, 20 : 15°C day : night temperature, and a photosynthetic photon fluence rate of 450 μmol m^{-2} s^{-1} at canopy height. Plants were allowed to acclimate to growth-chamber conditions for 1 wk before temperature treatments were imposed.

Treatments and experimental design

Control plants were maintained at a constant air/soil temperature of 20°C, while roots were exposed to 20°C in a water bath, and shoots were exposed to ambient air at 20°C in the growth chamber. For the high soil-temperature treatment, shoots were maintained at low air temperature (day : night 20°C), while roots were exposed to a constant day : night temperature of 37°C in the water bath described above. The temperature treatment was repeated in four water baths placed in the walk-in growth chamber. The entire root zone (a 40-cm-long sand column in a polyethylene bag) was kept in the water bath, while the grass canopy was kept above the water level, which was maintained at the top edge of the water bath. The water-bath temperature was controlled using an immersion-circulating heater. Root-zone temperature was monitored daily using thermocouples located in the root zone at a depth of 10 cm. The root-zone temperature at 10 cm depth during the study was maintained at 37°C for the high soil-temperature treatment and at 20°C for the control treatment.

Photochemical efficiency and shoot gas-exchange measurements

Leaf photochemical efficiency was estimated by measuring chlorophyll fluorescence, variable fluorescence/maximal fluorescence (F_v/F_m), with a leaf photochemical efficiency analyser (Fi_m 1500, ADC BioScientific, Hoddedson, UK). Intact leaves were covered in specially designed leaf chambers and allowed to adjust to the dark for 30 min before F_v/F_m was measured.

Net photosynthesis and dark respiration rates of shoots were measured using an open-flow infrared gas analyser (IRGA: Li-Cor 6400, Li-Cor, Lincoln, NE, USA). Net photosynthesis and dark respiration of shoots were measured by enclosing the whole canopy in a transparent Plexiglas chamber (15 × 10 × 10 cm) attached to the Li-Cor 6400. Both measurements began 3 h into the daily light period. Dark respiration was measured for plants enclosed in an opaque chamber following a 30-min dark-adjustment period.

Root-respiration measurement

At weekly intervals, following the initiation of the high-temperature treatment, roots were washed free of sand and transferred to 500 ml Erlenmeyer flasks containing the nutrient solution used to fertilize the plants as described above. Shoots were exposed to ambient air, while the root system was placed in an Erlenmeyer flask with 400 ml nutrient solution and a 150 m air space. The hydroponic solution in each flask was aerated via a circulating pump (Apollo Enterprises, Oxnard, CA, USA) maintaining an open-flow system. Flasks were submerged in a water bath to control root temperature at either 20 or 37°C, while shoots were exposed to the ambient air temperature (20°C) of the growth chamber. Following a 24-h period of temperature treatment, a closed-flow system was created by connecting the exit air from the Erlenmeyer flask to a circulating pump. The root system was sealed into the flask with a split rubber stopper around the stem. The rubber stopper was sealed with vacuum grease and Teflon tape to create air-tight conditions. The system was tested for leaks before each sampling. Air was sampled via a septum valve from each flask for measurements of CO_2 evolution every 10 min for a total period of 60 min, using 1-ml air-tight syringes. The air samples were analysed using gas chromatography.

The concentrations of CO_2 collected in the hydroponic system were measured using a thermal conductivity (TCD) gas chromatograph (GC-8AIT, Shimadzu Corporation, Kyoto, Japan) with a CO_2 column (80 11/2 Alltech Deerfield, IL, USA). The column temperature was set at 30°C, and the detector was a TCD set at 100 mA. A 0.5 ml sample was injected into an injection port. CO_2 concentrations were plotted vs time, and respiration rates were calculated from the plot regressions. In addition to the closed-flow system measurements of root respiration with gas chromatography, open-flow system measurements of CO_2 evolution were made using an IRGA (Li-Cor 6400). The Erlenmeyer's exit air was connected to the IRGA's sample inlet. The results were compared with results obtained using gas chromatography, and differences between the methods were not significant ($P > 0.1$). The data for respiration measurements using the closed-flow system are reported here.

Following root CO_2 sampling, plants were harvested and separated into shoots and roots, and analysed for root viability. Shoots and roots were then dried in an oven at 80°C for 3 d for dry weight determination.

Relative growth rate and root viability

Relative growth rates of roots and shoots were determined as the slope of the natural logarithm of root dry mass vs time, and expressed as mg g^{-1} d^{-1} (Hunt, 1982).

Root viability was determined by measuring dehydrogenase activity using the triphenyltetrazolium chloride (TTC)-reduction technique (Knievel, 1973). A 0.2 g sample of root fresh weight was incubated in 0.6% (v/v) TTC in phosphate buffer at 30°C for 24 h. Roots were rinsed with water and extracted with 95% ethanol at 60°C for an additional 4 h. The absorption of the extracts was measured at 490 nm to estimate the amount of live roots. Root viability was expressed as the percentage of live roots out of the total roots.

Nitrate uptake and assimilation

Plants were grown as described, except that 2 d before measurement of N uptake, plants were transferred from a

medium containing KNO_3 to one devoid of N. This protocol allowed the depletion of free NO_3^- in shoots and roots. Then, during a 2 h measurement period, plants were transferred to an aerated medium containing 0 or 200 μM KNO_3. Net NO_3^- uptake was assessed from the amount of NO_3^- analysed in the medium before and after the 2-h incubation. Following NO_3^--uptake measurements, plants were divided into shoots and roots, oven-dried and ground to a powder in a ball mill. The nutrient solutions from each plant were collected and kept at 4°C until they were analysed for NO_3^- concentration. The nutrient solutions and extracts of the powder were analysed for NO_3^- using an Alpkem RFA/2 auto-analyser (Perstorp Analytical, Silver Spring, MD, USA). Net NO_3^- accumulation in shoots and roots was calculated from the difference in NO_3^- content between plants that had received NO_3^- during the measurement period and those without NO_3^- supply. The rate of shoot and root NO_3^- accumulation was the amount of NO_3^- accumulated in shoots and roots during the measurement period divided by the incubation time (2 h). NO_3^- absorption was calculated from the depletion of NO_3^- accumulated from the nutrient solution after 2 h. Net nitrate assimilation was calculated as the difference between the rates of NO_3^- absorption and plant NO_3^- accumulation.

Carbon and nitrogen allocation

Dry-root samples were used to determine total C content with a C–H–N elemental analyser (EA 1108, Fisons Instruments, Beverly, MA, USA). Carbon allocation was calculated from total C content in roots and shoots, RGR, and daily C respired through shoot dark respiration and root respiration (Poorter *et al.*, 1990).

Dry-root samples were used to determine total N content with a C–H–N elemental analyser (EA 1108, Fisons Instruments). Nitrogen allocation was calculated from RGR, and total N content in roots and shoots.

Statistical analyses

The experiment was a completely randomized design with four replicates for each treatment and measurements made at weekly intervals. ANOVA was conducted (PROC GLM using repeated measures in SAS ver. 8.02, SAS Institute, Cary, NC, USA) to determine the effects of temperature and time and their interaction on root viability, RGR, gas-exchange rates, photochemical efficiency, and C and N allocation. Treatment means were separated using a least significance test at $P = 0.05$. The relationship between RGR and respiration was determined with linear regression equations. Significant differences between regression lines were tested using ANOVA. Probabilities of < 5% were deemed significant.

Results

Root viability, leaf photochemical efficiency and RGR in response to high soil temperature

Root viability decreased significantly ($P < 0.05$) in L-93 and Penncross after 7 d at high soil temperature (37°C), compared with that at 20°C. A significant ($P < 0.05$) decrease in root viability was not observed in thermal *A. scabra* until 14 d (Fig. 1). *Agrostis scabra* exhibited a less severe decrease in root viability than L-93 and Penncross throughout the entire experimental period. By 28 d at 37°C, root viability had decreased by 30% in Penncross and 21% in L-93, compared with plants grown at 20°C; however, in *A. scabra* root viability decreased by only 10%, and was significantly ($P < 0.05$) higher than root viability in both Penncross and L-93 after 28 d at 37°C (Fig. 1). Root viability did not change significantly ($P > 0.1$) over time or vary with species/genotypes at 20°C.

Photochemical efficiency (F_v/F_m) decreased ($P < 0.05$) by 15% in L-93 and 13% in Penncross, at 7 d of high soil temperature (Fig. 2). The decline in *A. scabra* was not observed until 14 d, when F_v/F_m had decreased by 10%, whereas after 14 d F_v/F_m had decreased by 22% in L-93 and 47% in Penncross (Fig. 2). *Agrostis scabra* exhibited a less severe decrease in F_v/F_m than L-93 and Penncross throughout the entire experimental period. By 28 d at 37°C, F_v/F_m had decreased by 63% in L-93 and 75% in Penncross, compared with plants grown at 20°C, whereas F_v/F_m decreased by 28% in *A. scabra* (Fig. 2). F_v/F_m did not change significantly ($P > 0.1$) over time or vary with species/genotypes at 20°C.

The RGR of roots and shoots decreased over time in all species/genotypes, irrespective of root temperature (Fig. 3a–f).

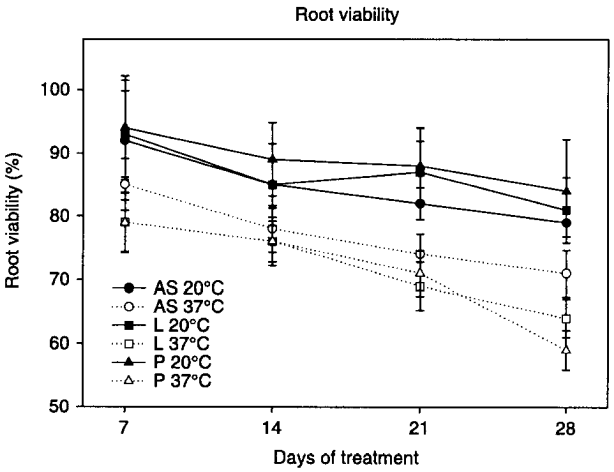

Fig. 1 Root viability expressed as percentage of live roots from total roots of two *Agrostis* species, *Agrostis scabra* (AS, circles) and two creeping bentgrass (*Agrostis stolonifera*) cultivars, L-93 (L, squares) and Penncross (P, triangles), in response to soil temperature: 20°C (solid lines, filled symbols) and 37°C (dotted lines, open symbols). Error bars, SE (n = 5–6).

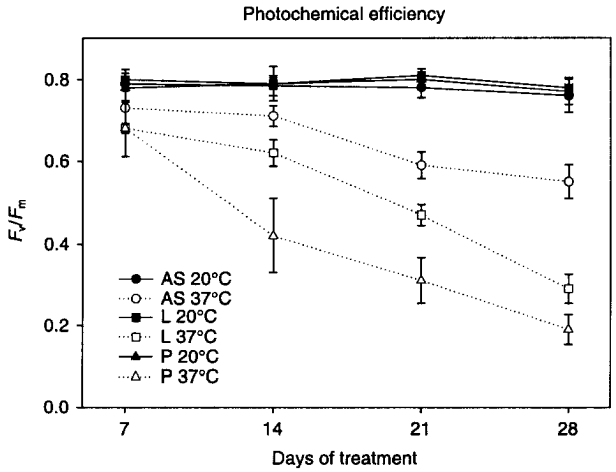

Fig. 2 Photochemical efficiency measured as F_v/F_m of two *Agrostis* species, *Agrostis scabra* (AS, circles) and two creeping bentgrass (*A. stolonifera*) cultivars, L-93 (L, squares) and Penncross (P, triangles), in response to soil temperature: 20°C (solid lines, filled symbols) and 37°C (dotted lines, open symbols). Error bars, SE (n = 5–6).

Agrostis scabra had a significantly ($P < 0.05$) higher RGR at 37°C than Penncross and L-93 throughout the entire experimental period for both roots and shoots. In Penncross and L-93, root RGR decreased by > 80% at 37°C compared with roots grown at 20°C (Fig. 3), while root RGR in *A. scabra* decreased by 65% (Fig. 3). Shoot RGR at 37°C (compared with 20°C) decreased by 90% at 7 d of treatment in Penncross and L-93, whereas in *A. scabra* shoot RGR decreased by 76% after 7 d at 37°C for the same treatment time.

Carbon assimilation and consumption in response to high soil temperature

Daily C-assimilation rate decreased over time in all species/genotypes, irrespective of root temperature (Fig. 4). Exposure to 37°C resulted in a significant ($P < 0.05$) decreases in C-assimilation rate in all species/genotypes; however, the severity of the decrease was highest in Penncross and lowest in *A. scabra*. At 7 d exposure to 37°C, the daily C-assimilation rate in Penncross had decreased by 26% (Fig. 4c), whereas in L-93 and *A. scabra* it had decreased by 17 and 12%, respectively, compared with plants exposed to 20°C (Fig. 4a,b).

Shoot dark respiration did not change significantly ($P > 0.05$) at high soil temperature in all species/genotypes during the initial 21 d of treatment (Fig. 5a–c). After 28 d, shoot dark respiration increased significantly ($P < 0.05$) by 15% in L-93 and 25% in Penncross, whereas it did not change significantly ($P > 0.05$) in *A. scabra* (Fig. 5).

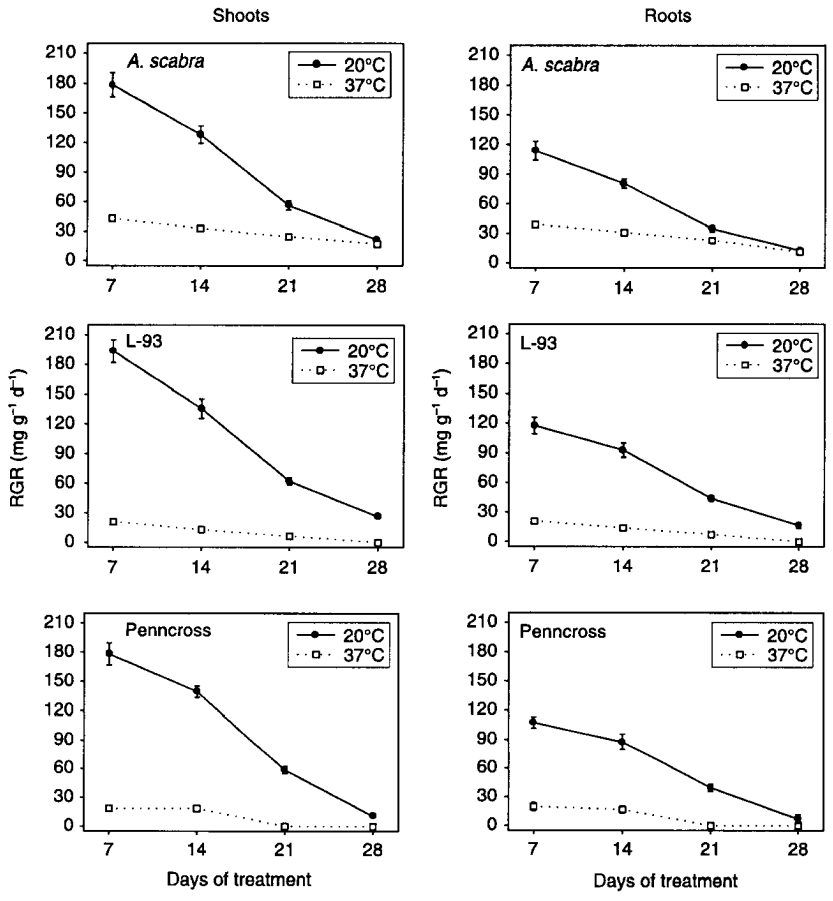

Fig. 3 Relative growth rate (RGR) of shoots and roots for two *Agrostis* species, *Agrostis scabra* and two creeping bentgrass (*A. stolonifera*) cultivars, L-93 and Penncross, in response to soil temperature: 20°C (solid lines, filled symbols) and 37°C (dotted lines, open symbols). Error bars, SE (n = 5–6).

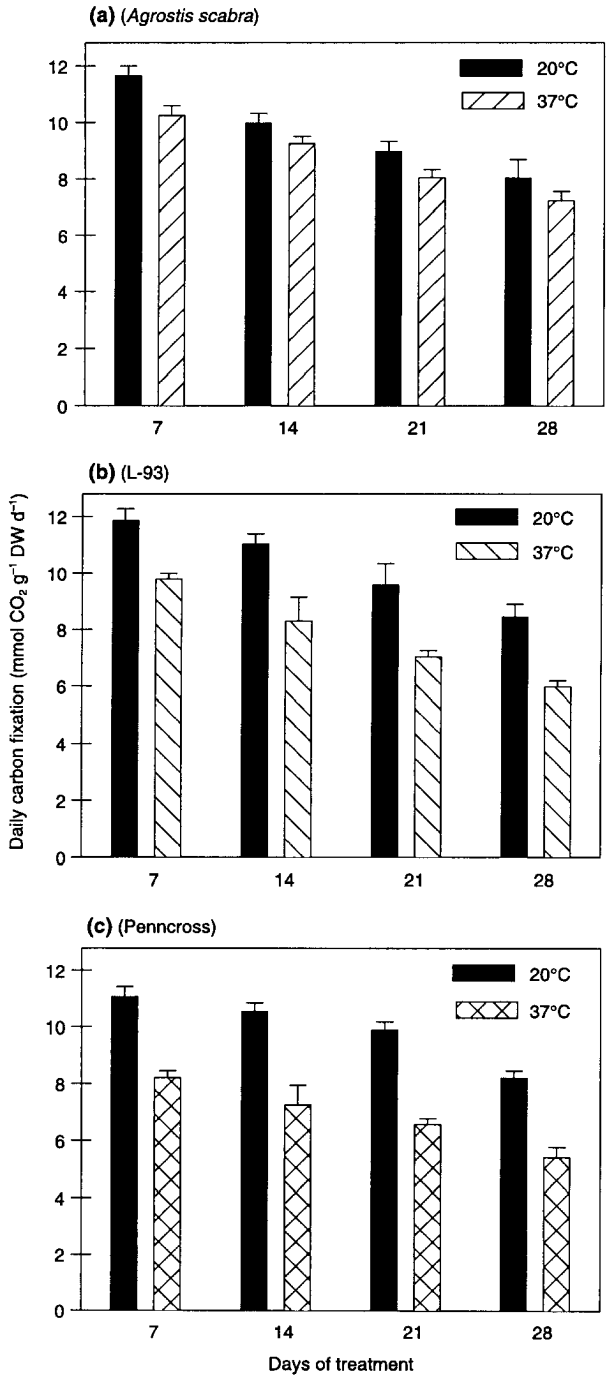

Fig. 4 Net photosynthesis expressed as daily carbon fixation of two *Agrostis* species in response to soil temperature (20°C, filled columns; 37°C, hatched columns). (a) *Agrostis scabra*; (b,c) two creeping bentgrass (*A. stolonifera*) cultivars: (b) L-93, (c) Penncross. Error bars, SE (n = 5–6).

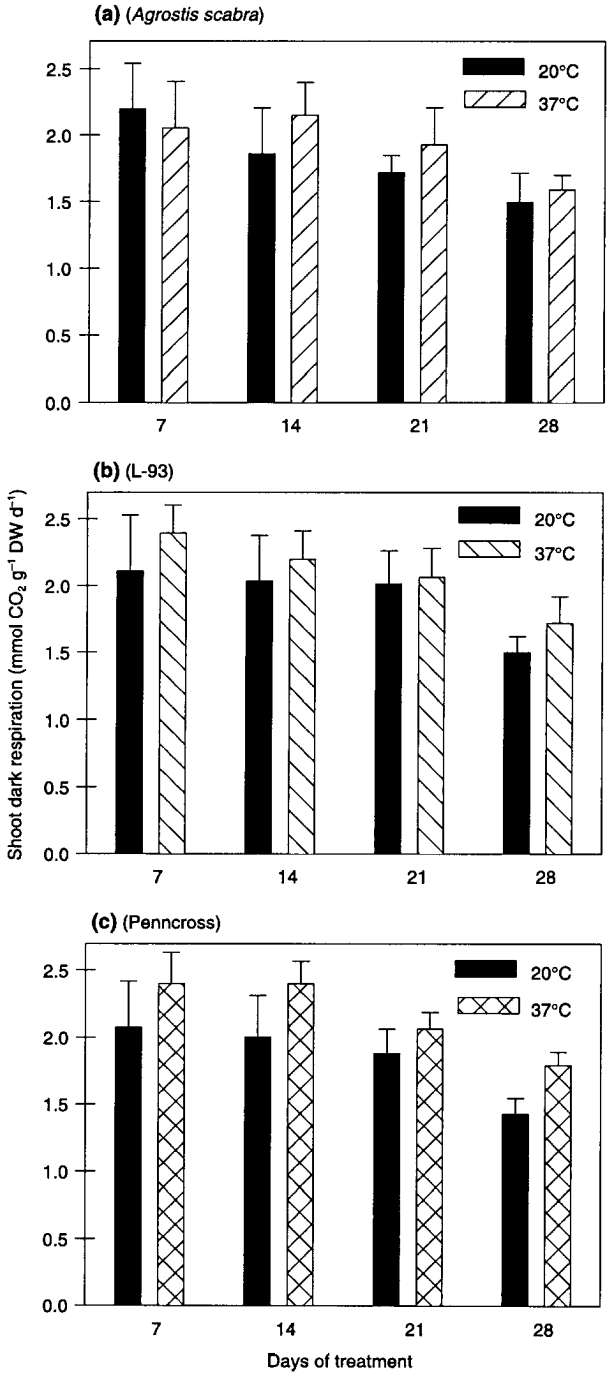

Fig. 5 Shoot dark respiration expressed as daily carbon evolution rate of two *Agrostis* species in response to soil temperature (20°C, filled columns; 37°C, hatched columns). (a) *Agrostis scabra*; (b,c) two creeping bentgrass (*A. stolonifera*) cultivars: (b) L-93, (c) Penncross. Error bars, SE (n = 5–6).

Carbon-consumption rates of roots increased significantly ($P < 0.05$) at high temperature in all species/genotypes, and varied with duration of exposure to high temperature and among species/genotypes (Fig. 6). The C-consumption rate increased 38% above that at 20°C, beginning at 7 d after exposure to 37°C for Penncross. The C-consumption rates of roots increased only after 14 d (by 20%) for L-93, and not until 21 d after exposure

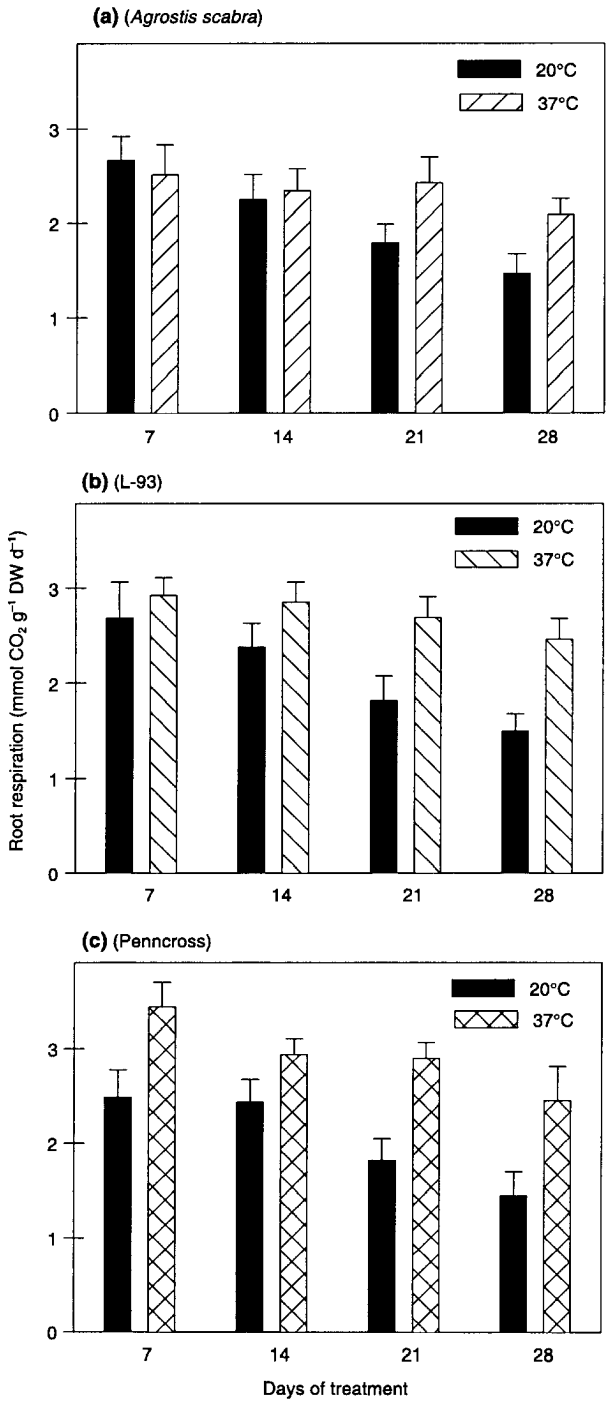

Fig. 6 Root respiration expressed as daily carbon evolution rate of two *Agrostis* species in response to soil temperature (20°C, filled columns; 37°C, hatched columns). (a) *Agrostis scabra*; (b,c) two creeping bentgrass (*A. stolonifera*) cultivars: (b) L-93, (c) Penncross. Error bars, SE (n = 5–6).

to high soil temperature for *A. scabra* (Fig. 6). The largest increase (70%) was found in Penncross after 28 d at 37°C, compared with 43% in *A. scabra* and 64% in L-93 (Fig. 6).

Carbon allocation

The C assimilated during photosynthesis is mainly allocated to four major sinks: shoot respiration, shoot growth, root respiration and root growth. The differences in C-allocation patterns among all species/genotypes are illustrated in Fig. 7. No differences in C-allocation patterns were observed at 20°C among species/genotypes (Fig. 7). Carbon allocation at 20°C was highest to shoot growth: 38, 39 and 36% for Penncross, L-93 and *A. scabra*, respectively. Allocation to root respiration in plants exposed to 20°C at 7 d was the lowest: 23% for Penncross and *A. scabra* and 18% for L-93 (Fig. 7).

On exposure to high soil temperature, allocation to shoot and root growth decreased significantly ($P < 0.05$) in all species/genotypes (Fig. 7). The largest decrease in C allocation to shoot growth after 7 d exposure to 37°C was in Penncross (61%), whereas the lowest decrease was in *A. scabra* (36%). Allocation to shoot growth in *A. scabra* was significantly ($P < 0.05$) higher than that in Penncross and L-93 at 37°C throughout the treatment period (Fig. 7). Carbon allocation to root growth changed among all species/genotypes and between the two temperatures, showing a trend similar to the changes in C allocation to shoot growth (Fig. 7).

Changes in C allocation to root and shoot respiration in response to 37°C were opposite to those for growth-allocation patterns. Most C was allocated to root respiration in all species/genotypes at 37°C; however, allocation to root respiration in *A. scabra* was significantly ($P < 0.05$) lower (48%) than those in Penncross and L-93 (93 and 107%, respectively; Fig. 7). The differences in C allocation to root respiration between *A. scabra* and both bentgrass cultivars were pronounced with prolonged high-temperature treatment (Fig. 7). Carbon allocation to growth at 37°C decreased, whereas allocation to respiration increased with stress duration in all species/genotypes. At 28 d of high-temperature treatment, allocation to growth in both Penncross and L-93 was near zero; however, C allocation to root and shoot growth in *A. scabra* was 15 and 10%, respectively (Fig. 7).

Nitrate assimilation

Nitrate-assimilation rates decreased over time in all species/genotypes, irrespective of root temperature (Fig. 8). Exposure to 37°C resulted in significant ($P < 0.05$) decreases in nitrate-assimilation rate in all species/genotypes; however the severity of decrease was highest in Penncross and lowest in *A. scabra*. At 7 d exposure to 37°C, nitrate assimilation in Penncross had decreased by 44% (Fig. 8), whereas in L-93 nitrate assimilation had decreased by 29% and in *A. scabra* by 15%, compared with plants exposed to 20°C (Fig. 8). At 28 d of exposure to 37°C, nitrate assimilation had decreased by 44% in both Penncross and L-93 compared with plants exposed to 20°C, whereas in *A. scabra* nitrate assimilation decreased by only 28% (Fig. 8).

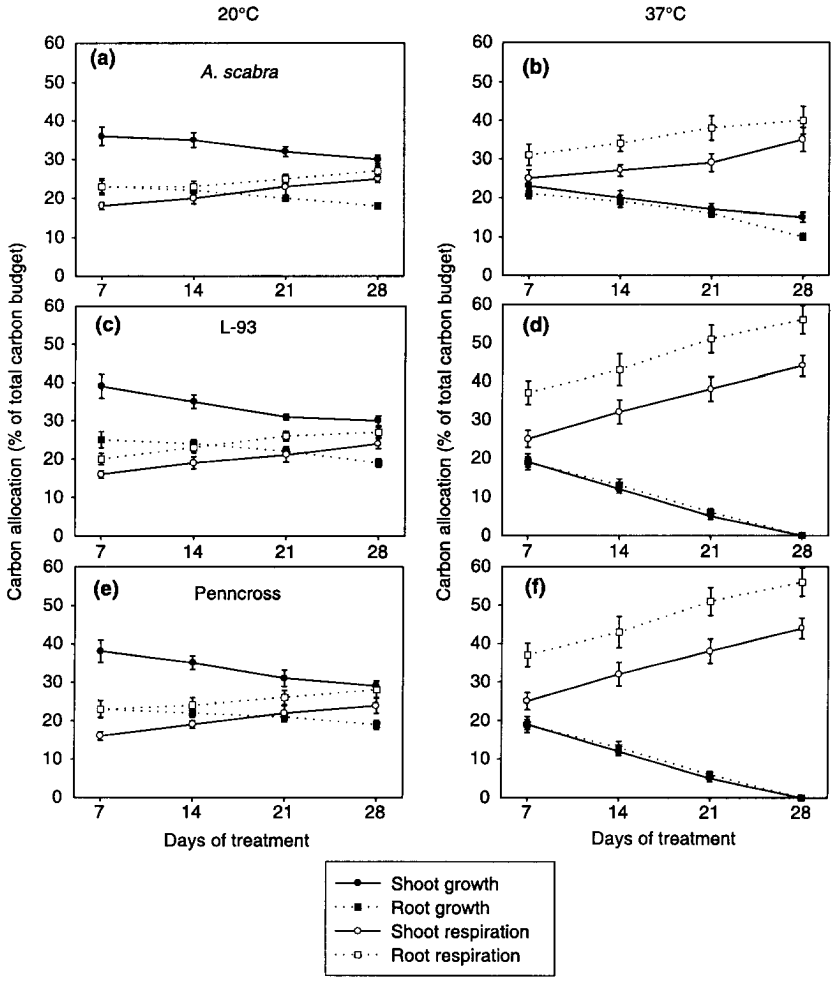

Fig. 7 Time course of carbon allocation during 28 d of treatment, as derived from gas-exchange, growth and total C measurements of two *Agrostis* species, *A. scabra* and two creeping bentgrass (*A. stolonifera*) cultivars, L-93 and Penncross, in response to soil temperature (20 and 37°C). Allocation to shoots and roots, solid and dotted lines, respectively; allocation to respiration and growth, open and filled symbols, respectively. Error bars, SE ($n = 5–6$).

Nitrogen allocation

Nitrogen allocation at 20°C did not vary among species/genotypes (Fig. 9). Nitrogen allocation to shoots at 20°C was 73, 72 and 70% for Penncross, L-93 and *A. scabra*, respectively. Nitrogen allocation between roots and shoots changed when roots were exposed to 37°C compared with 20°C (Fig. 9). At 7 d of exposure to 37°C, N allocation to the shoot had decreased to 58, 59 and 64% for Penncross, L-93 and *A. scabra*, respectively. Nitrogen allocation to shoots at 37°C was significantly ($P < 0.05$) higher in *A. scabra* than in Penncross or L-93 throughout the entire treatment period (Fig. 9).

Discussion

Cool-season plants are sensitive to high soil temperatures (Cooper, 1973). Pote *et al.* (2006) reported that soil temperatures of ≥ 23°C were detrimental to root growth and root activities of *A. stolonifera*. Carbon assimilation, chlorophyll fluoresence and plant viability were used as indicators for thermotolerance and for the isolation of thermotolerant mutants in many studies (Schreiber & Bilger, 1987; Mullarkey & Jones, 2000; Haldimann & Feller, 2004).

In the present study, root viability (Fig. 1); shoot and root RGR (Fig. 3); nitrate-assimilation rate (Fig. 8); F_v/F_m (Fig. 2); and photosynthesis (Figs 2,4) decreased for thermal *A. scabra* and both cultivars of creeping bentgrass following prolonged exposure to 37°C soil temperature; simultaneously, root respiration increased significantly ($P < 0.05$) at high soil temperature in all species/genotypes (Fig. 6). Comparing responses of the above physiological parameters to high soil temperature among thermal *A. scabra* and two cultivars of *A. stolonifera*, Penncross was the most heat-sensitive, and thermal *A. scabra* exhibited the highest tolerance to high soil temperature.

Changes in photochemical efficiency, C assimilation and respiration in response to environmental stresses are common in plants; they reflect metabolic adjustments, which include changes in C allocation and N/C balance (Bohnert & Sheveleva, 1998). Root-respiration rate increased while C-assimilation rate decreased at high soil temperature in both *Agrostis* species, in accordance with data in the literature on

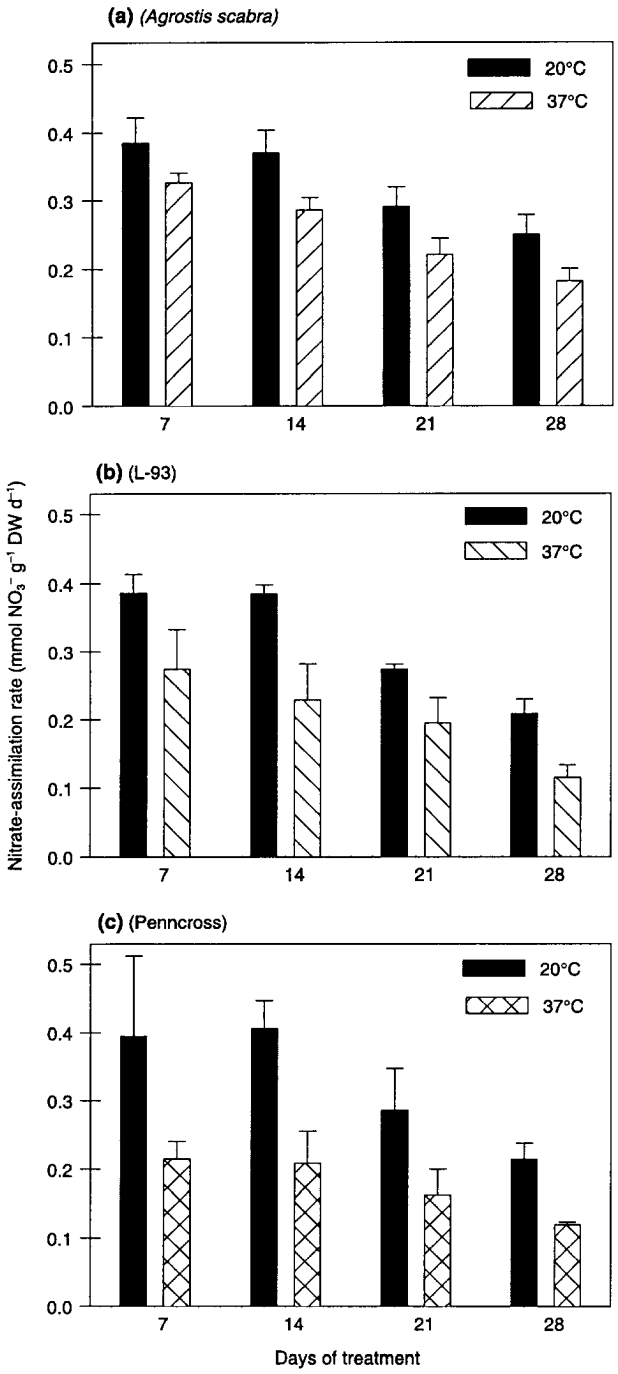

Fig. 8 Nitrate-assimilation rate of two *Agrostis* species in response to soil temperature (20°C, filled columns; 37°C, hatched columns). (a) *Agrostis scabra*; (b,c) two creeping bentgrass (*A. stolonifera*) cultivars: (b) L-93, (c) Penncross. Error bars, SE (n = 5–6).

other plant species (Lambers, 1985; Long & Woodward, 1988; Criddle *et al.*, 1994; Hamerlynck *et al.*, 2000; Martin & Stabler, 2002; Haldimann & Feller, 2004). However, root respiration of thermal *A. scabra* was considerably less sensitive to high soil temperature than that of *A. stolonifera*. Increase in C consumption through respiration may cause C starvation when C assimilation is inhibited, and may eventually lead to root death under high soil temperatures (Amthor, 1989). Therefore the control of a rise in root respiration in response to increasing soil temperature may conserve C and facilitate root survival of long-term exposure to high soil temperatures for the thermal grass. The maintenance of slow respiration rates during acclimation to high temperatures has been reported in other plant species (Gunn & Farrar, 1999). Alternatively, increased respiration rates may reflect higher activities of the alternative oxidase which, in turn, may prevent the accumulation of free radicals during stress (Wagner & Krab, 1995; Maxwell *et al.*, 1999). However, whether increases in respiration are involved in plant tolerance to high soil temperature is not clear.

Efficient C-allocation patterns may enhance plant tolerance to environmental stresses (Huang & Fu, 2000; Poorter *et al.*, 1995). Variation in growth and survival with temperature is associated with changes in C- and N-allocation patterns (Lambers *et al.*, 1998). We calculated C- and N-allocation patterns in response to high soil temperature based on the following assumptions: (1) the measured rates of CO_2 assimilation and respiration were correct estimates of the averaged values of the plant's daily C exchange; and (2) C assimilated through photosynthesis is predominantly allocated to four major sinks: shoot respiration, shoot growth, root respiration and root growth. In the current study, high soil temperature resulted in differential changes in the C- and N-allocation patterns between thermal *A. scabra* and two cultivars of *A. stolonifera*.

Carbon allocation to shoot and root growth decreased significantly ($P < 0.05$) in all species at high soil temperature (37°C) (Fig. 7). High-temperature tolerance in thermal *A. scabra* was correlated with a small reduction in C allocation to shoot and root growth. Carbon allocation to respiration increased in all species in response to 37°C. Under a range of environmental conditions that restrict plant growth, such as extreme temperatures (Rachmilevitch *et al.*, 2006) and severe nutrient limitation, as well as in genotypes with growth restricted caused by low endogenous phytohormone levels (Nagel & Lambers, 2002), C is allocated proportionally more to respiration. When comparing the present species, C allocation to respiration was lower in the heat-tolerant *A. scabra* than in the two cultivars of *A. stolonifera*. The low demand for C by root respiration in thermal *A. scabra* may be explained by its lower respiratory costs for maintenance and ion uptake (Rachmilevitch *et al.*, 2006), contributing to higher survival under high soil temperature conditions.

On exposure to high soil temperature, plants of the two *Agrostis* species exhibited differential patterns of N allocation to shoots and roots, although N allocation at 20°C did not differ significantly between species (Fig. 9). At 37°C, N allocation to shoot growth decreased and allocation to root growth increased in both species. This was in accordance with

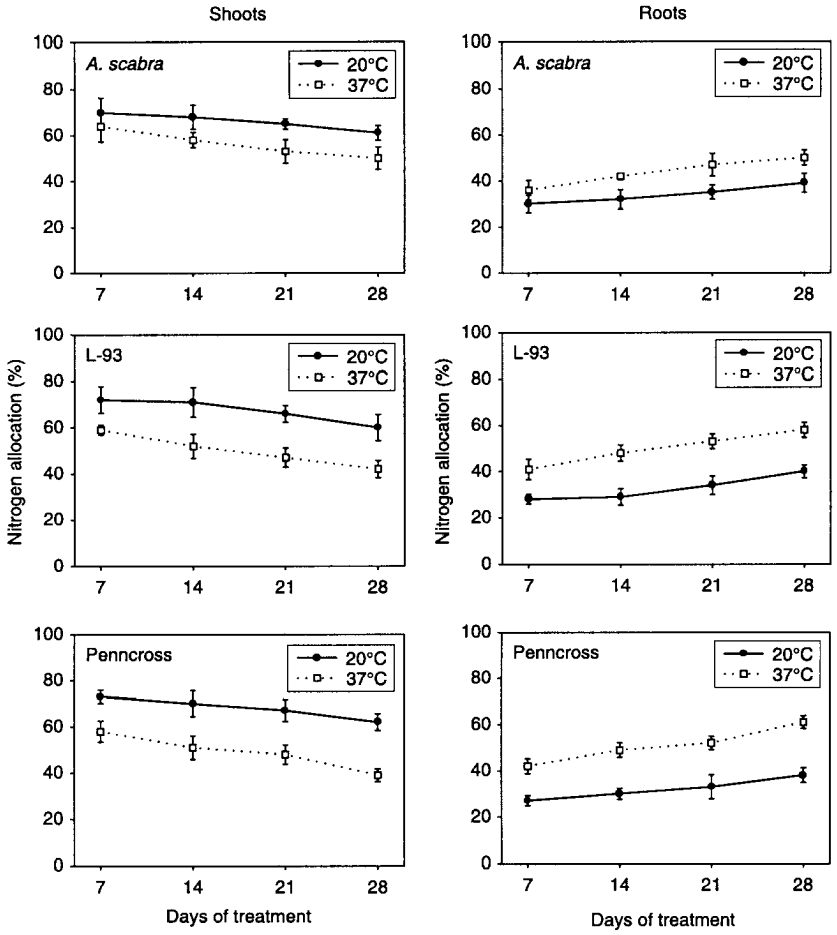

Fig. 9 Time course of nitrogen allocation to shoots and roots during 28 d of treatment, as derived from total N measurements, of two *Agrostis* species, *Agrostis scabra* and two creeping bentgrass (*A. stolonifera*) cultivars, L-93 and Penncross, in response to soil temperature (20°C, solid lines, filled symbols; 37°C, dotted lines, open symbols). Error bars, SE (n = 5–6).

the increase in investment costs in root biomass, as seen for whole plants exposed to extreme temperatures (Lambers *et al.*, 1998), or when only the root temperature was changed (Bowen, 1991; Lambers *et al.*, 1998). Species also varied in N-allocation pattern. Nitrogen allocation to shoots at 37°C was significantly ($P < 0.05$) greater in *A. scabra* than in Penncross and L-93 throughout the treatment period (Fig. 9). The higher N investment in shoots of thermal *A. scabra* may contribute to its better tolerance and growth under high soil temperatures, as demonstrated by its higher F_v/F_m and RGR (Figs 2,3). Poorter *et al.* (1990) reported that N allocation to shoots was greater in inherently fast-growing plants than in slow-growing ones.

In summary, root viability, RGR, F_v/F_m, C fixation and N assimilation decreased, but root respiration increased for both *Agrostis* species in response to increasing soil temperatures; however, thermal *A. scabra* exhibited higher tolerance to high soil temperature than both cultivars of *A. stolonifera*. Better tolerance of *A. scabra* to high soil temperature was related to more efficient C- and N-allocation patterns. *Agrostis. scabra* plants, which are adapted to high soil temperatures, had more C allocated for growth of roots and more N allocated for shoots when exposed to high soil temperatures, compared with both cultivars of *A. stolonifera*, therefore altering C and N expenditure may have biotechnological implications in increasing plant tolerance to high temperature stress.

Acknowledgements

The research was supported by a USDA grant (2003-00759). Thanks also go to Michelle Dacosta, Yan Xu and Emily Merewitz for critical review of the manuscript.

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