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Root production is determined by radiation flux in a temperate grassland community

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Abstract

Accurate knowledge of the response of root turnover to a changing climate is needed to predict growth and produce carbon cycle models. A soil warming system and shading were used to vary soil temperature and received radiation independently in a temperate grassland dominated by *Holcus lanatus* L. Minirhizotrons allowed root growth and turnover to be examined non-destructively. In two short-term (8 week) experiments, root responses to temperature were seasonally distinct. Root number increased when heating was applied during spring, but root death increased during autumnal heating. An experiment lasting 12 months demonstrated that any positive response to temperature was short-lived and that over a full growing season, soil warming led to a reduction in root number and mass due to increased root death during autumn and winter. Root respiration was also insensitive to soil temperature over much of the year. In contrast, root growth was strongly affected by incident radiation. Root biomass, length, birth rate, number and turnover were all reduced by shading. Photosynthesis in *H. lanatus* exhibited some acclimation to shading, but assimilation rates at growth irradiance were still lower in shaded plants. The negative effects of shading and soil warming on roots were additive. Comparison of root data with environmental measurements demonstrated a number of positive relationships with photosynthetically active radiation, but not with soil temperature. This was true both across the entire data set and within a shade treatment. These results demonstrate that root growth is unlikely to be directly affected by increased soil temperatures as a result of global warming, at least in temperate areas, and that predictions of net primary productivity should not be based on a positive root growth response to temperature.

Keywords: acclimation, belowground net primary production, grassland, *Holcus lanatus*, minirhizotrons, *Plantago lanceolata*, received photosynthetically active radiation, root demography, root respiration, root turnover, shading, soil warming, temperature

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Introduction

The Intergovernmental Panel on Climate Change predicts that the global mean temperature is likely to rise by between 1.4 °C and 5.8 °C during this century (Houghton *et al*., 2001). Wigley & Raper (2001) suggest that the most likely temperature rise during this period is 2.8 °C, with a 90% probability interval of 1.7 °C–4.9 °C. Soil temperatures are expected to reflect any rise in air temperature (Pollack *et al*., 1998), although local changes in cloudiness could complicate this in the short term (Dai *et al*., 1997). Warmer soil temperatures could potentially alter the rates of many biotic and edaphic processes, for example, plant root growth (Kaspar & Bland, 1992), root respiration (Boone *et al*., 1998) nutrient uptake (Tindall *et al*., 1990) and nitrogen mineralization (Niklinska *et al*., 1999).

It has often been reported that soil temperature is a major determinant of both root growth and root respiration (e.g. Lawrence & Oechel, 1983; Barber *et al*., 1988; Misra, 1999; Gavito *et al*., 2001). However,

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such studies have mostly used either controlled conditions where temperature is the only limiting factor on growth or annual crop plants that are unlikely to exhibit significant growth during cold periods. Furthermore, our previous field-based experiments have shown that root growth may often be independent of temperature. These studies, using either an altitudinal gradient (Fitter *et al.*, 1998) or a soil warming system (Fitter *et al.*, 1999) to obtain a range of soil temperatures, found that root turnover in natural plant communities was correlated with photosynthetically active radiation (PAR) flux rather than soil temperature. This relationship has also been observed in sunflower, for both field and controlled environment grown plants (Aguirrezabal *et al.*, 1994).

Measuring root turnover, roughly defined as the ratio of root number present at a time point to the number of roots produced up to that time, is problematic. Repeated coring at sufficiently short intervals is invasive and destructive. Minirhizotrons do allow root demography to be determined with precision, but many tubes are needed to study more than a few cubic millimeters of soil, and the collection and analysis of the images is very time consuming (Johnson *et al.*, 2001). However, it is important that accurate measurements of root turnover are generated, as any underestimate will result in a large underestimate of carbon flow into the soil.

Nutrient uptake accounts for the greater part of root respiration, while growth accounts for approximately 15–45% (Poorter *et al.*, 1991). Consequently, the temperature response of root respiration may not follow that of root growth. Furthermore, Atkin *et al.* (2000) demonstrated that the Q_{10} (the proportional increase in respiration for a 10 °C increase in temperature) and speed of acclimation of root respiration is critical when estimating the effects of global warming on CO_2 efflux from roots. Evidently, knowledge of long- and short-term root respiration responses to changing soil temperatures is vital in predicting both ecosystem net primary productivity (NPP) and belowground carbon storage.

Unfortunately, it is extremely difficult to obtain accurate measurements of respiration from *in situ* and intact roots. Most estimates of root respiration either use roots extracted from soil (e.g. Burton *et al.*, 1996; Gunn & Farrar, 1999) or measurements of total soil respiration with and without roots (e.g. Boone *et al.*, 1998). This area of the literature is conflicting, with several reports of root respiration acclimating to long-term changes in temperature, for example, in *Citrus* (Bryla *et al.*, 1997), *Bellis* and two species of *Poaceae* (Gunn & Farrar, 1999), and five boreal tree species (Tjoelker *et al.*, 1999); but no acclimation was observed in others, for example, *Acer* (Burton *et al.*, 1996; Zogg *et al.*, 1996) and *Alnus* (Kutsch *et al.*, 2001). Interpreting these results is difficult because phenology and other environmental factors, such as soil moisture, may correlate with soil temperature even where there is no causal link. In an attempt to tease out these relationships, Fitter *et al.* (1998) used stepwise regression to correlate several measured environmental parameters with root respiration of a grassland community and found a significant correlation with solar radiation but no relationship with soil temperature.

If received PAR is important in determining root growth, then any feedback of soil temperature into photosynthetic rates could further complicate analysis of studies examining such a link. Indeed, soybean (*Glycine max*) exposed to an increase in root zone temperature exhibits an increase in photosynthetic rates (Ziska, 1998). However, the opposite was found in bentgrass (*Agrostis palustris*) exposed to high soil temperatures (Xu & Huang, 2000), and radish (*Raphanus sativus*) showed no response to a 5 °C increase in root zone temperature (Kleier *et al.*, 2001). As well as being conflicting, these experiments were performed in controlled environment chambers with a constant nutrient supply and the observed responses may not be reflected in a natural environment due to other constraints on photosynthesis.

This paper describes a soil warming system used in conjunction with controlled levels of shading and environmental monitoring to study root responses to PAR and soil temperature on a time scale of weeks to months in a temperate grassland ecosystem. Minirhizotrons were used to examine root turnover and production non-destructively, while soil cores provided a second means of examining root growth and provided excised roots for measurement of root respiration. Photosynthetic measurements of the two dominant species at the site were used to look for possible feedback from root responses to the shoot. The principal objective was to estimate the relative importance of temperature and PAR in controlling root growth and respiration.

Materials and methods

Study site, shading and soil warming

The study site was a bare-soil area of the University of York Experimental Garden previously cultivated but free from fertilizer input for at least the previous 10 years. A split plot experiment was laid out, with 12, 2 m^2 shade treatment plots, each containing a 'heated' and an 'ambient' subplot.

Shading was provided by means of a 1 m high shade frame over eight of the 12 plots constructed from 2 cm square steel tubing to which variable types of shade mesh could be attached. During experiments, shading was maintained at two levels giving three light treatments in total: ambient, 'half' shade and 'full' shade. This generated a factorial design with four replicates for each of six treatment combinations.

Soil warming was provided by means of 1×0.5 m steel mesh grids, with a mesh size of 25×25 mm, which were pinned to the soil surface. A novel type of low voltage soil heating cable, as described by Ineson *et al*. (1998), was tightly attached to the mesh in loops. The use of a mesh allowed the heat to be spread more evenly than that by using a heating cable alone. Soil-heating regimes were controlled by custom-built electronic units, each of which averaged the readings of soil temperature thermistor probes at a depth of 25 mm, in both heated (three probes) and ambient (three probes) plots, to maintain ambient temperature or a constant temperature differential with respect to ambient, irrespective of the degree of controlled shading, at all times. Heating with such systems was detectable to approximately 200 mm, albeit to a greatly reduced extent at such depths (Ineson *et al*., 1998). A mesh was used in all 24 subplots irrespective of whether the soil was to be warmed.

Environmental monitoring

All environmental probes and monitoring equipment were supplied by Delta-T Devices (Cambridge, UK). A single type ST1 soil temperature probe was positioned at 2 cm depth in each of the 24 subplots. These were separate probes from those used for control of soil warming. AT1 air temperature probes were positioned at approximately 70 cm above the soil surface in six of the 12 plots and QS PAR probes at a similar height in the other six plots. Additionally, three ML2 soil moisture probes were used, which could be moved to any of the plots or subplots. A weather station was positioned in the centre of the site and monitored PAR, air temperature, air humidity, soil moisture, wind speed, wind direction and rainfall. A DL2e data logger recorded the output from all of these environmental probes at 30 min intervals.

Plant material

After the soil warming and monitoring systems were installed in July 1998, the site was seeded with a mixture of temperate northern grassland species. The mixture consisted of *Agrostis capillaries* (12%), *Cynosurus cristatus* (45%), *Festuca rubra* (25%), *Holcus lanatus* (5%), *Plantago lanceolata* (5%) and *Trifolium repens* (8%). The site was initially dominated by *P. lanceolata*, but once occasional cutting was implemented, *H. lanatus* became the dominant species. This change was largely complete before experiment 1 and no major alteration in species composition was observed during experiment 3. Cutting did not occur during experiments 1 and 2; during experiment 3 cutting always took place immediately after a harvest and occurred three times during the year. No attempt was made to control the species composition after seeding.

Root demography

A minirhizotron (250×22 mm glass tubes) was installed at each end of each subplot at an angle of 45°. The upper 25 mm of each tube was painted black and sealed with a rubber bung to prevent water ingress between imaging sessions. The tubes were engraved with spots at approximately 20 mm intervals along their length to allow images of the same portion of soil to be taken at multiple sample dates. Video images were taken at each spot with an Olympus OES swing-prism borescope connected to a WAT202D digital camera, which in turn was connected to a Sony GVD900 digital video recorder (all KeyMed Ltd., Southend-on-Sea, UK). The area in a single image was approximately 7 mm in diameter.

Images were captured from the digital video using a Snappy Deluxe video frame grabber (Insight UK, Workshop, UK) as tagged image format files (TIF). Images taken from a single spot over time could then be viewed simultaneously and each root in an image uniquely identified. Root numbers, changes in root numbers, root birth rates and root death rates could then be easily calculated. Images from approximately 40 mm depth from a single tube (chosen at random) from each plot were analysed in all experiments; during experiment 3 images from 110 mm depth were also analysed.

Root length determination and respiration

Live roots (determined visually by root colour) were extracted from 10 cm soil cores by washing with cold water. Soil particles adhering to the extracted roots were removed using forceps. The use of fine soil sieves (150 μm mesh) allowed virtually all the roots in a core to be retained. The total root length of these samples was determined using WinRhizo v. 3.10 running on a PC with a scanner capable of a resolution of 1200 dpi (Regent Instruments, Québec, Canada). The root samples were then used for root respiration measurements.

Subsequently, the roots were surface sterilized by dipping in a 2% sodium hypochlorite solution for 3–4 s (previously shown not to affect root respiration rates – Edwards, unpublished) and rinsed several times in deionized water. Respiration was then measured in deionized water using Hansatech CB1-D and Rank Brothers Dual Digital Model 20 oxygen electrodes (Hansatech Instruments, Kings Lynn, UK and Rank Brothers, Bottisham, UK). The output of these was logged on a PC using an NI-DAQ PC-LPM-16 analog-to-digital converter (National Instruments, Newbury, Berkshire) at 1 s intervals and respiration rates calculated by using a regression of the output against time. Once respiration measurements were completed, the root samples were dipped in liquid nitrogen and freeze-dried using an Edwards Modulyo freeze-drier (Edwards High Vacuum, Crawley, UK).

Chemical analysis of root samples

Freeze-dried root samples were ground to a fine powder using a 31-700 Hammer mill (Glen Creston Ltd, Stanmore, UK) and used for determination of carbon and nitrogen content with a Carlo-Erba CHN analyser (CE Instruments NA2100 Brewanalyser, ThermoQuest Italia S.p.A., Milan, Italy.

Experiments

The soil temperature of the heated plots was maintained at 3 °C above ambient in all experiments and the temperature of 'ambient' temperature shaded plots was maintained at that of 'ambient' non-shaded plots in order to remove any effects of the reduced insulation on soil temperature. All photosynthetic data were obtained using a LiCor 6400 Portable Photosynthesis System (Glen Spectra Ltd, Stanmore, UK). The spectral absorption of the Tildenet shade mesh (East Riding Horticulture, Sutton-on-Derwent, UK) used was examined using a PR1010 spectral radiometer (Macam Photometric Ltd, Livingston, UK), and was found to be neutral above 390 nm with no effect on red to far-red ratio.

Experiment 1

The first experiment was started once the vegetation had become established, 5 months after the site was seeded, and was intended to examine the effect of soil warming on plants coming out of winter dormancy, when root numbers could be expected to be increasing. Root images and root cores (1 core per subplot per harvest) were taken at weekly intervals from the beginning of March 1999 for 8 weeks. After removal of a core the hole was filled with root-free soil from the same site. Cores were never taken from a spot that had previously been cored. Soil heating and shading were applied on 19/3/99, 10 days after the start of the experiment and continued until 27/4/99. The 'full' shade treatment blocked 67% of ambient photosynthetic photon flux density (PPFD) and 'half' shade 48%. Root samples were used for root respiration measurements at the soil temperature at the time of harvest and two temperatures from 5 °C, 10 °C and 15 °C, with the temperature closest to the initial measurement (at extraction temperature) being omitted.

Experiment 2

The second experiment was intended to examine the effect of soil warming on plants entering winter dormancy, when root numbers could be expected to be decreasing. Root images and cores were again taken at 1-week intervals as for experiment 1, from mid-September 1999 for 8 weeks. Soil heating and shading were applied on 23/9/99, 10 days after the first harvest and continued until 1/11/99. The 'full' shade treatment was 53% and 'half' shade 46%. Root samples were used for root respiration measurements at three temperatures selected as for experiment 1. Root carbon and nitrogen content were then determined on these samples.

Experiment 3

The third experiment was used to follow the vegetation through an entire growing season in order to determine the longer-term effect of soil warming on root growth. Root images were taken at 2-week intervals from mid-January 2000 until mid-January 2001. During what was expected to be the peak of the growing season, May to September, the frequency was increased to 1-week intervals. The first root samples were extracted on 25/1/00 and further harvests were carried out at approximately 6-week intervals until 9/1/00. Soil heating and shading were applied on 2/2/00 and maintained for the duration of the experiment. The 'full' shade treatment was 86% and 'half' shade 70% (Fig. 1a). Root respiration was measured at soil temperature at the time of extraction and two temperatures from 5 °C, 10 °C, 15 °C and 20 °C, with the temperatures closest to and furthest from extraction temperature being omitted. Root samples from alternate harvests were then used for determination of carbon and nitrogen content.

Photosynthetic rates at saturating light levels (1000 μmol m^{-2} s^{-1}) were measured on single *H. lanatus* and *P. lanceolata* leaves from each plot on 7/4/00, 12/5/00, 15/7/00, 25/8/00 and 29/9/00. Measurements of light and CO_2 response curves were made on *H. lanatus* leaves on 17–18/7/00. All the leaves used were

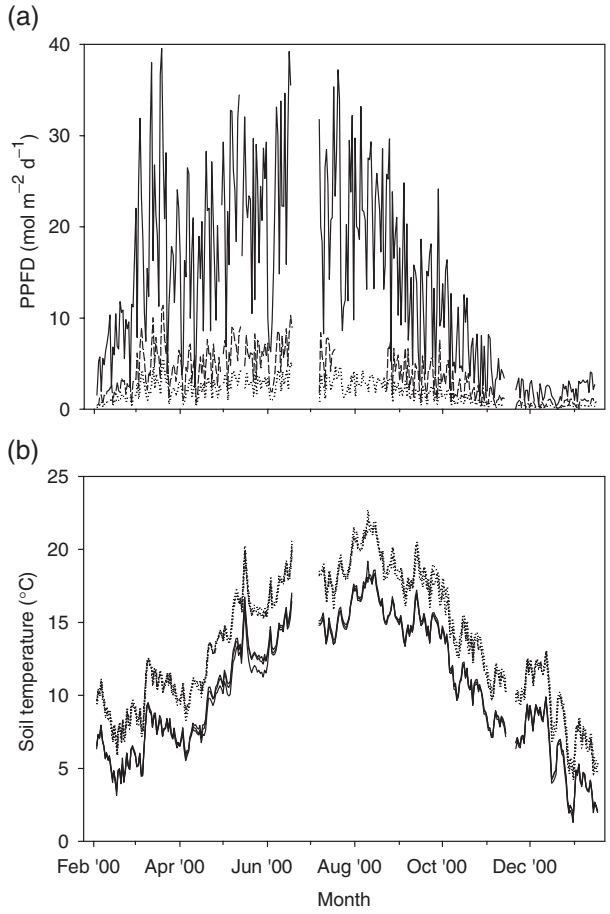

Fig. 1 Effect of treatments during experiment 3 on (a) PPFD at a height of 75 cm and (b) mean daily soil temperature at a depth of 2 cm. PPFD data are each a mean of two probes in two separate plots; solid line denotes no shading, dashed line 'half' shade and dotted line 'full' shade. Soil temperature data are each a mean of four probes in four separate sub-plots, solid lines denote three non-heated treatments and dotted lines three warmed treatments. Gaps in the data were due to loss of power to the logger.

subsequently harvested and the segment used for photosynthetic measurements cut out. Leaf area, fresh weight and dry weight of this segment were all determined.

Data analysis

All statistical analyses were generated using SPSS version 10 (SPSS Science, Woking, UK). Root demography data were analysed using repeated measures ANOVA, with shading and soil heating used as factors. Biomass data were analysed using a factorial ANOVA with factors as above and harvest date as a covariate. Only the data from experiment 3 were analysed for relationships with environmental measurements. Environmental data were grouped into means, minimums, maximums and/or totals, as appropriate for each measured environmental variable, for 1, 3 and 5 days prior to sampling, and relationships between these data and measured parameters were tested using stepwise regression. Only those environmental data where separate measurements were available for each treatment combination were used for the stepwise regressions; that is, soil temperature, radiation flux and air temperature. The mean data for a treatment combination at each harvest were used; apart from soil temperature, environmental data were not available for each individual plot. All r^2-values quoted refer to adjusted r^2.

Owing to the variability of root birth and death rates, the raw data and two harvest running means were used for regressions. These were repeated for birth rates but with spring data excluded, as root growth during this time was likely to be largely from stored reserves and consequently, many of the spring data were outliers. Additionally, data were examined on a seasonal and treatment basis.

CO_2 curves were used to estimate respiration during daylight (R_d), electron transport capacity (J_{max}) and RuBisCO activity (V_{cmax}) according to the model of Farquhar *et al*. (1980). These data were analysed using ANOVA.

Unless otherwise stated, the results are means ± standard error and $P < 0.05$ for all significant results.

Results

In all three experiments, at least one harvest was taken before treatments were applied. There were no pre-treatment differences for any of the measured variables. The shade frames were effective throughout the year and did not affect the proportional day-to-day variation in incident radiation (Fig. 1a). The soil-heating system performed flawlessly throughout all three experiments giving a mean warming effect of 2.7 °C at a depth of 2 cm (Fig. 1b). The maximum soil temperature was approximately 2 months later than maximum incident radiation (Fig. 1b cf. Fig. 1a). Shading had no effect on soil temperature, as shaded plots were warmed to match ambient plots. However, the air temperature at 75 cm above full-shade plots was, on average, 0.1 °C lower than above non-shaded plots irrespective of absolute air temperature. Soil moisture was monitored during experiment 3 only; neither soil warming nor shading affected the daily mean soil water content. For the entirety of winter and for extended periods during other seasons, the soil was at field capacity (0.31–0.34 m^3 water m^{-3} soil) in all measured plots. The

lowest soil moisture recorded was about 0.13 m^3 water m^{-3} soil, and this was also the same in all monitored plots.

Root demography

Experiments 1 and 2

Treatment effects on root demography were markedly different between the spring and autumn runs. No effects of shading were observed in either experiment, so the results from shaded and non-shaded plots were combined, except for the statistical analyses. All treatments exhibited a significant increase in absolute root numbers during spring, with control plots changing from 2.7 ± 0.9 to 6.8 ± 1.3 roots per image. Conversely, root numbers during the autumn were stable, dropping slightly in control plots from 4.5 ± 0.8 to 4.0 ± 0.8 roots per image during the experiment.

Soil warming during the spring run caused a significant increase in both the number of root births and the number of root deaths (Fig. 2a,b). Root births were affected by heating to a greater extent than root deaths leading to an increase in the absolute root number. As both births and deaths were increased, although the latter effect was small, root turnover was greater in the warmed plots. Heating during the autumn only had a significant effect on root deaths (Fig. 2c,d). Consequently, although root numbers were reduced, only the rate and not the total carbon input to the soil would be affected due to the lack of effect on births.

Experiment 3

When the entire root demography data set was analysed, using repeated measures ANOVA, no significant effect of depth (between 40 and 110 mm) was present for any of the variables measured; consequently, only the data for 40 mm depth (closest to the heating control depth) are presented.

Root numbers followed a seasonal pattern, with production being highest during spring and death highest in autumn, irrespective of treatment. This was

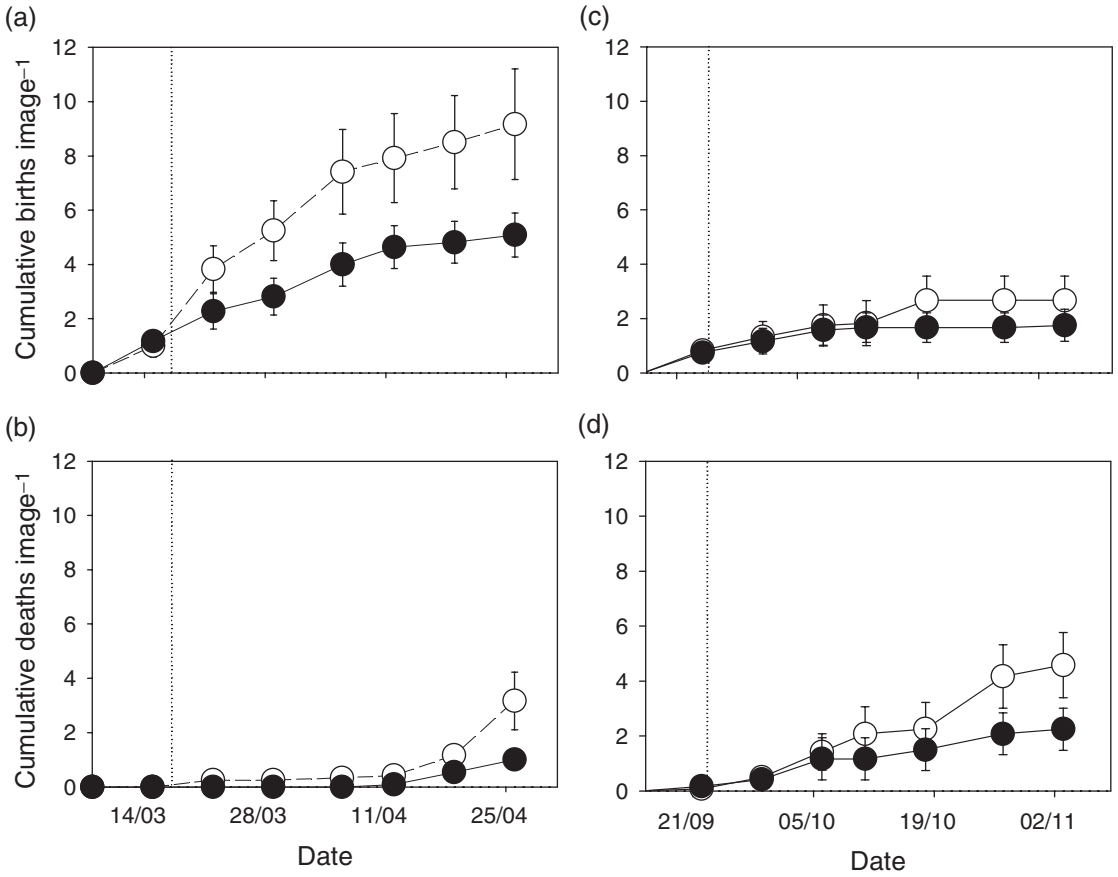

Fig. 2 Cumulative births (a,c) and deaths (b,d) during experiments 1 (a,b) and 2 (c,d) at a depth of 4 cm. Filled symbols represent ambient temperature plots and open circles heated plots. Each point is a mean of 12 plots ± standard error. Dotted lines denote start of heating treatments.

reflected in root accumulation, a measure of the net change in root number over time, calculated by subtracting cumulative root deaths from cumulative root births for each time point. Root accumulation in control plots increased throughout spring until early June and was then stable until it dropped rapidly to zero during autumn, indicating that the over-wintering root standing crop was the same as the previous year (Fig. 3a). Accumulation in non-shaded plots subject to soil warming (Fig. 3b) was very similar to the control plots for much of the year, with the exception that the period of root death in autumn was longer, resulting in a reduced root number throughout the following winter.

Although there were no differences between the two depths examined across the time course of the experiment, root numbers during winter were significantly higher at the 110 mm compared with the 40 mm depth in the heated plots.

Shading significantly reduced the maximum root accumulation in both warmed and ambient soil temperature plots, although there was little difference between the two shading treatments. Shading and heating effects during the autumn and winter appeared to be additive, with plots that received both heating and full shading having the lowest root accumulation values (Fig. 3b).

Birth and death rates of roots were calculated on a seasonal basis as the variability in the data necessitated a longer time frame than between single measurement dates. Birth rates were higher in spring than during the other seasons: the full-shade plots had a lower birth rate than control plots ($P = 0.03$) and half-shade plots marginally lower ($P = 0.09$). Death rates were less than half the birth rate in all treatments, explaining the observed increase in root number during spring (Fig. 4a). Summer birth rates were similar to spring rates in non-shaded plots but were less in shaded plots ($P = 0.04$). Birth and death rates were closely matched in all treatments; thus root numbers were stable during this period (Fig. 4b). The lowest birth rates were seen in autumn, which also had the highest death rates, although the death rates in the shaded plots reflected the lower number of roots present in those plots (Fig. 4c). Intriguingly, birth rates were actually slightly higher in winter than autumn and death rates much lower (Fig. 4d). However, shading during winter increased root deaths relative to the non-shaded plots, which exceeded birth rates in all except the control plots.

Biomass

Experiments 1 and 2

Across all plots root dry mass (DM) increased significantly during experiment 1 (Fig. 5a), corresponding to a large increase in root length (Fig. 5b) and, to a lesser extent, specific root length (SRL). Both heating and

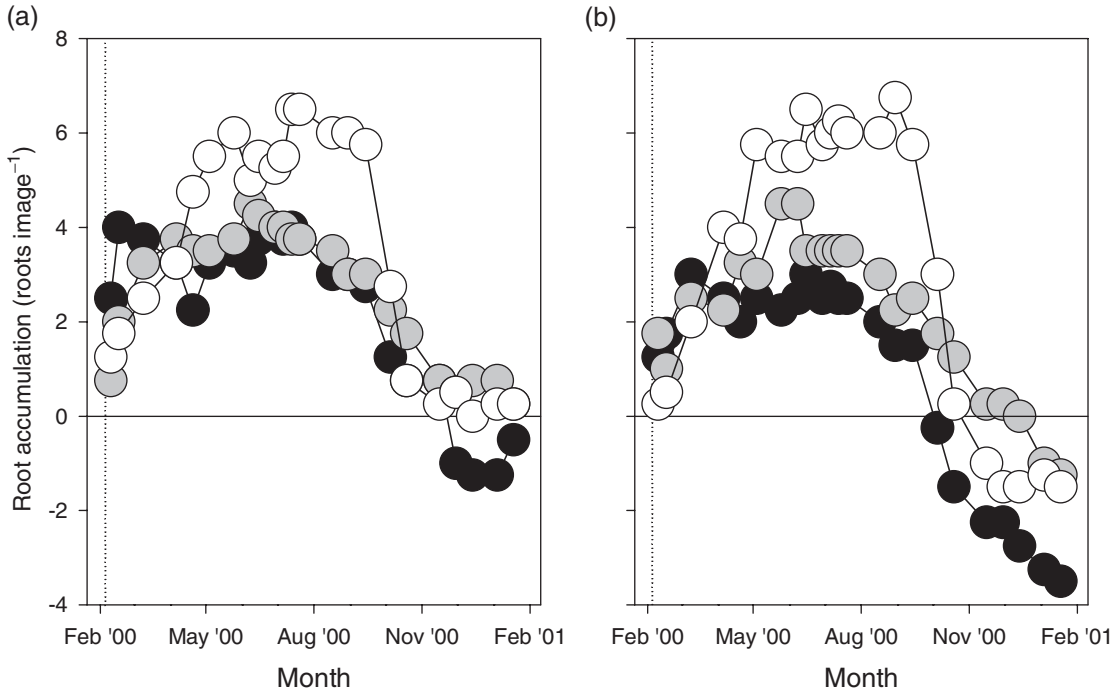

Fig. 3 Root accumulation at a depth of 4 cm during experiment 3, (a) in ambient temperature soil, (b) in warmed soil. Open symbols denote non-shaded, grey symbols 'half' shaded and black symbols 'full' shaded plots. Dotted lines represent start of treatments.

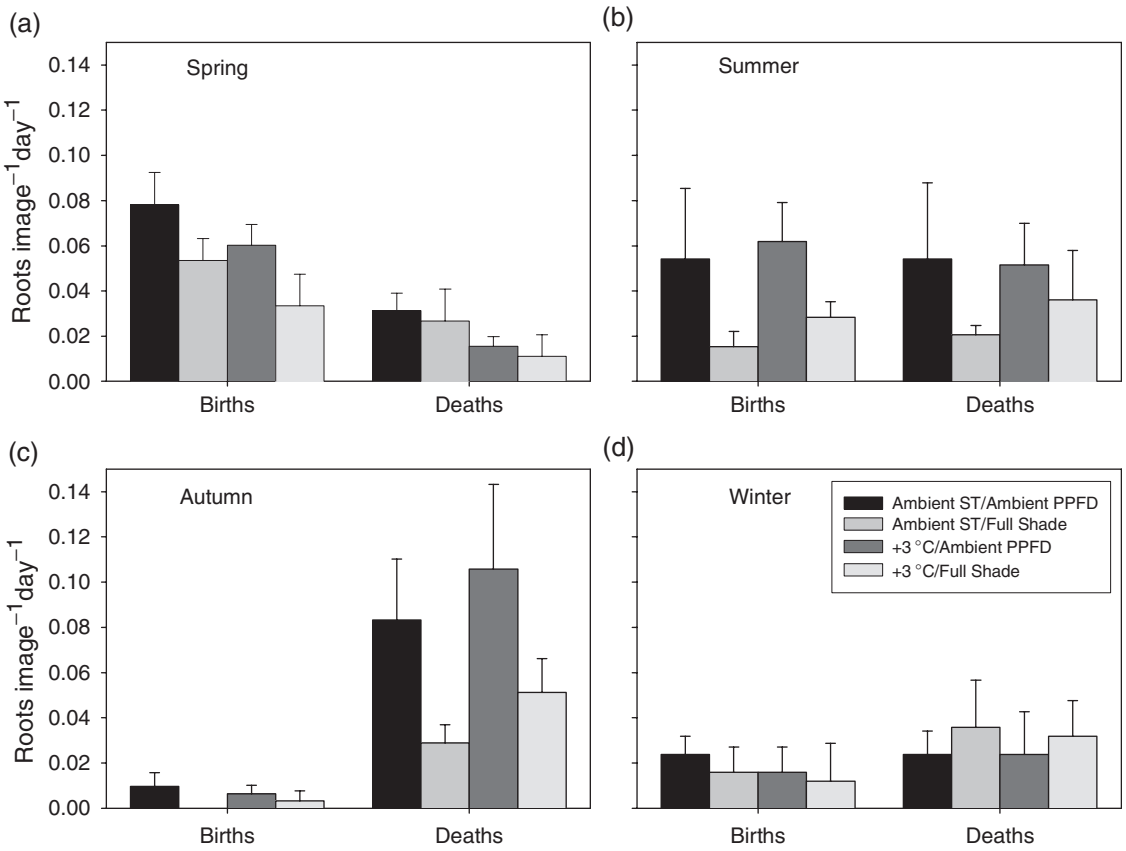

Fig. 4 Root birth and death rates at a depth of 4 cm in experiment 3 during (a) spring, (b) summer, (c) autumn and (d) winter: ■ represents non-shaded, non-heated plots, □ 'full' shaded, non-heated plots, ■ non-shaded, heated plots and □ 'full' shaded, heated plots. For clarity 'half' shade plots have been excluded.

shading significantly reduced the increase in root DM and length but had no effect on SRL.

There was no change in root DM in experiment 2 in the control plots, but root N concentration increased in all plots from approximately 0.5 to 0.8 mmol g^{-1}. Soil warming did not affect any biomass parameter, but root length was significantly reduced in shaded plots and SRL increased.

Shoot DM, leaf area and specific leaf area (SLA) all increased during the spring run, although there were no consistent effects of either shade or heating on shoot measurements. Shoot DM in the autumn run did not change with time in control plots, but decreased with time in shaded plots.

Experiment 3

In control plots, root DM increased during spring (from 0.54 to 0.98 mg cm^{-3}) and stayed high for most of the summer before decreasing steadily through autumn and winter (Fig. 6a). Across the year as a whole, there was a weakly significant reduction in root DM by shading ($P = 0.053$), most obvious in the summer, but no heating effect. However, there were strongly significant effects in the autumn and winter months where root DM dropped from 0.51 to 0.08 mg cm^{-3} in heated plots compared with 0.54 to 0.24 mg cm^{-3} in ambient temperature plots. Root length and DM were correlated throughout the year and across treatments ($r^2 = 0.283$, $P < 0.001$); most outlying samples had low SRL, suggesting that they probably contained segments of *Plantago* taproot. Consequently, root length responses were similar to root DM, doubling in 3 months during spring and early summer (Fig. 6b), but showed highly significant negative effects of both soil heating and shading ($P < 0.001$). SRL increased markedly during the year in response to shading, from around 25 to 70 m g^{-1}, whereas non-shaded plots maintained a similar SRL throughout the experiment. There was no significant effect of heating on SRL.

Root N concentration was approximately 1.0 mmol g^{-1} at the start and end of the experiment, but dropped to 0.6 mmol g^{-1} during the summer months. There were

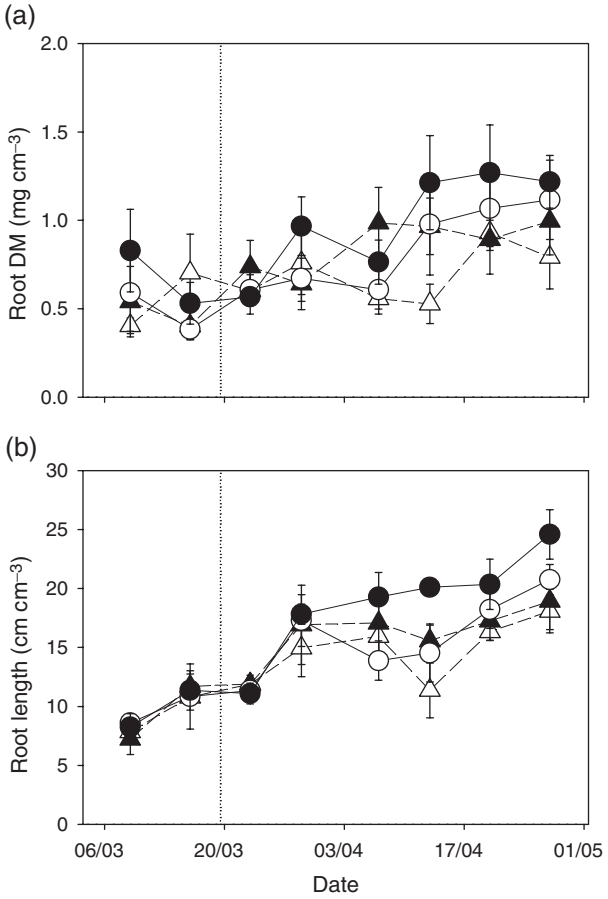

Fig. 5 Root dry mass (a) and length (b) per unit soil volume in the top 10 cm of soil during experiment 1. Filled symbols denote non-heated and open symbols heated plots, circles denote non-shaded and triangles 'full' shaded plots. Dotted lines represent start of treatments. Data for half-shade plots are omitted for clarity.

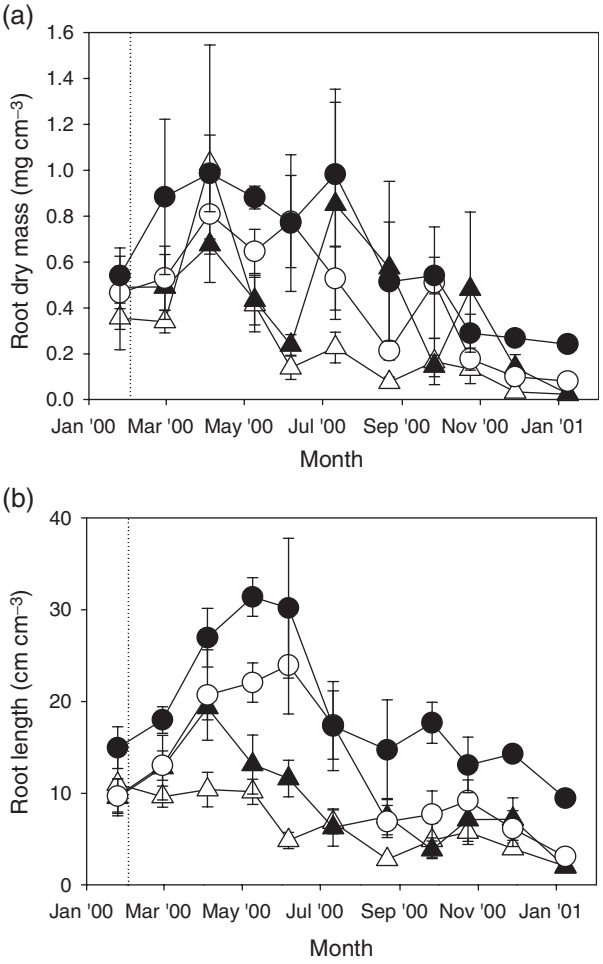

Fig. 6 Root dry mass (a) and length (b) per unit soil volume in the top 10 cm of soil during experiment 3. Filled symbols denote non-heated and open symbols heated plots, circles denote non-shaded and triangles 'full' shaded plots. Dotted lines represent start of treatments. Data for half-shade plots are omitted for clarity.

no treatment effects and root N concentration was unrelated to SRL.

Comparison with demographic data

The absolute root number and root DM across the whole data set were positively correlated in experiments 1 and 3 ($P = 0.002$ and < 0.001, respectively), but not in experiment 2. However, adjusted r^2-values were only 0.05–0.06. Examining the data sets on a treatment basis did not improve the fit.

Root respiration

Experiments 1 and 2

Respiration at extraction temperature rose slightly during experiment 1, from around 5–10 to 8–13 nmol $g^{-1} s^{-1}$ (Fig. 7a), despite soil temperatures, at the time of coring, only increasing significantly between the first and second harvests. However, during this time the Q_{10} of root respiration dropped from 2 to around 1.5, owing to a significant increase in respiration with time when measured at low temperature (5 °C) but a decrease when measured at high temperature (15 °C).

Conversely, root respiration at extraction temperature fell significantly during experiment 2, from 9–12 to 4–6 nmol $g^{-1} s^{-1}$ (Fig. 7b). After soil heating was applied, specific root respiration in the heated plots was higher than the ambient plots, but this effect was transient and had disappeared after 2 weeks. Owing to the increase in root N concentration in all treatments, these effects were even more pronounced when respiration was expressed on a nitrogen basis. However, there was no effect of treatment or time on Q_{10}.

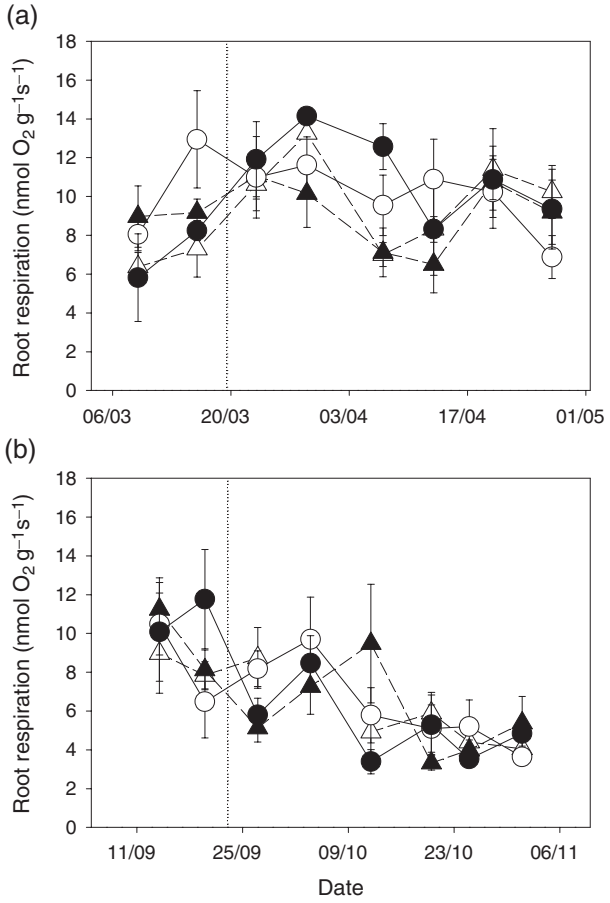

Fig. 7 Respiration rate of roots from the top 10 cm of soil during (a) experiment 1 and (b) experiment 2. Filled symbols denote non-heated and open symbols heated plots, circles denote non-shaded and triangles 'full' shaded plots. Dotted lines represent the start of treatments.

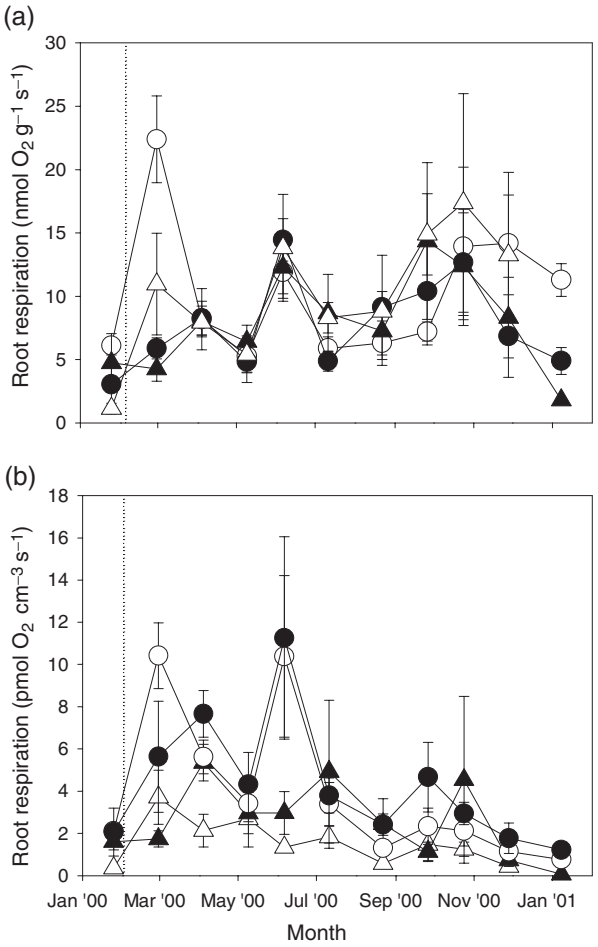

Fig. 8 Specific respiration rates (a) and expressed on a soil volume basis (b) of roots from the top 10 cm of soil during experiment 3. Filled symbols denote non-heated and open symbols heated plots, circles denote non-shaded and triangles 'full' shaded plots. Dotted lines represent the start of treatments.

Experiment 3

At the first harvest after soil warming was applied, heating increased specific root respiration at extraction temperature (Fig. 8a). However, this effect was transitory and throughout most of the experiment neither heating nor shading had any significant effect. Root respiration was rather variable, but in general increased from spring until late autumn, followed by a large and rapid decrease in winter to reach rates similar to those at the start of the experiment. Respiration rates from the final two harvests were higher in warmed plots than ambient, but sample numbers were lower as several heated plots did not have enough root mass in the soil cores to measure respiration.

Respiratory Q_{10} measurements were rather variable but dropped significantly from approximately 2.2–2.5 at the start of the experiment to below 1 in early May, then increased back to the initial values. Across the whole experiment, the average Q_{10} was between 1.5 and 2 in all treatments and there were no significant effects of the treatments applied.

When expressed on a soil volume rather than root weight basis, both shading and heating reduced root respiration except at the second harvest, where heating significantly increased rates (Fig. 8b). Respiration on a soil volume basis was highest in spring, then declined throughout the rest of the year.

Leaf gas exchange

Gas exchange measurements were only made during experiment 3. Photosynthesis at saturating light (A_{sat}) was not affected by the soil-heating treatment on any of the measurement dates in either *H. lanatus* or *P. lanceolata* (Table 1). Shading treatments had no effect on either A_{sat} measurements or light response curves

when expressed on an area basis, but on a weight basis there was a significant shade response in A_{sat} (Table 1) and photosynthetic rates at a fixed PPFD were between 25% and 68% higher in shaded plants. The difference in the results expressed on an area and weight basis was due to an increase in SLA of up to 50% in both species when shaded: from 32.5 to 42.6 $m^2 kg^{-1}$ in *H. lanatus* and 26.6 to 39.3 $m^2 kg^{-1}$ in *P. lanceolata*. The photosynthetic shade response varied through the year and was largest during midsummer, dropping to 25% by September. Leaf SLA responses were mirrored by an increase in percentage of leaf water content.

Soil heating also had no significant effect on the parameters derived from the CO_2 response curves for *H. lanatus*. R_d, $V_{c\,max}$ and J_{max} were all unresponsive to the change in root temperature (Table 1). However, shading increased both $V_{c\,max}$ and J_{max}.

Relationships with environmental variables

Instantaneous data

In all analyses of root birth rates, except where the spring data alone were examined, the first variable to enter was a measure of the total received radiation flux (Fig. 9). In each case, the *P*-value for the regression model was less than 0.001. The adjusted r^2 for these relationships ranged from 0.162 when the entire data set was used to 0.425 for the non-shaded plots with spring data excluded (Table 2a). When data were grouped by season, significant models could be fitted to summer and autumn data, but not to winter and spring data. A soil temperature measurement was only entered first in a single analysis: maximum soil temperature averaged over the preceding 3 days for a two harvest running mean for the spring data only. Indeed, from a total of 18 analyses, there was only one other regression model where any soil temperature measurement was entered at all.

In general, the relationships between death rate and environmental variables were poor, with several regressions producing no significant result (Table 2b). Across the data set as a whole, there was a significant positive relationship with minimum soil temperature averaged over the preceding 3 days, but the r^2 was only 0.193. For non-shaded treatments, only soil temperature was related to death rate, while for half-shade data, PPFD was the significant term; no model could be fitted to full-shade data. The strongest relationship was between death rates during autumn and a combination of a 3-day total PAR, 3-day minimum air temperature and 1-day mean soil temperature; no models could be fitted to the data for other seasons.

Root respiration at extraction temperature was significantly and positively correlated with maximum soil

Table 1 Leaf dark respiration rates and photosynthetic characteristics of *Holcus lanatus* and *Plantago lanceolata*

	H. lanatus					*P. lanceolata*
Treatment	R_n (nmol $g^{-1} s^{-1}$)	R_d (nmol $g^{-1} s^{-1}$)	$V_{c\,max}$ (μmol $g^{-1} s^{-1}$)	J_{max} (μmol $g^{-1} s^{-1}$)	A_{sat} (μmol $g^{-1} s^{-1}$)	A_{sat} (μmol $g^{-1} s^{-1}$)
Ambient light ambient soil temp.	26.7 ± 5.3^a	68.7 ± 17.5^a	1.11 ± 0.11^a	2.39 ± 0.06^a	0.35 ± 0.10^a	0.31 ± 0.02^a
Ambient light + 3 °C soil temp.	15.1 ± 5.2^a	65.2 ± 12.2^a	1.06 ± 0.09^a	2.28 ± 0.10^a	0.29 ± 0.06^a	0.32 ± 0.04^a
Full-shade ambient soil temp.	17.5 ± 5.0^a	64.3 ± 12.6^a	1.51 ± 0.18^b	3.32 ± 0.40^b	0.45 ± 0.07^b	0.42 ± 0.04^b
Full shade + 3 °C soil temp.	27.0 ± 5.1^a	50.9 ± 12.7^a	1.54 ± 0.13^b	3.09 ± 0.06^b	0.50 ± 0.01^b	0.47 ± 0.04^b

Data from 18/7/2000. Different letters denote significant differences.

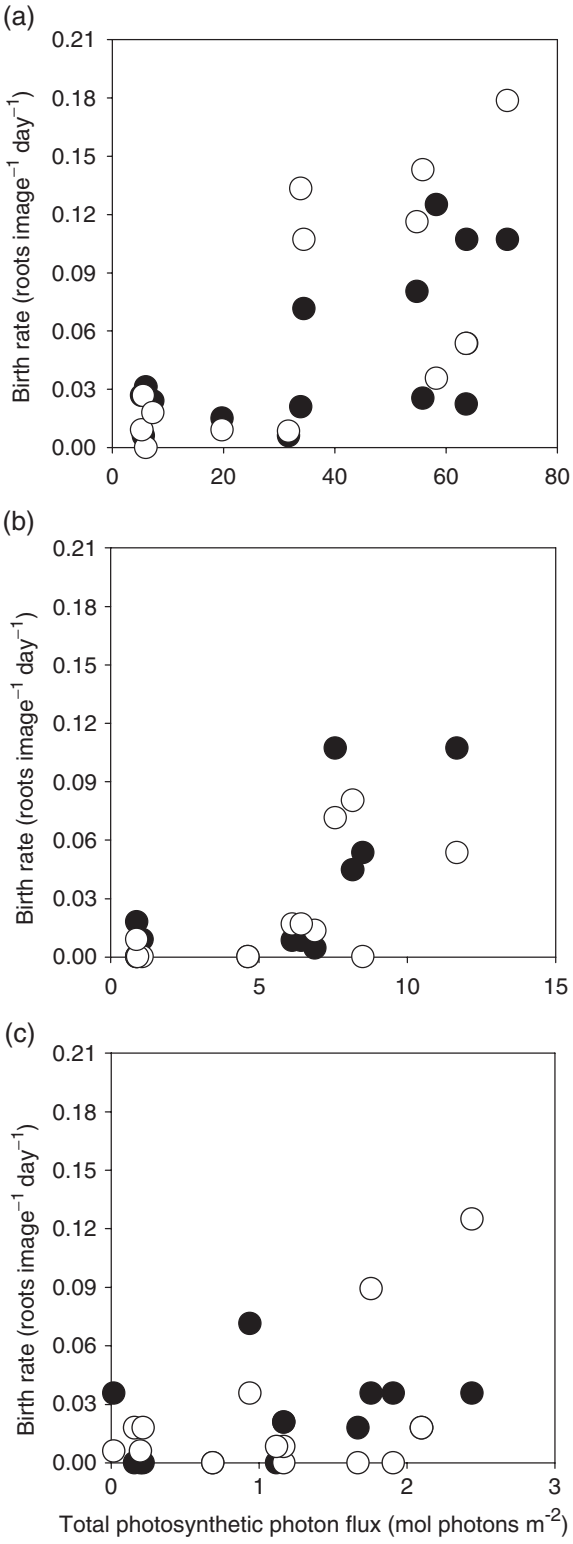

Fig. 9 Mean root birth rates, excluding spring data, in experimental plots warmed 3°C above ambient (open symbols) or not warmed (closed symbols) graphed against total received PPFD prior to root measurement; (a) non-shaded plots against PPFD over 5 days, (b) half-shade plots against PPFD over 3 days and (c) full-shade plots against PPFD over 1 day.

temperature and negatively with total received PAR (Table 2c). Daily maximum soil temperature varied between 5 °C and 20 °C; splitting the data set into two with an average soil temperature of above or below 11 °C revealed that while soil temperature was a significant factor when below 11 °C ($r^2 = 0.622$, $P < 0.001$), it was not when above 11 °C. However, the latter data set exhibited a negative correlation with received PAR ($r^2 = 0.389$, $P = < 0.001$); the relationship was stronger when the data were split into ambient or shaded results (Table 2c). The Q_{10} of root respiration was also negatively correlated with received PAR. Although this held true for the entire data set, the relationship was strongest when the non-shaded data were considered alone (Table 2d).

Non-instantaneous data

Root accumulation was positively correlated with maximum PPFD over a 3-day period ($r^2 = 0.584$, $P = < 0.001$) and to a lesser extent with maximum air temperature (Table 3a). However, splitting the data into the three shade treatments revealed much stronger relationships between PPFD and root accumulation, with r^2-values ranging from 0.871 for the non-shaded plots to 0.492 for the fully shaded plots (Table 3a). Similar highly significant relationships existed between absolute root numbers and PPFD, but the variation in the data was somewhat greater.

Total received PPFD over a 5-day period prior to measurement was the first variable to enter the stepwise regressions of root dry weight and root length. The relationships were significant and positive in each case. The second variables to enter were minimum air temperature and minimum soil temperature, respectively, and were both negative correlations (Table 3b). SRL was not well correlated with any variable when the whole data set was used, but was negatively correlated with PPFD in each case when shade treatments were analysed separately.

Discussion

The use of minirhizotrons allows root turnover as well as standing crop to be determined with minimal disturbance, something not possible using soil cores (Boehm, 1974; Hendrick & Pregitzer, 1993). However, coreporting of root biomass and root demographic data has occurred only sporadically in the literature (e.g. Fitter *et al.*, 1998; Tierney *et al.*, 2001) and direct comparison has been reported very rarely and is not straightforward to achieve (e.g. Bragg & Cannell, 1983). Such a comparison was not possible with the data presented here, as on no occasion were measurements for both data sets made on the same day. Furthermore, root biomass was

Table 2 Summary of stepwise regression models of measured variables with environmental data: PPFD (maximum and total received), air temperature (mean, minimum and maximum) and soil temperature (mean, minimum and maximum), each calculated over 1, 3 and 5 days: (a) root birth rates, (b) root death rates (c) root respiration and (d) respiratory Q_{10}

Data set	n	r^2	Variables entered	Coefficient	Probability
(a)					
All data	112	0.162	5-day total PPFD	0.0016	<0.001
			1-day min. air temperature	−0.0041	0.009
All data excluding spring	76	0.364	5-day total PPFD	0.0018	<0.001
Non-shaded data excluding spring	24	0.425	5-day total PPFD	0.0021	<0.001
Half-shade data excluding spring	24	0.241	3-day total PPFD	0.0055	0.009
Summer data only	28	0.465	1-day total PPFD	0.0069	<0.001
			5-day min. soil temperature	−0.0104	0.020
Autumn data only	24	0.330	5-day total PPFD	0.0019	0.002
(b)					
All data	112	0.193	3-day min. soil temperature	0.0032	0.006
			3-day max. PPFD	0.0331	0.009
Non-shaded data	36	0.240	5-day min. soil temperature	0.0071	0.001
Half-shade data	36	0.191	3-day total PPFD	0.0054	0.004
Autumn data only	24	0.568	3-day total PPFD	0.0914	0.001
			3-day min. air temperature	−0.0190	0.001
			1-day average soil temperature	0.0135	0.026
(c)					
All data	55	0.299	1-day max. soil temperature	0.0332	0.003
			3-day total PPFD	−0.445	<0.001
			5-day max. PPFD	9.156	0.002
Max. soil temp. <11 °C	23	0.622	1-day max. soil temperature	1.806	<0.001
			1-day max. PPFD	5.943	0.038
Max. soil temp. >11 °C	32	0.389	3-day total PPFD	−0.414	<0.001
			5-day max. PPFD	7.168	0.009
Non-shaded data where max. soil temperature>11°C	10	0.825	3-day total PPFD	−0.306	<0.001
Half-shade data where max. soil temperature>11°C	10	0.602	5-day total PPFD	−0.705	0.005
(d)					
All data	55	0.119	5-day total PPFD	−0.0025	0.004
			1-day max. PPFD	1.093	0.048
Non-shaded data	17	0.395	5-day total PPFD	−0.0032	0.003
Half-shade data	17	0.226	5-day total PPFD	−.0067	0.027
Full-shade data	19	0.166	1-day max. PPFD	8.960	0.042

PPFD = photosynthetic photon flux density.

taken from a root core to 10 cm depth, whereas root demographic data were obtained from images 7 mm in diameter at 4 cm depth. As a consequence of this, it would be likely that heating effects might be seen to a greater extent in the demography data as soil warming reduced with depth (Ineson *et al*., 1998). Despite this, regressions of root DM and root number did show a highly significant correlation, although the variability was large and the fit poor.

Belowground net primary production (BNPP) for the top 10 cm of soil can be calculated directly from root biomass measurements or by using estimates of root turnover (Table 4). A BNPP estimate based solely on standing biomass will usually be an underestimate (De Ruijter *et al*., 1996; Steele *et al*., 1997; Kajimoto *et al*., 1999). Therefore, the biomass method will typically also underestimate the importance of roots as a soil carbon source in estimates of ecosystem NPP. BNPP calculated from biomass data is also subject to greater error as a single erroneous measurement can cause an over- or underestimate (Caldwell & Virginia, 1989): BNPP of the full-shade/+3 °C soil temperature estimated from biomass is indeed larger than the demography-based estimate due to a very low initial measurement. Calculating BNPP from root demographic data not only includes root turnover but also reduces error by not being calculated from a single measurement.

The values of BNPP estimated from demographic data are comparable with those from other grassland

Table 3 Summary of stepwise regression models of measured variables with environmental data: PPFD (maximum and total received), air temperature (mean, minimum and maximum) and soil temperature (mean, minimum and maximum), each calculated over 1, 3 and 5 days: (a) root accumulation and (b) root harvest results

Data set	n	r^2	Variables entered	Coefficient	Probability
(a)					
All data	112	0.584	3-day max. PPFD	3.357	<0.001
			5-day max. air temperature	0.156	<0.001
Non-shaded data	36	0.886	5-day max. PPFD	5.517	<0.001
			5-day max. air temperature	0.127	0.003
Half-shade data	36	0.733	5-day max. PPFD	10.233	<0.001
Full-shade data	40	0.613	5-day total PPFD	0.695	<0.001
			3-day max. soil temperature	−0.126	0.023
Non-shaded data*	40	0.871	5-day max. PPFD	6.686	<0.001
Full-shade data*	40	0.492	5-day max. PPFD	20.966	<0.001
(b)					
Root dry weight – all data	56	0.347	5-day total PPFD	3.508	<0.001
			3-day min. air temperature	−4.850	0.030
Root dry weight – non-shaded data	18	0.551	5-day total PPFD	4.166	<0.001
Root dry weight – full-shade data	20	0.488	3-day total PPFD	5.824	<0.001
			3-day min. air temperature	−1.620	0.003
Root length – all data	56	0.657	5-day total PPFD	11.733	<0.001
			1-day min. soil temperature	−14.888	0.001
Specific root length – non-shaded data	18	0.448	3-day max. PPFD	−26486.2	<0.001
Specific root length – full-shade data	20	0.435	5-day total PPFD	−7820.9	0.001

Those marked * were regressions against a single variable only. PPFD = photosynthetic photon flux density.

sites (Gill *et al*., 2002). However, they clearly show that soil warming did not lead to an increase in BNPP, indicating that soil temperature *per se* does not drive root production in this system. This contradicts the conclusions of Gill & Jackson (2000), from a meta-analysis of 190 published studies, that root turnover increases exponentially with temperature. However, of the 190 studies, less than 10% were carried out with non-destructive observations and most of the rest used soil coring that does not give an accurate measure of root turnover (see above). More importantly, although Gill and Jackson's analysis considered precipitation as well as temperature it did not examine incident radiation, which is, in general, positively correlated with temperature and so the two factors could be confounded.

In order to predict community responses to temperature change it is necessary to separate the effects of received radiation and temperature. There are several soil warming studies in the literature (e.g. Saleska *et al*., 1999; Grogan & Chapin, 2000), but unfortunately few of these have examined root growth in any detail. Fitter *et al*. (1999) found that soil warming had little or no effect on standing root crop but did increase root turnover due to an increase in both root births and root deaths. The data presented here also show some effects on roots of a soil warming treatment. However, these seem to be dependent on the length of the experiment and season during which an experiment is conducted.

York is in a temperate region; consequently, it was expected that growth would differ between spring and autumn and that soil warming would potentially have the largest impact at these times. During the short-term spring experiment, warming increased both root turnover and absolute root number. However, despite the overall correlation between root DM and root number, heating reduced the increase in root DM observed during the spring run (Fig. 2 cf. Fig. 5). This discrepancy could have been due to (i) differing effects of heating at different soil depths; (ii) altered root distribution or (iii) a change in the size class distribution of roots, but the latter is unlikely since SRL was unaffected. It is impossible to say whether the effects of heating on root growth were direct or due to a change in edaphic conditions, such as increased N mineralization, which is known to promote localized root proliferation (Hodge *et al*., 1998). However, soil warming did not increase root births in the short-term autumn experiment, nor the long-term experiment.

Changes in birth and death rates during the long-term experiment (Fig. 4) can explain the pattern of root accumulation and loss through the seasons. Both demographic rates were similar throughout the spring and summer, with no relationship between root

Table 4 Belowground annual net primary production to 10 cm depth estimated from root mass in soil cores and root demographic data

	NPP – biomass ($g\,m^{-2}$)		NPP – demography ($g\,m^{-2}$)		
Treatment	Mean	SE	Mean	SE	% Difference
Ambient light/ambient soil temp.	64[a]	20	141[a]	51	119
Ambient Light/+3 °C soil temp.	59[a]	9	129[a]	29	118
Half shade/ambient soil temp.	36[a]	13	79[ab]	13	120
Half shade/+3 °C soil temp.	82[a]	16	88[ab]	9	6
Full shade/ambient soil temp.	54[a]	29	70[b]	21	28
Full shade/+3 °C soil temp.	67[a]	30	40[b]	5	−40

Biomass-based estimates were calculated by subtracting the initial root biomass (from 3/02/2000) from the maximum root biomass during the year estimated using a two-point running mean of harvest data. Demographic estimates were calculated by dividing the mean root biomass during the year with the mean root number and multiplying by the cumulative root births. The final column is the percentage difference between the two methods of calculation. Results are means of four plots with standard errors. Superscript letters denote significant differences ($P \leq 0.05$).

production and soil temperature. In contrast to spring, root DM did not increase during the summer months due to the higher root death rates. During autumn, there were virtually no root births, but very high death rates led to a loss of root DM. Nitrogen accumulated in the roots at this time in both the short- and long-term experiments. Many plants store N in roots over the winter (Bewley, 2002) and Burton *et al.* (2000) observed that forest roots with a higher N content had a longer lifespan and lower turnover. Over-wintering roots are typically longer lived than summer roots (Fitter *et al.*, 1998) and it is possible that there is also a relationship between longevity and N content in these roots, although neither experiment was continued for long enough to be certain. The higher root N could have been due to a higher rate of N uptake, perhaps in part due to the lower root density as root mortality increased. However, it is equally possible that roots with a lower N concentration had increased mortality relative to those with a high N content or that N was being recycled from aboveground parts of the plants.

During winter, despite root turnover occurring at only about a third of the summer rates, more roots appeared than autumn, again indicating that soil temperature was not controlling root production.

Soil heating had no significant effect on root birth rates, and although autumnal death rates were apparently greater in heated plots than in ambient plots, this difference was not significant either. However, root numbers and DM did drop significantly more with warming, so root death must have been greater in those plots. This was probably because of an extended period of root death rather than a change in root death rate itself. The same phenomenon was reported by Fitter *et al.* (1999), where root numbers dropped more between autumn and spring in heated plots than in ambient plots. The effect of the warming treatment on the extent of root death was less pronounced at the lower of the two depths examined. This was to be expected as the soil-heating treatment, while still present at this depth, reduced as depth increased.

One explanation for a reduction in root number with soil warming could be that warming roots reduced photosynthesis and hence carbon flow belowground. Roots may potentially influence photosynthetic rates by various means in response to changes in soil temperatures, for example, reduced transpiration rates (Wan *et al.*, 2002) or photosynthetic rates (but see Introduction). However, there was no discernible effect of the soil-warming treatment on photosynthetic rates or its components either directly or indirectly. Another possible cause of root death in the warmed plots would be a reduction in soil moisture (Dubrovsky *et al.*, 1998), but the lack of any treatment effect on soil moisture and the fact that the plots were at field capacity for most of the period of increased root death makes this explanation unlikely.

Shading reduced both root number and root DM (Figs 3 and 7). This effect was marginal during spring but pronounced during summer and autumn. At least part of the spring burst in root growth is due to stored carbohydrates and N (Millard & Proe, 1992; Tamura & Moriyama, 2001; Bates *et al.*, 2002); consequently, the effect of any reduction in photosynthesis caused by shading would be buffered. There was a much greater effect of shading during the summer months, with birth rates less than half those of the non-shaded plots,

explaining the large reduction in maximum root number. Throughout much of the year, the relationship between root birth and death rates was not altered by shade treatment. However, during winter the death rate in shaded plots continued at a rate similar to that in autumn, leading to a continued loss of roots and indicating that root birth and death may be under separate control.

Shading had little effect in either short-term experiment, except for small reductions in shoot DM and root length during the autumn run. However, the shade treatments were less severe than the long-term experiment and taking PAR measurements at a fixed height may have overestimated the degree of shading due to non-intercepted solar radiation reaching the plants at low solar angles.

Shade and temperature effects on root death during winter appear to be additive, with no loss of roots during winter in control plots, moderate loss in full-shade plots at ambient soil temperature or non-shaded heated plots but severe loss in the full-shade heated plots. In fact, the latter plots lost twice the number of roots that were gained during the growing season. Although it was the ratio of root births to deaths that determined root number, the root death rate was altered by the treatments to a greater extent than root births.

There are a number of reasons why root death rates in winter may have been higher in heated and shaded plots. Roots in shaded plots had a much greater SRL, which in many species would reduce longevity (Wells & Eissenstat, 2001; Gill *et al.*, 2002; Van der Krift & Berendse, 2002). Additionally, although the shaded plants exhibited acclimation to low light (see below), low winter light levels may have led to severe depletion of root carbohydrate reserves, especially since root respiration rates in winter were higher in heated plots than in control plots (Fig. 8), possibly due to respiratory sensitivity to low soil temperatures. However, these were also the measurements with the highest error because of small sample size. Root respiration also dropped during the short-term autumn experiment, but there was no difference in rates between heated and ambient plots, although minimum soil temperature was fairly high, 15 °C, in that experiment.

There is a large literature examining root respiratory responses to soil temperature but little that includes long-term measurements on roots grown in a field environment. A recent study of North American forests found no acclimation of roots to temperature (Burton *et al.*, 2002); in contrast, a study of grasslands in the UK found no relationship between temperature and respiration (Fitter *et al.*, 1998) and soil warming in Alaska did not affect root respiration (Johnson *et al.*, 2000), both implying acclimation. Although root respiration increased when the heating system was turned on, this effect was short lived, with heated roots fully acclimating within two weeks in experiment 2, and before the subsequent harvest in experiment 3. The initial response was probably an artefact of the sudden onset of soil warming (the 3 °C increase was achieved within an hour) and there was no further effect of heating on respiration rates in either of the short-term experiments or for much of the long-term experiment. Furthermore, there was no relationship between respiration and temperature when soil temperature was over 11 °C, so it appears that in this system root respiration only becomes temperature limited, or is unable to acclimate to temperature change, below around 11 °C.

Specific root respiration and its Q_{10} were both negatively correlated with PAR (Table 2c,d), but at different times of the year. The relationship was strongest during spring for Q_{10} when respiration rates were rising, together with an increase in root growth. A reduced sensitivity to temperature (i.e. a reduced Q_{10}) would enable this growth to continue during a period where temperatures may fluctuate. Specific root respiration also rose during late summer and autumn when light levels were falling. This coincided with an increase in root N concentration, so may have been due to respiration associated with high N uptake (but see above).

In the stepwise regression models of root data from the long-term experiment against environmental measurements (Tables 2–4), the majority of the significant relationships were with a measure of PAR rather than soil temperature; including births, deaths, respiration and DM. The closest relationship was between root accumulation and daily maximum PAR. While this relationship cannot be directly causal, as root accumulation is an integrative value over time and maximum PAR is not, it indicates that seasonal root growth is directly related to solar radiation rather than soil temperature, as the equivalent fit with temperature was poor.

Several of the fits (e.g. root DM, respiration and accumulation) between measured variables and PAR were improved by analysing the shade treatments separately, due to the relationships having different slopes, indicating that some acclimation to shading had occurred. Several factors could contribute to this, but photosynthesis is likely to be of particular importance. Indeed, on an area basis, shaded *H. lanatus* plants had higher photosynthetic rates, at all light levels but more so below 150 $\mu mol\, m^{-2}\, s^{-1}$ PPFD, as well as increased RuBisCO activity. Plants subject to low light conditions typically change the allocation of N from RuBisCO to light harvesting (Evans & Seeman, 1989) and increase

SLA (Björkman, 1981). A large change in SLA was seen in *H. lanatus* leaves, but there was little evidence of a reduced N allocation to RuBisCO. $V_{c\ max}$ is generally well correlated with leaf N (Evans, 1989), so it is likely that leaf N concentration was higher in shaded plants. Although detailed photosynthetic measurements were not made for *P. lanceolata*, A_{sat} measurements and SLA were higher in shaded plants than non-shaded plants, indicating that some acclimation of assimilation to shading also occurred in this species. These data support the hypothesis of Evans & Poorter (2001) that changes in SLA are more important to low light acclimation than reallocation of leaf N.

The results presented here demonstrate that root growth in this temperate lowland grassland ecosystem responds to radiation flux, and not to soil temperature as is often assumed in carbon cycle models (e.g. Century: Parton *et al*., 1993; TEM: McGuire *et al*., 1992). Furthermore, they show that root standing biomass is not directly related to total root production and consequently, carbon cycle models based on root biomass measurements will be inaccurate in their estimates of carbon movement from shoot to root and root to soil pools. They confirm earlier results from a high-altitude grassland (Fitter *et al*., 1998, 1999), and demonstrate the generality of the findings.

While the short-term experiments offer the possibility that climate warming occurring to a greater extent at different times of the year could have differing effects on root production and growth, the contrasting responses seen between the short- and long-term experiments show the necessity for extended study in natural ecosystems of global change scenarios. Furthermore, such studies should separate treatment effects, such as late season root loss with soil heating, from environmental controls, as these results contained no relationship between root number and soil temperature at any time of the year.

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References

Aguirrezabal LAN, Deleens E, Tardieu F (1994) Root elongation rate is accounted for by intercepted PPFD and source–sink relations in field and laboratory-grown sunflower. *Plant Cell and Environment*, **17**, 443–450.

Atkin OK, Edwards EJ, Loveys BR (2000) Response of root respiration to changes in temperature and its relevance to global warming. *New Phytologist*, **147**, 141–154.

Barber SA, Mackay AD, Kuchenbuch RO *et al*. (1988) Effects of soil temperature and water on maize root growth. *Plant and Soil*, **111**, 267–269.

Bates TR, Dunst RM, Joy P (2002) Seasonal dry matter, starch, and nutrient distribution in 'Concord' grapevine roots. *Hortscience*, **37**, 313–316.

Bewley JD (2002) Root storage proteins, with particular reference to taproots. *Canadian Journal of Botany*, **80**, 321–329.

Björkman O (1981) Responses to different quantum flux densities. In: *Physiological Plant Ecology I. Responses to the Physical Environment* (eds Lange OL, Nobel PS, Osmond CB, Ziegler H), pp. 57–107. Springer-Verlag, Berlin.

Boehm W (1974) Mini-rhizotrons for root observations under field conditions. *Zeitschrift Acker-Pflanzenbau*, **144**, 282–287.

Boone RD, Nadelhoffer KJ, Canary JD *et al*. (1998) Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature*, **396**, 570–572.

Bragg PL, Cannell RQ (1983) A comparison of methods, including angled and vertical minirhizotrons, for studying root growth and distribution in a spring oat crop. *Plant and Soil*, **73**, 435–440.

Bryla DR, Bouma TJ, Eissenstat DM (1997) Root respiration in citrus acclimates to temperature and slows during drought. *Plant, Cell and Environment*, **20**, 1411–1420.

Burton AJ, Pregitzer KS, Hendrick RL (2000) Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. *Oecologia*, **125**, 389–399.

Burton AJ, Pregitzer KS, Ruess RW *et al*. (2002) Root respiration in North American forests: effects of nitrogen concentration and temperature across biomes. *Oecologia*, **131**, 559–568.

Burton AJ, Pregitzer KS, Zogg GP *et al*. (1996) Latitudinal variation in sugar maple fine root respiration. *Canadian Journal of Forest Research*, **26**, 1761–1768.

Caldwell MM, Virginia RA (1989) Root systems. In: *Plant Physiological Ecology – Field Methods and Instrumentation* (eds Pearcy RW, Ehlringer J, Mooney HA, Rundel PW), pp. 367–398. Chapman and Hall, London.

Dai A, Del Genio AD, Fung IY (1997) Clouds, precipitation and temperature range. *Nature*, **386**, 665–666.

De Ruijter FJ, Veen EW, Van Oijen M (1996) A comparison of soil core sampling and minirhizotrons to quantify root development of field-grown potatoes. *Plant and Soil*, **182**, 301–312.

Dubrovsky JG, North GB, Nobel PS (1998) Root growth, developmental changes in the apex, and hydrolic conductivity for *Opuntia ficus-indica* during drought. *New Phytologist*, **138**, 75–82.

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia*, **78**, 9–19.

Evans JR, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell and Environment*, **24**, 755–767.

Evans JR, Seeman JR (1989) The allocation of protein nitrogen in the photosynthetic apparatus: costs, consequences and control. In: *Photosynthesis* (ed. Briggs WR), pp. 183–205. A.R. Liss, New York.

Farquhar GD, von Caemmerer S, Berry JA (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. *Planta*, **149**, 78–90.

Fitter AH, Graves JD, Self GK *et al*. (1998) Root production, turnover and respiration under two grassland types along an altitudinal gradient: influence of temperature and solar radiation. *Oecologia*, **114**, 20–30.

Fitter AH, Self GK, Brown TK *et al*. (1999) Root production and turnover in an upland grassland subjected to artificial soil warming respond to radiation flux and nutrients, not temperature. *Oecologia*, **120**, 575–581.

Gavito ME, Curtis PS, Mikkelsen TN *et al*. (2001) Interactive effects of soil temperature, atmospheric carbon dioxide and soil N on root development, biomass and nutrient uptake of winter wheat during vegetative growth. *Journal of Experimental Botany*, **52**, 1913–1923.

Gill RA, Burke IC, Lauenroth WK *et al*. (2002) Longevity and turnover of roots in the shortgrass steppe: influence of diameter and depth. *Plant Ecology*, **159**, 241–251.

Gill RA, Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. *New Phytologist*, **147**, 13–31.

Grogan P, Chapin FS (2000) Initial effects of experimental warming on above- and belowground components of net ecosystem CO_2 exchange in arctic tundra. *Oecologia*, **125**, 512–520.

Gunn S, Farrar JF (1999) Effects of a 4 °C increase in temperature on partitioning of leaf area and dry mass, root respiration and carbohydrates. *Functional Ecology*, **13**, 12–20.

Hendrick RL, Pregitzer KS (1993) The dynamics of fine-root length, biomass, and nitrogen-content in 2 northern hardwood ecosystems. *Canadian Journal of Forest Research*, **23**, 2507–2520.

Hodge A, Stewart J, Robinson D *et al*. (1998) Root proliferation, soil fauna and plant nitrogen capture from nutrient-rich patches in soil. *New Phytologist*, **139**, 479–494.

Houghton JT, Ding Y, Griggs DJ *et al*. (2001) *Climate Change 2001 – The Scientific Basis*. Cambridge University Press, Cambridge, UK.

Ineson P, Benham DG, Poskitt J *et al*. (1998) Effects of climate change on nitrogen dynamics in upland soils. 2. A soil warming study. *Global Change Biology*, **4**, 153–161.

Johnson LC, Shaver GR, Cades DH *et al*. (2000) Plant carbon–nutrient interactions control CO_2 exchange in Alaskan wet sedge tundra ecosystems. *Ecology*, **81**, 453–469.

Johnson MG, Tingey DT, Phillips DL *et al*. (2001) Advancing fine root research with minirhizotrons. *Environmental and Experimental Botany*, **45**, 263–289.

Kajimoto T, Matsuura Y, Sofronov MA *et al*. (1999) Above- and belowground biomass and net primary productivity of a *Larix gmelinii* stand near Tura, central Siberia. *Tree Physiology*, **19**, 815–822.

Kaspar TC, Bland WL (1992) Soil temperature and root growth. *Soil Science*, **154**, 290–299.

Kleier C, Farnsworth B, Winner W (2001) Photosynthesis and biomass allocation of radish cv. 'Cherry Belle' in response to root temperature and ozone. *Environmental Pollution*, **111**, 127–133.

Kutsch WL, Staack A, Wojtzel J *et al*. (2001) Field measurements of root respiration and total soil respiration in an alder forest. *New Phytologist*, **150**, 157–168.

Lawrence WT, Oechel WC (1983) Effects of soil temperature on the carbon exchange of taiga seedlings I. Root respiration. *Canadian Journal of Forest Research*, **13**, 840–849.

McGuire AD, Mellilo JM, Joyce LA *et al*. (1992) Interactions between carbon and nitrogen dynamics in estimating net primary productivity for potential vegetation in North America. *Global Biogeochemical Cycles*, **6**, 101–124.

Millard P, Proe MF (1992) Storage and internal cycling of nitrogen in relation to seasonal growth of sitka spruce. *Tree Physiology*, **10**, 33–43.

Misra RK (1999) Root and shoot elongation of rhizotron-grown seedlings of *Eucalyptus nitens* and *Eucalyptus globulus* in relation to temperature. *Plant and Soil*, **206**, 37–46.

Niklinska M, Maryanski M, Laskowski R (1999) Effect of temperature on humus respiration rate and nitrogen mineralization: Implications for global climate change. *Biogeochemistry*, **44**, 239–257.

Parton WJ, Scurlock JMO, Ojima DS *et al*. (1993) Observations and modelling of biomass and soil organic matter dynamics for the grassland biome worldwide. *Global Biogeochemical Cycles*, **7**, 785–809.

Pollack HN, Huang S, Shen P-Y (1998) Climate change record in subsurface temperatures: a global perspective. *Science*, **282**, 279–281.

Poorter H, Van der Werf A, Atkin OK *et al*. (1991) Respiratory energy-requirements of roots vary with the potential growth-rate of a plant-species. *Physiologia Plantarum*, **83**, 469–475.

Saleska SR, Harte J, Torn MS (1999) The effect of experimental ecosystem warming on CO_2 fluxes in a montane meadow. *Global Change Biology*, **5**, 125–141.

Steele SJ, Gower ST, Vogel JG *et al*. (1997) Root mass, net primary production and turnover in aspen, jack pine and black spruce forests in Saskatchewan and Manitoba, Canada. *Tree Physiology*, **17**, 577–587.

Tamura Y, Moriyama M (2001) Nonstructural carbohydrate reserves in roots and the ability of temperate perennial grasses to overwinter in early growth stages. *Plant Production Science*, **4**, 56–61.

Tierney GL, Fahey TJ, Groffman PM *et al*. (2001) Soil freezing alters fine root dynamics in a northern hardwood forest. *Biogeochemistry*, **56**, 175–190.

Tindall JA, Mills HA, Radcliffe DE (1990) The effect of root zone temperature on nutrient-uptake of tomato. *Journal of Plant Nutrition*, **13**, 939–956.

Tjoelker MG, Reich PB, Oleksyn J (1999) Changes in leaf nitrogen and carbohydrates underlie temperature and CO_2 acclimation of dark respiration in five boreal tree species. *Plant Cell and Environment*, **22**, 767–778.

Van der Krift TAJ, Berendse F (2002) Root life spans of four grass species from habitats differing in nutrient availability. *Functional Ecology*, **16**, 198–203.

Wan S, Luo Y, Wallace LL (2002) Changes in microclimate induced by experimental warming and clipping in tallgrass prairie. *Global Change Biology*, **8**, 754–768.

Wells CE, Eissenstat DM (2001) Marked differences in survivorship among apple roots of different diameters. *Ecology*, **82**, 882–892.

Wigley TML, Raper SCB (2001) Interpretation of high projections for global-mean warming. *Science*, **293**, 451–454.

Xu QZ, Huang BR (2000) Growth and physiological responses of creeping bentgrass to changes in air and soil temperatures. *Crop Science*, **40**, 1363–1368.

Ziska LH (1998) The influence of root zone temperature on photosynthetic acclimation to elevated carbon dioxide concentrations. *Annals of Botany*, **81**, 717–721.

Zogg GP, Zak DR, Burton AJ *et al*. (1996) Fine root respiration in northern hardwood forests in relation to temperature and nitrogen availability. *Tree Physiology*, **16**, 719–725.