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Response of root respiration and root exudation to alterations in root C supply and demand in wheat

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Abstract Rising atmospheric CO_2 concentrations have highlighted the importance of being able to understand and predict C fluxes in plant-soil systems. We investigated the responses of the two fluxes contributing to below-ground efflux of plant root-dependent CO_2, root respiration and rhizomicrobial respiration of root exudates. Wheat (*Triticum aestivum* L., var. Consort) plants were grown in hydroponics at 20°C, pulse-labelled with $^{14}CO_2$ and subjected to two regimes of temperature and light (12 h photoperiod or darkness at either 15°C or 25°C), to alter plant C supply and demand. Root respiration was increased by temperature with a Q_{10} of 1.6. Root exudation was, in itself, unaltered by temperature, however, it was reduced when C supply to the roots was reduced and demand for C for respiration was increased by elevated temperature. The rate of exudation responded much more rapidly to the restriction of C input than did respiration and was approximately four times more sensitive to the decline in C supply than respiration. Although temporal responses of exudation and respiration were treatment dependent, at the end of the experimental period (2 days) the relative proportion of C lost by the two processes was conserved despite differences in the magnitude of total root C loss. Approximately 77% of total C and 67% of ^{14}C lost from roots was accounted for by root respiration. The ratio of exudate specific activity to CO_2 specific activity converged to a common value for all treatments of 2, suggesting that exudates and respired CO_2were not composed of C of the same age. The results suggest that the contributions of root and rhizomicrobial respiration to root-dependent below-ground respiration are conserved and highlight the dangers in estimating short-term respiration and exudation only from measurements of labelled C. The differences in responses over time and in the age of C lost may ultimately prove useful in improving estimates of root and rhizomicrobial respiration.

Keywords Respiration · Rhizodeposition · Root exudation · CO_2 · Temperature · Light

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Introduction

Current concerns about rising atmospheric CO_2 and climate change have led to considerable effort

being applied to understanding C flow in the plant–soil system. This understanding is crucial if we are to predict future changes to the global C cycle. A number of techniques have been used to evaluate the magnitude of C fluxes and their response to a variety of environmental variables. Owing to the difficulty of establishing the origin and fate of C in a complex system, most of these studies of C flow in the plant–soil system have used C isotopes in order to separate different ages and origins of C and to follow the fate of any given portion of C fixed in photosynthesis (van Ginkel et al. 1997; Nguyen 2003; Kuzyakov and Larinova 2005). Arguably the most difficult fluxes to measure in the plant–soil system are respiratory losses of CO_2 from autotrophic and heterotrophic organisms and their relative contributions to the total below-ground CO_2 flux under non-sterile conditions in soil. Continuous labelling of plants with $^{14}CO_2$ or $^{13}CO_2$ is able to more-or-less separate the respiration of relatively recent plant C from that of pre-existing soil C (van Ginkel et al. 1997; Cotrufo and Gorissen 1997), although short-term processes such as exudation and root respiration cannot be distinguished from long-term processes such as root turnover (Killham and Yeomans 2001). Despite the use of a variety of approaches, the separation of the contribution of C respired directly by plant roots and that C respired by soil microorganisms following C loss from living roots as exudation has yet to be definitively achieved (Kuzyakov 2002; Kuzyakov and Larinova 2005). This investigation was predicated on the need to directly compare the amount and age (the time after photosynthetic fixation of C by plants) of C contributing to total below-ground C loss by root respiration and metabolism of root exudates. It also aimed to compare the relative response of the two fluxes to alterations of two environmental variables, temperature and light, with a view to using differences in the response to evaluate the contributions of the two fluxes to the total below-ground respiratory flux in soil.

Many investigations have attempted to evaluate the response of total below-ground respiration to temperature and light in non-sterile soil (Craine et al. 1999; Todorovic et al. 1999; Rustad et al. 2001; Kuzyakov and Cheng 2001). Similarly, the separate effects of the two environmental variables on root respiration and root exudation have been investigated to some extent (Bokhari and Singh 1974; Ferguson and Menge 1982; Pramanik et al. 2000; Uselman et al. 2000; Farrar 1980; Gloser et al. 1996; Lipp et al. 2003), although root respiration has received far more attention than root exudation. To our knowledge, this is the first investigation to concurrently measure total C and C of known-age in both root respiration and root exudation. Hydroponics were used because unless these fluxes are characterised in a system where the separate fluxes can be measured, it is not possible to investigate the relative magnitude and response to environmental variables of the two fluxes contributing to root-dependent below-ground respiration.

We know that root respiration responds strongly to temperature in the short term (Farrar 1988; Atkin et al. 2000; Atkin and Tjoelker 2003). We also know that rhizosphere microbes are C limited and respire labile C (e.g. root exudates) very rapidly (Jones 1999; Jones et al. 2004, 2005b; Boddy et al. 2006), even at low temperatures (Vinolas et al. 2001). Both root respiration and microbial respiration of labile substrates have been ascribed Q_{10}s of 2 (Pregitzer et al. 2000; Vinolas et al. 2001; Atkin and Tjoelker 2003), although responses of plant respiration are highly species and exposure-time specific (Gunn and Farrar 1999; Atkin et al. 2000; Covey-Crump et al. 2002; Atkin and Tjoelker 2003). When plants are growing in soil, below-ground respiration responds positively to both temperature and light (Boone et al. 1998; Craine et al. 1999; Rustad et al. 2001; Högberg et al. 2001; Bhupinderpal-Singh et al. 2003; Kuzyakov and Cheng 2001), although rhizomicrobial respiration may be more responsive to temperature than root respiration (Lee et al. 2003). Direct measurements of the responses of root exudation to the two environmental parameters have found that both temperature and light tend to increase root exudation (Bokhari and Singh 1974; Ferguson and Menge 1982; Pramanik et al. 2000; Uselman et al. 2000). Similarly, both temperature and light are strong determinants of root respiration (Farrar 1980; Gloser et al. 1996; Lipp et al. 2003).

It was our purpose to alter root demand for C by increasing or decreasing temperature relative

to the growth temperature (Atkin et al. 2005) and to limit root C supply by removing the capacity for plants to fix new C in photosynthesis. The flux of soluble carbohydrate through root C storage pools is very large relative to the size of the storage pools. Farrar and Williams (1990) estimated that the root soluble C pool in barley is only ca. 5 times as large as the quantity of sucrose delivered to the root each hour during photosynthesis. Thus, in the absence of new C input to the root, root storage pools in cereals are rapidly depleted (Farrar and Williams 1990). We hypothesised that root respiration and exudation would respond differently to alterations to root C supply and demand due to their different mechanistic causes. Root respiration is an unavoidable cost to a living plant (Wullschleger et al. 1994; Farrar 1999), whereas root exudation is thought generally to be a passive process driven by concentration differences between the root and the medium surrounding the root (Nguyen 2003; Jones et al. 2004). C compounds can be actively exuded, but this is generally in response to nutrient limitation or protection from toxic substances such as Al (Nguyen 2003; Jones et al. 2004). The exact nature of any benefit to the plant due to passive exudation remains uncertain (Nguyen 2003).

Pulse labelling of plants with $^{14}CO_2$ allows a portion of photosynthetically fixed C of known age to be followed through the plant and into exudates or respiration. Previous work has shown that ^{14}C is maximal in root exudates and root respiration after ca. 3 and ca. 1.5 h of shoot labelling respectively (Dilkes et al. 2004), but that a significant proportion of C lost root in exudates may have been fixed several days previously (Thornton et al. 2004). Combining pulse-labelling with measurements of unlabelled C efflux increases the resolution of measurements, giving two quasi independent measures of plant responses. It also allows the relative age of the C in the two routes of below-ground C loss to be investigated. The establishment of the age of C lost by respiration or exudation can be improved by the comparison of plants receiving light and those in the dark, since the relative responses of the ratios of total C loss to labelled C loss make it possible to distinguish between C fixed by the plant prior to, after and during ^{14}C labelling. Combining all these methods has allowed us both to compare the effects of light and temperature on root exudation and root respiration, and to investigate fundamental differences in their composition and response to alterations of root C supply and demand.

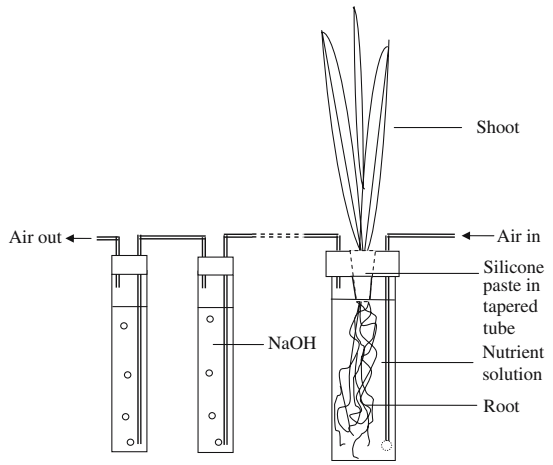

Fig. 1 Diagram of aerated vials used for growing plants and NaOH traps used to collect CO_2 lost in respiration

Materials and methods

Growth conditions and labelling

Captan-treated seeds of wheat (*Triticum aestivum* L., var. Consort) were germinated in the dark at 20°C on moist filter paper. Seedlings with roots ca. 3 cm long were transplanted into aerated vials (Fig. 1) each containing 40 ml of autoclaved full strength Long Ashton nutrient solution (Hewitt 1966). We did not attempt to maintain sterility by use of antibiotics due to their known deleterious effects on plant C partitioning (Neumann and Römheld 2001), although aeration was achieved using 0.2 μm filtered air. The nutrient solution was changed every third day at the start of the experiment and every second day during the week before labelling. All plants were grown in a climate-controlled growth cabinet (Sanyo-Gallenkamp, Fitotron PG660/C/RO/HQI, Loughborough, UK) at 20°C and 70% relative humidity, with a light intensity of 800 μmol m^{-2} s^{-1} PAR and a 12 h photoperiod.

Plants were labelled on day 24 after germination. Plant biomass was not measured at this time, but at the end of the experiment shoot and root weight were 0.9 ± 0.09 and 0.5 ± 0.03 g DW vial^{-1}, respectively (mean $\pm$ SEM; $n = 20$). Four hours before labelling the nutrient solution was exchanged and the root and shoot were sealed from each other with a 5 mm layer of NG 3170 silicone paste (Thauer & Co., Dresden, Germany). All 20 plants were labelled in a large polycarbonate labelling chamber (150 l), in which $^{14}CO_2$ was produced by addition of 3 ml 5 M H_2SO_4 to 3.7 MBq $Na_2^{14}CO_3$. The plants were allowed to assimilate $^{14}CO_2$ over a period of 1.5 h.

For three hours after the start of labelling, plants were maintained in the same light and temperature. The $^{14}CO_2$ was not trapped during the first 3 h. After 3 h, the nutrient solution was changed, the treatments were started and CO_2 trapping commenced. Plants were subjected to four treatments by the combination of two temperatures (15 or 25°C) with two light treatments (either no light or a continuation of the original 12 h photoperiod). The dark treatment was achieved by covering plants with an opaque box, which had shaded holes to permit air movement. Temperature treatments were achieved by the use of two separate, but identical, growth cabinets as described above. Only growth cabinet temperatures were monitored. Based upon previous measurements in similar experimental systems we assumed that root and shoot temperatures were the same as those in the climate-controlled cabinet.

The nutrient solution and CO_2 traps were changed twice daily, approximately at the beginning and end of the photoperiod. Collection periods were 0–7, 7–19, 19–32 and 32–43 h after the start of treatments.

Analyses

During the experiment, the CO_2 evolved from the root compartment was trapped in two trapping flasks arranged in series (Fig. 1), each containing 3 ml of 1 M NaOH, by continuous pumping (ca. 50 ml min^{-1}). The trapping system was checked with an IRGA (CIRAS 1, PP Systems, Hitchin, UK) before the experiment and captured >95% of the evolved CO_2. The ^{14}C activity captured in the NaOH solution and the ^{14}C-activity of nutrient solutions were measured by liquid scintillation counting in a Wallac 1404 ß-scintillation spectrometer (Wallac EG+G, Milton Keynes, UK), after mixing with HiSafe 3 scintillant (Fisher Scientific, Loughborough, UK). The total CO_2 captured in NaOH and the C content of nutrient solutions (root exudates) was measured in a Shimadzu TOC-V-TN analyser (Shimadzu Corp., Kyoto, Japan).

The experiment was conducted with five replicates for each of the four treatments. Statistical effects of treatment were evaluated using SPSS version 12.0 (SPSS, Chicago, USA). When comparing treatments within any given sampling time and looking for effects of combined treatments, we used a one-way ANOVA with post-hoc test. When looking for general effects of light and temperature or changes over time, we used the SPSS general linear model.

Results

Cumulative totals

Total respiratory C losses were increased 45% by light at 25°C ($P = 0.009$) and 59% by the higher temperature in the light ($P = 0.004$; Table 1). In contrast, respiratory losses of ^{14}C showed a reverse trend, being reduced by 30–51% (25 and 15°C, respectively) in the light. The specific activity of $^{14}CO_2$ was halved by light at both temperatures. The difference in the total respired C between the two temperatures in the light yielded a Q_{10} for respiration of 1.6. Total exuded C was increased 42% by light at 25°C and 94% at 15°C. Total exuded ^{14}C, however, was unaffected by any treatment. The specific ^{14}C activity of exuded C was therefore reduced by light at both temperatures (51% and 67% at 15 and 25°C, respectively).

Over all the treatments, four times as much C and ^{14}C was lost in respiration as in exudation over the duration of the experiment ($P < 0.001$ in both cases). The overall specific activity of exudates was approximately twice that of respiration ($P = 0.02$). The ratio of total respiration to total exudation was increased 96% by the higher

Table 1 Cumulative totals after 43 h of treatment for total C and ^{14}C, and specific ^{14}C activity

Temperature	15°C		25°C		P		
Light	Off	On	Off	On	Temp	Light	T × L
Total C							
Total respired (mg C plant^{-1})	24.1 ± 4	27.5 ± 1.8	29.6 ± 2	43.7 ± 4.5	0.005	0.02	ns
Total exuded (mg C plant^{-1})	6.3 ± 0.6	12.2 ± 2.1	6.6 ± 0.4	9.4 ± 1.5	ns	0.005	ns
Total respired/total exuded	3.8 ± 0.5	2.5 ± 0.4	4.5 ± 0.5	4.9 ± 0.6	0.006	ns	ns
^{14}C							
Total respired (kBq plant^{-1})	4.1 ± 1.2	2.0 ± 0.3	4.6 ± 0.3	3.2 ± 0.5	ns	0.02	ns
Total exuded (kBq plant^{-1})	1.5 ± 0.6	1.5 ± 0.5	2.6 ± 0.9	1.2 ± 0.4	ns	ns	ns
Total respired/total exuded	4.6 ± 1.6	2.1 ± 0.6	2.3 ± 0.4	5.5 ± 2.9	ns	ns	ns
Specific activity							
Respiration (Bq mg^{-1} C)	155 ± 25	71 ± 10	157 ± 14	71 ± 6	ns	<0.001	ns
Exudation (Bq mg^{-1} C)	223 ± 75	109 ± 21	402 ± 149	130 ± 42	ns	0.04	ns
Exudation/respiration	1.3 ± 0.4	2 ± 0.9	2.4 ± 0.6	1.7 ± 0.5	ns	ns	ns

Values are mean ± SEM; $n = 5$. Section "P" shows the significance level of the effects of treatment where "Temp" is temperature and T × L is an interaction between temperature and light

temperature regime in the light with no other effect of treatment. The ratios of respiration to exudation for ^{14}C and ^{14}C specific activity were not significantly affected by treatment.

Temporal dynamics in root respiration

Over the first 7 h of treatment application, total CO_2 losses from roots of plants at 25°C were double those from plants at 15°C ($P < 0.001$), but unaffected by light regime (Fig. 2). Between 7 h and 19 h, CO_2 losses were again increased (50%) at the higher temperature ($P < 0.001$). Further, plants in the dark tended to respire 10–20% (25°C and 15°C, respectively) less than those maintained in the light ($P < 0.06$). Between 19 h and 32 h, respiratory losses from plants in the dark at 25°C were halved in comparison to the previous collection period ($P < 0.001$) and to those from plants at 25°C held in the light ($P = 0.007$). In contrast, respiratory C losses from plants at 15°C were not significantly different from each other or from previous sampling periods. Between 32 h and 43 h, the respiration rate of plants in the dark at 15°C was 36% lower than in the previous sampling period ($P < 0.05$) whereas that of plants in the dark at 25°C was the same as that of plants in the dark at 15°C, but unchanged from the last sampling period. Plants in the light respired 57–87 % (15 and 25°C, respectively) faster than those in the dark ($P < 0.001$). Those at 25°C in the light respired faster than all other treatments ($P \leq 0.006$), but neither set of plants in the light were different from the previous sampling period.

The respiration rate of plants at 15°C in the light remained fairly constant over the whole experiment, whereas that of plants at 25°C in the light declined by 20% over the first three samplings ($P = 0.02$), but remained constant for the last two. Approximately 21 and 25 mg C plant^{-1} (15 and 25°C, respectively) was lost from roots before respiration dropped in dark treatments.

Total ^{14}C respiration rate and ^{14}C specific activity declined according to the usual first order exponential kinetics. Only in the 7–19 h sampling period was there a significant effect of treatment on total respired ^{14}C, when the rate of loss of $^{14}CO_2$ from plants in the dark was twice that of plants in the light ($P < 0.001$) with no effect of temperature. CO_2 specific activity was reduced 17–74% by light in all sampling periods ($P \leq 0.01$). At the first two sampling times, there appeared to be a 25–30% reduction of respired CO_2 specific activity for plants in the dark at the higher temperature, but the reduction was only significant at $P = 0.06$. However, at the third sampling (19–32 h) the response to temperature was reversed. The specific activity of CO_2 from plants in the dark at 25°C was 74% higher than that from plants in the dark at 15°C ($P = 0.001$).

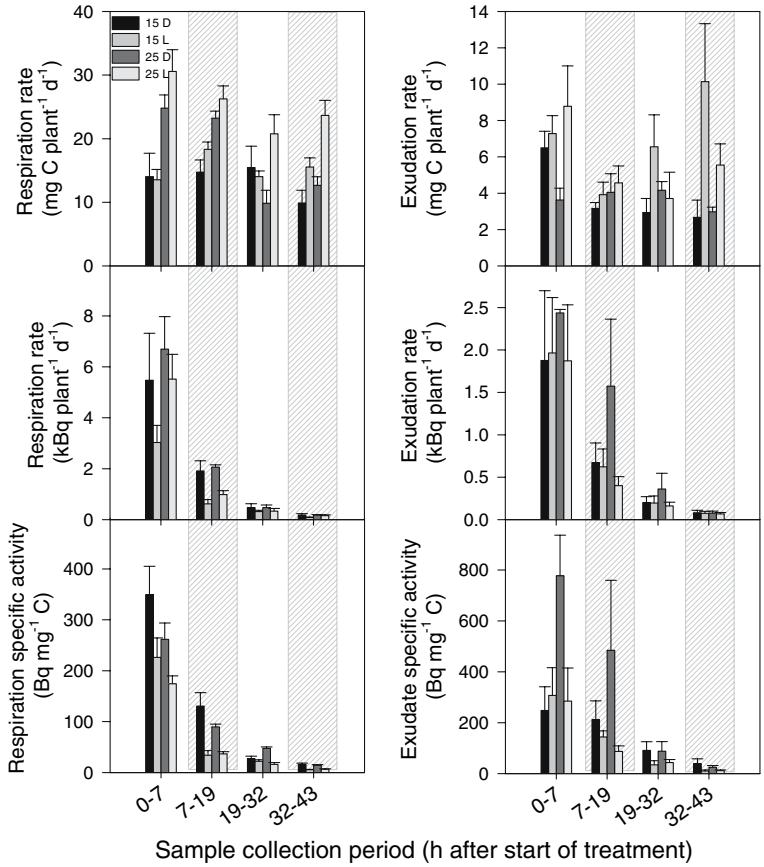

Fig. 2 Rate of respiration and exudation of total C and labelled ^{14}C, and CO_2 and exudate specific ^{14}C activity for individual sample collection periods. Time zero represents the start of treatments, 3 h after pulse-labelling. Values are mean ± SEM; $n = 5$. 15 D, 15 L, 25 D and 25 L represent 15°C dark, 15°C light, 25°C dark and 25°C light, respectively. Shaded areas approximate to dark periods in illuminated treatments

Temporal dynamics in root exudation

C losses in root exudation were subject to greater variation than C losses in root respiration (Fig. 2), regardless of treatment, so there were fewer statistically significant treatment effects. Over the first 7 h, the exudation rate of plants at 25°C in the dark was approximately half that of plants in the light at 25°C ($P < 0.02$), which was the same as that of plants at 15°C. At the second sampling, the exudation rate of all plants fell to the level of that from plants in the dark at 25°C at the first sampling. The exudation rate of plants in the dark remained the same for the remainder of the experiment regardless of temperature, whereas that of plants in the light returned to close to initial levels by the end of the experiment, 86–280% (15 and 25°C, respectively) higher than those of plants in the dark ($P = 0.01$). Approximately 6 mg C plant^{-1} was lost from plant roots at 15°C in the dark before the exudation rate fell and did not recover. Although the exudation rate of plants at 25°C in the dark had already dropped at 7 h, the amount of C lost before the decline must have been of the same order as for plants at 15°C in the dark.

Total exuded ^{14}C and the specific activity of exudates declined exponentially over time, but there were no statistically significant effects of treatment on total ^{14}C measurements. Over the whole experiment, the specific activity of exudates was reduced by light ($P = 0.009$), but not significantly affected by temperature ($P = 0.07$). During the first 7 h, the specific activity of exuded C was 150–200% higher at 25°C in the dark than in all other treatments ($P < 0.02$). Over 7–19 h there were no significant effects of treatment on C losses in root exudation. At the 19–32 h and 32–43 h samplings, the specific activity of exudates tended to be lower in illuminated plants ($P < 0.07$ and $P = 0.05$, respectively), however, there was no significant effect of temperature.

Partitioning of C between exudation and respiration

The ratio of C losses in respiration and exudation varied somewhat over time according to the temporal differences in the response of exudation and respiration to treatment (Fig. 3). At the end of the experiment (when treatment had taken effect in all groups), ratios converged to approximately the same value regardless of treatment. These values were similar to those derived from cumulative totals (Table 1) and mean values for the whole of the experiment. Using cumulative totals, after 43 h the ratio of respired C to exuded C was 4.0 ± 0.3 (mean $\pm$ SEM; $n = 20$), the ratio of respired ^{14}C to exuded ^{14}C was 3.6 ± 0.8, and the ratio of exudate specific activity to respiration specific activity was 1.8 ± 0.3. Using all times and treatments, the ratio of respired C to exuded C was 4.7 ± 0.3 (mean $\pm$ SEM; $n = 80$), the ratio of respired ^{14}C to exuded ^{14}C was 4.6 ± 1.0, and the ratio of exudate specific activity to respiration specific activity was 2.8 ± 0.5. At the final sampling time when the ratios had converged, the ratio of respired C to exuded C was 4.5 ± 0.6 (mean $\pm$ SEM; $n = 20$), the ratio of respired ^{14}C to exuded ^{14}C was 3.4 ± 0.8, and the ratio of exudate specific activity to respiration specific activity was 2.0 ± 0.3. Ratios of respired to exuded total C and ^{14}C were not significantly different. At 43 h when the ratios had stabilised, 77% of total C lost from roots and 69% of ^{14}C lost from roots was accounted for by respiration.

Discussion

Throughout the experiment, it was clear that removal of plant C input by shading and altering plant C demand by changing temperature altered total C and ^{14}C partitioning in different ways. Reducing root C supply by preventing photosynthetic C fixation prevented plants from diluting the ^{14}C label with newly fixed C or preferentially using more recent unlabelled C. Increasing plant temperature enhanced plant C demand and increased the use of ^{14}C when no new C was available in photosynthesis. These effects meant that,

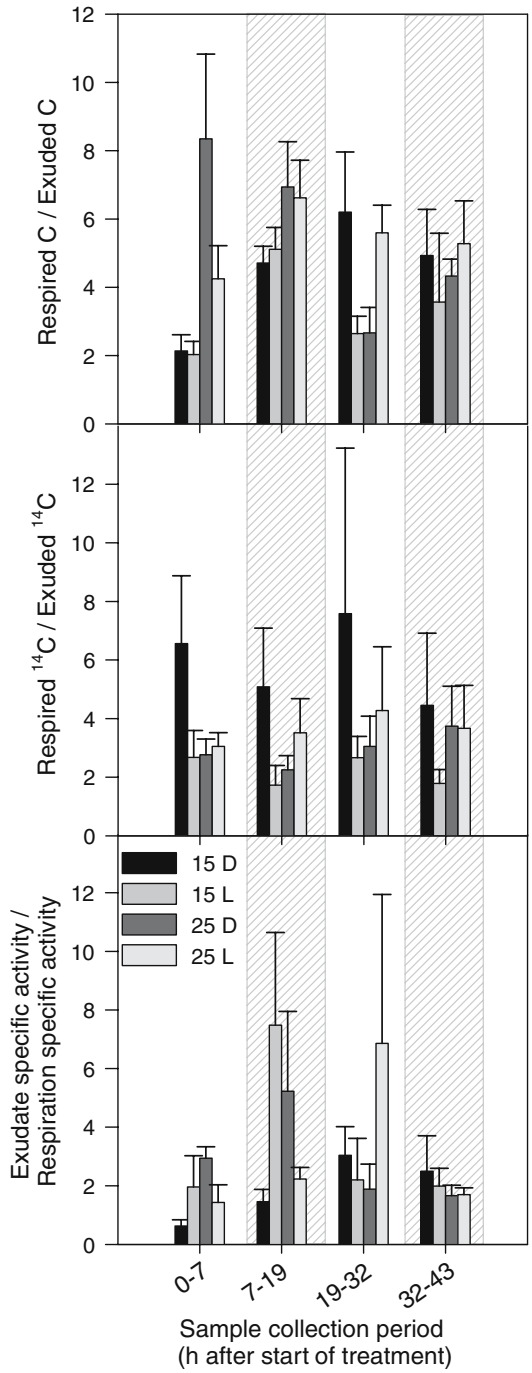

Fig. 3 Ratios of total C and ^{14}C respiration rate to exudation rate, and exudate specific ^{14}C activity to CO_2 specific ^{14}C activity for individual sample collection periods. Time zero represents the start of treatments, 3 h after pulse-labelling. Values are mean $\pm$ SEM; $n = 5$. 15 D, 15 L, 25 D and 25 L represent 15°C dark, 15°C light, 25°C dark and 25°C light, respectively. Shaded areas approximate to dark periods in illuminated treatments

broadly, ^{14}C loss was increased when total C loss was decreased, causing the specific ^{14}C activity to be particularly sensitive to the effects of treatment.

Total root respiration responded strongly to temperature when adequate C was available from photosynthesis ($Q_{10} = 1.6$). Total losses of CO_2 were only increased by light at 25°C because for the first 32 h of the experiment plants at 15°C were able to support respiration from pre-existing plant C. However, it is clear from the higher CO_2 specific activity at both temperatures that all the plants placed in the dark were more dependent on pre-existing C (^{14}C) than those in the light.

The response of total exudation to temperature differed from that of respiration. C losses in exudation did not increase in response to higher temperature, but were reduced more rapidly at 25°C than at 15°C when C fixation was prevented by placement in the dark. We suggest that this response of exudation to temperature in the dark reflected the temperature response of respiration rather than that of exudation itself. When demand for C in respiration was increased by increased temperature, less C was available for exudation. This difference in the response of exudation and respiration to temperature probably resulted from the different mechanisms of the two forms of root C loss. Because of the essential role of respiration in plant growth and maintenance, providing energy for processes such as nutrient uptake and C skeletons for plant metabolism (Wullschleger et al. 1994; Farrar 1999), it is maintained even when C availability is low. If, as is thought (Nguyen 2003; Jones et al. 2004), root exudation is a passive process driven by a diffusive gradient between root and soil solution, the lower exudation may simply have resulted from a reduction in the difference between soluble C concentrations in the root cytoplasm and the external medium. Indeed, it has been shown that root respiration can be maintained at an unchanged rate following a 47% reduction of root carbohydrate (Farrar 1999), which would necessarily reduce diffusive exudative losses.

The difference in response between the two root C loss pathways in response to the imposed treatments was also apparent in the subtle differences observed over time. Both respiration and exudation responded to the alterations to C supply and demand according to what might be expected from the demand on pre-existing plant C, but there was a temporal difference in the response. Respiration at 25°C rose to approximately twice that at 15°C within the first 7 h of the experiment regardless of the light treatment, showing that during the first 7 h existing plant C was adequate to support the increase in respiration. However, it is clear from the specific activity of respired CO_2 that light did then have an effect. Plants in the dark used more pre-existing plant C (^{14}C) than those in the light, hence specific activity was higher from plants maintained in the dark. At the same time, exudation itself did not increase or decrease in response to temperature, but increased C demand from respiration at 25°C caused total exudation to be reduced and specific activity to be increased in the absence of light at 25°C. The lack of a response of exudation to light at 15°C showed that in the first 7 h existing plant C was still adequate to maintain exudation. Root respiration was not significantly altered between the first two samplings, but there did appear to be an increase in the difference between light and dark treatments, which was visible in total respiration, respired ^{14}C and CO_2 specific activity, and showed that C supply was becoming limited in dark treatments. At the 7–19 h sampling there was a drop in total exudation in all treatments other than the 25°C dark treatment (which remained unchanged) relative to the previous sampling. We cannot explain the drop in lighted treatments, but the lower specific activity showed that again plants in the light were less dependent on ^{14}C. Moreover, exudation from plants in the light returned to close to original values by the end of the experiment, whereas that from plants in the dark remained the same at both temperatures. This suggests that exudation at 15°C in the dark was reduced during the 7–19 h sampling period due to the restriction of C supply by the maintenance of respiration, as exudation from plants at 25°C in the dark was after 7 h. The similarity of our estimates of the quantity of C lost before exudation declined in dark treatments at the two temperatures gives us further confidence that this conclusion is correct.

Respiration from roots of darkened plants did eventually show the same effect of C input removal as exudation, but the reduction in respiration rate in dark treatments did not occur until the 19–32 h and 32–43 h samplings at 25 and 15°C, respectively. Thus the response of respiration to the restriction of C supply took place at least 12 h later than the response of exudation. From the amount of C lost from roots before darkening had an effect on total C fluxes, we estimate that exudation is ca.4 times as sensitive to unreplaced root C loss as respiration. The decline in respiration rate of plants at 25°C in the light over the first three samplings was probably due to acclimation to the higher temperature (Gunn and Farrar 1999; Covey-Crump et al. 2002; Loveys et al. 2003), although respiration at 25°C always remained higher than that at 15°C.

The different temporal responses of the C loss pathways can be seen in the variation of the ratios of respiration rate-to-exudation rate and exudate specific activity-to-respired CO_2 specific activity. However, it can also be seen that the ratios of the two forms of C loss appeared to be ultimately conserved. At the 32–43 h sampling the ratios of respiration rate-to-exudation rate were similar to those determined from cumulative totals and statistically the same for total C or ^{14}C, with no difference between treatments. The ratio of exudate specific activity-to-respiration specific activity showed the convergence particularly clearly, also stabilising at the same value (ca. 2) as was determined from cumulative totals, with no difference apparent between treatments. We cannot be certain that these ratios would be conserved in the long term. However, the fact that the ratio of specific activities converged to a common ratio of 2 rather than 1 shows that the ratios of total C and ^{14}C were different. The difference in the specific activities is hard to explain unless it is assumed that respiration and exudation are dominated by fluxes from different root C pools e.g. different parts of the root, although the response of exudation to alteration of respiratory C demand suggests that the two pools are closely related. Further investigation will be necessary to fully explain these phenomena.

Assuming no significant microbial mineralisation of exuded C occurred, measurements of total C suggest that 77% of root-dependent belowground respiration in soil would be due to root respiration, and 23% to microbial respiration of exuded C. The proportion lost in respiration is higher than some previous estimates, but falls within the reported range of 23–81% (Kuzyakov and Larionova 2005), although no investigation of the two contributions has achieved a definitive separation in soil for any plant. The relatively high proportion of root C lost in respiration does suggest that some exudates may have been mineralised by microbes, incorporated into microbial biomass and/or taken back up by roots (Jones et al. 2005a, b) in this experiment. We do not suggest that our estimates represent the true values for root and microbial respiration in soil. The results do, however, show important qualitative differences between the two forms of root C loss. The twofold higher specific activity of exudates than respired CO_2 shows that the two forms of C loss did not consist of the same age of C and gives us some confidence that any mineralisation of exudates was probably minor.

Considerable within-treatment variation made it difficult to unequivocally distinguish between C fixed before labelling and C fixed after labelling. It is clear from the differences in specific activities due to darkening of plants that both exudates and respired CO_2 have some dependence on recent photosynthate. However, darkening appeared to have an effect on the specific activity of respired CO_2 (Fig. 2) more rapidly than it did on the specific activity of exudates at both temperatures. Furthermore, the ratio of exudate specific activity to CO_2 specific activity (Fig. 3) for plants in the dark at 15°C continued to increase until the 19–32 h sampling period. Thus, we suggest that the departure from unity of the ratio of exudate specific activity to CO_2 specific activity means that exudates were composed of older (fixed earlier) C than respiration. This is consistent with the large proportion of relatively old C found in exudates by Thornton et al. (2004), and the earlier maximum of ^{14}C in root respired CO_2 than in exudates found by Dilkes et al. (2004). If some of the C exuded in this experiment was mineralised by microbes, the difference between the specific activities would probably be larger in reality.

Many literature estimates of the relative contributions of root and microbial respiration to root-dependent below-ground respiration have been determined only from isotopically labelled C rather than total C (Kuzyakov and Larinova 2005). When our ^{14}C data are used to estimate the relative contributions of root and microbial respiration the values of 67% and 33% for root and microbes, respectively, are well within the range of reported estimates. Thus, the difference in the age of respired and exuded C suggests that pulse labelling alone is not sufficient for the separation of the two fluxes and may underestimate root respiration and overestimate rhizomicrobial respiration in short-term experiments.

Conclusion

Root respiration increased with increased temperature with a Q_{10} of 1.6 when sufficient C was available. In contrast, root exudation was not increased by temperature responding negatively to temperature when C supply was further limited by increased respiration. Exudation responded much faster to the removal of C inputs, being about four times more sensitive to unreplaced root C loss than respiration. Despite differences in the temporal response, the approximately fourfold faster C loss in respiration than in exudation was ultimately conserved despite alterations to the magnitude of root C loss. The origin of the C lost in exudation appeared to be older than that lost in respiration suggesting that respiration and exudation are dominated by losses from different root C pools and that caution should be exercised when comparing exudation and respiration using only measurements of isotopically labelled C. Subject to further investigation, the differences in temporal response and C age between exudation and respiration may prove useful in improving our estimates of the contributions of root and microbial respiration to root-dependent below-ground respiration.

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