Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric CO_2 and tropospheric O_3

Kurt S. Pregitzer[1], Andrew J. Burton[2], John S. King[3] and Donald R. Zak[4]

[1]Department of Natural Resources & Environmental Science, University of Nevada, Reno, NV 89512, USA; [2]Ecosystem Science Center, School of Forest Resources & Environmental Science, Michigan Technological University, Houghton, MI 49931, USA; [3]Department of Forestry and Environmental Resources, North Carolina State University, Raleigh, NC 27695, USA; [4]School of Natural Resources & Environment and Department of Ecology & Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA

Author for correspondence:
Kurt S. Pregitzer
Tel: +775 784 4000
Fax: +775 784 4583
Email: ksp@cabnr.unr.edu

Received: *27 March 2008*
Accepted: *21 May 2008*

Summary

• The Rhinelander free-air CO_2 enrichment (FACE) experiment is designed to understand ecosystem response to elevated atmospheric carbon dioxide ($+CO_2$) and elevated tropospheric ozone ($+O_3$). The objectives of this study were: to understand how soil respiration responded to the experimental treatments; to determine whether fine-root biomass was correlated to rates of soil respiration; and to measure rates of fine-root turnover in aspen (*Populus tremuloides*) forests and determine whether root turnover might be driving patterns in soil respiration.

• Soil respiration was measured, root biomass was determined, and estimates of root production, mortality and biomass turnover were made.

• Soil respiration was greatest in the $+CO_2$ and $+CO_2$ $+O_3$ treatments across all three plant communities. Soil respiration was correlated with increases in fine-root biomass. In the aspen community, annual fine-root production and mortality (g m^{-2}) were positively affected by $+O_3$.

• After 10 yr of exposure, $+CO_2$ $+O_3$-induced increases in belowground carbon allocation suggest that the positive effects of elevated CO_2 on belowground net primary productivity (NPP) may not be offset by negative effects of O_3. For the aspen community, fine-root biomass is actually stimulated by $+O_3$, and especially $+CO_2$ $+O_3$.

Key words: carbon allocation, carbon dioxide (CO_2), climate change, fine roots, global change, ozone (O_3).

New Phytologist (2008) **180**: 153–161

© The Authors (2008). Journal compilation © *New Phytologist* (2008)
doi: 10.1111/j.1469-8137.2008.02564.x

Introduction

Forest ecosystems contribute approximately half of global net primary productivity (NPP; Field *et al.*, 1998), and there is great interest in the ability of terrestrial ecosystems to accrue carbon (C), especially under predicted future conditions of elevated atmospheric carbon dioxide (CO_2) and ozone (O_3) (Karnosky *et al.*, 2005; Norby *et al.*, 2005; Grantz *et al.*, 2006). The interactive response of forest NPP to these gases (Karnosky *et al.*, 2003) will determine the terrestrial C balance (Thompson *et al.*, 2004), and changes in belowground NPP may be especially important, as they represent C inputs below the soil surface, where relatively stable soil C may eventually be formed.

Field exposure of forest ecosystems to elevated CO_2 often increases NPP for a variety of forest types (King *et al.*, 2005; Norby *et al.*, 2005) and these changes occur both above- and belowground (King *et al.*, 2005). One of the most common responses to elevated atmospheric CO_2 is increased fine-root production and biomass (Rogers *et al.*, 1994; Pregitzer *et al.*, 1995; Norby *et al.*, 2004). Concurrent with greater

belowground root production, these same experiments have shown increased soil respiration (King *et al.*, 2004).

However, exposure of forest ecosystems to elevated O_3 can reduce NPP, nullifying the positive effects of elevated CO_2 when the two gases are combined (Karnosky *et al.*, 2003, 2005). Again, these effects on NPP have been observed both above- and belowground (King *et al.*, 2005). Soil respiration also has declined with exposure to elevated O_3 in single gas exposures (Pregitzer *et al.*, 2006), but the interactive effects of both gases on soil respiration have not always followed the pattern observed for aboveground NPP. In aspen (*Populus tremuloides*) and aspen/birch (*Betula papyrifera*) forests at the Rhinelander free-air CO_2 and O_3 enrichment experiment (FACTS-II FACE) in Wisconsin (USA), soil respiration and belowground root biomass displayed similar responses to elevated CO_2 and elevated O_3 through the first 5 yr of exposure. The positive effects of elevated CO_2 were counteracted by the negative effects of elevated O_3 (King *et al.*, 2001; King *et al.*, 2005; Pregitzer *et al.*, 2006). However, in the sixth and seventh years of treatment exposure, soil respiration for the $+CO_2$ $+O_3$ treatment was greater than that for all other treatments, including $+CO_2$ alone (Pregitzer *et al.*, 2006). Interestingly, an experiment that exposed silver birch (*Betula pendula*) to factorial treatments of elevated CO_2 and O_3 in Finland also reported stimulation of soil respiration in the $+CO_2$ $+O_3$ treatment (Kasurinen *et al.*, 2004).

The first objective of this report was to quantify soil respiration during the 2005 through 2007 growing seasons to determine if the greatest flux of CO_2 from the soil to the atmosphere continued to occur in the $+CO_2$ $+O_3$ treatment. This result first became apparent during the 2003 and 2004 growing seasons (Pregitzer *et al.*, 2006). Higher rates of CO_2 efflux from the $+CO_2$ $+O_3$ treatment are counter-intuitive because they do not follow the pattern of overall growth response in this experiment (King *et al.*, 2005), nor do they correspond with results from the early years of exposure in which the greatest fine-root biomass and rates of soil respiration occurred in the $+CO_2$ treatment (King *et al.*, 2001). In dense, rapidly growing forests, in which rooting density in the soil is high, one would expect soil respiration to correspond with fine-root biomass, a pattern we clearly documented in earlier factorial elevated atmospheric CO_2 × nitrogen (N) availability experiments with *P. tremuloides* growing in open-top chambers (Pregitzer *et al.*, 2000). One of the possible explanations for greater CO_2 efflux from the $+CO_2$ $+O_3$ treatment is greater fine-root biomass in this treatment. It is possible that fine-root biomass in the $+CO_2$ $+O_3$ treatment has gradually increased and surpassed that of the other treatments. This would indicate that early responses in the experiment were transient, because fine-root biomass initially corresponded to overall whole-tree growth response and was greatest in the $+CO_2$ treatment (King *et al.*, 2001, 2005). Alternatively, it is possible that allocation of C to repair aboveground damage under elevated O_3 (Andersen, 2003) leads to reduced belowground allocation (Pell *et al.*, 1994; Grantz *et al.*, 2006) and shorter average root lifespan. A change in root turnover might enhance belowground litter production and stimulate microbial contributions to soil respiration. The second objective of this report was to quantify fine-root biomass and root turnover to determine whether either of these factors is responsible for greater soil respiration in the O_3 treatment combinations.

Materials and Methods

Research location and experimental design

The FACTS-II FACE project is located near Rhinelander, WI, USA (45°40.5′N, 89°37.5′E, 490 m elevation). The experiment is a randomized complete block design with three replicates of factorial CO_2 (ambient and elevated to 560 μl l^{-1}) and O_3 (ambient and elevated to 50 nl l^{-1}) treatments. Elevated CO_2 and O_3 treatments are maintained during daylight hours from bud-break in the spring until leaf senescence in the fall, a period that averages 145 d yr^{-1} (King *et al.*, 2004). The 12 (30-m-diameter) rings are fumigated using a free-air CO_2 enrichment (FACE) technology system that combines a gas monitoring system with a delivery system of blowers and vertical pipes placed around the plot perimeter (Dickson *et al.*, 2000). From 1998 to 2004, CO_2 concentration averaged 356 μl l^{-1} for the ambient and 534 μl l^{-1} for the elevated CO_2 treatment; O_3 concentrations averaged 36 nl l^{-1} for the ambient and 50 nl l^{-1} for the elevated O_3 treatment. The logic behind the ~×1.5 ambient concentration of $+O_3$ exposure and the performance of the exposure system are explained in detail by Karnosky *et al.* (2003, 2005).

Within each plot, three plant communities, aspen (*Populus tremuloides* Michx.), paper birch (*Betula papyrifera* Marsh.)/aspen, and sugar maple (*Acer saccharum* Marsh.)/aspen, are arranged in a split-plot design. In half of each plot, we planted five trembling aspen genotypes of differing CO_2 and O_3 responsiveness (Dickson *et al.*, 2000; Kubiske *et al.*, 2007). The other half of each plot is further divided into two quarters; one is planted with sugar maple and aspen and the other is planted with paper birch and aspen. The trees were planted at 1 × 1 m spacing in 1997. Trace gas exposure was initiated in May 1998.

Soil CO_2 efflux

During 2005, soil CO_2 efflux was measured biweekly from 19 April through 18 October using a dynamic chamber, infrared gas analyzer (IRGA) system (SRS-2 chamber and EGM-3 IRGA; PP Systems, Haverhill, MA, USA). In 2006, biweekly measurements were made from 22 May through 25 September, and in 2007, biweekly measurements were made from 14 May through 15 October. In 2006 and 2007, we used a new IRGA system (LI-8100 survey system; Li-Cor Biosciences, Lincoln, NE, USA). In all years, measurements were taken at 10 randomly located soil respiration collars within the inner

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)

10-m core area of each plant community within each plot. The respiration collars (10.2 cm diameter; schedule 40 PVC) were inserted 2.5 cm into the forest floor 2 wk before the beginning of the field season and were left in place for the remainder of the year. A portable thermometer was used to measure soil temperature (5 cm depth) concurrently with soil respiration measured at each collar. The IRGAs were calibrated daily with a certified CO_2 gas standard and all measurements were corrected for changes in atmospheric pressure. Estimates of the seasonal C flux associated with soil respiration were calculated by applying the observed flux on each measurement date to 2-wk periods extending from 1 wk before to 1 wk after the measurement. This produced an estimate of soil C efflux for the periods of 12 April–25 October 2005, 15 May–2 October 2006, and 7 May–22 October 2007.

The PP Systems soil respiration system can produce higher rates than those produced with other common commercially available systems (Jannsens *et al.*, 2000). We therefore cross-calibrated our PP Systems equipment with a Li-Cor 6400-9 system (Li-Cor Biosciences) for two dates in 2004. Rates obtained with the PP Systems instrument were 1.35 times those obtained with the Li-Cor. The relative differences among treatments were the same for the two instruments during this comparison. Relative differences among treatments measured by the PP Systems EGM in 2005 and the Li-Cor LI-8100 in 2006 and 2007 were also similar, and thus instrument choice should not affect our interpretation of treatment responses.

Root biomass

Samples for root biomass were collected in mid-July 2005 using 10 randomly located soil cores (4.8 cm diameter × 25 cm depth) in each plant community (30 cores per ring; 480 total cores). The cores were frozen immediately after field collection and taken to the laboratory for subsequent processing. After thawing, roots were sorted from the cores by hand. Live roots were distinguished by white, cream, red, tan or brown coloration and a smooth appearance. Dead roots had frayed, rough edges, were brittle, and often were dark brown or black in color. We did not stain roots to confirm assignment to the live vs dead categories. Herbaceous roots were identified as having greater average diameter than tree roots, white color, a lack of woody development in any root order, and a more limited branching system. Live tree roots were placed into one of four diameter classes: < 0.5, 0.5–1, 1–2, and > 2 mm. The roots were cleaned thoroughly using deionized water, and root mass was determined after oven drying for 48 h at 65°C. Subsamples from each root class and plant community within a plot were combusted at 500°C for 8 h to correct for mineral content not removed by washing.

The residual soil from the cores was elutriated (Hydropneumatic Root Washer; Gillison's Variety Fabrication, Benzonia, MI, USA) to capture very fine roots (< 0.5 mm) that were missed by hand sorting (Smucker *et al.*, 1982; Hendrick & Pregitzer, 1993; Burton *et al.*, 2004). The elutriated root slurry was placed in a clear plastic tray with a grid pattern on the bottom. This was then placed on a light table, and the total number of line intercepts was recorded. For a subset of these samples, all elutriated very fine roots (< 0.5 mm) were retrieved by hand from the line-intercept tray, dried and weighed. This allowed us to develop a relationship between line-intercept count and very fine root mass, which was used to convert all line-intercept counts to mass.

Production, mortality and biomass of aspen roots

To better understand fine-root demography in our experiment, we quantified biomass, production and mortality of aspen fine roots. We harvested fine roots from the aspen community in 2002 using a less elaborate protocol than the one employed in 2005. Although the two protocols differ in the diameter of the soil cores and elutriation of roots to recover small fragments, it is useful to compare the pattern of treatment response over two separate years. We also used the two sampling periods to estimate the flux (turnover) of C from the aspen root systems into the soil (g m^{-2} yr^{-1}) as explained later in this section. Estimates of fine-root (< 1 mm) biomass for 2002 were obtained from 10 randomly located soil cores (15 cm diameter × 25 cm depth) within each aspen plot (King *et al.*, 2005). Roots from these cores were collected by washing over a 1-mm mesh screen. These roots were dried to a constant mass, with subsamples combusted at 500°C for 8 h to correct for mineral content not removed by washing. These root samples were not elutriated, and thus the fine-root data from 2002 were increased by 39%, which is the average increase in fine-root capture attributable to elutriation of the 2005 root biomass cores determined in this study. Simple root washing techniques such as the one we employed in 2002 can significantly underestimate the biomass of fine roots (Ruess *et al.*, 2003). Fine-root biomass for the aspen plant community for 2003 and 2004 was needed for estimating the biomass involved in fine-root production and mortality using minirhizotron estimates of production and mortality rates (see the last paragraph in this section). These values were obtained by linearly interpolating between soil core measurements of root biomass in 2002 and 2005.

Fine-root (< 1 mm) production and mortality were measured from 15 September 2002 until 29 September 2004 using eight clear polybutyrate minirhizotron tubes (2 m length × 5.08 cm inside diameter) located in each aspen community (three replicate aspen plots per treatment; 12 plots × 8 minirhizotron tubes = 96 total minirhizotron tubes). Sampling for root longevity and turnover for the 3-yr period was limited to the aspen community because of the time-consuming nature of image processing and analysis. Minirhizotron tubes utilized in this study were installed in 1998 at a 45° angle. Rectangular, numbered image frames (0.9 × 1.3 cm) were scribed every

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

Table 1 Statistical significance of the effects of elevated CO_2, elevated O_3 and plant community on root biomass in July 2005 and growing season soil CO_2 efflux (12 April to 25 October 2005; 15 May to 2 October 2006; 7 May to 22 October 2007)

Source	< 1 mm roots	1–2 mm roots	> 2 mm roots	All roots	Seasonal CO_2 efflux 2005	Seasonal CO_2 efflux 2006	Seasonal CO_2 efflux 2007
Block	ns	ns	ns	ns	ns	0.094	ns
CO_2	**0.022**	**0.005**	0.083	**0.049**	< **0.001**	< **0.001**	**0.008**
O_3	ns	ns	ns	ns	ns	ns	ns
$CO_2 \times O_3$	ns	ns	ns	ns	ns	ns	ns
Community	**0.014**	ns	ns	ns	**0.031**	**0.002**	ns
$CO_2 \times$ community	ns	ns	**0.027**	0.071	**0.025**	ns	ns
$O_3 \times$ community	**0.029**	ns	ns	ns	ns	0.083	ns
$CO_2 \times O_3 \times$ community	ns	ns	**0.018**	0.074	ns	ns	ns

ns, not significant.

0.9 cm along a transect on the exterior surface of each minirhizotron tube before installation to enable videotaping of the same locations within the soil on all sampling dates (Hendrick & Pregitzer, 1992; Burton *et al.*, 2000).

Root video images were collected using a model BTC 1.125 Minirhizotron Research Color Camera (Bartz Technology Co., Santa Barbara, CA, USA) at *c.* 2-wk intervals from mid-May through late September in each year. Slightly longer intervals (3–4 wk) separated the early May 2003 and mid-October 2002 samplings from the more recent sampling date. During quantification of root demography, images from all dates for a given frame were displayed together to allow individual roots to be followed throughout their lifespan. This ensured that dead or missing roots did not 'reappear' as a new root at a later date with better image quality. Each root was given a unique identification number on the date it first appeared; on all subsequent image dates the root was reclassified as living or dead (based upon color and consistency in the image), and root length was recorded.

The effects of trace gas treatments on fine-root lifespan were assessed by studying the demography of fine roots (≤ 1.0 mm) from the 0–10 cm depth. Four randomly selected tubes per plot were analyzed, producing life-history data for over 1000 individual roots in each treatment (range 1048–1606). Fine-root production for each sampling interval was determined by summing the lengths (mm) of all new roots and adding the length growth of all previously existing roots. Fine-root mortality for each sampling interval was determined by summing the lengths of all roots that had died during that interval and adding root length lost by existing roots as a result of herbivory or dieback. Production and mortality for each plot were both expressed as root length per minirhizotron tube area observed (mm cm^{-2}). Rates of annual fine-root production and mortality (mm mm^{-1}) were estimated by dividing the root length production and root length mortality observed for a plot by the total live root length (mm cm^{-2}) observed in minirhizotrons on the plot in July, the time of year when root biomass samples were collected. To estimate the biomass involved in annual fine-root production and mortality, the production and mortality rates were multiplied by the July fine-root biomass estimated for 2003 and 2004 (already described). Minirhizotron data collected from 15 September 2002 to 15 September 2003 were used to estimate production and mortality for 2003. Data collected from 15 September 2003 to 14 September 2004 were used to estimate values for 2004. Data from the previous autumn were critical, as they allowed us to identify new roots in the first May images of the following year.

Statistical analyses

The effects of the trace gas treatments and plant community on seasonal soil CO_2 efflux for 2005, 2006 and 2007 and root biomass for 2005 were assessed using ANOVA for a split-plot randomized complete block design appropriate for the FACTS-II FACE experiment (King *et al.*, 2001). The individual years for seasonal soil CO_2 efflux were considered separately because of the change in instrumentation between 2005 and 2006 and different periods of measurement among years. The effects of trace gas treatments on fine-root production rate, mortality rate, production, biomass and mortality biomass in 2003 and 2004 for the aspen community were assessed using a repeated measures ANOVA for a randomized complete block design.

Results

Seasonal soil respiration for 2005, 2006 and 2007 was significantly greater under $+CO_2$, but was not significantly affected by $+O_3$ (Fig. 1, Table 1). The $+CO_2$ $+O_3$ treatment tended to have the greatest values for seasonal soil respiration across all community types (Fig. 1; 5–10% greater than $+CO_2$), but values for the $+CO_2$ $+O_3$ treatment were not significantly greater than those for $+CO_2$ alone (Table 1). Across treatments, seasonal soil respiration was significantly greater in the aspen community than for the birch/aspen and maple/aspen communities during 2005 and 2006, but not in 2007 (Table 1). Greater seasonal soil respiration in 2005 than in 2006 is attributable both to a longer period of measurement in 2005 and to the use of the PP Systems IRGA, which produced respiration rates at a given

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)

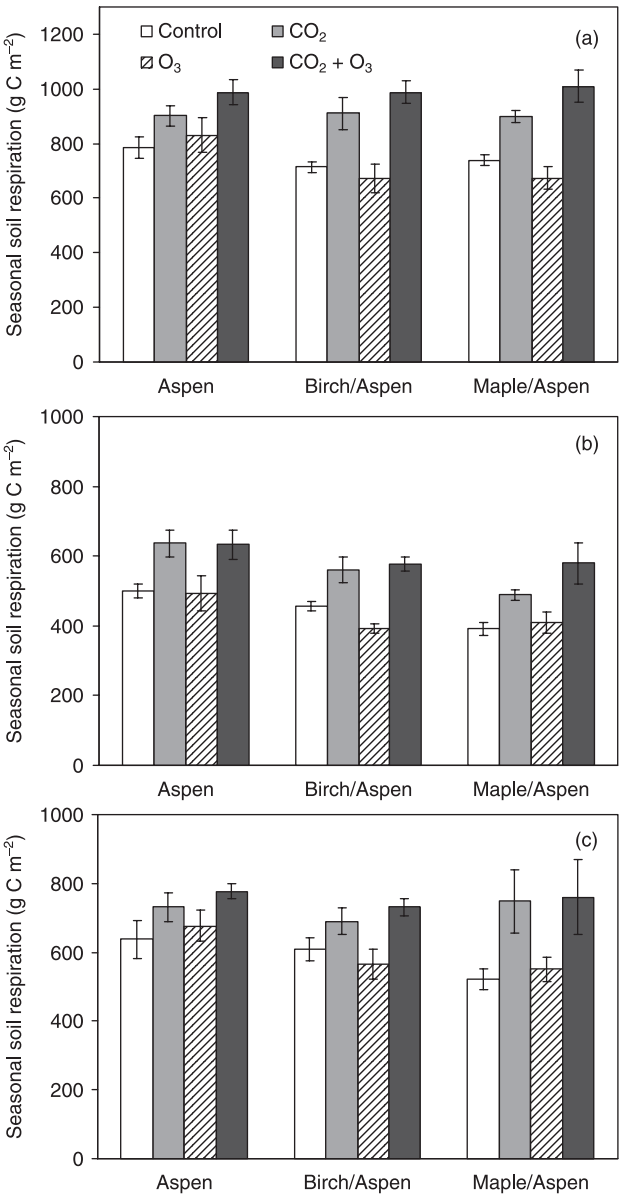

Fig. 1 Growing season seasonal soil respiration in (a) 2005, (b) 2006 and (c) 2007. Error bars are 1 SE of the mean (n = 3). Values from the different infrared gas analyzers (2005 vs 2006–2007) have not been adjusted (see the Materials and Methods and Discussion).

temperature in 2005 that were on average 25% higher than rates measured in 2006 by the Li-Cor IRGA system for the same temperatures (data not shown). Relative differences among treatments were similar for the two IRGA systems, with elevated CO_2 treatments (+CO_2 and +CO_2 +O_3) averaging 29% more seasonal CO_2 efflux in 2005 than the control and +O_3 treatments, 31% more in 2006, and 25% more in 2007.

In 2005, +CO_2 increased total root biomass across all the size classes measured, root biomass for roots < 1.0 mm in diameter, and root biomass for roots 1.0–2.0 mm in diameter (Table 1, Fig. 2). Elevated O_3 increased fine-root (< 1 mm) biomass in the aspen community only, and fine-root biomass (< 1.0 mm) in the aspen community was significantly greater than in the birch/aspen and maple/aspen communities (Table 1). Of the four treatments, the +CO_2 +O_3 treatment produced the highest coarse root (> 2 mm) biomass for the aspen and birch/aspen communities, whereas the +CO_2 treatment produced the greatest coarse root biomass for the maple/aspen community (Fig. 2 and $CO_2 \times O_3 \times$ community interaction in Table 1).

When fine-root biomass (< 1.0 mm) in the aspen community was compared across years (2002 and 2005), it was found that both +CO_2 and +O_3 significantly increased biomass and their effects were additive (Table 2, Fig. 3). Aspen fine-root (< 1.0 mm) production rates were not affected by +CO_2 or +O_3 (Tables 2, 3). Fine-root (< 1.0 mm) mortality rates (Table 3) were not affected by +CO_2; however, they were enhanced by +O_3 in 2003, but not in 2004 (Table 2). Overall, fine-root (< 1.0 mm) production and mortality rates (mm mm^{-1}; Table 3) showed no clear response to treatments, and thus fine-root (< 1.0 mm) survival was fairly consistent across treatments and years (Fig. 4). As a result, differences among treatments in annual fine-root production and mortality expressed on a mass basis (g m^{-2}; Table 3) were controlled primarily by treatment differences in standing fine-root biomass in the aspen community (Fig. 3). Biomass production was positively affected by both +CO_2 and +O_3 (Table 3; note marginal significance in Table 2) and was greatest in the +CO_2 +O_3 treatment (Table 3). Rates of biomass mortality were positively influenced by +O_3, but varied from year to year (Table 2). These results were driven by larger aspen standing fine-root (< 1.0 mm) biomass in the +O_3 and +CO_2 +O_3 treatments (Fig. 3).

Seasonal soil respiration in 2005 (Fig. 1) was correlated to < 2-mm root biomass ($r = 0.87$; $P < 0.001$) and < 1-mm root biomass ($r = 0.72$; $P = 0.008$) for that year. The tendency for the +CO_2 +O_3 treatment to have the greatest values for biomass of fine roots < 1.0 mm in diameter (Fig. 3) also occurred for seasonal soil C efflux (Fig. 1).

Discussion

Root biomass

Root biomass across all the size classes sampled was enhanced by elevated CO_2 (Table 1, Fig. 2), as has been observed throughout this experiment (King *et al.*, 2001, 2005). This CO_2 enhancement of belowground NPP has consistently occurred in conjunction with increased aboveground NPP, resulting in no changes in proportional C allocation to wood, foliage or roots as a result of elevated CO_2 (King *et al.*, 2005).

The response of root biomass to elevated O_3, however, has been changing over time and is no longer consistent with observations made during the early years of the experiment. All root biomass sampling previous to 2002 showed that O_3 exposure, alone or in combination with elevated CO_2, consistently

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

Source	Biomass	Production rate	Production biomass	Mortality rate	Mortality biomass
Block	ns	ns	ns	ns	ns
CO_2	**0.025**	ns	0.080	ns	ns
O_3	**0.046**	ns	0.060	ns	**0.049**
$CO_2 \times O_3$	ns	ns	ns	ns	ns
Year	**< 0.001**	ns	ns	ns	**0.034**
$CO_2 \times$ year	na	ns	ns	ns	ns
$O_3 \times$ year	**0.032**	ns	ns	**0.031**	**0.050**
$CO_2 \times O_3 \times$ year	**0.035**	ns	ns	ns	ns

ns, not significant.

Table 2 Statistical significance of the effects of elevated CO_2, elevated O_3 and year on fine-root (< 1 mm) biomass in 2002 and 2005 and fine-root production rate, mortality rate, production biomass and mortality biomass in 2003 and 2004 for the pure aspen (*Populus tremuloides*) community

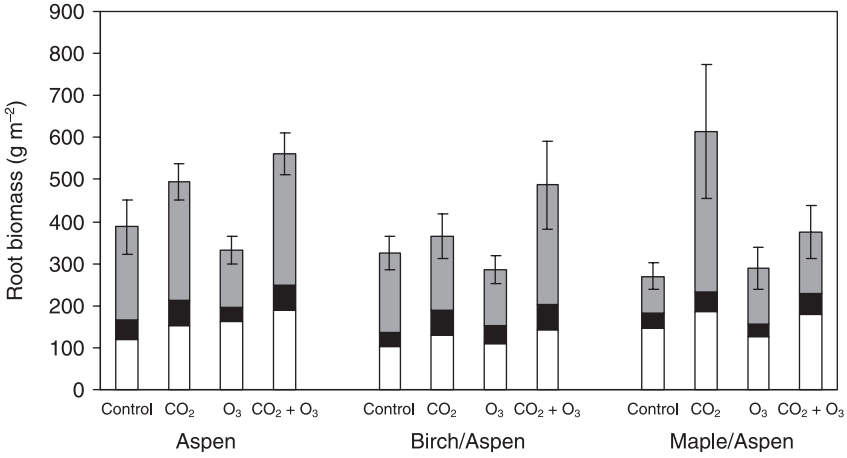

Fig. 2 Root biomass by size class (< 1 mm, white columns; 1–2 mm, black columns; > 2 mm, gray columns) and plant community (aspen, birch/aspen and maple/aspen) in 2005. Error bars are 1 SE of the mean for total root biomass (n = 3).

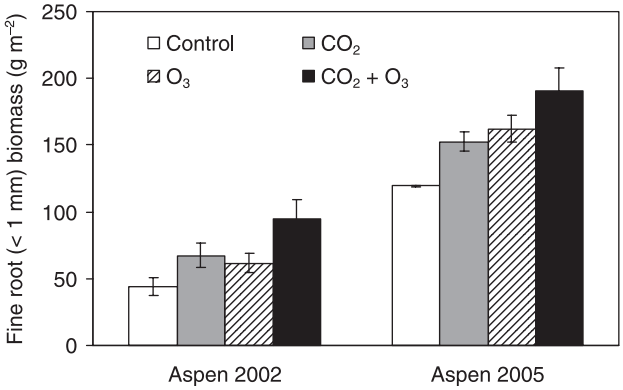

Fig. 3 Fine-root (< 1 mm) biomass for the aspen plant community for 2002 and 2005. Error bars are 1 SE of the mean (n = 3).

resulted in lower coarse root biomass for all plant communities and lower fine-root biomass for the birch/aspen and birch/maple communities (King *et al.*, 2001, 2005). However, in 2002 and 2005 $+O_3$ significantly increased fine-root (< 1.0 mm) biomass in the aspen community, and, in combination with $+CO_2$, increased coarse root biomass in both the aspen and birch/aspen communities. This response of root system biomass to elevated O_3 was not proportional to aboveground responses, where elevated O_3 resulted in significantly reduced NPP from the start of the experiment to 2004, and the NPP in the $+CO_2$ $+O_3$ treatment was similar to that for the control, and clearly lower than that for the $+CO_2$ treatment (Kubiske *et al.*, 2006). Through 2003, King *et al.* (2005) found no effect of $+O_3$ on biomass allocated to major above- and belowground plant parts in the FACTS-II experiment, with the possible exception of increased allocation to fine roots in the aspen community. The most recent data (Fig. 3) confirm the increase in aspen fine-root biomass under $+O_3$ and suggest that C allocation to fine roots (< 1 mm) in the two treatments involving $+O_3$ has been shifting over time.

Fine-root biomass can vary from year to year, but the fact that root biomass is highly correlated to soil respiration through time (see following discussion) suggests that the root biomass data are robust. Although vital staining was not used to confirm assignment of roots to the live vs dead categories, we doubt that this confounds our results because the criteria used to separate live vs dead roots were consistently applied across all treatments. Herbaceous root biomass is not reported, but it accounted for less than 2.7% of total fine-root (< 2.0 mm) biomass regardless of treatment (data not shown). These are some of the caveats that temper our results.

Our findings regarding the long-term response of root biomass to elevated O_3 are quite unusual, as glasshouse and

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)

Table 3 Fine-root (< 1 mm) productivity and mortality rates and biomass for the aspen (*Populus tremuloides*) plant community for 2003 and 2004

	Year	Control	CO_2	O_3	$CO_2 + O_3$
Production rate (mm mm^{-1})	2003	2.33 (0.25)	1.87 (0.05)	2.19 (0.36)	2.39 (0.13)
	2004	1.92 (0.19)	2.30 (0.45)	1.99 (0.18)	2.21 (0.55)
Mortality rate (mm mm^{-1})	2003	1.63 (0.20)	1.35 (0.11)	2.30 (0.29)	2.47 (0.35)
	2004	2.15 (0.21)	2.21 (0.17)	1.72 (0.13)	2.16 (0.27)
Production biomass (g m^{-2})	2003	161 (19)	179 (13)	206 (30)	300 (12)
	2004	181 (17)	281 (47)	259 (29)	351 (93)
Mortality biomass (g m^{-2})	2003	114 (21)	128 (7)	222 (41)	306 (34)
	2004	202 (16)	273 (19)	223 (29)	345 (60)

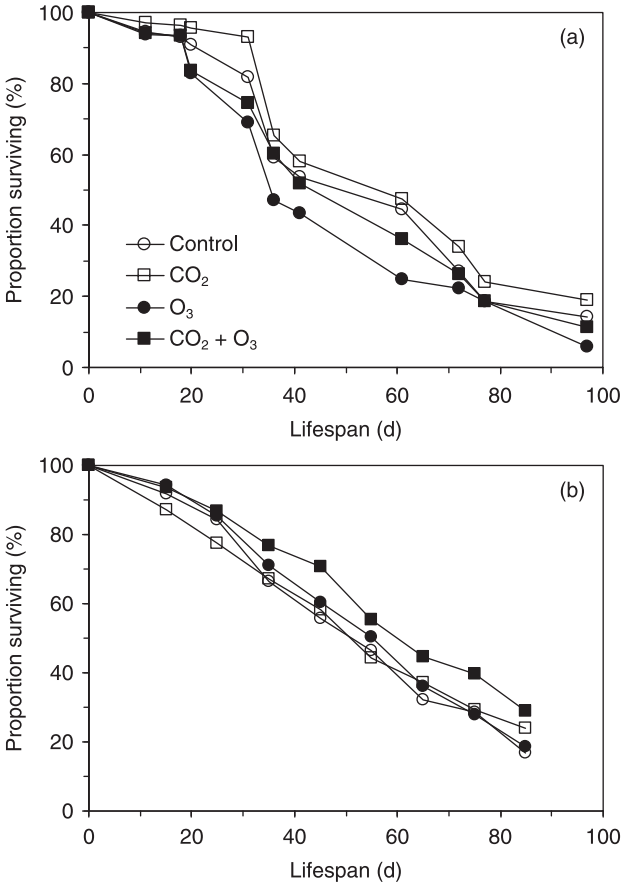

Fig. 4 Survival probabilities of fine roots initiated in (a) 2003 and (b) 2004 in the aspen plant community.

open-top chamber studies have typically shown that O_3 stress reduces C allocation to roots (Manning *et al.*, 1971; Gorissen & van Veen, 1988; Pell *et al.*, 1994; Rennenberg *et al.*, 1996; Andersen, 2003). Direct effects of elevated O_3 on leaves can include decreased activity and concentration of Rubisco, reduced photosynthesis, increased metabolic costs to synthesize antioxidant compounds and repair damaged leaves, and possibly decreased phloem loading (Andersen, 2003). Ozone can also decrease stomatal conductance and leaf lifespan. The net result of reduced assimilation and increased demand for nonstructural carbohydrates to repair damaged leaves is decreased availability of nonstructural carbohydrates for export to roots, which become a weaker sink in plants exposed to elevated O_3 (Andersen, 2003). Lower levels of root nonstructural carbohydrates and lower rates of root respiration (Coleman *et al.*, 1996; Grulke *et al.*, 2001) in short-term studies of plants exposed to elevated O_3 are in agreement with decreased C availability for roots. A meta-analysis by Grantz *et al.* (2006) similarly found that elevated O_3 elicited mostly negative responses in the root/shoot allometric coefficient, but they did report the existence of occasional positive responses in a variety of plant forms, including trees. Our longer term results from the FACTS-II experiment clearly indicate that the amount of C being allocated to aspen fine-root (< 1.0 mm) biomass under elevated O_3 is increasing over time relative to the control, especially in the $+CO_2$ $+O_3$ treatment, in contrast with most shorter term results, including our own from FACTS-II (King *et al.*, 2001).

There are several possible reasons for this increase in C allocation to fine roots after long-term $+O_3$ exposure, especially in the $+CO_2$ $+O_3$ treatment in the pure aspen community. For both the $+O_3$ and $+CO_2$ $+O_3$ treatments, competitive interactions among genotypes and species, that is, community dynamics, may dominate the cumulative ecosystem response. Because some genotypes of aspen and the different species (aspen, paper birch, and sugar maple) may be responding differently to $+O_3$ exposure, the mortality of individual trees may influence ecosystem responses. For example, demise of O_3-sensitive aspen genotypes and better overall survival of birch and maple, which are less sensitive to O_3 than aspen, may drive a 'stand dynamic' that results in dominance by genotypes and species more tolerant of prolonged exposure to elevated O_3. In the field, single-tree mortality is apparent in all three of the community types, and the tolerant aspen genotype that now dominates under $+O_3$ exposure actually grows faster in the $+O_3$ treatment than in the control treatment (Kubiske *et al.*, 2007). Exposure to O_3 has also increased the rate at which birch is becoming dominant in the birch/aspen community (Kubiske *et al.*, 2007, Zak *et al.*, 2007).

Compensatory growth of the O_3-tolerant survivors as they occupy space created by death of O_3-sensitive individuals may have resulted in greater fine-root biomass in the aspen community. This effect would probably be most pronounced for

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

the $+CO_2$ $+O_3$ treatment, in which the stimulatory effect of $+CO_2$ on net photosynthesis (Ainsworth & Long, 2005) could enable rapid growth of the O_3-tolerant survivors. In this case, the interaction treatment would be expected to begin behaving more similarly to the elevated CO_2 treatment (as it has). The stimulation of root biomass by this mechanism would occur at the same time as the soil is receiving C inputs from mortality of O_3-sensitive individuals, leading to increases in soil respiration for treatments involving $+O_3$ exposure. Our measurements of soil respiration in the aspen community are reasonably consistent with this mechanism, with the treatments receiving $+O_3$ now having rates similar to or higher than those of the corresponding treatments not receiving $+O_3$ (compare $+O_3$ to control and $+CO_2$ $+O_3$ to $+CO_2$ in Fig. 1). In earlier years, soil respiration for the treatments receiving $+O_3$ was lower than that for the corresponding treatments without O_3 (King *et al.*, 2001; Pregitzer *et al.*, 2006).

Fine-root production and mortality

We had initially hypothesized that decreases in average root lifespan and corresponding increases in root biomass turnover might be responsible for the observed increases in soil respiration in the $+CO_2$ $+O_3$ treatment. Minirhizotron observations of root lifespan, production rates and mortality rates for the aspen community provided little to no support for this hypothesis (Fig. 4, Table 3). Instead, these parameters did not differ among treatments, with the exception of higher fine-root mortality rates under elevated O_3 for one of the two years of observation (Table 2). Annual fine-root biomass production ($g\ m^{-2}$) and biomass mortality did increase significantly in response to $+O_3$ exposure and marginally in response to $+CO_2$ exposure (Tables 2, 3), but these changes were primarily the result of treatment response for root biomass, not changes in root lifespan or biomass turnover.

Soil CO_2 efflux and fine root biomass

The $+CO_2$ and $+CO_2$ $+O_3$ treatments had the greatest seasonal soil respiration across all plant communities from 2005 to 2007 (Fig. 1). These findings are identical to observations in 2003 and 2004 (Pregitzer *et al.*, 2006), and contrast with results from the first 5 yr of the experiment, in which soil respiration in the $+CO_2$ $+O_3$ treatment was similar to that for the control treatment and significantly lower than means in the $+CO_2$ treatment (King *et al.*, 2001; King *et al.*, 2004; Pregitzer *et al.*, 2006). We now know that this change has been accompanied by an increase in fine-root (< 1.0 mm) biomass in the $+CO_2$ $+O_3$ treatment, which now has the highest fine-root biomass in the aspen and birch/aspen communities (Fig. 3). Greater root biomass, in the absence of changes in root lifespan and biomass turnover (Tables 2, 3), has the potential to increase both the autotrophic root respiration component of soil respiration and root detrital inputs that contribute to the heterotrophic microbial portion of soil respiration. As a result, the biomasses of roots < 2 mm and < 1 mm were both highly correlated with growing season soil CO_2 efflux across plant communities and treatments. Similarly, Pregitzer *et al.* (2000) reported that mean soil respiration for aspen growing in open-top chambers was linearly related to fine-root (< 1 mm) biomass ($r^2 = 0.87$) and total root biomass ($r^2 = 0.96$) across four factorial combinations of soil N availability and atmospheric CO_2. The mean ratio of seasonal soil respiration in the $+CO_2$ $+O_3$ treatment to that in the elevated $+CO_2$ treatment (1.101) in 2005 is essentially the same as that found for relative differences in fine-root (< 1 mm) biomass (1.097).

Our evidence for increased belowground C allocation during the sixth through tenth years of $+O_3$ exposure at FACTS-II differs from most results typically obtained in shorter term studies, and, we believe, is an important advance in our understanding of how ecosystems respond over longer periods of exposure to elevated atmospheric CO_2 and O_3. The mechanism for this transient ecosystem response is not fully understood, but the loss of O_3-sensitive individuals followed by compensatory growth of O_3-tolerant individuals is one possibility. Whatever the mechanism, it appears that, in the longer term, increases in C allocation to roots are a potential response of forest ecosystems to elevated O_3, suggesting that the positive effects of elevated CO_2 on NPP may not necessarily be offset by negative effects of O_3, at least for belowground components of NPP. These possibilities, based on measurements from the longest running (10 yr) $+O_3$ field experiment in forests, should be considered by those assessing and modeling the potential effects of elevated tropospheric O_3 on C allocation and storage in forest ecosystems.

Acknowledgements

The authors thank Jennifer Eikenberry, Angela Piket and numerous undergraduate students who contributed to the field measurements and sample collection, Lynn Jarvi who provided invaluable help with processing the minirhizotron images, and David Karnosky, Jaak Sober and Wendy Jones for their assistance in operating the FACTS-II Aspen FACE facility. This research was supported by the US Department of Energy – Office of Biological and Environmental Research, through its Program for Ecosystem Research, the USDA Forest Service Northern Global Change Program and North Central Research Station, the National Science Foundation (DBI/MRI), USDA NRI Competitive Grants Program, and the University of Nevada Agricultural Experiment Station.

References

Ainsworth EA, Long SP. 2005. What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* **163**: 351–372.

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)

Andersen CP. 2003. Source-sink balance and carbon allocation below ground in plants exposed to ozone. *New Phytologist* **157**: 213–228.

Burton AJ, Pregitzer KS, Crawford JN, Zogg GP, Zak DR. 2004. Chronic NO_3^- additions reduce soil respiration in northern hardwood forests. *Global Change Biology* **10**: 1080–1091.

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

Coleman MD, Dickson RE, Isebrands JG, Karnosky DF. 1996. Root growth and physiology of potted and field-grown trembling aspen exposed to ozone. *Tree Physiology* **16**: 145–152.

Dickson RE, Lewin KF, Isebrands JG, Coleman MD, Heilman WE, Riemenschneider DE, Sober J, Host GE, Zak DR, Hendrey GR *et al.* 2000. *Forest Atmosphere Carbon Transfer and Storage (FACTS-II), the aspen free-air CO_2 and O_3 enrichment (FACE) project: an overview*. General Technical Report NC-214. St Paul, MN, USA: USDA Forest Service.

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. *Science* **281**: 237–240.

Gorissen A, van Veen JA. 1988. Temporary disturbance of translocation of assimilates in Douglas-firs caused by low levels of ozone and sulfur dioxide. *Plant Physiology* **88**: 559–563.

Grantz DA, Gunn S, Vu H-B. 2006. O_3 impacts on plant development: a meta-analysis of root/shoot allocation and growth. *Plant, Cell & Environment* **29**: 1193–1209.

Grulke NW, Andersen CP, Hogsett WE. 2001. Seasonal changes in above- and belowground carbohydrate concentration of ponderosa pine along a pollution gradient. *Tree Physiology* **21**: 173–181.

Hendrick RL, Pregitzer KS. 1992. The demography of fine roots in a northern hardwood forest. *Ecology* **73**: 1094–1104.

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

Jannsens IA, Kowalski AS, Longdoz B, Ceulemans R. 2000. Assessing forest soil CO_2 efflux: an in situ comparison of four techniques. *Tree Physiology* **20**: 23–32.

Karnosky DF, Pregitzer KS, Zak DR, Kubiske ME, Hendrey GR, Weinstein D, Nosal M, Percy KE. 2005. Scaling ozone responses of forest trees to the ecosystem level in a changing climate. *Plant, Cell & Environment* **28**: 965–981.

Karnosky DF, Zak DR, Pregitzer KS, Awmack CS, Bockheim JG, Dickson RE, Hendrey GR, Host GE, King JS, Kopper BJ *et al.* 2003. Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2: a synthesis of molecular to ecosystem results from the Aspen FACE project. *Functional Ecology* **17**: 287–307.

Kasurinen, A, Kokko-Gonzales P, Riikonen J, Vapaavuori E, Holopainen T. 2004. Soil CO_2 efflux of two silver birch clones exposed to elevated CO_2 and O_3 levels during three growing seasons. *Global Change Biology* **10**: 1654–1665.

King JS, Hanson PJ, Bernhardt E, DeAngelis P, Norby RJ, Pregitzer KS. 2004. A multi-year synthesis of soil respiration responses to elevated atmospheric CO_2 from four FACE experiments. *Global Change Biology* **10**: 1027–1042.

King JS, Kubiske ME, Pregitzer KS, Hendrey GR, McDonald EP, Giardina CP, Quinn VS, Karnosky DF. 2005. Tropospheric O_3 compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO_2. *New Phytologist* **168**: 623–636.

King JS, Pregitzer KS, Zak DR, Sober J, Isebrands JG, Dickson RE, Hendrey GR, Karnosky DF. 2001. Fine root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO_2 and tropospheric O_3. *Oecologia* **128**: 237–250.

Kubiske ME Quinn VS, Marquardt PE, Karnosky DF. 2007. Effects of elevated atmospheric CO_2 and/or O_3 on intra- and interspecific competitive ability of aspen. *Plant Biology* **9**: 342–355.

Kubiske ME, Quinn VS, Heilman WE, McDonald EP, Marquardt PE, Teclaw RM, Friend AL, Karnosky DF. 2006. Interannual climatic variation mediates elevated CO_2 and O_3 effects on forest growth. *Global Change Biology* **12**: 1054–1068.

Manning WJ, Feder WA, Papia PM, Perkins I. 1971. Influence of foliar ozone injury on root development and root surface fungi of pinto bean plants. *Environmental Pollution* **1**: 305–312.

Norby RJ, DeLucia EH, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJP, Ceulemans R *et al.* 2005. Forest response to elevated CO_2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences, USA* **102**: 18052–18056.

Norby RJ, Ledford J, Reilly CD, Miller NE, O'Neill EG. 2004. Fine-root production dominates response of a deciduous forest to atmospheric CO_2 enrichment. *Proceedings of the National Academy of Sciences, USA* **101**: 9689–9693.

Pell EJ, Temple PJ, Friend AL, Mooney HA, Winner WE. 1994. Compensation as a plant response to ozone and associated stresses: an analysis of ROPIS experiments. *Journal of Environmental Quality* **23**: 429–436.

Pregitzer KS, Zak DR, Curtis PS, Kubiske ME, Teeri JA, Vogel CS. 1995. Atmospheric CO_2, soil nitrogen and fine root turnover. *New Phytologist* **129**: 579–585.

Pregitzer KS, Loya W, Kubiske M, Zak D. 2006. Soil respiration in northern forests exposed to elevated atmospheric carbon dioxide and ozone. *Oecologia* **148**: 503–516.

Pregitzer KS, Zak DR, Maziasz J, DeForest J, Curtis PS, Lussenhop J. 2000. Interactive effects of atmospheric CO_2 and soil-N availability on fine roots of *Populus tremuloides*. *Ecological Applications* **10**: 18–33.

Rennenberg H, Herschbach C, Poole A. 1996. Consequences of air pollution on shoot-root interactions. *Journal of Plant Physiology* **148**: 296–301.

Rogers HH, Runion GB, Krupa SV. 1994. Plant responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* **83**: 155–189.

Ruess RW, Hendrick RL, Burton AJ, Pregitzer KS, Sveinbjornssön B, Allen MG, Maurer GE. 2003. Coupling fine root dynamics with ecosystem carbon cycling in black spruce forests of interior Alaska. *Ecological Monographs* **73**: 643–662.

Smucker AJM, McBurney SL, Srivanstava AK. 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. *Agronomy Journal* **74**: 500–503.

Thompson SL, Govindasamy B, Mirin A, Caldeira K, Delire C, Milovich J, Wickett M, Erickson D. 2004. Quantifying the effects of CO_2-fertilized vegetation on future global climate and carbon dynamics. *Geophysical Research Letters* **31**: L23211.

Zak DR, Holmes WE, Pregitzer KS, King JS, Ellsworth DS, Kubiske ME. 2007. Belowground competition and the response of developing forest communities to atmospheric CO_2 and O_3. *Global Change Biology* **13**: 2230–2238.

© The Authors (2008). Journal compilation © *New Phytologist* (2008)