

Effects of elevated CO₂ and N on tree–grass interactions: an experimental test using *Fraxinus excelsior* and *Dactylis glomerata*

J. M. G. Bloor*, L. Barthes and P. W. Leadley

Université Paris-Sud, Laboratoire d'Ecologie, Systématique et Evolution, UMR CNRS 8079, F-91405 Orsay Cedex, France

Summary

1. The invasion of grasslands by woody species is often associated with changes in environmental conditions, but few studies have addressed the impact of climate change on the competitive interactions between tree seedlings and herbaceous vegetation. We examined patterns of growth and morphology in *Fraxinus excelsior* seedlings germinating in the presence or absence of grass competition (*Dactylis glomerata*) at either low (380 p.p.m.) or high (645 p.p.m.) atmospheric carbon dioxide (CO₂), and at two levels of nitrogen (N) nutrition.
2. Elevated CO₂ had a positive effect on *Fraxinus* dry mass irrespective of N treatment, but the magnitude of growth response was small. In contrast, *Dactylis* dry mass showed a significant CO₂ × N interaction: no apparent response to elevated CO₂ in the low N treatment compared with a 25% dry mass increase in the high N, high CO₂ treatment.
3. *Dactylis* and *Fraxinus* showed greater responses to N compared with CO₂ in terms of dry mass, morphology and biomass allocation. The direction of *Fraxinus* responses to N varied depending on the trait examined and the grass competition treatment.
4. Both the competitive intensity (C_{int}) and the importance of the competition (C_{imp}) experienced by *Fraxinus* seedlings increased with an increase in N availability. Contrary to expectations, elevated CO₂ had no significant effect on either C_{int} or C_{imp}.
5. Plant plasticity may have important implications for the long-term success of tree seedlings in grasslands. Our results suggest that the combination of both grass and tree seedling responses to CO₂ may have indirect benefits for the persistence of woody invaders in high-nutrient grasslands under future atmospheric conditions.

Key-words: climate change, growth, morphology, plant competition, seedlings

Introduction

Over the last century, the invasion of grasslands by woody species has become increasingly widespread (Polley, Mayeux & Tischler 1996; Julien, Peltier & Balent 2006). Although woody seedlings are generally considered to be a weaker competitor for below-ground resources compared with grass vegetation (Wilson 1998), changes in habitat management and environmental conditions may modify the competitive balance between interacting grass and woody plants (Davis *et al.* 1999; Zavaleta 2006). Theoretical work has shown that changes in grassland community composition are likely to

accelerate due to land use change and future climate conditions (Sala *et al.* 2000). However, relatively few studies have explored the role of climate change on the interactions between grasses and tree seedlings.

Short-term atmospheric carbon dioxide (CO₂) enrichment has been shown to enhance photosynthetic rates, reduce stomatal conductance (and thus transpiration rates) and to stimulate plant growth in both grass and woody species (Bazzaz 1990; Poorter & Navas 2003; Ainsworth & Long 2005). A synthesis of results from free-air CO₂ enrichment studies with monocultures suggests that trees respond more to high CO₂ than do C3 grasses (Ainsworth & Long 2005). However, the effects of CO₂ on interspecific competition are complex and difficult to derive from the responses of individual species (Bazzaz & McConnaughay 1992; Navas *et al.* 1999; Poorter & Navas 2003). A few experimental and modelling studies

*Correspondence author. INRA, UR874-Agronomie, F63100 Clermont Ferrand, France. E-mail: juliette.bloor@clermont.inra.fr

with mixed tree–grass communities have suggested that high levels of CO₂ may facilitate grassland invasion by woody species (Polley *et al.* 1996; Bond, Midgley & Woodward 2003; Zavaleta 2006). In theory, elevated CO₂ environments may reduce competition between tree seedlings and herbaceous vegetation by lowering the soil water demand of the competing individuals (Davis, Wragge & Reich 1998, but see Wullschlegel, Tschaplinski & Norby 2002). Interspecific variation in response to elevated CO₂ may also alter the performance ranking among competing individuals, since the magnitude, duration and direction of plant responses to CO₂ are known to be highly variable among species (Poorter & Perez-Soba 2001; Poorter & Navas 2003). However, growth responses to elevated CO₂ may be constrained if other plant resources such as nitrogen (N) are in limiting supply (Arp *et al.* 1998; Bernacchi *et al.* 2000). Consequently plant CO₂ responses may depend on resource availability and soil type as well as species identity (Korner 2003; Poorter & Navas 2003).

Understanding the interactions between nutrient supply, plant competition and atmospheric CO₂ may provide valuable insights into grassland invasions under future climate conditions. Increased soil N availability can enhance the dominance of competitive plant species with a higher growth rate and taller stature (Aerts 1999). Hierarchies of competitive dominance can be further accentuated by species-specific variation in phenology and patterns of nutrient acquisition early in the growing season (Brooker 2006). Nonetheless, patterns of competition along productivity gradients remain difficult to predict (Goldberg *et al.* 1999; Gaucherand, Liancourt & Lavorel 2006). Different authors have argued that: (i) competition is more intense at high productivity where plant biomass is greater (Grime 1973); (ii) the intensity of competition (C_{int}) remains constant along productivity gradients due to shifts from root to shoot competition (Wilson & Tilman 1991); or that (iii) C_{int} depends on net resource supply and biotic effects on resource levels rather than on productivity *per se* (Goldberg & Novoplansky 1997; Davis *et al.* 1998). Furthermore, the importance of competition (C_{imp}) relative to other biotic or abiotic factors (*sensu* Welden & Slauson 1986) may also vary along productivity gradients (Brooker *et al.* 2005).

To our knowledge, no studies have characterized the competitive interactions between germinating tree seedlings and herbaceous vegetation grown under interactive soil N–atmospheric CO₂ treatments. We used tree–grass mesocosms in a greenhouse experiment to examine the effects of grass competition, nutrient availability and CO₂ on the seedling growth and morphology of an early successional temperate tree, *Fraxinus excelsior*. We also quantified C_{int} and C_{imp} to evaluate the relative influence of environmental conditions and biotic interactions on tree seedling establishment in a grassland matrix. The following questions were addressed: (i) How do grasses and tree seedlings respond to elevated CO₂ and N addition? (ii) Does grass competition affect tree seedling responses to elevated CO₂ or N? (iii) Do changes in atmospheric CO₂ and soil resources modify the competitive balance between grasses and tree seedlings?

Materials and methods

STUDY SPECIES

The tree species *F. excelsior* L. and the grass *Dactylis glomerata* L. were used as a model system to investigate tree seedling–grass interactions in successional communities. *Fraxinus excelsior* (common ash) is a moderately light-demanding, mid-sized deciduous tree which occurs abundantly on base-rich, damp soils (Marigo *et al.* 2000). *Fraxinus excelsior* occurs widely across Europe in a large variety of habitats, and is present along a broad range of altitudinal and climatic gradients. In addition it is an important pioneer species; expansion of *F. excelsior* populations has been greatly favoured by agricultural abandonment in recent times (Kerr & Cahalan 2004). *Dactylis glomerata* L. (cocksfoot) is a vigorously growing, strongly-competitive perennial grass common to a wide variety of grassy and woodland habitats worldwide. Previous work has found that both *F. excelsior* and *D. glomerata* grow rapidly under glasshouse conditions and show significant morphological plasticity in response to plant competition even in short-term experiments (Bloor, Leadley & Barthes 2008). Stratified tree seeds (average 1000 seed weight = 88.7 g) were obtained from Forestart Ltd, Hadnall, UK. Grass seed was obtained from Arbiotech, St Gilles, France.

EXPERIMENTAL DESIGN AND GROWTH CONDITIONS

Soil used in the experiment was loamy topsoil collected in the locality of the University of Paris XI (Orsay, France) in February 2006. Deep PVC pots (20 × 15 × 40 cm) were filled with a layer of expanded clay pellets (for improved drainage), then with 7.5 L of a 50 : 50 mix of sieved topsoil and locally-obtained river sand (following Bloor *et al.* 2008). Analysis of this soil–sand mix indicated a pH of 8.5, a total N content of 0.23 g kg⁻¹ (determined by dry combustion) and an organic matter content of 4.3 g kg⁻¹.

Forty-eight pots were assigned to 1 of 12 naturally-lit growth chambers (wooden frame and clear plastic walls, 65 × 65 × 100 cm high) set up inside a large glasshouse at the University of Paris XI (Orsay, France). Each chamber had its own airflow supplied by a pipe system; six chambers were ventilated with ambient air taken from outside the glasshouse, the remainder with ambient air enriched with a small amount of pure CO₂ (see Barnard *et al.* 2004 for a full description). Relatively constant differentials between ambient and elevated CO₂ treatments (264 ± 6 p.p.m.) were achieved by flowmeters regulated for each chamber at the start of the CO₂ enrichment and checked daily during the experimental period. CO₂ concentrations were monitored throughout the experiment using a portable carbon dioxide analyser (Carbocap GM 70, Vaisala, Helsinki, Finland); these measurements indicated an average CO₂ concentration of 381 (SE = 6 p.p.m.) and 645 p.p.m. (SE = 9 p.p.m.) in the ambient and elevated CO₂ chambers, respectively. No temperature difference was observed between the ambient and elevated CO₂ chambers (mean daily temperatures based on hourly measurements ranged between 11.7 °C and 23.1 °C over the course of the experimental period). Maximum daily photosynthetically active radiation (PAR) values recorded during the study ranged between 150 and 1680 µmol s⁻¹ m⁻².

Two competition treatments (with or without grass) and two nutrient treatments (low- and high N) were applied in a complete factorial arrangement within each growth chamber (one pot per treatment combination per chamber). For the grass competition treatment, *Dactylis* populations were established 45 days prior to the planting of *Fraxinus* seeds. On 23 February 2006, seeds of

Dactylis were sown into half of the experimental pots at a density of 2000 seeds m⁻², leaving clear a central 5 × 5 cm zone per pot. The grass began to emerge 10 days later, and the grass-free central zone was maintained by weeding where necessary. At the time of *Fraxinus* planting, each grass-sown pot had 30–35 grass plants and average grass height ranged from 18 cm to 25 cm in the different nutrient–CO₂ treatments. A high-nutrient treatment was established by supplementing half the pots with 200 mL of 7.9 mM ammonium nitrate solution at 2-week intervals; pots in the low-nutrient treatment received the equivalent amount of distilled water alone. Nutrient addition was applied from 20 March onwards when the *Dactylis* seedlings had fully emerged, and over the course of the experiment the high-nutrient pots received the equivalent of 100 kg N ha⁻¹ year⁻¹. This nutrient treatment was intended to ensure that soil N was non-limiting.

Stratified *Fraxinus* seed obtained at the start of April 2006 were kept in a bag with a small amount of moist compost in a darkened cool room (5 °C) until germination started to occur. On 11 April 2006, even-sized germinating *Fraxinus* seeds with a radicle < 0.5 cm long were randomly allocated to the different experimental treatments and planted individually into the centre of each pot at a depth of half a centimetre. *Fraxinus* seedlings were left to grow in the experimental treatments for 10 weeks, and all pots were watered regularly throughout the experimental period. At the end of the experimental period, PAR measurements were made above each *Fraxinus* seedling using gallium–arsenide sensors (JYP 1000, SDEC, France).

PLANT HARVESTING

On 14 June 2006, all plants were harvested and *Fraxinus* seedlings were carefully disentangled from *Dactylis* plants. Each of the harvested *Fraxinus* seedlings was measured to determine plant height, leaf number and leaf area (using a Delta-T area meter, Delta-T devices Ltd, Burwell, UK). In addition, seedling roots were washed and scanned to determine total root length (WinRhizo 2002, Regent Instrument Inc, Quebec, Canada). In the pots with grass, a subsample of *Dactylis* plants was taken from each pot to determine average plant height, leaf length and leaf area. All *Fraxinus* seedlings were oven-dried (60 °C for at least 72 h) to obtain dry mass values for the roots, stems and leaves. *Dactylis* plants were separated into root and shoot material per pot and similarly oven-dried to obtain dry mass values.

Based on harvest data, a number of variables were calculated per plant for *Fraxinus*: leaf area ratio (LAR; total leaf area per plant dry mass), specific leaf area (SLA; total leaf area per leaf dry mass) for both foliaceous cotyledons and true leaves, specific root length (SRL; root length per root dry mass), specific stem length (SSL; stem height per stem dry mass), leaf mass fraction (LMF; leaf dry mass per plant dry mass), stem mass fraction (SMF; stem dry mass per plant dry mass) and root mass fraction (RMF; root dry mass per plant dry mass). In addition, root : shoot ratios and SLA values were calculated on a pot basis for *Dactylis* in the different treatments.

COMPETITION INDICES

Indices of C_{int} and C_{imp} were derived using *Fraxinus* biomass at final harvest in the four different N–CO₂ treatment combinations. Competition intensity (C_{int}) was calculated following Callaway *et al.* (2002) as: $(B_{+C} - B_{-C})/x$, where B is *Fraxinus* biomass in the presence (+C) or absence (–C) of competition and x is the highest value of $(B_{+C}; B_{-C})$. C_{int} reflects the impact of competition irrespective of environmental factors and ranges from –1 to +1, with negative values indicating competition and positive values indicating facilitation.

Competition importance (C_{imp}) was calculated following Brooker *et al.* (2005) as: $(B_{+C} - B_{-C})/(\text{Max } B_{-C} - y)$, where $\text{Max } B_{-C}$ is the maximum value of B_{-C} along an environmental gradient and y is the lowest value of $(B_{+C}; B_{-C})$. $\text{Max } B_{-C}$ provides a reference at the least constraining point along an environmental gradient, against which the impact of competition can be scaled. Consequently C_{imp} provides a measure of the impact of competition relative to the impact of all the other factors in an environment; when abiotic constraints are unimportant relative to competition, C_{imp} tends to be –1, and when abiotic (or other biotic) factors are more important relative to competition, C_{imp} tends to be +1. Since the $\text{Max } B_{-C}$ value for *Fraxinus* in this study comes from the ‘high N, high CO₂’ treatment, the response of *Fraxinus* to gradients of CO₂ and N can be compared (Brooker *et al.* 2005).

DATA ANALYSIS

The experiment was analysed as a split-plot design following Zar (1999), with CO₂ treatment as the whole-plot factor, fixed- and among-growth chambers, and both N and competition treatments as fixed sub-plot factors within-growth chambers. Where necessary, data were transformed prior to analysis to conform to assumptions of normality and homogeneity of variances. Effects of CO₂, N and competition on *Fraxinus* growth were analysed using a GLM procedure and type III SS. Since the third order interaction was non-significant ($P > 0.2$ in all cases), this term was removed from the analysis. Effects of CO₂, N and competition on *Fraxinus* morphology were initially analysed with *Fraxinus* seedling dry mass as a covariable to allow for variation in plant traits with size. Dry mass proved to be non-significant in all analyses and this term was subsequently omitted from the model. In addition, split-plot GLM analysis was used to test for effects of CO₂ and N on *Dactylis* plant traits.

Effects of CO₂ and N on C_{int} and C_{imp} were examined using F statistics and a randomization procedure. Randomization tests evaluate statistical significance based on a reference distribution generated from the observed samples, and are appropriate for small data sets which do not meet assumptions of normality or assumptions of independence (Fontin, Jacquez & Shipley 2002). More recently, randomization tests have been recommended for the analysis of competition indices to overcome the difficulties of analysing ratios with standard statistical methods (Brooker *et al.* 2005). Tests based on a two-way ANOVA model and 10 000 randomizations were carried out following Fontin *et al.* (2002). All statistical analysis was carried out using SAS 8.2. (SAS Institute Inc., Cary, NC).

Results

DACTYLIS GROWTH AND MORPHOLOGY

Nitrogen had a significant effect on all *Dactylis* plant traits measured (Table 1, Fig. 1). Plants in the high N treatment had a greater height, biomass and leaf area, but a lower root : shoot ratio and SLA compared with low N plants. Moreover, light interception by *Dactylis* showed a significant increase with increasing N. In contrast, significant effects of CO₂ were only recorded for *Dactylis* biomass, leaf area, SLA and light interception (Table 1). CO₂ had a negative effect on leaf area, SLA and light interception in both N treatments (Fig. 1). The magnitude of leaf area reduction was greater in the high N treatment (18.8% vs. 14.8% on average), resulting

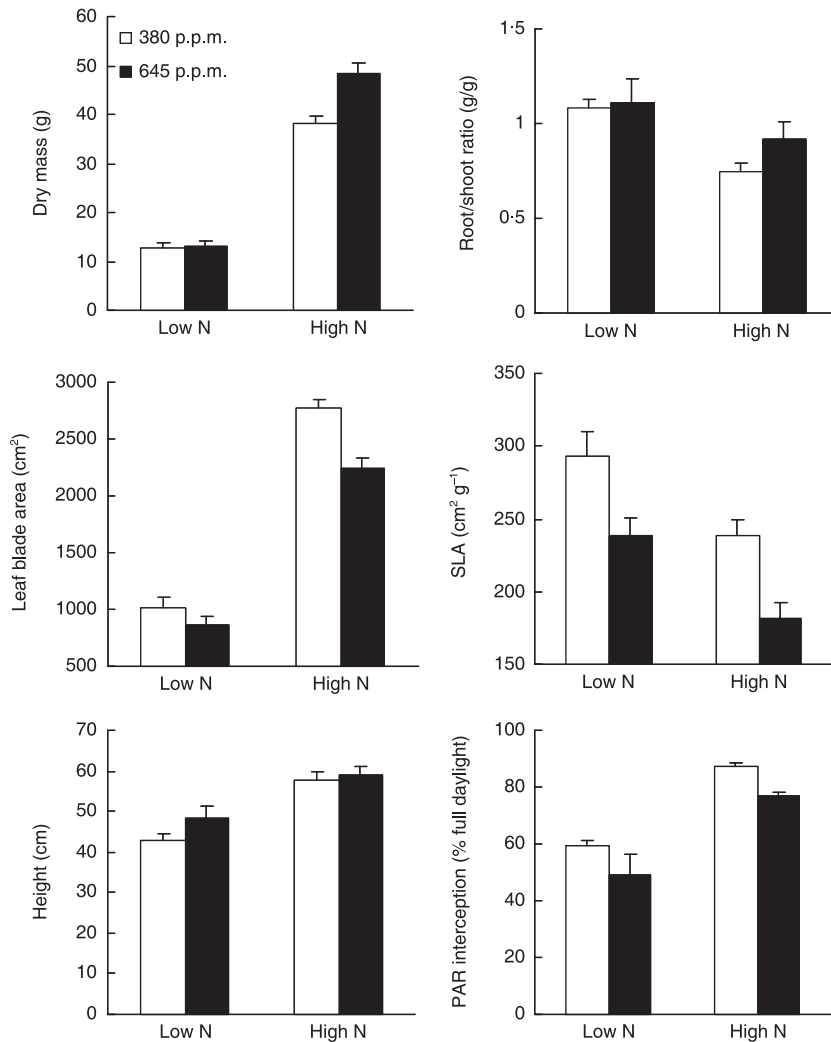


Fig. 1. Effects of N and CO₂ treatment on *Dactylis* plant traits. Means and SEs are shown ($n = 6$).

Table 1. Summary ANOVA results for plant traits of *Dactylis glomerata* grown under interactive CO₂ and N treatments (main effects df = 1, error df = 10). Levels of significance for F ratios are shown; bold values indicate $P < 0.05$

	Dry mass (g)	R : S ratio (g g ⁻¹)	Height (cm)	Leaf blade area (cm ²)	SLA (cm ² g ⁻¹)	% light interception
CO ₂	0.005	0.15	0.16	0.005	< 0.001	0.027
N	< 0.001	0.029	< 0.001	< 0.001	0.005	< 0.001
CO ₂ × N	0.009	0.48	0.35	0.014	0.94	0.94

in a significant CO₂ × N interaction (Table 1). Effects of CO₂ on *Dactylis* biomass also differed depending on N treatment (significant CO₂ × N interactions, Table 1). CO₂ had a significant positive effect on *Dactylis* biomass under high N (+25% on average), but no apparent effect in the low N treatment (Fig. 1). Consequently, the magnitude of the growth response to N was greater under elevated CO₂; the biomass difference between low- and high-N plants was threefold under ambient CO₂, but roughly fourfold greater under elevated CO₂.

FRAXINUS BIOMASS AND HEIGHT GROWTH

Ten weeks after germination, clear differences in seedling growth rates could be seen between treatments (Fig. 2). Grass

competition had a significant negative effect on the biomass and height of *Fraxinus* seedlings ($F_{1,10} = 267.5$, $P < 0.001$ and $F_{1,10} = 53.75$, $P < 0.001$ respectively). Seedling biomass was affected more negatively by grass competition than was height growth. The negative effect of grass competition on *Fraxinus* biomass was particularly pronounced in the high N treatment, with an average biomass reduction of 80% compared to control plants (vs. a 58% biomass reduction due to competition in the low N treatment).

Seedlings under elevated CO₂ showed a marginally significant biomass increase in all N and competition treatments (Fig. 2, $F_{1,10} = 3.48$, $P < 0.09$). In the absence of competition, N also had a significant positive effect on seedling biomass ($F_{1,10} = 8.08$, $P < 0.05$). This positive effect of N fertilization

Fig. 2. *Fraxinus* dry mass and seedling height for treatment combinations of CO₂ and N in the presence (+comp) and absence (–comp) of grass competition. Treatment codes are given by: c, ambient CO₂, 380 p.p.m.; C, elevated CO₂, 665 p.p.m.; n, low N; N, high N treatment. Means and SEs are presented ($n = 6$).

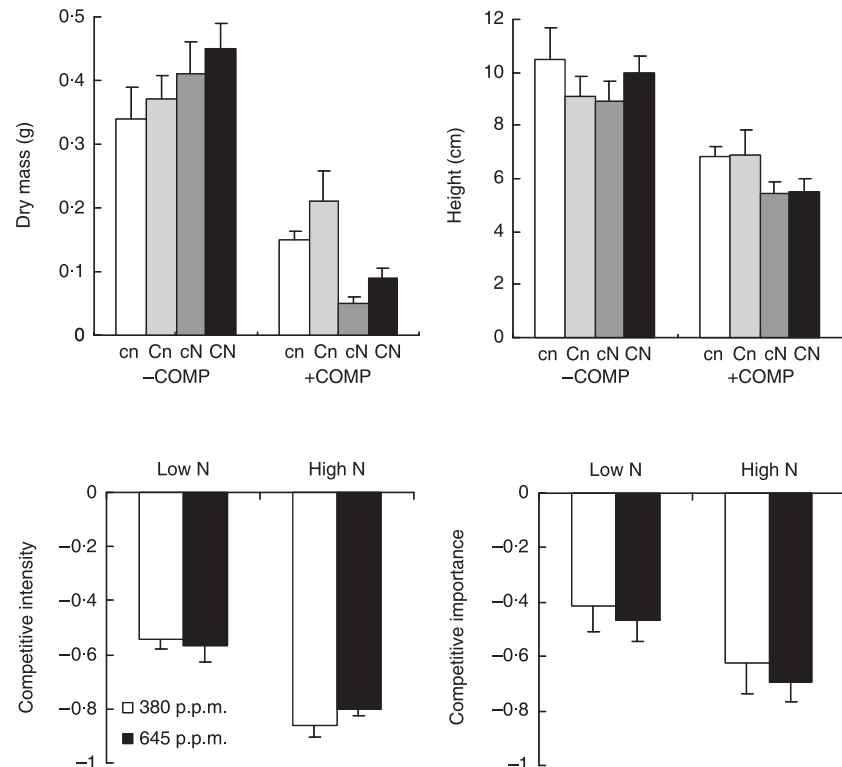


Fig. 3. Effects of N and CO₂ treatment on (a) the competitive intensity, and (b) the competitive importance relative to other factors experienced by *Fraxinus* seedlings grown with grass. Means (\pm SE) are shown; $n = 6$.

Table 2. Summary ANOVA results for plant traits of *Fraxinus excelsior* grown from seed under interactive CO₂ and N treatments in the presence or absence of grass competition (main effects df = 1, error df = 10). Levels of significance for F ratios are shown; bold values indicate $P < 0.05$

	LAR (cm ² g ⁻¹)	SLA _C (cm ² g ⁻¹)	SLA _L (cm ² g ⁻¹)	LMF (g g ⁻¹)	SSL (cm g ⁻¹)	SMF (g g ⁻¹)	SRL (cm g ⁻¹)	RMF (g g ⁻¹)
CO ₂	0.006	0.009	0.011	0.99	0.026	0.27	0.48	0.34
N	0.003	0.17	0.23	0.002	0.030	0.12	0.31	0.005
Comp	< 0.001	< 0.001	< 0.001	0.001	< 0.001	0.07	< 0.001	< 0.001
CO ₂ × N	0.55	0.46	0.91	0.44	0.47	0.96	0.65	0.37
CO ₂ × Comp	0.09	0.58	0.81	0.16	0.10	0.38	0.38	0.44
N × Comp	< 0.001	0.011	0.014	0.019	< 0.001	0.06	0.005	0.15

was reversed in the presence of grass competition, resulting in a significant competition × N interaction ($F_{1,10} = 23.11$, $P < 0.001$). Neither N nor CO₂ had any significant effect on seedling height ($F_{1,10} = 1.03$ and 0.27 , respectively, $P > 0.1$), but there was a tendency for shorter seedlings in the presence of grass competition in the high N treatment (Fig. 2).

INTENSITY AND IMPORTANCE OF GRASS COMPETITION

Indices of C_{int} confirmed a strong impact of grass competition on *Fraxinus* biomass in each of the different CO₂ and N treatments (negative C_{int} values, greater than -0.5 in all cases, Fig. 3). The intensity of competition showed a significant increase in response to increasing N ($F_{1,19} = 3.11$, $P < 0.001$). Nitrogen also had a significant positive effect on the C_{imp} ($F_{1,19} = 6.10$, $P < 0.05$). Neither significant effects of CO₂, nor significant N × CO₂ interactions, were found on either C_{int} or

C_{imp} . However, C_{int} did appear marginally reduced in the high N, high CO₂ treatment (Fig. 3). Across treatments, C_{int} and C_{imp} showed a significant positive relationship ($r^2 = 57.2$, $P < 0.001$).

FRAXINUS MORPHOLOGY AND BIOMASS ALLOCATION

Seven out of eight traits in *Fraxinus* seedlings showed a significant response to grass competition (Table 2). Grass competition was associated with a significant increase in LAR, SLA, SSL and SRL (Fig. 4). Grass competition was also associated with a shift in biomass allocation patterns; *Fraxinus* seedlings grown with grass had significantly lower RMF values but higher LMF values compared with seedlings grown without grass, particularly under N addition (Table 2, Fig. 4).

Fraxinus morphology showed significant responses to N, but in general the effects of N treatment varied depending on the level of competition experienced (significant

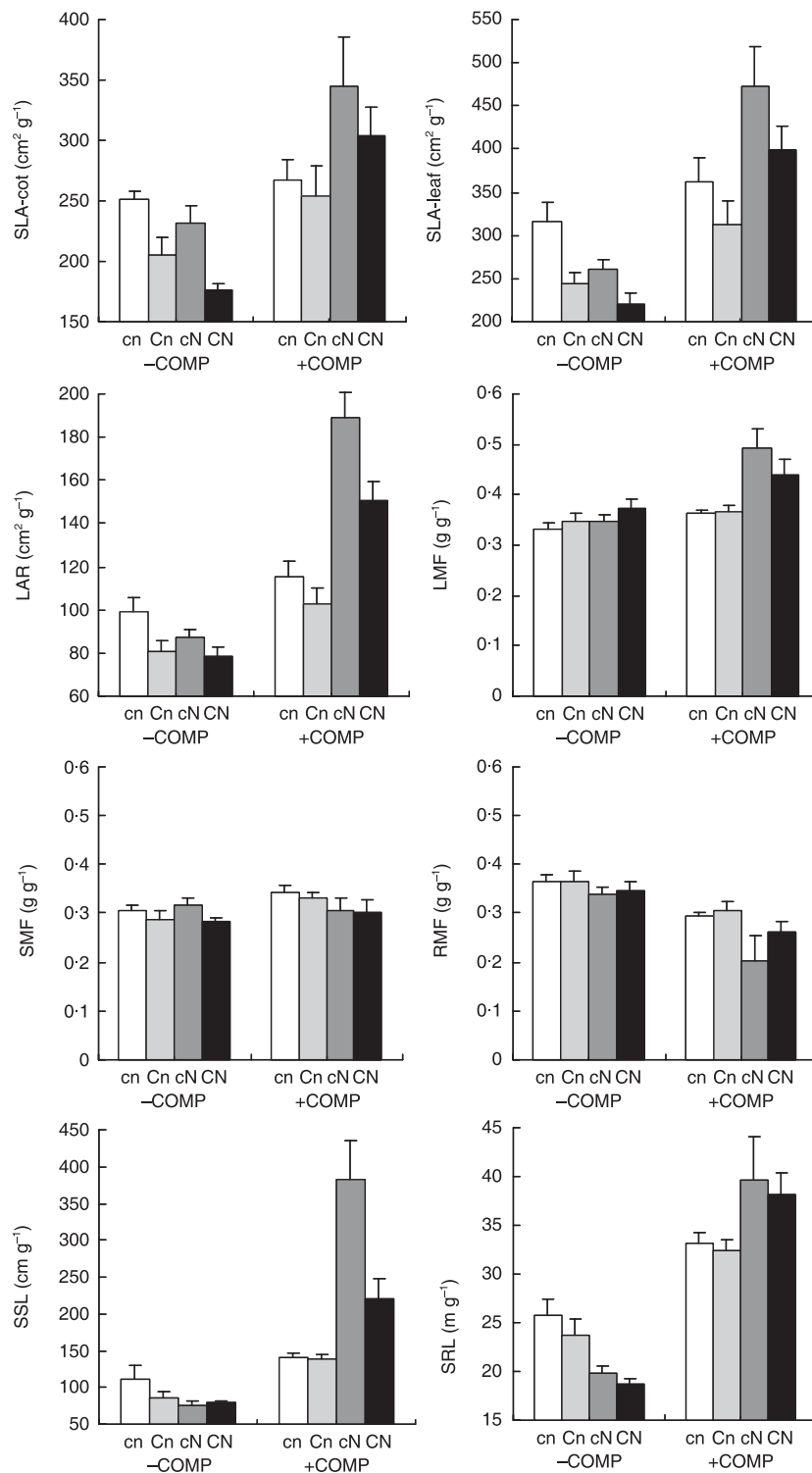


Fig. 4. *Fraxinus* seedling morphology in treatment combinations of CO₂, N and grass competition. Treatment codes are as follows: c, ambient CO₂, 380 p.p.m.; C, elevated CO₂, 665 p.p.m.; n, low N; N, high N treatment; -comp, no grass competition; +comp, with grass competition. Specific leaf area is presented for both photosynthetic cotyledons and true leaves (SLA-cot and SLA-leaf, respectively). Means and SEs are presented ($n = 6$).

competition \times N interactions, Table 2). The only exception was RMF which decreased with increasing N level irrespective of competition treatment (Fig. 4). In the absence of grass competition, an increase in N was associated with a significant decrease in LAR, SLA, SSL and SRL (Fig. 4). In the presence of grass competition however, an increase in N was associated with a significant increase in LAR, SLA, SSL and SRL. Furthermore, LMF showed a significant positive response to

increased N for seedlings grown in the grass competition treatment.

Fraxinus seedling morphology showed limited responses to CO₂ (Table 2, Fig. 4). An increase in CO₂ was associated with a significant decrease in both LAR and SLA, irrespective of the N or competition treatment (Table 2). Significant negative effects of CO₂ were also found for SSL; this latter result was driven by a large difference between low- and high-CO₂

seedlings grown with grass under high-nutrient conditions (Fig. 4). No significant effects of CO₂ were found on either root morphology or biomass allocation (Table 2).

Discussion

TREE AND GRASS GROWTH RESPONSES TO CO₂ AND N

Average biomass increases ranging from 38% to 63% and 16% to 45% have previously been documented for deciduous woody species and C3 grasses, respectively, in response to elevated CO₂ (for reviews see: Ceulemans & Mousseau 1994; Curtis & Wang 1998; Reich *et al.* 2001; Poorter & Navas 2003; Ainsworth & Long 2005). However, little information is available on the CO₂ growth responses of newly-germinated temperate tree seedlings. We found that elevated CO₂ had no effect on *Fraxinus* seedling height growth and only a marginally positive effect on seedling biomass.

During early plant development, growth responses to elevated CO₂ may be limited by morphological (Leadley & Reynolds 1989) or genetic constraints (Mohan, Clark & Schlesinger 2004). Furthermore, growth rates may be driven by seed reserves rather than resources autotrophically acquired by the seedling itself (Kitajima & Fenner 2000). Consequently, the positive effects of CO₂ on plant growth may be less pronounced for newly-germinated seedlings compared with older plants. Ontogenetic shifts in CO₂ responsiveness have been demonstrated for temperate tree seedlings growing in the forest understorey (Hattenschwiler & Korner 2000), and may explain the discrepancy between our results and those recorded elsewhere for 1-year-old *F. excelsior* seedlings (Cornelissen, Carnelli & Callaghan 1999; Broadmeadow & Jackson 2000). The considerable variation in woody seedling growth responses to elevated CO₂ observed in the literature could reflect interspecific differences in the duration of seed reserve dependency and the onset of seedling response to elevated CO₂, as well as differences in seedling age, study length and experimental growing conditions.

Unlike *Fraxinus*, *Dactylis* biomass showed a significant CO₂ × N interaction, suggesting that CO₂ fertilization responses are constrained by N limitation. Recent meta-analyses indicate no systematic differences in the CO₂ responses of C3 grasses and woody species, or between fast- and slow-growing species when growing in mixed species communities (Poorter & Navas 2003). The CO₂ × N interaction obtained here for *Dactylis* probably stems from intraspecific competition among the grass plants in each pot, and an attenuation of the CO₂ response at low-nutrient levels (Harmens *et al.* 2000; Roumet *et al.* 2002). Typically at low N supply, an initial stimulation of photosynthetic rates in response to high CO₂ results in an accumulation of non-structural carbohydrates followed by a down-regulation of the plant's photosynthetic capacity (Stitt & Krapp 1999, but see Lee *et al.* 2001). Whilst we did not measure photosynthetic rates in this study, CO₂ × N interactions and N limitation effects have previously been shown for the light-saturated photosynthesis of *D. glomerata* (Hymus, Baker & Long 2001).

Both *Fraxinus* and *Dactylis* showed a greater biomass response to high N compared with elevated CO₂, in agreement with previous studies on woody and herbaceous species (Bazzaz & Miao 1993; Navas *et al.* 1999; Bauer, Berntson & Bazzaz 2001).

EFFECTS OF N AND CO₂ ON TREE–GRASS COMPETITION

Grass-induced competition is typically associated with a dramatic reduction in woody seedling growth (Van Auker & Bush 1997; Wilson 1998). As expected, we found that *Dactylis* exerted a strong negative effect on *Fraxinus* seedling biomass. Both the C_{int} and C_{imp} imposed by *Dactylis* on *Fraxinus* seedlings increased with N addition. Furthermore, C_{int} and C_{imp} were strongly correlated across treatments. Our data corroborate work on herbaceous species which indicates that the C_{imp} increases along productivity or fertility gradients (Sammul *et al.* 2000; Brooker *et al.* 2005; Gaucherand *et al.* 2006), as proposed by Grime (1973). Patterns of C_{int} in relation to productivity gradients are less clear and are not necessarily correlated with C_{imp} (Welden & Slauson 1986; Sammul *et al.* 2000; Brooker *et al.* 2005). The increase in C_{int} that we observed between *Dactylis* and *Fraxinus* must in part reflect unequal growth responses to N input among different functional groups; here, as in other studies, N addition favoured the faster-growing herbaceous species more than the woody seedlings (Redente, Friedlander & McLendon 1992; Arp *et al.* 1998; Michelsen *et al.* 1999).

Previous work on the competitive interactions between *Dactylis* and *Fraxinus* seedlings has shown that below-ground competition is more important than above-ground competition under low N conditions (Bloor *et al.* 2008). However, as soil nutrient levels increase, light becomes the limiting factor to plant growth and shifts from root to shoot competition may occur (Tilman 1990; Cahill 1999). In this study, large increases in *Dactylis* above-ground biomass and leaf area in the high N treatment resulted in a significant reduction (32.7 ± 6.9%) in the PAR available to *Fraxinus* seedlings growing with grass compared to the low N treatment. Within the *Dactylis* competition treatment, *Fraxinus* seedlings showed greater carbon allocation to leaves at the expense of roots in response to N addition. Furthermore, N addition was associated with an increase in *Fraxinus* seedling SSL, SLA and LAR. The suites of seedling responses observed are consistent with an adaptive response to limiting light conditions. Given the symmetric nature of competition for below-ground resources, and the asymmetric nature of competition for light (Weiner 1990), an increase in the relative importance of shoot competition could magnify the C_{int} between species along a nutrient gradient.

Numerous studies suggest that variation in grass-induced competition is driven by differences in plant mass rather than differences in per-gram effects on resource availability (Brown, Scanlan & McIvor 1998; Wilson 1998; Peltzer & Kochy 2001). In view of the strong CO₂ × N interaction displayed by *Dactylis* biomass, we expected to find a corresponding CO₂ × N

interaction for the effect of grass competition on *Fraxinus*. Surprisingly, neither the competitive intensity nor C_{imp} effect exerted by *Dactylis* on *Fraxinus* showed a significant increase in response to CO_2 (either alone or in interaction with N). One possible explanation is that *Fraxinus* seedlings respond to *Dactylis* biomass in a non-linear manner, and that growth suppression occurs above a given *Dactylis* biomass threshold. Under shaded conditions in the woodland field layer, cohorts of suppressed *Fraxinus* juveniles may persist for a number of years 'waiting' for enhanced resource availability to complete their life cycle (Wardle 1961; Tapper 1992). Alternatively, elevated CO_2 may mitigate the impact of grass competition on *Fraxinus* under high N conditions via changes in plant traits.

It seems reasonable to suppose that changes in the morphology/physiology of the dominant *Dactylis* may have indirect effects on resource availability, whereas plasticity in the subordinate *Fraxinus* may enhance the capacity of young seedlings to acquire resources or tolerate prolonged periods in sub-optimal conditions. In the present study, *Dactylis* plants showed a greater leaf area reduction in response to elevated CO_2 under high N as opposed to low N conditions. Soil analysis conducted on pots with *Dactylis* also indicated higher soil water content in the high CO_2 , high N treatment compared with the low CO_2 , high N treatment (see Figure S1 in supplementary material). Coherent patterns of variation in *Dactylis* morphology and soil resource availability may be reinforced by concurrent physiological plasticity since increases in plant water use efficiency are often associated with elevated CO_2 (Arp *et al.* 1998; Lee *et al.* 2001; Korner 2003). In semi-arid environments, effects of N on competition between herbaceous vegetation and woody seedlings are thought to be mediated by concomitant changes in soil water availability (Davis *et al.* 1999). Our results suggest that plant-induced changes in water availability may also play a key role for tree seedling–grass interactions along N gradients in temperate environments.

Within the *Dactylis* competition treatment, only *Fraxinus* SSL showed a significant $CO_2 \times N$ interaction; seedlings in elevated CO_2 showed a greater reduction in SSL under high N compared to low N conditions. These results are consistent with the variation observed in *Dactylis* leaf area, since high SSL is typically associated with plant etiolation in low light. Overall, elevated CO_2 had a significant negative effect on *Fraxinus* LAR and SLA (either with or without *Dactylis*). Lower LAR and SLA values in response to elevated CO_2 are well documented among plant species (Cornelissen *et al.* 1999; Bruhn, Leverenz & Saxe 2000; Poorter & Perez-Soba 2002). Cornelissen *et al.* (1999) have suggested that the non-structural carbohydrate accumulation associated with a decrease in SLA may help woody plants maintain a positive carbon balance during periods of low light availability. Furthermore, lower SLA may reduce woody seedling susceptibility to invertebrate herbivory (Knepp *et al.* 2005). Decreased levels of herbivory under elevated CO_2 have recently been demonstrated for young *Baccharis* shrubs planted in grassland (Zavaleta 2006). *Fraxinus* seedling trait responses

to CO_2 may therefore promote seedling persistence in the face of grass competition.

Conclusions

Our results suggest that grass and tree seedling responses to increasing nutrient inputs reinforce the competitive inequality between grass vegetation and invading woody seedlings. In contrast, elevated CO_2 had little effect on the competitive balance between *Dactylis* plants and young *Fraxinus* seedlings. Nonetheless, the combination of grass and tree seedling plasticity in response to elevated CO_2 may have indirect benefits for the long-term growth and survival of woody seedlings competing with grass, particularly under high N conditions. Knowledge of species competitive responses must be coupled with an understanding of the spatial and temporal heterogeneity in grasslands in order to predict patterns of woody seedling proliferation under future atmospheric conditions.

Acknowledgements

We thank Annick Ambroise, Sandrine Fontaine, Jean-Louis Mabout, Audrey Niboyet and Lionel Saunois for technical assistance and help with plant harvesting. Thanks also to Catherine Picon-Cochard for use of the WinRhizo software, and to Denis Vile for advice on randomization procedures. This study was supported by a CNRS postdoctoral fellowship to J.M.G.Bloor and an IFB-GICC project grant.

References

- Aerts, R. (1999) Interspecific competition in natural plant communities: mechanisms, trade-offs and plant-soil feedbacks. *Journal of Experimental Botany*, **50**, 29–37.
- Ainsworth, E.A. & Long, S.P. (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*, **165**, 351–371.
- Arp, W.J., Van Mielerlo, J.E.M., Berendse, F. & Snijders, W. (1998) Interactions between elevated CO_2 concentration, nitrogen and water: effects on growth and water use of six perennial plant species. *Plant, Cell and Environment*, **21**, 1–11.
- Barnard, R., Barthes, L., Le Roux, X. & Leadley, P.W. (2004) Dynamics of nitrifying activities, denitrifying activities and nitrogen in grassland mesocosms as altered by elevated CO_2 . *New Phytologist*, **162**, 365–376.
- Bauer, G.A., Berntson, G.M. & Bazzaz, F.A. (2001) Regenerating temperate forests under elevated CO_2 and nitrogen deposition: comparing biochemical and stomatal limitation of photosynthesis. *New Phytologist*, **152**, 249–266.
- Bazzaz, F.A. (1990) The response of natural ecosystems to the rising global CO_2 levels. *Annual Review of Ecology and Systematics*, **21**, 167–196.
- Bazzaz, F.A. & McConnaughay, K.D.M. (1992) Plant–plant interactions in elevated CO_2 environments. *Australian Journal of Botany*, **40**, 547–563.
- Bazzaz, F.A. & Miao, S.L. (1993) Successional status, seed size and responses of tree seedlings to CO_2 , light and nutrients. *Ecology*, **74**, 104–112.
- Bernacchi, C.J., Coleman, J.S., Bazzaz, F.A. & McConnaughay, K.D.M. (2000) Biomass allocation in old-field annual species grown in elevated CO_2 environments: no evidence for optimal partitioning. *Global Change Biology*, **6**, 855–863.
- Bloor, J.M.G., Leadley, P.W. & Barthes, L. (2008) Responses of *Fraxinus excelsior* seedlings to grass-induced above- and below-ground competition. *Plant Ecology*, **194**, 293–304.
- Bond, W.J., Midgley, G.F. & Woodward, F.I. (2003) The importance of low atmospheric CO_2 and fire in promoting the spread of grasslands and savannas. *Global Change Biology*, **9**, 973–982.
- Broadmeadow, M.S.J. & Jackson, S.B. (2000) Growth responses of *Quercus petraea*, *Fraxinus excelsior* and *Pinus sylvestris* to elevated carbon dioxide, ozone and water supply. *New Phytologist*, **146**, 437–451.

- Brooker, R.W. (2006) Plant–plant interactions and environmental change. *New Phytologist*, **171**, 271–284.
- Brooker, R.W., Kikvidze, Z., Pugnaire, F.I., Callaway, R.M., Choler, P., Lortie, C.J. & Michalet, R. (2005) The importance of importance. *Oikos*, **109**, 63–70.
- Brown, J.R., Scanlan, J.C. & McIvor, J.G. (1998) Competition with herbs as a limiting factor in shrub invasion in grassland: a test with different growth forms. *Journal of Vegetation Science*, **9**, 829–836.
- Bruhn, D., Leverenz, J.W. & Saxe, H. (2000) Effects of tree size and temperature on relative growth rate and its components of *Fagus sylvatica* seedlings exposed to two partial pressures of atmospheric [CO₂]. *New Phytologist*, **146**, 415–425.
- Cahill, J.F. (1999) Fertilization effects on interactions between above- and below-ground competition in an old field. *Ecology*, **80**, 466–480.
- Callaway, R.M., Brooker, R., Choler, O. *et al.* (2002) Positive interactions among alpine plants increase with stress. *Nature*, **417**, 844–847.
- Ceulemans, R. & Mousseau, M. (1994) Effects of elevated atmospheric CO₂ on woody plants. *New Phytologist*, **127**, 425–446.
- Cornelissen, J.H.C., Carnelli, A.L. & Callaghan, T.V. (1999) Generalities in the growth, allocation and leaf quality responses to elevated CO₂ in eight woody species. *New Phytologist*, **141**, 401–409.
- Curtis, P.S. & Wang, X. (1998) A meta-analysis of elevated CO₂ effects on woody plant mass, form and physiology. *Oecologia*, **113**, 299–313.
- Davis, M.A., Wragg, K.J. & Reich, P.B. (1998) Competition between tree seedlings and herbaceous vegetation: support for a theory of resource supply and demand. *Journal of Ecology*, **86**, 652–661.
- Davis, M.A., Wragg, K.J., Reich, P.B., Tjoelker, M.G., Schaeffer, T. & Muermann, C. (1999) Survival, growth and photosynthesis of tree seedlings competing with herbaceous vegetation along a water-light-nitrogen gradient. *Plant Ecology*, **145**, 341–350.
- Fontin, M.-J., Jacquez, G.M. & Shipley, B. (2002) Computer-intensive methods. *Encyclopedia of Environmetrics* (eds A.H. El-Shaarawi & W.W. Piegorsch), pp. 399–402. John Wiley & Sons, Ltd, Chichester.
- Gaucherand, S., Liancourt, P. & Lavelle, S. (2006) Importance and intensity of competition along a fertility gradient and across species. *Journal of Vegetation Science*, **17**, 455–464.
- Goldberg, D.E. & Novoplansky, A. (1997) On the relative importance of competition in unproductive environments. *Journal of Ecology*, **85**, 409–418.
- Goldberg, D.E., Rajaniemi, T., Gurevitch, J. & Stewart-Oaten, A. (1999) Empirical approaches to quantifying interaction intensity: competition and facilitation along productivity gradients. *Ecology*, **80**, 1118–1131.
- Grime, J.P. (1973) Competitive exclusion in herbaceous vegetation. *Nature*, **242**, 344–347.
- Harmens, H., Stirling, C.M., Marshall, C. & Farrar, J.F. (2000) Is partitioning of dry weight and leaf area within *Dactylis glomerata* affected by N and CO₂ enrichment? *Annals of Botany*, **86**, 833–839.
- Hattenschwiler, S. & Körner, C. (2000) Tree seedling responses to *in situ* CO₂-enrichment differ among species and depend on understorey light availability. *Global Change Biology*, **6**, 213–226.
- Hymus, G.J., Baker, N.R. & Long, S.P. (2001) Growth in elevated CO₂ can both increase and decrease photochemistry and photosynthesis in a predictable manner. *Dactylis glomerata* grown in two levels of nitrogen nutrition. *Plant Physiology*, **127**, 1204–1211.
- Julien, M.-P., Alard, D. & Balent, G. (2006) Patterns of ash (*Fraxinus excelsior* L.) colonization in mountain grasslands: the importance of management practices. *Plant Ecology*, **183**, 177–189.
- Kerr, G. & Cahalan, C. (2004) A review of the site factors affecting the early growth of ash (*Fraxinus excelsior* L.). *Forest Ecology and Management*, **188**, 225–234.
- Kitajima, K. & Fenner, M. (2000) Ecology of seedling regeneration. *Seeds: The Ecology of Regeneration in Plant Communities* (ed. M. Fenner), pp. 331–360. CABI Publishing, Wallingford.
- Knepp, R.G., Hamilton, J.G., Mohan, J.E., Zangerl, A.R., Berenbaum, M.R. & DeLucia, E.H. (2005) Elevated CO₂ reduces leaf damage by insect herbivores in a forest community. *New Phytologist*, **167**, 207–218.
- Körner, C. (2003) Ecological impacts of atmospheric CO₂ enrichment on terrestrial ecosystems. *Philosophical Transactions of the Royal Society London, Series A*, **361**, 2023–2041.
- Leadley, P.W. & Reynolds, J.F. (1989) Effects of carbon dioxide enrichment on the development of the first 6 mainstem leaves in soybean. *American Journal of Botany*, **76**, 1551–1555.
- Lee, T.D., Tjoelker, M.G., Ellsworth, D.S. & Reich, P.B. (2001) Leaf gas exchange responses of 13 prairie grassland species to elevated CO₂ and increased nitrogen supply. *New Phytologist*, **150**, 405–418.
- Marigo, G., Peltier, J.P., Girel, J. & Pautou, G. (2000) Success in the demographic expansion of *Fraxinus excelsior* L. *Trees*, **15**, 1–13.
- Michelsen, A., Graglia, E., Schmidt, I.K., Jonasson, S., Sleep, D. & Quarmby, C. (1999) Differential responses of grass and a dwarf shrub to long-term changes in soil microbial biomass C, N and P following factorial addition of NPK fertilizer, fungicide and labile carbon to a heath. *New Phytologist*, **143**, 523–538.
- Mohan, J.E., Clark, J.S. & Schlesinger, W.H. (2004) Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO₂. *Global Change Biology*, **10**, 233–247.
- Navas, M.-L., Garnier, E., Austin, M.P. & Gifford, R.M. (1999) Effect of competition on the responses of grasses and legumes to elevated atmospheric CO₂ along a nitrogen gradient: differences between isolated plants, monocultures and multi-species mixtures. *New Phytologist*, **143**, 323–331.
- Peltzer, D.A. & Kochy, M. (2001) Competitive effects of grasses and woody plants in mixed-grass prairie. *Journal of Ecology*, **89**, 519–527.
- Polley, H.W., Mayeux, H.S. & Tischler, C.R. (1996) Are some of the recent changes in grassland communities a response to rising CO₂ concentrations? *Carbon Dioxide, Populations and Communities* (eds C. Körner & F.A. Bazzaz), pp. 177–195. Academic Press, San Diego.
- Poorter, H. & Navas, M.-L. (2003) Plant growth and competition at elevated CO₂: on winners, losers and functional groups. *New Phytologist*, **157**, 175–198.
- Poorter, H. & Perez-Soba, M. (2001) The growth response of plants to elevated CO₂ under non-optimal environmental conditions. *Oecologia*, **129**, 1–20.
- Poorter, H. & Perez-Soba, M. (2002) Plant growth at elevated CO₂. *The Earth System: Biological and Ecological Dimensions of Global Environmental Change* (eds H.A. Mooney & J.G. Canadell), pp. 489–496. John Wiley & Sons Ltd, Chichester.
- Redente, E.F., Friedlander, J.E. & McLendon, T. (1992) Response of early and late semiarid species to nitrogen and phosphorus gradients. *Plant and Soil*, **140**, 127–135.
- Reich, P.B., Tilman, D., Craine, J., Ellsworth, D., Tjoelker, M.G., Knops, J., Wedin, D., Naeem, S., Bahaeddin, D., Goth, J., Bengtson, W. & Lee, T.D. (2001) Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO₂ and N availability regimes? A field test with 16 grassland species. *New Phytologist*, **150**, 435–448.
- Roumet, C., Laurent, G., Canivenc, G. & Roy, J. (2002) Genotypic variation in the response of two perennial grass species to elevated carbon dioxide. *Oecologia*, **133**, 342–348.
- Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M. & Wall, D.H. (2000) Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770–1774.
- Sammul, M., Kull, K., Oksanen, L. & Veromann, P. (2000) Competition intensity and its importance: results of field experiments with *Anthoxanthum odoratum*. *Oecologia*, **125**, 18–25.
- Stitt, M. & Krapp, A. (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell and Environment*, **22**, 583–621.
- Tapper, P.-G. (1992) Demography of persistent juveniles in *Fraxinus excelsior*. *Ecography*, **15**, 385–392.
- Tilman, D. (1990) Constraints and tradeoffs: towards a predictive theory of competition and succession. *Oikos*, **58**, 3–15.
- Van Auken, O.W. & Bush, J.K. (1997) Growth of *Prosopis glandulosa* in response to changes in aboveground and belowground interference. *Ecology*, **78**, 1222–1229.
- Wardle, P. (1961) Biological flora of British Isles: *Fraxinus excelsior* L. *Journal of Ecology*, **49**, 739–751.
- Weiner, J. (1990) Asymmetric competition in plant populations. *Trends in Ecology and Evolution*, **5**, 360–364.
- Welden, C.W. & Slauson, W.L. (1986) The intensity of competition versus its importance: an overlooked distinction and some implications. *Quarterly Review of Biology*, **61**, 23–44.
- Wilson, S.D. (1998) Competition between grasses and woody plants. *Population Biology of Grasses* (ed. G.P. Cheplick), pp. 231–254. Cambridge University Press, Cambridge.
- Wilson, S.D. & Tilman, D. (1991) Components of plant competition along an experimental gradient of nitrogen availability. *Ecology*, **72**, 1050–1065.
- Wullschlegel, S.D., Tschaplinski, T.J. & Norby, R.J. (2002) Plant water relations at elevated CO₂: implications for water-limited environments. *Plant, Cell and Environment*, **25**, 319–331.
- Zar, J.H. (1999) *Biostatistical Analysis*. Prentice-Hall International, Inc., London.

Zavaleta, E.S. (2006) Shrub establishment under experimental global changes in a California grassland. *Plant Ecology*, **184**, 53–63.

Received 16 October 2007; accepted 22 January 2008
Handling Editor: Matthew Turnbull

Supplementary material

The following supplementary material is available for this article:

Fig. S1. Volumetric soil water content of pots sown with *Dactylis glomerata* and grown in a factorial treatment combination of N and CO₂. Means and SEs are presented

($n = 6$). Measurements were carried out at final harvest using a SM200 soil humidity probe (Delta T Devices, Burwell, UK).

This material is available as part of the online article from:
<http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2435.2008.01390.x>

(This link will take you to the article abstract).

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.