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The effects of elevated CO₂ on seed production and seedling recruitment in a sheep-grazed pasture

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Abstract Seed production and seedling recruitment were measured over 2 years under ambient (360 ppm) and elevated (475 ppm) atmospheric CO₂ in a free air carbon dioxide enrichment (FACE) experiment, carried out in a sheep-grazed pasture on dry, sandy soil in New Zealand. In both years elevated CO₂ led to more dispersed seeds of the grasses *Anthoxanthum odoratum*, *Lolium perenne* and *Poa pratensis*, the legumes *Trifolium repens* and *T. subterraneum* and the herbs *Hypochaeris radicata* and *Leontodon saxatilis*. The increased seed dispersal in *A. odoratum*, *H. radicata*, *Leontodon saxatilis* and *T. repens* reflected both more inflorescences per unit area and more seeds per inflorescence under elevated CO₂. The increased seed dispersal in *Lolium perenne*, *P. pratensis* and *T. subterraneum* was due solely to more inflorescences per unit area. The number of seedlings that emerged and survived to at least 7 months of age was increased by elevated CO₂ for *H. radicata*, *Leontodon saxatilis*, *T. repens* and *T. subterraneum* in both years and for *A. odoratum* and *Lolium perenne* in the first year. For species where increased seedling recruitment was noted, there was a significant positive correlation between seed production in summer and seedling emergence in the following autumn and winter, and sowing 200 extra seeds per species m⁻² resulted in more seedlings compared to unsown controls. Elevated CO₂ did not affect seedling survival in any species. There was no measurable effect of elevated CO₂ on canopy and soil surface conditions or soil moisture at the time of seedling emergence. The results suggest the dominant effect of elevated CO₂ on seedling recruitment in this pasture was an indirect one, reflecting effects on the number of seeds produced. The biomass of *H. radicata*, *Leontodon*

saxatilis, *T. repens* and *T. subterraneum* in the above-ground vegetation was greater under elevated than ambient CO₂. However, the size of individual seedlings and mature plants of these four species was unaffected by elevated CO₂. The results indicate an important way elevated CO₂ influenced plant species composition in this pasture was through changes in the pattern of seedling recruitment.

Keywords Climate change · Germination · Seedling emergence · Seedling survival

Introduction

How the species composition of plant communities will change under elevated atmospheric CO₂ is an important question to address in climate change research. Changes in species composition will reflect the effects of elevated CO₂ on the abundance of existing plants, as well as the recruitment of new plants from seeds. There have been many studies that have examined the impact of elevated CO₂ on the vegetative growth of plants (see reviews by Navas 1998; Pritchard et al. 1999) but few have considered how elevated CO₂ might influence seedling recruitment (Diaz et al. 1993; Morse and Bazzaz 1994; Taylor and Potvin 1997; Huxman et al. 1998).

One of the most obvious ways that elevated CO₂ might be expected to influence seedling recruitment is through effects on the number of seeds produced. Studies with agronomic plants have often shown that elevated CO₂ leads to increased grain yields (Lawlor and Mitchell 1991). Analyses of the components of grain yield have shown the increases are often due more to an increase in the number of structures (e.g. inflorescences, pods, seeds) than an increase in the size of structures (e.g. seed size; Lawlor and Mitchell 1991). In natural plant communities, effects of elevated CO₂ on the number of seeds produced have also been found, although the direction of the response is not as clear as in the agronomic studies (Curtis et al. 1994; Jackson et al. 1994; Farnsworth and

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Bazzaz 1995; Navas et al. 1997; Grünzweig and Körner 2000). So far, however, there has been little research on how a change in seed production under elevated CO₂ might influence seedling recruitment.

Whether or not a change in the number of seeds produced will impact on seedling recruitment will depend on the extent to which seedling recruitment is limited by the availability of seeds (Turnbull et al. 2000) and how other factors limiting recruitment such as inter- and intra-specific plant competition (Bazzaz and McConnaughay 1992) and seed and seedling predators (Arnone et al. 1995; Ledergerber et al. 1997) respond to elevated CO₂. For instance, an increase in the level of seed production under elevated CO₂ might be accompanied by an increase in the size and competitive ability of existing plants, thus resulting in little net effect on seedling recruitment.

Here, we report on a 2-year free air carbon dioxide enrichment (FACE) experiment examining the impact of elevated CO₂ on seedling recruitment in a sheep-grazed pasture on dry, sandy soil in New Zealand. In the experiment, we measured seed production of the principal species and examined whether CO₂-induced changes in seed production altered the number of seedlings recruiting. We related the data on seedling recruitment to data on the relative abundance of species in order to examine the extent to which shifts in species composition under elevated CO₂ were mediated by changes in seedling recruitment.

Materials and methods

Study site

The study was carried out from October 1997 to December 1999 in a 2.5-ha species-rich pasture on dry, sandy soil located at Bulls, Manawatu, New Zealand (40°14'S, 175°16'E). The pasture had been under permanent grass since at least 1940, during which time defoliation management had been grazing by sheep, cattle and goats, with occasional hay cuts taken. An inspection of the vegetation at the site in spring 1996 found 25 vascular plant species, with the C3 grasses *Agrostis capillaris* Sibth., *Anthoxanthum odoratum* L. and *Lolium perenne* L., the C4 grass *Paspalum dilatatum* Poir., the legumes *Trifolium repens* L. and *T. subterraneum* L. and the herbs *Leontodon saxatilis* Lam syn *Leontodon taraxacoides* and *Hypochaeris radicata* L. being predominant. All vascular plant species at the site were exotic. The soil at the site was a Pukepuke black sand (Cowie and Hall 1965) with a 0.25-m black, loamy, fine sand top horizon underlain by greyish-brown, fine sand-textured horizons. Soil tests taken at the site before the experiment began showed soil pH=5.8, K=0.15 cM(+) kg soil⁻¹, P=20 µg ml soil⁻¹ and S (sulphate)=7 µg ml soil⁻¹. The site was fertilized during the experiment with 30 g m⁻² of superphosphate in spring and 5 g m⁻² of potassium sulphate in spring, autumn and winter. The mean annual rainfall at the site from 1945 to 1995 was 874 mm. In both 1998 and 1999, rainfall during summer was below the 50-year average, with this being particularly pronounced in 1998 [summer (January to March) rainfall: 50-year average=185 mm, 1998=91 mm, 1999=143 mm]. No irrigation was conducted. In this paper, we define summer as comprising the 3-month period, December, January and February, autumn as March, April and May, winter as June, July and August and spring as September, October and November.

Experimental design

Six FACE rings, each 12 m in diameter, were marked out at least 20 m apart at the site in January 1997. Rings with similar vascular plant species composition and soil characteristics were paired to give three blocks. One ring in each block was randomly allocated to be fumigated with CO₂ and the other was left as an ambient CO₂ control. Each ring was ring-fenced to preclude sheep located in the surrounding field from grazing the rings when not desired. In order to assess pre-enrichment plant species composition, four 1 m×0.078 m quadrats were cut to 0.02 m above ground level in each ring in June 1997, sorted to species, oven-dried for 24 h at 95°C and weighed.

Carbon dioxide enrichment

Carbon dioxide enrichment was conducted using free air carbon dioxide enrichment technology (Lewin et al. 1992). The FACE system was installed in the pasture during the first 9 months of 1997, with enrichment beginning on 1 October 1997. The target enrichment concentration was 475 ppm CO₂ during the photoperiod. Enrichment only occurred during the photoperiod because the CO₂ concentration during the night frequently exceeded the target level. Fumigation was through 24 standpipes, each 0.05 m in diameter, located at equi-distance intervals on the perimeter of each ring. Pure CO₂ was piped to each elevated CO₂ ring, mixed with ambient air in the standpipes and blown across the ring from the upwind side. No air was blown across the ambient rings raising the question of "blower effects" (McLeod and Long 1999) in the CO₂ plus blower rings. However, to date, artefacts introduced by blowers have only been observed in response to night time fumigation (McLeod and Long 1999). In addition, it should be noted that there is no simple solution to testing for "blower effects". The recommended (McLeod and Long 1999) combination of CO₂ plus blowers, blower controls (blower with ambient air) and ambient (no blower) controls cannot test for blower effects because there is no CO₂ treatment from which blower×CO₂ interactions can be estimated. The CO₂ concentration (daily mean of the 1-min averages) of the enriched rings for the experimental period January 1998 to December 1999, measured during the photoperiod at 0.3 m above canopy height, was 474.6 ppm with a standard deviation of 12.6. The 1 min average values were within ±10% of the set point of 475 ppm for 83% of the time.

Sheep grazing procedure

Adult sheep intermittently grazed all rings. Grazing commenced when the total above-ground biomass, averaged across all rings (determined by a pasture meter), reached 1,800–2,000 kg dry weight ha⁻¹ and continued until the above-ground biomass was reduced to approximately 500–700 kg dry weight ha⁻¹. At some times of the year, due to poor growing conditions, the target biomass was not reached despite long regrowth intervals; in these circumstances the pastures were grazed in order to clear rank growth, in accordance with good grazing management practice. Both enriched and ambient rings were grazed at the same time and for the same duration (1–2 days). Grazing commenced on 3 October, 30 October, 24 November and 23 December 1997, 6 April, 25 June, 24 September, 12 November and 14 December 1998 and 15 February, 6 July, 2 September, 15 October and 1 December 1999. Rings were grazed by opening the gates into each ring and allowing a flock of approximately 200 sheep, which had been grazing in the surrounding field for 3–4 days, free access to all of the rings. Up to 15 sheep were observed grazing in one ring at any one time. This grazing procedure raises the possibility of sheep transferring seeds between ambient and elevated CO₂ rings in dung (Ozer 1979). However, as few seeds are excreted in dung within 1 day of ingestion (Ozer 1979), most seeds excreted during the time rings were grazed would have originated from the field surrounding the rings. Thus, we might expect all rings to receive a constant input of ambient CO₂ seeds and there to be little transfer of seeds from elevated to ambient CO₂ rings.

Measurements

Total and species biomass

One randomly selected 1 m \times 0.078 m quadrat was cut to 0.02 m above ground level from each compass quarter of each ring (four per ring) on the day prior to and on the day after each grazing. An additional cut was made on 2 September 1999 when no grazing occurred. The samples were bulked to give one sample per ring. The herbage was dried for 24 h at 95°C and weighed. A sub-sample of approximately 50 g from the bulked sample taken the day before grazing in November 1997, April, June and November 1998 and July, October and December 1999 was sorted into species prior to drying. The dry weight of the component species in the total sample was calculated by multiplying the proportion of each species (by mass) in the sub-sample by the total dry weight of the sample.

Species richness

The number of species in the entire area of each ring, excluding the outer 1 m, was recorded by visual inspection on 15 November 1997, 28 October 1998 and 1 December 1999. Each ring was searched until 5 min had elapsed since the last new species was found.

Inflorescences per unit area

One permanent 0.25 m \times 0.25 m quadrat was established in each compass quarter of each ring (four per ring) in November 1997. Inflorescences of all species were counted in each quadrat on 23 November and 23 December 1997, 12 January, 9 November and 15 December 1998 and 20 January 1999.

Seed mass and seeds per inflorescence

Inflorescences of *Agrostis capillaris*, *Anthoxanthum odoratum*, *H. radicata*, *Lolium perenne*, *Leontodon saxatilis*, *Poa pratensis* and *T. repens* were collected prior to seed dispersal during summer 1998 and 1999. At least 20 randomly selected inflorescences were collected per species in each ring. Inflorescences of *T. subterraneum* were only collected in the second summer. For *T. subterraneum*, where inflorescences (burs) are pushed below the ground, the peduncles of 50 burs per ring were marked with coloured insulation wire on 1 December 1998. On 20 December 1998, any marked burs remaining above the soil surface were collected and those buried were dug up. This process resulted in between 24 and 30 burs being sampled per ring; the rest were lost to grazing. For each species, fully formed seeds were extracted from each inflorescence and counted. The seeds from each inflorescence were weighed and divided by the number of seeds per inflorescence to determine mean seed mass. All seeds were then stratified in cold storage at 4°C for 7 days and subsequently used in germination tests.

Germination

Germination was tested for those species where seed mass had been measured, by sowing seeds on top of moistened filter paper in 0.09-m-diameter petri dishes. A total of 100 randomly selected seeds were sown per species per ring, except for *T. subterraneum* where only 30 seeds were sown per species per ring. Ten seeds were planted in each dish, giving ten (three for *T. subterraneum*) dishes per species per ring. Dishes were maintained at 25°C in constant light. Seeds were checked daily for 21 days, with the appearance of the radicle taken as an indication of germination.

Seed dispersal

Seed dispersal was measured by counting seeds trapped on adhesive paper (Trappit Yellow Sticky Traps, Agrisense-BCS Pontypridd,

Mid Clamorgan, UK) placed in the lower halves of 0.09-m-diameter petri dishes. Two seed traps were anchored to the ground surface close to each of the four permanent quadrats in each ring (eight per ring) on 24 December 1997, 10 January and 20 December 1998 and 12 January 1999. Those placed in December were collected after 2 weeks and those placed in January were collected after 3 weeks. All trapped seeds were identified to species and counted.

Seedling emergence and survival

Seedling emergence and survival were recorded in one permanent 0.25 m \times 0.25 m quadrat in each compass quarter of each ring (four per ring) at 1- to 3-month intervals. The quadrats were the same as those used for collection of inflorescence data. The dates of sampling were 12 January, 15 April, 20 May, 27 June, 21 September and 9 November 1998 and 12 February, 4 April, 9 May, 9 June, 24 August, 12 October and 12 November 1999. At each sampling date, all new seedlings were identified and their position marked on a map of the quadrat. At subsequent dates, previously marked seedlings were recorded as dead or alive, with missing seedlings assigned as dead. A herbarium containing seedlings of all the adult species found at the site was used to aid in seedling identification. It is noteworthy that seedlings emerging in CO₂-enriched rings could have originated either from seeds dispersed from parents grown under elevated CO₂ or seeds deposited in the seed bank before enrichment began. No attempt was made to differentiate between these two seed sources.

Seedling mass

The above-ground biomass of 20 randomly selected plants of the annual species *T. subterraneum* and *Cerastium glomeratum* L. was collected from each ring in mid-November 1998 and 1999. The above-ground biomass of at least 15 *T. repens* seedlings that emerged in April 1999 was collected from each ring in late December 1999. The *T. repens* seedlings sampled were found outside the permanent quadrats where records of seedling recruitment were made and were those surviving from 25 seedlings marked at emergence with a ring of insulated wire. All plants were dried for 24 h at 95°C and weighed. On 10 February 1999, the diameter of the basal rosette of at least 15 *H. radicata* and 15 *Leontodon saxatilis* plants in each FACE ring was measured.

Seed sowing experiment

Seeds of *Agrostis capillaris*, *Anthoxanthum odoratum*, *H. radicata*, *Leontodon saxatilis*, *Lolium perenne*, *Poa pratensis* and *T. repens* were sown together on 30 March 1998 into one 0.25 m \times 0.5 m quadrat located in each compass quarter of each ring (four per ring). A total of 25 seeds were sown per species into each quadrat (equivalent to 200 extra seeds per species m⁻²). The procedure was repeated with a new set of quadrats on 22 March 1999. The seeds sown were collected from the pasture surrounding the rings during the summer prior to sowing and so were developed on plants growing at ambient CO₂. Seedling emergence and survival were recorded in the same way and on the same dates as in the permanent quadrats where no seeds were sown.

Establishment microsite conditions

The canopy and ground surface conditions close to each recently emerged seedling were recorded at the April 1998 and April 1999 seedling emergence counts. A 2.5-mm-diameter pin was lowered vertically within 5 mm of each new seedling and the presence of a canopy was recorded if the pin touched a live leaf. The condition at the ground surface was also recorded in three categories: bare soil, dead litter, live vegetation (i.e. stolon, tiller base). The procedure was repeated at 200 randomly selected locations in each ring to give

an indication of the canopy and ground surface conditions across the whole experimental area. Volumetric soil moisture content was measured to a depth of 0.15 m by time domain reflectometry using a Trase System (Soil Moisture Equipment, Santa Barbara, Calif.). Soil moisture was measured at approximately 2-week intervals from the end of November 1997 to mid-January 1998 and then at approximately 10-day intervals from November 1998 to November 1999. At each date, 20 measurements were taken at randomly selected locations in each compass quarter of each ring (80 per ring).

Statistical analysis

Total biomass, biomass of principal species, seed mass, percentage soil moisture, percentage of ground area covered by the six microsite categories and the number of seeds per inflorescence were analysed by analysis of variance with block and CO₂ effects in the model. Analyses were carried out separately, and standard errors presented separately, for each date of measurement. The percentage data were arcsine square root-transformed before analyses.

The number of inflorescences at each measurement date, the total number of seeds trapped each summer, the total number of seedlings that emerged each year and the number of seedlings surviving at the end of each spring were analysed using log-linear models and Poisson errors with block and CO₂ effects in the model. Analyses were based on counts totalled across the replicate quadrats or seed traps in each ring. Survival of seedlings that emerged in autumn until the end of the following spring was analysed in each year using log-linear models with binomial errors. To examine if seed sowing increased seedling numbers, the difference in the number of seedlings present in November 1998 and Novem-

ber 1999 between sown and unsown quadrats was calculated for each ring, and compared to zero using a one-sample *t*-test. A separate *t*-test was conducted for ambient and elevated rings. The effect of elevated CO₂ on the magnitude of the difference in seedling numbers between sown and unsown quadrats was then analysed using log-linear models with Poisson errors.

The correlation between the number of dispersed seeds and the number of seedlings that emerged in the following season was examined by calculating the Spearman's rank correlation coefficient. To investigate if seedling emergence showed a different response to particular microsite types, we compared, using a χ^2 test, the observed pattern of seedling emergence in the six canopy-soil condition combinations with the expected distribution of seedlings over the six combinations. The expected distribution was based on the proportion of ground surface covered by each combination. Analysis was conducted on the data pooled across the three rings in the ambient and elevated CO₂ treatments and was carried out for the April seedling measurements each year.

Results

Total and species biomass

Total biomass was 41% higher in elevated than ambient CO₂ rings in October 1999 ($F_{1,2}=29.9$, $P=0.03$) but was unaffected by elevated CO₂ at the other harvest dates (Fig. 1A). Total biomass remaining after grazing was un-

Fig. 1 Mean (± 1 SE, $n=3$) biomass of all species combined (A), of principal species (B–G) and of non-legume dicots combined (H) in ambient (360 ppm, unfilled symbols) and elevated (475 ppm, filled symbols) atmospheric CO₂ rings between June 1997 and December 1999. In A circles represent pre-grazing values and squares post-grazing values. The values are from harvests of herbage cut to 0.02 m above ground level. Biomass was cut in four 0.078 m² quadrats in each of the three ambient and three elevated FACE rings and the four samples were bulked to give one sample per ring. The values at each harvest date have been offset so that the error bars can be seen. The non-legume dicots (H) consisted predominantly of *Hypochaeris radicata*, *Leontodon saxatilis* and *Rumex acetosella*

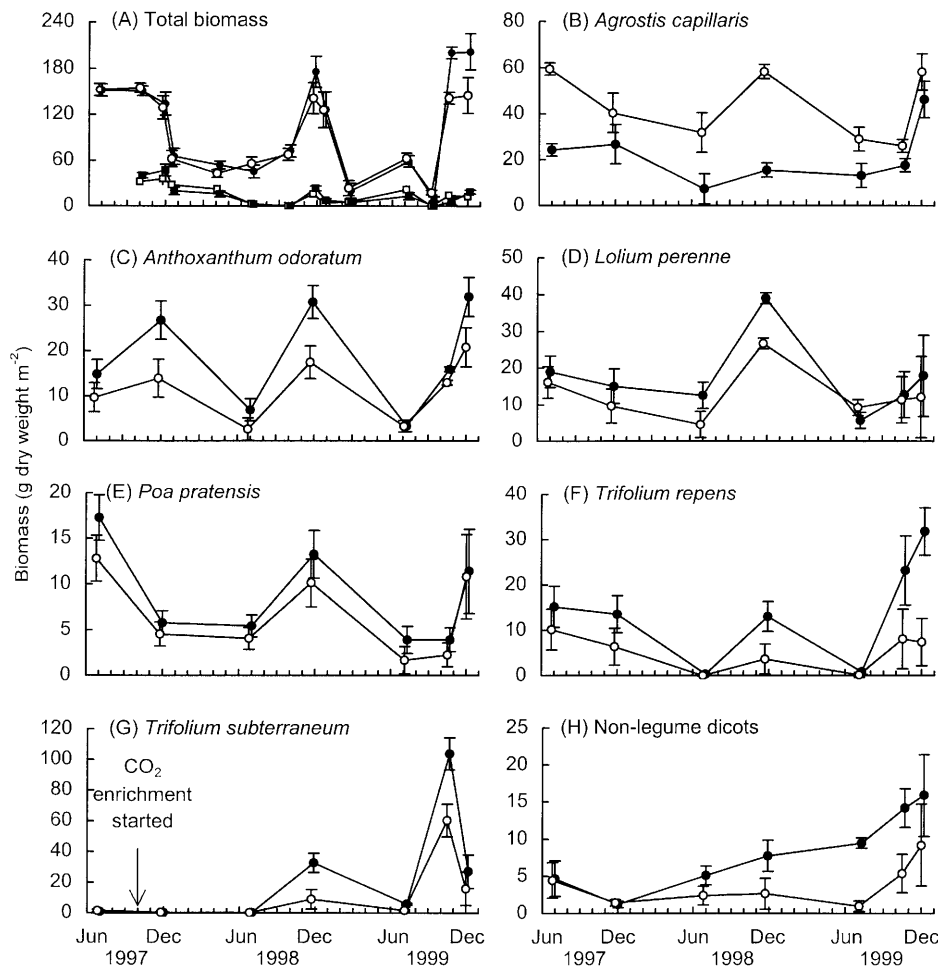
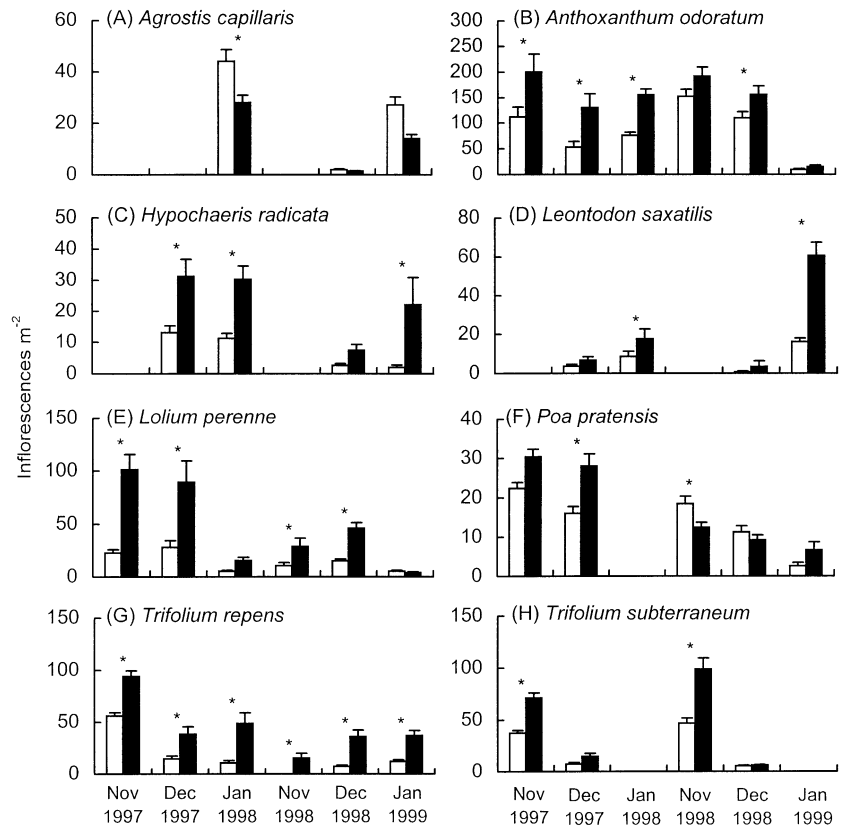


Fig. 2 Mean (± 1 SE, $n=3$) number of inflorescences m^{-2} in ambient (360 ppm, unfilled bars) and elevated (475 ppm, filled bars) atmospheric CO_2 rings in the first two summer periods after enrichment started. Summer 1997–1998=23 November and 23 December 1997 and 12 January 1998. Summer 1998–1999=12 November and 15 December 1998 and 20 January 1999. On each date, inflorescences were counted in four replicate $0.25 \text{ m} \times 0.5 \text{ m}$ quadrats in each of the three ambient and three elevated CO_2 FACE rings and the four values were totalled for statistical analyses. Means and error bars have been back-transformed from the log scale on which the analyses were carried out. Significant differences ($P < 0.05$) between ambient and elevated CO_2 rings at each date are highlighted with an asterisk



affected by elevated CO_2 at any time during the experiment (Fig. 1A).

Before enrichment began, *Agrostis capillaris* had higher biomass on rings assigned to ambient than elevated CO_2 ($F_{1,2}=112.5$, $P < 0.01$) (Fig. 1B). *Agrostis capillaris* biomass remained higher in elevated than ambient CO_2 rings until the end of the experiment with the difference significant in November 1998 ($F_{1,2}=94.8$, $P < 0.01$) but not at other dates. None of the other species showed significant differences in biomass between ambient and elevated CO_2 rings before enrichment began (Fig. 1C–H).

Anthoxanthum odoratum biomass was 90% higher in November 1997 and 76% higher in November 1998 in elevated than ambient CO_2 rings but the differences were not significant ($P=0.11$ for both) (Fig. 1C). *Lolium perenne* biomass was 46% higher in elevated than ambient CO_2 rings in November 1998 ($F_{1,2}=32.6$, $P=0.03$) but was unaffected by elevated CO_2 at other dates (Fig. 1D). *Poa pratensis* biomass was unaffected by elevated CO_2 enrichment throughout the experiment (Fig. 1E).

T. repens biomass was low during winter (July) in each year in both ambient and elevated CO_2 rings (Fig. 1F). During spring 1998 and 1999, the biomass of *T. repens* increased, with the increase being greater in elevated than ambient CO_2 rings. *T. repens* biomass was 260% higher in November 1998 ($F_{1,2}=11.1$, $P=0.08$) and 330% higher in December 1999 ($F_{1,2}=23.4$, $P=0.04$) in elevated than ambient CO_2 rings. The biomass of the annual legume *T. subterraneum* also increased dramatically during spring of 1998 and 1999, reflecting the growth of

seedlings that recruited during autumn. *T. subterraneum* biomass was 250% higher in November 1998 ($F_{1,2}=11.1$, $P=0.08$) and 330% higher in October 1999 ($F_{1,2}=23.4$, $P=0.04$) in elevated than ambient CO_2 rings.

The biomass of the non-legume dicots (mainly *H. radicata*, *Leontodon saxatilis* and *Rumex acetosella* L.) was greater on elevated CO_2 plots from November 1997 onwards. This difference was significant in July 1999 ($F_{1,2}=77.5$, $P=0.01$) and October 1999 ($F_{1,2}=20.1$, $P < 0.04$). Separation of non-legume dicots into individual species in October 1999 showed that *H. radicata* and *Leontodon saxatilis* had greater biomass in elevated than ambient CO_2 rings (dry weight DW) (*H. radicata* ambient=0.9 g DW m^{-2} , elevated=3.4 g DW m^{-2} , $F_{1,2}=22.2$, $P=0.04$; *Leontodon saxatilis* ambient 0.1 g DW m^{-2} , elevated=4.4 g DW m^{-2} , $F_{1,2}=28.2$, $P=0.03$), but that *R. acetosella* was unaffected by elevated CO_2 (ambient=3.7 g DW m^{-2} , elevated=5.9 g DW m^{-2} , $F_{1,2}=0.8$, $P > 0.1$).

Species richness

There was no difference in the number of species per ring between ambient and elevated CO_2 rings in November 1997 (ambient=19.3, elevated=19.7). The mean number of species per ring was greater in elevated than ambient CO_2 rings in both November 1998 (ambient=18.6, elevated=23.6) and December 1999 (ambient=22.3, elevated=26.6) but in neither case were the means significantly different ($P > 0.1$). The annual species *Geranium molle*

L., *Conyza albida* Sprengel and *Silene gallica* L., were only present in elevated CO₂ rings in November 1998 but were present in both ambient and elevated CO₂ rings in December 1999. The herb species *Sonchus asper* L. and *Taraxacum officinale* G. Weber were only present in elevated CO₂ rings in December 1999.

Inflorescences per unit area

Of the eight most abundant species, all except *Agrostis capillaris* had more inflorescences per unit area in elevated than ambient CO₂ rings on one or more census dates in each summer (Fig. 2). *Agrostis capillaris* had more inflorescences per unit area in ambient than elevated CO₂ rings in January 1998. Five other species not shown in Fig. 2 occurred frequently enough for statistical analysis on at least one date but these were unaffected by elevated CO₂ [mean number of inflorescences m⁻² (ambient/elevated): November 1997, *Trifolium dubium* (36.0/22.0); November 1998, *Cerastium glomeratum* (45.1/32.0), *R. acetosella* (41.2/31.2), *T. dubium* (36.6/24.6); January 1998, *Plantago lanceolata* (7.4/8.0), *Holcus lanatus* (13.4/21.0)].

Seeds per inflorescence and seed mass

There were more seeds per inflorescence in elevated than ambient CO₂ rings for *Anthoxanthum odoratum*, *Hypochaeris radicata*, *Leontodon saxatilis* and *Trifolium repens* in 1998 and 1999 (Table 1). *Trifolium repens* had greater seed mass in elevated CO₂ rings in summer 1999 but not in summer 1998 (Table 1). The seed mass of the other eight species sampled was unaffected by elevated CO₂ (Table 1).

Germination

The percentage of *Trifolium repens* seeds germinated after 21 days was greater for seeds developed at elevated than ambient CO₂ in both years (Table 1). Germination in the remaining six species was unaffected by elevated CO₂ (Table 1).

Seed dispersal

There were more seeds of *Anthoxanthum odoratum*, *Hypochaeris radicata*, *Lolium perenne*, *Leontodon saxatilis*, *Poa pratensis* and *Trifolium repens* collected from seed traps in elevated than ambient CO₂ rings in summer 1998 (Table 2). The same pattern was observed in 1999 except that there was no significant effect of elevated CO₂ for *Poa pratensis* (Table 2). Seed dispersal of *Agrostis capillaris* was unaffected by elevated CO₂.

Seedling emergence and survival

A total of 844 seedlings were counted in 1998 and 686 in 1999. Most seedlings in each year emerged in autumn (1998=738 seedlings, 1999=631 seedlings) and winter (1998=99 seedlings, 1999=50 seedlings) with few in spring (1998=7 seedlings, 1999=5 seedlings). In 1998, the number of *Anthoxanthum odoratum*, *Hypochaeris radicata*, *Lolium perenne*, *Leontodon saxatilis*, *Trifolium repens* and *Trifolium subterraneum* seedlings that emerged and the number that were still present in late spring were greater in elevated CO₂ rings (Table 2). By the time of the seedling count in late spring 1998, all marked *Trifolium subterraneum* plants had flowered. All of these plants died during summer 1999. The fate of

Table 1 The number of seeds per inflorescence, seed mass and percentage germination after 21 days of seeds taken from inflorescences collected in summer 1998 and 1999 from ambient (360 ppm) and elevated (475 ppm) atmospheric CO₂ rings. All values are means ($n=3$, $SED=1$ standard error of the difference)

* $P<0.05$; ** $P<0.01$; all others non-significant

^a Measurements of the number of seeds and seed mass were made on at least 20 inflorescences per species in each of the 3 ambient and 3 elevated rings and values were averaged for statistical analyses (ANOVA with 1 and 2 degrees of freedom)

^b Back-transformed from the arcsine square root scale, so no SED is given

^c Not recorded in 1999

	Seeds per inflorescence ^a			Seed mass (mg) ^a			Percentage germination ^b	
	360	475	SED	360	475	SED	360	475
Summer 1998								
<i>Agrostis capillaris</i>	107.3	100.7	10.8	0.06	0.06	0.01	12.4	14.8
<i>Anthoxanthum odoratum</i>	41.3	51.3	4.2*	0.43	0.56	0.04	10.4	14.1
<i>Hypochaeris radicata</i>	38.3	55.7	1.5**	0.85	0.90	0.09	30.9	25.0
<i>Leontodon saxatilis</i>	50.7	59.7	1.0*	0.61	0.64	0.04	26.5	24.3
<i>Lolium perenne</i>	13.0	12.7	1.2	1.51	1.58	0.08	32.5	37.7
<i>Poa pratensis</i>	76.3	88.3	9.1	0.18	0.22	0.06	8.7	11.3
<i>Trifolium repens</i>	10.3	33.3	5.3**	0.39	0.44	0.03	3.8	10.0*
Summer 1999								
<i>Agrostis capillaris</i>	110.7	122.7	12.1	0.07	0.06	0.01	16.9	15.0
<i>Anthoxanthum odoratum</i>	33.6	40.9	3.6*	0.45	0.48	0.05	12.7	16.4
<i>Hypochaeris radicata</i>	38.6	50.0	1.2*	0.61	0.71	0.10	35.2	29.7
<i>Leontodon saxatilis</i>	49.2	61.2	1.6*	0.36	0.31	0.03	32.7	25.7
<i>Lolium perenne</i>	28.2	32.6	3.1	1.45	1.53	0.10	28.6	31.9
<i>Poa pratensis</i>	89.3	95.3	8.1	0.21	0.23	0.05	12.6	9.7
<i>Trifolium repens</i>	39.3	59.4	4.3*	0.34	0.45	0.02*	8.7	15.3*
<i>Trifolium subterraneum</i> ^c	1.2	1.5	0.3	3.9	4.2	0.72	9.0	8.2

Table 2 The number of dispersed seeds in summer, seedlings that emerged in autumn, winter and spring, seedlings surviving at the end of spring, extra seedlings that emerged from seed sowing, and the percentage of seedlings surviving from emergence in autumn to the end of spring in ambient (360 ppm) and elevated (475 ppm)

atmospheric CO₂ rings in 1998 and 1999 (all values means, $n=3$). The Spearman's rank correlation coefficient (r_s) between the number of dispersed seeds and the number of seedlings that emerged in the following autumn to spring period is also shown

	Seeds dispersed m ⁻²		Seedlings emerged m ⁻²		Seedlings surviving m ⁻²		Percentage surviving ^b		Seed sowing (seedlings m ⁻²) ^a		r_s
	360	475	360	475	360	475	360	475	360	475	
1998											
<i>Agrostis capillaris</i>	746	596	18.7	16.7	10.0	8.7	56.6	45.1	4.0	2.0	0.08
<i>Anthoxanthum odoratum</i>	432	740*	18.0	45.3*	14.0	33.3*	74.3	78.4	10.6*	11.3*	0.89*
<i>Cerastium glomeratum</i> ^c			49.3	60.6	40.2	42.2	80.9	83.0			
<i>Hypochaeris radicata</i>	183	386**	5.7	12.7*	3.3	10.0*	63.2	78.4	11.3*	14.6*	0.89*
<i>Leontodon saxatilis</i>	104	373**	4.0	13.3*	2.0	10.0*	55.3	60.5	16.0*	11.4*	0.89*
<i>Lolium perenne</i>	137	281**	11.3	27.3**	8.0	17.3*	71.1	62.9	16.0*	19.3*	0.96**
<i>Poa pratensis</i>	144	320*	20.0	21.3	12.0	14.7	62.6	71.4	3.3	4.6	0.53
<i>Trifolium repens</i>	275	713**	23.3	66.7*	12.0	50.3*	51.3	42.2	24.6*	19.3*	0.90*
<i>Trifolium subterraneum</i> ^d			29.3	94.0*	16.0	60.0*	54.6	66.9			0.94*
1999											
<i>Agrostis capillaris</i>	576	550	9.4	14.7	6.7	7.3	72.3	57.0	4.6	4.0	0.04
<i>Anthoxanthum odoratum</i>	589	779	16.7	24.0	10.0	11.3	60.6	54.6	6.0	4.6	0.66
<i>Cerastium glomeratum</i> ^c			50.0	44.7	36.7	34.0	72.7	76.7			0.26
<i>Hypochaeris radicata</i>	157	392*	10.7	24.7**	5.3	15.3*	55.5	62.1	18.0*	14.7*	0.94*
<i>Leontodon saxatilis</i>	104	471**	9.3	23.3*	5.3	12.7*	54.3	56.2	17.3*	15.3*	0.94*
<i>Lolium perenne</i>	184	301*	9.3	13.3	7.0	9.3	71.6	75.3	6.7	6.0	0.26
<i>Poa pratensis</i>	203	340	10.7	13.3	8.0	9.3	78.8	71.4	4.6	4.0	0.32
<i>Trifolium repens</i>	184	569*	20.4	44.7*	10.7	27.3*	52.3	64.9	13.3*	16.6*	0.94*
<i>Trifolium subterraneum</i> ^d	56	148*	18.7	80.6**	10.6	51.3*	58.0	65.6			0.89*

* $P<0.05$; ** $P<0.01$; all others non-significant

^a Seed sowing values are the number of extra seedlings at the end of spring in sown compared to unsown plots. Asterisks for seed sowing indicate that the difference in seedling numbers between sown and unsown plots is greater than zero within each CO₂ treatment. Asterisks for correlation coefficients indicate significant correlations. Asterisks for other measurements indicate significant differences between ambient and elevated CO₂ treatments

^b Values back-transformed from logits. Seedling survival was unaffected by elevated CO₂

^c No seed dispersal data collected or seed sowing carried out. Correlation coefficient between inflorescence counts and seedling emergence

^d No seed dispersal data collected in 1998. Seed dispersal data in 1999 calculated by multiplying the total number of inflorescences m⁻² by the mean number of seeds per inflorescence. Correlation coefficient between inflorescences and seedling emergence in 1998 and seed dispersal and seedling emergence in 1999

Hypochaeris radicata and *Leontodon saxatilis* seedlings marked in 1998 was followed until the end of spring 1999. Few plants died in the second year and densities of marked plants were higher on elevated CO₂ plots (plants m⁻²: *Hypochaeris radicata* ambient=3.0, elevated=8.7; *Leontodon saxatilis* ambient=2.0, elevated=9.3). *Trifolium repens* seedlings marked in 1998 were tracked until the end of summer (February) 1999, at which time there were more seedlings in elevated (10.0 plants m⁻²) than ambient (42.6 plants m⁻²) CO₂ rings. At this time, most of the *Trifolium repens* seedlings were at least 9 months of age as they had emerged in April and May 1998. It was not possible to track the *Lolium perenne* and *Anthoxanthum odoratum* seedlings beyond the end of spring as fragmentation of plants made identification of individual plants impossible. There was no significant effect of elevated CO₂ on seedling emergence in *Agrostis capillaris* and *Cerastium glomeratum* in 1998.

In 1999, the number of *Hypochaeris radicata*, *Leontodon saxatilis*, *Trifolium repens* and *Trifolium subterra-*

neum seedlings that emerged and the number that were still present at the end of spring were greater in elevated CO₂ rings (Table 2). The number of *Agrostis capillaris*, *Anthoxanthum odoratum*, *Lolium perenne* and *Poa pratensis* seedlings that emerged was unaffected by elevated CO₂ in 1999.

Seedling survival from autumn until the end of spring was unaffected by elevated CO₂ in both years (Table 2). Seedlings of *G. molle*, *Conyza albida* and *Silene gallica* were only found in elevated CO₂ rings in 1998 but in both ambient and elevated CO₂ rings in 1999.

There was a significant positive correlation between the number of dispersed seeds and seedling emergence for *Anthoxanthum odoratum*, *Hypochaeris radicata*, *Lolium perenne*, *Leontodon saxatilis*, *Trifolium repens* and *Trifolium subterraneum* in 1998 (Table 2). In 1999, there was a significant positive correlation for *Hypochaeris radicata*, *Leontodon saxatilis*, *Trifolium repens* and *Trifolium subterraneum*.

Seed sowing

Seed sowing resulted in more seedlings compared to unsown controls for *Anthoxanthum odoratum*, *Hypochaeris radicata*, *Lolium perenne*, *Leontodon saxatilis* and *Trifolium repens* in 1998 but only for *Hypochaeris radicata*, *Leontodon saxatilis* and *Trifolium repens* in 1999 (Table 2). There was no significant effect of elevated CO₂ on the difference in seedling numbers between sown and unsown quadrats in either year.

Seedling and mature plant mass

There was no significant effect of elevated CO₂ on the mass (dry weight) of *Cerastium glomeratum* (1998: ambient=0.011 g, elevated=0.012 g; 1999: ambient=0.016 g, elevated=0.012 g), *Trifolium repens* (1999: ambient=0.14 g, elevated=0.12 g) or *Trifolium subterraneum* (1998: ambient=1.8 g, elevated=1.9 g; 1999: ambient=2.2 g, elevated=2.4 g) seedlings harvested at the end of spring. There was no significant effect of elevated CO₂ on the basal diameter of *Hypochaeris radicata* (ambient=12.0 cm, elevated=10.9 cm) and *Leontodon saxatilis* (ambient=13.1 cm, elevated=14.5 cm) rosettes in summer 1999.

Establishment microsite conditions

There was no significant effect of elevated CO₂ on canopy and soil surface conditions in autumn of either year (Fig. 3). There was a higher percentage of hits in the canopy-live vegetation category ($F_{1,4}=21.0$, $P=0.01$) and a lower percentage of hits in the canopy-litter category ($F_{1,4}=7.3$, $P=0.04$) in 1999 than 1998 (Fig. 3). In both years, seedlings emerged more frequently than expected in microsites with no canopy and soil or litter on the ground surface, and less frequently than expected in microsites with a canopy and litter or live vegetation on the ground surface (Table 3). This result was consistent across CO₂ treatments.

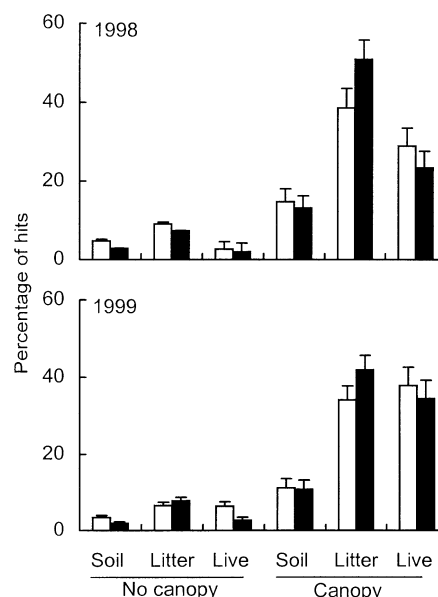


Fig. 3 Mean percentage (± 1 SE, $n=3$) of vertical pin hits in six combinations of canopy (canopy absent, canopy present) and soil surface conditions (soil, litter, live vegetation) in ambient (unfilled bars) and elevated (filled bars) atmospheric CO₂ in April 1998 and 1999. Means and error bars have been back-transformed from the arcsine square root percentage scale on which analyses were carried out. ANOVA based on 1 and 2 degrees of freedom showed no significant effect of elevated CO₂ for any combination

For soil measurements taken from October 1997 to February 1998 (spring/summer period), soil moisture was higher in elevated CO₂ rings on 27 November (ambient=8.6%, elevated=11.34%, $F_{1,2}=19.6$, $P=0.04$) and 12 December 1997 (ambient=7.4%, elevated 8.5%, $F_{1,2}=19.7$, $P=0.04$) but unaffected by elevated CO₂ on the other five dates. For soil measurements taken from November 1998 to November 1999, soil moisture was significantly higher in elevated CO₂ rings on 9 June 1999 and significantly lower in elevated CO₂ rings on 3 February 1999 (Fig. 4).

Table 3 Total number of seedlings that emerged in April 1998 and 1999 in microsites with or without a canopy and where conditions at the soil surface were either soil, litter or live vegetation

			No canopy			Canopy			χ^2 value ^b
			Soil	Litter	Live vegetation	Soil	Litter	Live vegetation	
1998	Ambient	Observed	10.0	19.0	3.0	11.0	16.0	5.0	68.7
		Expected ^a	2.8	5.4	6.1	8.2	22.8	18.8	
	Elevated	Observed	38.0	56.0	12.0	39.0	84.0	10.0	
		Expected	6.9	19.9	16.3	29.1	116.0	50.8	
1999	Ambient	Observed	20.0	24.0	6.0	10.0	15.0	6.0	201.6
		Expected	2.8	5.1	5.0	9.0	26.7	30.3	
	Elevated	Observed	37.0	44.0	9.0	23.0	50.0	14.0	
		Expected	3.2	14.3	5.4	18.5	75.6	60.0	

^a Expected values are proportional to the number of vertical pin hits in each microsite type from censuses conducted at the same time as emergence counts

^b The χ^2 values are for comparisons of observed with expected within each CO₂ treatment. All were significant at $P<0.01$ (1 degree of freedom)

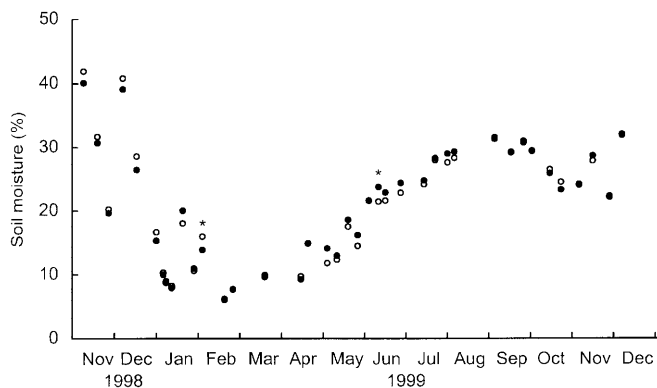


Fig. 4 Mean volumetric soil moisture content (%) ($n=3$) to 0.15 m soil depth in ambient (360 ppm, unfilled circles) and elevated (475 ppm, filled circles) atmospheric CO₂ rings. Means have been back-transformed from the arcsine square root percentage scale on which analyses were carried out. Soil moisture was measured in 80 locations in each of the 3 ambient and 3 elevated rings on each date and the 80 values were averaged for statistical analyses (ANOVA with 1 and 2 degrees of freedom). Asterisks denote a significant ($P<0.05$) effect of elevated CO₂ at each date

Discussion

Numerous studies have shown that elevated CO₂ can affect seed production in grassland plants (Curtis et al. 1994; Jackson et al. 1994; Farnsworth and Bazzaz 1995; Navas et al. 1995) but few have considered whether or not seedling recruitment will be altered (Spring et al. 1996; Taylor and Potvin 1997). In both years of our study, there were more dispersed seeds and seedlings of *Hypochaeris radicata*, *Leontodon saxatilis*, *Trifolium repens* and *Trifolium subterraneum* under elevated than ambient CO₂. The fact that there was a significant positive correlation between the number of dispersed seeds and seedling emergence for these four species in both years highlights increased seed production under elevated CO₂ as an important factor leading to the increased seedling recruitment. This argument is strengthened by the findings of the seed-sowing experiment; *Hypochaeris radicata*, *Leontodon saxatilis* and *Trifolium repens* all showed increased recruitment when extra seeds were sown. In the case of *Trifolium repens*, impacts of elevated CO₂ on seed provisioning may also have contributed to the increased recruitment as elevated CO₂-developed seeds were heavier and had higher germination.

Although the grasses *Anthoxanthum odoratum* and *Lolium perenne* showed increased seed dispersal in both years, they only showed increased seedling recruitment in the first year of the study. The fact that seed sowing resulted in increased seedling numbers in the first but not second year indicates that variation in establishment microsite conditions between years may have contributed to this result. The exact difference in establishment microsites between years that was important is unclear but it may reflect that there was greater area of pasture with a canopy and live vegetation at the soil surface in the second year. A further notable result concerning the

grasses is that seedling recruitment of *Poa pratensis* was unaffected by elevated CO₂ despite the species showing increased seed dispersal in CO₂-enriched plots. This result, combined with the observation that *Anthoxanthum odoratum* and *Lolium perenne* only showed increased seedling recruitment in the first year, highlights that single measurements of flowering or seed production may not reliably indicate the effect of elevated CO₂ on seedling recruitment for one or a range of species. Clearly, it is important to address, not only whether elevated CO₂ alters seed production, but under what conditions changes in seed production might be expected to affect seedling recruitment.

There was little evidence from the measurements taken in this study that differences in establishment microsite conditions between ambient and elevated CO₂ rings contributed to the effect of elevated CO₂ on seedling recruitment. Canopy and soil surface conditions in autumn, and biomass remaining after each grazing, factors that have previously been shown to influence seedling recruitment (Gross and Werner 1982; Bullock et al. 1994), were unaffected by elevated CO₂. Furthermore, seedling emergence in the six combinations of canopy and ground surface conditions measured in this study was similar in ambient and elevated CO₂ rings. In addition, soil moisture, which can have a marked impact on seedling recruitment (Defosse et al. 1997), and which might have been expected to be higher under elevated CO₂ due to improved water use efficiency (Owensby et al. 1999), was influenced little by elevated CO₂, particularly during the main emergence period in autumn. Of course, this does not rule out other differences in microsite conditions not measured in this experiment being important (e.g. extent of seed predation, soil nitrate levels). Note, however, that the number of extra seedlings arising from seed sowing was similar in ambient and elevated CO₂ rings; this argues that elevated CO₂ rings did not have microsite conditions that were markedly more conducive to seedling recruitment than ambient CO₂ rings.

There was no impact of elevated CO₂ on the survival of seedlings from emergence in autumn until the end of spring. This result is, perhaps, not surprising given that there was little impact of elevated CO₂ on above-ground biomass, a vegetation characteristic that has previously been shown to have a negative impact on seedling survival in established grasslands (Crawley and Brown 1995; Edwards and Crawley 1999). Although net photosynthesis in this study was increased under elevated CO₂ (P.C.D. Newton and H. Clark, unpublished data), above-ground biomass was greater in CO₂-enriched rings at the last measurement only.

A striking feature of the seedling data for this permanent pasture was the high number of seedlings that emerged and survived in both years. For example, in *Trifolium repens*, a perennial legume that often shows very little recruitment from seed (Chapman and Williams 1990), greater than 12 and up to 50 plants m⁻² survived to at least 9 months of age. There are two possible expla-

nations for the high seedling recruitment. First, the dry summer period in both years (although drier in 1998) resulted in low standing biomass and a high proportion of bare ground at the end of summer. This would have enhanced establishment microsite availability and reduced competition for emerging seedlings in autumn. Second, intermittent sheep grazing might have prevented biomass, in both ambient and elevated CO₂ rings, from reaching levels that reduced seedling survival.

The increased seed dispersal observed in *Anthoxanthum odoratum*, *Hypochaeris radicata*, *Leontodon saxatilis* and *Trifolium repens* under elevated CO₂ reflected both an increased number of inflorescences per unit area and an increased number of seeds per inflorescence. In the case of *Lolium perenne* and *Poa pratensis*, the increased seed dispersal was due solely to an increased number of inflorescences per unit area. It is likely that the increased inflorescence densities reflect that some species had greater abundance in the elevated than ambient CO₂ rings in the late spring/early summer period. This would be the case particularly in the second summer when biomass differences were evident (e.g. *Trifolium repens*, Fig. 1E). However, this does not explain the increased inflorescence densities observed when biomass was similar between ambient and elevated CO₂ rings (e.g. *Poa pratensis* and *Trifolium subterraneum* first summer) or the increased number of seeds per inflorescence. Possible explanations for these results include increased allocation to reproduction under elevated CO₂ (Fajer et al. 1991), or increased photosynthate production under elevated CO₂ leading to greater initiation and survival of inflorescences and seed primordia (Fischer and Aguilar 1976; Ackerson et al. 1984). An alternative explanation is that CO₂ exerted an influence on flower and inflorescence development that was independent of its effects on photosynthesis. For example, it has been suggested that the effects of elevated CO₂ on flowering may be related to the interaction between CO₂ and phytochrome (the pigment responsible for sensing light in photoperiod responses) or to the effects of CO₂ on ethylene synthesis (Zebian and Reekie 1998).

An important reason for conducting this seedling recruitment study was to provide an understanding of the processes that lead to shifts in plant species composition under elevated CO₂. Our study showed that the above-ground biomass of *Hypochaeris radicata*, *Leontodon saxatilis*, *Trifolium repens* and *Trifolium subterraneum* was greater in elevated than ambient CO₂ rings in both years. For the annual plant, *Trifolium subterraneum*, we consider that the increased seedling numbers under elevated CO₂, combined with the failure to detect any impact of elevated CO₂ on the size of flowering plants, constitutes reasonable evidence that greater seedling recruitment is the reason for this species having increased abundance in CO₂-enriched rings. Evidence also points towards increased seedling recruitment and plant numbers playing a crucial role for the other three species, which are all perennials. For *Hypochaeris radicata* and *Leontodon saxatilis*, differences in seedling numbers

persisted for over a year and there was no difference in the basal diameter of rosettes between ambient and elevated rings in summer 1999. For *Trifolium repens*, differences in seedling numbers persisted for at least 9 months and no difference in seedling size was noted. Furthermore, *Trifolium repens* regeneration appeared to be strongly dependent on seedling recruitment as little leaf material was present at the end of summer in either year (Fig. 1F), and recruitment of new shoots from buried stolons in this species during autumn and winter was negligible (P.C.D. Newton, unpublished data).

The perennial grass species, *Anthoxanthum odoratum* and *Lolium perenne*, both had higher biomass under elevated than ambient CO₂ during the first year of CO₂ enrichment but not during the 2nd year. The role of seedling recruitment in determining these patterns is suggested by the finding that seedling recruitment followed a similar pattern but it is not possible to ascertain its importance compared to differences in the growth, survival and size of existing plants.

Potvin and Vasseur (1997) found higher plant species richness in a pasture community under elevated than ambient CO₂. Higher species richness was also found under elevated CO₂ in this study, although the effect was not significant. The trend for increased species richness arose because several short-lived dicotyledon species were only found in elevated CO₂ rings: the annual plant species, *G. molle*, *Conyza albida* and *Silene gallica* in 1998 and *Sonchus asper* and *Taraxacum officinale* in 1999. The reason for these species only being present in enriched rings is unclear at this stage. The result could be due to CO₂ enrichment enhancing germination, growth and survival of these species, either directly (see Ziska and Bunce 1993) or indirectly through effects on microsite conditions and dominance of other species (Potvin and Vasseur 1997). Alternatively, the result may be an artefact of the experimental layout. For example, rings originally paired as replicates may have had different seed bank floras. In addition, soil disturbance during site preparation was greater around the outside of elevated CO₂ rings where pipes were inserted (P.C.D. Newton, unpublished data). These bare soil surfaces were colonized by annual species like *G. molle*, *Conyza albida* and *Taraxacum officinale*, so enhancing the seed rain of these species in elevated CO₂ rings. Long-term monitoring of the presence and dynamics of species will be required to ascertain whether the effect of elevated CO₂ is due to CO₂ enrichment or an artefact of the experimental protocol.

To clarify the role that seedling recruitment plays in determining plant community composition, the experimental protocol could be improved by more detailed studies of germination and growth of seedlings within the community. In particular, it would be useful to examine how germination and seedling growth and survival are influenced by the interaction of elevated CO₂ during seed development and elevated CO₂ after seed dispersal. For example, the current germination experiment was only conducted at one (ambient) level of CO₂, while the

seed sowing was conducted using ambient CO₂-developed seeds only. Recent studies have shown that interactions between elevated CO₂ during seed development and after seed dispersal can be important for plant growth. Bezemer et al. (1998), for instance, found tiller production of seedlings of the annual grass *Poa annua* L. was enhanced by elevated CO₂ for seeds collected from parent plants grown at elevated CO₂ for one generation, but not for seeds collected from parent plants grown at ambient CO₂. In a similar experiment, Huxman et al. (1998) found elevated atmospheric CO₂ increased the mass of seedlings of the annual grass *Bromus rubens* L. grown from ambient CO₂-developed seeds but not that of seedlings grown from elevated CO₂-developed-seeds. Work is now proceeding to examine whether these inter-generational differences are important for the species in this grassland.

In conclusion, the central result of this study is that elevated CO₂ affected seedling recruitment, with *Leontodon saxatilis*, *Hypochaeris radicata*, *Trifolium repens* and *Trifolium subterraneum* showing increased seedling numbers in CO₂-enriched plots. Evidence from correlation analyses between seed production and seedling emergence, and from seed-sowing experiments, indicates that the impact of elevated CO₂ on seedling recruitment was an indirect one, exercised through the effects of CO₂ enrichment on seed numbers. This should not be taken that we argue seedling recruitment will be affected by elevated CO₂ every year in this grassland or in every grassland community. The effect of elevated CO₂ will depend on how elevated CO₂ influences seed production and the extent to which recruitment is limited by the numbers of seeds that are dispersed (see Turnbull et al. 2000). Clearly, in years or in communities where seedling recruitment is not seed-limited, then CO₂-induced changes in seed production may not matter at all.

A further important result is that increased seedling recruitment was an important way in which species composition changed under elevated CO₂ in this pasture. Again, it is not our intention to argue that this is the only way species composition will change under elevated CO₂. Changes in plant survival and vegetative growth characteristics, such as tillering and branching, have been shown to affect species composition (Clark et al. 1997) and may well play a greater role in species composition change in communities where seedling recruitment is less prominent than the pasture studied here. Note, however, that altered patterns of seedling recruitment have largely been overlooked in generalizations of how plant communities respond to elevated CO₂ (Navas 1998). In order to unravel how the species composition of grassland communities will respond to elevated CO₂, future studies should consider how elevated CO₂ influences both the vegetative growth of plants and seedling recruitment.

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