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The impact of elevated CO_2 on plant-herbivore interactions: experimental evidence of moderating effects at the community level

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Abstract Surprisingly little research has been published on the responses to elevated [CO_2] at the community level, where herbivores can select their preferred food. We investigated the combined effects of atmospheric [CO_2] and herbivory on synthesised plant communities growing on soils of different fertility. Factorial combinations of two [CO_2] (350 or 700 μl l^{-1}), two fertility (fertilised or non-fertilised), and two herbivory (herbivores present or absent) treatments were applied to a standard mixture of seven fast- and eight slow-growing plants in outdoor microcosms. The herbivores used were the grain aphid (*Sitobion avenae*) and the garden snail (*Helix aspersa*). We measured plant biomass, foliar nitrogen and soluble tannin concentration, aphid fecundity, and snail growth, fecundity, and feeding preferences over one growing season. Elevated [CO_2] did not have a significant impact on (1) the combined biomass of fast-growing or slow-growing plants, (2) herbivore feeding preferences, or (3) herbivore fitness. There was, however, a significant biomass increase of *Carex flacca* (which represented in all cases less than 5% of total live biomass), and some chemical changes in unpalatable plants under elevated [CO_2]. The herbivory treatment significantly increased the biomass of slow-growing plants over fast-growing plants, whereas fertilisation significantly increased the abundance of fast-growing plants over slow-growing plants. Predictions on the effects of elevated [CO_2] based on published single-species experiments were not supported by the results of this microcosm study.

Key words Calcareous grasslands · Generalist herbivores · Global climate change · Microcosms · Carbon dioxide

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Introduction

The rise in atmospheric [CO_2] which is expected to continue well into the next century (Watson et al. 1996) has the potential to alter the composition of plant communities and their interactions with other trophic levels (Field et al. 1992; Vitousek 1994). In different ecosystems these effects are expected to vary according to the relative abundance of inorganic resources (light, water, nitrogen, phosphorus) (Bazzaz 1990; Lincoln et al. 1993; Koch and Mooney 1996), which in turn will be modified by land-use patterns (Grime 1996; Sage 1996). Any factor altering the supply of carbon or nitrogen, and therefore the amount and quality of plant tissue, has the potential to modify herbivore consumption and fitness and indirectly plant community composition (Mattson 1980; Crawley 1983; Peñuelas and Estiarte 1998).

Although there have been a number of studies on the effects of elevated [CO_2] and herbivory at the single-species level (e.g. Lincoln et al. 1986; Fajer et al. 1989; Lindroth et al. 1993, 1995; Salt et al. 1996; Docherty et al. 1997; Smith and Jones 1998), extremely little has been published on the responses of plant communities. The works of Arnone et al. (1995) on a generalist lepidopteran species feeding on mixtures of tropical plants, and of Ledergerber et al. (1997) on gastropods and grasshoppers feeding on natural calcareous grasslands are rare examples. Another aspect of major importance, hardly addressed in the literature, is the feedback effect of herbivory on plant performance (Hughes and Bazzaz 1997) and interspecific interactions (Salt et al. 1996; Traw et al. 1996) under CO_2 enrichment.

Community- and ecosystem-level experiments are particularly relevant in view of the fact that the responses to elevated [CO_2] of very simple systems, such as individually grown plants, monocultures, or one plant species + one specialist herbivore species, do not scale up well to results of experiments involving multispecies assemblages (Körner and Bazzaz 1996).

The main aim of the experiment described in this study was to analyse the effects of elevated [CO_2] on the interactions between a synthesised herbaceous plant community and two generalist herbivores, at two levels of soil fertility. From the existing literature several mechanisms can be predicted to have the potential to influence interactions between elevated CO_2, soil fertility, plant communities and herbivores. Carbon partitioning, mineral nutrient concentration and anti-herbivore defence vary strongly between species and habitats (Grime 1977; Coley 1983; Poorter and Bergkotte 1992; Grime et al. 1997). Fast-growing plants tend to have higher leaf nutrient concentration and they are more acceptable to generalist herbivores, and recover faster from defoliation than slow-growing plants. On the basis of the literature relating to single-species experiments (see Lindroth 1996; Poorter et al. 1997; Peñuelas and Estiarte 1998 for recent reviews), we expected elevated [CO_2] to decrease the nitrogen concentrations, and therefore decrease the nutritional quality, of plant tissue. We anticipated, therefore, that herbivore fitness would decrease at elevated [CO_2], especially under the low-fertility treatment. From models of plant functional types (Grime 1977; Bryant et al. 1983; Coley 1983), it is predicted that fast-growing plants will use extra carbon for growth, whereas slow-growing plants will tend to incorporate the surplus carbon into secondary metabolites. If this occurs herbivores might be expected to differentially consume fast-growing species in preference to slow-growing ones under elevated [CO_2]. Following this argument we expected the generalist herbivores used in our experiment to cause an increase in the abundance of slow-growing species. We also suspected that this effect would be most pronounced under low fertility conditions.

The experiment tested the following hypotheses: (1) elevated [CO_2] will alter the biomass of fast-growing species relative to that of slow-growing species directly by promoting growth, and indirectly through altered herbivory patterns; (2) elevated [CO_2] will modify the short-term carrying capacity for herbivores of herbaceous multispecies assemblages; and (3) plant and herbivore responses to elevated [CO_2] will depend on soil nutrient availability.

Materials and methods

Experimental design and set-up

The experiment was set up in 12 1.8-m^3 transparent chambers at Tapton Experimental Garden (University of Sheffield). Half of the chambers received CO_2 at ambient concentration, and the other half received elevated CO_2. Treatments were interspersed among chambers. Each chamber was split into four compartments using insect-proof fabric, which allowed free air circulation. Perspex doors were air-tight and ventilation holes were covered with the same material. A 40 l container with soil and plants (hereafter microcosm) was placed within each compartment. Factorial combinations of two levels of soil fertility and two levels of herbivory were applied to the four containers in each chamber. The four different combinations of treatments were randomly assigned within each chamber, following a split-plot design (Montgomery 1991) in which chambers were considered the main plots, and microcosms, the sub-plots. Each combination of treatments was replicated six times.

Establishment of plant communities

Natural topsoil (upper 10 cm) was collected from infertile calcareous grasslands at Buxton Experimental Field Site (Derbyshire), sieved, homogenised, and mixed with building sand in a 40:60 ratio. The substrate was placed into pots, and left outdoors for 60 days to allow recruitment from the seed bank. All emerging seedlings were eliminated by hand.

Depending on their most common way of propagation under natural conditions, 15 plant species were introduced in the form of seed or cuttings taken directly from field populations. The plant mixture was selected to include a broad selection of growth forms and functional types (Heal and Grime 1991), within the constraints imposed by both the regional flora and the experimental design (Table 1). Plants introduced as cuttings had a potential advantage over those establishing by seed because cuttings offered a large source of stored resources. In order to allow for the fact that the first shoots produced by the cuttings would be utilising stored carbohydrates produced under field CO_2 conditions, the initial flush of shoot growth was removed by cutting the vegetation to a uniform height of 10 cm 150 days after the planting/sowing date, after which the plant communities were maintained under ambient or CO_2-enriched air for 130 days.

Plant growth conditions

Plants grew under natural conditions of day length. Temperatures inside the chambers tracked ambient (although they tended to be higher, normally $< 2°C$, and up to 4°C on sunny days). No consistent variation in temperature was detected between the microcosms. Light intensity inside the chambers was ca. 30% lower than ambient, but still very high compared to artificially generated light in growth cabinets (Fraser and Grime 1997). Half of the chambers received ambient air ([CO_2] = 350 µl l^{-1}), and the other half received CO_2-enriched air ([CO_2] = 700 µl l^{-1}). The CO_2-enrichment and control system was modified from Spring et al. (1996). Microcosms under low-fertility treatment received 1 l tap water per week during the first 60 days, and 2.5 l during the last 70 days. Microcosms under the fertilisation treatment received 1 l full-strength Rorison solution per week during the first 60 days, and 1.5 l plus 1 l tap water per week during the last 70 days. All microcosms were sprayed daily with tap water.

At final harvest, live shoot material was sorted into species, oven-dried, and weighed. Since *Galium aparine* has a short life cycle and was senescent and decomposing at the time of the harvest, we counted total seed number per microcosm. Similarly, in the case of *Poa annua* (showing a lower degree of senescence), we counted total number of flowerheads per microcosm.

Foliar chemistry

Chemical analysis was carried out on fully expanded young leaves. Nitrogen concentration was determined using a Tecator FIA Star

Table 1 Plant species included in the artificial communities. Plant species were selected on the basis of Grime and Hunt (1975) and Grime et al. (1988). Seed densities per container were calculated following Burke and Grime (1996). Cuttings were introduced at a density of 10 cuttings per microcosm

	Family	Growth form	Introduced as	Seed density (no. m^{-2})
Fast-growing species				
Arrhenatherum elatius[a]	Poaceae	Grass	Seeds	112
Chamerion angustifolium[a]	Onagraceae	Non-legume dicot	Cuttings	–
Galium aparine	Rubiaceae	Non-legume dicot	Seeds	112
Lathyrus pratensis[a]	Fabaceae	Legume	Seeds	112
Poa annua	Poaceae	Grass	Seeds	440
Poa trivialis[a]	Poaceae	Grass	Cuttings	–
Urtica dioica[a]	Urticaceae	Non-legume dicot	Cuttings	–
Slow-growing species				
Brachypodium pinnatum[a]	Poaceae	Grass	Cuttings	–
Carex caryophyllea	Cyperaceae	Sedge	Cuttings	–
Carex flacca[a]	Cyperaceae	Sedge	Cuttings	–
Festuca ovina[a]	Poaceae	Grass	Seeds	332
Helianthemum nummularium	Cistaceae	Non-legume dicot	Cuttings	–
Helicotrichon pratense[a]	Poaceae	Grass	Cuttings	–
Lotus corniculatus[a]	Fabaceae	Legume	Seeds	220
Thymus praecox	Lamiaceae	Non-legume dicot	Seeds	–

[a] Species included in chemical analysis

5012 flow injection analysis system (Tecator AB, Sweden), after digestion of plant material in H_2SO_4, containing Salicylic acid (33 g l^{-1}), and using a $CuSO_4$, Li_2SO_4 catalyst (90:10). Soluble tannins were measured following Allen (1989), using tannic acid as standard.

Herbivores

The aphid *Sitobion avenae*, and the snail *Helix aspersa* were collected in the surroundings of Tapton Garden. These species were chosen on the basis of previous knowledge of their behaviour in microcosms and bioassays (Grime et al. 1996; Fraser 1996). Although they are both considered to be generalists, they show different feeding habits and growth rates. *S. avenae* is a metabolite-feeder on grasses, short-lived, with parthenogenetic and sexual reproduction and an extremely high reproductive rate (Dixon 1987). *H. aspersa* is a herbivore-detritivore tissue feeder, which tends to prefer dicotyledons, and shows low population growth rates (Godan 1983; Fraser 1996).

The herbivores were introduced in half of the microcosms 30 days after the beginning of the CO_2-enrichment treatment, at densities of 60 aphids and six juvenile snails (maximum shell diameter 20 mm) per microcosm. All the snails were individually identified. Their maximum shell diameter was measured before introduction and at the end of the experiment in order to estimate their growth. After 60 days from the beginning of the experiment, six extra (non-marked) adult snails were introduced. The first cohort of newly hatched snails was removed and the following cohorts were left to develop. Growth of marked snails [(final shell diameter $\times$ 100)/initial shell diameter], and number and shell diameter of newly hatched snails were measured in order to estimate snail fitness. No aphid fitness parameters at the whole-microcosm level were measured at the end of the experiment.

Aphid fecundity tests

Two fast-growing (*Poa annua* and *P. trivialis*) and two slow-growing (*Festuca ovina* and *Helicotrichon pratense*) grasses were selected in order to assess whether leaves of the same species growing under different [CO_2] and fertility treatment had an impact on aphid performance. These four grasses have been tested in a previous *S. avenae* population response experiment by Fraser (1996). The performance of aphids in this case was defined as the comparative fecundity of aphids on each plant species in independent trials. Midway through the growing season (August), one plant of each species was selected from each of the microcosms where herbivores were absent. A mesh (15 $\times$ 24 cm) clip-cage containing five aphids of equal size was secured onto a single blade of grass from each plant. After 2 weeks, the number of living aphids within each clip-cage was counted. Neither adults or juveniles, apterous or alate, nor the size of the aphids were differentiated. The total number of aphids produced was the sole criterion for measuring aphid fitness.

Snail preference tests

Multiple-choice experiments were conducted in order to test for snail preference among leaves of the same plant species grown under different [CO_2] and fertility treatments. We followed the general procedures described by Grime et al. (1996). Fully expanded young leaves were collected from all the species in all the herbivore-free microcosms, with the exception of *Thymus praecox* and *Helianthemum nummularium*, due to insufficient growth. For each plant species, we randomly allocated 18 100-mm^2 leaf fragments per combination of [CO_2] and fertility treatment (three fragments per microcosms), and 18 fragments of filter paper (reference material), to positions in a grid which formed the floor of a 60 $\times$ 30 $\times$ 20 cm plastic box. Leaf fragments were secured to each cell of the grid using pins. At the beginning of the experiment, 15 juvenile (maximum shell diameter $\leq$10 mm) snails were released into each box, at random positions. After 11 h, an extra ten adult snails were introduced. The snails were not allowed to feed in the 48 h preceding the experiment. Boxes were kept in the dark at 20°C in a growth cabinet for 60 h. All their inner surfaces were intermittently sprayed with tap water to keep plant fragments in a turgid condition and to encourage snail feeding. Percentage of leaf area removed was estimated visually at increasingly longer time intervals (30 min, and 1, 2, 5, 10, 12, 24, 36, 48, and 60 h since the starting time). All the estimations were made by the same observer.

Statistical analysis

We analysed all plant and herbivore variables using analysis of variance (SPSS; Norusis 1992), accounting for split-plot design

(Montgomery 1991). In the case of plant variables, we used the following model:

$$Y_{ijkl} = \mu + C_i + e_{ij} + F_k + H_l + C \times F_{ik} + C \times H_{il} + F \times H_{kl} + C \times F \times H_{ikl} + \varepsilon_{ijkl} \quad (1)$$

In the case of assessments of snail growth and fecundity in microcosms, and snail preference and aphid fecundity tests, we used the following model:

$$Y_{ijkl} = \mu + C_i + e_{ij} + F_k + C \times F_{ik} + \varepsilon_{ijkl} \quad (2)$$

In these models, $[CO_2]$ (C_i), fertility (F_k), and herbivory (H_l) levels, $[CO_2]$ × fertility interaction ($C \times F_{ik}$), $[CO_2]$ × herbivory interaction ($C \times H_{il}$), fertility × herbivory interaction ($F \times H_{kl}$), and $[CO_2]$ × fertility × herbivory interaction ($C \times F \times H_{ikl}$) are fixed effects, and main plot error (e_{ij}) and sub-plot error (ε_{ijkl}) are random effects. F tests for C_i were performed using e_{ij} as the error term. F tests for F_k, H_l, their interactions, and the $C \times F \times H_{ikl}$ interaction were performed using ε_{ijkl} as the error term.

Results

Plant biomass

Elevated $[CO_2]$ did not significantly affect total biomass, whereas herbivory and fertilisation treatments showed strong and interactive effects (Fig. 1a).

The establishment of *Thymus praecox* was highly inconsistent between microcosms; therefore this species was excluded from the statistical analysis. There was no significant effect of elevated $[CO_2]$ on total biomass of fast- (Fig. 1b) or slow-growing species (Fig. 1c). Total biomass of fast-growing species was higher under high-fertility conditions, and lower in the presence of herbivores, as compared with the control treatment. However the interaction was significant, i.e. the difference between herbivory treatments were larger under high fertility (Fig. 1b). Total biomass of slow-growing species was unaffected by herbivory treatments, but decreased under high fertility (Fig. 1c).

There was no significant effect of elevated $[CO_2]$ on individual fast-growing species (Table 2). The growth of *Lathyrus pratensis* tended to increase under elevated $[CO_2]$, but this trend was not statistically significant. In contrast, herbivory and fertilisation strongly affected the biomass and in some cases also the reproductive output of most fast-growing species, exceptions being *Chamerion angustifolium* and *L. pratensis*. Herbivory significantly decreased, and fertilisation significantly increased the shoot biomass of *Arrhenatherum elatius* and *P. trivialis*. *Urtica dioica* did not survive under the impact of low-fertility and herbivory; this was the only species that showed a significant interaction between the effects of herbivory and fertilisation. However, generally, the reduced biomass of microcosms with herbivores was much more pronounced proportionally under fertilised compared to unfertilised conditions.

Seed production per microcosm by *G. aparine* was unaffected by elevated $[CO_2]$, but was significantly reduced by herbivory, and promoted by soil fertilisation (Fig. 1d, Table 2). Similarly, the number of flowerheads of *P. annua* showed no changes under elevated $[CO_2]$, significantly decreased with herbivory, and marginally increased with fertilisation (Fig. 1e, Table 2).

Among the slow-growing species, *C. flacca* was the only species whose growth was significantly promoted by elevated $[CO_2]$ (Table 2). *Lotus corniculatus* showed a statistically non-significant tendency to increase under CO_2 enrichment. The shoot biomass of *Carex caryophyllea*, *C. flacca*, *F. ovina* and *Helicotrichon pratense* significantly decreased with soil fertilisation. Only *C. flacca* showed a significant biomass increase in the presence of herbivores; and there was no significant interaction between the responses to fertilisation and herbivory. In the case of *Brachypodium pinnatum*, there was a significant interaction between elevated $[CO_2]$ and herbivory. This species was not significantly affected by elevated $[CO_2]$ in the absence of herbivores, but showed a marked decrease when exposed to elevated $[CO_2]$ and herbivores. However, *B. pinnatum* is extremely unpalatable (Grime et al. 1988) and did not appear to be subjected to herbivore attack in this experiment. We suggest that the most likely explanation for the suppression of *B. pinnatum* at elevated $[CO_2]$ was increased dominance by *L. corniculatus*. Fertilisation significantly decreased, and herbivory increased, the biomass of a number of slow-growing species, but there was no significant interaction between these two factors.

Foliar chemistry

Foliar nitrogen concentration significantly decreased under elevated $[CO_2]$ in the cases of *C. angustifolium* (ca. 35%), *U. dioica* (ca. 22%), and *C. flacca* (ca. 16%), and remained unchanged in the other species analysed (Table 3). Fertilisation produced significant increases in leaf [N] of *A. elatius* (ca. 20%), *Chamerion angustifolium* (ca. 17%), *P. trivialis* (ca. 14%), *U. dioica* (ca. 21%), *B. pinnatum* (ca. 19%), and *F. ovina* (ca. 17%).

Foliar concentrations of soluble tannins were unaffected by any of the treatments in the species analysed, with the exception of *Chamerion angustifolium* and *L. pratensis*, in which there were significant increases under elevated CO_2 (ca. 27% and ca. 51%, respectively), and significantly decreased (ca. 14% and ca. 29%, respectively) under high soil fertility (Table 3).

Herbivore fitness

None of the variables used to estimate herbivore fitness was significantly ($P \leq 0.05$) affected by $[CO_2]$. Fertilisation significantly increased snail fecundity in microcosms ($P = 0.035$) and aphid fecundity on isolated shoots of fast- or slow-growing grasses ($P = 0.05$, 0.03, 0.05, and 0.06, of *P. trivialis*, *P. annua*, *F. ovina*, and *H. pratense*, respectively), but showed no significant effect on snail growth.

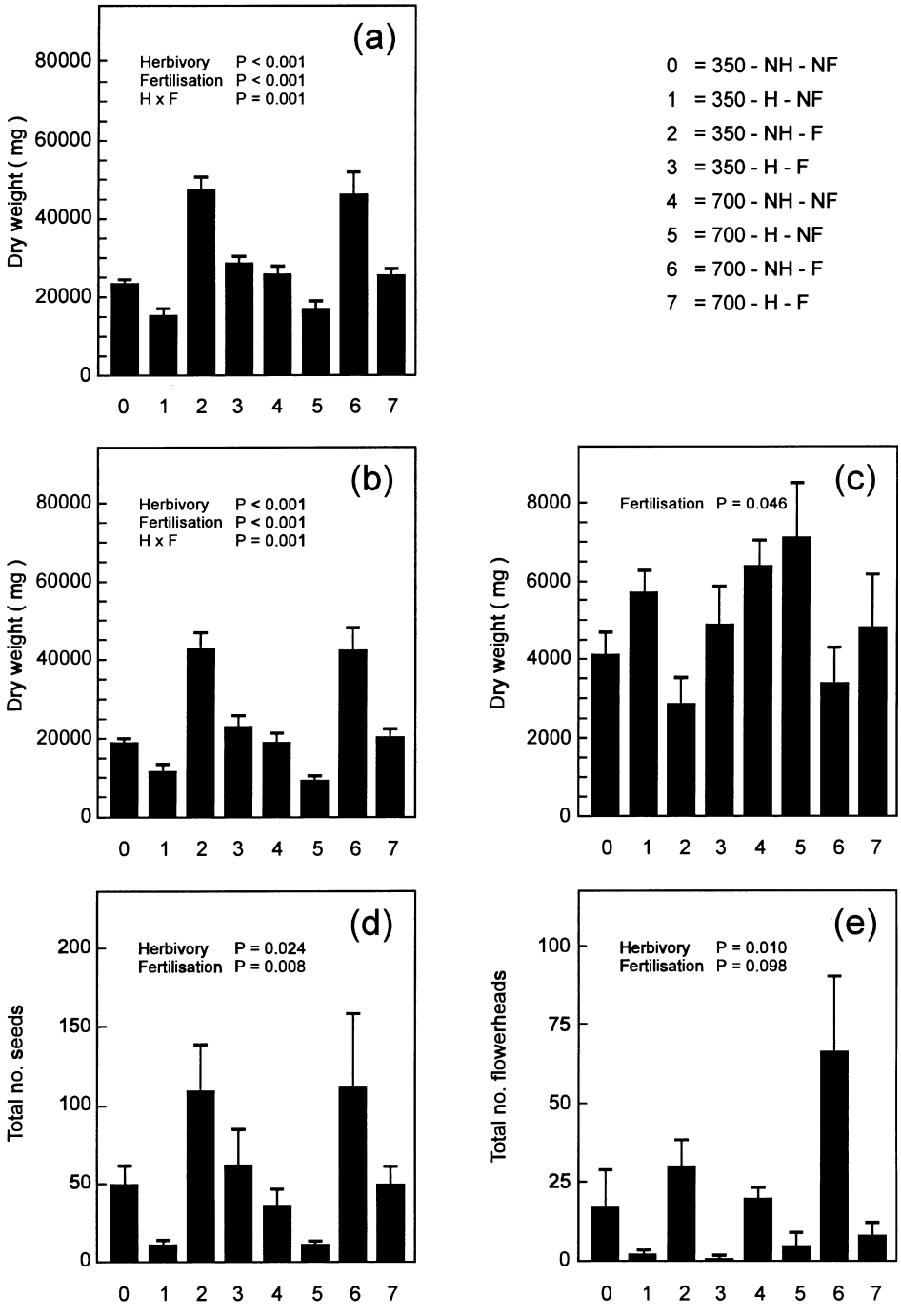

Fig. 1a–e Effects of elevated [CO_2], herbivory, and fertilisation on total live shoot biomass (**a**), biomass of fast-growing species (**b**), biomass of slow-growing species (**c**), and number of seeds of *Galium aparine* (**d**) and of flowerheads of *Poa annua* (**e**) per microcosm. Values are means + 1 SE. Numbers on *X* axis indicate different combinations of [CO_2] (350 or 700 µl l^{-1}), herbivory (*NH* herbivores absent, *H* herbivores present), and soil fertility (*NF* non-fertilised, *F* fertilised). Treatment effects not shown were not significant ($P > 0.05$; ANOVA)

Snail preferences

No significant differences ($P \leq 0.050$) were detected between the preferences of juvenile and adult snails; only results corresponding to the later group are presented. Three of the fast-growing species (*A. elatius*, *Chamerion angustifolium*, and *L. pratensis*), and all of the slow-growing species except *L. corniculatus*, were not consumed. Among the palatable species (*G. aparine*, *P. annua*, *P. trivialis*, *U. dioica*, and *L. corniculatus*), consumption of leaves grown under high [CO_2] by snails was not significantly different from consumption of leaves grown in ambient conditions. Leaves of potentially fast-growing plants were significantly preferred by snails when grown under high fertility conditions ($P = 0.007$, 0.003, 0.001, and 0.001 for *G. aparine*, *P. annua*, *P. trivialis*, and *U. dioica*, respectively). Snails did not discriminate between *L. corniculatus* leaves grown under high and low fertility conditions, and in this species only ca. 5% of the leaf area offered was consumed.

Table 2 Effects of [CO_2] (350 or 700 μl l^{-1}), herbivory (*NH* herbivores absent, *H* herbivores present), and soil fertility (*NF* non-fertilised, *F* fertilised) on shoot biomass (mg dry weight) or reproductive output (in the case of *Poa annua* and *Galium aparine*) of plant communities grown in microcosms (*P* values; ANOVA). – = single-factor effect not considered when interaction was significant. [CO_2] × Fertilisation and [CO_2] × Herbivory × Fertilisation interactions were not significant ($P > 0.05$; ANOVA). The establishment of *Thymus praecox* was highly inconsistent between microcosms; therefore this species was excluded from the statistical analysis

	Statistic	350-NH-NF	350-H-NF	350-NH-F	350-H-F	700-NH-NF	700-H-NF	700-NH-F	700-H-F	Results of ANOVA				
										[CO2]	Herbivory	Fertilisation	[CO_2] × herbivory	Herbivory × Fertilisation
Fast-growing species														
Urtica dioica	Mean	913	0	11106	5404	648	16	10741	3582	>0.050	–	–	0.650	0.007
	1 SE	159	0	3137	1987	124	16	2415	1149					
Chamerion angustifolium	Mean	4438	6463	2856	4507	5083	3470	2336	4648	>0.050	0.217	0.165	0.475	0.388
	1 SE	1260	1863	882	1477	1047	980	499	1143					
Arrhenatherum elatius	Mean	3495	2179	7698	4993	2631	2842	6761	3138	>0.050	0.025	0.002	0.691	0.560
	1 SE	788	475	1310	1917	549	357	1523	696					
Poa trivialis	Mean	8555	2429	19653	6744	7781	1973	16262	6925	>0.050	>0.001	>0.001	0.503	0.095
	1 SE	1161	423	2650	1375	1772	903	5887	1215					
Lathyrus pratensis	Mean	932	746	1120	891	1658	1260	2231	1011	>0.050	0.164	0.615	0.385	0.586
	1 SE	370	56	640	365	243	433	1225	204					
Poa annua (*no. of flowerheads*)	Mean	17	2	30	1	20	5	67	8	>0.050	0.010	0.098	0.389	0.133
	1 SE	12	1	8	1	3	4	23	4					
Galium aparine (*no. of seeds*)	Mean	50	11	110	62	36	11	112	50	>0.050	0.024	0.008	0.974	0.471
	1 SE	13	2	29	24	10	2	47	11					
Slow-growing species														
Lotus corniculatus	Mean	1191	1072	869	1723	2441	1522	2048	2048	>0.050	0.588	0.691	0.816	0.370
	1 SE	337	167	437	783	456	633	824	824					
Brachypodium pinnatum	Mean	609	1155	460	1289	604	772	364	728	–	–	0.516	0.040	0.213
	1 SE	118	179	45	269	140	135	83	118					
Helichotrichon pratense	Mean	548	860	267	360	687	885	447	280	>0.050	0.422	0.013	0.385	0.277
	1 SE	95	214	100	93	115	263	112	82					
Helianthemum nummularium	Mean	100	74	1	43	72	327	4	17	>0.050	0.364	0.124	0.418	0.577
	1 SE	77	18	1	28	20	237	3	11					
Carex caryophyllea	Mean	416	565	432	341	588	580	320	308	>0.050	0.972	0.023	0.666	0.397
	1 SE	74	85	174	66	80	104	82	50					
Carex flacca	Mean	607	895	324	674	1220	1054	366	920	<0.050	0.025	0.009	0.621	0.641
	1 SE	114	212	118	151	238	501	88	313					
Festuca ovina	Mean	597	1071	416	436	645	1000	378	493	>0.050	0.099	0.018	0.956	0.210
	1 SE	161	242	66	141	28	272	144	132					

Table 3 Leaf quality of the ten most abundant species at harvest. Results of ANOVA are indicated as positive (+), negative (−), and significant at $P \leq 0.05$ (**), at $P \leq 0.01$ (***), or non significant (*ns*). No significant two- or three way interaction between factors was detected. Values obtained by each species under each combination of treatments are available upon request

	Leaf [N] (mg g dry weight^{-1})		Soluble tannins (% dry weight)	
	[CO_2]	Fertilisation	[CO_2]	Fertilisation
Arrhenatherum elatius	ns	(+)***	ns	ns
Chamerion angustifolium	(−)***	(+)***	(+)***	(−)***
Lathyrus pratensis	ns	ns	(+)***	(−)***
Urtica dioica	(−)**	(+)***	ns	ns
Brachypodium pinnatum	ns	(+)***	ns	ns
Carex flacca	(−)**	ns	ns	ns
Festuca ovina	ns	(+)***	ns	ns
Helicotrichon pratense	ns	ns	ns	ns
Lotus corniculatus	ns	ns	ns	ns
Poa trivialis	ns	(+)***	ns	ns

Discussion

Our predictions of the effects of elevated [CO_2], based on reported results from previously published single-species studies, were not supported by the results of this multi-species microcosm study. Overall, elevated [CO_2] did not modify the biomass of fast-growing or slow-growing plants, or generalist herbivore interactions within the synthesised plant community. Furthermore, no major impacts were detected on herbivore feeding or herbivore fitness. Similar patterns were found by Arnone et al. (1995) and Ledergerber et al. (1997). It seems that the only significant [CO_2] effect was an increase in the biomass of *C. flacca*, and changes in the chemical composition of five species, under elevated [CO_2]. In considering the significance of this lack of response to elevated [CO_2] it is important to note that under the conditions prevailing in the experiment marked changes in community composition occurred as a consequence of soil fertilisation and exposure to herbivores. This confirms that the low responsiveness to [CO_2] did not arise from an innately low lability in the experimental assemblage. Longer experiments (Fraser 1996; Fraser and Grime 1997) have shown effects of fertilisation and herbivory on plant communities similar to those reported here. This further strengthens the conclusion that the low responsiveness to elevated [CO_2] was not due to a general inertia of the experimental assemblage. However, it may be argued that, should our experiment have run longer, elevated [CO_2] would induce changes in plant and herbivore performance. Significant elevated [CO_2]-induced changes have been detected in the biomass and/or chemistry of plants ranging from annuals to long-lived trees, and under conditions ranging from microcosm to field experiments (Körner and Arnone 1992; Díaz et al. 1993; Leadley and Stöcklin 1996; Ball and Drake 1997; Hirschel et al. 1997), after periods of similar or shorter duration. Our experiment also involved the longest period of plant-herbivore interaction under elevated [CO_2] reported to date. However, we cannot rule out the potential for some longer-term [CO_2]-induced effects on herbivory via shifts in species abundance. Changes in plant species relative abundance, which appear subtle after 4 months, may became more important with time, as shown by Potvin and Vasseur (1996) and by Stöcklin et al. (1998) for studies on the effect of [CO_2] over 3 years and two seasons, respectively. As pointed out by Arnone et al. (1995) generalist herbivore performance is unlikely to be affected by slight shifts in quantity or quality of leaf tissue of various species occurring under elevated [CO_2]. However, if the preferred plant species become less abundant with time, generalist herbivore insect populations may be adversely affected. For example, if the non-significant biomass increase of legumes and sedges of low palatability in combination with the decrease of palatable *U. dioica* were accentuated with time the availability of forage would be reduced, leading to a potential decrease in herbivore fitness.

Plant community composition

There was no significant direct effect of elevated [CO_2] on plant community composition. *C. flacca*, the only species significantly promoted by elevated [CO_2], represented in all cases less than 5% of total live biomass. Stimulation of *C. flacca* by elevated [CO_2] has been observed in multispecies assemblages by Leadley and Körner (1996); Stöcklin et al. (1997, 1998); and Spring (1997). Responsiveness to elevated [CO_2] in *C. flacca* may arise from possession of dauciform roots (Torrey and Clarkson 1975) which are suspected to confer an unusual ability to obtain phosphorus under conditions limiting the capacity of other species to respond to elevated CO_2. There were also positive, albeit non-significant, responses of the legumes *L. pratensis* and *Lotus corniculatus* to CO_2 enrichment. Growth stimulation of legumes by elevated [CO_2] has been often, though not universally, reported for mixed-communities (see Díaz 1996 for review). The increase of *L. corniculatus* biomass may explain the decreased growth of *B. pinnatum* under the elevated [CO_2] plus herbivory treatment. This species, however, represented less than 8% of total biomass in all treatments.

Herbivore responses

The lack of significant effect of elevated [CO_2] on herbivore preferences or fitness is consistent with the results

of the chemical analysis. Elevated [CO_2] was associated with decreased foliar [N] only in three species. Two of them (*Chamerion angustifolium* and *C. flacca*) were not palatable. In the case of *U. dioica*, the observed decrease in [N] was not associated with changes in snail fitness or in preferences in multiple-choice tests. Soluble tannins increased significantly in only two species (*Chamerion angustifolium* and *L. pratensis*), both of which are unpalatable under ambient [CO_2] levels.

Although a decrease of leaf [N] with a concomitant increase in herbivore consumption and decrease in herbivore fitness is considered a common response to CO_2 fertilisation (see Lincoln et al. 1993; Watt et al. 1995; Lindroth 1996 for review), many exceptions have been reported in the literature, involving both chewing and sucking insects. Lindroth et al. (1993) fed two species of lepidopteran herbivores on red oak grown under elevated [CO_2]. They found that [N] increased, and herbivore fitness was enhanced in one case and unaffected in the other. Lindroth et al. (1997), working with gypsy moth and quaking aspen, reported only marginal decrease in [N] of leaves, and prolonged development and increased consumption, but unchanged fecundity and final pupal weight, of herbivores. Williams et al. (1997) reared larvae of the red-headed pine sawfly and found no overall effects of elevated [CO_2] on herbivore fitness (larvae seemed able to compensate for the reduced quality of mature pine leaves by consuming larger amounts of chemically unaffected young leaves). Brooks and Whittaker (1998) found that the effects of elevated [CO_2] on larvae of the beetle *Gastrophysa viridula* reared on *Rumex obtusifolius* over three generations was minimal, despite a consistent decrease in leaf [N]. Working with sucking herbivores, Awmack et al. (1996) found no changes in [N] of winter wheat and increased infection by, but unchanged population growth rate of, *S. avenae* under elevated [CO_2]. Salt et al. (1996) reported no significant effect of elevated [CO_2] on the performance of shoot and root aphids and their forb host. Awmack et al. (1997) reported increased fitness of the aphid *Aulacorthum solani* reared on bean and tansy under elevated [CO_2]. Docherty et al. (1997) found no significant changes in [N] of tree leaves or in performance of generalist and specialist aphids and leafhoppers feeding on them, under elevated [CO_2].

In community-level studies, Thompson and Drake (1994) found decreased [N] in natural near-monocultures of *Scirpus olneyi*, together with decreased insect herbivore infection, under CO_2 enrichment. Arnone et al. (1995) found no changes in leaf quality of tropical plants, or lepidopteran consumption or fitness under elevated [CO_2]. Ledergerber et al. (1997) reported no changes in plant damage or herbivore density under CO_2 enrichment in field experiments involving calcareous grasslands, gastropods and grasshoppers. It is apparent that the number of studies and the range of species covered are still too limited to draw general conclusions, and that decrease of plant nutritional quality and decreased fitness and increased consumption by herbivores cannot be taken as a general rule (see Peñuelas and Estiarte 1998 for a review).

In our study, there was no significant effect of elevated [CO_2] on plant community composition mediated by herbivores. Furthermore, although the analysis of secondary metabolites was restricted to soluble tannins, we found no evidence of higher accumulation of these compounds by slow-growing plants than by fast-growing plants, as suggested by Lambers (1993). Therefore, we may infer that slow-growing plants of infertile conditions tend to exhibit low palatability as a constitutive feature rather than an attribute significantly enhanced (made lower) by elevated [CO_2].

All plant species whose biomass significantly increased under fertilisation were also significantly affected by herbivory. Furthermore, leaves grown under high-fertility conditions were preferred by snails. This is in accordance with models (e.g. Bryant et al. 1983), in which fast-growing species are expected to be more responsive to changes in the resource base of the system. The final yields of different species are likely to reflect both direct effects of consumption and fertilisation and indirect effects, such as plant regrowth after defoliation and plant-plant interactions (Crawley 1983). Although consumption was not directly measured, these results suggest it was higher in fast-growing plants growing under high-fertility conditions.

Implications for plant-herbivore interactions in the field: utility and limitations of the approach

To our knowledge, this experiment is the first reporting the outcome of generalist herbivory on multi-species plant assemblages under different [CO_2] and fertility conditions. The study involved herbivores of contrasting habits and growth rates, which were given the opportunity to choose among different kinds of plants, and exploited the plant community for more than one generation. Elevated [CO_2] experiments on the interaction between single plant and (usually specialist) insect herbivore species, and in most cases involving one fertility level, have provided invaluable information. However, our results, together with those from the very few examples available involving communities (e.g. Arnone et al. 1995; Lederberger et al. 1997), suggest that caution is needed in extrapolating these results beyond the individual to the impacts of elevated [CO_2] on the interactions between generalist herbivores and species-rich plant communities. Some of the effects of elevated [CO_2] which appear to be strong and consistent on individual species are often buffered or even reversed in systems including many species (see reviews by Koch and Mooney 1996 and Körner and Bazzaz 1996). On the basis of the evidence to date, elevated [CO_2] seems more likely to affect vegetation composition than short-term production or carbon storage. Shifts in species composition and relative abundance, which may seem subtle at first inspection, have the potential to trigger indirect changes at

the whole community or ecosystem level (see Körner et al. 1996 an references therein).

Community microcosms experiments are too artificial to allow direct extrapolation of results to real systems. However, together with good control and high replication of experimental conditions, they can provide interesting mechanistic insights into community and ecosystem processes, and they are useful "stepping stones" between single-species cabinet experiments and large-scale field experiments (Grime et al. 1987, see also reviews by Lawton 1995 and Fraser and Keddy 1997).

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