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Interacting effects of elevated CO_2, nutrient availability and plant species on a generalist invertebrate herbivore

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Abstract

By affecting plant growth and phytochemistry elevated CO_2 may have indirect effects on the performance of herbivores. These effects show considerable variability across studies and may depend on nutrient availability, the carbon/nutrient-balance in plant tissues and the secondary metabolism of plants. We studied the responses to elevated CO_2 and different nutrient availability of 12 herbaceous plant species differing in their investment into secondary compounds. Caterpillars of the generalist herbivore *Spodoptera littoralis* were reared on the leaves produced and their consumption and growth rates analysed. Elevated CO_2 resulted in a similar increase of biomass in all plant species, whereas the positive effect of fertilization varied among plant species. Specific leaf weight was influenced by elevated CO_2, but the effect depended on nutrient level and identity of plant species. Elevated CO_2 increased the C/N ratio of the leaves of most species. Caterpillars consumed more leaf material when plants were grown under elevated CO_2 and low nutrients. This indicates compensatory feeding due to lower tissue quality. However, the effects of elevated CO_2, nutrient availability and plant species identity on leaf consumption interacted. Both the effects of CO_2 and nutrient availability on the relative growth rate of the herbivore depended on the plant species. The feeding rate of *S. littoralis* on plant species that do not produce nitrogen-containing secondary compounds (NCSC) was higher under low nutrient availability. In contrast, in plants producing NCSC nutrient availability had no effect on the feeding rate. This suggests that compensatory feeding in response to low nutrient contents may not be possible if plants produce NCSC. We conclude that elevated CO_2 causes species-specific changes in the quality of plant tissues and consequently in changes in the preferences of herbivores for plant species. This could result in changes in plant community composition.

Keywords: C/N ratio, herbivory, insect growth rate, leaf consumption, palatability, plant growth, plant secondary compounds, plant species identity, specific leaf weight, *Spodoptera littoralis*

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Introduction

The concentration of atmospheric CO_2 is expected to increase in the next 100 years to 500–900 ppm (Houghton *et al.*, 2001). Elevated CO_2 has been shown to have a number of effects on the physiology, growth and reproduction of plants (Körner & Bazzaz, 1996; Körner, 2003). In general, plants tend to increase rates of photosynthesis, assimilation and development. However, these effects vary considerably among plant species and depend on other environmental factors (Jablonski *et al.*, 2002), which makes predictions of community responses to changes in atmospheric CO_2 difficult.

Insect herbivores are an omnipresent and often rather subtle cause of vegetational change in terrestrial ecosystems (Brown & Gange, 1992; Schädler *et al.*, 2004). The intensity of feeding by insect herbivores on different host plants depends on a number of plant traits which are known to be influenced by the CO_2-content of the atmosphere, including leaf thickness, tissue C/N ratio, and the secondary compound and water content

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of tissues (e.g. Ayres, 1993; Lincoln *et al.*, 1993; Watt *et al.*, 1995; Poorter *et al.*, 1996; Bezemer & Jones, 1998; Cotrufo *et al.*, 1998a). The growth, fecundity and population dynamics of herbivores may, therefore, be affected by changes in host plant quality due to elevated CO_2, potentially modifying their top-down effects on plant performance.

The carbon-nutrient balance hypothesis suggests that the allocation of resources to secondary compounds is directly controlled by the carbon-nutrient status of a plant (Bryant *et al.*, 1983; Bazzaz *et al.*, 1987, but see Hamilton *et al.*, 2001). Following this hypothesis, investment in carbon-based defences increases with increasing photosynthesis and decreasing nitrogen availability, whereas the opposite should increase reliance on nitrogen-based compounds. Accordingly, plants grown under elevated CO_2 are often characterized by increased contents of carbon-based secondary compounds and nitrogen depletion (Körner, 2000). This, in turn, may cause a lowered production of nitrogen-containing secondary compounds (hereafter called NCSCs), especially if nutrient availability is low (Cotrufo *et al.*, 1998b, Geiger *et al.*, 1999). Other studies, however, have indicated that elevated CO_2 leads to a reallocation of nitrogen to secondary metabolism in *Eucalyptus* trees because of a higher efficiency of photosynthesis (Gleadow *et al.*, 1998). Overall, this suggests that elevated CO_2 should reduce the suitability of plants, which produce NCSCs as hosts for herbivores. However, there may not be a negative effect of elevated CO_2 on the production of NCSCs if the availability of nitrogen is high (Matros *et al.*, 2006). The general applicability of the carbon-nutrient balance hypothesis has been doubted (Hamilton *et al.*, 2001). First, the synthesis of nitrogen-containing compounds is often costly in terms of carbon-based primary compounds, whereas the production of some carbon-based compounds may require a high investment into enzymes. Second, allocation of resources to plant growth may not always have priority over the production of secondary metabolites (Hamilton *et al.*, 2001). However, both the secondary chemistry and the nutrient content of a plant clearly affect herbivore performance (Mattson, 1980). It is, therefore, not surprising that growth and development of insect herbivores has often been found to be negatively affected by elevated CO_2 (Bezemer & Jones, 1998), although these effects vary considerably among specific insect–plant pairings and between feeding guilds. While leaf chewers and leaf miners tended to respond negatively to elevated CO_2, some phloem-feeders and whole-cell-feeders showed positive responses. However, due to a lack of studies, it is not clear how general these conclusions are (Bezemer & Jones, 1998).

Plant responses to elevated CO_2 are often suggested to be more accentuated in nutrient-enriched conditions (Goverde *et al.*, 2002; Körner, 2003 and references therein), because plant growth and development are often limited by the availability of nutrients. One may, therefore, expect that the influence of elevated CO_2 on plant–insect interactions is strongly modified by the nutrient status of a plant. For instance, the reduction in the concentration of tissue N in response to elevated CO_2 is more pronounced when plants are fertilized (Goverde *et al.*, 2002). Further, the high variability of herbivore responses to plants treated with elevated CO_2 may at least partly be due to differences in the nutritive status of plants used in different experiments. While some studies did not find interacting effects of nutrients and CO_2 (Hättenschwiler & Schafellner, 1999; Goverde & Erhardt, 2003) or weak effects (Kinney *et al.*, 1997; Williams *et al.*, 1997), Kerslake *et al.* (1998) found that elevated CO_2 cancelled out positive effects of fertilization on the pupal weight of a moth.

We investigated the effects of elevated CO_2 and nutrient availability on growth and leaf quality of a set of plant species differing in their ability to invest into secondary defence compounds and their effects on the feeding and growth of a generalist herbivore, the caterpillars of the noctuid moth *Spodoptera littoralis*. We hypothesize that elevated CO_2 will decrease the quality of species which do not produce NCSCs as a host plant for the herbivore more strongly than that of species which do produce NCSCs, but only under conditions of low nutrient availability.

Methods

Experimental setup

For our experiment, we used a set of 12 plant species from a wide taxonomic range (10 families) that differed in their ability to invest into secondary compounds (see Appendix A). In January 2003, seeds were sown into pots (9 cm×9 cm×12 cm) filled with standard potting soil (TKS 1, Floragard, Oldenburg, Germany, $110\,mg\,L^{-1}\,N$, $130\,mg\,L^{-1}\,P_2O_5$, $180\,mg\,L^{-1}\,K_2O$). Depending on the plant species, seedlings were thinned to two to seven individuals per pot to obtain approximately the same amount of biomass. Plants were grown in four closed tents (1 m×1 m×1 m) made from translucid plastic foil in a walk-in growth chamber. Light was provided $16\,h\,day^{-1}$ by high-pressure sodium lamps (FP Riburo, Powerstar HQI-T, Osram, Germany) and high-pressure mercury lamps (Eyelightning, MT 400DL/BH, Eye, Japan) resulting in a light intensity of ca. 200 μmol photons $s^{-1}\,m^{-2}$ in the tents. The temperature in the tents was kept at 22 °C during the day and 15 °C at night.

Two chambers were maintained at ambient CO_2 (375 ppm) and two at elevated CO_2 (725 ppm). Airflow was 400 L h^{-1} in every each tent. CO_2 levels were controlled using an infra-red gas analyser (Anagas CD 98, Environmental Instruments, Leamington, UK).

After germination four pots per species were placed into each tent. Two pots per tent and species were fertilized once per week with 50 mL of a 0.3% solution of an NPK-fertilizer (8-8-6, Wuxal super, Aglukon, Düsseldorf, Germany). In total, the experiment consisted of 192 pots (12 species × two levels of fertilization × two levels of CO_2 × four replicates). Every week, the CO_2 treatment of each tent was changed, and the pots were moved accordingly. In addition, pots were randomly exchanged between the two tents that received the same CO_2 treatment to avoid pseudoreplication and tent effects.

Measurement of plant growth

After 5 weeks of growth the height and number of leaves were determined for each plant. In three of the species (*A. githago, Chenopodium bonus-henricus, Digitalis purpurea*) some of the leaves had already become yellow and we determined the proportion of yellow leaves. The chlorophyll content of three leaves of the same age per pot was measured with a hand-held chlorophyll metre (SPAD-502, Minolta, Japan). The three measures were then averaged for each pot and analysed. The values measured by the instrument are related by a second order polynomial to the actual chlorophyll content of a leaf (Richardson *et al*., 2002) and we report chlorophyll content values obtained using the equation for total chlorophyll content given by Richardson *et al*. (2002).

To investigate specific leaf weight (SLW) a predefined leaf of the tallest plant per pot (e.g. the third leave from the apex in *Nicotiana tabacum*) was harvested, scanned, dried and weighed. The area of the leaf images was determined with Adobe Photoshop 6.0. SLW was calculated as the ratio of leaf dry mass (mg) over leaf area (cm^2).

Plants were cut at ground level, dried at 80 °C for 24 h, and weighed. One leaf per pot was ground in a mill and its carbon and nitrogen content was analysed with a CHNS-932 Elemental Analyzer (Leco Corporation, St Joseph, MI, USA).

Palatability trials and relative growth rate of Spodoptera *caterpillars*

Leaf palatability was assayed after 5 weeks of plant growth using the caterpillars of the cotton leafworm *S. littoralis* (Boisduval) (Lepidoptera: Noctuidae). *S. littoralis* is a serious pest of crops in North Africa, the Mediterranean and the Middle East (Sadek, 2003). The caterpillars of this moth attack host plants belonging to at least 40 plant families (Brown & Dewhurst, 1975).

Two-week-old caterpillars of *S. littoralis* (instars 2 and 3) were weighed and placed individually into plastic tubes. Two discs of 20 mm diameter were taken from one fully expanded, nonsenescent leaf per pot avoiding the midrib and weighed. One of the discs was placed together with a piece of wet filter paper into a plastic tube (diameter 4.5 cm, length 8 cm). A 2-week-old caterpillar of *S. littoralis* (instars 2 or 3) was weighed and placed into the tube. The tube was then closed by a pad of cotton wool at one end and by a perforated plastic cap at the other. The second disc taken was used for the determination of the water content of the leaves. The test ran for 24 h at 24 °C with a photoperiod of LD 16/8. After the tests, the remaining leaf material was dried at 80 °C to weight constancy.

After the feeding test, the caterpillars were provided with 200–400 mg of fresh leaf material from the same plants as in the palatability trial. After a further 6 days the experiment was terminated and the caterpillars were weighed. The leaves were dried at 80 °C for 24 h, and weighed. Relative growth rate (RGR) of the caterpillars was calculated as $\mathrm{RGR} = (\ln(w_e) - \ln(w_i))/d$, where w_e is the mass of the caterpillar at the end of the experiment, w_i the initial mass and d the duration of the experiment in days. The leaf dry mass consumed by the caterpillars was calculated as the difference between initial dry mass (calculated as fresh mass × [1 − water content]) and remaining dry mass of the leaves at the end of the palatability trials.

Statistical analyses

The effects of plant species, CO_2 concentration and fertilization on plant growth and growth of caterpillars were analysed by three-way ANOVAs. The effects of the studied factors on consumed leaf material were analysed by a three-way ANCOVA with initial herbivore fresh weight as a covariate (Raubenheimer & Simpson, 1992; Horton & Redak, 1993). SLW and C/N ratio are known to influence feeding by herbivores (Schädler *et al*., 2003). We, therefore, initially tested the effects of both factors on leaf consumption and RGR of caterpillars by including them as covariates in the analyses. However, every covariate, which did not show a significant effect on these traits was eliminated from the analyses. All variables were tested for homogeneity of variance and normality of residuals. Only data on plant biomass has to be log-transformed to satisfy both criteria. In a further analysis, plant species were grouped into species, which produce NCSC and species, which do not. The effects of NCSC, elevated CO_2, fertilization and

Table 1 ANOVA results of the effects of plant species, elevated CO_2 and fertilization on aboveground biomass, specific leaf weight (SLW), C/N ratio and chlorophyll content of 12 plant species

	Biomass			SLW			C/N			Chlorophyll		
Source of variation	df	MS	*F*	df	MS	*F*	df	MS	*F*	df	MS	*F*
Species (S)	11	2.14	60.9***	11	17.90	7.7***	11	1699.58	63.4***	11	0.20	55.1***
CO_2	1	0.44	12.5***	1	22.2	9.5**	1	908.35	33.9***	1	0.05	12.4***
Fertilizer (F)	1	8.09	229.2***	1	0.1	0.1	1	3562.71	132.8***	1	0.35	98.2***
$S \times CO_2$	11	0.04	1.2	11	10.55	4.5***	11	167.20	6.2***	11	0.01	2.6***
$S \times F$	11	0.77	21.9***	11	9.84	4.2***	11	469.00	17.5***	11	0.02	4.5***
$CO_2 \times F$	1	0.01	0.4	1	0.8	0.3	1	0.35	0.1	1	<0.001	0.06
$S \times CO_2 \times F$	11	0.04	1.1	11	4.81	2.1*	11	33.52	1.3	11	0.002	0.6
Residual	144	0.03		143	2.32		135	26.82		140	0.004	

Residual degrees of freedom differ due to different numbers of replicates.
Significance levels are indicated by $^*P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

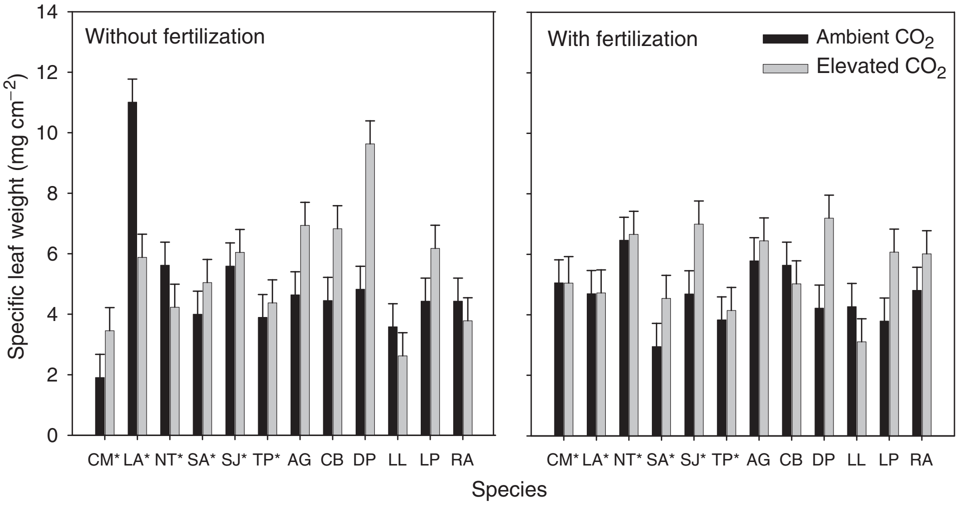

Fig. 1 Effect of elevated CO_2 and fertilization on the specific leaf weight of 12 plant species (means ± standard error). AG, *Agrostemma githago*; CB, *Chenopodium bonus-henricus*; CM, *Conium maculatum*; DP, *Digitalis purpurea*; LP, *Lolium perenne*; LA, *Lupinus angustifolius*; LL, *Lupinus luteus*; NT, *Nicotiana tabacum*; RA, *Rumex acetosella*; SJ, *Senecio jacobea*; SA, *Sinapis alba*; TP, *Trifolium pratense*. *Plant species that produce nitrogen-containing secondary compounds.

plant species (nested within NCSC) on leaf consumption and growth rate of caterpillars were tested using a nested general linear model in SAS 9.

Results

Plant growth

Across all species elevated CO_2 increased the aboveground biomass of the plants by 25% (Table 1). This effect did not differ significantly among species and was not influenced by fertilization. Not surprisingly, the application of fertilizer also increased the biomass, but the various studied species responded differently to this treatment (significant species × fertilization interaction, Table 1).

SLW increased with elevated CO_2 when averaged across all investigated plant species (Fig. 1, Table 1). However, magnitude and direction of this effect varied among the plant species and were also affected by fertilization (significant three-way interaction, Table 1, Fig. 1). For instance, the SLW of *D. purpurea* strongly increased under elevated CO_2 in the unfertilized treatment, whereas the SLW of *Lupinus angustifolius* decreased. With fertilization, however, these effects were much weaker.

On average, elevated CO_2 increased the C/N ratio of leaves by 23.5%, but the effect was not consistent among

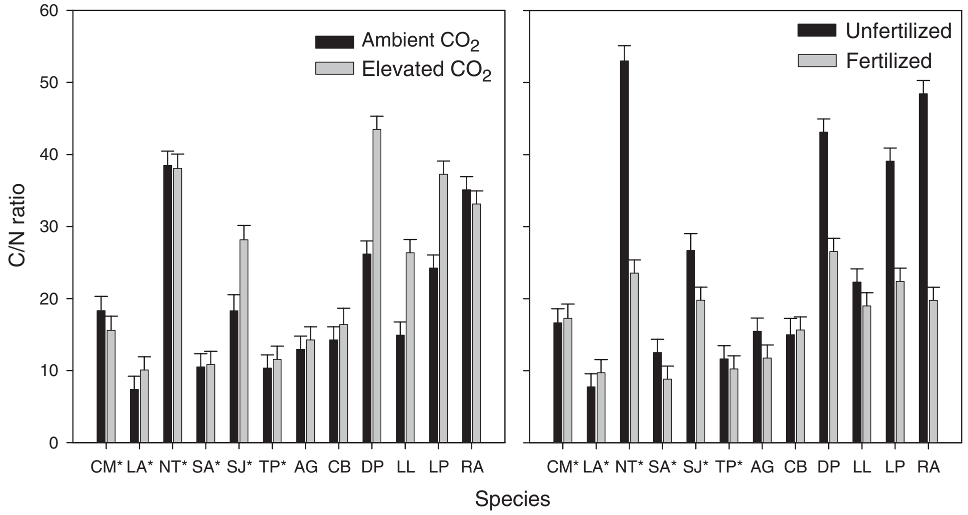

Fig. 2 Effect of elevated CO_2 (left) and fertilization (right) on the C/N ratio of leaves of 12 plant species (means ± standard error). See Fig. 1 for abbreviations of plant species. *Plant species that produce nitrogen-containing secondary compounds.

Table 2 ANOVA results of the effects of plant species, elevated CO_2 and fertilization on the number of leaves, plant height and proportion of yellow leaves

	Number of leaves			Height			% yellow leaves		
Source of variation	df	MS	*F*	df	MS	*F*	df	MS	*F*
Biomass	1	9.49	1604.9***	1	15943.5	579.1***	—	—	—
Species (S)	11	1.77	298.54***	8	7272.0	264.2***	2	0.137	10.54***
CO_2	1	0.02	3.58$^{(*)}$	1	43.1	1.56	1	0.104	8.00**
Fertilizer (F)	1	0.18	29.73***	1	36.76	1.34	1	0.022	1.70
S × CO_2	11	0.01	1.71$^{(*)}$	8	29.90	1.09	2	0.102	7.91***
S × F	11	0.04	7.52***	8	79.82	2.90***	2	0.030	2.32
CO_2 × F	1	0.01	0.03	1	12.78	0.46	1	0.002	0.16
S × CO_2 × F	11	0.004	0.61	8	17.02	0.62	2	0.075	5.80**
Residual	143	0.006		107	27.53		36	0.01	

Aboveground biomass was included as a covariate for the analyses of number of leaves and height. The residual degrees of freedom differ due to different numbers of replicates.

Significance levels are indicated with $^{(*)}P<0.1$, $^{**}P<0.01$ and $^{***}P<0.001$.

species (Fig. 2, Table 1). While in some species there was a strong increase in the C/N ratio of leaves with elevated CO_2 (*D. purpurea, Senecio jacobea, Lolium perenne, Lupinus luteus*), most species did not respond. When averaged across all species, fertilization decreased C/N ratio by 34.4%. Again, this effect varied depending on plant species identity (Fig. 2, Table 1). The chlorophyll content of leaves decreased on average under elevated CO_2 by 11.6%, but increased with fertilization by 43.6%. The effect of both factors differed between the studied plant species (Table 1).

The CO_2 level also influenced the growth form of the plants. Under elevated CO_2 plants produced more leaves (ANOVA, $F_{1,44} = 11.6$, $P = 0.0008$) and grew higher (ANOVA, $F_{1,108} = 4.41$, $P = 0.038$). The increase in leaf production was not merely an effect of increased size, because the effect of CO_2 level remained, if biomass was included as a covariate (Table 2). In contrast, the CO_2 effect on plant height was only due to a general effect on size.

Three plant species showed signs of senescence and developed yellow leaves during the experiment. Elevated CO_2 influenced the proportion of yellow leaves per plant, indicating changes in phenology, but this effect was not consistent among species (Table 2). While two plant species (*A. githago* and *C. bonus-henricus*) produced more yellow leaves under elevated CO_2,

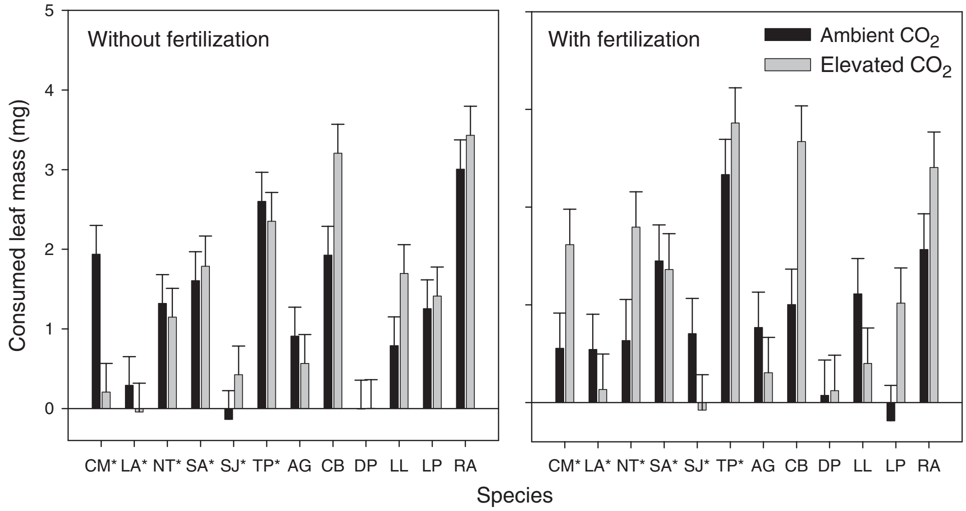

Fig. 3 Effect of elevated CO_2 (left) and fertilization (right) on the leaf mass of different plant species consumed by caterpillars of *Spodoptera littoralis* (means ± standard error). Consumed leaf mass was adjusted for the effect of initial herbivore mass. See Fig. 1 for abbreviations of plant species. Negative values can result in hardly consumed species if the water content of their leaves was slightly lower than in the reference leaf discs. *Plant species that produce nitrogen-containing secondary compounds.

D. purpurea produced less (significant species × CO_2 interaction, Table 2).

Palatability trials and RGR of Spodoptera *caterpillars*

The caterpillars responded differently to each plant species (Fig. 3, Table 3). Across the investigated plant species, the amount of consumed leaf material was increased by elevated CO_2 by 24.9%, and reduced by fertilization by 22.9%). However, the effects of elevated CO_2 varied among species (CO_2 × species, Table 3), and there was also a significant CO_2 × fertilization × species interaction (Table 3).

In some plant species, leaves grown under elevated CO_2 were more consumed when the plants were unfertilized but were less consumed with fertilization (*S. jacobea, L. luteus*), whereas for other species the trend was reversed (*N. tabacum, C. maculatum*). The amount of consumed leaf material decreased with SLW ($r = -0.24$, $P = 0.0008$). Inclusion of this variable as a covariate in the analysis, however, did not change the results qualitatively.

The RGR of the caterpillars depended strongly on the plant species they were fed with (Table 4, Fig. 4). Fertilization increased RGR by 105% across all plant species. The effect differed, however, between plant species (significant fertilization × species interaction, Fig. 4, Table 4). Elevated CO_2 had no consistent effect on the RGR of the caterpillars, but influenced the RGR of caterpillars feeding on individual plant species differently. The RGR of caterpillars fed with plants of *S. jacobea* and *L. angustifolius* grown at elevated CO_2 was considerable lower than that of caterpillars fed with material grown at ambient at CO_2, whereas for other species (*L. perenne, L. luteus*) the opposite was the case. Across all treatments, the RGR of caterpillars fed with a species was positively correlated with the leaf mass of this species consumed ($r = 0.68$, $P = 0.016$).

Table 3 ANCOVA results for the effects of initial herbivore mass, plant species, fertilization and elevated CO_2 on leaf mass consumed by the herbivores

Source of variation	df	MS	*F*
Herbivore mass	1	5.42	10.41**
Species (S)	11	12.46	23.96***
Fertilizer (F)	1	3.30	6.35*
CO_2	1	1.78	$3.46^{(*)}$
S × F	11	0.91	$1.75^{(*)}$
S × CO_2	11	1.19	2.29*
CO_2 × F	1	0.85	1.64
S × CO_2 × F	11	1.36	2.62**
Residual	143		

Significance levels are indicated with $^{(*)}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

The RGR of caterpillars decreased with increasing C/N ratio of the leaf tissue ($r = -0.35$, $P < 0.0001$). When leaf C/N ratio was included as a covariate in the model, the overall effect of fertilization was no longer significant, indicating that positive effects of fertilization on RGR of the caterpillars were mediated by an increased availability of nitrogen in the food (lower leaf C/N ratio). All other effects did not change qualitatively after adjusting for the effects of leaf C/N ratio.

The nested analyses with species nested in the factor NCSC yielded very similar results. More interestingly, there was a significant interaction between the ability of a species to produce NCSC and nutrient availability on leaf consumption by caterpillars (Table 5). Caterpillars consumed less leaf material of plants grown under high nutrient availability, but only if the plants did not produce NCSC (Fig. 5).

Discussion

Effects on plant growth

Elevated CO_2 levels can be regarded as a form of 'atmospheric' fertilization and can, therefore, be expected to generally increase plant photosynthesis, growth and reproduction. Our results add to the literature, which reports positive effects of elevated CO_2 on the biomass of plant species. In contrast to the results of other authors (e.g. Goverde *et al.*, 2002; Jablonski *et al.*, 2002) this effect did not differ substantially among the 12 plant species in our study. It has been suggested (Goverde *et al.*, 2004) that positive effects of elevated CO_2 on plant biomass may be particularly strong in legumes because of their nitrogen fixation, but this was not the case in our study. The effects of elevated CO_2 and nutrient availability on the aboveground biomass of plants were also additive. This is in contrasts to the results of previous studies (e.g. Stöcklin & Körner, 1998; Matthies & Egli, 1999), which found stronger effects of elevated CO_2 on fertilized plants.

Table 4 ANOVA results of the effects of plant species, elevated CO_2 and fertilization on the relative growth rate of *Spodoptera littoralis* caterpillars

Source of variation	df	MS	*F*
Species (S)	11	0.082	10.49***
Fertilizer (F)	1	0.093	11.83**
CO_2	1	0.003	0.35
S × F	11	0.024	3.06**
S × CO_2	11	0.028	3.57***
CO_2 × F	1	0.001	0.17
S × CO_2 × F	11	0.006	0.74
Residual	141	0.008	

Significance levels are indicated with $^{**}P<0.01$ and $^{***}P<0.001$.

The general increase of SLW and C/N ratio and the decrease in chlorophyll content of leaves under elevated CO_2 is in accordance with the results of a number of earlier studies (Ayres, 1993; Lincoln *et al.*, 1993; Watt *et al.*, 1995; Poorter *et al.*, 1996; Bezemer & Jones, 1998; Cotrufo *et al.*, 1998a, b). It is thought that under elevated CO_2 increased photosynthesis and photosynthate production leads to the increased production of structural carbohydrates which enhance leaf toughness and, therefore, SLW (Coviella & Trumble, 1999). However, in our study this effect differed among plant species.

Elevated CO_2 can have a number of effects on plant architecture and morphology (Pritchard *et al.*, 1999). In our study, both plant height and biomass increased under elevated CO_2 and the number of leaves per unit of biomass generally increased with elevated CO_2 and nutrient availability. This may be explained by higher rates of cell division in meristems caused by an in-

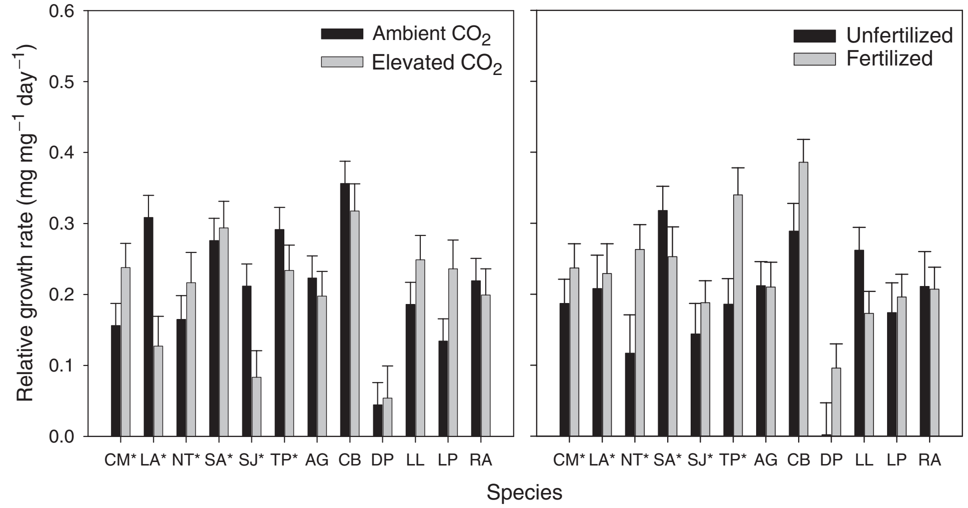

Fig. 4 Effect of elevated CO_2 (left) and fertilization (right) on the relative growth rate of caterpillars of *Spodoptera littoralis* feeding on different plant species (means ± standard error). See Fig. 1 for abbreviations of plant species. *Plant species that produce nitrogen-containing secondary compounds.

Table 5 Results of nested ANCOVA and ANOVA of the effects of nitrogen-containing secondary compounds (NCSC), plant species, fertilization and elevated CO_2 on consumed leaf mass und relative growth rate of caterpillars of *Spodoptera littoralis*

	Consumed leaf mass			Relative growth rate	
Source of variation	df	MS	*F*	MS	*F*
Herbivore mass	1	8.41	16.35***		
$NCSC^A$	1	0.92	0.07	1.53×10^{-2}	0.17
Species $(S)^a$	10	13.53	26.29***	8.89×10^{-2}	11.37***
Fertilizer (F)	1	3.30	6.42*	9.03×10^{-2}	11.54***
CO_2	1	1.60	$3.11^{(*)}$	1.86×10^{-3}	0.24
$NCSC \times F^B$	1	3.82	6.22*	1.01×10^{-3}	0.04
$S \times F^b$	10	0.61	1.19	2.66×10^{-2}	3.40***
$NCSC \times CO_2^C$	1	2.71	2.86	7.98×10^{-3}	0.27
$S \times CO_2^c$	10	0.95	1.85*	3.05×10^{-2}	3.90***
$CO_2 \times F$	1	0.84	1.64	1.91×10^{-3}	0.24
$NCSC \times CO_2 \times F^D$	1	0.02	0.02	4.53×10^{-5}	0.01
$S \times CO_2 \times F^d$	10	1.47	2.86**	6.37×10^{-3}	0.81
Residual	142	0.51		7.82×10^{-3}	

Plant species was nested within NCSC. Terms indicated by upper case letters were tested against the term with the corresponding lower case letter, all other terms were tested against the residual mean square.
Significance levels are indicated with $^{(*)}P<0.1$, $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

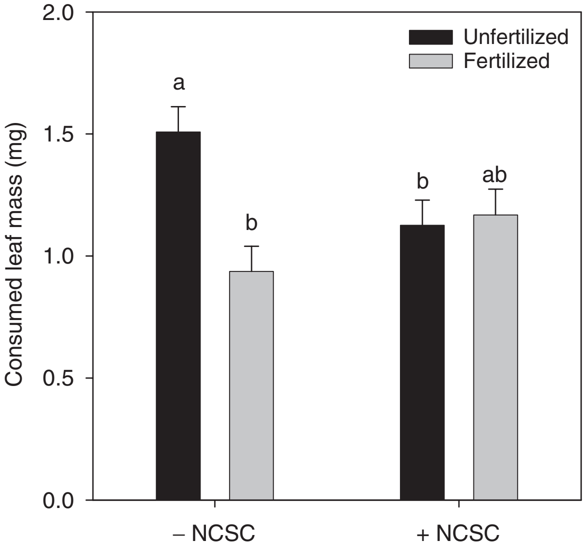

Fig. 5 Leaf mass consumed by caterpillars of *Spodoptera littoralis* feeding on plant species that produce nitrogen-containing secondary compounds (+NCSC) or not (−NCSC) and were either fertilized or not (means ± standard error). Effects are adjusted for initial herbivore mass. Bars with different letters indicate significantly different means ($P<0.05$, Tukey's HSD).

creased availability of assimilates (Kinsman *et al.*, 1997). However, most of the studies reviewed by Pritchard *et al.* (1999) did not correct for the effects of increased plant biomass. Finally, it has been pointed out that elevated CO_2 may accelerate the development of plants (Coleman *et al.*, 1993). Accordingly, the number of senescent leaves was increased in two (*Agrostemma githago* and *C. bonus-henricus*) of the three plant species with senescent leaves in our study. However, the third species (*D. purpurea*) produced fewer senescent leaves, indicating slower development. While studies involving a few species from the same family sometimes did not find a species × CO_2 interaction on leaf traits like SLW and C/N-ratio (Goverde & Erhardt, 2003), our study with 12 species from 10 families suggests that leaf traits of plants from the same family would respond similarly to elevated CO2 because of similar physiological traits, while SLW, C/N-ratio and chlorophyll content of less related species should respond differently despite similar trends of biomass change.

Effects on a generalist herbivore

Consumption, growth and development of phytophagous invertebrates depend strongly on the nutritional quality of plant tissue (Mattson, 1980). Given that elevated CO_2 affects the content of nitrogen and carbohydrates in plants, indirect effects on herbivores should be expected and have in fact been demonstrated by numerous studies (see Lincoln *et al.*, 1993; Watt *et al.*, 1995; Bezemer & Jones, 1998; Körner, 2003 for a review). In accordance with the nutrient balance hypothesis (Bryant *et al.*, 1983), the C/N ratio of plant tissue was generally lower under elevated CO_2 in our study and food quality for herbivores, therefore, lower. In fact, a reduction of the nitrogen concentration in leaves is a common response of a wide range of plant species to elevated CO_2 (Yin, 2002). However, in a review of available studies on plant–insect interactions under elevated atmospheric CO_2, Bezemer & Jones (1998) concluded that leaf-chewing insects generally are able to compensate for a reduced quality of tissues by increasing their consumption. In accordance with this conclusion, leaf consumption was increased by elevated CO_2 and decreased by fertilization in our study. However, the effect of elevated CO_2 on leaf consumption varied depending on nutrient availability and plant species. Some recent studies have found no compensatory feeding under elevated CO_2 and suggested post-ingestive mechanisms to compensate for diminished food quality (Barbehenn, 2004a, b; Cleland, 2006). Herbivores may experience different concentrations of chemicals in excised leaves compared with leaves attached to plants because of induced defences which were not considered in our study (Schmelz *et al*., 2001).

Leaf consumption by *S. littoralis* was also influenced by interacting effects between the presence of NCSCs in the plant species and nutrient availability. While the feeding rate on plant species, which do not produce NCSC was higher without fertilization, no differences where observed in plant species which do so. This may indicate that compensatory feeding in response to low nutrient content is not possible if plants produce NCSCs because of a limited ability of the herbivores to deal with the toxic compounds. In plant communities, herbivores may respond to a changed host plant quality by a shift of their food preferences rather than by compensatory feeding (Peters *et al*., 2000; Agrell *et al*., 2006), although in most studies no preference shifts were observed (Arnone *et al*., 1995; Traw *et al*., 1996; Ledergerber *et al*., 1997, 1998; Díaz *et al*., 1998). However, our study was not designed to investigate the effects of CO_2 and fertilization on the food choice of larvae given a range of possible host plants, but to test the effects of changed food quality due to these factors on the feeding behaviour and growth on host plants with different defence strategies. In contrast to the expectation, we derived from the carbon-nutrient hypothesis, we did not find interacting effects between the presence of NCSC and elevated CO_2 on leaf consumption or growth rate of the herbivore, suggesting that elevated CO_2 did not change the effect of NCSC on the herbivore. From our experiment, we can, therefore, not conclude that the effect that elevated CO_2 will have on the host quality of a plant for a generalist herbivore will depend on the plant's ability to produce NCSCs, as suggested by the carbon-nutrient balance hypothesis.

Most studies did not find changes in the growth rate of leaf-chewing insects in response to elevated CO_2 (Bezemer & Jones, 1998; Barbehenn *et al*., 2004a). In contrast, in our study elevated CO_2 influenced the RGR of second and third instar larvae of *S. littoralis*, but the effects depended on the plant species they fed on. While the RGR of the insects was increased by elevated CO_2 in some species (most notably in *L. perenne* and the nonalkaloid line of *L. luteus*), it was reduced in others (most notably in *L. angustifolius* and *S. jacobaea*). The two first species do not produce any secondary compounds, whereas the latter two produce NCSCs. In contrast, the carbon-nutrient balance hypothesis predicts an increase of defence compounds for species that rely mainly on carbon-based defences, and negative effects on the growth of herbivores should be expected for these species. However, even within species, which are able to produce NCSC and those which are not, direction and magnitude of the effect of CO_2 on growth rate differed. Accordingly, the effect of CO_2 on the growth rate of the herbivore did not depend on the plant's ability to produce NCSC. However, effects on RGR are not necessarily indicative for effects that CO_2 and nutrient additions may have on overall fitness.

Consumption by herbivores may further be affected by associations between plants and microbes. For instance, infection by the endophytic fungus *Neotyphodium lolii* often provides the basis for acquired chemical defence against herbivores by alkaloids (Porter, 1994), and Hunt *et al*. (2005) demonstrated that alkaloid production in *L. perennne* infected with *N. lolii* is affected by both elevated CO_2 and nutrient availability.

In conclusion, the results of our study suggest that the effects of elevated CO_2 on leaf consumption and growth rate of herbivores are not predictable using the framework of the carbon-nutrient balance hypothesis. Instead, the effects of CO_2 and nutrient availability on herbivore performance depended on species identity. This may have consequences for plant community structure under elevated CO_2. In natural systems the feeding pressure by herbivores may shift to alternative host plants whose tissue quality is not negatively affected by elevated CO_2. The effects of invertebrate herbivory on plant community composition and diversity may, therefore, change in a high CO_2 environment, but are difficult to predict.

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Appendix A

Table A1 List of plant species used in the experiments with information on important secondary compounds known to occur in the plant tissue (taken from Hoppe, 1958; Roth *et al.*, 1994, and the toxicological data base of the University of Zürich at www.vetpharm.unizh.ch)

Abbreviation	Host species	Family	Secondary compounds
AG	*Agrostemma githago*	Caryophyllaceae	Steroid saponins
CB	*Chenopodium bonus-henricus*	Chenopodiaceae	Saponines
CM	*Conium maculatum*	Apiaceae	Alkaloids (e.g. coniin)* [0.4–2.0%]
DP	*Digitalis purpurea*	Scrophulariaceae	Glucosides [0.2–0.4%]
LP	*Lolium perenne*	Poaceae	None
LA	*Lupinus angustifolius*	Fabaceae	Alkaloids*
LL	*Lupinus luteus*	Fabaceae	None (low-alkaloid line) [<0.04%]
NT	*Nicotiana tabacum*	Solanaceae	Alkaloids (nicotin)* [0.05–4.0%]
RA	*Rumex acetosella*	Polygonaceae	Oxalate [0.05–1.5%]
SJ	*Senecio jacobea*	Asteraceae	Pyrrolizidine alkaloids* [0.2%]
SA	*Sinapis alba*	Brassicaceae	Glucosinolates (sinalbin)* [2.5%]
TP	*Trifolium pratense*	Fabaceae	Cyanogenic glucosides*

Taxonomy of plants follows Schmeil & Fitschen (1996) and Roth *et al.* (1994). Species are in alphabetical order.
*Nitrogen-containing compounds.