Original article

High plant species diversity indirectly mitigates CO_2- and N-induced effects on grasshopper growth

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ABSTRACT

We examined how elevated atmospheric $[CO_2]$ and higher rate of nitrogen (N) input may influence grasshopper growth by changing food plant quality and how such effects may be modified by species diversity of the plant community. We reared grasshopper nymphs (*Melanoplus femurrubrum*) on *Poa pratensis* from field-grown monocultures or polycultures (16 species) that were subjected to either ambient or elevated levels of CO_2 and N. Grasshopper growth rate was higher on *P. pratensis* leaves grown in monocultures than in polycultures, higher on *P. pratensis* grown under elevated than under ambient $[CO_2]$, and higher on *P. pratensis* grown under elevated than under ambient [N]. The higher growth rate observed on *P. pratensis* exposed to elevated $[CO_2]$ was, however, less pronounced for polyculture- than monoculture-grown *P. pratensis*. Growth rate of the grasshoppers was positively correlated with leaf [N], [C], and concentration of soluble carbohydrates + lipids. Concentration of non-structural carbohydrates + lipids was higher in leaves grown under elevated than under ambient $[CO_2]$, and the difference between *P. pratensis* grown under ambient and elevated $[CO_2]$ was greater for monoculture- than polyculture-grown *P. pratensis*. In addition, leaf N concentration was higher in *P. pratensis* grown in monocultures than in polycultures, suggesting that plant species richness, indirectly, may influence insect performance by changed nutritional value of the plants. Because we found interactive effects between all factors included ($[CO_2]$, [N], and plant species diversity), our results suggest that these parameters may influence plant–insect interactions in a complex way that is not predictable from the sum of single factor manipulations.

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1. Introduction

Various human activities cause terrestrial ecosystems to experience multiple environmental changes such as reduced species diversity, increased concentration of atmospheric CO_2 ($[CO_2]$), and increased nitrogen (N) deposition (Vitousek et al., 1997; Sala et al., 2000). Three important anthropogenic changes, a loss in plant biodiversity, elevation of atmospheric $[CO_2]$ and increased N deposition, are likely to occur simultaneously, so interactive effects among them seem likely. Thus, increased knowledge of their combined effects on insect performance may be necessary in order to understand and

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predict future effects of global changes on insect populations as well on plant communities.

The individual effects from elevated $[CO_2]$ (Watt et al., 1995; Bezemer and Jones, 1998) and [N] (Mattson, 1980; White, 1993) on insect herbivores are relatively well known, but their combined effects, especially in relation to plant species diversity, are less well documented or understood. In general, plants with high N concentration, and low carbon (C) concentration are considered to be of high food quality for insects, and positive effects on insect performance, such as shorter development time, larger adult body mass, and higher reproductive output following increased [N] of food plants are well documented (Mattson, 1980; Ritchie, 2000). Hence, performance of grasshoppers can be expected to increase on plants grown under increased N input.

Plants grown under elevated $[CO_2]$ often have lower [N] and higher [C] than plants grown under ambient conditions (Reich et al., 2001a; Yin, 2002), and insect performance can thus be expected to be poorer under elevated $[CO_2]$. Accordingly poor performance of insects feeding on such plant material has also been reported (Watt et al., 1995; Bezemer and Jones, 1998; Agrell et al., 2000). However, insects may to some extent compensate for lower food [N] by increasing their food intake (e.g. Bezemer and Jones, 1998) or increasing their post-ingestive assimilation efficiency of the ingested food (Barbehenn et al., 2004a) and thereby maintain their growth rate. In addition, plants grown under elevated $[CO_2]$ may contain higher concentrations of carbohydrates (Barbehenn et al., 2004b), which may be beneficial for insects (Ayers, 1993), so even without compensatory feeding the response to elevated $[CO_2]$ may not necessarily be negative. However, not all types of carbohydrates are easily converted into energy by insects. Structural carbohydrates, found in cell walls, such as cellulose, lignin and hemicellulose (neutral detergent fiber) are of less importance, or may even negatively influence growth rates of insects. In contrast, non-structural carbohydrates, such as starch and sugars, and lipids are energy rich and may positively influence the growth rate of insects. Thus, the growth response of insects may range from negative to positive depending on which fraction of carbohydrates is most affected by elevated $[CO_2]$ and how food [N] is affected. The growth response will also vary depending on which of the above mentioned factors is most important for the performance of the individual insect.

Altered plant species diversity is known to influence the growth and feeding pattern of insects (Di Giulio and Edwards, 2003; Scherber et al., 2006). Most studies addressing the effects of plant species diversity for insect performance have focused on the importance of direct effects of altered species diversity, involving the quantity of preferred food plants and the importance of multiple food plant species for maintaining high performance. In addition to such direct effects, diversity may also potentially influence insect performance indirectly by changing the nutritional quality of the food plants. In N limited environments, plants growing in more productive polycultures may have lower [N] than plants growing in less productive monocultures (Reich et al., 2001b). This response appears to be largely due to enhanced resource competition for e.g. N among plants (Reich et al., 2001b, and unpublished data), which may result in lower concentration of nutrients in plant tissue. Hence, it may be hypothesized that increased plant diversity may reduce food quality and thereby the growth rates of insects feeding on plants from diverse plant communities. This raises the question how representative results from manipulation experiments using monoculture systems are for natural, more species rich systems? This question is relevant, given that many studies examining insect performance in relation to increased N input and, especially, elevated $[CO_2]$ have used plants grown in monocultures (e.g. Johnson and Lincoln, 1991; Goverde et al., 1999; Agrell et al., 2000; Barbehenn et al., 2004a).

The combined effects from altered plant species diversity, elevated $[CO_2]$, and increased N input on food quality and corresponding effects on insect performance may be complicated to predict, as interactive effects between factors may offset the individual effects. For example, increased N input may offset the potential lowered [N] in plants grown in species diverse plant communities or at elevated $[CO_2]$.

To examine how growth of grasshoppers differs between diets of different chemical composition that correspond to those imposed by elevated $[CO_2]$ and [N], and level of plant species diversity, we provided grasshopper nymphs with leaves of the grass *Poa pratensis* L. grown in monocultures or polycultures that were exposed to ambient or elevated $[CO_2]$ or [N] in a free-air CO_2 enrichment (FACE) experiment in Minnesota, USA. We measured differences in leaf total [C], [N], concentration of neutral detergent fiber and concentration of non-structural carbohydrates + lipids on the grass provided to the grasshoppers to determine if differences in grasshopper growth were associated with differences in food plant quality.

2. Materials and methods

2.1. Experimental design

To examine how growth of grasshoppers differed between diets with different chemical composition we provided *Melanoplus femurrubrum* (De Geer) nymphs (4th instar) with *P. pratensis* grown in monocultures or in polycultures that were exposed to ambient or elevated CO_2 and N conditions. We used plant material from the BioCON experiment (Biodiversity, Carbon dioxide, and Nitrogen effects on ecosystem functioning, http://www.lter.umn.edu/biocon/) located at Cedar Creek Natural History Area in east-central Minnesota, USA ($45°N$, $93°W$). The experiment consists of six circular areas (rings) each containing 61 2 by 2 m plots that were planted with seeds of 1, 4, 9 or 16 species (total of 12 g seed m^{-2}) in 1997. Since 1998, three of the six rings have been exposed to elevated $[CO_2]$ ($560 \, \mu mol \, mol^{-1}$) using a free-air CO_2 enrichment system (FACE) and the three remaining rings represent ambient $[CO_2]$ ($370 \, \mu mol \, mol^{-1}$). Within each ring, half of the plots are fertilized with NH_4NO_3 corresponding to N addition of $4 \, g \, m^{-2} \, year^{-1}$, applied over three dates each growing season (for a more detailed description of the experiment see Reich et al. (2001b) or the above mentioned web page). In the present study we used fully expanded leaves of *P. pratensis* from monoculture plots and from 16 species plots (polycultures) that had been exposed to all combinations of ambient and elevated $[CO_2]$ and [N]. By this we obtained eight different

types of *P. pratensis*, i.e. food types, from a total of 48 polycultures (12 of each treatment) and eight monocultures (two of each treatment). These eight food types were then used in a feeding experiment to estimate differences in grasshopper growth rate.

2.2. Feeding experiment

In mid-July 2003, we collected 80 *M. femurrumbrum* nymphs (4th instar) from an abandoned agricultural field near the Bio-CON experiment. Each nymph was placed individually in semitransparent plastic jars (1 L) and put in an incubator (28°C and 14:10 photoperiod). The nymphs were starved for 20 h and then weighed prior the start of the feeding experiment. The nymphs were randomly sorted into eight groups and we provided the grasshoppers in each group with one type (food type) of *P. pratensis* leaves (see Section 2.1). We used a mix of leaves from all plots with the same treatment. Hence the individual grasshoppers and not the treatment plots within the BioCON experiment are the replication unit. We provided the nymphs with fresh leaves (c. 0.2–0.4 g day^{-1}) for a 10-day period. The daily amount of grass provided to the grasshoppers was about twice the amount consumed in 1 day. New leaves were collected from each of the plots within the BioCON experiment every morning. To prevent the plant material from drying the leaves were, after cutting, immediately put into water-filled plastic florist vials and transferred to the nearby lab facility.

To estimate consumed biomass (ingested biomass), the fresh weight of the grass was measured before and after 24 h in the jar with a grasshopper. To control for potential weight loss of the leaves due simply to desiccation, we had three control jars without any nymphs that were treated in the same way as the jars with nymphs. The average weight loss from these control jars was then used as a correction factor when we estimated the consumption rate. On average the weight loss from desiccation was small (~1%). The plant material that was not consumed after 24 h was collected, and the pooled material from each jar during the 10-day trial was dried to constant weight and used to estimate plant dry mass. The relationship between dry (Y) and fresh (X) weight of the grass (linear regression: $Y = 0.41X - 0.039$, d.f. = 52, $R^2 = 0.86$, $P < 0.001$) was used to calculate the initial dry weight biomass that we used for calculations of consumption rate and assimilation by the grasshoppers. After the 10-day trial period was over the grasshoppers were starved for 4 h and then weighed. The frass in the jars was collected and weighed after drying to constant weight (40–45°C for 48 h).

During the experimental period some of the grasshoppers died. All data presented are based on grasshoppers that survived the entire experimental period. Consumption and growth rates are expressed as the total consumption and weight gain during the entire trial period divided by 10, i.e. consumption and growth rate per day. The final number of grasshoppers in each treatment was as follows. Monocultures: low N, amb. $CO_2 = 5$; low N, elev. $CO_2 = 9$; high N, amb. $CO_2 = 5$; high N, elev. $CO_2 = 9$. Polycultures: low N, amb. $CO_2 = 8$; low N, elev. $CO_2 = 6$; high N, amb. $CO_2 = 5$; high N, elev. $CO_2 = 8$.

2.3. Response parameters

We used three response variables (adapted from Waldbauer, 1968) to describe the grasshopper performance; growth rate (GR, mg day^{-1}), gross growth efficiency or efficiency of conversion of ingested food (ECI), and assimilation efficiency (AD). ECI is mg gained biomass per g ingested biomass. AD, which is approximately equal to digestibility, is expressed as assimilated biomass (ingested biomass − frass mass) per g ingested biomass. All calculations were made on dry mass.

2.4. Chemical analyses

The plant material collected from jars following feeding was dried (40–45°C), ground and analyzed for [C], [N], [neutral detergent fiber (NDF)], and [non-structural carbohydrates + lipids (NSC+)], all expressed as % of dry weight. Total nitrogen and carbon were analyzed following standard methods on a 4010 ECS element analyzer (Costech Analytical Technologies Inc., CA, USA). The protein fraction was estimated by conversion from plant N concentration using the average conversion factor for monocots of 4.37 reported by Yeoh and Wee (1994). Concentration of non-structural carbohydrates and lipids and neutral detergent fiber (NDF) was analyzed following standard methods on an Ankom^{200} autoanalyzer (Ankom Technology, Macedon, NY, USA). The dried, ground plant material (0.45–0.55 g) was placed in pre-manufactured mesh bags (F57, ANKOM Technology) and weighed (M_0). Mesh bags were soaked in acetone for 3–5 min, then dried at 100 °C to a constant weight (M_1) to determine the acetone-dissolved fraction ($1-M_1/M_0$), or non-structural carbohydrates, lipids and proteins. We assumed that proteins in the plants were primarily soluble so non-structural carbohydrates and lipids (NSC+) was determined by multiplying the acetone-dissolved fraction by $(1 - \text{protein fraction})$. Bags were then agitated in a pre-mixed neutral detergent solution (ANKOM Technology) at pH 7.0. The NDF solution contained 30 g sodium lauryl sulfate, USP; 18.61 g ethylenediaminetetraacetic disodium salt, dehydrate; 6.81 g sodium tetraborate decahydrate; 4.56 g sodium phosphate dibasic, anhydrous; and 10 mL triethylene glycol, in 1 L of distilled H_2O. After agitation for 75 min in the NDF solution the bags were rinsed for 5 min in a solution (80 °C) containing 1900 mL water and 4 mL alpha-amylase (FAA, ANKOM Technology). This procedure was repeated three times. After drying to constant weight, M_2 (100 °C, 4 h), the NDF fraction was determined as $(M_1 - M_2)/M_0$.

2.5. Statistical analyses

As a test of whether plant material fed to individual grasshoppers differed according to its treatment origin, leaf concentration of N, total C, NDF, and NSC+ we used ANOVA with the treatments of the BioCON experiment from which we obtained *P. pratensis* leaves (level of plant diversity, [N], and [CO_2]) as factors. We assessed grasshopper response with ANCOVA for three response variables, GR, ECI, and AD, with level of plant diversity, [N], and [CO_2] as factors. For growth rate we used the initial weight of the nymphs as covariate. For gross growth efficiency (ECI), we used grasshopper growth as response variable with ingested biomass covariate, and for

assimilation efficiency (AD), we used frass production as response variable with ingested biomass as covariate. Note that in all analyses, the individual grasshopper, or the plant material provided to an individual grasshopper, and not the plots within the BioCON experiment is the replication unit.

3. Results

3.1. Food plant chemistry

Leaf [N] was on average 9% lower in *P. pratensis* from polycultures than from monocultures (diversity effect), but there was no difference between leaves grown under ambient or elevated [CO_2] or [N] (Table 1, Fig. 1). Total [C] was slightly, but significantly, higher in leaves grown under elevated [CO_2] from monocultures, but lower in leaves from polycultures, causing a significant interaction between [CO_2] and plant species diversity (Table 1, Fig. 1). Compared to ambient conditions, leaf [C] was on average lower under elevated [CO_2] and [N], but tended to be higher in *P. pratensis* exposed to the combined treatment of elevated [CO_2] and [N] ($CO_2 \times$ N interaction: Table 1, Fig. 1).

In contrast to total [C], there were larger differences in concentration of non-structural carbohydrates (hereafter referred to as NSC+) and neutral detergent fiber (hereafter referred to as NDF) between food types (Fig. 1). On average, leaves grown under elevated [N] had lower concentrations of NSC+ than leaves grown under ambient [N], with no difference between mono- and polyculture-grown *P. pratensis* (N-effect: Table 1, Fig. 1). Moreover, leaves grown under elevated [CO_2] had higher concentrations of NSC+ than leaves grown under ambient [CO_2] (CO_2 effect: Table 1, Fig. 1), and this difference was greater for monoculture- (+17%) than polyculture-grown (+5%) *P. pratensis* (div. $\times CO_2$ interaction: Table 1, Fig. 1). There was, however, no overall difference in concentration of NSC between *P. pratensis* from monocultures and polycultures (Table 1, Fig. 1). Leaves grown under elevated [N] had lower
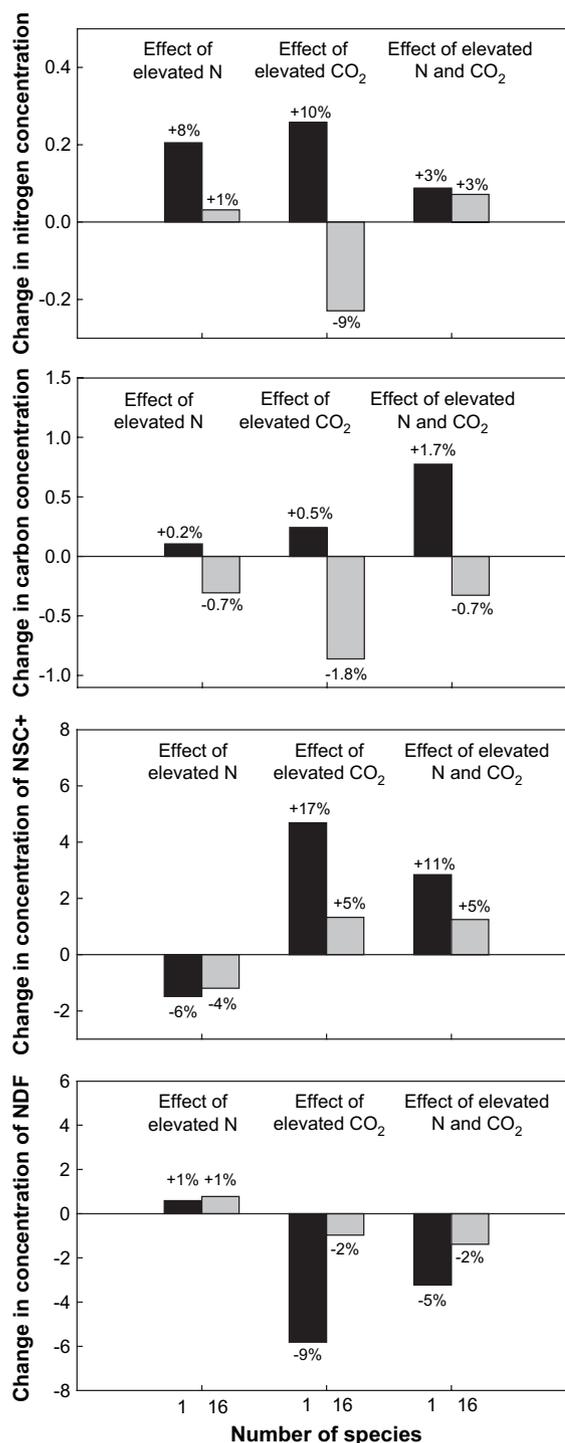

Fig. 1 – Change in chemical composition (compared with ambient levels of both CO_2 and N) in response to elevated [N] alone, to elevated [CO_2] alone, and to the combination of elevated [N] and [CO_2] for *P. pratensis* leaves grown in plots containing 1 (monoculture) and 16 (polyculture) plant species. From top to bottom; change in leaf nitrogen concentration, change in leaf total carbon concentration, change in leaf concentration of soluble carbohydrates + lipids (NSC+), and change in concentration of neutral detergent fiber (NDF). All concentrations are expressed as % of dry mass.

Table 1 – Results of the ANOVAs on differences in chemical composition between the different food types (type of *P. pratensis*). Individual response variables were: N, nitrogen concentration; C, total carbon concentration; and NSC+, concentration of non-structural carbohydrates, starch, and lipids. Plant species diversity, level of CO_2 and N was were used as factors

Source	d.f.	[N] F ratio	[C] F ratio	[NSC+] F ratio	[DNF] F ratio
Diversity	1	5.85*	0.083	0.86	26.99***
CO_2	1	0.014	0.002	52.14***	147.93***
Fertilization (N)	1	0.82	1.33	14.01***	25.41***
Div. $\times CO_2$	1	0.66	5.79*	2.85(*)	16.80***
Div. $\times$ N	1	0.54	0.30	0.21	0.11
$CO_2 \times$ N	1	0.07	2.85(*)	0.32	0.18
Div. $\times CO_2 \times$ N	1	2.54	0.31	0.04	4.10*
Error	43				

P values are represented by the following symbols: (*), $0.10 > P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[NSC+] than leaves grown under ambient [N] (N effect: Table 1, Fig. 1), with no difference between mono- and polyculture-grown *P. pratensis* (Table 1, Fig. 1).

Concentration of neutral detergent fiber (NDF) was negatively correlated with [NSC+] (Pearson correlation: $r = 0.78$) and the differences between food types were largely reflected by the differences just described for [NSC+] (Fig. 1). Compared to ambient conditions, leaves grown under elevated [CO_2] had lower [NDF] and leaves grown under elevated [N] had higher [NDF] (Table 1, Fig. 1). For elevated [N], there was no difference between mono- and polyculture-grown leaves (Table 1, Fig. 1), but there was a large difference in [NDF] between mono- and polyculture-grown leaves exposed to elevated [CO_2] ($CO_2 \times$ N interaction: Table 1, Fig. 1). Compared to ambient conditions, elevated [CO_2] resulted in 9% lower [NDF] for monoculture-grown leaves, but only 2% lower [NDF] for polyculture-grown leaves (Fig. 1). In contrast to [NSC+], there was a significant difference in [NDF] between mono- and polyculture-grown *P. pratensis*, with lower [NDF] in monoculture- than polyculture-grown *P. pratensis* (div. effect: Table 1, Fig. 1). In addition, there was a significant three-way interaction between all three main factors (div. $\times$ CO_2 $\times$ N interaction: Table 1, Fig. 1). This was caused by a smaller difference in [NDF] between mono- and polyculture-grown leaves when exposed to both elevated [CO_2] and [N] (3%) compared to when exposed to only elevated [CO_2] (7%).

3.2.   Response of the grasshoppers

The feeding experiment showed that grasshopper response differed depending on the food type they were provided. Growth efficiency (biomass gained per g consumed) was higher for grasshoppers provided *P. pratensis* grown under elevated than ambient [CO_2] and higher for grasshoppers provided *P. pratensis* grown in mono- than polycultures (CO_2 and div effects: Table 2, Fig. 2). Compared to ambient conditions, growth efficiency was 105% higher on CO_2 exposed leaves from monocultures, compared to 0.5% lower on leaves from polycultures (div. $\times$ CO_2 interaction: Table 2, Fig. 2). Although growth efficiency tended to be higher on N fertilized leaves there was no significant effect of N addition (Table 2, Fig. 2). There was, however, a significant interaction between the N and CO_2 treatments ($CO_2 \times$ N interaction: Table 2), as the positive effect on growth efficiency from the combined treatment of CO_2 and N was smaller than expected from the single treatments (Fig. 1).

Overall the grasshoppers had higher consumption rates on *P. pratensis* grown in monoculture than polyculture plots (div. effect: Table 2, Fig. 2). There was, however, no difference in consumption rate between leaves grown under ambient and elevated [CO_2] (Table 2, Fig. 2). In general, consumption rate was higher for leaves grown under elevated [N] (N effect: Table 2, Fig. 2). This effect was, however, not independent of plant species richness of the plots, i.e. there was a significant diversity $\times$ N interaction (Table 2). Compared to ambient conditions, consumption rate was 47% higher on leaves grown under elevated [N] when grown in monocultures but 18% lower when grown in polycultures (Fig. 2). This difference was however smaller when elevated [N] was combined with

elevated [CO_2] causing a significant three-way interaction (div. $\times$ CO_2 $\times$ N interaction: Table 2, Fig. 2).

Overall grasshopper growth rate was higher on leaves grown in mono- than polycultures (div. effect: Table 2, Fig. 2), higher on leaves grown under elevated than ambient [CO_2] (CO_2 effect: Table 2, Fig. 2), and higher on leaves grown under elevated than ambient [N] (N effect: Table 2, Fig. 2). The higher growth rate of grasshoppers provided leaves grown under elevated [CO_2] was much stronger for monoculture- than polyculture-grown *P. pratensis* (div. $\times$ CO_2 interaction: Table 2). Compared to ambient conditions, growth rate on leaves grown under elevated [CO_2] was 118% higher on *P. pratensis* from monocultures and 2% lower on *P. pratensis* from polycultures (Fig. 2). In addition, the growth response of grasshoppers provided leaves exposed to the combined treatment of elevated [CO_2] and [N] tended to be less positive than expected from the individual treatments of elevated [CO_2] and [N] ($CO_2 \times$ N interaction: Table 2, Fig. 2).

Compared to growth efficiency, assimilation efficiency differed less between treatments, but was higher for grass grown in polycultures than in monocultures, indicating that leaves from polycultures had higher digestibility than leaves from monocultures (div. effect: Table 2, Fig. 1).

To get a better understanding of how leaf chemistry influenced grasshopper growth we ran multiple regressions with growth efficiency and growth rate as dependent variables and leaf [N], [C], [NSC+], and [NDF] as predictors. Since [NSC+], and [DNF] was correlated (indicated with high colliniarity), we included either [NDF] or [NSC+] depending on which of the two produced the highest explanatory power. The regressions revealed that growth efficiency (ECI) was positively influenced by high leaf [C] and negatively influenced by high leaf [NDF] (ECI = 13.84[C] − 4.35[NDF] − 323.86, $R^2_{adj.}$ = 0.31; $F_{2,48}$ = 12.39; $P < 0.001$; $P_{[C]}$ = 0.002; $P_{[NDF]} < 0.001$) and growth rate (GR) was positively influenced by high leaf [N], [C], and [NSC+] (GR = 0.48[C] + 1.04[N] + 0.13[NSC] − 26.70, $R^2_{adj.}$ = 0.31; $F_{2,48}$ = 8.36; $P < 0.001$; $P_{[C]}$ = 0.008; $P_{[N]}$ = 0.003; $P_{[NSC+]}$ = 0.005). Given the noted effects of diversity on leaf [N] and [NDF], and of [CO_2] and N addition on [NSC+] and [NDF], these relations between leaf chemistry and grasshopper growth indicate the likely causal paths by which the treatments influence grasshopper growth.

4.   Discussion

We found that both grasshopper growth and plant chemistry differed substantially depending on growth conditions for the food plant *P. pratensis*. Differences in chemical composition between the different food types used in this study correspond to how changes in plant species diversity, elevated [CO_2] and increased N input have influenced plant chemistry in general, and *P. pratensis* chemistry specifically in the BioCON experiment (Reich et al., 2001a, b, and unpublished), suggesting that chemical differences between the food types used in the present study are comparable to differences induced by these global changes.

High growth rate of grasshoppers provided *P. pratensis* grown under elevated [CO_2], especially evident for monoculture leaves, was clearly derived from high growth efficiency (ECI). The ECI on leaves exposed to elevated [CO_2] was higher for

Table 2 – Results of the ANCOVAs on grasshopper performance. Plant species diversity, level of CO_2 and N were used as factors. For gross growth efficiency (ECI) GR was the response variable with ingested biomass covariate. For growth rate (GR) the initial weight of the grasshopper was used as covariate, and for assimilation efficiency (AD) frass production was the response variable with ingested biomass as covariate

Source	d.f.	ECI	GR	CR	AD
		F ratio	F ratio	F ratio	F ratio
Ingested biomass	1	40.37***	–	–	–
Initial weight	1	–	7.14*	0.73	–
Frass production	1	–	–	–	107.05***
Diversity	1	5.99*	25.08***	12.12**	7.18**
CO_2	1	10.91**	7.78**	0.04	0.17
Fertilization (N)	1	0.98	4.30*	5.09*	0.09
Div. $\times CO_2$	1	17.39***	8.30**	0.20	0.64
Div. $\times$ N	1	1.24	0.48	7.02*	1.85
$CO_2 \times$ N	1	5.31*	3.38(*)	0.01	0.81
Div. $\times CO_2 \times$ N	1	0.51	2.37	9.18**	0.61
Error	44				

P values are represented by the following symbols: (*), $0.10 > P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

monoculture- than polyculture-grown *P. pratensis*. This was probably a consequence of higher leaf [N] and [NSC+] and lower leaf [NDF] in monoculture- than polyculture-grown leaves, which may explain the interactive effect between CO_2 and diversity on grasshopper growth rate. This indicates that plant species richness, indirectly, may influence grasshopper growth by changing the nutritional value of the food plant, while elevated [CO_2] may influence growth by changing the energetic value of the food plant.

In contrast to the grasshoppers feeding on leaves exposed to elevated [CO_2], the higher growth rate on leaves grown under elevated [N] was not primarily caused by higher ECI. Instead the high growth rate was a consequence of higher food intake. Exactly what stimulated the increased consumption rate is unclear, but the increased amount of processed feed may have been a consequence of high [NDF] in leaves grown under elevated [N].

The finding that grasshopper growth was higher on grass grown under elevated than under ambient [CO_2] seems to contradict the general perspective that elevated [CO_2] should reduce food quality and insect performance (Watt et al., 1995; Bezemer and Jones, 1998; Agrell et al., 2000). Most examples of reduced insect performance following elevated [CO_2] have been associated with decreased plant [N] and increased concentration of total carbon, or increased concentration of carbon based secondary metabolites such as different types of phenolics (e.g. Bezemer and Jones, 1998; Agrell et al., 2000). However, considering that in the present study we found only minor differences in [N] and total [C], but larger differences in [NSC+] and [NDF] between *P. pratensis* grown under ambient and elevated [CO_2], our results may actually not contradict the general perspective outlined above. Moreover, many, and perhaps most, studies that have examined effects of elevated [CO_2] on insect performance have used experiments in which plants have been exposed to higher [CO_2] than we used in this experiment ($\geq$700 vs. 560 μmol mol^{-1}). If the effect on plants and subsequent effects on insect growth is non-linearly related to the [CO_2], we cannot exclude the possibility that the positive growth response that we recorded could have been smaller or even reversed if we used a higher [CO_2].

High performance by insects feeding on plants with high [N] and low C:N ratio is well documented (Mattson, 1980; White, 1993), but higher performance on plants with high concentration of non-structural carbohydrates (NSC) is less well documented, and our result contradicts other studies that have found no positive effects on grasshopper growth when reared on a diet with a high concentration of NSC (Zanotto et al., 1993; Barbehenn et al., 2004a). Our result is, however, in accordance with the finding that increased weight gain and faster development rate may be higher for Lepidoptera larvae feeding on plants in which elevated [CO_2] have induced high levels of NSC (Goverde et al., 2002). In that case the weight gain was primarily related to higher lipid concentration of the insects, which also may have been the case for the grasshoppers in this study.

The higher [NSC+] and lower [NDF] in *P. pratensis* grown under elevated [CO_2] suggest the possibility that elevated atmospheric [CO_2] may induce changes in concentration of both structural (cell wall carbohydrates) and non-structural carbohydrates. Increased concentration of NSC as a response to elevated [CO_2] has been found for other C_3 grasses (Barbehenn et al., 2004b), indicating that this may be a common response of C_3 grasses following elevated [CO_2]. The changed [NDF] in response to elevated [CO_2] is, however, in contrast to the general view that elevated [CO_2] has little or no effect on plant [NDF] (Peñuelas and Estiarte, 1998). An interesting, and important, result of the present study is that although concentration of NSC+ and NDF differed substantially between the different food types, there were only minor differences in total [C]. Since grasshopper growth response differed between food types this implies that not only the quantity, but also the quality of carbon matters, and that insect growth may not always be easily predicted from the stoichiometric relationship of element ratios, such as C:N, between plants and herbivores.

4.1. Plant species richness

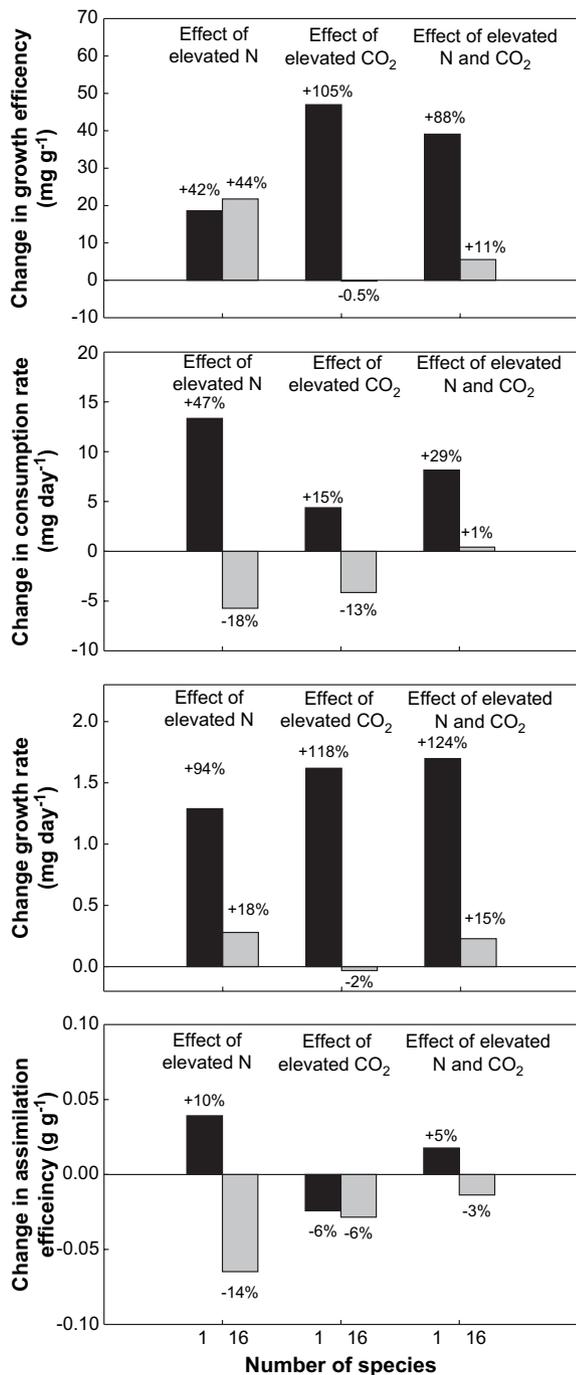The finding that grasshopper growth differed between *P. pratensis* leaves from mono- and polycultures suggests that

species richness of a plant community, in addition to the direct effects from changes in relative availability of preferred food species, also indirectly may influence insect growth by altering food plant quality. This also implies that previously reported direct effects of plant species richness on insect performance (Di Giulio and Edwards, 2003; Scherber et al., 2006) to some extent may have been derived from indirect, diversity-induced chemical changes of the food plants.

The higher growth rate of grasshoppers feeding on *P. pratensis* grown in monocultures than polycultures that we observed was probably derived from high N concentration and lower [NDF] in this food type. The higher [N] and lower [NDF] of *P. pratensis* from monocultures than from polycultures may also be the reason for the higher growth efficiency and corresponding growth rate of grasshoppers provided *P. pratensis* grown in monocultures than polycultures under elevated $[CO_2]$.

Differences in [N] between plants from polycultures and monocultures may be a consequence of intense resource competition in more diverse plant communities (Reich, unpublished data). Such heightened resource competition for N has also been suggested as a mechanism to explain why biomass yield may increase with increasing plant species richness (Hille-Ris Lambers et al., 2004). The lower responsiveness of polyculture-grown *P. pratensis* to elevated $[CO_2]$ may, indirectly, be related to heightened resource competition for N in polycultures. Photosynthetic enhancement following elevated $[CO_2]$ may be lower than expected if relative leaf [N] is reduced (Lee et al., 2001; Strengbom and Reich, 2006). If lower leaf [N] in polyculture plots results in lower than expected effects of elevated $[CO_2]$ on plant photosynthetic rates, responsiveness to elevated $[CO_2]$ should be lower in poly- than monocultures. The greater responsiveness of monoculture-grown *P. pratensis* to elevated $[CO_2]$ is in accordance with what has been found for non-nitrogen fixing dicots in the BioCON experiment (Novotny et al., 2006), suggesting that this phenomenon is not just restricted to *P. pratensis*.

5. Conclusions

In conclusion, the finding that plant species diversity may mitigate indirect effects on insect growth from elevated $[CO_2]$ and [N] demonstrates that predicting insect responses to global changes such as elevated $[CO_2]$ and [N] from plant monoculture systems may be complicated, as conclusions from such experiments may not be valid for the mixed species plant communities typically found in most extant temperate grassland. Our results also highlight the problems associated with predicting the effects on insect performance from the sum of single factor manipulations.

Acknowledgements

Fig. 2 – Change in grasshopper responses (compared with ambient levels of both CO_2 and N) when provided *P. pratensis* grown under elevated [N] alone, under elevated $[CO_2]$ alone, and under the combination of elevated [N] and $[CO_2]$. Data shows change in growth efficiency expressed as biomass gain per amount ingested biomass (dry mass), change in consumption rate (mg day^{-1}), change in growth rate expressed as biomass gain per day (dry mass), and change in assimilation efficiency (approximately digestibility of the grass) expressed as conversion of ingested biomass into biomass (dry mass). Data based on grasshoppers surviving the entire experimental period.

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