

Elevated Atmospheric CO₂ Triggers Compensatory Feeding by Root Herbivores on a C₃ but Not a C₄ Grass

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Abstract

Predicted increases in atmospheric carbon dioxide (CO₂) concentrations often reduce nutritional quality for herbivores by increasing the C:N ratio of plant tissue. This frequently triggers compensatory feeding by aboveground herbivores, whereby they consume more shoot material in an attempt to meet their nutritional needs. Little, however, is known about how root herbivores respond to such changes. Grasslands are particularly vulnerable to root herbivores, which can collectively exceed the mass of mammals grazing aboveground. Here we provide novel evidence for compensatory feeding by a grass root herbivore, *Sericesthis nigrolineata*, under elevated atmospheric CO₂ (600 μmol mol⁻¹) on a C₃ (*Microlaena stipoides*) but not a C₄ (*Cymbopogon refractus*) grass species. At ambient CO₂ (400 μmol mol⁻¹) *M. stipoides* roots were 44% higher in nitrogen (N) and 7% lower in carbon (C) concentrations than *C. refractus*, with insects performing better on *M. stipoides*. Elevated CO₂ decreased N and increased C:N in *M. stipoides* roots, but had no impact on *C. refractus* roots. Root-feeders displayed compensatory feeding on *M. stipoides* at elevated CO₂, consuming 118% more tissue than at ambient atmospheric CO₂. Despite this, root feeder biomass remained depressed by 24%. These results suggest that compensatory feeding under elevated atmospheric CO₂ may make some grass species particularly vulnerable to attack, potentially leading to future shifts in the community composition of grasslands.

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Introduction

The largest annual increase in global atmospheric CO₂ emissions in the last 50 years occurred during 2010 [1]. Such increases will impact on ecological communities and the species interactions within them. For example, it is widely observed that elevated atmospheric CO₂ concentrations (eCO₂) reduces the nutritional quality of plants for herbivores [2]. A meta-analysis of over 100 published studies demonstrated that while both carbon (C) and nitrogen (N) increased in both roots and shoots, C increased at an accelerated rate relative to N and led to an average increase in C:N ratios of 11%, effectively reducing nitrogen concentrations in both roots and shoots [3], either by dilution or reallocation [4]. Since N is the limiting factor in most herbivore diets [5], herbivores may respond to this decline in host quality by compensatory feeding, whereby the herbivore eats more plant biomass in an attempt to acquire adequate nutrition [4,6]. Indeed, Stiling and Cornelissen's [2] meta-analysis reported that relative consumption by insect herbivores increased by 17% and total consumption by 19%, when feeding on plants under eCO₂. Given that eCO₂ can cause similar increases in C:N ratios in the roots as in the shoots [3], it is surprising that feeding responses of root herbivores to such changes in chemistry have not been examined [7]. To our knowledge, only four studies have investigated the effects of eCO₂ on root feeding insects [8–11] and none have investigated this for grasses.

Root herbivores are major components of many ecosystems, having the capacity to shape the community structures of other herbivores and plant communities [12,13]. Grassland systems can be particularly vulnerable to root herbivores [14]. For instance, in some pasture systems it is not uncommon for the collective biomass of root herbivores to exceed that of grazing mammals aboveground [15]. In addition to covering over 40% of the planet's land surface area [16], grasslands are responsible for storing over one third of global terrestrial carbon stocks [17]. Grasslands often comprise of C₃ and C₄ grass species; C₃ grasses are usually superior hosts for herbivores compared with C₄ grasses, but they also are more strongly affected by elevated CO₂, generally showing greater increases in C and reductions in N concentrations than C₄ plants [18,19]. This occurs because Rubisco, the initial carboxylating enzyme to facilitate the assimilation of CO₂ into carbohydrates operates below its maximum capacity at current CO₂ concentrations in C₃ plants, so has the greater capacity to respond to eCO₂ [4,20]. Because of this, C₃ plants may be disproportionately subject to compensatory feeding under eCO₂. This has rarely been tested for aboveground herbivores (e.g. [21,22]), and never, to our knowledge, for root herbivores.

This study characterised how eCO₂ affected a C₃ (*Microlaena stipoides*) and a C₄ (*Cymbopogon refractus*) grass species, and how any changes in grass traits affected the feeding behaviour and performance of a root feeding insect, the scarab *Sericesthis*

nigrolineata Boisduval (Coleoptera: Scarabaeidae). We hypothesised that: (1) *M. stipoides* would be nutritionally superior (higher N, lower C:N) than *C. refractus* under ambient CO₂ (aCO₂), (2) under eCO₂, both grasses would become inferior hosts for the root herbivore due to lower N and higher C:N, and this effect would be more pronounced for *M. stipoides* than *C. refractus*; (3) under eCO₂, insects would consume more root tissue through compensatory feeding, with the biggest increase on *M. stipoides* and (4) scarab performance (body mass) would be better on *M. stipoides* than *C. refractus*, but would decline at eCO₂.

Materials and Methods

Chambers

Six glasshouse chambers, three maintained at aCO₂ of 400 μmol mol⁻¹ and the other three at eCO₂ (600 μmol mol⁻¹), were used. These chambers (3 m×5 m×3 m; width×length×height) with UV transparent plexiglass (6 mm thick) walls and roof were naturally lit throughout the experiment. Daytime air temperature was regulated to reach a midday peak of 24°C and fall to 21°C at midnight (±4°C) at night time. Humidity was controlled at 60% (±6%). CO₂ levels were controlled via the monitoring and control system PlantVisorPRO (Carel Industries, Padova, Italy). Briefly, CO₂ levels within each chamber were monitored by a CO₂ probe (GMP222, Vaisala, Vantaa, Finland), with CO₂ (food grade, AirLiquide, Australia) injected from pressurized cylinders through solenoid valves. Before entering a chamber, CO₂ was passed through a Purafil column to eliminate possible ethylene contamination.

Experimental procedure

Grasses were planted in 90 pots (100 mm diameter) containing 850 g of air-dried and sieved (1 mm) soil, which was loamy-sand with low (0.7%) organic matter (full details given in [23]). These were randomly assigned to the six climate chambers (15 in each) and watered daily to maintain soil water content at 15%, which was verified with a two-rod moisture probe (Hydrosense, Campbell Scientific, Australia). No nutritional supplement was provided. After 10 weeks, five of the plants for each species were selected and shoots and roots were separated, oven dried (40°C) and weighed. For the remaining 10 plants, a single seedling comprising small section of roots and grass blades (*c.* 2–3 g fresh mass) was teased apart and transferred into bioassay cages (Fig. 1) constructed from 90 mm Petri dishes filled with soil (details as above). Cages were a variation of similar bioassay cages used for measuring root damage by root feeding insects in other studies [24,25]. One side had an

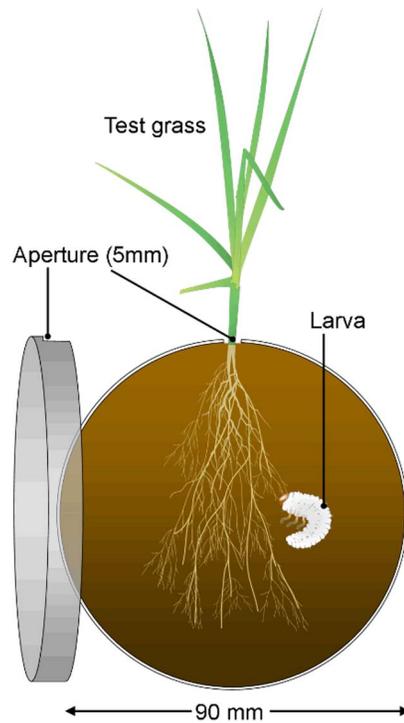


Figure 1. Bioassay cage used to determine root consumption and change in body mass of larval scarab beetles.
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aperture, through which the grass blades were left exposed. Dishes were wrapped in tinfoil and stored vertically in the chambers. Moisture was maintained by delivering 2–3 ml water daily via the aperture. After 3 d, a single second instar larva was weighed and placed inside half of the cages, selected at random. Insects were from an established culture at UWS previously obtained from a site containing a range of C₃ and C₄ grasses [26]. After 7 d, the larva was removed and re-weighed. Roots were snap-frozen, freeze-dried and weighed. Material was milled and analysed for C and N concentrations using a LECO TruSpec[®] CHN analyser.

Statistical analysis

Analysis of variance (ANOVA) tests, in which chamber (and hence the three replicates of CO₂ treatment) were included as block terms to avoid pseudo-replication, were used. Plant biomass,

Table 1. Plant biomass responses to ambient and elevated CO₂ conditions.

Grass species	Atmospheric CO ₂ concentration	Plant biomass (g)		
		Total	Shoot	Root
<i>Cymbopogon refractus</i> (C ₄)	400	3.31 ± 0.23	2.51 ± 0.15	0.79 ± 0.09
	600	2.91 ± 0.19	2.31 ± 0.16	0.59 ± 0.05
<i>Microlaena stipoides</i> (C ₃)	400	2.49 ± 0.16	1.81 ± 0.11	0.67 ± 0.07
	600	2.27 ± 0.12	1.67 ± 0.10	0.60 ± 0.05
CO ₂ (F _{1,4})		F = 1.56, P = 0.280	F = 1.82, P = 0.248	F = 0.85, P = 0.409
Grass species (F _{1,108})		F = 17.79, P < 0.001	F = 26.23, P < 0.001	F = 1.11, P = 0.297
CO ₂ × grass species (F _{1,108})		F = 0.31, P = 0.582	F = 0.05, P = 0.822	F = 1.42, P = 0.239

Statistically significant effects indicated in **bold**.
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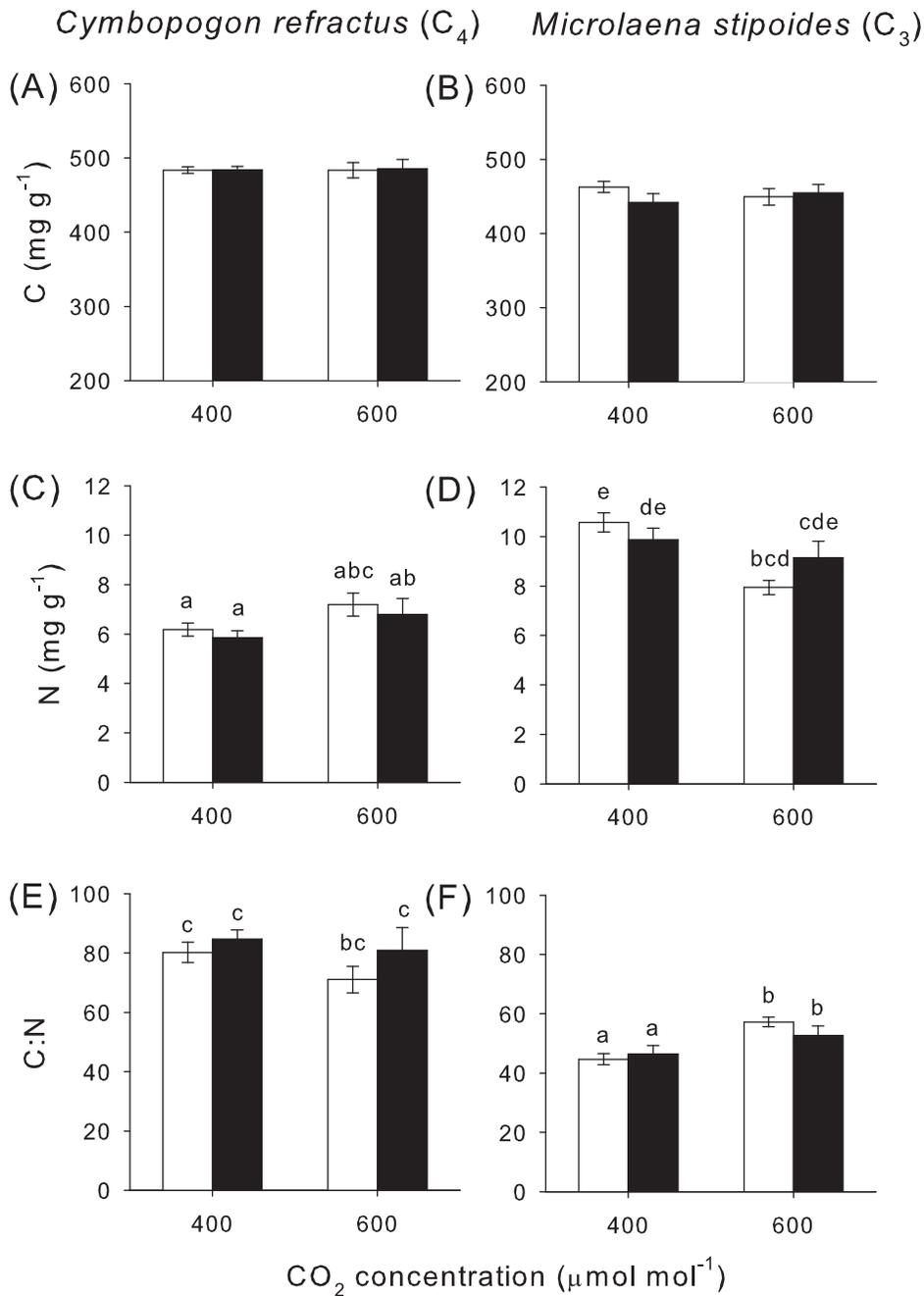


Figure 2. Carbon and nitrogen concentrations in grass roots. (A–B) Carbon (C), (C–D) nitrogen (N) concentrations and (E–F) C:N ratio in roots of C₃ (*Microlaena stipoides*) and C₄ (*Cymbopogon refractus*) grass species with (closed bars) and without (open bars) larval feeding. Mean \pm S.E. shown, N = 15. Lowercase superscripts indicate significant differences between treatments. doi:10.1371/journal.pone.0090251.g002

root consumption and larval mass was analysed with a two-way ANOVA (grass species and CO₂, with an interaction of each term) with initial larval mass included as a covariate in the latter case. Chemistry was analysed with three-way ANOVAs (grass species, CO₂ and insect presence, with interactions of each term). Differences between treatments were determined using least square mean tests when significant interactions between CO₂ and grass species existed. Unless indicated otherwise all analysis was conducted on untransformed data using Genstat (version 14, VSN International, UK).

Results

Plant responses

Plant biomass was unaffected by eCO₂, for either grass species, although *C. refractus* plants were significantly bigger than *M. stipoides* largely due to higher shoot mass (Table 1). Concentrations of root C were higher in *C. refractus* (Fig. 2A) than *M. stipoides* (Fig. 2B), but largely unaffected by other variables (Table 2). In contrast, root N concentrations were higher in *M. stipoides* than *C. refractus* (Fig. 2 C–D), but in this case there was also a significant interactive effect of CO₂ and grass species (Table 2). In particular,

Table 2. Summary of statistical analysis for carbon and nitrogen concentrations.

Responses	Fixed effects													
	CO ₂		Insects		CO ₂ ×Insects		Grass species		CO ₂ ×Grass species		Grass species×Insects		CO ₂ ×Grass species×Insects	
	F _{1,4}	P	F _{1,108}	P	F _{1,108}	P	F _{1,108}	P	F _{1,108}	P	F _{1,108}	P	F _{1,108}	P
Root Carbon ¹ – Fig. 1A–B	0.01	0.962	0.25	0.617	1.09	0.299	23.28	<0.001	0.01	0.922	0.48	0.490	0.83	0.363
Root Nitrogen ¹ Fig. 1C–D	0.43	0.547	0.17	0.680	1.53	0.219	89.91	<0.001	17.08	<0.001	1.20	0.276	2.35	0.129
C:N – Fig. 1E–F	0.23	0.653	1.09	0.299	0.01	0.933	109.62	<0.001	8.15	0.005	2.39	0.125	1.15	0.286

Statistically significant effects indicated in **bold**.

¹Arcsine square root transformation applied prior to analysis.

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eCO₂ reduced root N concentrations in *M. stipoides*, but not *C. refractus* (Fig. 2C–D). Roots of *C. refractus* had a higher C:N ratio than *M. stipoides* (Fig. 2E–F). Again there was a significant interaction between eCO₂ and grass type (Table 1); eCO₂ caused an increase in root C:N in *M. stipoides* (Fig. 2F), but not in *C. refractus* (Fig. 2E).

Insect responses

CO₂ did not affect root consumption overall (Fig. 3A) but consumption rose significantly on *M. stipoides* under eCO₂. No difference in root consumption was seen on *C. refractus* under either CO₂ treatment (Fig. 3A). The final mass of larvae was higher on *M. stipoides* than *C. refractus* at aCO₂, but under eCO₂ conditions larval mass was reduced to levels seen for those feeding on *C. refractus* (Fig. 3B).

Discussion

This study demonstrates that eCO₂ negatively affects a grass root herbivore when feeding on *M. stipoides*, a C₃ grass, but not on *C. refractus*, a C₄ grass. Elevated CO₂ caused bigger reductions in *M. stipoides* quality than in *C. refractus*, lowering N concentrations and increasing the C:N ratio and root consumption by insects. This increase in herbivore damage may make *M. stipoides* more susceptible to herbivory under predicted climate change than other competing species, with consequences for the composition of grassland systems. As hypothesised, we found that *M. stipoides* was a better quality host that *C. refractus*, and that eCO₂ had a bigger impact on *M. stipoides*. Our findings also matched our predictions that herbivore performance on *M. stipoides* would be worse under eCO₂ and compensatory feeding would take place, but we did not find that increased feeding compensated for this decrease in performance.

C₃ and C₄ grasses differ in physiological, anatomical and chemical traits which are thought to make C₃ grasses more susceptible to herbivory than C₄ grasses, giving rise to the C₃-C₄ hypothesis [27]. This states that herbivores should select and perform better on C₃ rather than C₄ plants because they find them easier and more nutritious to consume. Compared to C₃ grasses, C₄ grasses tend to be lower in protein, which is also less accessible to herbivores as it is stored in bundle sheath cells, and possess higher levels of structural carbohydrates, making them less suitable host plants for folivores [27,28]. The lower demands and uptake of N by C₄ grasses compared to C₃ grasses may similarly result in lower root N concentrations in C₄ grasses and make them less

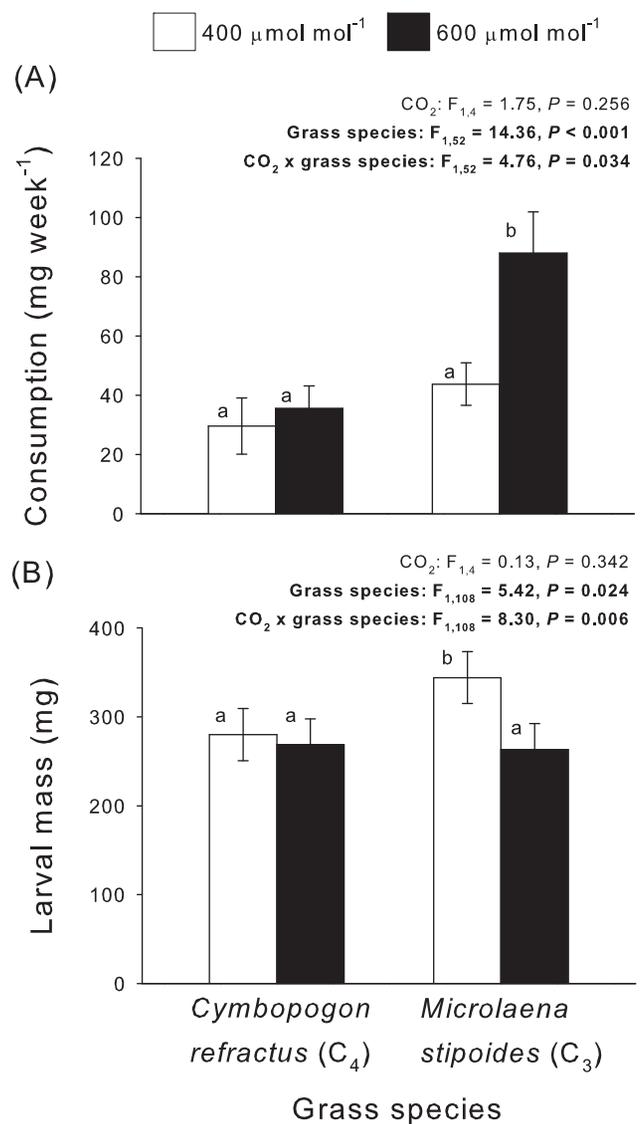


Figure 3. Feeding and performance (body mass) of larvae. (A) Root consumption and (B) larval mass when reared on *M. stipoides* and *C. refractus* under ambient and elevated CO₂. Mean ± S.E. shown, N = 15. Lowercase superscripts indicate significant differences between treatments. Statistically significant terms indicated in bold. doi:10.1371/journal.pone.0090251.g003

favourable for root herbivores [26]. Under aCO₂, *M. stipoides* represented a superior host for root herbivores than the C₄ grass *C. refractus*, in line with the C₃-C₄ hypothesis [27]. While tests with further species are needed, these findings provide some initial support for the C₃-C₄ hypothesis potentially operating below-ground as well as aboveground.

Despite evidence for compensatory feeding on the *M. stipoides* at eCO₂, insect performance remained depressed at eCO₂, suggesting that increased levels of herbivory were not enough to compensate for reduced plant quality. Similar effects occur for aboveground herbivores; Stiling and Cornelissen [2] concluded that most insect herbivores were generally unable to redress the problem of reduced food quality, and their abundance typically fell by 21% under eCO₂. Compensatory feeding imposes extra energy requirements [6] which may be particularly demanding for soil-dwelling herbivores that have to physically burrow through the soil to access new root tissue [29]. Further work is needed to understand mechanisms of compensatory feeding for belowground herbivores, but the fact that they could not adequately compensate for deterioration in food quality suggests that energy constraints and thresholds for host plant quality may play a role. For example, the costs and benefits associated with compensatory feeding are likely to vary with both host quality and the impact of changes in

quality on herbivore development and may only be possible above a quality threshold [30].

Compensatory feeding on C₃ grasses might be particularly damaging since we observed no significant increases in plant biomass in response to eCO₂. In their review, Hovenden and Williams [31] also note that Australian grasses are generally unresponsive to eCO₂ in terms of growth, so they may be prone to higher herbivory levels without the advantage of enhanced growth rates seen in many other plants under eCO₂. Our observations suggest that eCO₂ may contribute to compositional changes in grass communities if C₃ grasses are disproportionately damaged by root herbivores.

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Author Contributions

Conceived and designed the experiments: SNJ. Performed the experiments: SNJ GL. Analyzed the data: SNJ GL. Contributed reagents/materials/analysis tools: SNJ SEH. Wrote the paper: SNJ SEH.

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