Contingency in ecosystem but not plant community response to multiple global change factors

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Summary

• Community and ecosystem responses to global environmental change are contingent on the magnitude of change and interacting global change factors. To reveal whether responses are also contingent on the magnitude of each interacting factor, multifactor, multilevel experiments are required, but are rarely conducted.

• We exposed model grassland ecosystems to six levels of atmospheric CO_2 and six levels of nitrogen enrichment, applying the latter both chronically (simulating deposition) and acutely (simulating fertilization). The 66 treatments were maintained for 6 months under controlled growing conditions, with biomass harvested every 28 d and sorted to species.

• Aboveground plant productivity responses to CO_2 were contingent on nitrogen amount, and the responses to nitrogen amount were dependent on whether applications were chronic or acute. Specifically, productivity responses to increasing CO_2 concentrations were accentuated with higher nitrogen enrichments, and productivity was greater when higher nitrogen enrichments were applied acutely. Plant community composition was influenced only by nitrogen enrichment, where the co-dominant grass species with the greatest leaf trait plasticity increasingly dominated with higher nitrogen amounts.

• Community processes are considered to be unpredictable, but our data suggest that the prediction of the impacts of simultaneous global changes is more complex for ecosystem processes, given that their responses are contingent on the levels of interacting factors.

Introduction

Plant communities, and the ecosystems of which they are a part, face multiple global changes, including elevated atmospheric CO_2 concentrations ($[CO_2]$) and nitrogen (N) enrichment. An increasing number of multiple-factor global change experiments have been established to test whether community and ecosystem responses to one global change factor are modified by a second (Reich *et al.*, 2006b; Templer & Reinmann, 2011). These multifactor experiments reveal that the effects of one global change on community and ecosystem processes, such as plant abundance and productivity, are often contingent (*sensu* Schmitz, 2010) on a second global change (Dukes *et al.*, 2005; Reich *et al.*, 2006a; Kardol *et al.*, 2010; Morgan *et al.*, 2011). Given the logistical and economic constraints, multifactor experiments are usually performed at two levels of a specific factor, such as at ambient vs elevated levels. However, many of these factors vary across time and space in their magnitude, and this variation can alter their effects on community and ecosystem processes (Phoenix *et al.*, 2012). To investigate the effects of these varying

magnitudes, single-factor but multilevel experiments have been established; these experiments reveal nonlinearities in community and ecosystem responses across increasing availabilities of global change factors, such as $[CO_2]$ and N enrichment (Gill *et al.*, 2002; Granados & Körner, 2002; Bradford *et al.*, 2008; Fay *et al.*, 2009). Furthermore, meta-analyses suggest that the combined effects of these factors vary nonadditively across levels. For example, ecosystem responses to elevated $[CO_2]$ are highest in studies in which N addition rates are greatest (van Groenigen *et al.*, 2006). To determine definitively whether these nonadditive interactions are level dependent requires multifactor, multilevel global change experiments.

Managed grasslands comprise 70% of agricultural lands, and global changes that influence their productivity by changing forage amount and quality will influence human food production (Soussana & Lüscher, 2007). Elevated $[CO_2]$ is expected to increase forage amount where N is not limited (e.g. through fertilization), but impacts on forage quality are more uncertain as they are dependent on foliar trait plasticity (e.g. N concentration), as well as plant community composition (e.g. legumes vs

grasses; Soussana & Lüscher, 2007). Many of the C_3 species from managed grasslands were introduced by European settlers to the New World and so are common on multiple continents, across different fertilizer managements and under widely varying levels of N deposition (Hunt *et al.*, 1991, 1993). Although it is recognized that acute (e.g. fertilizer) vs chronic (e.g. atmospheric deposition) N enrichment is likely to influence community and ecosystem processes differently (Bernot & Dodds, 2005; Phoenix *et al.*, 2012), direct experimental tests appear to be lacking. Further, in the context of multifactor global change experiments, it appears unknown whether the regime (i.e. acute vs chronic) of N amendment will modify interactive effects of N with elevated $[CO_2]$.

We assessed community (species dominance) and ecosystem (aboveground net primary productivity, ANPP) responses to multiple global change factors using model grassland ecosystems. We applied six levels of $[CO_2]$ across six levels of N enrichment, with the latter added following either a chronic or acute regime to simulate deposition or fertilization, respectively. Although chronic and acute regimes varied in the [N] per addition, we made 56 chronic vs four acute additions across the 28 wk of the experiment, ensuring that chronic and acute treatments of the same N enrichment level (e.g. 70 kg N ha^{-1} yr^{-1}) received the same total amount of N. We expected a three-way interaction between $[CO_2] \times$ N enrichment $\times$ N regime, where N enrichment would reduce N limitation and hence yield greater ANPP gains with increasing $[CO_2]$, which, in turn, would alleviate moisture limitations through improved water use efficiency and so give greater ANPP responses to N enrichment. We expected these gains to be greater with acute vs chronic N addition, because we reasoned that the higher concentrations at which the acute additions were added would permit the plants to acquire more of the added N for growth. This expectation was based on the fact that plants switch from high- to low-affinity N uptake kinetics at higher N concentrations, and low-affinity systems have much higher maximal rates (Kraiser *et al.*, 2011). We also expected community composition to be determined by a three-way interaction, with productivity gains being mirrored by abundance increases in those species with higher relative growth rates (RGRs; representative of improved pastures and higher competitive ability) and decreases in those species with lower RGRs. We examined additional response variables (e.g. specific leaf area (SLA), foliar N concentration, root biomass) across a subset of the experimental ecosystems to attempt to explain the composition and productivity responses across the full experiment.

Materials and Methods

Model ecosystems

Experimental microcosms were constructed and maintained following protocols similar to those described previously for the same soils and plant communities (Maestre *et al.*, 2005; Maestre & Reynolds, 2007). The microcosms consisted of 38-cm-deep, 10-cm-diameter PVC tubing that contained a soil

profile (from the base) of 2 cm of gravel, 28 cm of mineral (surface A horizon) field soil and 4.5 cm of organic (H horizon) field soil mixed 1 : 1 with commercial sphagnum peat. Field soils were collected from The Duke Forest Teaching and Research Laboratory (Durham, NC, USA) from the top 30 cm of mineral horizon of a sandy loam of the White Store series. Herbaceous communities that contain the study species grow on this soil series, but we collected the soils under oak-dominated forest canopy to minimize the herbaceous seed bank. Soils were homogenized using a cement-mixer, and hand sorted to remove large stones, macrofauna and roots > 2 mm in diameter. We used field soils to provide complex soil microbiota and mesofauna communities, and to maintain the N limitation typical of many field conditions. These approaches permit the interpretation of plant responses to varying N availability that would not be possible with the use of common glasshouse growing materials (Hodge, 2004).

The experimental plant communities comprised two individuals of five species that are common, co-occurring, semi-natural grassland species: *Lolium perenne* L., *Holcus lanatus* L., *Poa pratensis* L., *Anthoxanthum odoratum* L. and the legume *Trifolium repens* L. (Hunt *et al.*, 1991, 1993; Staddon *et al.*, 1999; Joshi *et al.*, 2000). The high-RGR species (*L. perenne* and *H. lanatus*) are most abundant in intensively managed, improved pastures, and the two species with lower RGRs (*P. pratensis* and *A. odoratum*) occur more often in less improved systems. The species are distributed broadly across multiple continents, including North America and Europe, as well as oceanic islands, such as New Zealand, and occur in multiple habitats, including pastures, lawns and roadsides. Seeds were obtained from commercial suppliers and germinated in a glasshouse on different days to ensure all seedlings were at the same size and stage when planted (two-leaf seedling). Planting positions for the 10 seedlings were allocated randomly, but the pattern was maintained across all microcosms, at a density of 1270 individuals m^{-2}. Seedlings that died within the first 2 wk of planting were replaced.

The communities were established and maintained in growth chambers of the Duke University Phytotron. Plants were first grown for 2.5 months because we wanted to investigate treatment effects on established communities, and seedling responses often differ from those of mature plants (Warren & Bradford, 2011). Treatments were then applied for 6 months and, across the full 8.5 months, the walk-in growth chambers had a day : night air temperature of 21 : 12°C, relative humidity of 70 : 85% and a 16-h photoperiod. Environmental conditions were gradually ramped up and down between day and night across a simulated 2-h dawn and dusk. To prevent high-light shock, during the first week of plant establishment, full light intensity was limited to 500 μmol m^{-2} s^{-1}, to 750 μmol m^{-2} s^{-1} in the second week, and then maintained at 1000 μmol m^{-2} s^{-1} across the experiment. To establish the plants, microcosms received four additions of a dilute, modified Hoagland's solution. This was to reduce nutrient limitation of plant development, given that the mineral soils were Ultisols, which are characterized by low base status and inherent fertility. From these Hoagland solution additions, each unit received 1.8 kg N ha^{-1} yr^{-1}, which pro-

vided < 20% of the N added at the lowest level of N enrichment (described later). In addition, KH_2PO_4 and $CaSO_4$ were added in solution every 4 wk across the 8.5-month growing period to minimize the possibility that other limiting macronutrients would obscure interactions between N enrichment and the CO_2 and N regime treatments. These additions provided per microcosm element amounts equivalent to 32 kg K ha^{-1}, 25 kg P ha^{-1}, 40 kg Ca ha^{-1} and 32 kg S ha^{-1}. Chamber [CO_2] was maintained at 375 ppm (i.e. ambient) across the 2.5-month establishment period. Microcosms were watered 3 d a week with 100 ml of water and 2 d a week with 75 ml of water, giving a rate equivalent to precipitation of 1553 mm yr^{-1} and virtually no water loss from the base of the experimental units.

After 1.5 months of growth, plants were cut to 6 cm above the soil surface and then cut again, to 6 cm, 28 d later, following the cutting regime employed across the full experiment (see 'Response variables' below). Microcosms were assigned to one of five experimental blocks based on the dry foliar biomass recovered (block 1 had microcosms with highest biomass, and block 5 those with least biomass). Each one of the 66 global change treatments (Table 1 and described below) was randomly assigned to a microcosm within each block, giving 330 (66 treatments × 5 blocks) experimental units.

Global change treatments

Experimental treatments were maintained across 6 months, which, under controlled environment conditions, is equivalent to two 3-month growing seasons. Treatments consisted of six levels of [CO_2], spanning a gradient from sub- to super-ambient (i.e. 280, 325, 375, 450, 550 and 700 ppm, with 375 ppm representing ambient; Table 1). These concentrations were manipulated at the scale of a growth chamber and so, to avoid chamber artifacts, microcosms (and the associated [CO_2]) were rotated every 2 wk

(Granados & Körner, 2002). Each [CO_2] was crossed with six levels of N enrichment, ranging from lower rates typical of N deposition to higher rates typical of fertilization (i.e. 0, 10, 35, 70, 130 and 240 kg N ha^{-1} yr^{-1}; Table 1). To simulate N deposition, N was applied chronically by twice-weekly additions across the 28 treatment weeks (i.e. 56 additions; Table 1). These chronic N additions replaced the 75-ml water additions that occurred twice a week and, to minimize leaching losses, the microcosms were not watered the day after the 75-ml additions. To simulate N fertilization, N was applied four times across the 28 wk, replacing one of the 75-ml water additions, in weeks 1, 9, 17 and 25. The N addition regime ensured two things. First, that N was added at concentrations representative of deposition, except for the highest rate (Bradford et al., 2001). For the addition rates of 0, 10, 35, 70, 130 and 240 kg N ha^{-1} yr^{-1}, the chronic treatments had concentrations of 1.87, 6.54, 13.1, 24.3 and 44.9 mg N l^{-1}, respectively. This means that the concentrations of the 10, 35 and 70 kg N ha^{-1} yr^{-1} chronic treatments all fell below the threshold (i.e. 14 mg N l^{-1}) at which plants switch from high- to low-affinity uptake kinetics for NH_4^+ and NO_3^- (Kraiser et al., 2011). In contrast, all the acute treatments were above this threshold and, across the range of N enrichment rates, the acute treatments had concentrations of 26.2, 91.6, 183, 340 and 628 mg N l^{-1}, respectively. Second, the addition regime ensured that chronic and acute treatments of the same rate (e.g. 70 kg N ha^{-1} yr^{-1}) received the same amount of N across the 28 wk, so that the effect of N regime (chronic or acute) could be separated from N rate effects. We should note, however, that the chronic N addition rates of 130 and 240 kg N ha^{-1} yr^{-1} were established to match the acute, fertilizer-type additions; even the most extreme N deposition rates rarely exceed 100 kg N ha^{-1} yr^{-1}. Similarly, N fertilizer is not generally added at the lower N enrichment rates that we used; these rates are more typical of deposition.

Table 1 Full experimental design showing the 66 treatments (numbers 1–66) testing for interactive effects across six levels of atmospheric CO_2 concentration (280–700 ppm) and six levels of nitrogen (N) enrichment (0–240 kg N ha^{-1} yr^{-1}), with the latter applied following a chronic or acute regime

[CO_2] (ppm)	N rate (kg N ha^{-1} yr^{-1}) and regime (chronic, acute)					
	0	10 chronic, acute	35 chronic, acute	70 chronic, acute	130 chronic, acute	240 chronic, acute
280	1	7, 37	13, 43	19, 49	25, 55	31, 61
325	2	8, 38	14, 44	20, 50	26, 56	32, 62
375	3	9, 39	15, 45	21, 51	27, 57	33, 63
450	4	10, 40	16, 46	22, 52	28, 58	34, 64
550	5	11, 41	17, 47	23, 53	29, 59	35, 65
700	6	12, 42	18, 48	24, 54	30, 60	36, 66
Values of n for reported interaction and main effects[1]						
[CO_2] × N rate effect (pool across N regime)	5	10	10	10	10	10
N rate × N regime effect (pool across [CO_2])	30	30, 30	30, 30	30, 30	30, 30	30, 30
N rate effect (pool across [CO_2], N regime)	30	60	60	60	60	60

[1]The replication (n) for each of the treatments 1–66 is five, giving 330 experimental units (66 × 5). When investigating two-way interactions or main effects of the global change factors, experimental units can be pooled across the factors not involved in the effect being explored. For example, to investigate the N rate × N regime effect, one can pool across the six levels of [CO_2], so that, for 10 kg N ha^{-1} yr^{-1} added chronically, n = 30 (6 × 5; i.e. the sum of the experimental units from treatments 7–12).

Response variables

Plants were cut to 6 cm above the soil surface every 28 d, simulating periodic and unselective grazing/mowing of the pasture species. The cut foliar biomass was sorted to species and litter (i.e. senesced foliage), and the biomass (oven dried at 60°C to constant mass) was measured to estimate ANPP and species composition (by mass). However, two species contributed either all or the majority of the biomass above 6 cm per microcosm (see later), so that these measures of composition scored the relative dominance of the lower (*A. odoratum*) vs higher (*H. lanatus*) RGR co-dominant species. In total, there were seven experimental harvests across 196 d, generating 2310 samples (330 units × 7 harvests). Biomass for the community and by species was summed across these seven harvests for the statistical analyses, giving cumulative ANPP. The primary reason for summing these numbers was because, for the same N enrichment rate, the chronic and acute regimes only equaled each other in the amount of N applied by the end of the experiment.

To help to interpret the ANPP and community dominance responses, we measured a suite of additional variables on a subset of the microcosms. These microcosms were drawn from blocks 1–3 (i.e. $n = 3$), and received the following treatments: 280, 375, 550 or 700 ppm [CO_2] crossed with 0, 70 or 240 kg N ha^{-1} yr^{-1}, with the N applied chronically or acutely. This gave 60 microcosms (3 replicates × 4 [CO_2] × 2 N enrichments × 2 N regimes + 12 microcosms receiving water only (i.e. 0 kg N ha^{-1} yr^{-1})). For these 60 microcosms, volumetric soil moisture was measured every 2 wk with a handheld Hydrosense Soil Water Content Measurement System (Campbell Scientific Inc., Logan, UT, USA). Then, as part of the last harvest, a suite of leaf traits, soil pH and root and shoot variables were measured.

We focused on *A. odoratum* and *H. lanatus* for the assessment of leaf traits because these two species accounted for between 83.7% and 99.5% of the cumulative ANPP per microcosm. The other three species persisted below 6 cm, but were highly subordinate across all treatments, and only rarely contributed to the biomass above 6 cm. Leaf area (for the determination of SLA) was determined for blades > 6 cm above the soil surface immediately after cutting using an LI-3100c Leaf Area Meter (LiCor Inc., Lincoln, NE, USA). After drying at 60°C to constant mass, the leaf blades were weighed and then ball milled to a fine powder for the determination of total C, N and stable N isotope ratios using an NA1500 CHN Analyzer (Carlo Erba Strumentazione, Milan, Italy) coupled to a continuous-flow, isotope ratio mass spectrometer (IRMS; Thermo, San Jose, CA, USA). For the IRMS, the analytical precision was ± 0.1 δ^{15}N‰ and working standards were calibrated to atmospheric N$_2$. Total N and C contents permitted us to determine foliar %N and C : N ratios. The δ^{15}N values were used to estimate each species' ability to acquire amended N, estimated as the percentage recovery of a ^{15}N tracer added in the last 4 wk of the experiment during the regular N additions. The tracer was 5.5 atom% ^{15}NH$_4$^{15}NO$_3$ (Isotec Inc., Miamisburg, OH, USA) and was used for the remaining eight chronic N amendments and the final acute amendment, so

that we could measure what proportion of the ^{15}N tracer was recovered in the plant biomass. The proportion of added tracer N recovered in plant tissue was calculated as follows:

$$(\text{Plant}_N \cdot ((\text{Atom\%}^{15}\text{N}_{treat} - \text{Atom\%}^{15}\text{N}_{nat})/(\text{Atom\%}^{15}\text{N}_{amend} - \text{Atom\%}^{15}\text{N}_{nat})))/\text{Amend}_N,$$

where Plant_N is the biomass N of the plant tissue of interest, $\text{Atom\%}^{15}\text{N}_{treat}$ is the atom%^{15}N value of the same tissue, $\text{Atom\%}^{15}\text{N}_{nat}$ is the atom%^{15}N value of the tissue in the microcosms receiving only water (i.e. natural abundance values), $\text{Atom\%}^{15}\text{N}_{amend}$ is the atom%^{15}N value of the added N and Amend_N is the mass of N added either chronically or acutely in the last 4 wk of the experiment. We used atom% and not δ^{15}N values, given that we were dealing with substantially enriched materials (Fry, 2006).

Foliar biomass was cut at the soil surface (0–6 cm) of the subset of 60 microcosms, dried at 60°C to constant mass and weighed. Roots were treated similarly after washing clean of soil. This shoot and root material was ball milled to a fine powder and total C, N and stable N isotope ratios were determined as described above, so that we could determine the total mass of shoot and root C and N, C : N ratios and the proportion of added tracer N acquired by the plant community. Lastly, duplicate subsamples of soils that had been passed through a 2-mm mesh were used to determine pH, measured after mixing soil with water 1 : 1 by volume (Allen, 1989).

Statistical analysis

We fitted linear mixed models (LMMs) to investigate the ecosystem (cumulative ANPP across the harvests) and community (proportion of *A. odoratum* biomass of the cumulative ANPP) responses to the global change treatments with block fit as a random factor. Given that *A. odoratum* and *H. lanatus* dominated the community biomass above 6 cm, composition responses were tested with the proportion of both species and the models returned the same results; therefore, we report results for the proportion of *A. odoratum*. Given that N was added neither chronically nor acutely at the 0 kg N ha^{-1} yr^{-1} amendment rate, the inclusion of these 30 microcosms in the analyses precluded the investigation of an N rate by N regime interaction. Because overall treatment effects were similar whether or not we included these microcosms, and that responses at 0 vs 10 kg N ha^{-1} yr^{-1} were essentially equivalent (see the Results section), we report results for the 300 microcosms in which we had a full factorial design ([CO_2] × N rate × N regime).

The LMMs were fitted assuming a Gaussian error distribution ('identity' link function) and using the Laplace approximation in the 'lme4' package for the 'R' statistical program (R Development Core Team, 2011). We selected minimally adequate models using the lowest Akaike information criterion (AIC). Variance inflation factors of < 1 indicated that collinearity was low between model variables. The same approaches were used to assess responses in the 60 microcosms in which we carried out more intensive sampling.

The F statistic is not considered to be accurate for the 'lme4' package, given fixed denominator degrees of freedom, making P values anti-conservative (Baayen et al., 2008; Zuur et al., 2009). To overcome this, we used a Markov Chain Monte Carlo (MCMC) approach in the 'languageR' package (Baayen, 2007) to estimate coefficients and P values for retained parameters in LMMs. All reported P values are quasi-Bayesian, rather than the classical frequentist P values. We considered coefficients with $P < 0.05$ to be significant and coefficients with $P < 0.10$ to be marginally significant (Hurlbert & Lombardi, 2009).

Results

For cumulative ANPP across the seven harvests, the best-fit model retained significant interaction terms for [CO$_2$] × N rate ($P = 0.0000$) and N rate × N regime ($P = 0.0014$). The [CO$_2$] × N rate interaction appeared to be a product of the fact that responses to increasing levels of [CO$_2$] and N were generally greater when the other resource was in higher supply. For example, ANPP was c. 2.3 times greater at 700 ppm [CO$_2$] when the N rate was 240 as opposed to 10 kg N ha^{-1} yr^{-1}, but it less than doubled between the same N rates at 280 ppm [CO$_2$] (Fig. 1). Equally, at 240 kg N ha^{-1} yr^{-1}, ANPP increased by c. 1.4 times across 280–700 ppm [CO$_2$], but by only c. 1.2 times across the same [CO$_2$] levels for 10 kg N ha^{-1} yr^{-1} (Fig. 1). Also evident is that the shape of the ANPP response across [CO$_2$] appeared to be strongly dependent on the N rate. For example, with 0 kg N ha^{-1} yr^{-1}, maximum ANPP was recorded across 375–450 ppm, but, at 240 kg N ha^{-1} yr^{-1}, the maximum occurred across 450–550 ppm, and, at 70 kg N ha^{-1} yr^{-1}, the maximum ANPP occurred at 700 ppm (Fig. 1).

The N rate × N regime interaction arose because ANPP under acute N amendment did not appear to differ from ANPP under chronic N amendment at the lowest N rates, but became progressively greater with increasing N rate (Fig. 2). At 240 kg N ha^{-1} yr^{-1}, acute additions resulted in c. 1.15 times greater ANPP than chronic additions, giving a mean difference of 126 g m^{-2} (Fig. 2).

In contrast with the ANPP responses, there were no interactions between the CO$_2$ and N treatments on community dominance. However, as for ANPP, the community response was dependent on the N enrichment rate. The best-fit LMM only retained this factor and its positive coefficient ($P = 0.0001$) indicated the shifting biomass dominance from H. lanatus to A. odoratum with increasing N rate (Fig. 3).

We evaluated a suite of additional response variables on a subset of 60 microcosms to help to explain the ANPP and community composition responses across the full experiment. Before using these variables, we checked that ANPP (estimated for the subset as the standing aboveground biomass at the final harvest, permitting direct comparison with the other plant biomass variables measured for the subset) and community dominance responded similarly to the full experiment. This was true for ANPP, where there was a [CO$_2$] × N rate interaction ($P = 0.07$), with ANPP responses to increasing [CO$_2$] or N rate being greater when the level of the other resource was greater (Table 2). This same response appeared to be mirrored in the whole-community shoot and root C biomass values (Table 2). Perhaps facilitating these synergistic effects between [CO$_2$] and N rate, [CO$_2$] was a significant predictor of soil moisture ($P = 0.0001$), which, for example, increased steadily from 16% to 24% at the highest N rate as [CO$_2$] increased from 280 to 700 ppm (Table 2). Equally, acidification of mineral soil pH with increasing N appeared to be muted by higher [CO$_2$] ([CO$_2$] × N rate interaction: $P = 0.062$). Other response variables appeared to be less directly tied to the [CO$_2$] × N rate interaction on ANPP, but were typical for single factor [CO$_2$] and N rate effects, such as increased ($P = 0.0001$) and
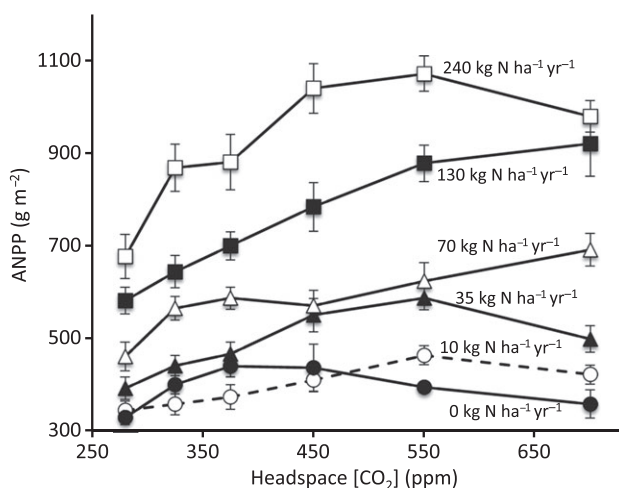
Fig. 1 Interactive effects of atmospheric CO$_2$ concentration and nitrogen (N) enrichment rate on aboveground net primary productivity (ANPP). Shown are the means ± 1SE ($n = 10$, data pooled across N regime: Table 1). Note that $n = 5$ for the 0 kg N ha^{-1} yr^{-1} values. To aid visual interpretation, mean values within an N enrichment level are connected by solid or dashed lines.
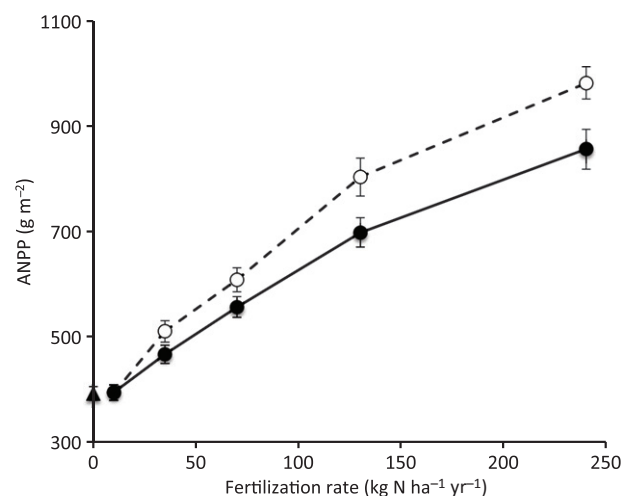
Fig. 2 Interactive effects of nitrogen (N) enrichment rate and regime (chronic, closed circles; acute, open circles) on aboveground net primary productivity (ANPP). ANPP for plants watered but without N amendment is shown by the closed triangle. Shown are means ± 1SE ($n = 30$, data pooled across CO$_2$ concentration: Table 1).
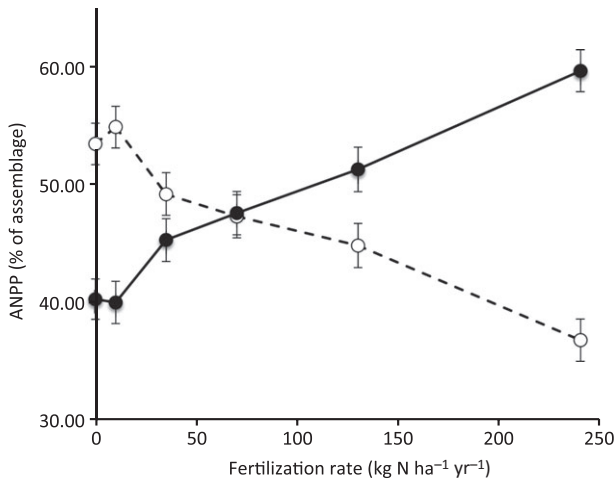
Fig. 3 Effect of nitrogen (N) enrichment rate on plant community composition, represented as the proportional biomass contribution to aboveground net primary productivity (ANPP) of the co-dominant species *Anthoxanthum odoratum* (closed circles) and *Holcus lanatus* (open circles). Shown are means ± 1SE ($n = 60$, data pooled across CO_2 concentration and N regime: Table 1). Note that $n = 30$ for the 0 kg N ha^{-1} yr^{-1} values because data could only be pooled across CO_2 concentration.

reduced ($P = 0.0001$) shoot C : N ratios with increasing [CO_2] and N, respectively (Table 2). Further, shoot N ($P = 0.0001$) and the proportion of ^{15}N-labeled amendment acquired by the shoots ($P = 0.0001$) generally increased with N rate (Table 2). Root responses were more idiosyncratic: root C : N ratios appeared to be relatively unresponsive to [CO_2] and N rate ($P > 0.01$), the proportion of ^{15}N-labeled amendment acquired

by roots was lower ($P = 0.0001$) at 240 vs 70 kg N ha^{-1} yr^{-1}, and root mass N was dependent on [CO_2] and N rate (interaction: $P = 0.02$). This interaction occurred because root mass N was unaffected by N rate at certain [CO_2] (e.g. 375 ppm), but at others (e.g. 550 and 700 ppm) responded positively to at least the highest N level (Table 2).

Unlike the [CO_2] × N rate interaction, the N rate × N regime interaction for the subset did not mirror the full experiment as it was not significant ($P = 0.13$) and, indeed, there was little difference in standing ANPP between chronic and acute treatments (Table 3). The absence of a chronic vs acute difference was reflected in the majority of the other variables, with the only obvious effects being of N rate itself, which decreased pH ($P = 0.0001$), soil moisture ($P = 0.03$) and shoot C : N ratios ($P = 0.0001$), and increased masses of shoot and root C and N ($P = 0.0001$ in all four instances; Table 3). The only variable for which chronic vs acute treatments differed was the proportion of the ^{15}N-labeled amendment recovered in the shoots; it was *c.* 1.7 times greater when added acutely ($P = 0.0001$), and this acute vs chronic difference was slightly larger at 240 vs 70 kg N ha^{-1} yr^{-1} (N rate × N regime interaction: $P = 0.0001$; Table 3).

For community dominance, the subset of 60 microcosms behaved almost identically to the full experiment, with increasing N rate shifting biomass dominance from *H. lanatus* to *A. odoratum* ($P = 0.0002$, Table 4). At higher N rates, both species had higher foliar %N ($P = 0.0001$ for both species), lower foliar C : N ratios (*A. odoratum*, $P = 0.0001$; *H. lanatus*, $P = 0.03$) and higher SLA (Table 4), although the increase was only significant for *A. odoratum* (*A. odoratum*, $P = 0.06$; *H. lanatus*, $P = 0.22$).

Table 2 Atmospheric CO_2 concentration and nitrogen (N) enrichment rate effects on soil and community variables for the subset of 60 microcosms[1]

[CO_2] (ppm)	N rate (kg N ha^{-1} yr^{-1})	pH[2]	Moisture (%)[3]	ANPP (g m^{-2})[4]	Shoot mass C (g m^{-2})	Root mass C (g m^{-2})	Shoot mass N (g m^{-2})	Root mass N (g m^{-2})	Shoot C : N	Root C : N	Shoot ^{15}N (%)[5]	Root ^{15}N (%)
280	0	4.66 + 0.02	20 ± 0.3	339 ± 26	372 ± 15	820 ± 89	5.1 ± 0.2	15 ± 1.5	74 ± 3.2	56 ± 0.8	na	na
	70	4.55 + 0.01	20 ± 0.3	488 ± 46	434 ± 39	859 ± 33	5.7 ± 0.6	18 ± 1.6	77 ± 3.7	50 ± 4.1	39 ± 4.4	29 ± 3.3
	240	4.40 + 0.03	16 ± 0.8	673 ± 32	632 ± 43	1104 ± 69	10.0 ± 0.6	22 ± 2.2	63 ± 3.0	51 ± 3.7	48 ± 6.4	27 ± 1.3
375	0	4.66 + 0.05	23 ± 1.4	445 ± 42	337 ± 16	719 ± 66	3.7 ± 0.3	15 ± 2.7	92 ± 2.8	49 ± 8.3	na	na
	70	4.57 + 0.03	22 ± 0.4	579 ± 32	434 ± 24	869 ± 41	5.2 ± 0.4	15 ± 0.9	84 ± 3.3	60 ± 2.9	36 ± 7.5	26 ± 1.1
	240	4.48 + 0.04	21 ± 0.8	883 ± 87	692 ± 45	988 ± 66	10.9 ± 0.6	16 ± 1.4	64 ± 3.8	61 ± 1.5	46 ± 5.3	21 ± 2.1
550	0	4.75 + 0.02	22 ± 0.4	411 ± 6	401 ± 53	737 ± 111	3.7 ± 0.5	12 ± 2.7	109 ± 6.0	67 ± 5.3	na	na
	70	4.69 + 0.04	24 ± 0.4	654 ± 64	443 ± 13	871 ± 66	5.0 ± 0.1	17 ± 2.5	89 ± 3.2	54 ± 4.5	36 ± 4.9	28 ± 2.1
	240	4.55 + 0.05	22 ± 0.9	1119 ± 50	675 ± 60	1187 ± 81	9.5 ± 0.5	24 ± 1.6	71 ± 4.9	51 ± 3.4	49 ± 6.6	25 ± 1.7
700	0	4.63 + 0.04	26 ± 3.7	342 ± 28	377 ± 26	893 ± 215	4.0 ± 0.2	18 ± 4.7	94 ± 4.1	52 ± 7.2	na	na
	70	4.66 + 0.04	24 ± 0.7	742 ± 44	504 ± 35	1008 ± 82	5.8 ± 0.6	19 ± 1.4	90 ± 7.7	52 ± 2.7	39 ± 5.6	30 ± 1.0
	240	4.54 + 0.03	24 ± 0.5	947 ± 46	705 ± 49	1467 ± 67	9.2 ± 0.4	31 ± 2.8	76 ± 3.0	49 ± 4.0	41 ± 6.1	28 ± 0.9

[1]Shown are means ± 1SE. For the subset of 60 microcosms (i.e. experimental units), each treatment had $n = 3$. Data presented here are pooled across the N regime, so that $n = 6$ (i.e. 2 N regimes × 3 units), except for the 0 kg N ha^{-1} yr^{-1} values, where $n = 3$.
[2]pH values were back-transformed from [H$^+$] and so, given the asymmetry in the errors, only positive error values are shown.
[3]Moisture is reported as the volumetric soil water content.
[4]Aboveground net primary productivity (ANPP), and shoot and root mass C and N, are based on the standing biomass at the final experimental harvest.
[5]Proportion of the ^{15}N tracer added that was recovered in the shoot and root tissues at the final experimental harvest.
na, not applicable.

Table 3 Nitrogen (N) enrichment rate and N regime effects on soil and community variables for the subset of 60 microcosms[1]

N regime	N rate (kg N ha^{-1} yr^{-1})	pH[2]	Moisture (%)[3]	ANPP (g m^{-2})[4]	Shoot mass C (g m^{-2})	Root mass C (g m^{-2})	Shoot mass N (g m^{-2})	Root mass N (g m^{-2})	Shoot C : N	Root C : N	Shoot ^{15}N (%)[5]	Root ^{15}N (%)
na	0	4.67 + 0.02	23 ± 1.0	384 ± 18	372 ± 15	792 ± 61	4.1 ± 0.2	15 ± 1.5	92 ± 4.2	56 ± 3.2	na	na
Chronic	70	4.59 + 0.03	22 ± 0.8	604 ± 29	489 ± 34	964 ± 66	5.9 ± 0.6	19 ± 1.5	86 ± 3.7	51 ± 2.2	29 ± 3.4	29 ± 1.6
Acute	70	4.63 + 0.03	23 ± 0.5	657 ± 48	433 ± 16	890 ± 32	5.3 ± 0.3	16 ± 1.6	83 ± 3.7	58 ± 2.4	48 ± 2.4	27 ± 1.2
Chronic	240	4.48 + 0.03	21 ± 1.1	904 ± 69	696 ± 35	1135 ± 64	9.6 ± 0.4	24 ± 2.4	73 ± 2.7	50 ± 3.0	34 ± 2.4	26 ± 1.5
Acute	240	4.50 + 0.04	20 ± 1.0	908 ± 53	657 ± 33	1238 ± 75	10.3 ± 0.4	23 ± 1.6	64 ± 2.6	56 ± 1.8	58 ± 1.5	25 ± 1.2

[1]Shown are means ± 1SE. For the subset of 60 microcosms (i.e. experimental units), each treatment had n = 3. Data presented here are pooled across CO_2 treatments, so that n = 12 (i.e. 4 [CO_2] × 3 units).
[2]pH values were back-transformed from [H$^+$] and so, given the asymmetry in the errors, only positive error values are shown.
[3]Moisture is reported as the volumetric soil water content.
[4]Aboveground net primary productivity (ANPP), and shoot and root mass C and N, are based on the standing biomass at the final experimental harvest.
[5]Proportion of the ^{15}N tracer added that was recovered in the shoot and root tissues at the final experimental harvest.
na, not applicable.

Table 4 Effect of nitrogen (N) enrichment rate on soil, community and leaf variables of the co-dominant species *Anthoxanthum odoratum* (Ant) and *Holcus lanatus* (Hol) for the subset of 60 microcosms[1]

N rate (kg N ha^{-1} yr^{-1})	Spp.	pH[2,3]	Moisture (%)[4]	ANPP (g m^{-2})[5]	Comp. (%)[6]	Foliar [N] (%)	Foliar C : N	SLA (cm^{-2} g^{-1})	Foliar ^{15}N (%)[7]
0	Ant	4.46 + 0.02	23 ± 1.0	384 ± 18	37 ± 3.2	0.96 ± 0.06	46 ± 2.2	79 ± 8.0	na
70	Ant	4.34 + 0.02	23 ± 0.4	616 ± 30	44 ± 3.3	1.06 ± 0.03	42 ± 1.3	106 ± 8.1	5.8 ± 0.6
240	Ant	4.21 + 0.02	21 ± 0.7	906 ± 43	59 ± 3.1	1.29 ± 0.05	35 ± 1.4	109 ± 5.7	8.6 ± 0.9
0	Hol	na	na	na	57 ± 3.2	0.97 ± 0.06	44 ± 2.3	117 ± 17.1	na
70	Hol	na	na	na	51 ± 3.1	1.01 ± 0.04	43 ± 1.9	134 ± 9.1	2.0 ± 0.4
240	Hol	na	na	na	38 ± 3.1	1.14 ± 0.07	40 ± 2.4	139 ± 7.7	1.9 ± 0.5

[1]Shown are means ± 1SE. For the subset of 60 microcosms (i.e. experimental units), each treatment had n = 3. Data presented here are pooled across CO_2 and N regime treatments, so that n = 24 (i.e. 4 [CO_2] × 2 N regimes × 3 units). Note that n = 12 for the 0 kg N ha^{-1} yr^{-1} values because data could only be pooled across CO_2 treatments.
[2]pH values were back-transformed from [H$^+$] and so, given the asymmetry in the errors, only positive error values are shown.
[3]pH, moisture and aboveground net primary productivity (ANPP) values are only given in Ant rows because they apply to the whole microcosm level, whereas species were within a microcosm.
[4]Moisture is reported as the volumetric soil water content.
[5]ANPP, foliar [N] and specific leaf area (SLA) are based on the standing biomass, above 6 cm from the soil surface, at the final experimental harvest.
[6]Comp. is the proportional contribution of each species to ANPP at the final experimental harvest.
[7]Proportion of the ^{15}N tracer added that was recovered in the shoot and root tissues at the final experimental harvest.
na, not applicable.

A significant N rate × species interaction for foliar %N (P = 0.0032) and C : N (P = 0.0028) revealed that *A. odoratum* showed greater relative increases in these leaf traits: for example, its foliar %N increased *c.* 1.34 times from 0 to 240 kg N ha^{-1} yr^{-1}, but only *c.* 1.18 times for *H. lanatus* (Table 4). For both species, SLA responded most between 0 and 70 kg N ha^{-1} yr^{-1}, and foliar N (and C : N) most between 70 and 240 kg N ha^{-1} yr^{-1}, revealing marked differences and nonlinearities in the response size of different leaf traits across N rate. In addition, although, on average, *H. lanatus* was still more abundant than *A. odoratum* at 70 kg N ha^{-1} yr^{-1}, the latter acquired almost three times as much N from the ^{15}N-labeled amendments, and even greater amounts at 240 kg N ha^{-1} yr^{-1} (N rate × species interaction: P < 0.05; Table 4).

Discussion

Our hypothesis was that we would see a three-way interaction between [CO_2], N rate and N regime, with greatest ANPP and shifting composition to dominance by the species with higher RGR (e.g. *H. lanatus*) at the highest [CO_2] and N rate, when N was applied acutely. Our rationale for this hypothesis was that elevated [CO_2] responses would be N limited, as is often observed with natural soils (Reich *et al.*, 2006a,b; Leakey *et al.*, 2009;

Drake *et al.*, 2011; Luo *et al.*, 2011). Hence, we expected N enrichment to remove this limitation, favoring species of improved pastures, and that higher soil solution N concentrations from acute additions would make N most available to the plants (Kraiser *et al.*, 2011). Greater ANPP with higher N rates, especially when applied acutely (Fig. 2), and greater relative increases in productivity with increasing $[CO_2]$ at higher N rates (Fig. 1), seemed to confirm these expected mechanisms. However, ecosystem responses to the global change factors were simpler than projected, with only two-way interactions between $[CO_2]$ and N rate, and N rate and N regime (Figs 1, 2). Further, the community dominance only responded to N rate and, in contrast with our expectations, by shifting dominance to a species with lower RGR and more typical of less improved pasture (Fig. 3).

Multifactor global change experiments are still rare, especially longer term experiments (Dukes *et al.*, 2005; Reich *et al.*, 2006b; Dawes *et al.*, 2011; Luo *et al.*, 2011), and multifactor, multilevel experiments are rarer still (Lewis *et al.*, 2010). Given the cost and logistics of such experiments, one proposed solution is to use mechanistic insights from single-factor experiments to construct models that project ecosystem response and feedback to global environmental change (Ostle *et al.*, 2009). Our observations contribute empirical data highlighting the limitation of this approach, where combinations of factors yield ecological responses that may not be predicted from single-factor responses (Luo *et al.*, 2011). In our case, the surprise was less contingency than anticipated, where, although we observed N limitation of the $[CO_2]$ response, and higher productivity with acute vs chronic N, we did not observe a three-way interaction across our factors on the ecosystem or community variables. Perhaps, most importantly, our data support the idea that both the level and regime of applied N matters in determining the system response (Phoenix *et al.*, 2012). Meta-analyses reveal that the effects of elevated $[CO_2]$ on productivity may be greater under higher vs lower N addition (van Groenigen *et al.*, 2006) and we provide direct empirical support for this (Fig. 1). Further, many experimental studies add N at concentrations and frequencies not representative of deposition (Phoenix *et al.*, 2012), and we show that productivity responses differ with acute vs chronic N addition, especially at the higher addition rates typical of many experiments (Neff *et al.*, 2002; Mack *et al.*, 2004; Bradford *et al.*, 2008). Lastly, low rates of N addition, whether chronic or acute, are rarely investigated (Phoenix *et al.*, 2012), but our data suggest that low rates still drive community change (Fig. 3).

We find general support for the idea that ANPP responses to $[CO_2]$ are N limited, whether by initial N availability or progressive N limitation (PNL; Reich *et al.*, 2006b; Luo *et al.*, 2011). The phenomenon of PNL might explain why productivity under 0 kg N ha^{-1} yr^{-1} peaked at ambient $[CO_2]$ (Fig. 1), especially given the pronounced increase in shoot C : N at higher $[CO_2]$ (Table 2). Such an increase might feed back to bind N in organic detrital forms that are less available for plant growth. Higher soil moistures with higher $[CO_2]$ (Table 2) are consistent with the expectation that C_3 plants have higher water use efficiencies under elevated $[CO_2]$ (Morgan *et al.*, 2011); this reduced moisture limitation (despite higher ANPP), combined

with reduced N limitation through N addition, probably explained why the highest productivity was observed with higher $[CO_2]$ and N rate (Fig. 1). This interaction may also have been facilitated by the fact that N addition was less acidifying at higher vs lower $[CO_2]$ concentrations (Table 2), where acidification can decrease plant performance given the increased mobility of toxic ions in soils (Phoenix *et al.*, 2012). We cannot uncouple the relative contributions of moisture, N and H^+ availabilities in determining ANPP responses to $[CO_2]$ × N rate, but our data do suggest that the interaction is probably driven by more than just N limitation.

Our data confirm the expectation that the productivity of fertilized grasslands will increase with rising atmospheric $[CO_2]$ (van Kessel *et al.*, 2000; Soussana & Lüscher, 2007). However, such an increase is accompanied by declines in forage quality (Table 2; shoot data), and these declines in quality are expected to alter biotic interactions and foodweb structure aboveground (Reich *et al.*, 2006a; Tylianakis *et al.*, 2008; Antoninka *et al.*, 2009). Notably, root responses to $[CO_2]$ × N rate were idiosyncratic across traits and factor levels, with changes in C : N ratios and total N amount at some $[CO_2]$ but not others, and lower acquisition of the ^{15}N tracer under higher vs lower N rates (Table 2). These differences between the foliar and root responses to our global change treatments question whether aboveground responses to global changes are a suitable proxy for belowground responses. If they are not a suitable proxy, then to understand the consequences of multiple global changes for root–herbivore-based foodwebs requires that we redress the paucity of investigations into root mass and stoichiometric responses to $[CO_2]$ and N enrichment (Antoninka *et al.*, 2009; Anderson *et al.*, 2010).

Data from the subset of 60 microcosms added little to our understanding of what drives the N rate × N regime interaction on ANPP (Fig. 2). The N rate showed large and expected consequences on variables such as ANPP, shoot C : N and pH. However, unlike ANPP in the full experiment, N regime had little influence on ANPP in the subset and, indeed, little influence on shoot and root N dynamics, pH and moisture (Table 3). The lack of effect on ANPP may have been a result of reduced statistical power (as our *n* dropped from five to three per treatment) and/or because ANPP for the subset of microcosms was based on the standing plant biomass at the final experimental harvest, but calculated across harvests 2–8 for the full experiment from the biomass clipped 6 cm above the soil height. The only variable influenced by the N regime was the amount of fertilizer acquired by the shoots, estimated through the percentage recovery of the ^{15}N tracer, which was higher under acute N (Table 3). This greater acquisition under the acute regime might explain why ANPP responses to acute additions in the full experiment increasingly diverged from chronic additions as the N rate increased (Fig. 2). The most plausible mechanism to explain this divergence is that plants switch from high- to low-affinity uptake kinetics as soil solution NH_4^+ and NO_3^- concentrations increase (Kraiser *et al.*, 2011). Low-affinity kinetics are associated with first-order and much higher rates of N uptake, meaning that plants should acquire N for growth more rapidly when soil

solution N concentrations are higher. However, other than ANPP, our data suggest that many soil and plant variables are relatively insensitive to the N regime (Table 3). Therefore, although the single to few large doses of N applied in many field studies may poorly represent N deposition to ecosystems (Phoenix *et al.*, 2012), they may still permit valid inferences about N deposition effects on a range of response variables. To test this possibility robustly will require large-scale field manipulations that investigate directly N regime effects.

Lawton (1999) infamously argued that community ecology is a mess, opining that contingency in local processes makes community responses to perturbations overwhelmingly complicated, and hence unpredictable. In reply to Lawton, emerging syntheses in community ecology (Vellend, 2010) outline frameworks for making general statements about fundamental processes that underlie community dynamics at local scales, but remain pessimistic about our ability to predict how particular processes shape a specific community. However, we observed less 'mess' in our community than in ecosystem data, with the community response only driven by N rate and not contingent on [CO_2] or N regime (Fig. 3). The greater phenotypic plasticity across N rate in *A. odoratum* leaf traits, associated with light interception (SLA) and photosynthetic rates (%N), provides the most parsimonious explanation for why it increased in relative abundance at the expense of *H. lanatus* (Fig. 3 and Table 4). Such a clear mechanistic interpretation was not obvious for the ecosystem data, but, as in the ANPP response, the importance of the magnitude of a global change factor in determining its relative effect size is evident. For example, the most abrupt shift in community dominance was between 10 and 35 kg N ha^{-1} yr^{-1} (Fig. 3), showing not only that low additional N loadings associated with N deposition can influence community processes (Phoenix *et al.*, 2012), but also that they can have the greatest relative effects, making even 'clean' systems vulnerable to N deposition. Leaf traits also exhibited different sensitivities across different N levels, with SLA increasing most from 0 to 70 kg N ha^{-1} yr^{-1}, and foliar %N from 70 to 240 kg N ha^{-1} yr^{-1} (Table 4). These results demonstrate that interpretations of functional leaf trait responses to a global change factor might need to carefully consider the level at which the factor is applied.

We used a multispecies plant community and field soils, which presumably included beneficial and pathogenic soil organisms, but our work was still shorter term (6 months) and applied factors in a stepwise increase. In longer term studies, shifts in plant community composition can eventually negate positive effects of a factor, such as [CO_2], on productivity (Langley & Megonigal, 2010), and incremental or continuous increases in [CO_2] have the potential to yield very different productivity and community consequences compared with stepwise increases (Luo & Reynolds, 1999; Klironomos *et al.*, 2005). Nevertheless, none of the longer term field studies have assessed grassland community responses to [CO_2] × N rate at more than two levels, nor have they looked at the N regime. Yet, we demonstrate that level and regime can alter ecosystem and community responses, making the magnitude and shape of field responses uncertain across space and time as the amount and nature of the factors vary.

Notably, as observed in [CO_2] gradient studies (Gill *et al.*, 2002; Fay *et al.*, 2009), we saw some of the strongest responses to [CO_2] between sub- to ambient concentrations and, at some N rates, a decline in ANPP between 550 and 700 ppm [CO_2] (Fig. 1). Such nonlinearities and even declines at [CO_2] around 700 ppm have been highlighted (Berntson & Bazzaz, 1998; Granados & Körner, 2002), but are not represented in the modeling efforts to project vegetation responses to rising atmospheric [CO_2] (Ostle *et al.*, 2009). There is a clear need for multifactor, multilevel field studies to permit us to assess uncertainty in model projections, but the required number of experimental treatments and replicates (66 and 330, respectively, in our study) may preclude their establishment given the logistics and cost to set up and maintain so many plots. If their establishment is unlikely, data from laboratory experiments, such as ours, should be used in efforts to quantify certainty bounds for model projections of global change effects on communities and ecosystems.

The paucity of two-level (ambient and elevated), multifactor experiments makes the prediction of future responses to global environmental change highly uncertain (Tylianakis *et al.*, 2008; Ostle *et al.*, 2009). This uncertainty is magnified further by the lack of multifactor, multilevel experiments, because the outcome of interacting global changes on communities and ecosystem processes may be dependent on the amount of the factor (van Groenigen *et al.*, 2006). We find direct experimental support for this possibility, showing that amount matters in determining interactive and main effects of factors, and that nonadditivity and nonlinearity in ecosystem responses are likely to be the norm rather than the exception. The call has been made for more long-term, multifactor field experiments to clarify how global environmental changes will influence terrestrial systems (Luo *et al.*, 2011). Our results suggest that this clarity will only be achieved if these long-term studies include multiple levels of the global change factors.

Acknowledgements

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