LETTERS

Ecosystem response to elevated CO_2 levels limited by nitrogen-induced plant species shift

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Terrestrial ecosystems gain carbon through photosynthesis and lose it mostly in the form of carbon dioxide (CO_2). The extent to which the biosphere can act as a buffer against rising atmospheric CO_2 concentration in global climate change projections remains uncertain at the present stage[1–4]. Biogeochemical theory predicts that soil nitrogen (N) scarcity may limit natural ecosystem response to elevated CO_2 concentration, diminishing the CO_2-fertilization effect on terrestrial plant productivity in unmanaged ecosystems[3–7]. Recent models have incorporated such carbon–nitrogen interactions and suggest that anthropogenic N sources could help sustain the future CO_2-fertilization effect[8,9]. However, conclusive demonstration that added N enhances plant productivity in response to CO_2-fertilization in natural ecosystems remains elusive. Here we manipulated atmospheric CO_2 concentration and soil N availability in a herbaceous brackish wetland where plant community composition is dominated by a C_3 sedge and C_4 grasses, and is capable of responding rapidly to environmental change[10]. We found that N addition enhanced the CO_2-stimulation of plant productivity in the first year of a multi-year experiment, indicating N-limitation of the CO_2 response. But we also found that N addition strongly promotes the encroachment of C_4 plant species that respond less strongly to elevated CO_2 concentrations. Overall, we found that the observed shift in the plant community composition ultimately suppresses the CO_2-stimulation of plant productivity by the third and fourth years. Although extensive research has shown that global change factors such as elevated CO_2 concentrations and N pollution affect plant species differently[11–13,] and that they may drive plant community changes[14–17], we demonstrate that plant community shifts can act as a feedback effect that alters the whole ecosystem response to elevated CO_2 concentrations. Moreover, we suggest that trade-offs between the abilities of plant taxa to respond positively to different perturbations may constrain natural ecosystem response to global change.

The progressive nitrogen limitation (PNL) hypothesis[7] suggests that N additions should enhance CO_2 effects on plant productivity. However, only a limited number of studies have provided direct experimental evidence that N addition actually sustains or enhances the CO_2 response of productivity[3,7]. In a pine forest, N addition amplified the CO_2 effect on woody tissue increment[5]. A $CO_2 \times N$ experiment in a grassland reported that a positive $CO_2 \times N$ interaction emerged after three years, indicating that N addition amplified the effect of elevated CO_2 on productivity[6]. In managed ryegrass swards, N addition yielded larger CO_2 responses, an effect that strengthened over time on a relative basis, but diminished in terms of absolute magnitude[18].

As originally articulated, the PNL hypothesis does not explicitly consider the effects that elevated CO_2 and added N can have on the ecosystem-level response through changes in species composition. The role of changing species composition in regulating PNL is not clear. For instance, a plant community response to a step change in CO_2 and N addition in forest FACE (free-air CO_2 enrichment) studies could take decades. The Cedar Creek FACE study occurs in an herbaceous community in which plant composition is dynamic, but the number of possible plant species is restricted in order to maintain experimental diversity treatments[6]. On the other hand, an annual grassland of unmanipulated composition elicited no effect of N on CO_2 response[19].

We hypothesized that differences in individual species responses to elevated CO_2 and N could feed back to regulate the whole ecosystem response to these global change factors. To test this, we manipulated atmospheric CO_2 concentration and soil N availability factorially (four treatment groups, $n = 5$) in a herbaceous brackish wetland[10] where plant community composition is capable of responding rapidly to environmental change. As in many temperate ecosystems, plant productivity is typically N-limited in brackish tidal wetlands. Yet, unlike in other unmanaged, herbaceous ecosystems, the plant community structure is naturally simple (species richness across our study plots = 3), allowing for both realistic ecological phenomena and tractable analysis of species interactions.

We found a significant $CO_2 \times N \times year$ interaction on total aboveground biomass (Table 1), indicating that the manner in which N addition modified the CO_2 stimulation of biomass changed through time. As predicted by the PNL hypothesis, N addition tended to enhance the aboveground biomass CO_2 response in the first year of the study (2006), suggesting N limitation of the CO_2 response (Fig. 1a). However, the magnitude of the response changed in the second year (2007) and reversed in the third and fourth years of treatment (2008 and 2009; Fig. 1a). At the same time, N fertilization increasingly stimulated C_4 grass (*Spartina patens* and *Distichlis spicata*) biomass from 2006 through to 2009 in the ambient CO_2 treatment (Supplementary Fig. 1). Even though the combination of elevated CO_2 and N continued to stimulate the dominant C_3 sedge, *Schoenoplectus americanus*, the magnitude of the CO_2-stimulation declined (Fig. 1c), while added N continued to strongly stimulate C_4 grass biomass throughout the study (Supplementary Fig. 1). Taken together, these results point to a novel finding that N-driven changes in species composition—expansion of C_4 grasses in this case—limited the whole ecosystem response to elevated CO_2.

The PNL hypothesis predicts that N addition will enhance the magnitude of the CO_2 effect on productivity by relieving the N limitation that develops over time in a plant community that initially responds positively to elevated CO_2. However, changes in the availability of resources such as soil N can also modify plant community composition, which also has considerable consequences for ecosystem productivity[20,21]. As predicted by the PNL hypothesis, elevated CO_2 decreased porewater ammonium concentration (that is, $[NH_4]$) by 20% with no added N and by 30% with N addition in the first year (Fig. 2), presumably by increasing C_3 plant uptake, and perhaps microbial

Table 1 | Treatment effects on plant growth and soil N availability

Effect	C_3 biomass	C_4 biomass	Root production	Total aboveground biomass	Pore water [NH_4]
CO_2	<**0.001**	**0.004**	**0.013**	0.754	**0.014**
N	0.778	<**0.001**	**0.004**	<**0.001**	<**0.001**
$CO_2 \times$ N	**0.036**	0.095	0.759	0.957	0.355
Year	0.395	**0.053**	0.898	0.072	<**0.011**
$CO_2 \times$ year	0.349	0.138	0.325	0.073	0.612
N $\times$ year	**0.005**	**0.047**	0.214	0.965	**0.004**
$CO_2 \times$ N $\times$ year	0.697	**0.007**	0.709	**0.009**	0.564
Pretreatment	0.933	**0.029**	**0.026**	0.899	<**0.001**
Pretreatment $\times$ year	0.873	0.075	0.998	0.060	**0.001**

Results from a repeated-measures multivariate analysis of covariance (MANCOVA: two-tailed, $n = 5$). P-values less than or equal to 0.05 are bold. Pretreatment refers to the covariate data from 2005 used in analyses.

immobilization, of N. This response may explain why elevated CO_2 alone reduced C_4 biomass (CO_2 effect, $P = 0.004$, Table 1). However, N fertilization favoured the expansion of C_4 grasses, as has been shown in many other wetland ecosystems[22,23]. The negative effect of N addition on *S. americanus* strengthened over time (N $\times$ year, $P = 0.005$). In a N-limited ecosystem, it is unlikely that N addition would negatively affect *S. americanus* in isolation; nor is it likely that elevated CO_2 would negatively affect the production of C_4 grasses in isolation. Instead, these relationships suggest that there was a negative interaction between the C_4 and C_3 plants. Had we maintained C_3 purity by removing C_4 species, the ecosystem might have continued to exhibit the strong positive effect of N on biomass CO_2 response observed in the first year. We propose that N addition stimulated C_4 biomass, increasing competition with the C_3 species for other resources such as light. The result was reduced growth of *S. americanus*, which ultimately limited the response of the whole ecosystem to elevated CO_2.

The implications of our results for other ecosystems are predicated on ecological theory. Strategies for acquiring resources require physiological and evolutionary trade-offs such that optimization for capture of one resource may preclude optimal capture of another[24,25]. For instance, plants that maintain a higher root-to-shoot mass ratio typically compete more effectively for limiting soil resources, whereas plants that allocate relatively more mass aboveground optimize light capture[25,26]. At our wetland site, fine root production was on average twice as high in stands of *S. americanus* than in stands of the C_4 grass *S. patens* over the past 20 years[16]. Allocation to nutrient-acquiring roots instead of light-acquiring shoots represents a trade-off that should confer an advantage to *S. americanus* in N acquisition under conditions of low N availability. This view is consistent with the

observation that porewater nitrogen concentration (that is, [N]) was over eight times higher in pure *S. patens* stands than in *S. americanus* stands in a previous study at the site[27]. Greater allocation to roots affords *S. americanus* enhanced ability to acquire N when porewater [N] is low. But, when N is made abundant by N addition, *S. americanus* appears to be unable to respond as strongly as the grasses.

The properties that underlie trade-offs in resource acquisition may also engender trade-offs in plant response to resources altered by global change. If strong N-responders are not also strong CO_2-responders, and increased growth of some species negatively affects other species, then the combination of elevated CO_2 and elevated N is likely to diminish the magnitude of ecosystem response to elevated CO_2, as in our study. However, if N addition favoured the expansion of a plant species that responds more strongly to CO_2 than the average species in the ecosystem, then the resultant community shift could have the opposite effect, amplifying the whole ecosystem CO_2 response. Our results provide a stark example wherein the species that responded most strongly to N-addition were C_4 grasses, which respond weakly to CO_2. Similar effects may occur in ecosystems composed entirely of C_3 species, provided that functional groups, species or genotypes respond differently to elevated CO_2 and N.

Findings from past studies support the notion of trade-offs among plant responses to different global change factors. Meta-analytic studies suggest that grasses (either including or excluding C_4 grasses) tend to respond strongly to N but poorly to CO_2; legumes respond strongly to CO_2 but poorly to N; and trees respond intermediately to both[11–13]. Similarly, limited data from individual ecosystems suggest that plant functional group CO_2 responses relate negatively to N responses. In a species-rich prairie community, relative abundance of C_3 grasses declined in response to elevated CO_2 but increased strongly with added N, while N-fixers exhibited the opposite pattern,
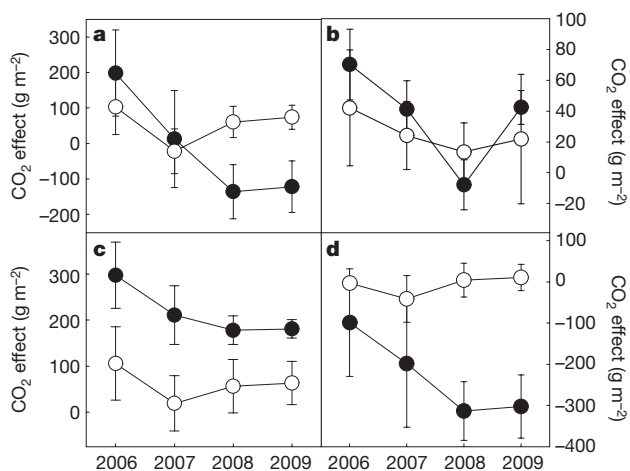

Figure 1 | The magnitude of the CO_2 effect. The effect of CO_2 on total aboveground biomass (**a**), fine root productivity (**b**), C_3 biomass (**c**) and C_4 biomass (**d**) from 2006 to 2009 was calculated as elevated CO_2 mass minus ambient CO_2 mass, with added N (filled symbols) and without added N (open symbols). Error bars represent combined standard error of the mean calculated as $\sqrt{(\text{s.e.m.}_{\text{ambient}})^2 + (\text{s.e.m.}_{\text{elevated}})^2}$.
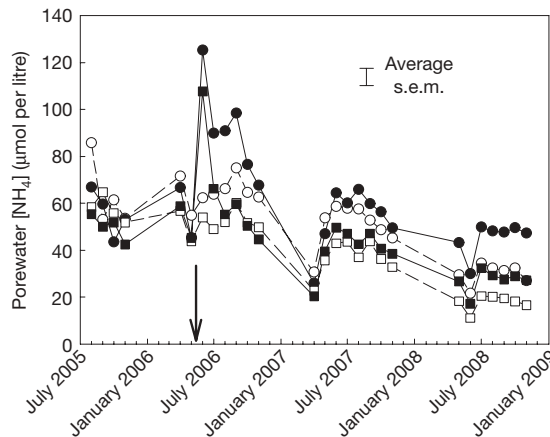

Figure 2 | Porewater ammonium concentrations over four growing seasons averaged over three depths. After treatments were initiated in May 2006 (arrow), the N-fertilized treatments (closed symbols) are higher than the unfertilized (open symbols), and ambient CO_2 plots (circles) are higher than elevated CO_2 plots (boxes).

and forbs responded intermediately to both perturbations[15]. In a serpentine grassland, grasses responded strongly to N but negatively to CO_2, while forbs were negatively affected by N, and showed no clear response to CO_2 (ref. 17). In the present study, trade-offs between elevated CO_2 response and elevated N response are related, at least partly, to the distribution of C_3 and C_4 photosynthetic pathways among species. As such, our results are most directly relevant to ecosystems with a mixture of C_3-dominated and C_4-dominated plant communities, including tidal marshes and grasslands. Further testing is needed to establish whether evolutionary trade-offs between the optimal acquisition of different resources can be generalized across functional groups, species or genotypes to predict long-term ecosystem responses to multiple interacting global change factors.

The PNL hypothesis predicts that N should become less available in ecosystems exposed to elevated CO_2 as biomass and litter accumulates. Yet elevated CO_2 studies have reported no consistent CO_2 effects on estimates of soil N availability[3,28] either owing to great error in estimates[3], or because CO_2-stimulated plant activity could liberate additional soil N to compensate[29]. In the present study, constantly saturated soils afforded a direct assessment of N availability without the fluctuating and heterogeneous soil moisture effects that can introduce considerable variability into nutrient availability estimates in upland ecosystems. Two observations from the porewater nutrient data bear on the theory of PNL. First, we observed a rapid drawdown of soil porewater $[NH_4]$ with initiation of CO_2 treatment in May 2006 (Table 1, Fig. 2). This rapid response indicates that N limitation, triggered by elevated CO_2, was not driven initially by slow-acting feedbacks that are commonly purported to drive PNL[3,7]. Second, N-addition enhanced N availability in the elevated CO_2 + N treatment (Fig. 2) and, yet did not sustain the initial CO_2-stimulation of biomass (Fig. 1a). Our results demonstrate that enhancing N availability does not necessarily enhance CO_2 effects in a mixed-species ecosystem, even though it may do so for an individual species in isolation.

We observed a decrease in soil N availability under elevated CO_2, and an initial positive $CO_2 \times N$ interaction, both consistent with N limitation of CO_2 response. However, continued soil N addition ultimately diminished the CO_2 effect by favouring a community shift towards species that do not respond strongly to elevated CO_2 (Fig. 3). These treatment responses may change beyond the four growing seasons as the current plant community continues to reorder, or as the present species are replaced by others[30]. For example, the ecosystem response to elevated CO_2 and N reported here could change
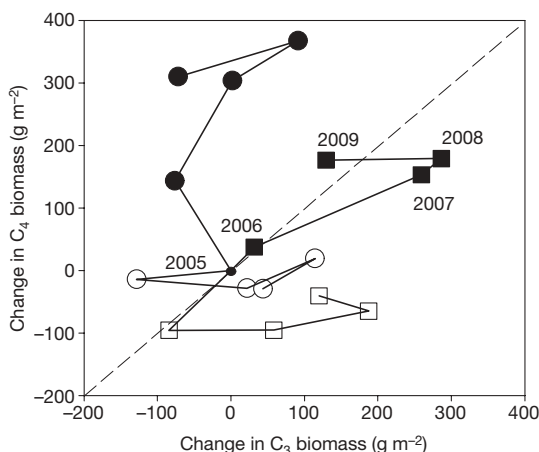
dramatically with the encroachment of non-native genotypes of the C_3 species, *Phragmites australis*, a widespread invasive plant in North American tidal wetlands.

The relatively rapid responses of this dynamic plant community may foreshadow changes in more slowly responding ecosystems as atmospheric CO_2 and N pollution accumulate in the long-term future. Increasing anthropogenic N may ultimately cause shifts in plant communities that alter the CO_2 fertilization effect on global productivity. Even where PNL acts on plants in the short term, longer-term community dynamics may obviate the stoichiometric N limitation of the ecosystem CO_2 response. If so, models that incorporate a positive effect of anthropogenic N on the elevated CO_2 response may overestimate future carbon uptake in terrestrial wildlands.

METHODS SUMMARY

The experiment was carried out in a brackish marsh on the Rhode River, a sub-estuary of Chesapeake Bay (see Supplementary Methods). The site was dominated by a C_3 sedge, *Scheonoplectus americanus*, and two C_4 grasses, *Spartina patens* and *Distichlis spicata*. Twenty 3.3-m^2 plots of intact marsh were enclosed in octagonal, open-top chambers (2 m in height). Plots were randomly assigned to one of four treatment groups ($n = 5$): ambient CO_2, ambient CO_2 + N, elevated CO_2 and elevated CO_2 + N. Pure CO_2 was injected into the blower stream of the elevated chambers to achieve a target concentration of 720 p.p.m. On five occasions, approximately monthly during each growing season, half of the plots were fertilized with NH_4Cl at a rate of 5 g N m^{-2} (an annual rate of $25 \text{ g N m}^{-2} \text{ yr}^{-1}$) to simulate tidal N loading in a marsh that would be heavily polluted compared to the study site.

In July 2005, and every July and October thereafter, aboveground biomass was estimated using allometry and stem density counts for *S. americanus* and biomass clipping for *S. patens* and *D. spicata*. Total root productivity was estimated each year using three root ingrowth cores in each plot. Nine porewater wells were placed in each plot, three at each of three depths: 15, 30 and 75 cm. Porewater was sampled approximately monthly throughout the growing season and analysed for ammonium concentration. In these anaerobic soils, porewater nitrate is typically below detection limits and does not contribute substantially to total mineral [N].

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Figure 3 | The trajectories of plant biomass according to contributions from C_3 and C_4 plants in each treatment group. Open circles, ambient CO_2; open boxes, elevated CO_2; closed circles, ambient CO_2 + N; closed boxes, elevated CO_2 + N. Values represent the annual treatment means of biomass measured in July of each study year relative to mean biomass in July 2005, the year before treatments were initiated. All lines begin at the origin, which represents mean biomass for each treatment group in July 2005.

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