Global Change Biology

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Global Change Biology

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Cover: Jasper Ridge Global Change Experiment plots with irrigation in progress. Elevated nitrogen and precipitation treatments altered phosphorus demand in these California annual grasslands (see Menge & Field, pp. 2582–2591; photograph by Nona Chiariello).

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Alcamo J, Kreileman E, Krol M *et al.* (1998) Global modelling of environmental change: an overview of IMAGE 2.1. In: *Global Change Scenarios of the 21st Century: Results from the IMAGE 2.1 Model* (eds Alcamo J, Leemans R, Kreileman E), pp. 3–71. Pergamon, Oxford.

Llorens L (2003) *Plant ecophysiological responses to experimentally drier and warmer conditions in European shrublands.* Unpublished PhD thesis, Universitat Autonoma de Barcelona, Barcelona.

Hill JK, Thomas CD, Huntley B (2001). Climate and recent range changes in butterflies. In: *"Fingerprints" of Climate Change – Adapted Behaviour and Shifting Species Ranges* (eds Walther G-R, Burga CA, Edwards PJ), pp. 77–88. Kluwer Academic/Plenum Publishers, New York.

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Simulated global changes alter phosphorus demand in annual grassland

DUNCAN N. L. MENGE* and CHRISTOPHER B. FIELD†

*Earth Systems Program, Stanford University, Stanford, CA 94305, USA, †Department of Global Ecology, Carnegie Institution of Washington, Stanford, CA 94305, USA

Abstract

In the Jasper Ridge Global Change Experiment – an annual grassland with elevated carbon dioxide (CO_2), nitrate deposition, temperature, and precipitation – we used six indices of phosphorus (P) limitation to test the hypothesis that global changes that increase net primary production (NPP) increase P demand or limitation. All indices indicated that nitrate deposition, the only factor that stimulated NPP, increased P demand or limitation: (1) soil phosphatase activity increased by 14%; (2) P concentration in green and (3) senescent leaves of the dominant grass genus, Avena, dropped by 40% and 44%, respectively; (4) N:P ratios in green and (5) senescent Avena widened by 99% and 161%, respectively; and (6) total aboveground plant P decreased by 17% with elevated nitrate deposition. The other three factors, which did not stimulate NPP, did not increase P demand: based on two indices, enhanced precipitation decreased P demand (11% decrease in phosphatase activity, 19% increase in total aboveground P), and there was no evidence that elevated CO_2 or temperature altered P demand. In a meta-analysis to assess the generality of P constraints on growth increases from global change factors, we found that six of 11 N-limited ecosystems responded to N deposition with enhanced P limitation or demand, but did not detect significant effects of elevated CO_2 or warming.

Keywords: Avena barbata, Avena fatua, climate change, CO_2, global change, grassland, Jasper Ridge, N:P ratio, nitrogen deposition, nitrogen saturation, phosphatase, phosphorus

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Introduction

Atmospheric carbon dioxide (CO_2) concentrations, nitrogen (N) deposition, and global average temperature are increasing, and precipitation patterns are changing as a result of anthropogenic emissions of greenhouse gases (IPCC, 2001). Because they share a common set of causes, these four global change factors are strongly linked at the global scale, with potentially important differences in spatial pattern (IPCC, 2001). CO_2, N, and water frequently limit plant growth (Field et al., 1992), whereas warming can either increase or decrease primary production. Thus, increasing the availability of any factor or combination of factors could increase primary production, potentially driving terrestrial carbon storage and offsetting some of the carbon released from fossil fuel combustion (McGuire et al., 2001). However, increased growth must have some limit. Any stimulation will saturate at some level, as a result of inadequate supply of another essential resource, an environmental constraint, or intrinsic growth potential (Field et al., 1992). In particular, the supply of phosphorus (P) may function as a secondary limit, for it too often limits primary production (Chapin, 1980; Vitousek & Farrington, 1997). Local availability of P is controlled by a combination of substrate composition and age, climate, and vegetation (Vitousek, 2004), with inputs from dust deposition being important in some cases (Chadwick et al., 1999; Smil, 2000). Importantly, anthropogenic changes in patterns of P supply are not necessarily linked to CO_2 and NO_x emissions as are temperature and precipitation. Due to this uncoupling, we hypothesize that P availability may constrain the growth-enhancing effects of CO_2, N (Huenneke et al., 1990; Vitousek & Farrington, 1997), and/or precipitation.

Correspondence: Present address: Duncan N. L. Menge, Department of Ecology and Evolutionary Biology, Princeton University, 106A Guyot Hall, Princeton, NJ 08544, USA, tel. +1 609 258 6883, fax +1 609 258 1334, e-mail: dmenge@princeton.edu

We examined the effects of elevated CO_2, nitrate deposition, precipitation, and temperature on biological P demand in an ecosystem limited by N but not CO_2, water, or temperature (Zavaleta et al., 2003; Dukes et al., 2005). Our work took place in the Jasper Ridge Global Change Experiment (JRGCE), which maintains all 16 possible combinations of ambient and elevated atmospheric CO_2, nitrate deposition, temperature, and precipitation in intact annual grassland, allowing us to determine single- and multiple-factor effects (Zavaleta et al., 2003). The elevated levels of all factors fall in the range of possibility for later decades of the 21st century in California (IPCC, 2001; Dukes et al., 2005). Since the dominant species are annuals, each year represents a new generation, allowing us to detect effects driven through multigeneration changes in population or community structure.

We measured six indices relevant to P demand in the JRGCE. The first index addresses ecosystem-level P demand with an assay for potential soil (extracellular) phosphatase activity. Extracellular phosphatases are enzymes produced and secreted by plants and microbes that catalyze the hydrolysis of ester bonds; they release phosphate from organic matter so it is available for uptake (Speir & Ross, 1978). Phosphatase production by both plants and microbes increases in response to P limitation in many systems (Spiers & McGill, 1979; Dracup et al., 1984; Sinsabaugh et al., 1993; Tadano et al., 1993; Barrett et al., 1998; Fries et al., 1998; Olander & Vitousek, 2000; Treseder & Vitousek, 2001). However, not all P-limited systems respond with increased phosphatase production (Speir & Ross, 1978), so we examined five other indices.

The five other P demand indices are the concentration of P and the N:P ratio in green and senescent foliage, and the total amount of aboveground P. The ratio of N:P can indicate which element is more limiting (Koerselman & Meuleman, 1996), although critical values for limitation vary with species (Drenovsky & Richards, 2004; Gusewell, 2004). Senescent tissue chemistry reveals nutrient inputs to litter, which partially control future plant nutrient availability. Since tissue chemistry measurements are more sensitive to changes in nutrient availability in fast-growing plants (Chapin, 1980), we used a fast-growing grass genus, Avena, which comprises ~27% of net primary production (NPP) in the JRGCE (in the 2001 harvest). [The two species of Avena in the JRGCE, A. fatua and A. barbata (Zavaleta et al., 2003), were not sorted to species in this harvest and are, therefore, pooled for our analyses. Hereafter, they are referred to collectively by their genus name.] Finally, assuming that Avena tissue P data from each plot reflect P content for all species, we estimated treatment effects on aboveground P pools.

Collectively, these varied aspects of P nutrition provide a window on interactions between anthropogenic global changes and P limitation in the JRGCE. We hypothesized that manipulated factors that tend to increase NPP would increase P demand, which could potentially constrain growth increases, and thus carbon storage.

To understand how widespread this mechanism may be globally, we analyzed published P cycle responses to elevated CO_2, N, precipitation, or temperature in 16 ecosystems ranging from desert to rainforest and from tropical to arctic.

Methods

Study site

The JRGCE lies in the foothills of the Central California (CA) coast range (37°24'N, 122°14'W). It experiences a Mediterranean-type climate, with a cool, wet winter (the growing season) and hot, dry summers. Introduced annual grasses (A. fatua, A. barbata, and Bromus hordeaceus) and forbs (Geranium dissectum and Erodium botrys) dominate the plant community. The soil is a fine, mixed Typic Haploxeralf developed from Franciscan complex alluvium sandstone. Detailed site and climate descriptions can be found elsewhere (Zavaleta et al., 2003).

Experimental design

The JRGCE is a four-way factorial split-plot design (Shaw et al., 2002). Within each of the eight randomized blocks, there are four plots 1 m in radius, each of which is divided into four quadrants. The four manipulated factors are atmospheric CO_2, temperature, precipitation, and nitrate deposition. Atmospheric CO_2, manipulated at the plot level, is elevated from ambient (~370 ppm) to ambient +300 ppm with a ring of free-air emitters surrounding each plot, using the mini-FACE approach (Miglietta et al., 1996). Temperature, also a plot-level treatment, is elevated by ~1 °C at canopy height by infrared heaters (80 W m^{-2}), with dummy heaters over unheated plots to reproduce shading or other non-treatment effects of the heaters (Zavaleta et al., 2003; Dukes et al., 2005). Precipitation, a quadrant-level treatment, is elevated to 150% of ambient with drip (1998–2000) or spray (2001–2003) irrigation following each rain event, with two additional simulated rain events, extending the rainy season by approximately 20 days (Zavaleta et al., 2003). Nitrate deposition, also applied at the quadrant level, is elevated by 7 g NO_3-N m^{-2} yr^{-1} above the background rate of <1 g N m^{-2} yr^{-1} (Weiss, 1999). Nitrate is applied as $Ca(NO_3)_2$, with an initial application of 2 g m^{-2} in solution directly following the

first autumn rain (to mimic the pulse of accumulated dry N deposition that occurs with the first rains after the dry summer), and an additional $5 \, \text{g m}^{-2}$ applied as a slow-release fertilizer (Nutricote 12-0-0; Agrivert, Riverside, CA, USA) in January (Zavaleta et al., 2003; Dukes et al., 2005). Treatments have been applied throughout each growing season since the 1998–1999 growing season.

Sampling

We took soil cores from the JRGCE in March 2002, May 2002, and January 2003. Core depth and diameter in March and May were 15 cm and 22 mm; in January they were 5 cm and 11 mm. Phosphatase activity from 0 to 5, from 5 to 10, and from 10 to 15 cm was statistically indistinguishable, although there was a tendency toward decreased activity with depth (data not shown). We completed each round of coring within 3 days, stored the soil samples at $4 \, ^{\circ}\text{C}$, and processed soils within 3 weeks of sampling. Foliar tissue samples were harvested from the JRGCE on May 16, 2001, approximately 30 weeks after germination. Following the harvest, Avena samples were dried for 24 h at $70 \, ^{\circ}\text{C}$ and stored at room temperature. For chemical analyses, we ground samples to 20 mesh in a Wiley mill or cut them with scissors if samples were too small to grind.

Phosphatase assays and tissue chemistry

Phosphatase assay techniques followed the outline of Tabatabai & Bremner (1969). We incubated soils (with roots removed) at pH 5.0 (acetate buffer) or 7.0 (TRIS buffer) with 5.0 mM para-nitrophenyl phosphate (presumed to be saturating concentration) for 120 min, stopping the reaction with NaOH. This assay measures the maximum enzyme activity rate (V_{max}), which will rarely, if ever, be realized in natural soils with low P availability. Our interpretation of the measurement, therefore, assumes that the biotic P demand response is to produce more phosphatases, not different (e.g. higher affinity) phosphatases. Phosphatase activity was calculated from spectrophotometric readings (Beckman DU70, Fullerton, CA, USA) at 410 nm (color-corrected for sample and substrate controls) of the reaction product para-nitrophenol. It is likely that both plants and microbes contributed to the measured phosphatase activity. We cannot exclude the possibility that treatments induced P limitation in either plants or microbes, but not both (Sundareshwar et al., 2003).

Dry, ground plant tissue was sulfuric acid (Kjeldahl) digested for nutrient analysis. At no more than 10 days following digestions, samples were colorimetrically analyzed for total P and total N concentration on an Alpkem RFA/2 continuous flow analyzer (Clackamas,

OR, USA). For details on both techniques see Menge (2003). To quantify the aboveground plant P pool we assumed that, for each tissue type (green or senescent) in each quadrant, all species had the same P concentration as Avena, filling in the few gaps in tissue P data with treatment means. Green and senescent biomass data used to calculate the plant P pools were from the same 2001 harvest as Avena tissue chemistry data (Zavaleta et al., 2003).

JRGCE Statistical analyses

Data from the JRGCE were analyzed with a split-plot general linear model (GLM) in SAS 9.1, with two levels (ambient and elevated) each for CO_2, temperature, precipitation, and nitrate (Zavaleta, 2001). All data were transformed (logarithmically or square-root) for statistical analyses when necessary to meet homoskedasticity and normality assumptions (using Bartlett's test for homoskedasticity; Sokal & Rohlf, 1995).

Meta-analysis

To investigate published effects of global change on P demand or limitation in a relatively unbiased way, we searched Web of Science in June 2006 with the keys 'phosphorus and global change,' 'phosphorus and warming,' and 'phosphorus and CO_2 and (enrichment or elevated),' then used those hits and the references therein to compile our initial database. Other search keys we tried suggested that these captured most of the relevant literature. We then restricted the database to manipulative experiments in intact terrestrial ecosystems in which experimentally increasing a global change factor elevated NPP (or some similar measure such as basal area), and in which some P cycle response to the manipulations was measured and presented with statistics. The final database comprised 24 studies (including this study) from 16 ecosystems, listed in Table 1. None of these studies included a precipitation manipulation that increased NPP, so we present only CO_2, N, and temperature effects.

The aspects of the P cycle measured varied from study to study, and are listed for each study in Table 1. Since each study used different metrics that are not quantitatively comparable, and many studies did not report effect sizes (only P-values), we used a vote-counting meta-analysis to test our hypothesis (Hedges & Olkin, 1980; Gurevitch & Hedges, 1999). Our method follows the outline of Hedges & Olkin (1980), with changes as follows. We scored effects on P limitation or demand as positive (significant increases in those measures with a [+] superscript in Table 1 or significant decreases in those with a [−] superscript), negative, or not

Table 1 Sources, ecosystems, manipulations, and measurements used in meta-analysis of P cycle responses to global change

Source	Ecosystem	Manipulation(s)	P cycle measurement(s)
Aerts et al. (1992)	Sweden bog, 1 year	NH_4NO_3: + 2	[-]Plant [P], [+]N : P
Clarholm (1993)	Sweden spruce forest, 20 years	NH_4NO_3: + 6	[-]Microbial P, [+]soil phosphatase activity
Vitousek et al. (1993)	Hawaii forest, 2 years	Urea/NH_4NO_3: + 5–10	[+]Growth response to P after N fertilization
Chapin et al. (1995)	Alaska tundra, 3–9 years	Temperature: + 3–5 °C	[-]Soil KCl PO_4, [-]resin PO_4
Moorhead & Linkins (1997)	Alaska tundra, 3 years	CO_2: + 300 ppm	[+]Root and [+]soil phosphatase activity
van Duren et al. (1997)	Belgium fen, 2 years	CO $(NH_2)_2$: + 20	[+]Growth response to P after N fertilization
Vitousek & Farrington (1997)	Hawaii forest, 2 years	Urea/NH_4NO_3: + 10	[+]Growth response to P after N fertilization
Niklaus et al. (1998)	Switzerland grassland, 4 years	CO_2: + 250 ppm	[-]Plant P pool, [+]N : P
Ajwa et al. (1999)	Kansas tallgrass prairie, 9 years	NH_4NO_3: + 10	[+]Soil acid, alkaline phosphatase activity
Jonasson et al. (1999)	Sweden subalpine heath, Sweden fellfield, 5 years	Temperature: + 0.4–5.0 °C	[-]Total soil, [-]inorganic soil, [-]microbial, [-]plant P pools
van Wijnen & Bakker (1999)	Netherlands salt marsh, 3 years	NH_4NO_3: + 5, 25	[+]Growth response to P after N fertilization
Olander & Vitousek (2000)	Hawaii forest, 11 years	Urea/NH_4NO_3: + 10	[+]Soil phosphatase activity
Finzi et al. (2001)	North Carolina forest, 2 years	CO_2: + 200 ppm	[-]Foliar, [-]litter [P], [+]P resorption, [-]litter P pool
Treseder & Vitousek (2001)	Hawaii forest, 11 years	Urea/NH_4NO_3: + 10	[+]Root phosphatase activity
Ebersberger et al. (2003)	Switzerland grassland, 6 years	CO_2: + 250 ppm	[+]Soil phosphatase activity
van Heerwaarden et al. (2003)	Sweden subarctic bog, 3 years	NH_4: + 10	[-]Plant green, [-]senescent [P], [+]N : P, [+]P resorption efficiency
Drenovsky & Richards (2004)	California desert, 2 years	NH_4NO_3: + 105 g N $plant^{-1}$ yr^{-1}	[+]Growth response to P after N fertilization, [-]plant [P]
Finzi et al. (2004)	North Carolina forest, 4 years	CO_2: + 200 ppm	[-]Foliar [P], [+]canopy N : P
Niklaus & Körner (2004)	Switzerland grassland, 6 years	CO_2: + 250 ppm	[-]Aboveground, [-]litter, [-]root P pools
Øien (2004)	Norway fens, 1 year	NH_4NO_3: + 12	[+]Growth response to P after N fertilization
Henry et al. (2005)	California annual grassland (JRGCE), 6 years	NO_3: + 7	[+]Soil phosphatase activity
Niinemets & Kull (2005)	Estonian meadow, 1 year	NH_4NO_3: + 2, 5, 10, 20	[+]Growth response to P after N fertilization
Finzi et al. (2006)	North Carolina forest, 6 years	CO_2: + 200 ppm	[+]Soil phosphatase activity
This study	California annual grassland (JRGCE), 3–5 years	NO_3: + 7	[+]Soil phosphatase activity, [-]Avena [P], [+]Avena N : P, [-]Total aboveground P

All N additions are given in g N m^{-2} yr^{-1} unless otherwise stated. In the 'ecosystem' column, the number of years of treatment is given after the type of ecosystem. Evidence that the manipulation increased NPP (or some similar measure) is in the source listed except for Moorhead & Linkins (1997) (Oechel et al., 1994); Ajwa et al. (1999) (Baer et al., 2003); Finzi et al. (2001, 2004, 2006) (DeLucia et al., 1999); van Heerwaarden et al. (2003) (Richardson et al., 2002); Henry et al. (2005) and this study (Zavaleta et al., 2003; Dukes et al., 2005 for both). A[+] to the left of a measurement denotes that an increase in the level of that measurement indicates an increase in P limitation or demand. A[-] denotes that an increase in the level of that response indicates a decrease in P limitation or demand. JRGCE, Jasper Ridge Global Change Experiment; P, phosphorus; CO_2, carbon dioxide.

significant, using statistics in the published works (with $\alpha = 0.05$ and two-tailed tests), then analyzed the data by each measure, each study, and each ecosystem. A 'sig-

nificant' study effect indicates that the majority of the measures had that effect, and likewise a significant ecosystem effect indicates a majority of studies in the

ecosystem had that effect (ties were given half an effect). Although not all measures and not all studies are equal, measures in any given study and studies within each ecosystem were given equal weight in this analysis. Our statistical null model was that each measure, study, or ecosystem was a random draw from a binomial distribution with $P = 0.025$ for an increase (because this was the cutoff used in the published studies); results showing more increases than expected by chance were deemed significant.

Results

Soil phosphatase activity

Soil phosphatase activity in the JRGCE control plots ranged from 0.69 to 3.33 μmol pNP g dry soil^{-1} h^{-1} (0.58–4.33 for manipulated plots). Addition of nitrate increased soil phosphatase activity (March $P = 0.082$, May $P = 0.005$, January $P < 0.001$), whereas enhancing precipitation decreased it (March $P = 0.012$, May $P = 0.010$, January $P < 0.001$) (Table 2, Fig. 1f). All other effects and interactions were insignificant ($P > 0.06$ at all time points).

Avena tissue chemistry

Nitrate deposition decreased tissue P concentration in green and senescent *Avena* ($P < 0.001$, Table 2, Fig. 1a and b). Increased precipitation decreased P concentration in senescent ($P = 0.029$, Fig. 1b) but not in green *Avena* ($P = 0.131$, Fig. 1a). Main effects of temperature and CO_2 and all interactions were not significant for green or senescent foliar P concentration in *Avena* ($P > 0.17$). Nitrate deposition increased the N : P ratio in both green and senescent *Avena* ($P < 0.001$, Table 2,

Fig. 1c and d). No other main effects or interactions were significant for green or senescent *Avena* N : P ($P > 0.06$).

Whole plot P

Assuming *Avena* tissue chemistry is representative of the entire community in each quadrant, precipitation increased the aboveground plant P pool ($P = 0.043$, Table 2, Fig. 1e) and N decreased it ($P = 0.024$). No other main effects or interactions were significant ($P > 0.08$).

Meta-analysis

Of the studies in which elevated CO_2 increased NPP, 29% (2/7) resulted in increases in some index of P limitation or demand (Table 3). P limitation increased in Alaska tussock tundra but not in Swiss calcareous grassland or North Carolina pine forest. The proportions of studies and ecosystems (0.29, 0.33) showing a positive P limitation response were substantially greater than the null expectation (0.05), but due to the small sample size ($n = 7$, 3) these responses were not significant at $P < 0.025$ ($P = 0.044$, 0.14). Of the studies in which elevated N increased NPP, 59% (10/17, $P < 0.001$) showed a P limitation increase in response to N deposition, corresponding to six of the 11 (55%, $P < 0.001$) ecosystems: California grassland, Sweden bog, intermediate-aged Hawaii montane forest, Estonia meadow, Norway fen, and Kansas prairie showed increased P limitation or demand, whereas California desert, Sweden forest, young Hawaii montane forest, Netherlands salt marsh, and Belgium fen did not. P limitation or demand did not increase in either of the two studies in which warming increased NPP (Table 3).

Table 2 ANOVA table for Jasper Ridge Global Change Experiment (JRGCE) data, showing P-values only

Treatment	Phosphatase			Green *Avena*		Senescent *Avena*		
	March	May	January	[P]	N : P	[P]	N : P	AGP pool
C	0.087†	0.878	0.643	0.279	0.764	0.885	0.965	0.756
T	0.757	0.334	0.587	0.393	0.246	0.278	0.368	0.320
R	0.012*	0.010**	<0.001***	0.131	0.643	0.029*	0.938	0.043*
N	0.082†	0.005**	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	0.024*
R × N	0.068†	0.939	0.178	0.546	0.089†	0.348	0.063†	0.586
C × T × R	0.278	0.133	0.077†	0.219	0.200	0.750	0.970	0.461
T × R × N	0.186	0.918	0.306	0.539	0.590	0.308	0.244	0.085†

Interactions are only shown if they have at least marginally significant ($P < 0.10$) effects on one or more variables.
Treatment abbreviations: C, carbon dioxide; T, temperature; R, rain (precipitation); N, nitrate deposition. AGP pool indicates the total plant aboveground P, assuming *Avena* tissue chemistry reflects community tissue chemistry.
†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
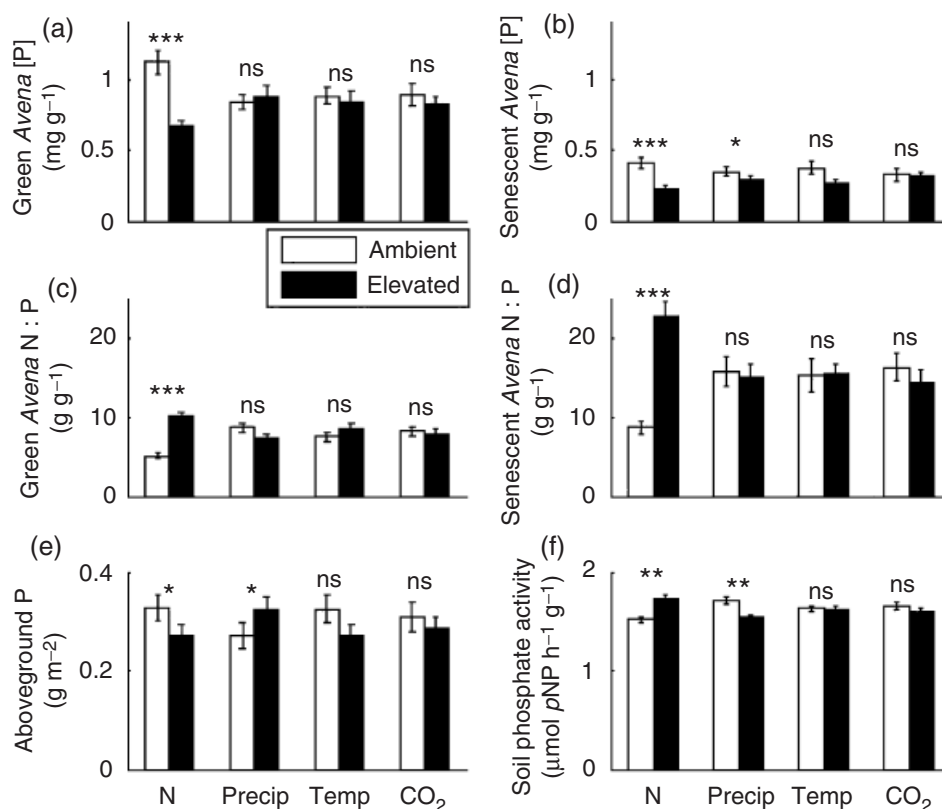
Fig. 1 Main effects of nitrate deposition, precipitation, temperature, and carbon dioxide (CO_2) on measures of phosphorus (P) demand or limitation in the Jasper Ridge Global Change Experiment (JRGCE). Each pair of bars represents all experimental units in the JRGCE: white bars show means ± SE of all experimental units with ambient level of the corresponding factor, black bars show means ± SE of elevated experimental units. Significance of main factors only is shown: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; ns $P > 0.10$. (a) and (b) show P concentration in green and senescent *Avena* foliage, respectively. (c) and (d) show N:P ratio in green and senescent *Avena* foliage, respectively. (e) shows the total P in aboveground plant biomass, assuming *Avena* P chemistry in each quadrant is representative of the entire community in that quadrant. (f) shows soil phosphatase activity, where the data are pooled from the three assay periods.

Discussion

CO_2, temperature, and interactions

Previous results from the JRGCE have shown that elevating atmospheric CO_2 does not increase NPP (Dukes *et al.*, 2005), and can even suppress the positive effects of heat, precipitation, and N on NPP (Shaw *et al.*, 2002). One proposed explanation for this suppression was limitation by a soil nutrient, probably P (Shaw *et al.*, 2002). However, none of the indices in our study suggests that P demand sufficiently increased under elevated CO_2 to bring it into the limiting range. In studies from other ecosystems where CO_2 was limiting, there was a tendency for P limitation or demand to increase, but we found too few such studies in natural ecosystems for this effect to be significant in our meta-analysis.

Increasing temperature by $\sim 1\,^{\circ}\mathrm{C}$ in the JRGCE did not increase NPP (Dukes *et al.*, 2005), and accordingly,

our study revealed no evidence of P limitation. Our full factorial design was set up to detect nonadditive effects, but we found none for P demand, as no interaction term was significant at the 5% level. Neither of the two published studies where warming increased NPP showed an increase in P limitation.

Precipitation

Increasing precipitation by 50% decreased soil phosphatase activity in the JRGCE by $\sim 11\%$, agreeing with results from the 2004 growing season (Henry *et al.*, 2005). Together with the increase in total aboveground P, this indicates that increasing precipitation moved the ecosystems away from, not toward, P limitation. In open-top chambers at Jasper Ridge, an increase in soil moisture (which resulted from decreased transpiration in elevated CO_2 chambers) stimulated N mineralization (Hungate *et al.*, 1997), and it is possible the same

Table 3 Meta-analysis: effects of global change manipulations on P limitation or demand

Effect on P limitation or demand	# Measures of P limitation or demand	# Studies	# Ecosystems
CO_2			
Increase	3†	2†	1
No effect	10	5	2
Decrease	2	0	0
% that increased	20	29	33
N			
Increase	16***	10***	6***
No effect	11	7	5
Decrease	0	0	0
% that increased	59	59	55
Temperature			
Increase	1	0	0
No effect	12	2	3
Decrease	1	0	0
% that increased	7	0	0

'Increase' and 'decrease' indicate significant effects of the relevant variable on a measure of P limitation or demand, as reported in the source. Study effects were deemed significant if a majority of the measures in a study were significant; similarly, ecosystem effects were deemed significant if a majority of the studies in that ecosystem were significant. Ties (an equal number of significant and nonsignificant effects) were split between 'no effect' and 'increase' or 'decrease.' Although some studies manipulated more than one variable, only main effects are shown.

***$P < 0.001$; †$P < 0.05$ on one tail; $P > 0.05$ for all other numbers.

P, phosphorus; CO_2, carbon dioxide.

mechanism – increased mineralization with increased soil moisture – occurs with P. The decreased phosphatase activity and increased aboveground P may also reflect an increase in P diffusivity through soil to roots and microbes – which is frequently the rate-limiting step in plant P uptake (Chapin, 1980) – and/or an increase in the number and size of anaerobic microsites in the soil, which could stimulate iron reduction and mobilize iron-bound P.

Another possible mechanism of decreased P demand with increased precipitation is increased N loss, through leaching or denitrification (Parkin, 1987). Unlike the Hungate et al. (1997) study, where increased soil moisture occurred during water-stressed periods (through decreased transpiration), precipitation in this experiment is supplemented at the time of natural precipitation, when water stress is least likely and N leaching losses and denitrification are most likely. An increase in N losses would help to explain both the decreased P demand and the lack of NPP response to precipitation (Dukes et al., 2005). The increased aboveground P may have been offset by decreased root P, if root P (not measured) followed root biomass (Dukes et al., 2005). Thus, it is possible that the decreased P demand follows from increased N losses. However, increased N losses and increased mineralization of both N and P are not mutually exclusive, and could act in concert. We were not able to examine precipitation effects on P limitation in the meta-analysis because no precipitation studies met our criteria.

Nitrate deposition

Previous data from the JRGCE have shown that nitrate addition enhances grass (including *Avena*) (Zavaleta et al., 2003), shoot, and total NPP over many years (Dukes et al., 2005), including the years of our study, qualifying it as the best candidate factor to increase P demand. The strongest effect we observed in the JRGCE was the addition of nitrate: our data show decreases in P concentrations and widened N:P ratios in green and senescent *Avena*, increased phosphatase activity, and even a decrease in total aboveground plant P, perhaps a consequence of decreased root allocation under N deposition (Dukes et al., 2005). Because we did not fertilize with P we cannot conclusively show P limitation, but all the evidence lines up. Critical N:P values – that determine the cutoff for N or P limitation – for terrestrial plants tend to range from 10 to 20 in terrestrial foliage (Drenovsky & Richards, 2004; Gusewell, 2004), and thus the shift in *Avena* N:P ratios from 5 to 10 with N fertilization is consistent with P limitation under elevated N deposition. Soil phosphatase activity in the JRGCE increased by 14% with elevated N deposition, indicating an increase in net ecosystem P demand. Decreases in P concentration (40% for green, 44% for senescent) indicate P stress to plants, and the decrease in total aboveground P (17%), consistent with the N:P ratios, suggests this increased demand has not been met.

Earlier nutrient work at Jasper Ridge yielded a different pattern. A fertilization study with mesocosms found that, on sandstone (the same substrate as the JRGCE), (1) PK addition after N fertilization (the N × PK interaction) decreased *Avena* shoot biomass relative to N alone, (2) no other species showed a significant N × PK interaction, and (3) no species had a direct response to PK fertilization, suggesting that P is not limiting (Joel et al., 2001). The earlier study involved breaking up rock and soil to fill the mesocosms (as opposed to the JRGCE, which is on natural soils). Given the results from our study and others in the JRGCE (e.g. Henry et al., 2005), we now hypothesize that this process released a pulse of rock-derived nutrients

(including P), alleviating any potential limitation by these nutrients.

Increasing N deposition in the JRGCE pushed the ecosystem toward P limitation, probably as a consequence of decreased N limitation. This shift is one manifestation of N saturation (Aber et al., 1989). Chronic exposure to increased N deposition can cause a shift from N to P limitation in multiple European systems (Aerts & Chapin, 2000) and annual grassland on serpentine-derived soil in California (Huenneke et al., 1990; Joel et al., 2001), as well as the annual grassland on sandstone-derived soil in this study, suggesting a general trend. Results from our meta-analysis support this general trend: six of the 11 previously N-limited ecosystems (in 10 of 17 studies) responded to N fertilization with increased P limitation or demand. These studies come from biogeographically diverse locations (there were P-limited and non-P-limited ecosystems from almost all the geographic areas of study: California, Hawaii, Sweden, and Northern Europe), suggesting that local controls on P cycling – soil type, recent disturbance, etc. – are important. This analysis indicates that globally increased N deposition (Vitousek et al., 1997) may shift many ecosystems toward P limitation, potentially causing N saturation and its associated problems (Aber et al., 1989) in many locations worldwide.

Conclusions

The treatments in the JRGCE simulate a range of possible futures. Only N deposition increased plant growth, and it increased P limitation to the dominant grass and ecosystem-level P demand. Precipitation decreased P demand, possibly by increasing P supply or by increasing N loss more than P loss (or both). Neither CO_2 nor temperature affected P demand or limitation. If P supply is just sufficient for normal NPP, future NPP increases, from any source, could be constrained by P availability. A meta-analysis revealed that N deposition is the most likely such source, causing increases in P limitation or demand in six of 11 ecosystems from across the world.

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References

Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen saturation in Northern forest ecosystems. Bioscience, 39, 378–386.

Aerts R, Chapin FS III (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Advances in Ecological Research, 20, 1–67.

Aerts R, Wallén B, Malmer N (1992) Growth-limiting nutrients in Sphagnum-dominated bogs subject to low and high atmospheric nitrogen supply. Journal of Ecology, 80, 131–140.

Ajwa HA, Dell CJ, Rice CW (1999) Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. Soil Biology and Biochemistry, 31, 769–777.

Baer SG, Blair JM, Collins SL, Knapp AK (2003) Soil resources regulate productivity and diversity in newly established tallgrass prairie. Ecology, 84, 724–735.

Barrett DJ, Richardson AE, Gifford RM (1998) Elevated atmospheric CO_2 concentrations increase wheat root phosphatase activity when growth is limited by phosphorus. Australian Journal of Plant Physiology, 25, 87–93.

Chadwick OA, Derry LA, Vitousek PM, Huebert BJ, Hedin LO (1999) Changing sources of nutrients during four million years of ecosystem development. Nature, 397, 491–497.

Chapin FS III (1980) The mineral nutrition of wild plants. Annual Review of Ecology and Systematics, 11, 233–260.

Chapin FS III, Shaver GR, Giblin AE, Nadelhoffer KJ, Laundre JA (1995) Responses of arctic tundra to experimental and observed changes in climate. Ecology, 76, 694–711.

Clarholm M (1993) Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizers. Biology and Fertility of Soils, 16, 287–292.

DeLucia EH, Hamilton JG, Naidu SL et al. (1999) Net primary production of a forest ecosystem with experimental CO_2 enrichment. Science, 284, 1177–1179.

Dracup MNH, Barrett-Lennard EG, Greenway H, Robson AD (1984) Effect of phosphorus deficiency on phosphatase activity of cell walls from roots of subterranean clover. Journal of Experimental Botany, 35, 466–480.

Drenovsky RE, Richards JH (2004) Critical N : P values: predicting nutrient deficiencies in desert shrublands. Plant and Soil, 259, 59–69.

Dukes JS, Chiariello NR, Cleland EE et al. (2005) Responses of grassland production to single and multiple environmental changes. PLoS Biology, 3, e319.

Ebersberger D, Niklaus PA, Kandeler E (2003) Long term CO_2 enrichment stimulates N-mineralisation and enzyme activities in calcareous grassland. Soil Biology and Biochemistry, 35, 965–972.

Field CB, Chapin FS III, Matson PA, Mooney HA (1992) Responses of terrestrial ecosystems to the changing atmosphere: a resource-based approach. Annual Review of Ecology and Systematics, 23, 201–235.

Finzi AC, Allen AS, DeLucia EH, Ellsworth DS, Schlesinger WH (2001) Forest litter production, chemistry, and decomposition following two years of free-air CO_2 enrichment. Ecology, 82, 470–484.

Finzi AC, DeLucia EH, Schlesinger WH (2004) Canopy N and P dynamics of a southeastern US pine forest under elevated CO_2. *Biogeochemistry*, **69**, 363–378.

Finzi AC, Sinsabaugh RL, Long TM, Osgood MP (2006) Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. *Ecosystems*, **9**, 215–226.

Fries LLM, Pacovsky RS, Safir GR, Kaminski J (1998) Phosphorus effect on phosphatase activity in endomycorrhizal maize. *Physiologia Plantarum*, **103**, 162–171.

Gurevitch J, Hedges LV (1999) Statistical issues in ecological meta-analyses. *Ecology*, **80**, 1142–1149.

Gusewell S (2004) N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist*, **164**, 243–266.

Hedges LV, Olkin I (1980) Vote-counting methods in research synthesis. *Psychological Bulletin*, **88**, 359–369.

Henry HAL, Juarez J, Field CB, Vitousek PM (2005) Interactive effects of elevated CO_2, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. *Global Change Biology*, **11**, 1808–1815.

Huenneke LR, Hamburg SP, Koide R, Mooney HA, Vitousek PM (1990) Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. *Ecology*, **71**, 478–491.

Hungate GA, Chapin FS III, Zhong H, Holland EA, Field CB (1997) Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. *Oecologia*, **109**, 149–153.

IPCC (2001) *Working Group 1, Third Assessment Report*. Cambridge University Press, Cambridge, UK.

Joel G, Chapin FS III, Chiariello NR, Thayer SS, Field CB (2001) Species-specific responses of plant communities to altered carbon and nutrient availability. *Global Change Biology*, **7**, 435–450.

Jonasson S, Michelsen A, Schmidt IK, Nielsen EV (1999) Responses in microbes and plants to changed temperature, nutrient, and light regimes in the arctic. *Ecology*, **80**, 1828–1843.

Koerselman W, Meuleman AFM (1996) The vegetation N : P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, **33**, 1441–1450.

McGuire AD, Sitch S, Clein JS *et al.* (2001) Carbon balance of the terrestrial biosphere in the twentieth century: analyses of CO_2, climate and land use effects, with four process-based ecosystem models. *Global Biogeochemical Cycles*, **15**, 183–206.

Menge DNL (2003) *Phosphorus! Or: anthropogenic global environmental changes and phosphorus limitation: interactions and implications*. Honors thesis, Stanford University, Stanford, CA.

Miglietta F, Giuntoli A, Bindi M (1996) The effect of free air carbon dioxide enrichment (FACE) and soil nitrogen availability on the photosynthetic capacity of wheat. *Photosynthesis Research*, **47**, 281–290.

Moorhead DL, Linkins AE (1997) Elevated CO_2 alters belowground exoenzyme activities in tussock tundra. *Plant and Soil*, **189**, 321–329.

Niklaus PA, Körner C (2004) Synthesis of a six-year study of calcareous grassland responses to *in situ* CO_2 enrichment. *Ecological Monographs*, **74**, 491–511.

Niklaus PA, Leadley PW, Stöcklin J, Körner C (1998) Nutrient relations in calcareous grassland under elevated CO_2. *Oecologia*, **116**, 67–75.

Niinemets Ü, Kull K (2005) Co-limitation of plant primary productivity by nitrogen and phosphorus in a species-rich wooded meadow on calcareous soils. *Acta Oecologica*, **28**, 345–356.

Oechel WC, Cowles S, Grulke N *et al.* (1994) Transient nature of CO_2 fertilization in Arctic tundra. *Nature*, **371**, 500–503.

Øien D (2004) Nutrient limitation in boreal rich-fen vegetation: a fertilization experiment. *Applied Vegetation Science*, **7**, 119–132.

Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry*, **49**, 175–190.

Parkin TB (1987) Soil microsites as a source of denitrification variability. *Soil Science Society of America Journal*, **51**, 1194–1199.

Richardson SJ, Press MC, Parsons AN, Hartley SE (2002) How do nutrients and warming impact on plant communities and their insect herbivores? A 9-year study from a sub-Arctic heath. *Journal of Ecology*, **90**, 544–556.

Shaw MR, Zavaleta ES, Chiariello NR, Cleland EE, Mooney HA, Field CB (2002) Grassland responses to global environmental changes suppressed by elevated CO_2. *Science*, **298**, 1987–1990.

Sinsabaugh RL, Antibus RK, Linkins AE, McClaugherty CA, Rayburn L, Repert D, Weiland T (1993) Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. *Ecology*, **74**, 1586–1593.

Smil V (2000) Phosphorus in the environment: natural flows and human interferences. *Annual Review of Energy in the Environment*, **25**, 53–88.

Sokal RR, Rohlf FJ (1995) *Biometry*, 3rd edn. W.H. Freeman, New York.

Speir TW, Ross DJ (1978) Soil phosphatase and sulphatase. In: *Soil Enzymes* (ed. Burns RG), pp. 197–250. Academic Press, London, UK.

Spiers GA, McGill WB (1979) Effects of phosphorus addition and energy supply on acid phosphatase production and activity in soils. *Soil Biology and Biochemistry*, **11**, 3–8.

Sundareshwar PV, Morris JT, Koepfler EK, Fornwalt B (2003) Phosphorus limitation of coastal ecosystem properties. *Science*, **299**, 563–565.

Tabatabai MA, Bremner JM (1969) Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, **1**, 301–307.

Tadano T, Ozawa K, Sakai H, Osaki M, Matsui H (1993) Secretion of acid phosphatase by the roots of crop plants under phosphorus-deficient conditions and some properties of the enzyme secreted by lupin roots. *Plant and Soil*, **155/156**, 95–98.

Treseder KK, Vitousek PM (2001) Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology*, **82**, 946–954.

van Duren IC, Boeye D, Grootjans AP (1997) Nutrient limitations in an extant and drained poor fen: implications for restoration. *Plant Ecology*, **133**, 91–100.

van Heerwaarden LM, Toet S, Aerts R (2003) Nitrogen and phosphorus resorption efficiency and proficiency in six sub-arctic bog species after 4 years of nitrogen fertilization. *Journal of Ecology*, **91**, 1060–1070.

van Wijnen HJ, Bakker JP (1999) Nitrogen and phosphorus limitation in a coastal barrier salt marsh: the implications for vegetation succession. *Journal of Ecology*, **87**, 265–272.

Vitousek PM (2004) *Nutrient Cycling and Limitation: Hawai'i as a Model Ecosystem*. Princeton University Press, Princeton, NJ.

Vitousek PM, Aber JD, Howarth RW *et al.* (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, **7**, 737–750.

Vitousek PM, Farrington H (1997) Nutrient limitation and soil development: experimental tests of a biogeochemical theory. *Biogeochemistry*, **37**, 63–75.

Vitousek PM, Walker LR, Whiteaker LD, Matson PA (1993) Nutrient limitation to plant growth during primary succession in Hawaii Volcanoes National Park. *Biogeochemistry*, **23**, 197–215.

Weiss SB (1999) Cars, cows, and checkerspot butterflies: nitrogen deposition and management of nutrient-poor grasslands for a threatened species. *Conservation Biology*, **13**, 1476–1486.

Zavaleta ES (2001) *Influences of climate and atmospheric changes on plant diversity and ecosystem function in a California grassland*. PhD thesis, Stanford University, Stanford, CA.

Zavaleta ES, Shaw MR, Chiariello NR, Thomas BD, Cleland EE, Field CB, Mooney HA (2003) Grassland responses to three years of elevated temperature, CO_2, precipitation, and N deposition. *Ecological Monographs*, **73**, 585–604.