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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 (CO₂), 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 CO₂ 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 CO₂ or warming.

Keywords: Avena barbata, Avena fatua, climate change, CO₂, global change, grassland, Jasper Ridge, N:P ratio, nitrogen deposition, nitrogen saturation, phosphatase, phosphorus

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Introduction

Atmospheric carbon dioxide (CO₂) 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). CO₂, 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 CO₂ and NOₓ emissions as are temperature and precipitation. Due to this uncoupling, we hypothesize that P availability may constrain the growth-enhancing effects of CO₂, 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 CO₂, nitrate deposition, precipitation, and temperature on biological P demand in an ecosystem limited by N but not CO₂, 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 CO₂, 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 CO₂, 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 CO₂, temperature, precipitation, and nitrate deposition. Atmospheric CO₂, 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 NO₃-N m⁻² yr⁻¹ above the background rate of <1 g N m⁻² yr⁻¹ (Weiss, 1999). Nitrate is applied as Ca(NO₃)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 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 °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 °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 (\(V_{\text{max}}\)), 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. Although 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 \(^\ast\) superscript in Table 1 or significant decreases in those with a \(^\ast\ast\) superscript), negative, or not

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

<table>
<thead>
<tr>
<th>Source</th>
<th>Ecosystem</th>
<th>Manipulation(s)</th>
<th>P cycle measurement(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerts et al. (1992)</td>
<td>Sweden bog, 1 year</td>
<td>NH₄NO₃: +2</td>
<td>*Plant [P], *N:P</td>
</tr>
<tr>
<td>Clarholm (1993)</td>
<td>Sweden spruce forest, 20 years</td>
<td>NH₄NO₃: +6</td>
<td>*Microbial [P], *soil phosphatase activity</td>
</tr>
<tr>
<td>Vitousek et al. (1993)</td>
<td>Hawaii forest, 2 years</td>
<td>Urea/NH₄NO₃: +5–10</td>
<td>*Growth response to P after N fertilization</td>
</tr>
<tr>
<td>Moorman &amp; Körner (1997)</td>
<td>Alaska tundra, 3 years</td>
<td>NH₄NO₃: +10</td>
<td>*Root and *soil phosphatase activity</td>
</tr>
<tr>
<td>van Duren et al. (1997)</td>
<td>Belgium fen, 2 years</td>
<td>CO (NH₄)₂: +20</td>
<td>*Growth response to P after N fertilization</td>
</tr>
<tr>
<td>Vitousek &amp; Farrington (1997)</td>
<td>Hawaii forest, 2 years</td>
<td>Urea/NH₄NO₃: +10</td>
<td>*Growth response to P after N fertilization</td>
</tr>
<tr>
<td>Ajwa et al. (1999)</td>
<td>Kansas tallgrass prairie, 9 years</td>
<td>NH₄NO₃: +10</td>
<td>*Soil acid, alkaline phosphatase activity</td>
</tr>
<tr>
<td>van Wijnen &amp; Bakker (1999)</td>
<td>Sweden fellfield, 5 years</td>
<td>NH₄NO₃: +5,25</td>
<td>*Growth response to P after N fertilization</td>
</tr>
<tr>
<td>Olander &amp; Vitousek (2000)</td>
<td>Hawaii forest, 11 years</td>
<td>Urea/NH₄NO₃: +10</td>
<td>*Soil phosphatase activity</td>
</tr>
<tr>
<td>Treseder &amp; Vitousek (2001)</td>
<td>Hawaii forest, 11 years</td>
<td>Urea/NH₄NO₃: +10</td>
<td>*Root phosphatase activity</td>
</tr>
<tr>
<td>Ebersberger et al. (2003)</td>
<td>Switzerland grassland, 6 years</td>
<td>CO₂: +250 ppm</td>
<td>*Soil phosphatase activity</td>
</tr>
<tr>
<td>Drenovsky &amp; Richards (2004)</td>
<td>California desert, 2 years</td>
<td>NH₄NO₃: +105 gN plant⁻¹ yr⁻¹</td>
<td>*Growth response to P after N fertilization, *plant [P]</td>
</tr>
<tr>
<td>Finzi et al. (2004)</td>
<td>North Carolina forest, 4 years</td>
<td>CO₂: +200 ppm</td>
<td>*Foliar [P], *canopy N:P</td>
</tr>
<tr>
<td>Öien (2004)</td>
<td>Norway fens, 1 year</td>
<td>NH₄NO₃: +12</td>
<td>*Growth response to P after N fertilization</td>
</tr>
<tr>
<td>Henry et al. (2005)</td>
<td>California annual grassland (JRGCE), 6 years</td>
<td>NO₃: +7</td>
<td>*Soil phosphatase activity</td>
</tr>
<tr>
<td>Niinemets &amp; Kull (2005)</td>
<td>Estonian meadow, 1 year</td>
<td>NH₄NO₃: +2, 5, 10, 20</td>
<td>*Growth response to P after N fertilization</td>
</tr>
<tr>
<td>Finzi et al. (2006)</td>
<td>North Carolina forest, 6 years</td>
<td>CO₂: +200 ppm</td>
<td>*Soil phosphatase activity</td>
</tr>
<tr>
<td>This study</td>
<td>California annual grassland (JRGCE), 3–5 years</td>
<td>NO₃: +7</td>
<td>*Soil phosphatase activity, *Avena [P], *Avena N:P, *Total aboveground P</td>
</tr>
</tbody>
</table>

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). * denotes that an increase in the level of that measurement indicates an increase in P limitation or demand. ** 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; CO₂, carbon dioxide.

significant, using statistics in the published works (with α = 0.05 and two-tailed tests), then analyzed the data by each measure, each study, and each ecosystem. A ‘significant’ 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 \( \mu \)mol \( pNP \) 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Phosphatase</th>
<th>Green <em>Avena</em></th>
<th>Senescent <em>Avena</em></th>
<th>AGP pool</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.087†</td>
<td>0.878</td>
<td>0.643</td>
<td>0.279</td>
</tr>
<tr>
<td>T</td>
<td>0.757</td>
<td>0.334</td>
<td>0.587</td>
<td>0.393</td>
</tr>
<tr>
<td>R</td>
<td>0.012*</td>
<td>0.010**</td>
<td>&lt;0.001***</td>
<td>0.131</td>
</tr>
<tr>
<td>N</td>
<td>0.082†</td>
<td>0.005**</td>
<td>&lt;0.001***</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>R ( \times ) N</td>
<td>0.068†</td>
<td>0.939</td>
<td>0.178</td>
<td>0.546</td>
</tr>
<tr>
<td>C ( \times ) T ( \times ) R</td>
<td>0.278</td>
<td>0.133</td>
<td>0.077†</td>
<td>0.219</td>
</tr>
<tr>
<td>T ( \times ) R ( \times ) N</td>
<td>0.186</td>
<td>0.918</td>
<td>0.306</td>
<td>0.539</td>
</tr>
</tbody>
</table>

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.

†\( P < 0.10 \); *\( P < 0.05 \); **\( P < 0.01 \); ***\( P < 0.001 \).
**Discussion**

**CO₂, temperature, and interactions**

Previous results from the JRGCE have shown that elevating atmospheric CO₂ 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 CO₂ to bring it into the limiting range. In studies from other ecosystems where CO₂ 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 ~1 °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 ~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 CO₂ chambers) stimulated N mineralization (Hungate *et al.*, 1997), and it is possible the same
are shown. Studies manipulated more than one variable, only main effects between ‘no effect’ and ‘increase’ or ‘decrease.’ Although some number of significant and nonsignificant effects) were split the studies in that ecosystem were significant. Ties (an equal larly, ecosystem effects were deemed significant if a majority of measures in a study were significant; simi- reported in the source. Study effects were deemed significant if relevant variable on a measure of P limitation or demand, as ‘Increase’ and ‘decrease’ indicate significant effects of the Temperature (e.g. Henry studies met our criteria. 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 terres- trial 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 dif- ferent 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

<table>
<thead>
<tr>
<th>Effect on P</th>
<th>% that increased</th>
<th># Studies</th>
<th># Ecosystems</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO2</td>
<td>Increase 16***</td>
<td>10***</td>
<td>6***</td>
</tr>
<tr>
<td>N</td>
<td>Increase 3†</td>
<td>2†</td>
<td>1</td>
</tr>
<tr>
<td>Temperature</td>
<td>Increase 1</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

'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 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; CO2, carbon dioxide.
(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., 1990) 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 CO₂ 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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