Phosphorus!

or:

Anthropogenic global environmental changes and phosphorus limitation: interactions and implications

by

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ABSTRACT

Atmospheric carbon dioxide (CO₂), nitrogen deposition, temperature, and precipitation are all increasing due to human activities. As these are essential for growth, many studies have predicted and shown that increasing these four factors increases plant production. The availability of another essential element, phosphorus, is not increasing as broadly as are the other four factors, and thus may constrain the fertilization effects of CO₂, nitrogen, or precipitation. In a manipulated annual grassland with all combinations of elevated CO₂, nitrate deposition, temperature, and precipitation, three pathways of the phosphorus cycle were measured to assess this possible constraint. 1) The phosphorus concentration and the nitrogen: phosphorus ratio in green Avena, 2) the phosphorus concentration and nitrogen: phosphorus ratio in senescent Avena, and 3) soil phosphatase activity were measured in all plots. In general, these measures revealed an increase in phosphorus limitation with increased nitrate deposition and a decrease in phosphorus limitation with increased precipitation. In a second study, a global soil map and a simulation model that estimates global net primary production were used to assess the spatial coverage of potential phosphorus limitation to plant growth. Phosphorus limitation is heavy in the tropical regions of the globe, which account for the majority of the world’s plant growth. The Northern temperate regions also exhibit the potential for phosphorus limitation.
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PART 1

INTRODUCTION
Natural ecosystems are complex and fascinating. The quest to understand how they function is well underway, but there is much left to discover in ecosystem ecology. Aside from being an intriguing scientific endeavor, ecosystem ecology is pertinent to every human on Earth. Natural ecosystems provide us with myriad goods and services, without which our lives would differ substantially (Daily and Ellison 2002). As well as reaping great benefits from ecosystems, humans affect all ecosystems of the world (Vitousek et al. 1997). Land transformation, species extinctions, climate change, and increased water use are but a few of the profound impacts of human activity (Vitousek et al. 1997).

From the standpoint of humans, not all of our changes are detrimental. Agriculture, for example, is a huge land transformation, but it has sustained the world population of over six billion people. With each benefit, however, comes a suite of costs. Many of the costs of anthropogenic environmental change are not immediate – they take time to develop. When the costs mature, they can be immense. In the case of agriculture, the same fertilizer that is intended to increase crop yields leaks into waterways, fertilizing aquatic organisms. The subsurface decomposition of these blooming populations can remove virtually all oxygen from the water, causing the death of many animals and the subsequent destruction of fisheries (CENR 2000). In the most widely publicized case of this eutrophication, a “dead zone” larger than the state of Massachusetts forms from spring until fall in the Gulf of Mexico. This area used to be one of the most productive fisheries in the United States (Jane Lubchenco, personal communication, April 28, 2003).
A number of environmental phenomena have been linked to human activity. Some of the direct effects have been studied at length, and are understood well enough to be incorporated into policy decisions (e.g. ozone depletion by CFCs and the Montreal Protocol). However, indirect effects, long-term effects, interactive effects, and feedbacks are less well known. As the eutrophication example illustrates, indirect effects can be quite costly. To detect these types of effects, one can either wait for a disaster to occur and figure out the cause, or one can investigate interactions between factors that are known to be important on their own. Of course, not all higher-order effects can be predicted, and it is likely that not all important single factors have been identified, but experimentation is necessary to make progress. It will always be a challenge to balance the short-term and better-known benefits with the longer-term and less-well-known detriments, but the better known the effects are, the more informed decisions can be.

Biogeochemical cycles, or the movement of elements through and within ecosystems, have been studied extensively in the context of anthropogenic global changes (Vitousek 1994). In particular, carbon dioxide and nitrogen have received much attention in recent decades. However, phosphorus – one of the two most limiting soil nutrients, along with nitrogen (Aerts and Chapin 2000) – has received less attention in global change studies (eutrophication being the exception). This may be because the terrestrial effects of anthropogenic alterations to the P cycle are more local than other global change factors such as carbon dioxide and nitrogen (Chapin et al. 2002). Because of the spatial confinement of changes to its cycling and its importance in biology, phosphorus provides a fixed background against which to test the effects of our activities.
This thesis addresses questions about interactions between the phosphorus cycle and four anthropogenic global change factors: increasing carbon dioxide, temperature, precipitation, and nitrogen deposition. To what extent will phosphorus constrain plant growth under these conditions? How does plant phosphorus demand respond to manipulations of these factors? How important might phosphorus constraint be on a global scale? What are the implications of this constraint for global change policy?

To address the mechanistic questions, this study investigates phosphorus cycling in an annual grassland that has been subjected to manipulations of carbon dioxide, temperature, precipitation, and nitrate deposition. Annual grasslands permit the study of thousands of individuals across multiple generations, making them a good system for studying biologically long-term effects. To address the global question this study uses a global soil map and a simulation model to estimate the extent of phosphorus constraint. Before addressing these questions, though, some background must be clarified. Chapter 2 explains in more detail the causes and known consequences of rising carbon dioxide, temperature, precipitation, and nitrogen deposition. Chapter 3 explores the terrestrial phosphorus cycle and its relation to plant nutrition. Chapter 4 then presents the investigations into the effects of the four global change factors on the phosphorus cycle in the natural grassland ecosystem. Chapter 5 presents the extent of phosphorus constraint on global net primary production, as estimated by the simulation model. Chapter 6 summarizes these investigations and explores a way in which these findings could apply to policy and management decisions.
PART 2

BACKGROUND INFORMATION
CHAPTER 2
CARBON DIOXIDE, TEMPERATURE, PRECIPITATION,
AND NITRATE DEPOSITION

Introduction

Increases in atmospheric carbon dioxide, global temperature, precipitation, and nitrogen deposition are four of the best-understood – and most important – human-caused global change factors (Vitousek 1994). Although they are by no means the extent of our influence, they affect virtually all natural ecosystems (Vitousek et al. 1997), they are relatively simple to manipulate, and their causes are well known. This chapter first provides commentary on the central role of resource limitation, and then offers an overview of current knowledge about these four factors, emphasizing both the certainty of the changes and the numerous feedbacks between them.

Multiple resource limitation

Plants require many resources to grow, including light, carbon, water, and soil nutrients. Without any one of these essential resources, they cannot grow. However, limitation by one of these resources does not necessarily imply absence of the resource, nor does it imply cessation of growth. It means that plants could benefit from addition of the limiting resource, for they could then spend more resources and energy on growth, reproduction, or acquisition of other limiting resources. A key concept to understand this distinction is resource allocation\textsuperscript{1}.

The way in which plants take up and lose various types of resources differ. Light and carbon are both aboveground resources, and water and nutrients are belowground

\textsuperscript{1} This term derives it name from microeconomic theory, and means the same in economics and plant ecophysiology.
resources. Specialized mechanisms have evolved for light capture, CO₂ uptake, water uptake/retention, and nutrient uptake/retention. These mechanisms are often costly to the plant, so evolution has shaped resource allocation to deal most efficiently with all of them simultaneously (Chapin et al. 2002). The time scales over which these mechanisms operate can vary widely. For example, on scales as brief as minutes, plants regulate the openness of their stomata to facilitate CO₂ uptake but minimize water loss. On diurnal to weekly time scales, they produce enzymes to increase nutrient uptake (e.g. phosphatases, which will be discussed at length later). On seasonal to yearly time scales, they retranslocate nutrients out of senescent tissue to minimize nutrient loss. They continually grow upwards towards light and downwards/laterally towards water sources and/or pockets of nutrients. Within each time scale, plants can adjust to environmental conditions in an attempt to maximize efficiency. This plasticity in allocation is the basis for the multiple resource limitation hypothesis, which states that optimal allocation leads to equal limitation by all classes of essential resources (Bloom et al. 1985).

Increased limitation by one resource means a plant must allocate more towards acquiring that resource. As an energetic result, it cannot grow as much, for energy and resources spent acquiring the limiting resource could otherwise have been spent on growth. If the resource were to vanish completely, growth would cease, but this is rare for essential resources. They typically are limiting because they are present in low concentrations (e.g. CO₂ in the air, N and P in some soils, or water in arid zones), difficult to access (N₂, clay-bound P), or difficult to retain (water).
Carbon dioxide

The concentration of carbon dioxide (CO₂) in the atmosphere has risen by 30% in the past 300 years; half of this increase has occurred in the past forty years (Chapin et al. 2000). Both the rate of increase and the present atmospheric concentration (~370 ppm) are unparalleled over the past 400,000 years (Chapin et al. 2002); the correlation between this trend and the human population explosion is striking. Isotopic data from ice cores and direct measurement confirm that the rise is due to human activity, notably through fossil fuel emissions, deforestation, and cement production (Vitousek 1994). Indeed, annual CO₂ emissions from fossil fuel combustion alone exceed the annual rise in atmospheric CO₂ (Vitousek 1994). There is no question that atmospheric CO₂ is rising, and that human activity is the reason (Vitousek 1994). Efforts to curb CO₂ emissions are underway, but societal inertia will prevent CO₂ stabilization for centuries (IPCC 2001). Plausible estimates for the year 2100 extend from about 550 to 1000 ppm (IPCC 2001). Understanding the consequences of this now unavoidable increase is essential to allow policy makers to reduce emissions and anticipate consequences.

The known physical and biological effects of rising CO₂ are numerous. The physical effect of greenhouse warming is better understood, and will be discussed in the following section. Biological responses to increased atmospheric CO₂ include increased photosynthesis, shifts in competitive advantage, and changes in carbon storage.

Carbon dioxide is fixed during photosynthesis, and thus is the source of virtually all carbon in organisms. Because it is essential for growth, and yet rare in the atmosphere, CO₂ is frequently limiting to plant growth. Increasing the amount of CO₂ in the atmosphere increases the gradient of CO₂ from outside to inside leaves, speeding the
rate of photosynthesis (Chapin et al. 2002, Greer et al. 1995). Increasing the rate of photosynthesis (an instantaneous measure), though, does not necessarily increase gross photosynthesis (a time-integrated measure). Although some species do increase gross photosynthesis, others photosynthesize faster, but for a shorter period of time each day. Known as **down-regulation**, this strategy has the effect of conserving more water\(^2\), and thus can be very beneficial to plants in water-stressed environments (Chapin et al. 2002). Consistent with the multiple resource limitation hypothesis, elevated CO\(_2\) in nutrient-poor environments should induce increased allocation to nutrient uptake, for carbon limitation has been lessened (Vitousek 1994).

Some plants are able to adjust their allocation or physiological strategies faster or more completely than other plants, leading to competitive advantages. Predicted results of this are shifts in species’ ranges and loss of biological diversity as slowly or poorly adapting plants are outcompeted (Chapin et al. 2000). As an example, plants with different photosynthetic pathways (“C\(_3\)” and “C\(_4\)” ) respond quite differently to CO\(_2\) enrichment. The enzymes that fix CO\(_2\) during C\(_3\) and C\(_4\) photosynthesis, **rubisco** and **PEP carboxylase**, respectively, differ in their carbon fixation efficiencies (Chapin et al. 2002). PEP carboxylase is very efficient, so C\(_4\) photosynthesis is saturated at present CO\(_2\) levels. Thus, increasing CO\(_2\) favors C\(_3\) plants, and will likely lead to a decrease in the range of C\(_4\) plants (Chapin et al. 2002).

Increasing photosynthesis increases gross carbon input to the biosphere and gross CO\(_2\) removal from the atmosphere. But how ubiquitous is this “**CO\(_2\) fertilization**” effect? Down-regulation and CO\(_2\)-saturated photosynthesis (C\(_4\)) are two established

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\(^2\) Water is always lost during photosynthesis, for leaves are water-saturated, and the pathway through which CO\(_2\) enters and water exits leaves is the same (stomata).
constraints on the extent of CO₂ fertilization. Nutritional or environmental constraints can also limit CO₂ fertilization (Field et al. 1992).

Even when CO₂ fertilization is an important factor in carbon uptake, net carbon storage may not increase. Heterotrophic respiration increases with CO₂ fertilization, for more litter is available for decomposition. Thus, net CO₂ removal from the atmosphere may be dampened by respiration inputs. The net result of all feedbacks to increasing atmospheric CO₂ is not precisely known, and likely will not be the same in all ecosystems. One study has shown an increase in carbon storage above and beyond plant production (Hamilton et al. 2001). Another has shown an increase in carbon storage, though the magnitude was not proportional to the increase in photosynthesis (Hungate et al. 1997). Yet another has found that CO₂ enrichment leads to a decrease in carbon storage (Shaw et al. 2002). One important difference between this last study and others is that it manipulates three factors in addition to CO₂, capturing interacting effects as well as single-factor effects.

Temperature

Global mean temperature has risen by more than 0.6° C in the past 100 years, with an accelerating increase similar to the CO₂ trend (IPCC 2001). Increased greenhouse warming³, caused by the rise in CO₂ and other gases⁴ that absorb and scatter infrared radiation, is a primary driver of this temperature increase (IPCC 2001). Plausible predictions for future global temperature increase range from 1° C to over 5° C

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³ Greenhouse warming is essential for life as we know it, for without it Earth’s mean temperature would be 32° C lower than it is today (NOAA 2003). However, it is the rapid increase and eventual amount of warming that are of concern.

⁴ CO₂, methane (CH₄), nitrous oxide (N₂O), and water vapor (H₂O) are four of the many greenhouse gases with strong links to human activity. CH₄, N₂O, and H₂O all much stronger greenhouse effects per molecule than does CO₂, but have received less attention because they are either much more diluted (CH₄ and N₂O), or have a much smaller residence time (CH₄ and H₂O).
by 2100 (IPCC 2001). Regional and local temperature changes are much greater, especially in the interior of continents, due to the difference in heat capacity between land and water (IPCC 2001). Predictions for 2100 range as high as a mean increase of 8° C for much of North America and Eurasia (IPCC 2001). Seasonal temperature swings are also predicted to widen (IPCC 2001).

The implications of global warming have been widely publicized. Sea level rise is unavoidable, as thermal expansion of the oceans and melting of the polar ice caps are well understood, straightforward physical phenomena. Some monitored areas have already seen an increase in sea level of ~200 mm in the past 100 years (IPCC 2001), and the global rise over the next 100 years is estimated to be 200-700 mm (IPCC 2001). Hundreds of millions of people worldwide could be flooded by a sea level rise of 400 mm combined with storm surges (IPCC 2001).

Global warming will also cause range shifts or expansion of many tropical species, including pests that hinder crop production and diseases such as malaria. The increased number of biological invasions due to human activity exacerbates this phenomenon. Shifts in the ranges and **phenology** of temperate species have already been documented, and are likely to continue (Root et al. 2003, Parmesan and Yohe 2003). All of these will likely reduce biological diversity (Chapin et al. 2000). Other predicted biological effects include increasing decomposition, and thus respiration, releasing more CO₂, acting as a positive feedback to global warming. The list goes on.
Precipitation

Temperature and precipitation are the two most important climatic factors for biology. In fact, the first global plant production model used these alone to predict the spatial distribution of productivity (Lieth 1975) – this model was astoundingly accurate given its simplicity. Humans have altered patterns of precipitation as well as temperature, but these vary in space and time much more than do temperature patterns. The frequency and amplitude of drought/flood cycles will change much more than the globally averaged precipitation (IPCC 2001). An example of this is the increase in the frequency of the El Niño Southern Oscillation (ENSO) cycle, which increases droughts and floods on both sides of the Pacific Ocean. El Niño causes increased droughts on the Asian side of the Pacific and increased floods on the American side, and vice versa for La Niña (the other end of the oscillation).

Increased variability in precipitation will have considerable effects on ecosystems. The consequences of more severe droughts are readily understood. Crop yields will fall, especially in places where irrigation is not readily accessible. Natural ecosystems will also grow less. Hardier species may gain a competitive advantage, or grazing regimes may change, again potentially altering species diversity. Fire frequency will increase with droughts, affecting agricultural ecosystems, natural ecosystems, and humans alike. Increased precipitation may stimulate plant growth, but it may also have many negative effects. If it comes in the form of large storms, as many climate models predict, the potential for damage to both ecosystems and human infrastructure is large (IPCC 2001).
The nitrogen cycle

Nitrogen gas (N\textsubscript{2}) accounts for about 80\% of our atmosphere. However, since the two atoms are connected by a triple bond (N≡N), a lot of energy is required to make N\textsubscript{2} biologically useful. Since \textbf{N fixation} is so energetically expensive, species that do it are most often symbiotic bacteria that trade the N they fix for C fixed by plants or other bacteria. These symbioses are not ubiquitous in terrestrial ecosystems, for when N availability rises they are outcompeted by species that can more efficiently utilize other resources (Vitousek and Howarth 1991). And thus, nitrogen is growth-limiting in many ecosystems (Vitousek and Howarth 1991).

Humans have more than doubled the total amount of N fixed annually (Vitousek 1994). Two main pathways of this increase are fossil fuel combustion and intentional N fixation for fertilizer (Vitousek 1994). Fossil fuel combustion (coal and oil) releases nitrogen oxides (NO\textsubscript{x}) along with CO\textsubscript{2} and other gases. Fertilizers include both ammonium (NH\textsubscript{4}\textsuperscript{+}) and nitrate (NO\textsubscript{3}\textsuperscript{−}).

The locality of these two pathways differs greatly. Fossil fuel combustion emits NO\textsubscript{x} into the atmosphere, where its mean residence time ranges from less than a day to ten days (IPCC 2001). NO\textsubscript{x} can affect areas as far away as thousands of kilometers (Peter Vitousek, personal communication, May 16, 2003). Fertilizer is meant to stay on crops to increase yields, but often too much is applied, or it is applied at a suboptimal time (Matson et al. 1998). In these cases losses through \textbf{leaching} to groundwater are great, for nitrate is highly mobile in soil (and ammonia is relatively mobile) (Brady and Weil 2002). Losses of fertilizer to the atmosphere through \textbf{denitrification} and ammonia
volatilization are also substantial (Matson et al. 1998). NH₃ in the atmosphere has a relatively short residence time, so the effects are relatively local (Chapin et al. 2002).

The ramifications of released NOₓ are numerous. NOₓ is involved in the creation of tropospheric ozone, which damages all organisms (IPCC 2001). Since ozone is so reactive, its effects are strongest near the source of production, i.e. near urban and industrial areas. Thus, the human health risk is substantial (Burtraw et al. 1997). NOₓ emissions also have a large impact on ecosystems. Along with SO₂, NOₓ is the cause of acid rain, which has been detrimental to many ecosystems in the United States Northeast and Europe (Brady and Weil 2002). Acid rain lowers soil pH and increases levels of toxic compounds in soils, leading to a decline in the health of affected terrestrial ecosystems. As the acids and toxic compounds are filtered into waterways and concentrated, effects on aquatic ecosystems are even more drastic (Brady and Weil 2002). In soils with neutral or high pH or high acid-buffering capacity, NO₃⁻ addition (one of the forms of NOₓ) can stimulate plant growth. N fertilization, similar to CO₂ fertilization, favors some species more than others, leading to a decline in biodiversity (Tilman 1987).

N lost through leaching (from deposition of fossil fuel combustion or from direct fertilizer application) proceeds to waterways, eventually being deposited in lakes or oceans. Here it can cause eutrophication, which was discussed in Chapter 1. Denitrification can also cause problems. Complete denitrification of NO₃⁻ (to N₂ gas) is benign, but incomplete denitrification (to N₂O, NO, or NO₂) is not. N₂O is a potent greenhouse gas with a residence time of 120 years (IPCC 2001). NO and NO₂ are NOₓ, which was discussed above.
Ammonia volatilization acts to fertilize non-agricultural ecosystems, increasing growth. Again, species with a high N demand will have a competitive advantage in this scenario. These species are typically fast-growing, weedy species, and are often invasive. Invasive species often bring many problems of their own, so facilitating their establishment can be disastrous.

**Conclusion**

The number of possible effects of these factors and the number of connections between them are vast. Human activity is the primary cause of all these changes, and although the human population growth rate is slowing, the total population continues to increase, and will probably not plateau until it reaches nine or ten billion people. As more developing countries increase their affluence, impact will rise even more. To reduce and/or deal with the impact, we need to continue exploring the consequences of these changes, especially the less-known multi-factor effects.

The next chapter describes another biogeochemical cycle, that of phosphorus. This cycle has also been altered by human activity, but differs in many key ways from the carbon, water, and nitrogen cycles described above. Subsequent chapters show results of some investigations into interactions and feedbacks between the four global change factors described in this chapter and the phosphorus cycle.
CHAPTER 3
THE PHOSPHORUS CYCLE

Introduction

This chapter briefly explains the cycling of phosphorus (P) through terrestrial ecosystems, the importance of P to all organisms, and anthropogenic changes to the P cycle. Although P is also extremely important to aquatic ecosystems (if not more, in the case of lakes), terrestrial ecosystems are the focus of this thesis. The first section of this chapter discusses the three classes of soil P (soluble inorganic, organic, and insoluble inorganic P), emphasizing the speed of cycling and their relation to plant nutrition. The next section focuses on the biological functions of P. The following section explores the causes and spatial distribution of P limitation. The final section explores similarities and differences between alterations to the P cycle and the other four global change factors presented in the Chapter 2.

The terrestrial phosphorus cycle

Figure 3.1 provides a conceptual overview of the terrestrial phosphorus cycle. Soluble inorganic P typically appears as phosphate\(^5\), and is by far the most accessible form of P. Phosphate enters the soil through decomposition of organic phosphorus, weathering of inorganic minerals, or direct input from animal excretion. Phosphate is not as mobile as nitrate or ammonium, but it can still leach out of the soil and into groundwater, especially when plant growth is phosphate-saturated. This is the main loss.

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\(^5\)Phosphate ion is PO\(_4^{3-}\), and takes on various forms of reduction at different pH values. Below pH 2, most phosphate is H\(_2\)PO\(_4\); between pH 2 and pH 7, most is H\(_3\)PO\(_4\); between pH 7 and 12, most is HPO\(_4^{2-}\); and above pH 12 most is PO\(_4^{3-}\). Soil pH values below 2 or above 12 are rare, so most phosphate in natural settings is H\(_2\)PO\(_4\) or HPO\(_4^{2-}\).
of P from terrestrial ecosystems\(^6\). Like nitrogen, leached P enters waterways, and can cause eutrophication in lakes, which are often P-limited (Brady and Weil 2002). Soluble P is the most rapidly cycling form of phosphorus. When it is in high demand, plants and microbes take it up quickly. When it is not in high demand (or if it escapes organismal uptake), it leaches out (leaving the ecosystem) or adsorbs to minerals (becoming insoluble P).

Figure 3.1: The terrestrial phosphorus cycle. Large text in boxes indicates pools of P. Solid arrows indicate fluxes of P, whereas dashed arrows indicate factors that allow fluxes. Arrow size indicates speed of flux. Small text indicates fluxes and mechanisms. Sizes of pools are not indicated, for they vary substantially with soil type and plant community. Note that there is no outside source of P when net dust deposition is zero or negative. Also note that P is lost from the system through leaching, and that mineralization to occluded P is an irreversible sink. See text for details.

\(^6\)Except when harvesting removes a significant portion of the biomass, which contains P.
**Organic phosphorus** (simply P in organic matter) comes from dead plant, animal, and microbial tissue\(^7\). The majority of organic P is in phosphate groups, and is in a chemical form that can be readily cleaved from the organic matter (ester-bonded). Due to this chemical conformation and the relatively rapid input of organic matter to most ecosystems, organic phosphorus is usually the largest contributor to the phosphate pool. However, two complications prevent ready access to this source. One, organic matter often has a complex spatial structure. Thus, much of the nutritional content (including P) is not readily accessible due merely to its spatial conformation. Two, cleavage of ester bonds requires significant activation energy. There are, of course, ways to deal with these two problems. Soil animals ingest organic matter for energy, in the process reducing it to smaller chunks. Microbial decomposers (bacteria and fungi) and plants then secrete enzymes into the soil that catalyze the hydrolysis of the ester bonds, releasing phosphate. There are many forms of these enzymes, and they are collectively referred to as **phosphatases**. The relative rates of these decomposition processes and the pool size of organic P determine plant P availability in the short term.

The third form of P in soil is **insoluble inorganic P**. This “fixed” phosphorus is the most difficult for organisms to access. However, rock minerals are the ultimate source of P. Before there is any organic matter, organisms must derive their P from rocks. Fixed P binds tightly to stable mineral structures\(^8\) by covalent bonds. Insoluble, mineral phosphorus can be divided into **occluded P** and **non-occluded P**. As the names suggest, occluded P is so tightly bound it is completely unavailable (barring complete

\(^7\) Directly after mortality, plant tissue is considered “litter” (and animal tissue is considered “carcass”). When this breaks down enough so its former physical structure cannot be identified visually, it becomes “organic matter.”

\(^8\) At low pH, P binds to aluminum (Al), iron (Fe), and manganese (Mn); at high pH it binds to calcium (Ca).
physical soil disturbance), whereas non-occluded P is less tightly bound (Chapin et al. 2002). Weathering of rock releases non-occluded P, but is limited by environmental conditions (precipitation) and input of organic acids. It is a slow process, taking upwards of millions of years. Fixation is the reverse of weathering, binding soluble phosphate to aluminum, iron, or calcium, which can be occluded P. Finally, soil particles that are small enough can enter and leave the soil system as dust, though this exchange is usually quite small.

**Biological importance and plant nutrition**

P is required by all organisms for energy, structure, and reproduction. The conversion of \( \text{ATP}^9 \) to \( \text{ADP}^9 \) is the source of energy in most biochemical reactions. Cell membranes separate cells from each other and the surrounding environment, and contain many phospholipids. The nucleic acids DNA\(^9\) and RNA\(^9\) have phosphate “backbones” to form the double helix structure. Phosphorus is essential for all of these compounds, and thus is classified as a “macronutrient,” or a nutrient required in large quantities, for all organisms. Plants have an additional P requirement in NADPH\(^9\), which is used in the light harvesting reactions of photosynthesis. The proportions of P to other required nutrients vary among different classes of organisms, but all organisms require it in large amounts (Sterner and Elser 2002).

Plants acquire soluble phosphate through their roots, but many steps are involved in phosphate uptake. Diffusion and bulk flow through soil solution bring phosphate to root surfaces (Aerts and Chapin 2000). Root growth and proliferation in pockets of high P availability also increases phosphate uptake (Aerts and Chapin 2000). Extracellular

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\(^9\) ATP: adenosine triphosphate. ADP = adenosine diphosphate. DNA = deoxyribonucleic acid. RNA = ribonucleic acid. NADPH = reduced nicotinamide adenine dinucleotide phosphate.
phosphatase production accelerates phosphate release, increasing the amount available to plants (Aerts and Chapin 2000). **Mycorrhizal associations** also aid in plant P uptake. In this symbiosis plants trade carbon to mycorrhizal fungi for nutrients and water; P is the nutrient for which these relationships are most beneficial for plants, due to its relative immobility (Aerts and Chapin 2000). Mycorrhizal fungi are better at taking up P because their **hyphae** have much more surface area per volume than do roots. They may also be able to take up forms of P other than phosphate (e.g. dissolved organic phosphorus), giving plants access to more phosphorus pools.

**Phosphorus limitation in space and time**

All organisms need phosphorus in large quantities, yet it is typically not abundant. Due to differences in the rates of P weathering, organic P release, mineral P immobilization, and biotic P uptake, it is often growth limiting. As a general pattern, highly weathered soils are P-depleted, for all non-occluded P has been released, and the remaining mineral phosphorus is occluded. In these highly weathered soils, organic phosphorus is the main source of plant nutrition (Brady and Weil 2002). This can also be lost to the mineral structure, though, as released phosphate may bind to soil minerals before it reaches roots or hyphae. In such cases, atmospheric dust can be the most important input of P, but this input is typically much smaller than demand (Chadwick et al. 1999).

Soils also vary considerably in P availability due to **parent material**, which affects both total P in the soil and **phosphorus fixation capacity** (Brady and Weil 2002). Soil pH, another determinant of P availability, is a function of parent material, weathering, and acid input (both biological and atmospheric). At low soil pH Al, Fe, and Mn fix the vast majority of P, whereas at high pH Ca fixes most P.
Plant communities themselves can affect the extent of P limitation. Some groups of plants require more P than others, raising the amount of P that must be available to overcome P limitation (Aerts and Chapin 2000). Species with low P demand are typically slow growing and stress tolerant (often late successional species, e.g. boreal firs), whereas species with high P demand are typically fast growing and opportunistic (often edge or invasive species, e.g. agricultural species) (Aerts and Chapin 2000). Plant communities also vary in their P uptake capacity. Mycorrhizal plant species obtain a large portion of their P from the symbiotic fungi, and thus are less likely to be P limited than non-mycorrhizal species (Aerts and Chapin 2000). Though most plant species are mycorrhizal, some that inhabit disturbed soils (i.e. invasives) are not (Aerts and Chapin 2000).

All of these factors – weathering, soil chemistry, and plant community – vary across both time and space. Through succession soils weather, organic matter accumulates, and plant communities change. Different geological origins (e.g. basalt vs. granite) and processes (erosion, landslides) also produce different soils and plant communities. And, of course, land transformation by humans affects phosphorus dynamics by disturbing soil structure and altering plant community structure.

**Human changes to the phosphorus cycle**

As with nitrogen, humans have drastically altered phosphorus inputs to ecosystems (Smil 2000). By mining phosphorus for fertilizer, humans have increased the quantity of P cycling through ecosystems by 20 to 30% annually (Chapin et al. 2002). Locally, land transformation and soil disturbance render soil-bound P more available.

However, since P has no gaseous form\(^\text{10}\), human effects are limited to agricultural,

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\(^{10}\) P actually does have a gaseous form, phosphine (PH\(_3\)), but it reacts violently with oxygen. Since it does not stay in the atmosphere for any substantial period of time, it is inconsequential to P cycling.
highly disturbed, and aquatic systems. As opposed to the nitrogen cycle, precipitation, warming, and atmospheric CO₂, anthropogenic changes to the terrestrial P cycle are local. As carbon, nitrogen, water, and phosphorus are all essential for growth, increasing the availability of C, N, and water while maintaining the availability of P may not increase growth much if P is not abundant. Consistent with the multiple resource limitation hypothesis, plants may have to allocate more to P acquisition, reducing the growth increase.

This line of reasoning is the basis for much of this thesis. Chapter 4 analyzes the effects of altering the four global change factors on the P cycle in an annual grassland ecosystem, and Chapter 5 explores the potential for P limitation on a global scale with a simulation model.
PART 3

ORIGINAL WORK
CHAPTER 4

PHOSPHORUS IN THE GRASS:

THE JASPER RIDGE GLOBAL CHANGE EXPERIMENT

Introduction

Anthropogenic activities are resulting in multiple global scale changes, many of which co-occur and interact. Changes in factors such as N deposition, temperature, and precipitation vary substantially on relatively short spatial and temporal scales. Other factors such as atmospheric CO₂ do not vary significantly on short time scales, but are changing on longer time scales. Thus, situations in nature exist where one, two, three, and all of four of these factors are changing concurrently. An important scientific challenge is to understand the impact of the effects of all possible combinations of the factors. Studying only single-factor effects can be misleading if the factors interact. If single-factor effects are used to inform policy decisions, yet combined effects differ from the sum of single effects, the policy will not target the “true” effect.

The Jasper Ridge Global Change Experiment (JRGCE) manipulates carbon dioxide, temperature, precipitation, and nitrogen deposition in all possible combinations. Set in an annual grassland, the experiment spans multiple generations and entire plant communities at relatively small spatial and temporal scales. As is the case in non-agricultural areas, there is no intentional phosphorus deposition in the JRGCE, though dust from a nearby gravel road may reach the plots. Preliminary fertilization experiments near the JRGCE have suggested the potential for phosphorus limitation, though nitrogen is likely more limiting (Chris Field, personal communication, January 2011). The experiment is a randomized block design, so P inputs from dust should affect all treatments equally.
This setting is almost ideal for investigating the effects of global change factors on phosphorus limitation, for small changes may push the ecosystem more towards N or P limitation.

What are the single and multiple factor effects of increasing carbon dioxide, temperature, precipitation, and nitrogen deposition on phosphorus cycling in this grassland? One could imagine many possible answers, and even conflicting effects for the same factor. For example, as indicated in Chapter 2, increased CO₂ could stimulate plant growth, diluting plant tissue P, thus increasing P limitation. Alternatively, increased growth could increase N limitation more than P limitation (because N is required in larger quantities than P for photosynthesis), resulting in a decrease in relative P limitation. In a third scenario, increased CO₂ could lead to down-regulation of photosynthesis, increasing soil moisture, and thus facilitating P diffusion to roots. Increased soil moisture could also increase P leaching, increasing P limitation, or increase N leaching more than P leaching, decreasing relative P limitation. The possibilities for consequences of changes in the other single factors are numerous and equally complex, not to mention the sixteen combinations of all four factors. Outlining all possible hypotheses would be tedious at this point, and therefore the relevant mechanisms will be explained in the discussion, when actual results can be interpreted.

Plants play a large role in the P cycle, via numerous pathways (see Figure 3.1); unfortunately, time did not permit the study of all interactions. For this study, three complementary measures were chosen to investigate P dynamics in this annual grassland, each of which addresses a specific question about the mechanisms of P limitation (Figure
4.1). The three areas are current P nutrition in plants, plant inputs to organic P, and ecosystem-level demand for P.

First, how do increased CO₂, N deposition, precipitation and temperature affect plant P uptake? The concentration of P in aboveground plant tissue that was alive at the time of harvest (hereafter: green tissue) reveals current P nutrition in relation to the sum of all other elements. For example, a decrease in green tissue P would indicate a decrease in P uptake per unit biomass, and thus would indicate increased P limitation. However, this measure does not distinguish between absolute P uptake and relative P uptake. For example, if a treatment decreased tissue P content, but decreased N content more, P
limitation would decrease relative to N limitation, despite a decrease in tissue P content. As N is the other limiting soil nutrient in the Jasper Ridge grasslands, it was also measured in these analyses. The ratio of N:P in plant tissue\textsuperscript{12} has been shown to be a good indicator of which element is more limiting (Koerselman and Meuleman 1996), i.e. it shows P nutrition relative to N. Using this ratio to determine relative N or P limitation assumes that either N or P is limiting in the study ecosystem (Aerts and Chapin 2000); this assumption has been met in the Jasper Ridge grasslands. Both total P content and N:P can vary substantially across plant functional groups (Sterner and Elser 2002), so the same taxon, \textit{Avena} spp.\textsuperscript{13}, was used across all treatments and replications. \textit{Avena} was chosen because it is a dominant grass in the JRGCE, and is present in the majority of the experimental plots. Using tissue chemistry of one species to ascertain ecosystem P limitation has its limitations, however, as will be discussed later.

Second, how do the treatments affect the P quality of organic inputs to the soil? To address future plant P nutrition, one must know the inputs of P to the system. The chemistry of senescent tissue\textsuperscript{14}, which becomes litter once it drops, reveals the nutrition of plant inputs to organic matter and the decomposition cycle. As discussed in Chapter 3, the cycling of organic P through organisms is orders of magnitude more rapid than weathering of mineral P, and thus organic P is the most important input for P nutrition in the short term. A decrease in senescent tissue P content would indicate a future decrease in soil P availability, and thus would increase P limitation to future generations after seed reserves are spent. Similarly, an increase in senescent tissue N:P would indicate

\textsuperscript{12} Please pardon the confusing nature of using N:P for P limitation (instead of P : N, which would be more intuitive). N:P is used here to follow the convention of the field, facilitating comparisons across studies.

\textsuperscript{13} Two species of \textit{Avena, fatua} and \textit{barbata} are present in the JRGCE; however, they are difficult to sort at the time of harvest, and thus are pooled.
proportionally less P available to future generations, and an increase in relative P limitation. As for green tissue, both P concentration and N:P ratio were measured in this study. To facilitate comparisons to the green tissue chemistry, senescent *Avena* was chosen for chemical analysis. Analyzing only *Avena* in senescent tissue has the same problems as in green tissue: even though it is a dominant, *Avena* comprises only a fraction of the plant biomass, leaving much of the plant biomass and all non-plants unstudied. Analyzing tissue chemistry in all plant, animal, and microbial species has logistic difficulties, so another method was chosen to address ecosystem level P nutrition.

Third, how do the global change treatments affect ecosystem-level demand for phosphorus? At any given time a very small fraction of soil P is in the easily accessible form of phosphate. To access the large organic pool, plants and microbes produce and secrete extracellular phosphatases (Tarafdar and Jungk 1987, Pant and Warman 2000), which catalyze the release of phosphate from organic matter (Speir and Ross 1978). Thus, soil phosphatase activity reveals ecosystem-level P demand (Juma and Tabatabai 1978, Spiers and McGill 1979, Dracup et al. 1984, Kroehler and Linkins 1988, Sinsabaugh et al. 1993, Tadano et al. 1993, Kolari and Sarjala 1995, Barrett et al. 1998, Fries et al. 1998, Olander and Vitousek 1999). An increase in phosphatase activity would indicate an increase in P demand, and therefore P limitation. If demand is not met by supply, P is limiting to growth; even if P supply does keep pace with demand, the resources spent on producing more phosphatases could have been spent on growth or reproduction; this too indicates increased P limitation.

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14 During **senescence**, annual plants retranslocate nutrients out of leaves, stems, and roots, and put them in seeds (Chapin et al. 2002).
These three questions (P uptake, P organic quality, and ecosystem-level demand for P) will be used to investigate the short-term P dynamics as depicted in Figure 4.1. However, none of these measures is a foolproof determinant of P limitation. For example, phosphatase activity measurements do not distinguish between actual vs. potential soil enzyme activity\textsuperscript{15} (Sinsabaugh 1994). Temporal\textsuperscript{16} issues may also confuse interpretations of enzyme activity (Sinsabaugh 1994). To ensure the validity of using these three techniques to address various aspects of P limitation in the field experiment, P limitation was induced in a controlled pot experiment. The time scale of this study was much shorter than the JRGCE manipulations, so any temporal constraints in the JRGCE would be magnified in the pots. Environmental conditions of the pots replicated the JRGCE as closely as possible. Green \textit{Avena} P and N, senescent \textit{Avena} P and N, and soil phosphatase activity were then measured in the pots to test for response to P limitation. If these respond as predicted to P limitation in the pots, they can be used to address the various aspects of P limitation in the JRGCE with more certainty.

**Methods**

\textit{Pot study}

**Setup**

Soil was obtained from a plot adjacent to the JRGCE, \textbf{homogenized}, and divided into twelve one-gallon circular gardening pots. The parent material and mineral composition is the same in both the pots and the JRGCE, but the process of transportation

\textsuperscript{15} To measure soil enzyme activity, one must homogenize the soil. In the process, enzymes that were bound to the mineral structure of the soil are released and counted as part of “soil enzyme activity,” even though they are actually inactive in the soil. An assumption of most phosphatase studies is that the active enzyme levels in the soil are proportional to measured (potential) enzyme activity.

\textsuperscript{16} Extracellular enzymes can remain in soil after the organisms that produce them have died, for they typically have a residence time of months. Thus, short-term studies measure P demand of both pre- and post-manipulation (Sinsabaugh 1994).
disturbed the soil structure. Each pot was filled to within 5 cm of the surface. The two dominant grasses from the JRGCE, *Avena fatua* and *Bromus hordeaceus* (both annual exotics), were planted as monocultures in six pots each. Seeds were planted 1 cm below the soil surface and watered to induce germination. The plants germinated in mid-September, avoiding confounding seasonal effects. Pots were placed outside in full sunlight and watered regularly throughout the study. Neither disease nor pests were apparent. Ca(NO₃)₂, the form of nitrate fertilizer used in the JRGCE plots, was applied to all twelve pots in solution at 7.0 g * m⁻² N.

*Treatments*

For each species, three pots were chosen at random to be the phosphorus enriched treatments (+ P), and the remaining three were designated ambient P treatments (– P). P was applied as Ca₃(PO₄)₂ in solution, also at 7.0 g * m⁻² P.

*Harvest for aboveground NPP and tissue chemistry*

*Avena* was harvested February 1st, and *Bromus* was harvested between February 3rd and 6th, when they were setting seed. This is the same phenological period used in the JRGCE analyses (Shaw et al. 2002). All harvested material was oven-dried at 70° C to constant mass before weighing. Aboveground NPP was calculated as the sum of green tissue, senescent tissue, litter, and seeds. Litter was included because the pots were devoid of plant material prior to planting, so any plant material present was part of the year’s production. Only *Avena* was used for tissue chemistry analysis, for only *Avena*

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17 At densities of 9500 plants/m² for *Bromus* and 3000 plants/m² for *Avena*, consistent with other studies of monoculture of these species in this soil type (Nona Chiariello, personal communication, September 9, 2003).

18 Within the range of possible germination dates of the species in the JRGCE.
samples from the JRGCE were available. Soils under both species were used for phosphatase measurements.

*Soil sampling*

Cores for phosphatase measurements in pots were taken November 2\textsuperscript{nd} and November 23\textsuperscript{rd}, 2002. Depth of coring was 5 cm, and core diameter was 11 mm.

*Jasper Ridge Global Change Experiment*

*Experimental design*

The JRGCE is in Stanford University’s Jasper Ridge Biological Preserve (37°24′N, 122°14′W). It is in a Mediterranean climate, with cool, wet winters (growing season) and hot, dry summers. The JRGCE plant community is predominantly exotic annual grassland on sandstone-derived soil. Annual grasses (*Avena* species and *Bromus hordeaceus*) and forbs (*Geranium dissectum* and *Erodium botrys*) dominate the plant community. The experiment is a four-way full factorial complete block design. There are thirty-two plots one meter in radius, each of which is divided into four quadrants. The four manipulated factors are atmospheric CO$_2$, temperature, precipitation, and nitrate deposition. Each of the sixteen combinations of these treatments is replicated eight times for a total of 128 experimental units. Atmospheric CO$_2$, applied at the plot level, is elevated from ambient (~370 ppm) to ~680 ppm with a ring of free-air emitters surrounding each plot. Heat input, also applied at the plot level, is 80 W * m$^{-2}$, supplied by infrared heaters above each plot. This raises air temperature by ~1° C at canopy height. Precipitation, applied at the quadrant level, is elevated to 150% ambient by drip-irrigation and sprinklers at the time of rain, with two additional rain events simulated each spring. Nitrogen is also applied at the quad level. 7 g * m$^{-2}$ * yr$^{-1}$ nitrogen is applied
as Ca(NO₃)₂, with an initial application of 2 g * m⁻² directly following the first rain and an additional 5 g * m⁻² applied as a slow-release fertilizer in January. Treatments have been applied during each growing season since the 1998-1999 growing season.

Plant tissue sampling

Foliar tissue samples were harvested from the JRGCE on May 16, 2001. Samples were sorted in the days following harvest. Analyses for this study were conducted on green and senescent Avena.

Soil sampling

Cores from the JRGCE were taken during 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. Coring for each month was completed within three days.

Tissue chemistry

Sample handling and preparation

Between the time of harvest and these analyses, Avena samples were stored at room temperature in paper bags, which were in airtight bins. For chemical analysis, samples of 50 mg or greater were ground to 20 mesh in a Wiley mill. If less than 50 mg of material was available, samples were cut to small pieces manually with scissors to mimic grinding but reduce tissue loss.

Kjeldahl digests

The general form of the Kjeldahl acid digestion method was used for analysis of total P and total N in foliar tissue. In the exact form of the technique used here, tissue samples were placed in 75 mL volumetric digest tubes, followed by boiling stones and
5.0 mL warm sulfuric acid digest solution\textsuperscript{19}. They were then heated by a 210° C heating block for 30 seconds, removed, and amended with 2 mL hydrogen peroxide. Samples were once again placed on the heating block, which ramped from 210° C to 410° C over two hours. The block remained at 410° C for another two hours, during which time the samples were completely digested to solution. After two hours at 410° C, samples were removed from the heating block, cooled, and diluted to constant volume with distilled water. Two blanks per forty samples were prepared, differing only in that tissue was not added to the tubes. At no more than ten days following the digestions, samples were analyzed for total phosphorus and total nitrogen concentration on an Alpkem RFA/2 continuous flow analyzer (uses \textit{colorimetric} analysis).

\textit{Calculations}

Data were converted from (mg P or N) / (L solution) to (mg P or N) / (g plant tissue) by first subtracting blanks for the corresponding round of digests, then multiplying by (75 mL solution) / (g plant tissue). Most samples had 50 mg plant tissue, but some had less. Samples with less than 25 mg were removed from analysis.

\textit{Phosphatase assays}

\textit{Soil sample handling}

All soil samples were stored at 4° C. Pot study samples were processed within one week; JRGCE samples within three weeks. Soil phosphatase activity remains stable over 28 days when stored at 4° C (Pettit et al. 1977).

\textsuperscript{19} Digest solutions were 19 parts 12 M sulfuric acid, 10 parts potassium sulfate, and 2 parts cupric sulfate, and were heated on a hot plate and stirred by a magnetic stirrer.
**Assay procedure**

Phosphatase assay techniques follow the general outline of Tabatabai and Bremner (1969), but incorporate alterations made by Malcolm (1983), Sinsabaugh et al. (1993), and others. The exact technique used here is presented below.

Once removed from the refrigerator, soils were homogenized by hand and subsampled to acquire the desired amount of soil. When possible, samples were sifted to remove roots that could contribute intracellular phosphatases when blended. When samples were too wet to sift, roots were removed by hand. One subsample for each sample was put in a drying tin, weighed, and put in the oven. After drying at 105°C for a week they were reweighed and water weight content was determined. A 2.0 g subsample to be used for the assay was combined with 60 mL of buffer, and then blended for 60 seconds with a laboratory blender. The resulting **homogenates** were pipetted into **microcentrifuge tubes**. In each microcentrifuge tube 750 µL of homogenate was combined with 750 µL of 5.0 mM p-nitrophenyl phosphate (pNPP), a synthetic phosphatase **substrate**. This concentration of pNPP was used to ensure substrate saturation and **zero-order kinetics** so measured activity would vary linearly with incubation time. The pH of the substrate solution was measured to make sure it did not depress the pH of the buffer. Three or four analytical replicates of each sample were prepared, along with one or two sample controls (sample homogenate and buffer; no substrate) per sample and four substrate controls per four samples (buffer and substrate; no sample homogenate). Controls correct for background coloration in the sample and the substrate, which varies, and could confound coloration due to enzyme activity without

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20 Intracellular phosphatases are used for myriad biochemical functions, including non-nutritional functions, and do not necessarily correlate to P demand.
controls (Malcolm 1983). Each sample was incubated for 120 minutes on a shaker table to allow the enzymes to catalyze the hydrolysis reaction. Samples were at room temperature (20° C) during incubation. After incubation, each 2 mL microcentrifuge tube was centrifuged for 90 seconds at 10,000 rpm. 750 µL of the supernatant from each tube was pipetted into culture tubes, followed by 75 µL of sodium hydroxide (NaOH, which stops the reaction and develops the color) and 3 mL of distilled water. Each tube was vortexed, then contents were transferred to transparent plastic cuvettes. pNP-OH, formed when NaOH encounters pNP, is yellow. Thus, colorimetric analysis yields absorbance of the solution, from which phosphatase activity can be calculated. After zeroing with distilled water, the absorbance of each sample was measured using a spectrophotometer at 410 nm.

Activity calculations

Activity was calculated as:

Phosphatase activity (µmol pNP converted h\(^{-1}\) g DM\(^{-1}\)) =

\[ \frac{\text{OD}}{[(\text{EC/µmol/mL})/(1.5 \text{ mL/assay}) \times (2 \text{ hours incubation time}) \times (\text{g DM/mL sample homogenate}) \times (0.75 \text{ mL homogenate /assay})]} \]

where EC is the extinction coefficient\(^{21}\), OD is optical density, and DM is dry soil mass. The EC is calculated by making dilutions of a 1.00 µmol/mL solution of pNP in the buffer. These dilutions were treated as normal samples. Plotting the absorbance of the dilutions against their concentration yields a standard curve. The slope of the linear regression line of this curve is the extinction coefficient. Optical density was calculated by subtracting the corresponding means of the sample and substrate controls from the

\(^{21}\) Extinction coefficient: a property of the particular substrate used to normalize activity across multiple rounds of assays.
mean of each sample\textsuperscript{22}. Dry soil mass is calculated by multiplying the wet weight used for the assay by dry matter content of the soil (oven dried soil mass divided by wet soil mass).

**Statistical analyses**

All statistical analyses were run with Systat 10, except for tissue chemistry data from the JRGCE (SAS version 8) and all \( F_{\text{max}} \) and Bartlett’s tests (Excel 1997). Pot biomass and phosphatase activity were both analyzed with paired t-tests, comparing + P to – P treatments in both species. Since the sample size for each treatment/species combination was low (\( n = 3 \)), I pooled biomass and phosphatase results to increase statistical power. Green and senescent *Avena* phosphorus concentration and N:P ratio in the pots were analyzed with a one-way model I ANOVA, with P treatment as the factor. Phosphatase activity and tissue chemistry data from the JRGCE were analyzed with a split-plot general linear model (GLM), with two levels each for CO\(_2\), heat, water, and nitrate (Zavaleta 2001). Care was taken in all experiments to follow the assumption of random sampling in both analysis of variance and the t-test. Homoscedasticity of each dataset was tested using the \( F_{\text{max}} \) test or Bartlett’s test for homoscedasticity (if close) (Sokal and Rohlf 1995). Normality was estimated visually, using histograms and normal quantile plots. Data were transformed if necessary – all were homoscedastic and apparently normally distributed for statistical analyses. Pot N and P data were square root transformed; pot biomass, pot phosphatase, pot N:P, JRGCE phosphatase, N, P, and N:P data were all logarithmically transformed. Though data were transformed for statistical analysis, data presented in tables and figures are untransformed.

\textsuperscript{22} Three subsamples were taken from each sample preparation and measured.
Results

Pot study

Phosphorus fertilization in the pots increased aboveground biomass of both Avena and Bromus by the time of harvest (Pr^{23} = 0.0036, Figure 4.2).

![Effect of phosphate addition on aboveground biomass in pots](image)

Figure 4.2 Effect of phosphate addition on aboveground biomass of Avena and Bromus in pots, harvested February 2003. N = 3 for each bar. Error bars are ± 1 SE. **: Pr < 0.01.

Both green and senescent Avena phosphorus concentration increased in P-amended plots (Pr < 0.001 for green, Pr = 0.0105 for senescent, Figure 4.3). Addition of phosphorus also decreased the N:P ratio in both green and senescent Avena (Pr < 0.001 for both green and senescent, Figure 4.4).

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^{23} Instead of the conventional “P” to denote probability, “Pr” will be used here to avoid confusion with phosphorus and precipitation.
Figure 4.3: Effect of phosphate addition on Avena [Phosphorus] in pot study. Error bars are ± 1 SE. *: Pr < 0.05; ***: Pr < 0.001.
Phosphorus addition decreased phosphatase activity on November 23rd (Pr = 0.015, Figure 4.5). The trend was the same on November 2nd, but results were not statistically significant (Pr = 0.118, Figure 4.5).
Figure 4.5: Effect of P limitation on phosphatase activity of pooled *Avena* and *Bromus* in pots, cored November 2\(^{nd}\) and 23\(^{rd}\), 2002. \(n = 6\) for each point. Error bars are ± 1 SE of pooled data. ns: Not significant; *: \(P < 0.05\).

**Jasper Ridge Global Change Experiment**

*Green tissue chemistry*

Enriching nitrate deposition decreased tissue phosphorus concentration in green *Avena* (Pr < 0.001, Table 4.1, Figure 4.6). Precipitation, temperature, and CO\(_2\) did not significantly affect P content (Table 4.1). The effects of the factors on green *Avena* P concentration were direct and uncomplicated by interacting with one another (Pr ≥ 0.22 for all interactions, Table 4.1). N deposition increased N:P ratio in green *Avena* (Pr < 0.001, Table 4.1, Figure 4.7). Although the effect of N tended to depend on P, this trend was not significant (N x P interaction: Pr = 0.089, Table 4.1, Fig. 4.8). In contrast, neither CO\(_2\) nor temperature had direct (main effects) or indirect effects (through interactions) on the N: P ratio in green *Avena*. 

![Graph showing effect of phosphate addition on phosphatase activity in pots](image)
Table 4.1: Split plot general linear model table for green *Avena* tissue P concentration and N:P ratio in JRGCE. Factors are C: carbon dioxide; T: temperature; P: precipitation; N: nitrate deposition. All main effects and interactions are shown. F statistics and probabilities are given. †: Pr < 0.10; *: Pr < 0.05; **: Pr < 0.01; ***: Pr < 0.001.

![Green Avena [Phosphorus] in JRGCE](image)

Figure 4.6: Main effects of N deposition, precipitation, temperature, and CO₂ on tissue phosphorus content of green *Avena* harvested in May 2001. Each bar is the mean of all experimental units that included the ambient or enriched factor (38 < n < 52). For example, the bar on the far left includes the eight groups: control, P, T, TP, C, CP, CT, and CTP, whereas the leftmost bar for the enriched groups includes the eight groups: N,
PN, TN, TPN, CN, CPN, CTN, and CTPN. Error bars are ± 1 SE of pooled data. ns: Not significant; ***: Pr < 0.001.

Figure 4.7: Main effects of N deposition, precipitation, temperature, and CO₂ on N:P ratio of green Avena harvested in May 2001. Each bar is the mean of all experimental units that included the ambient or enriched factor (38 < n < 53). For example, the bar on the far left includes the ambient or enriched factor (38 < n < 53). For example, the bar on the far left includes the eight groups: control, P, T, TP, C, CP, CT, and CTP, whereas the leftmost bar for the enriched groups includes the eight groups: N, PN, TN, TPN, CN, CPN, CTN, and CTPN. Error bars are ± 1 SE of pooled data. ns: Not significant; ***: Pr < 0.001.
Figure 4.8: Effects of N deposition and Precipitation ("P" treatment) on N:P ratio of green Avena harvested in May 2001. Each bar is the average of four groups (19 < n < 27). For example, the “control” bar includes treatments: control, T, C, and CT; and the “N” bar includes treatments N, TN, CN, and CTN. Error bars are ± 1 SE of pooled data. ns: Not significant; †: Pr < 0.10; ***: Pr < 0.001.

Senescent tissue chemistry

Increased N deposition decreased the concentration of phosphorus in senescent Avena (Pr < 0.001, Table 4.2, Figure 4.9). Increased precipitation also decreased the concentration of phosphorus in senescent Avena (Pr = 0.029, Table 4.2, Figure 4.9). In contrast, neither CO2 nor temperature directly (main effect) or indirectly (interactions) affected senescent Avena phosphorus concentration (Table 4.2). Elevated N deposition also increased the N:P ratio in senescent Avena (Pr < 0.001, Table 4.2, Figure 4.10). Although the effect of N on the N:P ratio of senescent Avena tended to depend on P, this trend was not statistically significant at the 5% level (N x P interaction: Pr = 0.063, Table 4.2, Figure 4.11). As with P concentration, neither CO2 nor temperature had direct (main
effect) or indirect (through interactions) effects on N:P ratio of senescent *Avena* (Table 4.2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>F value</th>
<th>Pr</th>
<th>F value</th>
<th>Pr</th>
</tr>
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<tbody>
<tr>
<td>C</td>
<td>0.02</td>
<td>0.885</td>
<td>0.00</td>
<td>0.965</td>
</tr>
<tr>
<td>T</td>
<td>1.22</td>
<td>0.278</td>
<td>0.84</td>
<td>0.368</td>
</tr>
<tr>
<td>P</td>
<td>5.47</td>
<td>0.029*</td>
<td>0.01</td>
<td>0.938</td>
</tr>
<tr>
<td>N</td>
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<td>&lt; 0.001***</td>
<td>100.52</td>
<td>&lt; 0.001***</td>
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<tr>
<td>CxT</td>
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<tr>
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<td>0.08</td>
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<tr>
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<td>0.250</td>
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</tr>
<tr>
<td>TxP</td>
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<td>0.528</td>
<td>0.10</td>
<td>0.750</td>
</tr>
<tr>
<td>TxN</td>
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<tr>
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<td>0.348</td>
<td>3.83</td>
<td>0.063†</td>
</tr>
<tr>
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<td>0.00</td>
<td>0.970</td>
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<td>1.20</td>
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</tr>
<tr>
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<tr>
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</tr>
<tr>
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<td>0.315</td>
<td>1.03</td>
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</table>

Table 4.2: Split plot general linear model table for senescent *Avena* tissue P concentration and N:P ratio in JRGCE. Factors are C: carbon dioxide; T: temperature; P: precipitation; N: nitrate deposition. All main effects and interactions are shown. F statistics and probability values are given. †: Pr < 0.10; *: Pr < 0.05; **: Pr < 0.01; ***: Pr < 0.001.
Figure 4.9: Main effects of N deposition, precipitation, temperature, and CO₂ on tissue phosphorus content of green *Avena* harvested in May 2001. Each bar is the mean of all experimental units that included the ambient or enriched factor (52 < n < 58). For example, the bar on the far left includes the eight groups: control, P, T, TP, C, CP, CT, and CTP, whereas the leftmost bar for the enriched groups includes the eight groups: N, PN, TN, TPN, CN, CPN, CTN, and CTPN. Error bars are ± 1 SE of pooled data. ns: Not significant; *: Pr < 0.05; ***: Pr < 0.001.
Figure 4.10: Main effects of N deposition, precipitation, temperature, and CO₂ on N:P ratio of green *Avena* harvested in May 2001. Each bar is the mean of all experimental units that included the ambient or enriched factor (51 < n < 58). For example, the bar on the far left includes the eight groups: control, P, T, TP, C, CP, CT, and CTP, whereas the leftmost bar for the enriched groups includes the eight groups: N, PN, TN, TPN, CN, CPN, CTN, and CTPN. Error bars are ± 1 SE of pooled data. ns: Not significant; ***: Pr < 0.001.
Figure 4.11: Effects of N deposition and Precipitation (“P” treatment) on N:P ratio of green *Avena* harvested in May 2001. Each bar is the average of four groups (25 < n < 30). For example, the “control” bar includes treatments control, T, C, and CT; the “N” bar includes treatments N, TN, CN, and CTN, and so on. Error bars are ± 1 SE of pooled data. ns: Not significant; †: Pr < 0.10; ***: Pr < 0.001.

*Soil phosphatase activity*

Throughout the mid- to late-growing season enriching precipitation decreased phosphatase activity (Pr ≤ 0.012) and nitrate deposition increased phosphatase activity (Table 4.3, Figure 4.1224). The effect of N in March 2002 was marginally significant (Pr = 0.08), but was strong in both May 2002 (Pr = 0.005) and January 2003 (Pr = 0.001). The effect of CO₂, the precipitation x nitrate deposition interaction, and the CO₂ x temperature x precipitation interaction all approach significance in one month only (Table 4.3).

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24 Phosphatase data in Figure 4.12 are presented as means across months because effects of treatments were similar for January 2003, March 2002, and May 2002.
Table 4.3: Split plot general linear model results for soil phosphatase activity in the JRGCE during January 2003, March 2002, and May 2002 (mid growing season, late growing season, and flowering period, respectively). Factors are C: carbon dioxide; T: temperature; P: precipitation; N: nitrate deposition. All main effects and interactions are shown. F statistics and probability values are given. †: Pr < 0.10; *: Pr < 0.05; **: Pr < 0.01; ***: Pr < 0.001.
Figure 4.12: Main effects of CO₂, heat, water, and nitrate on phosphatase activity in the JRGCE. Data from January 2003, March 2002, and May 2002 are pooled, for the results are similar. n = 64 for each point, where each point is a mean of that plot for the three months. Each bar is the average of eight experimental groups. Error bars are ± 1 SE. ns: Not significant; **: Pr < 0.01.
Figure 4.13: Effects of N deposition and Precipitation on phosphatase activity in JRGCE. Only data from March 2002 are shown. Each bar is the average of four groups (n = 32). For example, the “control” bar includes treatments control, T, C, and CT; the “N” bar includes treatments N, TN, CN, and CTN, and so on. Error bars are ± 1 SE of pooled data. ns: Not significant; †: Pr < 0.10; *: Pr < 0.05.

Discussion

Pot study

Biomass

The intent of the pot study was to test methods of phosphorus analysis on a system known to be P-limited. As expected, addition of P increased the biomass of both study species (Figure 4.2), indicating the potential for P to limit growth of these species in Jasper Ridge soil when N limitation is removed. This result is even more convincing than is apparent from these data, for the physical disruption of the soil structure from transporting the soil from the field site to the pots most likely released bound P into more readily-soluble P, decreasing the potential for P limitation.
Green tissue chemistry

The increase in *Avena* tissue phosphorus concentration in P-amended pots (Figure 4.3) could result from either increased P use or luxury consumption; both indicate P limitation (Aerts and Chapin 2000). This confirms the validity of using green P concentration to denote P limitation in *Avena*. Although the N:P ratio decreased in the pots (Figure 4.4), this may not be meaningful. Relative P limitation will not be less than relative N limitation, for N is not limiting. To use the tissue N:P ratio to address relative limitation, the following discussion relies on a literature survey (Aerts and Chapin 2000).

Senescent tissue chemistry

Senescent tissue chemistry will be used as a predictor of future P limitation, not an indicator of current P limitation like green tissue chemistry and phosphatase activity. The P concentration in senescent tissue decreased with P limitation, implying a smaller return of P to the soil with P limitation (Figure 4.3). Although the N:P ratio also increased with P limitation (Figure 4.4), N fertilization confounds interpretation of this as it does for green tissue, for this system is no longer co-limited by N and P.

Soil phosphatase activity

Soil phosphatase activity increased in response to P limitation, but this change was not evident until the middle of the growing season (Figure 4.5). This result confirms the validity of using phosphatase activity to denote P limitation in the mid to late growing season. A possible explanation for the seasonal trend is that the plants were still growing on seed reserves, and thus had not yet fully developed the P-limitation response. Accordingly, JRGCE phosphatase measurements were taken in the mid to late growing season. Another possible explanation for the late onset of the phosphatase response is
that the release in P due to soil disruption of the pots reduced the need to produce phosphatases until later in the season. Soil disruption is not as much of an issue in the JRGCE, so phosphatase measurements should be more sensitive in the JRGCE than in the pots.

**Jasper Ridge Global Change Experiment**

For the discussion of the JRGCE results, please refer to Table 4.4 for a summary or back to the figures and tables in the Results section.

<table>
<thead>
<tr>
<th>Detectable treatment effects in the JRGCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>P limitation</td>
</tr>
<tr>
<td>CO₂</td>
</tr>
<tr>
<td>Temp</td>
</tr>
<tr>
<td>Precip</td>
</tr>
<tr>
<td>N x Precip</td>
</tr>
</tbody>
</table>

Table 4.4: Summary of detectable treatment effects in the Jasper Ridge Global Change Experiment. Columns 2 through 6 are the different measures of P cycling tested in this study. The first row is the direction that would indicate increased P limitation based on data from the pot study and the literature. All main effects are listed, as well as the interaction that had a nearly significant effect. “-” indicates the effect was not detected (Pr > 0.10). Pr is the probability value. Probabilities for phosphatase activity were from data pooled from all three time periods except the Nitrate deposition x Precipitation interaction (‡), for which the probability of March 2002 (the only significant time period) is given. “X” indicates an antagonistic interaction. The last row denotes the specific aspect of P cycling targeted by each technique.

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There is, of course, natural soil disruption due to soil animal activity, but the magnitude is much less than the artificial disruption imposed by removing and transporting the soil.
Nitrate deposition

Adding 7 g * m⁻² * yr⁻¹ of nitrogen decreased the amount of phosphorus in green and senescent *Avena* from 2001, increased the mean N:P ratio in green and senescent *Avena* from 2001, and increased soil phosphatase activity throughout 2002-2003 (Table 4.4). The null hypothesis of no effect of N deposition on P cycling in this system can be rejected. The proposed alternative hypothesis is that P limitation increases due to a lessening of N limitation. By removing N limitation, the soil resource limitation has been bumped over to phosphorus.

Is there another hypothesis that could also explain these data? It is possible that the increase in tissue N:P could be due to a strongly N-limited species responding to increased N availability with luxury consumption, acquiring it when it’s “cheap” and storing it for later use. This would both increase N:P and decrease P concentration by diluting everything else relative to N. However, this does not explain the increase in phosphatase activity. Though enzymes require large proportions of N, luxury consumption would result in N storage, not use. There is no reason to produce proportionately more phosphatases unless P is limiting. Conversely, one could explain the increase in phosphatase activity as a consequence of increased enzyme production capacity: more N, more enzymes. However, a proportional increase in phosphatase activity without P limitation should yield a proportional increase in P availability, and thus increased P uptake. My results showed a decrease in tissue P concentration beyond
the proportional decrease expected from the increase in N\textsuperscript{26}, so this alternative hypothesis is not supported.

These data strongly suggest that atmospheric N deposition increases P stress at both the species and ecosystem levels by lessening N limitation. Phosphorus content in one of the dominant grasses declines while N increases (Figure 4.14), P cycling becomes tighter as the input from plant mortality decreases, and the ecosystem as a whole is spending more resources to obtain P. What are the implications of this result? In the short-term, growth has increased in response to N fertilization (Shaw et al. 2002). However, the long-term response may differ. Long-term exposure to increased atmospheric N deposition has been shown to cause a shift from complete N limitation to complete P limitation (Aerts and Chapin 2000). The Jasper Ridge system is currently co-limited by N and P, and thus a shift to P limitation could occur relatively soon if N deposition continues to increase. This effect is even more likely in similarly co-limited areas with higher N deposition (e.g. parts of Europe and the Northeastern U.S., Aerts and Chapin 2002).

The ramifications of this shift in nutrient limitation from N to P are numerous. Without adequate P, plants cannot produce enough RNA or NADPH, which are required for protein production and photosynthesis, respectively. The growth response to N fertilization would, therefore, saturate, and N uptake would not increase commensurate to N deposition. Effects of increasing inputs of N to soil systems have already been discussed at length, and include increased soil acidity (increasing soil P fixation capacity,

\textsuperscript{26} With added nitrogen, [N] changed from 5.05 to 6.35 mg/g. Taking this 1.3 mg increase into account, the expected value for [P] in the N-amended plots would be \([P_{\text{ambient}}] \times (1000/1001.3)\), or 1.13/1001.3 = 1.126. The observed [P] in the N-amended plots was 0.677.
acting as a feedback to P limitation), increased N leaching (possibly leading to eutrophication), increased N$_2$O release back into the atmosphere (increasing greenhouse warming), increased N volatilization (shifting the effects further downwind), and shifts in community composition to species that respond more rapidly to N fertilization (see Chapter 2).

*Precipitation*

Increasing precipitation by 150% significantly decreased soil phosphatase activity throughout the middle to late growing season, indicating decreased P demand. This indicates first of all that P was limiting in the ambient precipitation treatment, providing further evidence for co-limitation between N and P. Why would increased precipitation alleviate P limitation? Two mechanisms seem likely: 1) Increased water availability increases decomposition and ion diffusion rates, which release more nutrients into the soil and increase ion transport to roots, thus reducing belowground resource limitation (N, P, and water limitation). 2) Increased water flux through the ecosystem increases nutrient loss through leaching. Since nitrate and ammonium are more mobile than phosphate, losses of N should be greater than losses of P, decreasing relative P limitation.

Both of these hypotheses would account for the decrease in phosphatase activity with added precipitation. However, increased decomposition should also lessen N limitation, which was not the case for green *Avena* in the JRGCE (Figure 4.14). N content decreased significantly with increased precipitation (Pr = 0.039). Hence, these data support the second hypothesis: precipitation increases loss of N from the soil more than P, decreasing P limitation relative to N, though not necessarily increasing the amount of P taken up by plants. The changes in N and P content in senescent *Avena*
further support this hypothesis (Table 4.4, Figure 4.14): litter inputs from 2001 decreased with increased precipitation (Pr = 0.012 for N). Thus, the amount of both N and P in organic matter decreased, most likely increasing soil nutrient limitation.

Figure 4.14: Effect of precipitation on green and senescent Avena N concentration in the JRGCE. Each bar represents means of eight treatments (38 < n < 58). Error bars are ± 1 SE of pooled data. *: Pr < 0.05.

Nitrate deposition x Precipitation interaction

The above discussion assumes that the main effects are additive. This is not always the case. The point of studying all combinations of these four factors was to see if the effects of any factor varied in relation to the other factors. The interaction between precipitation and N deposition was marginally significant for green Avena N:P (Pr = 0.089), senescent Avena N:P (Pr = 0.063), and March phosphatase activity (Pr = 0.068). Treatments with both enriched N deposition and precipitation had lower N:P ratios than would be expected from the sum of the single-factor effects, i.e. the interaction was
antagonistic. Thus, P limitation may not increase commensurate to the single-factor N deposition prediction when precipitation concurrently increases.

In the real world, N deposition is much less variable on a monthly and annual time scale than is precipitation, and thus the strength of the interaction in natural systems would probably vary temporally. Temporal variation in the magnitudes of these effects suggests that both main effects and interactions can be important, as each will take effect at some point.

A possible explanation for this interaction is that increased precipitation increases nitrate leaching through the soil much more than phosphate leaching. If this is the case, although relative P limitation decreases with added precipitation, N limitation increases due to increased N losses. Another possible explanation is that increased precipitation speeds the release of P via phosphatases more than the release of N via decomposition. If this were the case, though, precipitation should have directly affected tissue phosphorus uptake (i.e., the main effect should have been significant), which it did not. Thus, loss of N through leaching is the more likely explanation. This increases the potential for eutrophication, as more N makes its way into the water system.

Temperature

Increasing temperature by ~1°C had no effect on any of the aspects of P cycling studied here, suggesting that in the short-term at least, P cycling is not affected by warming.

CO₂

Elevating CO₂ to ~680 ppm did not affect green or senescent tissue chemistry or soil phosphatase activity. Previous results from the JRGCE have shown that enriching
CO₂ suppresses the positive effects of heat, precipitation, and nitrogen on net primary productivity (Shaw et al. 2002). When applied alone, CO₂ increased NPP, agreeing with the results of numerous elevated CO₂ studies. However, when applied in combination with other treatments, it decreased NPP. One proposed explanation was that a soil nutrient (e.g. phosphorus) limited NPP under these combined factors (Shaw et al. 2002). Data presented here suggest that phosphorus limitation does not increase under elevated CO₂ in the JRGCE. Thus, another mechanism (or set of mechanisms) must account for the depressive effect of CO₂ on NPP.

**Summary**

Nitrate deposition and precipitation, alone and in combination, altered the P cycle in the JRGCE. Nitrate deposition alone increases relative P limitation by increasing N availability, decreasing relative N limitation. Precipitation alone decreases relative P limitation, probably by increasing N loss more than P loss, increasing relative N limitation. The combined effect is to increase N output from the system even more: both increased N accumulation and increased leaching per unit N will increase flux of nitrate through the system. Which factor (or combination of factors) has a larger effect on P cycling depends partially on the magnitude of the effects and partially on the temporal nature of the factors in reality. The effect of N deposition was larger than that of precipitation, and despite the interaction, the combined effect was to increase N content (and decrease P limitation), suggesting an eventual switch to P limitation. The seasonality of precipitation patterns indicates that both main effects and interactive effects will appear in the real world.
Neither warming nor CO₂ enrichment affected P cycling, according to these data. These are the more commonly-studied global change factors, but these data imply that studying CO₂ and warming alone will omit some potentially important consequences of anthropogenic activity. The next chapter scales down in complexity, omitting many important factors. However, it will scale up in spatial coverage, examining the global patterns of P limitation and the effect of P limitation patterns on global net primary production using a simulation model.
CHAPTER 5

PHOSPHORUS IN THE WORLD: THE CASA BIOSPHERE MODEL

Introduction

Fossil fuel combustion releases ~5.6 Pg (1 Pg = 10\textsuperscript{15} grams) C \text{* yr\textsuperscript{-1}} into the atmosphere annually, but the net annual increase in atmospheric CO\textsubscript{2} is ~3.5 Pg C \text{* yr\textsuperscript{-1}}. Where does the rest of the carbon go? This problem of the “missing sink” for carbon is intriguing to both ecosystem ecologists and policy makers. If we understand where and why carbon is taken up, and we can encourage that pathway, we may be able to increase CO\textsubscript{2} uptake from the atmosphere, thereby decreasing the amount of global warming and its associated problems. Provided there are no side effects of our “encouragement,” this is an attractive option. Alternatively, if the pathway is unsustainable, we cannot count on continued carbon storage at present levels, and the need to reduce emissions becomes even more urgent.

The exact location and mechanisms of the missing sink are a contentious issue. Such a large-scale problem requires modeling estimates, for there is no practical way to measure the carbon flux through all pathways across the globe. There are many different modeling approaches, most of which make different assumptions, use different inputs, and come to slightly different conclusions. However, there is now a general consensus that the majority of the carbon sink is terrestrial, and that it is in North America and Europe (Schimel et al. 2000). Major pathways are likely reforestation of previously logged and/or agricultural areas (Schimel et al. 2000, Friedlingstein et al. 1995), increased growth due to N deposition (Friedlingstein et al. 1995), and increased growth due to CO\textsubscript{2} fertilization (Friedlingstein et al. 1995, Schimel et al. 2000).
Why are South America, Africa, Asia, and Oceania not major carbon sinks as well? A few answers have been proposed. First, reforestation is largely a Northern Hemisphere phenomenon, because until recently the most widespread logging was in North America and Europe, so these are the areas that have more regrowth potential (Schimel et al. 2000). Second, the tropics now account for the majority of the world’s deforestation, which releases CO₂ into the atmosphere. The gross release from deforestation could balance (or exceed) the gross uptake via fertilization. Third, there is more N deposition in the Northern temperate zones than anywhere else, providing more fertilization. However, CO₂ and N fertilization should affect more than just the Northern Hemisphere. Atmospheric CO₂ is more or less equivalent across the globe at any given time (Vitousek 1994), and thus should affect all ecosystems. N deposition is not as homogenous, but it still affects much of the globe. NOₓ emissions from fossil fuel combustion most heavily influence Europe and Eastern North America, but agricultural N fertilization – and the resulting volatilization of ammonia and NOₓ – is important in both temperate and tropical zones (Vitousek 1994). The balance of these mechanisms is not precisely known, and it is quite possible that there may be other mechanisms prohibiting substantial carbon storage in the tropics.

In particular, phosphorus limitation may constrain the fertilization effects of CO₂ and N in many tropical areas, preventing an increase in growth similar to that seen in the Northern temperate zones. As discussed in Chapter 3, the ultimate source of phosphorus is rock and mineral weathering, and atmospheric inputs are limited to dust transport. Aeolian dust inputs, though important in highly weathered areas, are typically well below P requirements for optimal growth (Chadwick et al. 1999). Tropical soils are typically
highly weathered, for they receive high inputs of precipitation (Brady and Weil 2002). Although P is applied as fertilizer in the tropics with N, it does not volatilize, preventing fertilization by P proportional to that of N in non-agricultural areas. This logic suggests that P constraint may be an important factor in explaining the spatial patterns of net carbon storage. This chapter will explore the role of P limitation on a global scale.

What are the spatial patterns of P constraint to plant production? The idea presented above is a plausible qualitative answer. To give a quantitative answer, though, is more complicated, for there are many variables that determine phosphorus constraint. Soils differ in the amount of P they contain, the rate at which they lose P, and the proportions of P in the soil that are available to plants. Dust inputs vary across space and time with climatic patterns. Plant communities vary in the amount of P required per unit biomass, their growth rates, and their P acquisition and retention efficiencies. A full, global-scale investigation of these parameters would require a substantial amount of time and resources. However, given some explicit assumptions, it is possible to explore the potential for P limitation to plant production across the globe. There are three steps involved in this process: determining P availability, plant production without P stress, and plant production with P stress.

First, what is the spatial variability in biologically available P? There is no global map of P availability, but it can be estimated from soil type (Friedlingstein et al. 1995). The global coverage of soil type is well documented (Nachtergaele 2000) and provides a decent mapping to P availability (Friedlingstein et al. 1995). Increased

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27 Biologically available P is defined here as soluble P, which makes the assumption that fluxes between organic P and soluble P are in equilibrium. Even if P limitation increases, an increase in phosphatase production represents a substantial resource investment, indicating a constraint to NPP.
weathering decreases the amount of P in a soil and changes soil type (Brady and Weil 2002). In general, highly weathered soils have very high levels of immobile aluminum and iron oxides and low levels of mobile, exchangeable ions. In these soils most phosphorus has either become irreversibly bound to the immobile clay minerals or has leached out.

Using soil type to denote P availability requires assumptions of homogeneity. This study assumes equal and negligible P input to non-agricultural systems (through dust or human activity), and similarity across all soils of a particular type. On a small spatial scale these assumptions are obviously untrue, but integrated over large regions they are assumed to average out.

The second step is to determine the spatial variability in plant production without P limitation. The Carnegie-Ames-Stanford Approach, or CASA biosphere model, is used here. CASA is a simulation model that estimates global net primary production (NPP) at one degree spatial resolution using satellite remote sensing, meteorological datasets, and soil textural properties (Potter et al. 1993). CASA does not explicitly take nutritional constraints into account, for it was built to estimate current NPP and carbon pools. It was assumed that the remote sensing inputs would capture the effects of nutrient limitation, for canopy cover could not exceed that allowed by nutrients (Potter et al. 1993). To explore future NPP potential, though, requires knowledge of nutritional constraints.

Third, what is the effect of P availability on NPP? Using the soil map, CASA was modified to incorporate phosphorus stress. Consistent with the multiple resource limitation hypothesis, this study assumes that P limitation does not shut down growth
entirely. The maximum effect of P stress is set to be a 20% reduction in NPP. Since the spatial patterns of P limitation are the target of this study, rather than absolute NPP, this rather arbitrary number will not hinder the interpretation.

Plants vary substantially in their P nutrition parameters. With one exception, this study assumes that all plant types are equal. Though this is obviously untrue, using soil type may capture some of the variation. Soil type partially determines plant community, plant community partially determines soil type, and the environmental controls on both are similar. The one exception mentioned above is that this study assumes all agricultural land is fertilized with P, alleviating any P stress that would otherwise impede production.

This simple approach omits many important processes and variables, but the extent of P limitation is worthy of exploration. This rather crude approach intends to highlight broad patterns, laying the groundwork for future studies. As discussed in previous chapters, interactions between various dynamic factors should not be ignored. Although this study does not explicitly take other changing factors into account, potential implications of these results will be discussed in the context of other factors.

**Methods**

**Soil map**

Soil data were obtained from the website of the USDA’s National Resources Conservation Service (NRCS 2003). The data are global in coverage, arranged as an array in two-minute resolution (58,320,000 points). The input data for this map come

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28 NPP is defined as gross photosynthesis (carbon uptake) minus plant respiration (Field et al. 1995). Conceptually, it is the amount of carbon taken out of the atmosphere that may be used for growth, herbivory, or plant storage.

29 USDA: United States Department of Agriculture
from FAO-UNESCO’s\textsuperscript{30} Soil Map of the World combined with a soil climate map from the NRCS’s Soil Survey Division (NRCS 2003). The FAO-UNESCO Soil Map of the World was originally based on field survey measurements taken from 1961-1981, and has been updated and improved each year after 1981 as better measurements are taken and amassed (Nachtergaele 2000). Due to technological differences between countries, the accuracy of the map differs across space (Nachtergaele 2000). And, as with any global scale project, some detail is lost in extrapolating from field measurements to entire grid cells. In general, though, the map agrees quite well with independent, smaller-scale soil maps, and is considered the best available global soil map (Nachtergaele 2000).

The NRCS map is laid out as an array of soil types, according to the USDA’s soil taxonomy system (NRCS 2003). Since there is no direct correlation between the FAO data and soil suborder, the NRCS assigned each combination of soil climate and FAO classification a USDA soil type based on the best available information (NRCS 2003). Classification is to soil suborder, with additional values for rock, ice, and shifting sand.

*Phosphorus availability by soil type*

There were not sufficient data in the literature to determine relative P availability values to the resolution of soil suborder, so soil orders were used\textsuperscript{31}. Relative P availability values for six of the twelve soil orders are listed in Table 5.1. Brady and Weil (2002) compiled these data from multiple sources, all of which used similar techniques. Each value is representative of its order, though there is variability within soil orders.

\textsuperscript{30} FAO: Food and Agricultural Organization; UNESCO: United Nations Educational, Scientific, and Cultural Organization

\textsuperscript{31} For reference, soil order is taxonomically analogous to “phylum” in biology, whereas suborder is analogous to “class.”
Table 5.1: Phosphorus availability for six soil orders, based on data put together by Brady and Weil (2002). The data are concentrations of phosphate in solution.

<table>
<thead>
<tr>
<th>Soil Order</th>
<th>P availability (mg P/L soil solution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxisol</td>
<td>0.001</td>
</tr>
<tr>
<td>Andisol</td>
<td>0.001</td>
</tr>
<tr>
<td>Ultisol</td>
<td>0.003</td>
</tr>
<tr>
<td>Mollisol</td>
<td>0.010</td>
</tr>
<tr>
<td>Vertisol</td>
<td>0.100</td>
</tr>
<tr>
<td>Histosol</td>
<td>1.000</td>
</tr>
<tr>
<td>Gelisol</td>
<td>.</td>
</tr>
<tr>
<td>Entisol</td>
<td>.</td>
</tr>
<tr>
<td>Inceptisol</td>
<td>.</td>
</tr>
<tr>
<td>Aridisol</td>
<td>.</td>
</tr>
<tr>
<td>Alfisol</td>
<td>.</td>
</tr>
<tr>
<td>Spodosol</td>
<td>.</td>
</tr>
</tbody>
</table>

Why do these orders exhibit these patterns? Two main controls on P availability in soils are 1) the degree of weathering and 2) the phosphorus fixation capacity. Oxisols and Ultisols are the most and next-most weathered soils, respectively, and are characterized by substantial aluminum and iron oxide composition. Thus, there has been ample time for P to leach out, and the phosphorus fixation capacity is high, keeping the remaining mineral P occluded. Though andisols (volcanic ejecta) are only slightly weathered, they have an extremely high P fixation capacity due to their high content of amorphous silicates and oxides (Brady and Weil 2002). Amorphous silicates and oxides have a very high surface area to volume ratio, and fix P very rapidly. Mollisols are less weathered than Oxisols and Ultisols, so they have lost and occluded less P than the more weathered soils. Vertisols are less weathered than Mollisols. Histosols are organic-dominated soils, which have very low P fixation capacity.

What about the orders not listed above? They may not have been listed because data are not available, or because there is too much variability within an order to give a “representative” value. Briefly, here is an outline of the soil types and their potential for
P availability based on their degree of weathering and fixation capacity. Gelisols are soils that are characterized by year-round permafrost. These soils are not heavily weathered, and are unlikely to be low in P availability. Entisols are the least developed soils, meaning either very recent deposition/formation or extremely low weathering rates. Either way, they are unlikely to be low in available P. Inceptisols are the next most weathered forms, also unlikely to be low in available P. Aridisols are extremely dry soils. Similarly, they have not been subjected to much weathering, and are unlikely to be P-depleted. Alfisols are moderately weathered soils typically found in cool to hot humid areas, and thus are likely to be somewhat depleted. Spodosols are more weathered than alfisols, and are typical of coniferous forests. Being both substantially weathered and highly acidic, spodosols likely have relatively low P availability. P availability input values chosen here for alfisols and spodosols vary between ultisols and vertisols (which bracket alfisols and spodosols in terms of weathering). Alfisols are less weathered than spodosols, so they are never more P-depleted than spodosols in this study. Table 5.2 lists the input values used in this study. From high to low P availability, they are numbered 1 through 5.
Table 5.2: Distribution of P availability values used as inputs in this study. Orders oxisol through histosol are based on data put together by Brady and Weil (2002). Remaining data are values chosen for this study based on relative weathering rates and P fixation capacity of the soil orders.

Phosphorus limitation by soil type

To convert phosphorus availability to phosphorus stress, P requirements must be combined with P availability. To account for the variability within species, a range of values was used in this study. Requirements for optimal growth of various crop species range from 0.005 mg P/L to 0.3 mg P/L (Brady and Weil 2002). However, crop species can have higher nutrient demands than wild species, for they have been artificially selected for fast growth rates (Chapin 1980). Brady and Weil (2002) list 0.2 mg P/L as the concentration at which most plants can grow to 95% yield, hence it is chosen as the upper bound here. This study uses 0.005, 0.01, 0.1, and 0.2 mg P/L as the P demand values. All combinations of P demand and P availability were run; there were twenty scenarios, listed 1-1 through 4-5. 1-1 has the lowest demand and highest availability; it is the most conservative estimate. 4-5 has the highest demand and lowest availability; it is the most liberal estimate. In the results and discussion sections, only scenarios 1-1, 2-4,
3-3, and 4-5 are presented. Scenarios 1-1 and 4-5 are presented to give the full range of plausible possibilities and denote the level of uncertainty; scenarios 2-4 and 3-3 are more plausible, central tendency estimates.

A linear response to P availability was assumed. The equation used to calculate P stress is:

$$P(x) = 1 - \left( S_{\text{max}} \times (D - \text{MIN}(P_i, D)) / (D - P_{\text{min}}) \right)$$  \hspace{1cm} (1)

where P(x) is the phosphorus stress value for a spatial location “x”, S_{\text{max}} is the maximum stress that phosphorus can impose (set to be 20%, or 0.2), D is the demand cutoff (ranges from 0.005 to 0.2 by scenario, as described above), P_i is the phosphorus availability for that soil order in mg P/L, and P_{\text{min}} is the minimum phosphorus availability across all soil orders (always 0.001 mg P/L). P(x) values range from 0.8 to 1.0, meaning the maximum change due to phosphorus limitation will be 80% of what NPP would be without P limitation. Table 5.3 shows P stress input values for the four scenarios in order of most to least conservative.

<table>
<thead>
<tr>
<th>Soil Order</th>
<th>Scenario 1-1</th>
<th>2-4</th>
<th>3-3</th>
<th>4-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxisol</td>
<td>0.800</td>
<td>0.800</td>
<td>0.800</td>
<td>0.800</td>
</tr>
<tr>
<td>Andisol</td>
<td>0.800</td>
<td>0.800</td>
<td>0.800</td>
<td>0.800</td>
</tr>
<tr>
<td>Ultisol</td>
<td>0.900</td>
<td>0.844</td>
<td>0.804</td>
<td>0.802</td>
</tr>
<tr>
<td>Mollisol</td>
<td>1.000</td>
<td>1.000</td>
<td>0.818</td>
<td>0.809</td>
</tr>
<tr>
<td>Vertisol</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.900</td>
</tr>
<tr>
<td>Histosol</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Gelisol</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Entisol</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Inceptisol</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Aridisol</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Alfisol</td>
<td>1.000</td>
<td>0.844</td>
<td>0.818</td>
<td>0.802</td>
</tr>
<tr>
<td>Spodosol</td>
<td>1.000</td>
<td>0.844</td>
<td>0.818</td>
<td>0.802</td>
</tr>
</tbody>
</table>

Table 5.3: Phosphorus limitation input values for four scenarios. 0.8 is the most limiting scenario in all cases, but the relative spacing and number of soil orders differ across the scenarios. See text for calculations and explanations. Note that in all scenarios oxisols, andisols, and ultisols are limiting, 2-4 includes limitation by spodosols, 3-3 includes limitation by mollisols and alfisols as well, and 4-5 also includes limitation by vertisols.
Converting to global phosphorus limitation

Using the values described in the previous section, the NRCS global soil map was converted to a phosphorus limitation file (see Appendix 2 for code). Since CASA runs on one-degree resolution, the soil map had to be condensed from the original two-minute resolution. Each one-degree point is an arithmetic mean of the 900 or fewer points land that comprise it, retaining the spatial precision of the soil map. If there are oceanic points in the 900 points that comprise a cell, they are not included in the average. Points that the soil map lists as ice, rock, or sand receive P limitation values of 1 (not P limited). All points that are cultivated\(^{32}\) are also given P limitation values of 1, regardless of soil type.

The CASA Biosphere Model

Inputs into the CASA model include remote sensing data (NDVI\(^{33}\)), meteorological data (temperature and precipitation), and soil characteristics (C, N, and texture). The model runs on one-degree spatial resolution, and thus there are approximately 14,700 land cells (64,800 cells total). For a full description of the model see Potter et al. (1993)\(^ {34}\). CASA estimates NPP with the basic equation

\[
NPP(x,t) = \text{APAR}(x,t) \times \varepsilon(x,t)
\]

where APAR is absorbed photosynthetically active radiation and \(\varepsilon\) is light use efficiency. The “\(x\)” and “\(t\)” subscripts denote space and time, in one-degree spatial and monthly temporal resolution, respectively. APAR is calculated from NDVI. The light use efficiency parameter, \(\varepsilon\), is calculated as

\(^{32}\) According to a global vegetation type map used in CASA

\(^{33}\) NDVI, or normalized difference vegetation index, is calculated as \((\text{NIR} - \text{VIS}) / (\text{NIR} + \text{VIS})\), where NIR is near infrared light and VIS is visible light. Because photosynthetic pigments absorb visible but not infrared light, this is a remote index of canopy coverage (Chapin et al. 2002).

\(^{34}\) For full model code see http://globalecology.stanford.edu/DGE/George/george.html.
\[ \varepsilon(x,t) = T_1(x,t) * T_2(x,t) * W_\varepsilon(x,t) * \varepsilon^* \]  

(3)

\( \varepsilon^* \) is the maximum light use efficiency, calibrated by empirical NPP estimations and assumed to be the same for all plants. It is assumed that variation in community types will show up in remote sensing inputs, hence \( \varepsilon^* \) is a scalar. \( T_1, T_2, \) and \( W_\varepsilon \) are temperature and water stress terms.

**Putting phosphorus into CASA**

To incorporate P limitation into CASA, equation (3) was modified to be

\[ \varepsilon(x,t) = T_1(x,t) * T_2(x,t) * W_\varepsilon(x,t) * P(x) * \varepsilon^* \]  

(4)

where \( P(x) \) is the phosphorus stress term from equation (1) and Table 5.3. \( P(x) \) does not vary temporally because input values are fixed for each spatial cell. P availability will vary seasonally in reality with decomposition rate and nutrient pulses, but this equation makes a steady state assumption by necessity. The maximum light use efficiency term, \( \varepsilon^* \), is not recalibrated here, so absolute numbers in output data are less meaningful.

CASA outputs NPP data as \( g \ C \ * \ yr^{-1} * \ m^{-2} \). To remove the area component and sum across latitudinal bands, programs were written (see Appendix 3 for example code). All visualization was done using Research Systems’ ENVI 3.6 software.

**Results**

**Phosphorus limitation**

Figures 5.1 through 5.4 show the global phosphorus limitation for scenarios 1-1, 2-4, 3-3, and 4-5, respectively. The equatorial regions of South America and Africa are heavily phosphorus limited in all scenarios. Scenario 2-4, which includes spodosols as P-limited (as well as oxisols, andisols, and ultisols), shows increased limitation in Scandinavia, Eastern Canada, and Southeast Asia as well as the equatorial regions of
Africa and South America. Scenario 3-3, which includes alfisols and mollisols, shows heavier limitation in Northern Canada and Eurasia. Nearly all equatorial regions are extremely heavily limited. Scenario 4-5, the most limited scenario, including heavy limitation is oxisols, andisols, ultisols, mollisols, alfisols, and spodosols, and some limitation in vertisols, shows similar spatial patterns to scenario 3-3; each patch of P limitation is slightly larger in 4-4.

Figure 5.1: Global phosphorus stress for scenario 1-1. Color scale is from white to light red to dark red to black, in order of increasing phosphorus stress. Oceanic points are not included in this analysis.
Figure 5.2: Global phosphorus stress for scenario 2-4. Color scale is from white to light red to dark red to black, in order of increasing phosphorus stress. Oceanic points are not included in this analysis.

Figure 5.3: Global phosphorus stress for scenario 3-3. Color scale is from white to light red to dark red to black, in order of increasing phosphorus stress. Oceanic points are not included in this analysis.
Phosphorus stress varies with latitude (Figure 5.5). All scenarios show strong limitation in the equatorial regions, and scenarios 2-4, 3-3, and 4-5 show increasing limitation in the range of 60° N. Under no scenario did phosphorus stress exceed 21% of its maximum value across an entire latitudinal band.
Without P stress, NPP estimations from the CASA model are the same as can be found in Potter et al. (1993). Generally, NPP is high in the tropics. Equatorial South America, equatorial Africa, and the Indonesia/Papua New Guinea region have the highest NPP (Figure 5.6). Although NPP per unit area is not as high, there is also a spike in total NPP from 60° N to 40° N due to the large total land area in that strip (Figure 5.7). CASA estimates global annual NPP to be 50.51 Pg C * yr⁻¹. The Northern Hemisphere accounts for 30.22 Pg C * yr⁻¹, and the Southern Hemisphere 20.29 Pg C * yr⁻¹. 25.38 Pg C * yr⁻¹ are sequesterated in the tropics (between 20° N and 20° S).
Figure 5.6: Global terrestrial NPP patterns as estimated by CASA, integrated over a year. Color scale is a rainbow pattern, with purple low and red high. Black on land indicates no NPP.

Figure 5.7: Global terrestrial NPP by latitude, as estimated by CASA. Positive latitudes are North, negative are South. Vertical axis units are $10^{15}$ g C * yr$^{-1}$. This figure includes no phosphorus stress.
With P stress

The overall pattern of NPP with P stress is similar to the pattern of NPP without P stress. The maximum possible reduction for any given cell was chosen to be 20%, so this is not surprising. The tropics account for the majority of Earth’s terrestrial NPP, and the Northern Hemisphere far eclipses the Southern Hemisphere (Figure 5.8). In the tropics all phosphorus limitation scenarios depict a strong reduction in NPP (Figure 5.9), whereas in the Northern Hemisphere spike only the more liberal scenarios deviate strongly from the non-P-stressed estimate (Figure 5.10). The largest deviations are in the tropics (the most liberal P reduction in the Northern Hemisphere is only half of the most conservative reduction in the tropics), and all scenarios show this deviation (Figure 5.11).

Figure 5.8: Global terrestrial NPP by latitude, as estimated by CASA. Positive latitudes are North, negative are South. Vertical axis units are $10^{15}$ g C * yr$^{-1}$. The black (top) line has no phosphorus stress. The blue, green, orange, and red lines have increasing P stress.
Figure 5.9: Global terrestrial NPP by latitude, as estimated by CASA. Positive latitudes are North, negative are South. Vertical axis units are $10^{15}$ g C * yr$^{-1}$. The black (top) line is without phosphorus stress. The blue, green, orange, and red lines have increasing P stress, as described in the Methods section. Only 20° N to 20° S are shown. Note that all P-stressed lines dip well below the non-P-stressed line, and are relatively similar.
Figure 5.10: Global terrestrial NPP by latitude, as estimated by CASA. Positive latitudes are North, negative are South. Vertical axis units are $10^{15}$ g C yr$^{-1}$. Note that the NPP range is different from Figures 5.7, 5.8 and 5.9. The black (top) line is without phosphorus stress. The blue, green, orange, and red lines have increasing P stress, as described in the Methods section. Only 60° N to 40° N are shown. Note that the P-stressed lines differ in the degree of dip below the non-P-stressed line.
Figure 5.11: Differences between different P-limited scenarios and non-P-limited scenarios. Vertical axis values are the difference between NPP in a P-limited scenario and a non-P-limited scenario, expressed as a proportion of the maximum difference between NPP in each scenario and the non-P-limited scenario.

**Discussion**

Before discussing the implications of these results, the assumptions and limitations of this study must be emphasized. CASA calibrates its NPP estimates with empirical data. The modified model used here was not recalibrated, so absolute NPP values estimated by this study cannot be interpreted meaningfully. The spatial patterns of P stress, though, were the target, and can be interpreted. Another potentially important omission is mobilization of P through erosion. Humans exert a strong influence on many of Earth’s ecosystems; land use change and the resulting soil erosion are important in many areas (Vitousek et al. 1997). Erosion mobilizes P from its occluded mineral form, which could alleviate the stresses presented here. Ideally, using soil classification as the basis for P input should account for this, for newly eroded and deposited soils (entsols).
are set to be non-P-limited. However, it is not likely that worldwide soil testing keeps up with the rate of erosion, so the input soil map probably does not include all newly disturbed soils. Another potentially important assumption is temporal homogeneity in P availability. Seasonal fluctuations change the availability of nutrients in many areas as nutrients build up during dry periods and get flushed through soils with the onset of rains. This could bias estimates of P constraint, but temporally sensitive data were not available.

Keeping the above limitations in mind, the results of this study are quite interesting. Although there are differences between the scenarios, some general patterns are quite consistent. All estimates predict a strong phosphorus constraint in the tropics (20° N – 20° S), and virtually no phosphorus constraint from 70° – 90° N, 20° – 40° N, and 20° – 90° S. The central and liberal estimates (shown here as scenarios 2-4, 3-3, and 4-5) also predict substantial phosphorus constraint from 40° – 60° N.

This model suggests that phosphorus limitation may be an important factor preventing an increase in carbon storage in the tropics similar to that estimated in North America and Europe. P stress affects areas of the Northern latitudes, but it affects Asia, Scandinavia, and Canada much more strongly than the United States and non-Scandinavian Europe, which are estimated to be the largest contributors to the carbon sink (Schimel et al. 2000). These areas are likely to be either N-limited or co-limited by N and P, which would explain the increase in growth with N deposition.

As anthropogenic activity continues to increase the amount of N available for use by plants, phosphorus constraint will increase in importance. N deposition in areas that are already strongly P-limited, e.g. the Amazon and Congo, will most likely cause
substantial N accumulation, increasing N leaching. If the N deposition is in the form of NO\textsubscript{x}, it could also augment the acidification of these regions.

Results from Chapter 4 suggest that amendment of a limiting nutrient (in this case N) increases the relative limitation of a co-limiting nutrient (in this case P). If this effect is general, it is likely to be important in areas that are now co-limited by N and P. Assuming that mildly P-limited areas are co-limited by N and P, this would encompass much of North America, Eurasia, and the outer edges of the tropical “hotspots” of P limitation. In these areas chronic N deposition may remove N limitation, shifting to completely P-limited systems, as has been documented in parts of Sweden, Denmark, and the Netherlands (Aerts and Chapin 2000). One implication of this switch would be a decrease in the extent of net carbon storage, for steady state would be approached soon after a switch to sole limitation by phosphorus. Others have suggested that the terrestrial carbon sink is only temporary (Steffen et al. 1998); P limitation may be a reason. Other implications would be increased acidification as NO\textsubscript{x} accumulates in the soil, increased N leaching, and the cascading effects of these processes described in Chapters 2 and 4.

The other significant results from Chapter 4 were the effects of increased precipitation and the interaction between N deposition and increased precipitation. In both cases precipitation decreased P limitation, probably by increasing N loss. The role this mechanism on a global scale is difficult to estimate. Precipitation change is predicted to vary substantially across regions (IPCC 2001). Areas that increase in precipitation and are co-limited by N and P (possibly Central and Southeast Asia, the boundary between the Sahara and the Congo, and the Northeastern U.S.) could respond similarly to this interaction, but the alleviation of water limitation could offset the
decreases in NPP due to nutrient stress. The role of this interaction at large spatial scales is difficult to determine, given the number of variables that influence it.

Results from Chapter 4 did not show any effect of CO$_2$ on the pools and fluxes of the P cycle that were measured. However, CO$_2$ had an overall suppressive effect on NPP in the JRGCE (Shaw et al. 2002). Many other empirical studies have shown a fertilization effect of CO$_2$, and even though the majority of these studies were single-factor studies, CO$_2$ fertilization is generally accepted to be an important mechanism of increased carbon storage. If this is the case, the extent of CO$_2$ fertilization depends on nutritional constraints (Friedlingstein et al. 1995). Results presented here suggest that CO$_2$ fertilization will have less of an effect in the tropics and the high northern latitudes if P stress is the only other constraint in question.

**Summary**

Assuming a linear response to phosphorus availability, and using the inputs described here, phosphorus availability heavily constrains terrestrial NPP in equatorial regions. These regions account for the majority of terrestrial NPP. High northern latitudes are likely also affected by P stress. If the results of Chapter 4 can be extrapolated to similar systems, continued N deposition has the potential to shift systems co-limited by N and P to exclusive limitation by phosphorus. Affected areas are probably parts of North America and Europe, which are currently a net sink for carbon. A shift to P limitation in these systems would both lessen the carbon sink and allow N to accumulate in the soil or move to aquatic systems.

The next chapter summarizes the results presented in this and previous chapters, then explains how these results could potentially be applied to current policy.
PART 4

POLICY AND CONCLUSION
CHAPTER 6

PHOSPHORUS IN POLICY: WHAT DOES THIS ALL MEAN?

General summary

Nitrogen and phosphorus are the two most commonly limiting soil nutrients to terrestrial primary production. Fossil fuel combustion deposits nitrogen in the form of NO$_x$ onto ecosystems across the Northern Hemisphere. Both the results presented in Chapter 4 and previous research in other ecosystems (see Aerts and Chapin 2000) show that N deposition concurrently alleviates N limitation and increases P limitation. The fertilization response to N deposition should decrease with this shift as the demand for N decreases. A complete shift to P as the sole limiting soil resource would prevent any growth response to N deposition.

A shift from nitrogen limitation to phosphorus limitation can only happen if the ecosystem in question has the potential for phosphorus limitation. If N becomes abundant, yet P is also abundant, production will become limited by some other resource. The coverage of P limitation in the Northern Hemisphere is uncertain, according to the results of Chapter 5. The less conservative scenarios show heavy P limitation to primary production in much of the Northern Hemisphere, whereas the more conservative scenarios show little to no P limitation in the Northern temperate zones. Assuming the truth lies somewhere near the middle of this range, P is potentially limiting to plant production in a considerable portion of the Northern Hemisphere. In every scenario the tropical regions of the world show heavy P limitation.

Fertilization by nitrogen deposition is currently predicted to be one of the major contributors to net carbon storage in the Northern Hemisphere (Schimel et al. 2000). The
present work suggests that this is but a temporary effect in many areas due to phosphorus limitation. The time scale for a shift to exclusive P limitation depends on the specific system in question, but it has been shown to have occurred in the past few decades in Sweden (Aerts and Chapin 2000). The currently accelerating rate of N deposition will only increase the speed of transition.

Precipitation has increased over much of the world in the last century, and is predicted to continue increasing in the coming century (IPCC 2001). Results from Jasper Ridge (Chapter 4) suggest that elevated precipitation (alone and in combination with N deposition) improves relative phosphorus nutrition, yet decreases relative nitrogen limitation. The proposed mechanism for this result is an increase in N loss due to increased leaching. Although this mechanism decreases relative P limitation, the net effect on carbon storage is negative because more of the deposited N leaves the rooting zone. This mechanism is much faster than the shift to P limitation, since it acts as soon as water can filter through the soil. The spatial variation in precipitation and system-specific characteristics (e.g. the extent of water limitation) make predicting the extent of this mechanism complicated. The net effect on carbon storage predicted by these mechanisms, taking both N deposition and precipitation into account, is slowing of carbon storage due either to increased N loss with precipitation or a shift towards P limitation with N deposition. Less carbon is taken up for a shorter period of time.

**NOx policies**

The terrestrial biosphere is currently a net sink for carbon, but this sink is only temporary (Steffen et al. 1998). N fertilization will eventually saturate (due to P limitation, another resource limitation, or biophysical constraints), as will forest regrowth
(Field and Fung 1999). The fertilization effect is the only unintended benefit of NOx emissions, but this benefit is not sustainable. Unintended costs of NOx emissions are numerous. Ozone production, acid precipitation, biodiversity losses, and acidification of soils and lakes are among the health and environmental problems resulting from NOx emissions; these have sparked political interest in NOx reductions.

The Clean Air Act Amendments (CAAA) of 1990 included NOx emissions among the chemical species targeted for reductions. Although power plants would have to invest a substantial amount to reduce their emissions, a benefit-cost analysis of the 1990 CAAA showed that projected health and recreational benefits of reducing SO2 and NOx emissions would far exceed the costs of reductions (Burtraw et al. 1997). It should be noted that avoided ecosystem damages and the resulting benefits were not taken into account in this analysis. The fertilization feedback effect is the only ecological benefit of NOx emissions, because it removes N from the soil and CO2 from the atmosphere, decreasing damages from its source, NOx emissions, and from associated problems (e.g. global warming). The transitory nature of the fertilization effect, however, decreases this benefit: when N demand saturates (through P limitation or some other means), the magnitudes of all negative effects of NOx and CO2 increase. Results from this thesis suggest that N saturation due to P limitation alone is quite possible in much of the land that is currently a sink for carbon. Thus, the need to decrease NOx emissions is more pressing than ever.

Two sets of bills that would amend the CAAA are currently in Congress. The Clean Power Act of 2003 (S.366) and the Clean Smokestacks Act of 2003 (H.R.2043) were introduced to the Senate on February 12, 2003, and the House of Representatives on
March 8, 2003, respectively (Library of Congress 2003). These bills would reduce NO\textsubscript{x} emissions from included facilities to at least 75% below 1997 levels by 2009 (to a maximum emissions cap of 1.51 million tons per year). Covered facilities include all fossil-fuel-fired electric generators that have a nameplate capacity of 15 MW or greater (Library of Congress 2003). An alternative bill, the Clear Skies Act of 2003 (S.485 and H.R.999), was introduced into the Senate and House of Representatives on February 27, 2003 (Library of Congress 2003). This bill would reduce NO\textsubscript{x} emissions from included facilities to at least 67% below 2000 emissions (to a maximum emissions cap of 1.7 million tons per year) by 2018, with a checkpoint of 2.1 million tons by 2008. It includes all fossil-fuel-fired electric generators that have a nameplate capacity of 25 MW or greater (Library of Congress 2003).

Although there has been no explicit benefit-cost analysis of the environmental impacts of NO\textsubscript{x} emissions, it is highly unlikely that further reductions would have a net cost. The only benefit of increased emissions is enhanced growth, which is a fleeting effect. This suggests that the Clean Power Act/Clean Smokestacks Act of 2003 are superior to the Clear Skies Act of 2003. They would effect more NO\textsubscript{x} emissions reductions for more power plants in a shorter time. Along with the myriad health and recreational benefits described in Burtraw et al. (1997)\textsuperscript{35} and well-known ecological benefits (reduced biodiversity loss, acidification, and ecological ozone damage), these reductions could slow the shift towards P limitation, prolonging the period over which the terrestrial United States is a carbon sink. Prolonging the shift would have the effect of buying time for new technology innovations or new policies to address a root of the

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\textsuperscript{35} They found that combined reductions of SO\textsubscript{2} and NO\textsubscript{x} from the 1990 CAAA substantially reduced mortality, morbidity, and visibility; benefits from mortality reductions alone offset the costs of reductions.
problem (e.g. reliance on fossil fuels). The Clean Power and Clean Smokestacks Acts of 2003 also include significant reductions in CO₂, essentially targeting one of these roots.

If the Clean Power and Clean Smokestacks Acts of 2003 pass, NOₓ emissions from power plants will be reduced to 1.51 million tons of NOₓ by 2009. However, all of the policies mentioned above include only fossil-fuel-fired electric power plants. Automobiles account for 49% of all NOₓ emissions in the United States, while electric power plants account for only 27% (EPA 2003). Thus, the policies outlined here only affect about one quarter of all NOₓ emissions in the U.S.

**Conclusions**

With complete cessation of NOₓ emissions unlikely and N volatilization from fertilizer and burning even less likely to decrease, a shift towards phosphorus limitation in many areas of the Northern Hemisphere is quite likely in the next century. This switch will exacerbate the effects of NOₓ emissions, as less N will be taken up into the biosphere when it is no longer limiting. Increased precipitation could also decrease terrestrial N uptake by increasing N leaching from soils. This would further increase the amount of N reaching aquatic systems. Although there is proportionately less N deposition in the tropics, the heavy P limitation already in place there may prevent an increase in N uptake proportional to the amount of N deposited.
APPENDIX 1
PHOSPHATASE ACTIVITY DETAILS AND REVISIONS

Introduction

Phosphatases are divided into phosphomonoesterases, phosphodiesterases, and phosphotriesterases, which have one, two, and three active sites for phosphate hydrolysis, respectively. Previous studies have shown that these three classes are all highly correlated, and that phosphomonoesterases are typically present in much higher concentrations than the other two (Eivazi and Tabatabai 1976, Malcolm 1983). Therefore, this study isolates phosphomonoesterases to represent all phosphatases. This assumes that all three classes have the same response to the various manipulations in the JRGCE and in the pots.

Depth of soil cores may influence phosphatase activity measurements. Since the majority of soil organisms and roots are near the surface, phosphatase activity should be higher near the surface. A literature review by Speir and Ross (1978) shows that in most studies this is the case. To test this phosphatase activity was measured at different depths.

Phosphatases produced by different groups of organisms and in different environments catalyze hydrolysis at different optimal pH levels (Speir and Ross 1978, Malcolm 1983, Kroehler and Linkins 1988). Plants typically produce phosphatases that work optimally in acidic conditions (acid phosphatases). Microbes are more adaptive, typically producing phosphatases that work optimally at the pH of their environment. However, some plants have been shown to produce phosphatases that work best under neutral conditions (Dracup et al. 1984, Kroehler and Linkins 1988, Tadano et al. 1993,
Hysek and Sarapatka 1998, Fries et al. 1998), and thus using pH optimum to determine the source is not precise. To optimize activity measurements, the effects of pH on enzyme activity were tested, and the optimum pH level was chosen to the maximize signal: noise ratio.

**Methods**

*Depth study*

To examine the effects of depth on soil phosphatase activity, soil was cored from a plot adjacent to the Jasper Ridge Global Change Experiment (JRGCE) in August 2002. Soil type was the same in this plot and in the JRGCE. Due to the possibility of intracellular phosphatase contamination, litter was cleared away from the coring site and the top millimeter of soil was not used as part of the sample. Core depth and diameter were 15 cm and 22 mm, respectively, each of which was divided into 5 cm depth fractions (0-5, 5-10, and 10-15 cm). Four 15 cm cores were taken (n = 4). Samples were processed within one day of coring. Assay procedure and activity calculations were the same as in Chapter 4.

*pH study*

To examine the effect of pH on phosphatase activity three cores were taken from the same plot as the depth study in August 2002 (depth 5 cm, diameter 22 mm). Each core was homogenized and divided into ten subsamples. Assays were conducted from pH 5.0 to pH 9.0 at 0.5 unit intervals on these subsamples. For pH 5.0 to 7.0 assays, 50 mM acetate buffer was used (buffering range 3.3 to 5.8); for pH 7.0 to 9.0 assays, 50 mM TRIS buffer was used (buffering range 7.0 to 9.0). pH was measured at intermediate time
steps during analysis to confirm buffer pH readings. Samples were processed within three days. Assay procedure and activity calculations were the same as in Chapter 4.

**Statistical analyses**

Depth and pH phosphatase studies were both analyzed with one-way model I analysis of variance (ANOVA), with depth (three levels) and pH (nine levels) as the factors. The Bonferroni post-hoc test and correction was used to determine differences between levels of pH (Sokal and Rohlf 1995). Depth phosphatase data were square root transformed to improve normality and homoscedasticity; pH phosphatase data were normally distributed and homoscedastic without transformation.

**Results and discussion**

**Depth**

The depth of sample used had a marginally significant effect on phosphatase activity for assays at pH 5.0 and pH 7.0 (Table A1.1). The trend was for phosphatase activity to be higher in shallow samples, agreeing with previous results from the literature (Figure A1.1). Coring is a disruptive measure, so any way to reduce impact is desirable. Since activity does not decrease with depth, all remaining studies used 5 cm cores.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F value</th>
<th>Pr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>0.0478</td>
<td>2</td>
<td>0.0239</td>
<td>3.8463</td>
<td>0.062</td>
</tr>
<tr>
<td>Error</td>
<td>0.0559</td>
<td>9</td>
<td>0.0062</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F value</th>
<th>Pr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>0.0515</td>
<td>2</td>
<td>0.0257</td>
<td>3.136</td>
<td>0.0926</td>
</tr>
<tr>
<td>Error</td>
<td>0.0739</td>
<td>9</td>
<td>0.0082</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A1.1: Anova tables for effect of depth on phosphatase activity in cores taken from a plot adjacent to the JRGCE. Assays were conducted at both pH 5.0 and pH 7.0, for some of the assays for the JRGCE and pot study were conducted at 5.0 (March 2002 and May 2002) and some at 7.0 (January 2003 JRGCE, pots).
The pH of the buffer used to assay for phosphatase had a highly significant effect on phosphatase activity (Table A1.2). Peak activity occurred at neutral pH for both buffers, with higher activity at the acidic end of the range than the alkaline end. Activity at pH 7.0 was statistically indistinguishable from activity at pH 6.0, 6.5, and 7.5. Phosphatase activity is highest at neutral pH, which happens to be the soil pH at the JRGCE. That activity is higher in the acidic range than in the alkaline range, yet highest at neutral pH suggests that both microbes and plants are contributing substantially to total soil phosphatase activity. These data suggest that phosphatase activity is an ecosystem-level measure of P demand. Based on these results all subsequent assays were conducted
at pH 7.0, since this pH maximized phosphatase activity readings and fell within the range of the TRIS buffer.

<table>
<thead>
<tr>
<th>Analysis of Variance: Effect of pH on phosphatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Error</td>
</tr>
</tbody>
</table>

Table A1.2: Anova table for effect of pH on phosphatase activity in cores taken from a plot adjacent to the JRGCE. Cores were taken to 5 cm.

![Effect of pH on phosphatase](image)

Figure A1.2: Effect of pH of assay on phosphatase activity in soil taken from Jasper Ridge. N = 3 for each point/color combination (pH 7.0 has two overlapping y-values, for each of which N = 3). Error bars are ± 1 SE. The effect of pH on phosphatase activity was significant at P < 0.001. Phosphatase activity from pH 5.0 to pH 7.0 was assayed with acetate buffer; activity from pH 7.0 to pH 9.0 was assayed with TRIS buffer. Letters represent differences between treatment levels as revealed by Bonferroni post-hoc tests. Points with the same letter are not significantly different from each other.
APPENDIX 2

CONVERTING THE SOIL MAP TO A PHOSPHORUS STRESS FILE

/* File: plimit.c
* -------------
* This program takes a soil map input file (at 2-minute resolution)
* and assigns phosphorus limitation values to each soil order. It
* then compresses the map to one degree resolution, removes ocean
* points (using CASA's landmask) and creates an output text file
* for use in CASA.
*/

#include <stdio.h>
#include <stdlib.h>
#include <ctype.h>
#include <assert.h>

/* #defines: soil type values
* --------------------------
* These are the phosphorus limitation values, and should be the
* main changes for different CASA runs.
* I'm now initializing them based on the figure in the soil textbook,
* assigning values of 1 to types not listed (conservative estimate).
* I'm using a linear limitation curve with 0.8 as the lowest value
* (for 0.001 mg P/L).
*/
#define OCEAN           -1.00
#define SANDROCKICE     1.000
#define OXISOL          0.800
#define ANDISOL         0.800
#define ULTISOL         0.802
#define MOLLISOL        0.809
#define VERTISOL        0.900
#define HISTOSOL        1.000
#define GELISOL         1.000
#define ENTISOL         1.000
#define INCEPTISOL      1.000
#define ARIDISOL        1.000
#define ALFISOL         0.802
#define SPODOSOL        0.802

/* #defines: file sizes
* --------------------
* These are the sizes, used for various for loops, of 1) the original
* 2-min resolution file, 2) the half-compressed 2-min by 1-deg array,
* 3) the 1-deg resolution file, and 4) the land-only points.
*/
#define BIG 58320000
#define MED 1944000
#define SML 64800
#define LND 14628
/* #defines * -------- * These are random values used in compressing or converting ASCII * numbers to meaningful integers. */

#define NUMCOLS 360
#define NUMROWS 30
#define ASCIISPACE 32
#define ASCIIZERO 48

/* #defines: file names * ------------------- * These are the files used in the program, and should be in the same * directory as the executable. Suborder is the soil map I downloaded * from the USDA NRCS website. Both vegtype and landmask come from CASA. */

#define SOILFILE "suborder.img"
#define VEGTYPE "vegtype.txt"
#define MASKFILE "landmask.txt"

/* Function Prototypes */
static double *TypeToStress(void);
static double *Compress(double *twominPlimit);
static double *CompressUp(double *temp);
static double *ModifyAgriculture(double *onedegPlimit);
static double *RemoveOcean(double *onedegPlimit);
static void ArrayToFile(double *landOnly, char *outname);
static double StressFactor(int ch);

/* Function: main * ------------- * Main calls functions to assign limitation values, compress * the file, remove oceanic points, and write the outfile. It * also takes care of freeing the arrays. */

int main(int argc, char *argv[])
{

    twominPlimit = TypeToStress();
    temp = Compress(twominPlimit);
    free(twominPlimit);
    onedegPlimit = CompressUp(temp);
    free(temp);
    onedegPlimit = ModifyAgriculture(onedegPlimit);
    landOnly = RemoveOcean(onedegPlimit);
    free(onedegPlimit);
    ArrayToFile(landOnly, argv[1]);
    free(landOnly);
    return 0;
}

/* Function: TypeToStress
This function converts each original soil type (in two minute resolution) to an array of phosphorus limitation values (each value a double).

```c
static double *TypeToStress(void)
{
    int i, ch;
    double *twominPlimit;
    FILE *infile;

    infile = fopen(SOILFILE, "r");
twominPlimit = malloc(BIG*sizeof(double));
assert(twominPlimit);
for (i = 0; i < BIG; i++) {
    ch = getc(infile);
twominPlimit[i] = StressFactor(ch);
}
fclose(infile);
return twominPlimit;
}
```

This function compresses each set of thirty points to one point, essentially compressing the x component of the minute resolution to degree resolution. The limitation value is an arithmetic mean of the limitations contained within. It keeps track of how many land points there are, and only averages the land points.

```c
/* Function: Compress
 * ----------
 * This function compresses each set of thirty points to one point, essentially compressing the x component of the minute resolution to degree resolution. The limitation value is an arithmetic mean of the limitations contained within. It keeps track of how many land points there are, and only averages the land points.
 */

static double *Compress(double *twominPlimit)
{
    double *temp;
    int i, *numPoints;

    temp = malloc(MED*sizeof(double));
numPoints = malloc(MED*sizeof(int));
assert(temp);
assert(numPoints);
for (i = 0; i < MED; i++) {
    temp[i] = 0;
    numPoints[i] = 0;
}
for (i = 0; i < BIG; i++) {
    if (twominPlimit[i] != OCEAN) {
        temp[(i/30)] = temp[(i/30)] + twominPlimit[i];
        numPoints[(i/30)]++;
    }
}
for (i = 0; i < MED; i++) {
    if (numPoints[i] != 0) {
        temp[i] = temp[i]/numPoints[i];
    }

    return temp;
}
```
else temp[i] = OCEAN;
}
free(numPoints);
return temp;
}

/* Function: CompressUp
 *  ---------------
 *  This function does the y component of compressing minutes
 *  to degrees, again averaging the values. Again, it keeps
 *  track of how many points are being averaged, omitting ocean
 *  points. One current problem is that it doesn't distinguish
 *  between half ocean/half land points from Compress() and full
 *  land points (treats them all as all land). This may skew the
 *  average towards certain points. However, I'm quite sure the
 *  land mask removes virtually all of these points (if not all).
 */

static double *CompressUp(double *temp)
{
    double *onedegPlimit;
    int i, *numPoints, index;

    onedegPlimit = malloc(SML*sizeof(double));
    assert(onedegPlimit);
    for (i = 0; i < SML; i++) {
        onedegPlimit[i] = 0;
        numPoints[i] = 0;
    }
    for (i = 0; i < MED; i++) {
        if (temp[i] != OCEAN) {
            index = ((i%NUMCOLS) +
                      ((i/(NUMCOLS*NUMROWS))*NUMCOLS));
            onedegPlimit[index] =
            onedegPlimit[index] + temp[i];
            numPoints[index]++;
        }
    }
    for (i = 0; i < SML; i++) {
        if (numPoints[i] != 0) {
            onedegPlimit[i] =
            onedegPlimit[i]/numPoints[i];
        }
        else onedegPlimit[i] = 1;
    }
    free(numPoints);
    return onedegPlimit;
}

/* Function: ModifyAgriculture
 *  ---------------------------
 *  This function modifies the one-degree resolution P limitation
 *  file to account for the fact that agricultural areas are not
 *  likely to be limited by phosphorus (they're fertilized).
 */

static double *ModifyAgriculture(double *onedegPlimit)
{ 
    FILE *vegetation;
    int i, j, k, ch, type[2], veg;

    vegetation = fopen(VEGTYPE, "r");
    for (i = 0; i < SML; i++) {
        for (j = 0; j < 2; j++) type[j] = -1;
        k = 0;
        /* read each number into type */
        while (1) {
            ch = getc(vegetation);
            if (ch == ASCIISPACE) break;
            ch = ch - ASCIIZERO;
            type[k] = ch; k++;
        }
        /* convert the type to an actual integer */
        if (type[1] == -1) { veg = type[0]; }
        else veg = ((type[0] * 10) + type[1]);
        /* make P limitation 1 if it's cultivated */
        if (veg == 12) onedegPlimit[i] = 1;
    }
    fclose(vegetation);
    return onedegPlimit;
}

/* Function: RemoveOcean */
/* ---------------------
* This function uses the ASCII version of CASA's landmask to
* convert the soil file to land-only points. I call getc twice
* in each for loop because the landmask has spaces in between
* each mask value.
*/
static double *RemoveOcean(double *onedegPlimit)
{
    FILE *landmask;
    int i, ch, numLand = 0;
    double *landOnly;

    landOnly = malloc(LND*sizeof(double));
    assert(landOnly);
    landmask = fopen(MASKFILE, "r");
    for (i = 0; i < SML; i++) {
        ch = getc(landmask) - ASCIIZERO;
        if (ch == 1) {
            landOnly[numLand] = onedegPlimit[i];
            numLand++;
        }
    }
    getc(landmask);
    fclose(landmask);
    return landOnly;
}

/* Function: ArrayToFile */
/* ---------------------
* This function makes the compressed, modified array into an
static void ArrayToFile(double *landOnly, char *outname)
{
    FILE *outfile;
    int i;

    outfile = fopen(outname, "w");
    for (i = 0; i < LND; i++) {
        fprintf(outfile, "%g ", landOnly[i]);
    }
    fclose(outfile);
}

/* Function: StressFactor
 * ---------------
 * This function, called from TypeToStress, returns the stress
 * value for each array point, based on the #defines at the top
 * of the program.
 */

static double StressFactor(int ch)
{
    switch (ch) {
    case 0:
        return OCEAN; break;
    case 1:
    case 2:
    case 3:
        return SANDROCKICE; break;
    case 5:
    case 6:
    case 7:
        return GELISOL; break;
    case 10:
    case 11:
    case 12:
    case 13:
        return HISTOSOL; break;
    case 15:
    case 16:
    case 17:
    case 18:
        return SPODOSOL; break;
    case 20:
    case 21:
    case 22:
    case 23:
    case 24:
    case 25:
    case 26:
        return ANDISOL; break;
    case 30:
    case 31:
    case 32:
    case 33:
case 34:
    return OXISOL; break;
case 40:
case 41:
case 42:
case 43:
case 44:
case 45:
    return VERTISOL; break;
case 50:
case 51:
case 52:
case 53:
case 54:
case 55:
case 56:
    return ARIDISOL; break;
case 60:
case 61:
case 62:
case 63:
case 64:
    return ULTISOL; break;
case 70:
case 71:
case 72:
case 73:
case 74:
case 75:
case 76:
    return MOLLISOL; break;
case 80:
case 81:
case 82:
case 83:
case 84:
    return ALFISOL; break;
case 85:
case 90:
case 91:
case 92:
case 93:
case 94:
    return INCEPTISOL; break;
case 95:
case 96:
case 97:
case 98:
case 99:
    return ENTISOL; break;
default:
    return -1.00; break;
}
APPENDIX 3

CONVERTING NPP OUTPUT FROM g*m⁻²*yr⁻¹ TO g*yr⁻¹ BY LATITUDE

/* File: nppAnal.c
 * ---------------
 * This file does the calculations for converting npp infiles
 * (text files) to output text files by latitude. It first
 * opens the infile, then reads the data into an array of
 * "cell" structures that hold latitude, longitude, and npp
 * data. It then converts the npp data from g/m2/yr to g/yr,
 * assuming the earth is a sphere. It then sums total npp
 * across each latitudinal degree. Finally, it writes an
 * output file of npp data by latitude.
 */

#include <math.h>
#include <stdio.h>
#include <stdlib.h>
#include <assert.h>

/* #defines
 * --------
 * These are some useful numbers used in the program.
 * NUMCELLS is the number of one-degree latitude by longitude
 * cells in the input file. NUMLAT and NUMLNG are the number
 * of degrees of latitude and longitude on Earth, respectively.
 * MAXLAT and MINLNG are the coordinates of the upper-left-hand
 * corner of the raster array. ASCIISPACE and ASCIIZERO are
 * the ascii values of " " and "0", respectively. PI is pi.
 * R is the radius of the earth in meters. The outfile name
 * the file that will be written after all conversions and
 * summations are done.
 */
#define OUTFILENAME     "../../Outfiles/npp0_8.txt"
#define NUMCELLS        64800
#define NUMLAT          180
#define NUMLNG          360
#define MAXLAT          89.5
#define MINLNG          -179.5
#define ASCIISPACE      32
#define ASCIIZERO       48
#define PI              3.14159
#define R               6371000

/* structs
 * -------
 * The one struct used in this program is called cell, and
 * holds the latitude, longitude, and npp value (which will be
 * converted from g/m2/yr to g/yr). Longitude is not used in
 * this program, but is kept for reference and debugging
 * purposes.
 */

typedef struct {


double lat;
double lng;
double npp;
} cell;

/* Function Prototypes */

static cell *GetData(char *datafile);
static void RemoveArea(cell *npparray);
static cell *SumOneDegree(cell *npparray);
static void WriteOut(cell *latnpp);
static double ToDouble(char buff[], int numChars);
static double DoubleRec(char buff[], int numBefore, int numChars,
    int i, double *value);
static double TenX(int x);
static double Max(double x, double y);
static double Min(double x, double y);

/* Function: main
 * --------------
 * Main calls function to read in the data, convert from g/m2/yr
to g/yr, sum across each latitudinal degree, and write the
*outfile. The string argument it takes is the infile name,
passed in when the program is run (to run, type "programname
*nostress.txt" - program name will be ./a.out unless it is
*renamed after compiling).
*/

int main(int argc, char *argv[])
{
    cell *npparray, *latnpp;

    npparray = GetData(argv[1]);
    RemoveArea(npparray);
    latnpp = SumOneDegree(npparray);
    free(npparray);
    WriteOut(latnpp);
    free(latnpp);
    return 0;
}

/* Function: GetData
 * ---------------
 * This function reads the data from the input file into an
* array of cell structs, which it returns to main.
*/

static cell *GetData(char *datafile)
{
    int i, j, ch;
    cell *npparray;
    FILE *infile;
    char buff[10];

    npparray = malloc(NUMCELLS*sizeof(cell));
    assert(npparray);
    infile = fopen(datafile, "r");
    for (i = 0; i < NUMCELLS; i++) {
j = 0;
while (1) {
    ch = getc(infile);
    if (ch == ASCIISPACE) break;
    buff[j] = (char) ch;
    j++;
}
npparray[i].npp = ToDouble(buff, j);
npparray[i].lat = MAXLAT - (i/360);
npparray[i].lng = MINLNG + (i%360);
    return npparray;
    fclose(infile);
}

/* Function: RemoveArea
 * ---------------
 * This function removes the area component of the npp
 * data, leaving it in g C/yr. Thanks to Jonathan and
 * Pat for the math help in figuring out the area of
 * latitudinal strips.
 */
static void RemoveArea(cell *npparray)
{
    int i;
    double cellarea, phia, phib;
    for (i = 0; i < NUMCELLS; i++) {
        phia = ((npparray[i].lat + 0.5) * (PI/180));
        phib = ((npparray[i].lat - 0.5) * (PI/180));
        cellarea = ((2*PI*R*R*(Max(sin(phia), sin(phib))
                        - Min(sin(phia), sin(phib))))/NUMLNG);
        npparray[i].npp *= cellarea;
    }
}

/* Function: SumOneDegree
 * --------------
 * This function takes the global raster array at one
 * degree resolution and returns the latitudinal sum,
 * still in the cell *format. Longitude is set to be 0,
 * for it's not actually used.
 */
static cell *SumOneDegree(cell *npparray)
{
    int i;
    cell *latnpp;
    latnpp = malloc(NUMLAT*sizeof(cell));
    assert(latnpp);
    for (i = 0; i < NUMLAT; i++) {
        latnpp[i].npp = 0;
        latnpp[i].lng = 0;
    }
    for (i = 0; i < NUMCELLS; i++) {
latnpp[(i/NUMLNG)].npp += npparray[i].npp;
latnpp[(i/NUMLNG)].lat = npparray[i].lat;
}
return latnpp;
}

/* Function: WriteOut
 * ------------------
 * This function takes the 180-element array and writes
 * an outfile of npp data in g C/yr/latitude.
 */
static void WriteOut(cell *latnpp)
{
    int i;
    FILE *outfile;

    outfile = fopen(OUTFILENAME, "w");
    for (i = 0; i < NUMLAT; i++) {
        fprintf(outfile, "%g\n", latnpp[i].npp);
    }
    fclose(outfile);
}

/* Function: ToDouble
 * ------------------
 * This function takes an array of characters and
 * returns a double, calling a recursive function
 * to do the actual calculations.
 */
static double ToDouble(char buff[], int numChars)
{
    int i, numBefore;
    double value = 0;

    if (numChars == 1) return 0;
    numBefore = 0;
    i = 0;
    while (1) {
        if (buff[i] != '.') numBefore++;
        if (buff[i] == '.') break;
        i++;
    }
    return DoubleRec(buff, numBefore, numChars, 0, &value);
}

/* Function: DoubleRec
 * -------------------
 * This function eventually returns the double value
 * converted from the input character array.
 */
static double DoubleRec(char buff[], int numBefore, int numChars, int i, double *value)
{
    if (i == numChars) return *value;
if (numBefore != 0) {
    *value += ((int) (buff[i] - ASCIIZERO) * 
              (TenX(numBefore)));
}
DoubleRec(buff, (numBefore - 1), numChars, i + 1, value);

/* Function: TenX
 * ---------------
 * A power function should be in math.h, but it does not appear to
 * be. Thus, I write one myself. True, it's not mathematically
 * correct, but given the way it's called it works.
 */
static double TenX(int x)
{
    switch (x) {
    case -10: return 0.0000000001; break;
    case -9: return 0.000000001; break;
    case -8: return 0.00000001; break;
    case -7: return 0.0000001; break;
    case -6: return 0.000001; break;
    case -5: return 0.00001; break;
    case -4: return 0.0001; break;
    case -3: return 0.01; break;
    case -2: return 0.1; break;
    case -1: return 1; break;
    case 0: return 10; break;
    case 1: return 100; break;
    case 2: return 1000; break;
    case 3: return 10000; break;
    case 4: return 100000; break;
    case 5: return 1000000; break;
    case 6: return 10000000; break;
    case 7: return 100000000; break;
    case 8: return 1000000000; break;
    case 9: return 10000000000; break;
    }
}

/* Functions: Max, Min
 * -------------------
 * These function return the maximum and minimum
 * of two double values.
 */
static double Max(double x, double y)
{
    if (x > y) return x;
    return y;
}
static double Min(double x, double y)
{
    if (x < y) return x;
    return y;
}
### Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>410 nm</strong></td>
<td>The wavelength of yellow light. This is the wavelength used in phosphatase assays, for it is the color of pNP.</td>
</tr>
<tr>
<td><strong>Absorbance</strong></td>
<td>A physical property of a substance defining the quantity of a certain wavelength of light absorbed.</td>
</tr>
<tr>
<td><strong>Buffer</strong></td>
<td>A salt solution used to maintain constant pH. Used in phosphatase assays.</td>
</tr>
<tr>
<td><strong>C3</strong></td>
<td>The “normal” photosynthetic pathway that fixes carbon dioxide on a molecule with three carbon atoms. Catalyzed by Rubisco.</td>
</tr>
<tr>
<td><strong>C4</strong></td>
<td>Another photosynthetic pathway that fixes carbon dioxide first with PEP carboxylase, then transfers this carbon to another chamber to carry out “normal” photosynthesis. Useful in low CO2 environments due to its efficiency at drawing down CO2 concentrations in the leaf.</td>
</tr>
<tr>
<td><strong>CO2 fertilization</strong></td>
<td>Increased primary production due to increasing atmospheric CO2.</td>
</tr>
<tr>
<td><strong>Colorimetric</strong></td>
<td>Uses color. In this case, both Kjeldahl digest solutions and phosphatase assays use colorimetric analysis because the products of the reactions have a certain color, the intensity of which determines the quantity of P, N, or phosphatase.</td>
</tr>
<tr>
<td><strong>Culture tube</strong></td>
<td>A cylindrical glass tube, very common in bio labs. 10 mL tubes were used for phosphatase assays.</td>
</tr>
<tr>
<td><strong>Cuvette</strong></td>
<td>A clear plastic container used for measuring absorbance of liquids.</td>
</tr>
<tr>
<td><strong>Down-regulation</strong></td>
<td>The process of decreasing time spent photosynthesizing, usually in response to increased atmospheric CO2.</td>
</tr>
<tr>
<td><strong>Ester-bonded</strong></td>
<td>A chemical bond: O – P – O. This is the form in which phosphate groups usually attach to organic matter.</td>
</tr>
<tr>
<td><strong>Eutrophication</strong></td>
<td>The process of aquatic fertilization due to agricultural fertilizer runoff or leaching. Stimulates algal growth in lakes or oceans, leading to increased subsurface decomposition and zones of extremely low oxygen.</td>
</tr>
<tr>
<td><strong>Exotic</strong></td>
<td>Synonymous with “invasive.” A species not native to the area.</td>
</tr>
</tbody>
</table>
Foliar  Pertaining to the leaf. “Foliar tissue” is leaf tissue.

Forb  A low-lying, broad-leafed plant, e.g. clover. Forbs and grasses are the components of grasslands.

Green tissue  Plant tissue that was alive at the time of harvest, as opposed to senescent tissue or litter.

Heterotrophic  Relating to an organism that does not produce energy from abiotic sources. All animals and fungi, and many bacteria and archaea are heterotrophic. Opposite of autotrophic, which for the purposes of this thesis means plants and photosynthetic bacteria.

Homogenate  The solution containing liquid buffer and a soil sample.

Homogenize  To mix up so subsample is identical.

Hydrolysis  In this case, addition of water to an ester bond to release phosphate. Catalyzed by phosphatases.

Hyphae  Fungal extensions that have extremely high surface area to volume ratios. Useful in nutrient and water uptake in soil.

Insoluble inorganic phosphorus  The type of phosphorus that is bound to soil minerals. Divided up into occluded and non-occluded phosphorus.

JRGCE  The Jasper Ridge Global Change Experiment. For full explanation see Chapter 4.

Leaching  The process of movement downward through soil, often to groundwater.

Luxury consumption  Uptake of resources above and beyond that which can be immediately used. Often a sign of limitation, indicating “stockpiling.”

Microcentrifuge tube  A small tube that can hold, in this case, 2 mL of solution. Put in a microcentrifuge, which spins the tubes until the solids are separated from the liquids.

mM  A measure of concentration: millimolar; a thousandth of a molar, which is defined as one mole per liter (one mole = Avogadro’s number)

Monoculture  A planting of one species over an area.

Multiple resource limitation hypothesis  The hypothesis that plants are simultaneously limited by all classes of essential resources. See Chapter 2 for details.
Mycorrhizal association  A symbiotic relationship between plants and fungi where plants trade carbon they fix during photosynthesis for nutrients taken up by the mycorrhizal fungi.

N fixation  The process of cleaving and reducing N₂ gas to two H-bonded N molecules.

Non-occluded phosphorus  Mineral-bound phosphorus that can be accessed through weathering or organic acid leaching.

NPP  Net primary production, defined as gross photosynthesis minus plant respiration (see Field et al. 1995 for full discussion).

Occluded phosphorus  Mineral-bound phosphorus that cannot be accessed through weathering or organic acid leaching. Can only be accessed through substantial physical disturbance of the soil.

Organic phosphorus  Phosphorus bound to organic matter in the soil.

Parent material  The rock from which a soil was born.

PEP Carboxylase  The enzyme used to initially fix carbon in C₄ photosynthesis.

Phenology  The timing of biological events. Phenological events include germination, flowering, migration, etc.

Phosphatases  Enzymes that cleave phosphate groups off of ester bonds with hydrolysis. In this paper phosphatases refer to soil phosphatases that plants and microbes secrete into the soil to access organic phosphorus pools.

Phosphorus fixation capacity  The extent to which a soil can bind phosphorus as insoluble inorganic P.

Plasticity  The ability of one genotype to change its allocation strategy in response to environmental conditions.

pNPP  Poly-nitrophenyl phosphate, the synthetic substrate used in phosphatase assays. Not to be confused with NPP, which is net primary production.

ppm  Parts per million, typically expressed as microliters per liter for concentrations.

Retranslocation  The process of removing nutrients from senescing tissues and using them in living tissue or seeds.
Rubisco  Ribulose bisphosphate carboxylase oxygenase, the enzyme that fixes carbon in C3 photosynthesis.

Senescence  The process of severing leaves, branches, etc. that are no longer needed. Usually involves retranslocation of nutrients out of senescent tissue, hence senescent foliar tissue has a brown appearance.

Soluble inorganic phosphorus  Phosphorus that comes readily into solution, or phosphate.

Spectrophotometer  A machine that measures the absorbance of a solution at a given wavelength.

Stomata  Holes in the bottom of leaves through which carbon dioxide, water vapor, and other gases travel. The plant typically regulates the degree of openness.

Substrate  A compound on which an enzyme acts.

Supernatant  The liquid contents in a centrifuged tube.

Volatilization  The process of coming into a gaseous phase – used here mostly for ammonia and NOx.

Vortex  To spin a culture tube and homogenize its solution.

Zero-order kinetics  The part of the curve where enzyme activity relates linearly to substrate concentration.
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