Nutrient Manipulations in Terrestrial Ecosystems

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Introduction

Nutrient addition experiments are the ecosystem manipulations that are undertaken most frequently, because they are relatively easy and inexpensive to perform. In addition, because of the widespread nature of nutrient limitation in terrestrial ecosystems (Vitousek and Howarth 1991), these experiments almost always show dramatic results. As with much of ecology, the roots of nutrient manipulation experiments come from agriculture, where fertilizer trials are regularly used to determine which nutrients most strongly limit plant growth and what the biological and economic returns are for different levels of nutrient addition.

Ecological Questions Addressed by Nutrient Addition

Most early nutrient addition experiments in ecology addressed questions that were similar to those of agronomists: Are nutrients limiting to plant growth, or which nutrients most strongly limit plant growth in a particular location? These experiments demonstrate that nutrients are among the factors that limit plant growth in most terrestrial ecosystems (Chapin 1980; Vitousek and Howarth 1991; Shaver et al. 1986), and that the particular nutrients that are limiting change through space and time. The extension of these site-specific patterns of nutrient limitation across the landscape have led to an understanding of large-scale ecological issues such as changes in nutrient availability and limitation through succession and soil development (Chapin et al. 1994; Vitousek and Farrington 1997; Walker and Syers 1976). Nutrient addition experiments have also been used to elucidate the roles of nutrients in determining plant community dynamics (Wedin and Tilman 1993; Berendse et al. 1992; Willems et al. 1993; Bobbink 1991), microbial characteristics (Lovell and Hatch 1998; Clarholm 1993; Stamford et al. 1997), and ecosystem processes (Bremer and Kurkmar 1997, Magill et al. 1997).

By using isotopically labeled fertilizer, it is possible to track the quantity of added nutrients that is readily available for plant uptake, versus the amount that is adsorbed, leached, lost as gas, immobilized by microbes, or incorporated into soil organic matter. Isotopes also enable us to follow the dynamics of the added nutrients through time, tracking how long they are retained and the mechanisms of retention. By comparing plant uptake of labeled (fertilizer-derived) and unlabeled (native soil-derived) nutrients, we can gain important insights into how nutrient additions alter nutrient cycling and the role of native soil nutrients in plant nutrition. Also, by tracing the relative uptake of labeled nutrients by different plant species, we can determine how plants compete for resources at different nutrient levels.

The role of nutrients as a control over ecosystem processes has recently received more attention due to concern over the effects of atmospheric deposition of nitrogen (N), and to a lesser extent, sulfur, (S), on ecosystems. Nutrient deposition can have profound effects on plant community composition (Aerts and Berendse 1998; Gunn 1995; Hogg et al. 2000).
plant production (Aber et al. 1993), microbial communities (Arnolds 1989; Munzenberger et al. 1995), and biogeochemical cycles (Aber et al. 1993; Feijtel et al. 1989; Vitousek et al. 1997; van Breemen and van Dijk 1988; Friedland et al. 1991). Nutrient addition experiments are critical tools in helping ecosystem ecologists determine critical thresholds of system responses to increased atmospheric deposition. For example, N or S deposition can acidify the soil, leading to aluminum toxicity and a loss of essential nutrients such as calcium (Ca), which has been linked to the decline of forest systems (Lawrence et al. 1995; Fernandez and Russtad 1990; Forsius et al. 1995; Wilson and Skeffington 1994).

The increase in nutrient deposition has made an understanding of ecosystem nutrient limitation and its thresholds critical in mitigating human perturbations of the global system. The ability of a system to retain N plays a crucial role at the regional and global scales. Nitrogen leaching into groundwater pollutes streams and lakes, while N trace gas losses can increase tropospheric ozone and contribute to stratospheric ozone depletion and global warming (Schlesinger 1997). The response of plant biomass to nutrient additions is critical in determining the capacity of terrestrial systems to serve as a carbon (C) sink for elevated carbon dioxide (CO₂) emissions.

Nutrient addition experiments are becoming increasingly important in addressing applied issues with important economic and health consequences. For example, Scandinavian tundra and forest tundra are typically dominated by lichens, which serve as the major winter food for reindeer, which in turn are an important cultural and economic resource for Saami people. Nutrient addition experiments show that lichens are extremely sensitive to N deposition. The results of these experiments suggest that the pollutants carried northward from eastern Europe seriously threaten the reindeer industry of Scandinavia (Woodin 1997).

The capacity of ecosystems to retain or “filter” nutrients is also an important applied issue that can be addressed by nutrient addition experiments. Wetlands and riparian zones have been suggested as natural filters for nutrient-rich waters from agriculture or sewage treatment plants (Jensen et al. 1994; Rogers et al. 1991; Daniels and Gilliam 1996). Once the nutrient retention capacity of any ecosystem is exceeded, the nutrients leach into groundwater. Groundwater with a high nitrate concentration is a health concern, because the nitrate can be converted to nitrite, which is toxic in low-oxygen environments (such as a human fetus, where fetal hemoglobin is a strong competitor for oxygen) (Bouchard et al. 1992; Fan and Steinberg 1996).

Nature of Nutrient Limitation

It is not always clear exactly what is meant by the phrase nutrient limitation; it has been used to describe the response of various components of the overall ecosystem to nutrient additions. Historically, the most common test of nutrient limitation is to determine if the growth of a given plant community is limited by a particular nutrient. In this case, the initial growth response of plants indicates whether this plant community is nutrient-limited, and biomass measurements must be taken before the plant community changes. However, even though the overall community biomass may respond to one nutrient, some component species may be limited by others (Bobbink 1991; Jones and Martin 1964), and thus nutrient limitation within a given site can vary among patches with different species composition.

Altered nutrient supply usually changes competitive balance, leading to changes in species composition. For example, in Alaskan tussock tundra, most species responded favorably to nutrient addition for the first three years of the experiment, but after nine years, tall shrubs, which benefited most from the nutrient addition, produced a dense canopy that reduced the growth of mosses and evergreen shrubs (Chapin et al. 1995). Körner (Körner 1995; Körner and Larcher 1988; Körner 1989; Körner 1999) argues that this competitive exclusion of low-nutrient-adapted species following addition of nutrients implies that these species are not nutrient-limited. We argue that the concept of nutrient limitation for individual species or a given community is most useful as a gauge of the initial growth response of plants to nutrient addition prior to changes in competitive balance. In contrast, the changes in the plant community in response to nutrient additions must be incorporated into the concept of nutrient limitation of an ecosystem, since
the community shifts are a critical factor determining the response of the overall ecosystem net primary productivity (NPP) and biogeochemical processes to nutrient additions (Bowman et al. 1993). Thus, for questions addressing the overall ecosystem capacity to fix C, the nutrient saturation point of an ecosystem, or the overall response of a system to nutrient deposition—the duration of the experiment should be long enough to incorporate these community shifts, which can be the most significant mechanism driving overall ecosystem response to nutrient additions.

Commonly Limiting Nutrients

Which nutrients are limiting can often be predicted based on the age of the system, climate (especially how weathered or leached the soil is), soil texture, parent material, and vegetation type. Most nutrient limitation experiments focus on nitrogen (N), phosphorus (P), and potassium (K). Nitrogen is the most commonly limiting nutrient to plant growth in temperate terrestrial systems (Vitousek and Howarth 1991). Phosphorus tends to be limiting in the lowland wet tropics (Tanner et al. 1998), on very old soils (Vitousek and Farrington 1997), and in legume-rich systems (Jones et al. 1983, 1990). Potassium tends to be limiting in areas of high precipitation, or very late in soil development (Tisdale et al. 1993). While N, P and K may be the most commonly limiting nutrients, it is not uncommon to find productivity limited by other nutrients such as calcium (Ca), magnesium (Mg), and sulfur (S). For example, California grasslands are often assumed to be N-limited, but many sites respond to P and S fertilization (Jones et al. 1983). Nitrogen, P, and K fertilizers often contain these other nutrients, so that some responses to these fertilizers may partially reflect responses to Ca, Mg, and S. This is especially true for legume-rich sites, which are often limited by nutrients such as S and P (Jones and Martin 1964; Jones et al. 1970).

Single Versus Multiple Nutrient Limitation

The simplest view of environmental limitation is that growth is limited by a single resource at any one time; another resource becomes limiting only when the supply of the first resource is increased above the point of limitation (Liebig’s law of the minimum). Several processes contribute to the multiple resource limitation observed in most ecosystems: (1) Plants adjust both root/shoot allocation and nutrient uptake capacity to maximize capture of (and minimize limitation by) the most strongly limiting resource (Chapin et al. 1987; Rastetter and Shaver 1992). (2) Changes in environment (e.g., rainstorms, pulses of nutrient supply) change the relative abundance of resources so that different factors limit NPP at different times. (3) Different species in an ecosystem are limited by different resources, so that ecosystem-scale NPP responds to addition of more than one resource. Each of these processes contributes to the response of ecosystems to multiple resources.

Experimental Design

General Approach

The design of nutrient-addition experiments must be tailored to the question addressed. A single application of nutrients at high addition rates (e.g., 10 to 20 g N or P m⁻²) is the simplest test of whether production of the ecosystem (or its dominant species) is limited by a particular nutrient at the time of nutrient application. The lack of response to low nutrient-addition rates could reflect lack of limitation by the nutrient applied or failure of the plants to gain access to the added nutrient due to chemical fixation (which frequently happens with phosphorus) or biological immobilization by soil microbes or vegetation on the ground surface (Chapin et al. 1986).

Experiments intended to examine the sensitivity of ecosystems to atmospheric deposition should use application rates that approximate natural deposition, which can range from considerably less than 1 g m⁻² yr⁻¹ to greater than 10 g m⁻² yr⁻¹ in industrialized areas. Low deposition rates typically occur on the west coasts of continents and other places remote from pollution sources. These additions should be added repeatedly to determine long-term response. One problem with these experiments is that control plots inevitably receive ambient deposition, so one can only test the impact of atmospheric deposition that is higher than ambient levels.
Experimental Setup

The appropriate design for nutrient addition experiments is similar to that of most field experiments. For example, many experiments use a randomized block design, in which replicate blocks are set out in "representative patches" of the ecosystem, with buffer strips between treatment plots. Each treatment is then randomly assigned within blocks. The size of the buffer strips depends on the mobility of the nutrient and the lateral root extension of the dominant plants. The most difficult task in implementing this design is in deciding what is a representative patch, and what is the domain over which the results can be generalized. The results of the experiment can only be rigorously generalized to the experimental area. For example, if the experiment is set out on a hillside, and patches of bare ground are avoided, the results can be generalized only to vegetated components of the ecosystem on that hillside. This would be an appropriate design for looking at the effect of nutrients on the competitive interactions among plants. In a study of this sort, inclusion of bare patches would only increase the variance without adding insight about competitive processes (Hobbs and Chapin 1998). If the experiment is intended to be generalized to a large region, either the replicate blocks must be dispersed over the entire region or the experiment must be repeated over the entire region. For example, to generalize about whether nutrient limitation changed through postglacial succession at Glacier Bay, Alaska, plots were distributed throughout a 100-km length of the bay, with 10 replicate blocks per successional stage but only one replicate block in each location (Chapin et al. 1994). In another series of studies conducted by several research groups, similar nutrient addition treatments were applied to tussock tundra throughout its range in Alaska, Britain, and Scandinavia (Tamm 1954; Goodman and Perkins 1968; Shaver and Chapin 1980; Shaver and Chapin 1995), demonstrating that growth of the dominant species (Eriophorum vaginatum) in this vegetation type was consistently nutrient-limited, but that the identity of the limiting nutrient varied regionally.

The extent of replication necessary in a nutrient-addition study depends on the magnitude and variability of nutrient limitation and the size of the nutrient addition. For experiments in which expected effects are large, a sample size of 4 to 6 may be adequate. However, if effects may be more subtle, as in isotope addition experiments or experiments simulating atmospheric deposition, or if the experiment deliberately incorporates substantial landscape heterogeneity (to increase the generalizability of results), larger sample sizes (e.g., 8 to 10) are essential. The actual experimental design chosen is usually a compromise between what is desirable and what is feasible.

Experimental Design

There are many factors to consider in designing a nutrient addition experiment, and the choices are specific to the question and system. There are many controls on the effectiveness of a given nutrient addition, including climate, soils, the potential of the vegetation to respond to nutrients, land use, date of germination relative to date of fertilization, limitation of other nutrients, and decreases in biomass due to diseases and herbivores (FAO 1987). These factors could confound the interpretation of nutrient-addition experiments by affecting the availability of nutrients added and the measurements of biomass response. These confounding factors can be minimized by carefully deciding the form of nutrient to add, the amount to add, the frequency and timing of additions, and when to measure the response variables.

The question to be addressed should guide which of these complicating issues to focus on. For example, when determining which nutrients are limiting at a given site, it is critical to add the different nutrients in forms with similar availabilities. Most N additions (ammonium [NH₄⁺], nitrate [NO₃⁻], urea) are immediately soluble and available for plant uptake. It would not be valid to compare the biomass response to these soluble N fertilizers with response to rock phosphate or elemental S, which have much slower nutrient supply rates. In addition, factors such as adsorption, trace gas loss, and leaching can prevent any of the added nutrients from being available to plants. These factors should be accounted for, and addition rates of different nutrients should be adjusted to ensure similar supply rates. This is especially crucial when comparing nutrient limitation across sites. Site differences in hydrology, soil adsorption, pH, and vegetation can
have large effects on partitioning, adsorption, volatilization, and leaching. Thus, a constant rate of fertilizer application may not add a consistent amount of nutrients to the plant-available pool, and site differences in response to fertilization may be due to the different availability of added nutrients, rather than due to differences in nutrient limitation.

The way that nutrients are added can influence plant uptake (Malhi et al. 1989), and the distribution of biomass and nutrients (Imo and Timmer 1992), which can have large impacts on the interpretation of results. The following sections present some general guidelines for designing a nutrient addition experiment, but for a complete review, Tisdale et al. (1993) is an invaluable reference.

Time Scale of Response

The interpretation of nutrient-addition experiments requires a clear understanding of the sequential response of different ecosystem processes to nutrient addition (Table 19.1) so that the appropriate response variable and time scale can be chosen.

1. After nutrients are applied, they must first reach the plant. If application rates are high, this movement to the plant may be virtually instantaneous. However, with low application rates, nutrients may be immobilized by surface mosses or soil microbes and never reach plants in detectable amounts. We expect chemical or biological immobilization of nutrients to be most pronounced in low-fertility soils.

2. Following plant uptake of nutrients, tissue nutrient concentrations typically increase before there is a growth response. If growth is seasonally programmed, is limited by the number of meristems, or occurs slowly due to environmental constraints, growth may lag behind the increased tissue concentration by as much as a year (Shaver and Chapin 1995). Still other plant responses, such as flowering, depend on initiation of floral meristems and are generally delayed even more than the growth response.

3. The initial growth response of plants reflects the limitation of growth of that given plant assemblage by nutrient supply.

4. The altered nutrient supply generally changes competitive balance (Tilman 1988), so that plants that are superior competitors in fertile soils begin to out-compete and may eventually eliminate species that were initially abundant under the original low-nutrient conditions. Incorporating these community shifts gives a measure of the overall ecosystem’s potential to respond to nutrient additions.

Addition Rates

As discussed above, the experimental question will determine the magnitude of nutrient additions. However, deciding on addition rates becomes more difficult when considering the variation in the factors that influence the availability of added nutrients. In order to detect if a given nutrient is limiting across a number of sites, it may be advisable to add large amounts of fertilizer to ensure that plants can access some of the added nutrients on all sites. There is no need to be concerned about fertilizer additions being available to plants in deposition studies because differences in adsorption and loss are important variables that determine how a system responds to deposition. Thus, for deposition studies, a low, constant fertilization rate is desired across sites.

How to Add?

The timing of addition can be important in determining the availability of nutrient additions to plants. High loss rates can result from additions at times of high precipitation or low plant uptake. In general, the decision of when to add fertilizer should be dictated by the question, and then by a balance between timing of fertilizer availability, plant demands, and environmental conditions that affect loss.

### Table 19.1. Typical time course of ecosystem responses to nutrient additions.

<table>
<thead>
<tr>
<th>Process</th>
<th>Time scale</th>
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</thead>
<tbody>
<tr>
<td>Diffusion through soil</td>
<td>Seconds to days</td>
</tr>
<tr>
<td>Microbial immobilization</td>
<td>Hours to year</td>
</tr>
<tr>
<td>Increased tissue concentration</td>
<td>Days to years</td>
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<tr>
<td>Increased plant growth</td>
<td>Weeks to years</td>
</tr>
<tr>
<td>Altered competitive balance</td>
<td>Months to decades</td>
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<tr>
<td>Altered species composition</td>
<td>Years to decades</td>
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</tbody>
</table>
Nutrients are typically added in solid form, although this can burn the surface vegetation. For example, mosses, lichens, and many evergreen shrubs are sensitive to high nutrient addition rates. However, addition of dissolved nutrients in a water solution is time consuming and can create artifacts if moisture is potentially limiting to growth.

Use of slow-release fertilizer (e.g., Osmocote or rock phosphate) is an excellent way to provide nutrients gradually, rather than in a single pulse. These fertilizers differ in the forms of nutrients present (ammonium vs. nitrate), the combinations of nutrients supplied, and in the rate of release. In other cases, pulsed nutrient input (as simulated by addition of soluble nutrients) may be more typical of natural ecosystem patterns (Cui and Caldwell 1997; Bilbrough and Caldwell 1997).

Ideally, the nutrients should be added to that component of the ecosystem where the processes naturally occur. For example, if the purpose is to trace N uptake by plants and soil microorganisms, isotopically labeled N should be distributed uniformly through the rooting zone rather than applied to the surface, where it might contaminate the plants or be immobilized by surficial vegetation (algae or mosses) or litter microbes. These experiments are most readily done with microcosms, in which the isotope can be injected into the soil using long "spinal-tap" needles that are soldered closed at the end but have holes drilled in the side of the needle (Jackson et al. 1989; Hungate et al. 1997).

Nadelhofer et al. (1995) have applied $^{15}$N to the litter layer of forests to follow the fate of nutrients returned to the forest floor in litterfall.

Form of Nutrients Added

Nutrients should be added in a chemical form that reasonably approximates the form that is available in that ecosystem. For example, N might be added as nitrate in ecosystems with high nitrification rates or as urea or $\text{NH}_4^+$ in ecosystems with low nitrification rates. In N deposition experiments, it is important to choose the form of N that is being deposited at the study sites (e.g., $\text{NO}_3^-$ in the eastern United States, $\text{NH}_4^+$ in the Netherlands), because these different forms of N can have very different effects on ecosystem and community response (Crabtree and Bazzaz 1993; Vaast and Zasoski 1992). The form of added nutrients is important, because inappropriate choices can create unintended side effects. For example, addition of ammonium chloride or sodium nitrate can cause chloride or sodium toxicity, create osmotic problems at high levels, or alter soil pH. Two options are to add ammonium nitrate or to add two ammonium forms which differ in their pH effects (see below). Which form is chosen also determines the fate and retention of fertilizer, and effects on ecosystem and community dynamics.

Many fertilizers contain multiple elements. For example, most commercial phosphate fertilizers are made with sulfuric acid and contain enough sulfate to act as S fertilizer in S-deficient soils. When choosing which form of fertilizer to use, pay careful attention to the other nutrients that are in the fertilizer; nutrients are commonly complexed to forms of other limiting nutrients (K, N, P, S, Ca), which could also play an important role in biomass response.

Practicality and economy are two other important considerations when choosing the appropriate form of fertilizer. For example, studies requiring large P inputs would necessitate as much as a 30-cm-deep layer of rock phosphate to achieve the desired P additions! Nutrient addition experiments that focus on agriculture or rangeland applications need to consider economy as well. Osmocote, or any other expensive nutrient source, would never be used by growers.

Nitrogen

Control of Fertilizer Nitrogen Availability

The major processes that decrease the fertilizer N available to plants are microbial immobilization, leaching loss, trace gas losses through nitrification and denitrification, ammonia volatilization, and adsorption of $\text{NH}_4^+$ on soil particles. To minimize loss through leaching and denitrification, it is best to avoid additions of N during very wet times. Ammonia ($\text{NH}_3$) volatilization can lead to substantial losses of added N, as high as 25 to 50% (Tisdale et al. 1993). Ammonia losses are especially high in systems with high soil pH, soil moisture around field capacity, high evaporation, and substantial plant residue at the surface of the soil. In such systems, N should be added as $\text{NO}_3^-$ rather than as $\text{NH}_4^+$ or urea.
Fixation of $\text{NH}_4^+$ on minerals is not a permanent loss—it is often a temporary retention mechanism that decreases nitrification and leaching loss. Ammonium fixation on soil is most common on coarse clays and fine silt, especially on vermiculite and illite. Fixation increases with freezing and drying. Soils with high concentrations of K and Ca tend to have very low $\text{NH}_4^+$ adsorption.

**Common Nitrogen Fertilizers**

Nitrogen is commonly added as $\text{NH}_4^+$ salts, $\text{NO}_3^-$ salts, or urea. Ammonium nitrate ($\text{NH}_4\text{NO}_3$) is a very common form of N fertilizer in nutrient addition experiments. Additions of this form ensure high N availability, without the confounding factor of adding other mineral nutrients, and it has little effect on soil pH. Disadvantages of using $\text{NH}_4\text{NO}_3$ are that it is more prone to denitrification and leaching than $\text{NH}_4^+$ salts, it must be stored carefully because it can absorb moisture and cake very easily, and, if it comes into contact with oxidizable C sources (such as fuel), it is very explosive, so it must be handled with care.

Ammonium salts have the best system retention of N in most cases, but they reduce pH and should not be used in acidic soils, except in combination with urea, which has the counteracting effect of raising pH. Care must also be taken in applying $\text{NH}_4^+$ salts to calcareous soils (Kissel et al. 1985); $\text{NH}_4^+$ salts that precipitate with Ca (fluoride ($\text{F}^-$), sulfate ($\text{SO}_4^-$), phosphate ($\text{PO}_4^-$)) can stimulate $\text{NH}_3$ volatilization and nitrification, leading to high loss rates of the N added. However, $\text{NH}_4^+$ additions with chloride ($\text{Cl}^-$), $\text{NO}_3^-$, and iodide ($\text{I}^-$) are suitable for application to calcareous soils (Tisdale et al. 1993). In addition, at high pH, additions of $\text{NH}_4^+$ can lead to the accumulation of nitrite ($\text{NO}_2^-$), which is toxic to plants and microbes. Ammonium sulfate is the most acidic N addition (Boswell 1985), and should be avoided in low pH soils. Ammonium phosphate can be used as a fertilizer in situations in which both N and P additions are required. Ammonium chloride ($\text{NH}_4\text{Cl}$) can be problematic because some plants are sensitive to it at relatively low concentrations, and at high concentrations, chloride can be toxic to all plants. This is especially an issue in long-term additions, or areas where $\text{Cl}^-$ can accumulate quickly (such as arid regions with high evaporation).

Nitrate salts are not as acid as ammonium salts. Nitrate accompanied by base cations (e.g., sodium ($\text{Na}^+$), $\text{Ca}^{2+}$) can increase pH, and should be avoided in basic soils. Nitrate fertilizers are very soluble and mobile and are subject to high loss rates through leaching and denitrification. However, in some systems, long-term fertilization studies have seen higher plant yields in response to $\text{NO}_3^-$ than $\text{NH}_4^+$ due to the negative effect of $\text{NH}_4^+$ on soil acidity (Koren'kov 1983). Common sources of nitrate are $\text{NaNO}_3$, $\text{KNO}_3$, and $\text{Ca(NO}_3)_2$.

Urea is one of the most common forms of N additions. It is relatively inexpensive and easy to handle, it has much less tendency to absorb moisture and cake than ammonium nitrate, and it is not prone to explosion. However, there are many problems associated with urea additions, and careful attention must be paid to the characteristics of a system before deciding that its use is appropriate. Urea should never be added to soils with high pH (pH greater than 7.5). As with ammonium applications, high pH can lead to nitrite accumulation, as well as high rates of ammonia volatilization. This can occur with urea applications even in acid or neutral soils, because urea can substantially increase soil pH (Bremner 1995).

Another problem associated with urea application is that it often contains biuret, an impurity that inhibits germination and seedling growth. Because of this, as well as the toxicity of high concentrations of $\text{NH}_3$ to plants, urea should not be added just before germination, or early in the growing season. Urea losses can be minimized by subsurface applications, rather than a broadcast surface application.

Another important option for N additions is slow-release fertilizers, which are the easiest way to apply N gradually and continuously. Slow-releasing N is available in a number of different forms, but it is most commonly supplied as urea, particularly in the slow-releasing fertilizers that do not also supply P and K (Hauck 1985).

**How to Add Nitrogen Fertilizers**

As discussed above, the form of N chosen will dictate what conditions minimize losses and negative effects of fertilizer N. In general, N fertilization is optimized when added at the time of peak plant uptake. Nitrate additions should be avoided in areas with high rainfall, or that are very wet, in order to
Phosphorus

Control of Phosphorus Fertilizer Availability

The availability of fertilizer P to plants is limited mostly by its solubility and its adsorption to soil minerals. Adsorption is especially high in soils with high clay contents and in weathered acid soils, which tend to have high concentrations of aluminum oxides and iron oxides. Adsorption is higher on 1:1 clays such as kaolinite than on 2:1 clays, such as montmorillonite. The pH is a critical factor determining adsorption. Although different minerals adsorb P maximally at different pHs, in most soils P availability is maximal at pH 5.5 to 6.5. The presence of divalent cations also can substantially increase P adsorption. For example, clays saturated with Ca⁺⁺ have higher adsorbed P than those with Na⁺. Thus, calcareous soils often have low P availability. Phosphate can also compete with other ions for adsorption sites, so its adsorption is decreased in the presence of OH⁻, H₂SiO₄⁻, SO₄²⁻, and MoO₄²⁻. Phosphorus adsorption is more permanent than N adsorption. Once P is adsorbed, it does not become available to plants at any great quantities. To make up for this, P fertilization must usually be high in order to cause any appreciable increase in P availability. For example, it is common to add P at half the rate that N is added, although plant tissue contains about 14 times more N than P.

In addition to adsorption, it is also necessary to consider precipitation of the phosphate ions. Adsorption usually occurs once phosphate enters the bulk soil, whereas precipitation occurs close to fertil- izer granules. High concentrations of calcium, aluminum and iron could cause substantial precipitation, while the use of other fertilizer salts, such as (NH₄)₂SO₄, NH₄NO₃, NH₄Cl, KNO₃, K₂SO₄, and KCl, can decrease P precipitation.

It is important when comparing the response of growth to P fertilization among different sites, that one consider these different factors regulating P availability.

Common Phosphorus Fertilizers

There are two main types of P fertilizer: a water-soluble form that is immediately available to plants, and P in rock phosphate, which is slowly available in acid conditions.

Rock phosphate is a good way to provide a small, continuous supply of P to a system. The P supply to plants is optimized if rock phosphate is finely ground and mixed into the soil, but it is only effective in acid soils (pH <6.0). It is most effective in systems with warm, moist soils, and long growing seasons. Application rates must be high to achieve effective fertilization. It should be noted, however, that long-term studies have shown that even after 4 to 10 years, a single application of superphosphate can enhance P availability to a greater extent than fertilization with rock phosphate (Bolland et al. 1989).

The water-soluble forms offer the advantage of immediate availability of P, and are much more comparable to forms of other nutrient additions such as NH₄NO₃ and KCl. In general, there are very small differences in supply rate between the different water-soluble P fertilizers. Water-soluble P is often added as single superphosphate or triple superphosphate, which are calcium orthophosphates. These work well in nutrient limitation experiments as long as the site is not calcium limited. These have no effect on soil pH, and almost all of the P is water soluble and plant available. Single superphosphate contains substantial S, which is beneficial for combined-nutrient additions, but is not a satisfactory way to test for P limitation in soils that may also be S limited. Triple superphosphate, on the other hand, has very little S (0 to 1%) and is much better for such applications. Phosphorus is often added as ammonium phosphates, but this is not a satisfactory way to add P if trying to test for N versus P limitation.

Phosphorus can also be added as a slow release fertilizer. For example, Osmocote produces P₂O₅ pellets.
How to Add Phosphorus Fertilizer

Even with the best P fertilization management, plants often take up less than 25% of added P (Tisdale et al. 1993). In high-fixing soils, rather than adding P in one application, several smaller, more frequent applications can maximize plant P availability. If adding a water-soluble form, it is desirable to minimize contact with the soil, so large granules should be used. In contrast, very fine granules maximize release of phosphate from water-insoluble fertilizers (Young et al. 1985).

The best time to add water-soluble P is just before germination, because P availability rapidly decreases with time after fertilization (Bramley and Barrow 1992).

Potassium

Control of Fertilizer Potassium Availability

The availability of K fertilizer is determined by many different factors, and different soils can be drastically different in the amount of fertilizer needed to increase exchangeable K concentrations. Potassium, like NH₄⁺, can be temporarily fixed onto soils. However, at very high pH, soil fixation can be permanent as K becomes trapped between the collapsed silicate layers of clays. Wet soils substantially increase K fixation, and K is gradually released as the soil dries. Adsorption characteristics of soils for K are opposite to those of P; the highest K fixation is seen in 2:1 clays with high illite, while 1:1 clays, such as kaolinite, do not fix substantial amounts of K. Fine soil textures increase fixation onto soil, while aluminum ion (Al³⁺) concentrations decrease fixation. In very acid soils, high concentrations of Al³⁺ and magnesium ions (Mg²⁺) inhibit plant K⁺ uptake, and could interfere with growth response to fertilization. Leaching losses of K are relatively small in most soils, but could be substantial in sandy soils, or humid tropical soils with high rainfall.

Assessment of K limitation is more complicated than for other nutrients because it is not a simple function of K available in soil solution, but is more dependent on the ratio of K⁺ to Ca²⁺ and Mg²⁺, which compete with K⁺ for entry into the plants. So in soils with high calcium and magnesium, higher amounts of K must be added to become available to plants. In addition, when adding a combination of nutrients that includes K, be sure to avoid adding other nutrients complexed with Ca or Mg (such as CaSO₄).

Common Potassium Fertilizers

All commonly used forms of K fertilizer are equally effective in supplying plant-available K. The major factor determining the appropriate choice depends on the other nutrients being tested or added. Potassium is most commonly added as KCl (muriate of potash), which is readily dissolved in water. There are some plants that are intolerant to Cl⁻, in which case another form must be used. Potassium chloride also has limited applications in long-term fertilization, because Cl⁻ accumulation can be detrimental to plant growth or microbial activity. Potassium sulfate (K₂SO₄) is often used in Cl⁻-sensitive situations and is an appropriate choice for systems where S is not limiting to growth.

Potassium carbonate (K₂CO₃), potassium bicarbonate (KHCO₃), and potassium hydroxide (KOH) are very effective fertilizers, and when used on acid soils, they will decrease the leaching of cations. Potassium phosphates are good for gradual applications because they have more controlled solubility than the other forms of K fertilizer. Other forms that are commonly used are potassium magnesium sulfate and potassium nitrate. Long-term or high rates of K additions usually use potassium nitrate (KNO₃) or potassium pyrophosphate (K₄P₂O₇) in order to avoid salt accumulation problems. Unfortunately, because these are complexed with the two most commonly limiting nutrients, these forms can’t be used to separately test K, N, and P limitation.

How to Add Potassium Fertilizer

Potassium fertilizers are most effective if added just before or at the time of germination or onset of growth. Higher leaching losses can occur if K is applied long before plant uptake will occur.

Sulfur

Control of Sulfur Fertilizer Availability

Sulfate (SO₄²⁻), like nitrate, can be readily lost through leaching, especially in soils where monovalent cations predominate (K⁺, Na⁺). Adsorption
can be high in soils with high clay content and high concentrations of aluminum oxides and iron oxides. Adsorption increases at low pH, and is greater in soils with kaolinite than with illite or montmorillonite. This adsorption is readily reversible and will only affect short-term S availability, and probably increase long-term availability by preventing S leaching. Sulfate can also be removed from the plant-available pool by precipitating with calcium carbonate (CaCO₃).

**Common Sulfur Fertilizers**

Like phosphorus, S has two main forms of fertilizer, a readily available water-soluble form and a slowly available insoluble form.

Sulfate additions are immediately plant available, and supply rates are comparable with NH₄NO₃, KCl, and water-soluble P additions. Different forms of soluble S are approximately equal in their efficiency to supply S to plants, unless complexed with zinc (Zn), copper (Cu), or Mn. All forms of water-soluble S are complexed with other important nutrients (N, P, iron [Fe], Ca, K, Mg), so careful consideration of other limiting nutrients in a system must be made before deciding on which form of S fertilizer to use. Gypsum (CaSO₄) is one of the most common ways to add sulfur. It is also possible to add S as single superphosphate if a combined application with P is desired. In order to minimize S leaching loss, applications of sulphate should be made close to the time of peak plant uptake.

The second main form of S fertilizer is elemental sulfur (S₀), which is a water-insoluble solid. When finely ground and mixed with soils, it is oxidized to SO₄²⁻ by microbes. The relative effectiveness of S₀ application is determined by the particle size added, how and when it is added, the S₀ oxidizing characteristics of soil, and environmental conditions. Finer-size particles supply SO₄²⁻ at a faster rate. A mixture of S₀ particle sizes is sometimes desirable in order to achieve both rapid and long-lasting S availability. Dissolution of SO₄²⁻ is maximized if the pellets undergo wet-dry or freeze-thaw cycles. A way to maximize release of plant-available S is to use irregularly shaped, porous granules. Alternatively, S₀ can be applied with bentonite, a clay that absorbs water and causes the S₀ granules to disintegrate.

The timing of additions is crucial; S₀ must be added as far ahead of germination and growth initiation as possible (Beaton et al. 1985). Even when adding S₀ with bentonite or porous granules, a few months are needed for substantial release of plant-available S. Elemental sulfur applications are more efficient if they are incorporated into the soil. A possible problem associated with S₀ additions is acidification of the soil, but this can be avoided by making sure that the fertilizer is uniformly distributed (Beaton et al. 1985).

**Isotopes**

The use of isotopically labeled fertilizer allows ecologists to ask more refined, mechanistic questions. Isotopes can serve as an invaluable tool to check the efficiency of nutrient additions by tracking the fate of fertilizer additions, and thus helping to determine if the lack of response of a system to nutrient additions is mediated by low fertilizer availability due to adsorption, loss, or microbial immobilization (Drury and Beauchamp 1991). Isotopes can also be used to look at the long-term fate and availability of fertilizer (Preston and Mead 1994). Reciprocal transplants of litter from plots fertilized with labeled and unlabeled nutrients can help track recycling of fertilizer nutrients from the litter (Power et al. 1986).

Isotopes can also elucidate the mechanisms of ecosystem response to nutrient additions. Community composition changes may be explained by differences in resource competition at different nutrient levels, which can be determined by comparing labeled fertilizer uptake by different species (Nannipieri et al. 1985; Chang et al. 1996). Comparing amounts of labeled and unlabeled nutrients in soil and plant pools can elucidate how nutrient additions alter native soil nutrient availability (Pilbeam et al. 1997; Hart et al. 1986; Clinton and Mead 1994).

Isotopes can be particularly useful for studying system response to nutrient deposition. Isotopically labeled nutrients can elucidate the pathways and controls over nutrient movement through ecosystems, and can point to the mechanisms of ecosystem retention and loss (Nadelhoffer et al. 1995; Buchmann et al. 1996; Koopmans et al. 1996).
Isotopically labeled fertilizers are most commonly enriched. The minimum level of enrichment needed depends on how subtle the effects of a treatment are, and the rates of cycling and loss of nutrients, especially in relation to how long after addition the effects must be detected. For short-term experiments looking at more obvious changes, the less expensive isotopically depleted fertilizers or low-enrichment fertilizers can be used (see Power et al. 1986).

Nitrogen and S have stable isotopes that are valuable in fertilizer-labeled studies as well as in tracing the fate of atmospheric deposition. The radioactive N isotope has too short of a half-life to be useful in fertilizer applications. Phosphorus and S also have radioactive forms. The P isotope is short-lived, and is generally only used for short-term partitioning experiments to detect trace levels of native organic N uptake. The S isotope is used to determine the effect of S fertilization on S dynamics (Eriksen 1996; Ghani et al. 1993).

Nitrogen-15 is the most common isotopically labeled fertilizer used. It has been used in many studies to elucidate fate (Nannipieri et al. 1985; Bristow et al. 1987), adsorption (Foster et al. 1985), and long-term versus short-term fertilizer availability and dynamics (Preston and Mead 1994; Chabrol et al. 1988; see Schimel 1993 for a good review). It has also been used to trace the fate of fertilizer nutrients in litter over time (Power et al. 1986; Muller 1988). (For a review of N fertilizer methods, see Hauck et al. 1994.)

The use of N fertilizers has led to interesting findings. For example, it is often assumed that microbial immobilization of fertilizer is a short-term mechanism for ecosystem retention, and microbial N will eventually turn over and become available to plants. Several studies tracing the fate of N fertilizer did not see this microbial N being made available to plants, even over an extended period of time (Proctor and Mead 1994; Rutherford and Juma 1992). Another surprising result from studies was that the ecosystem retention of N in a jack pine forest was dominated by chemical fixation of NH₃, which was substantially higher than microbial immobilization (Foster et al. 1985).

While isotopes can be invaluable tools, interpretations must be made carefully. Studies of N fertilizers have often shown that fertilizing increases plant uptake of unlabeled soil N, which has been attributed to increases in soil organic N mineralization (Pilbeam et al. 1997) and increases in plant uptake rates (Leon et al. 1995). These increases in native organic N uptake may not necessarily be due to increased availability or uptake, but may be an artifact of fertilizer application. Microbes may have immobilized the flux of N fertilizer, allowing native soil N that would normally be immobilized to be taken up by plants. This pool substitution could lead to an overestimate of the effect of fertilizer on N cycling, and an underestimate of nutrient uptake by plants in response to fertilization. (Hart et al. 1986; Jenkinson et al. 1985). Interpretation of isotope data must also consider isotope fractionation in uptake and soil processes, especially when using depleted, or low-enrichment fertilizer. This is especially true in systems with even small amounts of NH₃ volatilization, which discriminates very strongly against N, and will enrich the soil pool relative to the unlabeled pool. In such systems, the use of N rather than urea or ammonium is recommended (Nomnik et al. 1994).

These problems in interpretation of isotopic fertilization experiments can be minimized by using trace levels of highly enriched label to trace the natural movement of nutrients in ecosystems. For example, to resolve the question of whether the increase in unlabeled plant N described above is an artifact of fertilizer additions, or a result of N addition altering N cycling, unlabeled fertilizer additions could be followed by trace amounts of highly enriched N to document nutrient partitioning and gross rates of nutrient transformations. Tracer additions should be high enough for the isotope to be detectable for the duration of the experiment, but not so high as to perturb natural process rates by "fertilization" (Jackson et al. 1989; Davidson et al. 1991). Low-level N addition can determine rates of microbial immobilization in the absence of roots (Davidson et al. 1991; Hart et al. 1994); the parti-
tioning of inorganic N among plant uptake, microbial uptake, and chemical fixation in the presence of plants (Jackson et al. 1989; Norton and Firestone 1996; Recous et al. 1992; Hart et al. 1993); or the time course of N movement from the inorganic pool to microbes (including mycorrhizae) to plants (Buchmann et al. 1996).

Isotopes can be used in a variety of approaches. If the main objective of the study is to track fertilizer fate, these isotopically labeled fertilizers should be used. However, if the main objective of the study is to document changes in nutrient cycling in response to fertilization, it would be better to fertilize with unlabeled nutrients, and then add trace amounts of labeled nutrients to track the movement of the overall nutrient pool through the system, or to determine gross rates of nutrient transformations.

Alternatives to Nutrient Addition Experiments

Nutrient addition experiments are not the only way to assess nutrient limitation in ecosystems. For example, the N/P ratio in plant tissues is an excellent indicator of the type of nutrient limitation (Redfield 1958; van den Driessche 1974; Ingestad 1979; Aerts et al. 1992; Koerselman and Muelemann 1996). A plot of P concentration against the N concentration in unfertilized plots was an effective predictor of the biomass response to N vs. P (Fig. 19.1) (Koerselman and Muelemann 1996). At N/P ratios >16, the community biomass was P-limited, whereas at N/P ratios <14, N limited plant growth in all but one study. At N/P ratios between 14 and 16, plants responded to both N and P. The N/P ratio was a better predictor of nutrient response than was tissue concentration, which varied 3-fold for N and 16-fold for P. There was no clear relationship between the nutrient concentration in plant tissue and the nature of nutrient limitation (Koerselman and Muelemann 1996).

The effects of nutrients on ecosystem dynamics can also be elucidated through nutrient depletion experiments. Carbon-rich (low-nutrient) substrates, such as sawdust or starch, can be added to increase the C/N ratio of soils, leading to microbial immobilization of nutrients and a reduction in nutrient availability to plants (Yarie and Van Cleve 1996). Studies of 15N fertilization have indicated that this N immobilized by microbes may not become available to plants, even after an extended period of time (Rutherford and Juma 1992; Jonasson et al. 1996). However, this approach should be used with caution, because labile C additions can also stimulate nutrient cycling and availability.

Other methods to decrease nutrients are harvesting of litter or plants, burning, and topsoil removal (Marrs 1985). These methods are appropriate only for certain questions and have substantial ecosystem effects in addition to the reduction in nutrient supply.

Another approach to studying the effects of nutrient additions has been to take advantage of depositional gradients of N and S. This approach has been used in many studies to look at the effects of deposition on plant communities and ecosystem processes (Randlett et al. 1992). Isotopes can be particularly helpful here, because N and S from atmospheric deposition have different isotopic sig-
Summary and Conclusions

Nutrient addition experiments can be used to address a variety of questions, including the role of nutrients in mediating ecosystem processes, nutrient effects on plant and microbial community dynamics, the limitations on plant growth and ecosystem production, and the response of ecosystems to increased atmospheric deposition of nutrients.

When designing a nutrient addition experiment, it is critical to minimize the impact of the fertilizer additions on soil pH, salt toxicity, and the supply of other potentially limiting nutrients that are not being tested (e.g., S, Ca). It is also important to understand whether a lack of response to fertilization is due to the absence of limitation for that nutrient, or the failure of the nutrient additions to become available to plants. This is especially important when comparing responses of plant growth to nutrient additions across sites, where factors controlling fertilizer availability could differ substantially.

Isotopes are an invaluable tool for attaining a mechanistic understanding of the fate and dynamics of nutrient additions. They can be used to test how much of the fertilizer becomes available to plants, elucidate mechanisms of nutrient retention and loss, detect changes in soil nutrient dynamics, and trace competitive uptake between different plant species and microbial groups. The incorporation of isotope methods into nutrient addition experiments should solidify our understanding of the controls of nutrients over all aspects of the ecosystem.

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Cover illustration: Diagram developed by A. T. Austin and O. E. Sala representing the different scales of study in ecosystem science. The questions asked by the ecosystem scientist will determine the scale of study and the variables to be evaluated, which range from interpretation of satellite imagery at the global scale to microbial populations in a milliliter of water or a gram of soil. A multidisciplinary approach and a broad range of methodology are necessary to address the newest challenges in ecosystem science.