Soil nitrogen dynamics under simulated global changes in a California annual grassland

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Submitted to the Earth Systems Program,
Stanford University, in Partial Fulfillment of
the Requirements of Master of Science

September 22, 2002

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Dedication

To my grandfather

Clark Kerr

for teaching me the value
of education and hard work,
and for setting an unforgettable example of
a life lived for the benefit of humankind.

Abstract

Soil nutrient availability plays a key role in ecosystem ecology and may be directly impacted by global change. This study used ion exchange resin bags to measure nitrate (NO_3^-) and ammonium (NH_4^+) availability in a California annual grassland under four global change treatments: warming, elevated CO_2, increased precipitation, and nitrogen deposition. The only significant treatment effect averaged across the entire growing season was nitrogen deposition, which increased both nitrate and ammonium availability by approximately 50%. However, many treatment effects showed seasonal variation. At the end of the growing season, increased precipitation significantly increased availability of both nitrate and ammonium. Warming had a positive effect on ammonium availability at the end of the growing season, with a significant interaction between warming and CO_2. Elevated CO_2 alone had no effect on ammonium availability and caused a slight decrease in nitrate availability. Our results suggest that the overall consequence of anthropogenic global change will be to increase soil nitrogen availability in this ecosystem, at least temporarily, but with complex seasonal and treatment interactions.

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List of Abbreviations

ANOVA   analysis of variance

CO2   elevated carbon dioxide (abbreviation in ANOVA table)

FACE   free-air carbon dioxide enrichment

HEAT   elevated temperature (abbreviation in ANOVA table)

IER   ion exchange resin

JRGCE   Jasper Ridge Global Change Experiment

N   nitrogen deposition (abbreviation in ANOVA table)

PVC   polyvinyl chloride

SEM   standard error of the mean

WATER   increased precipitation (abbreviation in ANOVA table)

Introduction

Soil nutrients, more often than any other environmental factor, limit the growth of plants and soil microorganisms. As a result, soil nutrients have wide-ranging and often surprising effects on ecosystem function – for example, by changing plant community composition (Vitousek et al., 1997) or by limiting the response of an ecosystem to elevated CO_2 (Zak et al., 2000*a*). Human-induced global change is likely to affect soil nutrient availability, but much remains to be learned about the magnitude and even the direction of these effects.

Nitrogen deserves particular attention. An essential component of all living cells, and the nutrient required in greatest amount by plants (Taiz and Zeiger, 1998), nitrogen is also the most commonly limiting nutrient in terrestrial ecosystems (Stark, 2000). Nitrogen has been affected by human activity, both directly and indirectly, more than any other major nutrient. Although the nitrogen cycle has been studied for many decades, we have only begun to investigate how it will be impacted by global change.

Principles of soil nitrogen availability

A variety of biotic and abiotic factors affect soil nutrient availability, including nutrient input, soil particle size, soil moisture, acidity, salinity, and the activity of roots and microbes (Mengel and Kirkby, 2001; Binkley and Vitousek, 1989). Although availability of most nutrients is determined by inorganic equilibria (Fitter and Hay, 2002), nitrogen availability is largely under biological control[1].

The dominant N pool in the soil nitrogen cycle (represented in Figure 1) is organic N. Plants, however, cannot easily take up organic N, and therefore depend on inorganic N for most of their nitrogen supply (Kaye and Hart, 1997). Inorganic N is supplied primarily by microbes through the process of *mineralization* (Williams et al., 2001). Between 50 and 99% of mineralized N is immediately taken up again by microbes in a process called *immobilization* (Binkley and Vitousek, 1989). The remainder (net mineralization) is generally considered to be available to plants. Since mineralization and immobilization occur simultaneously, plants may

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[1] Nitrogen has both important similarities to and important differences from other nutrients. The phrase "nutrient availability" will be used whenever a generalization is appropriate; otherwise, "nitrogen availability" will be used (in reference to inorganic nitrogen unless otherwise specified).

have an adequate N supply if the microbial turnover rate is high enough, even if there is little N available at a given instant (Hu et al., 1999).

Inorganic N occurs in two main forms: ammonium (NH_4^+) and nitrate (NO_3^-) (Mulvaney, 1996). Since plants must convert nitrate to ammonium before assimilating it (Taiz and Zeiger, 1998), plants are thought to use ammonium preferentially to nitrate, though this is still debated (Foth and Ellis, 1997). However, ammonium binds to the cation exchange sites on clay particles (Brady and Weil, 2001), making it much less mobile than nitrate and therefore more difficult to acquire. The relative importance of each of these ions varies considerably from ecosystem to ecosystem.

Nitrate and ammonium are transported through the soil by two major processes: mass flow (water movement), and diffusion. Mass flow dominates when soil moisture and transpiration are high, and when nutrients are abundant; diffusion dominates under dry or nutrient-limited conditions. Both mass flow (Foth and Ellis, 1997) and diffusion (Fitter and Hay, 2002) have been shown to be important for nitrate; diffusion is usually more important for ammonium.

Rather than simply waiting for nutrient transport to take place, soil organisms can play an active role in nutrient uptake. For example, plants can alter fine root production, mycorrhizal colonization, and rhizosphere chemistry in order to obtain more nutrients (Binkley and Vitousek, 1989). Fine root production and root nutrient uptake kinetics can also be controlled in response to changing environmental conditions, but little is known about these processes (Zak et al., 2000b; BassiriRad, 2000).

Soil organisms can compete directly with each other for nutrients. For example, plants and microbes compete for inorganic nitrogen, though the outcome
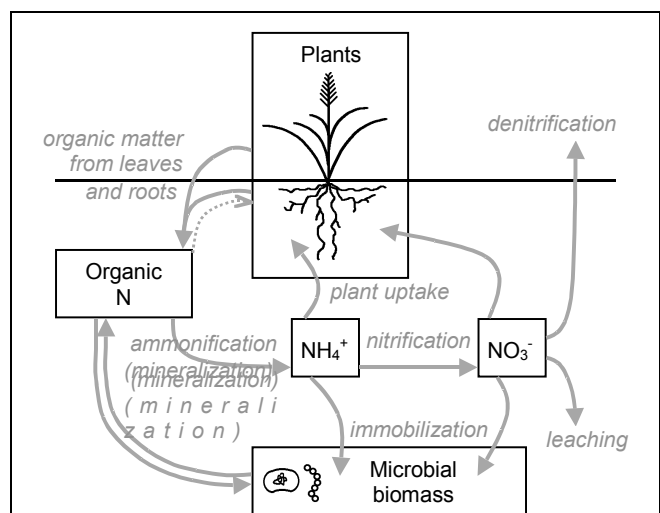

Figure 1. Model of soil nitrogen transformations. Each step in the soil nitrogen cycle may be affected by global change. Adapted from Kaye and Hart (1997) and Brady and Weil (2001).

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of the competition can be hard to predict (Kaye and Hart, 1997). Microbes are usually C-limited, but when the C:N ratio in litter exceeds 20:1, they are likely to become N-limited and remove most of the available inorganic N (Fitter and Hay, 2002), reducing the amount available to plants. The balance of competition may depend on the ratio of plant to microbial biomass (Zak et al., 2000*a*). On the other hand, soil microbes can also increase N availability, not only through mineralization, but also by fixation and mycorrhizal association.

The complex physical and biological processes which determine soil nitrogen availability can also make it difficult to measure in a realistic way. Total soil N content, as well as net and gross mineralization, can be measured in laboratory assays; however, these measures do not always accurately reflect N availability in the field (Binkley and Vitousek, 1989). For this study, we chose to use ion exchange resin bags, because they can overcome some of the limitations of other techniques.

Measuring nitrogen availability with ion exchange resin bags

Binkley and Matson (1983) pioneered the technique of using ion exchange resin (IER) bags to measure nutrient availability. There are a great diversity of IER techniques, and some confusion about how to compare their results (Skogley and Dobermann, 1996), but they all work on the same basic principle.

Ion exchange resins are solid polymer beads with a large internal surface area which is permanently charged (see Appendix 3 for more information). Their numerous ion exchange sites allow them to readily take up any free ions in the soil, making them useful in soil nutrient availability studies. IER bags have several advantages over other soil nutrient assays: they are exposed to ambient conditions, are in intimate contact with the rhizosphere, and cause minimal disturbance to the soil (Arnone, 1997).

In many ways, ion exchange resins work similarly to plant roots. The amount of inorganic nitrogen they capture depends on a variety of factors, including mineralization and immobilization, water flow, and diffusion rates (Binkley and Vitousek, 1989; Skogley and Dobermann, 1996). However, there are some important differences: unlike soil organisms, resin bags have no active uptake mechanisms, so nutrient transport processes are relatively

more important to resin bags than to plants or microbes (Stark, 2000). Furthermore, water may flow differently through resin bags than through bulk soil (Torbert and Elkins, 1992).

Also, it is not known how effectively IER bags compete with plants and microbes for nutrients (Stark, 2000). IER is thought to capture N that has been mineralized but not immediately taken up by soil organisms (Gloser et al., 2000) – meaning that although high levels of captured N indicate high availability, low levels do not necessarily indicate low availability (Binkley and Vitousek, 1989). Previous work at our field site showed that resin bags collected much less inorganic N in vegetated plots than in bare plots, indicating that plants competed with resin bags for N (S. Thayer, unpublished data). However, plants and microbes also experience the effects of nutrient competition, so resin bag data may still provide an accurate measure of the soil nutrient environment.

The validity of data from resin bags may partly depend on their depth in the soil. Resin bags at the same depth as the majority of root biomass are likely to experience a nutrient environment similar to that of the plants. However, resin bags located below most root biomass may only receive nutrients that have been lost by leaching (Niklaus et al., 2001). Despite these drawbacks, resin bags at the right depth should still provide a fairly accurate measure of *relative* nutrient availability, which was the main purpose of this experiment.

Global change factors and their effect on soil nitrogen

The nitrogen cycle is complex and largely controlled by biological processes, making it especially susceptible to the effects of global change. Since most soil N transformations are controlled by a distinct community of microbes, the effects of global change on N availability partly depend on which microbial communities are affected (Hu et al., 1999). Also, in some cases, such as anthropogenic nitrogen deposition, global change can affect nitrogen availability directly. The effects of four major global change factors on nitrogen availability are outlined below; for a more complete literature review, see Appendix 1.

Nitrogen deposition

Human activities (including N fertilization, fossil fuel combustion, N-fixing crops, and land use change) have approximately doubled the rate of N input into the terrestrial N cycle

(Vitousek et al., 1997), and rates are expected to increase. The straightforward effect of N deposition is to increase soil nitrogen availability, as multiple studies have confirmed (e.g. Hungate et al., 1997*a*; Mikan et al., 2000).

However, N addition can also have indirect effects on nitrogen availability: for example, differences have been detected in microbial community composition between high-N and low N soil (e.g. Zak et al., 2000*a*). Thayer et al. (2002) found that N deposition in an annual grassland actually caused a decline in the total N content of plant biomass, due to the decrease in abundance of a nitrogen-fixing forb (*Lotus purshianus*). Also, N deposition usually causes downregulation in plant N uptake (BassiriRad, 2000), though little is known about interactions with other global change factors.

Elevated carbon dioxide

Atmospheric carbon dioxide (CO_2) is expected to approximately double (to 700 ppm) by the year 2100 (IPCC, 2001). Plant responses to elevated CO_2 have been extensively studied over the last two decades, but many plant parameters have shown highly variable responses (Luo et al., 1999). Microbial responses are even less well understood, making it difficult to predict the effects of CO_2 on soil nitrogen availability.

Initially, two hypotheses were proposed. Diaz et al. (1993) found that elevated CO_2 led to an increase in microbial N and a nitrogen deficiency in plants, even on fertile soil. They concluded that elevated CO_2 led to an increase in root exudates, thereby enhancing microbial growth and N immobilization. However, Zak et al. (1993) found significantly greater soil N availability under elevated CO_2, apparently because additional soil carbon stimulated the microbes that carry out mineralization.

It now appears that both responses can occur under natural conditions, but what determines the direction of the response is not fully understood. Furthermore, there is disagreement on which response is more common: Hu et al. (1999) state that soil nitrogen availability generally decreases under elevated CO_2, whereas Finzi (2002) states that CO_2 usually has no effect on soil nitrogen. According to Zak et al. (2000*b*) and Niklaus et al. (2001), results are currently too inconsistent to draw any conclusions.

The mechanisms by which CO_2 affects nitrogen availability are still unclear, but may include fine root production, chemistry of root exudates, and litter C:N ratio (Zak et al., 2000*b*). Hungate et al. (1997*b*) found that elevated CO_2 increased N mineralization indirectly by increasing soil moisture. There are also indications that elevated CO_2 can affect mycorrhizal colonization (Rillig et al., 1997), root distribution and root longevity (Arnone et al., 2000), and kinetics of root nutrient uptake (BassiriRad, 2000). More research is needed to determine the relative importance of these effects.

Warming

Average global temperatures are expected to continue to rise due to human-induced global warming. In the western United States, where our study site is located, average air temperature is predicted to increase by 3 - 5° C over the next 100 years (IPCC, 2001), with corresponding increases expected in the length of the growing season.

The most straightforward effect of warming is to increase N mineralization and diffusion (Foth and Ellis, 1997). However, more complex effects are also likely. Decomposition rates may increase, causing faster nutrient release, but increased rates of microbial immobilization may cancel out this effect. In addition, warming has been shown to affect species composition of plant communities (Harte and Shaw, 1995) which in turn can lead to changes in litter quality and decomposition rates. Another unknown factor is the effect of temperature on kinetics of root nutrient uptake, which may depend on nutrient supply (BassiriRad, 2000). Finally, interactions between warming and precipitation can affect nutrient availability (Shaw, 1995), underscoring the need to examine global change factors in combination.

Increased precipitation

Global climate change is expected to increase precipitation in some areas and decrease it in others. In the Western US, precipitation is predicted to increase in the winter months by 5 - 20% over the next 100 years (IPCC, 2001). Soil moisture has also been shown to increase as an indirect effect of elevated CO_2, and (in at least one instance) as a result of increased temperature (Zavaleta, 2002).

It is unclear what effect this will have on soil nitrogen availability. Water plays a crucial role in mass transport and diffusion of nutrients (Mengel and Kirkby, 2001), but excess water flow

can cause leaching (especially of nitrate). Soil moisture also has the indirect effect of facilitating microbial growth, but it is unknown whether mineralization (Hungate et al., 1997*b*) or immobilization will be the dominant effect. Effects may vary from region to region, depending on the extent to which water is a limiting resource.

<p style="text-align:center">∗   ∗   ∗</p>

In summary, each of the four global change factors outlined above has been shown to have a measurable effect on soil nitrogen availability, but the magnitude and even the direction of the effect is often difficult to predict. Additional complexity is introduced when global change factors occur in combination. Furthermore, most studies to date have focused on artificial ecosystems (single species; microcosms or mesocosms with disturbed soil; greenhouses or growth chambers; etc.). Although such experiments have been extremely valuable in advancing understanding, some of their results may not be applicable in field conditions (Zak et al., 2000*b*). For this reason, we chose to examine the effects of global change on nitrogen availability in a natural ecosystem.

Objectives and hypotheses

The aim of this study was to examine the effects of global change factors, singly and in combination, on soil nitrogen availability in a natural ecosystem over the course of a growing season. Our study site was the Jasper Ridge Global Change Experiment (JRGCE), an annual grassland in northern California. The four global change treatments we chose to apply were elevated CO_2, warming, increased precipitation, and nitrogen deposition.

We expected nitrogen deposition to be the dominant effect. Previous resin bag work at the JRGCE demonstrated an overwhelming effect of N deposition, with resin bags in N-treated plots showing nitrate and ammonium concentrations hundreds of times higher than those untreated plots (S. Thayer, unpublished data). We also expected increased precipitation to have a positive effect on nitrogen availability, both because of the role of soil moisture in ion transport and decomposition, and because our field site is thought to be water-limited for much of the year (Thayer et al., 2002). We expected a similar positive effect for warming, especially because it has been shown to increase soil moisture at the JRGCE through the mechanism of early senescence (Zavaleta, 2002).

Previous experiments at Jasper Ridge have shown elevated CO_2 to have a variety of effects on soil nitrogen. Hungate et al. (1997*a*, *b*) observed both increases and decreases in net N mineralization. Thayer et al. (2002) found an increase in the C:N ratio of plant tissue under elevated CO_2, which would be likely to suppress decomposition and thus nitrogen supply. Furthermore, a microcosm experiment at Jasper Ridge (Hu et al., 2001) showed that elevated CO_2 increased plant N uptake, reducing soil-available N. If plants or microbes, or both, experience increased growth under elevated CO_2, resin bags are likely to encounter fewer free nutrients. However, recent data from the JRGCE indicates that elevated CO_2 reduces total plant biomass (J. Dukes, pers. comm.). Given these varied results, we were unsure what effect to expect from elevated CO_2.

Methods

The Jasper Ridge Global Change Experiment

The Jasper Ridge Global Change Experiment (Figure 2) is located in the eastern foothills of the Santa Cruz Mountains near Stanford, California (elevation 120 m; latitude 37° 24' 11" N, longitude 122° 14' 29" W). The climate is Mediterranean, with cool, wet winters and warm, dry summers. Nearly all the annual precipitation (60 cm) occurs between November and April, which is the growing season for annual plants. Peak aboveground biomass is usually reached in late April, and most annuals have senesced by the end of May.

The Jasper Ridge Global Change Experiment (JRGCE) was established in 1998 to examine the long-term effects of human-induced global change on a natural ecosystem. The site is two acres of annual grassland on a gently sloping hillside, undisturbed for 25 years previously. The community is maintained as close to its natural state as possible, with the exception of several manipulations necessary to protect the equipment and keep the environment consistent between plots. These manipulations

Figure 2. The Jasper Ridge Global Change Experiment.

include deer fencing, gopher trapping, and removal of large perennial plants.

The 32 field plots (Figure 3) are circles 2 m in diameter, each divided into four quadrants to make a total of 128 experimental units. Each plot is unenclosed above ground and has a 50 cm barrier below ground. The following four global change factors are manipulated, each at two different levels:

Figure 3. Close-up of experimental plot (dominated by *Avena* and *Sisyrinchium*). The heat lamp hangs overhead, and CO_2 supply tubes ring the outer edge.

- Atmospheric carbon dioxide (ambient, 680 ppm)
- Temperature (ambient, ambient + 80 W $\cdot$ m^{-2}) [2]
- Precipitation (ambient, 1.5 × ambient) [3]
- Nitrogen deposition (ambient, ambient + 7 g $\cdot$ m^{-2} $\cdot$ year)

These four factors are applied in a full factorial design (for a total of 16 treatments, with 8 replicates each), allowing investigation of every possible interaction between the factors. CO_2 is applied with a free-air carbon dioxide enrichment system; warming with an overhead heat lamp; precipitation with a sprinkler system, and nitrogen in the form of liquid and solid fertilizer[4]. This is achieved with a split-plot design: carbon dioxide and warming are applied at the plot level, and increased precipitation and nitrogen deposition are applied at the quadrant level. Thus, each of the 128 quadrants receives a particular combination of global change treatments that stays consistent throughout the experiment. (See Appendix 2 for a diagram of the experimental layout.)

A mixture of native and introduced species populate the site. As in most California grassland communities, introduced species dominate. Common introduced species include *Avena*
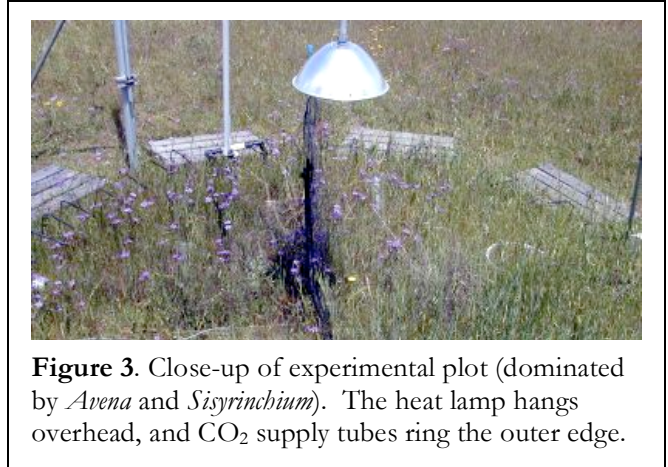
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[2] The warming treatment results in a temperature increase of approximately 2° C in the canopy and approximately 1° C at the soil surface.

[3] The precipitation treatment is extended 2 weeks beyond the last natural rain of the season.

barbata and *A. fatua* (wild oat), *Bromus hordeaceus* (soft chess), *Geranium dissectum* (cut-leaved cranesbill), *Erodium botrys* (long-beaked storksbill), *Crepis vesicaria* (beaked hawk's-beard), *Plantago lanceolata* (ribwort plantain), and *Convolvulus arvensis* (field bindweed). Native species include *Briza minor* (little quaking grass) and *Danthonia californica* (California oatgrass). Nearly all the species are annual, though there are a few perennials (including *Sisyrinchium bellum*, blue-eyed grass). Several are nitrogen fixers (such as *Vicia sativa*, common vetch). The community is composed exclusively of C_3 plants.

The soil at the site is derived from sandstone formed 30 - 50 million years ago (E. Miller, pers. comm.). The soil is only moderately fertile and is thought to be nutrient-limited, probably by nitrogen (Joel et al., 2001) and possibly also by phosphorus (C. Field, pers. comm). Water is also thought to be an important limiting factor during the dry season (Thayer et al., 2002). A summary of soil characteristics is presented in Table 1.

Table 1. Soil characteristics of the JRGCE.	
Soil type	Silty clay loam
Depth	Unknown (>180 cm)
Percent clay	40 – 50% in top 30 cm
Classification	Clayey, mixed, thermic Typic Haploxeralf
Soil series	Francisquito (Kashiwagi, 1985)
Cation exchange capacity	3.5 – 4.0 cmol / kg
Base saturation	~100%
pH	Neutral (6.5- 7.0)

Resin bag use and extraction

Our methods closely follow those described by Stark (2000). Resin bags were made from a mixture of cation exchange resin (Dowex HCR-W2 Na+ form) and anion exchange resin (Bio-Rad AG-1-X8), blended to give a mixture with a 1-to-1 cation to anion exchange capacity. (See Appendix 3 for ion exchange resin specifications). Approximately 1.5 g of resin was placed in a piece of fine nylon mesh (pantyhose material), and the top was closed with a cable tie. Each bag was then tagged with a small metal ID tag. Finally, a string (cotton or synthetic) was attached to the top of each bag.

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[4] Nitrogen is applied in two separate treatments: 2 g · m^{-2} in the form of calcium nitrate (liquid) after the first rains in November, followed in January by 5 g · m^{-2} in the form of slow-release nitrate fertilizer.

Before placing the resin bags in the field, we charged the ion exchange sites by soaking the bags in 5 M NaCl for 24 hours[5], followed by soaking in deionized water for 24 hours. We then spun the resin bags to remove excess water and placed each resin bag in an individual plastic bag for transport to the field. In the field we placed each resin bag ($n = 128$) in the plot and quadrant corresponding to the number on its ID tag (i.e., each individual bag was placed in the same quadrant every time, throughout the year).

Resin bags were buried for 7 days at a time, with 11 measurements throughout the season. For our first three measurements, during the early growing season (November – December), we buried the bags near the soil surface (1 - 2 cm deep) in order to measure the zone in which the roots were actively growing. We pushed a small amount of soil aside to make a hole, placed the resin bag in the hole, and replaced the soil, leaving the resin bag surrounded by soil on all sides (Figure 4).
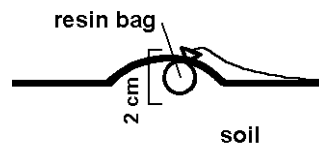

Figure 4. Diagram of early-season resin bag placement.

For the remaining eight measurements, during the middle to late growing season (January – May), we modified this technique in order to keep the resin bags near the middle of the rooting zone. We placed the resin bags in PVC tubes (interior diameter 15 mm) that extended approximately 16 cm below the soil surface at a slight angle (Figure 5). The tubes were open at the end, allowing only the bottom of the resin bag to come into contact with the soil. This resulted in a smaller, but more consistent, exposed surface area. The top of the tube was capped to prevent downward water flow.
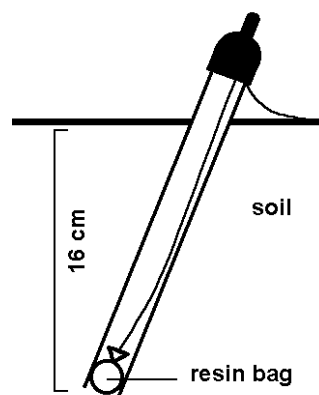
Figure 5. Diagram of late-season resin bag placement.

After removing the resin bags from the ground, we washed them with deionized water to remove any soil contamination. Collected ions were then extracted from the resin bags by placing each bag in 15 mL of 2 M KCl solution and placing on a shaker table for 30 minutes.

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[5] We chose to use Na^+ and Cl^- as counterions for our resin bags for the following reason: In natural systems, it is desirable to avoid using H^+ and OH^- as counterions in order to avoid changing the pH during the process of ion exchange, and thereby affecting the solubility of certain elements (Skogley 1996).

(Strings were removed from the bags before this step was carried out.) Extraction efficiency was not taken into account (see Appendix 3 for details). Samples of the solution were transferred to 5 mL autoanalyzer vials, and stored at either 2° C or -6° C until analysis.

We measured the concentration of nitrate and ammonium in the samples by means of a continuous flow analyzer (Alpkem RFA/2, manufactured by O.I. Corporation, College Station, TX, USA). We attempted to measure phosphate by the same method, but the phosphate concentration of most samples was below the detection limit (see Appendix 6).

Units and correction factors

Ion flux could not be calculated per weight (or volume) of soil, because the ions that were transported into the resin bag originated from an unknown volume of soil (Stark, 2000). We investigated both the possibility of calculating flux per exposed surface area of the resin bag, and per gram of resin. (To achieve the latter calculation, we removed and weighed the resin from each resin bag at the end of the experiment. Bags varied slightly in weight, with a mean of 1.4 g and a standard deviation of 0.16 g.)

Resin bag weight showed a slight correlation with nitrate collected at the surface ($r^2 = 0.23$), but no correlation with NO_3^- collected at depth ($r^2 = 0.002$). Ammonium showed no correlation with resin bag weight at either depth ($r^2 = 0.07$ at surface, $r^2 = 0.003$ at depth). This indicated that resin bag weight was somewhat important for nitrate capture when the bags were surrounded by soil and experiencing direct water flow, but not important when the bags were placed in the tubes.[6]

It was not possible to calculate a similar correlation for surface area, because we could not accurately determine surface areas of individual resin bags. We estimated that the shallow resin bags (surrounded on all sides by soil) had an exposed surface area approximately 7.5

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[6] Weight would have played a greater role in determining number of ions collected if all the exchange sites on the resin were becoming saturated, but we do not believe this was the case. Values varied greatly between individual resin bags (see Appendix 4 for an example of raw data), indicating that most of the bags were not reaching their full capacity. Previous work supports the assumption that it takes much more than one week for 1.5 g resin bags to reach saturation in this ecosystem (S. Thayer, unpublished data).

times greater than the deep resin bags. The shallow bags did collect more ions (on average, 6.5 times more nitrate, and 3.9 times more ammonium).

Figure 6. Comparison between weight correction and surface area correction for nitrate flux over the growing season.

However, it is unclear to what extent this was a result of surface area, and to what extent it reflected greater nitrogen availability at a shallower depth and at the beginning of the growing season. Correcting values for exposed surface area greatly affected the relationship between the first three measurements and all subsequent measurements. The correction led to a seasonal trend which appeared unrealistic, with nitrogen flux showing an abrupt jump with the change in method (Figure 6).

Based on this information, we decided to express ion flux relative to resin weight (nmol · g resin^{-1} · day^{-1}). Surface area is also known to play an important role in ion capture by IER devices (Skogley and Dobermann, 1996), but we had no way of separating the effects of surface area from the other conditions experienced by the shallow resin bags.

Although the change in methods partway through the growing season does make it more difficult to interpret overall seasonal trends in nitrogen flux, there is no reason to believe that the global change treatment effects would be influenced by a change in resin bag depth[7]. The focus of this study was responses to global change, rather than seasonal dynamics, and we

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[7] Data collected at the end of the 2001 – 2002 growing season indicated that our 16-cm-deep resin bags were below about 80% of the final root biomass (L. Moore, unpublished data). Had this information been available earlier, it would have been preferable to place the resin bags nearer the middle of the rooting profile.

believe that our methods provided a valid relative measure of biologically available nitrate and ammonium.[8]

Statistics

Values of nitrate flux and ammonium flux were calculated for each resin bag (corresponding to each of the 128 quadrants in the JRGCE). These values were averaged over the whole year (11 measurements), as well as over the early growing season (November – December 2001; 3 measurements), the middle growing season (December 2001 – February 2002; 4 measurements), and the late growing season (March – May 2002; 4 measurements).

The averages were natural-log-transformed and analyzed with an analysis of variance (ANOVA) model, specifically created by Erika Zavaleta and others for the split-plot randomized complete block design of the Jasper Ridge Global Change Experiment. (See Appendix 5 for complete ANOVA results.) We ran another set of analyses using raw data with resin bag weight as a covariate, but those results are not reported here as they were nearly identical to the results for the corrected data. Based on the full ANOVA results, we also performed a two-factor ANOVA on treatments which contained only CO_2 and warming.

All ANOVAs were performed with Systat 10 (standard version; copyright 2000 by SPSS); basic statistics and correlations were calculated with Microsoft Excel 97 (copyright 1996 by Microsoft Corporation). We defined $p < 0.05$ as significant and $p < 0.1$ as marginally significant.

Results

Background variability in nitrogen flux

The level of background variability in nitrogen flux was considerable, as is frequently the case (Mulvaney, 1996). Correlations were often weak even for the same ion in the same location on consecutive sampling dates, ranging from $r^2 = 0.1$ to $r^2 = 0.7$ (see Figure 7). It appears that both nitrate and ammonium experience large temporal fluctuations, and possibly also that our

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[8] In this paper, the words "flux" and "availability" are used interchangeably, as they have comparable meanings in the context of ion exchange resin bags (though not necessarily in the context of soil organisms).

sampling methods may be prone to error[9].

Ammonium flux and nitrate flux did not appear to be correlated spatially. We were unable to detect any correlation between nitrate and ammonium collected by individual resin bags averaged over the year ($n = 128$; $r^2 = 0.048$; see Figure 8). That is, a quadrant that was high in nitrate overall was not necessarily also high in ammonium. (This lack of spatial correlation was seen even when nitrate-fertilized plots were considered separately from unfertilized plots.)

Temporal correlation of the two ions was somewhat more complex. Ammonium showed no correlation with nitrate on a date-by-date basis when averaged between all plots ($n = 11$ sampling dates, $r^2 = 0.016$) or between all control plots ($n = 11$, $r^2 = 0.008$). However, ammonium and nitrate did show a positive correlation when ammonium was compared to nitrate from the previous sampling date ($n = 10$, $r^2 = 0.306$) or from two sampling dates previously ($n = 9$, $r^2 = 0.809$). These patterns were similar when only control plots were used ($r^2 = 0.891$ and 0.930, respectively).
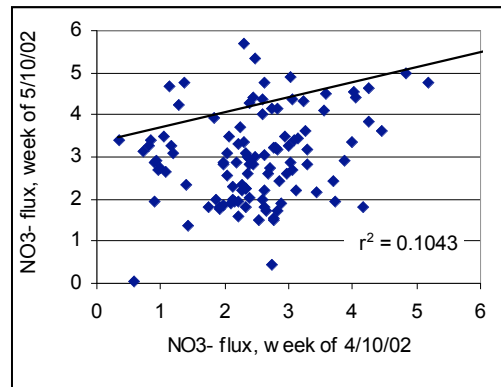
Figure 7. Correlation between nitrate in individual resin bags on two consecutive measurement dates. A weak correlation exists, but nitrate availability appears to be affected by many other factors besides physical location and global change treatment.
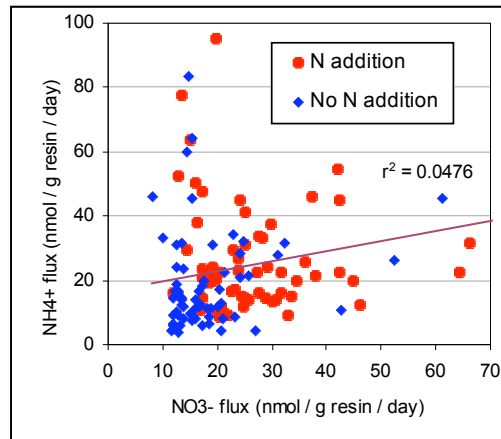
Figure 8. Correlation between nitrate and ammonium by resin bag ($n = 128$), yearly averages. Each resin bag (which corresponds to an individual quadrant) shows little relationship between the nitrate it collects and the ammonium it collects over the year.

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[9] We were able to ascertain that the variability did not come from the laboratory analysis of our samples (running a batch of 128 samples on the autoanalyzer twice resulted in an r^2 of 0.983). See Appendix 4 for an example of raw data.

This indicates the presence of a process in the soil which converts nitrate to ammonium over a period of weeks, most likely nitrate immobilization and subsequent ammonification by microbes (Brady and Weil, 2001). However, if the date of peak availability for each ion (early in the growing season) was not included in the correlations, the relationships became much weaker ($r^2 \approx 0.1$). Microbial conversion of nitrate to ammonium may only be important during the pulse of nutrients at the beginning of the growing season.

Seasonal patterns and effects of environmental variables [10]

Nitrogen availability decreased markedly as the growing season went on (Figure 9). Peak availability for nitrate occurred at the first measurement date (11/01/01), and peak availability of ammonium occurred at the second measurement date (11/14/01). Availability of both ions stayed low from December onward, with nitrate continuing to decrease throughout the growing season but ammonium increasing slightly toward the end of the growing season.
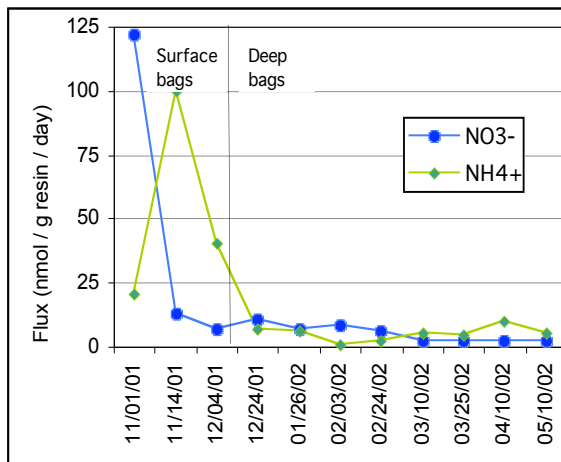
Figure 9. Seasonal trends in nitrate flux and ammonium flux, control plots only (treatment plots show a similar pattern).

Rainfall may explain some of the variation in nitrogen availability throughout the growing season, because soil moisture facilitates the transport and uptake of mineral nutrients, and because wetting of soils after a dry period often causes a peak in mineralization rates (Hungate et al., 1997*a*). Indeed, rainfall was high at the beginning of the growing season when nitrogen availability was also high (Figure 10). However, it may not be a causal relationship (the peak for nitrate occurs some time before the peak rainfall period, and both ions begin dropping off well before the rainfall disappears). The correlation between rainfall and nitrogen availability over the whole growing season is very weak ($r^2 = 0.04$ for nitrate and 0.19 for ammonium).

Temperature is known to have an effect on the diffusion of ions (Foth and Ellis, 1997), but there was no clear relationship between average air temperature and nitrogen flux (Figure 11). The slight increase in ammonium availability at the end of the growing season may be partly related to the increase in temperature, but correlations between temperature and nitrogen flux over the growing season were very low ($r^2 = 0.014$ for nitrate and 0.076 for ammonium).

Finally, we looked at the relationship between end-of-year biomass and nitrogen availability (Figure 12). No correlation was apparent between nitrogen flux and above-ground biomass ($r^2 = 0.001$ for nitrate and 0.023 for ammonium); below-ground biomass ($r^2 = 0.012$ and 0.001), or total biomass ($r^2 = 0.005$ and 0.003). There was also no relationship between nitrogen availability and root/shoot ratio ($r^2 = 0.022$ for nitrate and 0.006 for ammonium). It is possible that biomass at different times in the growing season would have shown different patterns, but seasonal biomass data was not available.
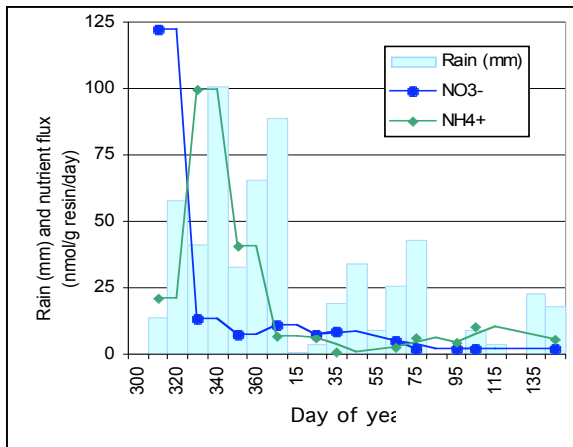
Figure 10. Seasonal trends in rainfall over the 2001-2002 growing season. Though both rainfall and nutrient availability peak early in the growing season, nutrient availability begins to decline while rainfall is still high, making it unlikely that the relationship is directly causal.
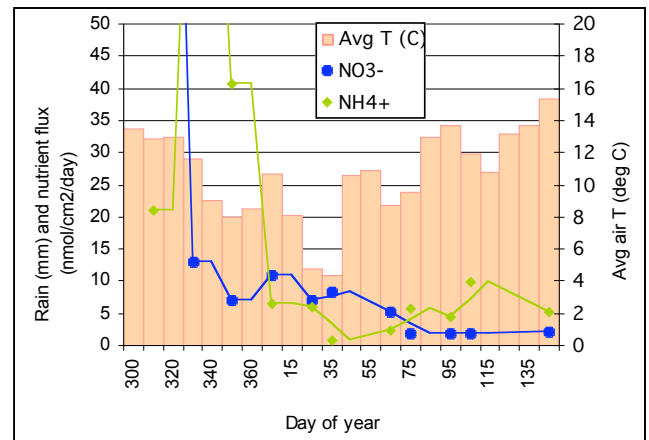
Figure 11. Seasonal trends in air temperature over the 2001 - 2002 growing season. There appears to be no correlation between temperature and nutrient availability, with the exception of a slight peak in ammonium late in the growing season that may be partly due to warmer temperatures.

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[10] In correlations of nutrient flux with environmental variables (temperature, rainfall, and total biomass), only control samples were used, in an attempt to accurately characterize the natural dynamics of the ecosystem.

Global change effects

Yearly averages

Analysis of variance on averages of all treatments (Figures 14 and 15) showed that nitrogen deposition was the only global change effect that significantly altered soil nitrogen availability. Under addition of nitrate fertilizer, nitrate availability increased by an average of 50.0% and ammonium availability increased by an average of 44.0% (Figure 13).

Figure 12. Relationship between net primary production and nitrogen availability throughout the season. (Above-ground biomass and below-ground biomass, examined separately, also failed to show a correlation with nitrogen availability.)

Nitrate availability showed no response to elevated CO_2 or warming, and a slight positive response to increased precipitation ($p = 0.61$). Ammonium availability showed a small increase in response to warming and increased precipitation ($p = 0.41$ and 0.62) and no response to elevated CO_2. (See Appendix 5 for complete ANOVA results.)

Seasonal effects

The seasonal results revealed a more complex story. N deposition still dominated the global change effects, but warming and increased precipitation showed significant effects. Increased precipitation showed opposite effects at the beginning and at the end of the growing season (Figures 16 and 17): in the early growing season, it slightly decreased availability of both nitrate and ammonium ($p = 0.28$ for nitrate and 0.66 for ammonium), whereas at the end of the growing season, it increased the availability of both ions ($p = 0.01$ for nitrate and 0.03 for ammonium). Warming increased
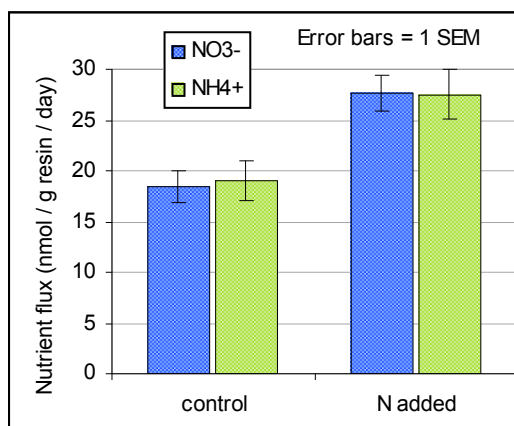

Figure 13. Effect of N deposition on nitrate and ammonium availability. As expected, N addition causes a large increase in availability ($p < 0.001$), of nearly equal magnitude for both ions.
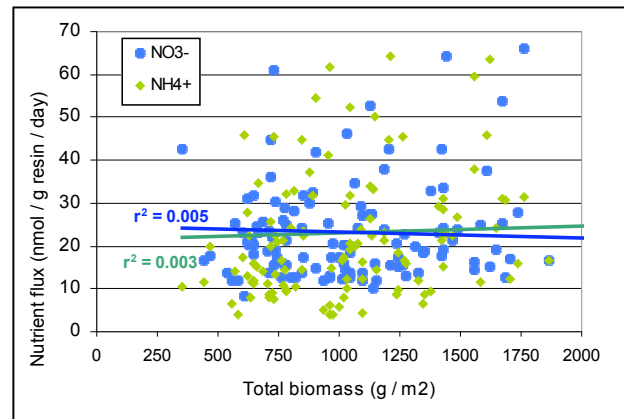
Figure 14. Effects of global change treatments on nitrate availability (yearly averages). The dominant effect is a "zigzag" pattern due to N deposition (treatments 2, 4, 6, 8, 10, 12, 14 and 16). Elevated CO_2, warming, and increased precipitation do not appear to have a strong effect on nitrate availability averaged over the growing season.
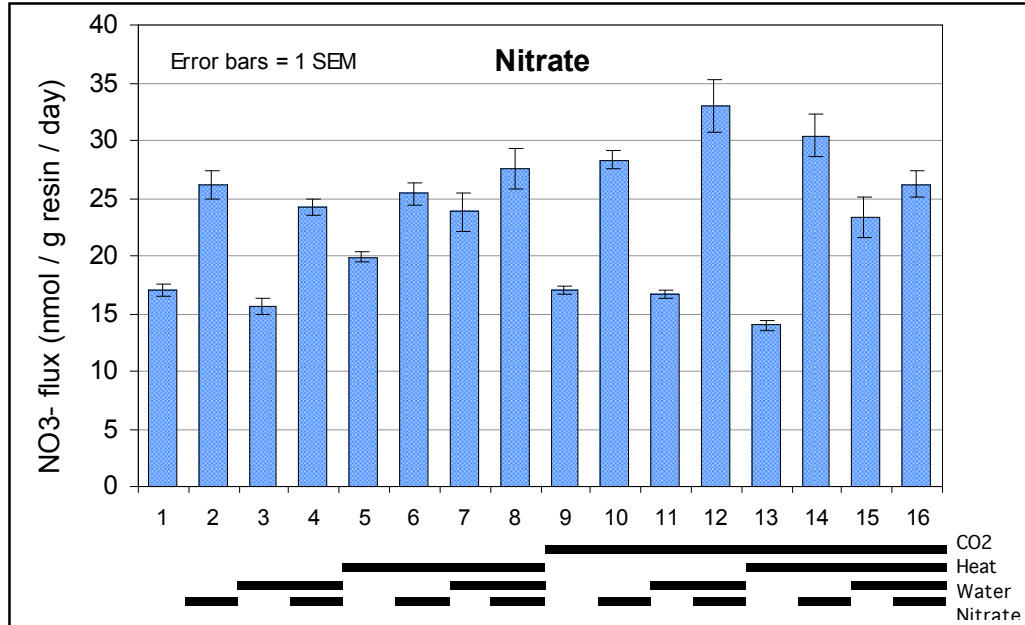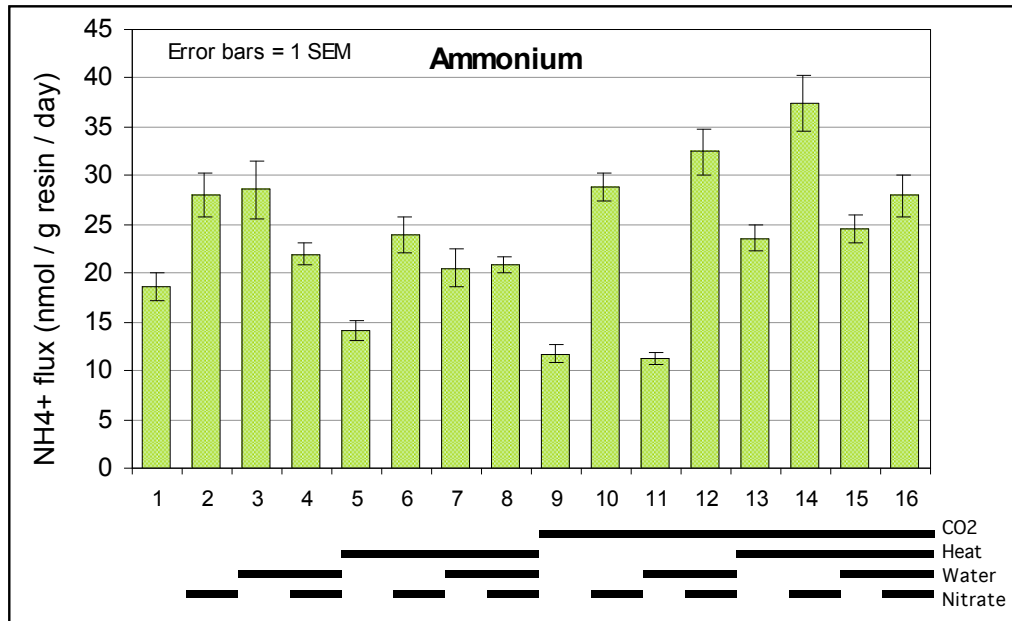

Figure 15. Effects of global change treatments on ammonium availability (yearly averages). As with nitrate, a zigzag pattern due to N deposition can be seen. Effects of the other three global change factors cannot be easily detected at this level of detail.
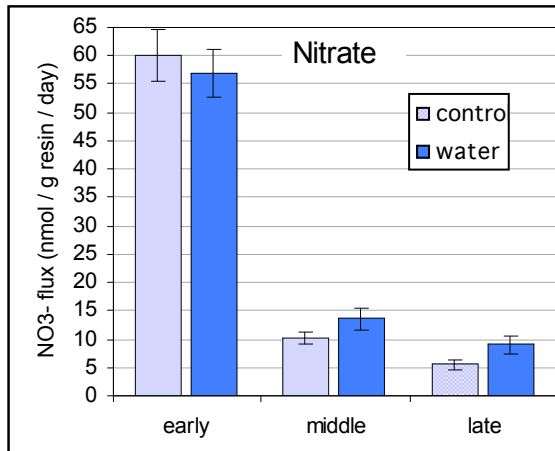
Figure 16. Effect of increased precipitation on nitrate availability throughout the growing season. In the early growing season, water addition slightly decreases nitrate availability, but the effect reverses direction in the middle and late growing season. (Error bars = ± 1 SEM.)
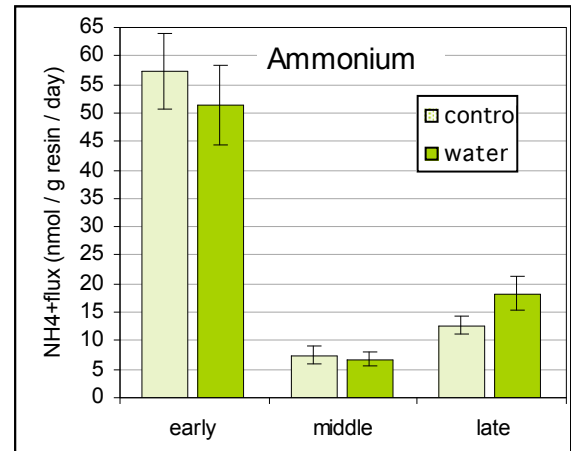
Figure 17. Effect of increased precipitation on ammonium availability throughout the growing season. The same pattern can be seen as for nitrate: water addition decreases availability in the early growing season, but increases availability in the late growing season. (Error bars = ± 1 SEM.)

ammonium availability at the end of the growing season ($p < 0.01$) but had no effect on nitrate availability. Elevated carbon dioxide had no effect on either ammonium or nitrate at any stage of the growing season.

Interaction effects

Although CO_2 and warming did not cause significant effects on their own, their interaction approached significance for ammonium ($p < 0.07$ for yearly average, $p = 0.05$ for late growing season). The interaction of warming and elevated CO_2 increased the availability of ammonium more than would be expected from the additive effects of the two factors. However, the availability of nitrate was not affected by this interaction.

Nitrate showed a significant $CO_2 \times$ water interaction ($p < 0.09$ for middle growing season, $p < 0.04$ for late growing season. CO_2 tended to suppress nitrate availability in the absence of water and increase nitrate availability in the presence of water. Nitrate also showed a $CO_2 \times$ water $\times$ N interaction during the early growing season ($p < 0.05$), but this effect was not repeated at any other time (though the direction of the effect was consistent throughout the year).

Finally, ammonium availability was affected by a CO_2 × heat × N interaction, both averaged over the whole year ($p = 0.05$) and during the early and middle growing season ($p < 0.06$ and 0.10, respectively). However, the direction of this interaction was not consistent between the early and the middle growing season.

A closer look at CO_2 and warming

Effects of warming and CO_2 on nitrogen availability were, for the most part, too subtle to be detected in the 4-factor ANOVA, so we ran a 2-factor ANOVA on treatments only including CO_2 and warming (Figure 18). Complete results are included in Appendix 5. Averaged over the year, both CO_2 and warming slightly increased ammonium availability but decreased nitrate availability (no effects significant; $p < 0.07$ for effect of CO_2 on nitrate). The interaction of CO_2 and warming increased ammonium availability more than would be expected from their additive effects ($p < 0.07$), and decreased nitrate availability more than would be expected ($p < 0.05$). The CO_2 × warming effect for nitrate only occurred in the early growing season ($p < 0.01$), not in the middle growing season or the late growing season ($p > 0.9$). This was the only prominent seasonal effect.

Discussion

Single-factor effects

Nitrogen deposition strongly increased availability of both nitrate and ammonium throughout the growing season[11]. This effect occurred regardless of the presence of other global change factors: there was no factor or combination of factors that could negate the effects of N deposition. It is not surprising that the effect of nitrogen deposition overwhelmed the effects of the other global change treatments, though the magnitude of the effect was less than in a previous study at this site (S. Thayer, unpublished data). It will be some time before anthropogenic nitrogen deposition could equal the level of deposition in this experiment at a widespread scale, but should that occur, soil nitrogen availability will be dramatically affected.
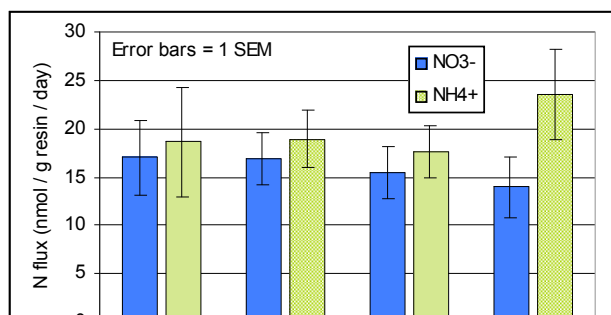
ilability to N addition in the middle of the
< 0.3). However, this lack of a clear
s were near the detection limit in the middle
ess meaningful.

It appears that water is in excess at the beginning of the growing season, so that additional water contributes to nitrogen leaching and therefore lower nitrogen availability. However, were this the case, it would be expected to have a greater negative effect on nitrate than on ammonium, which was not observed. In any case, it seems clear that water is limiting at the end of the growing season, thus additional water makes nitrogen more available. It is possible that the late-season water effect is more important for resin bags than for plants or microbes (because resin bags depend more on mass transport to pick up ions).

Warming had no effect on nitrate, but had a substantial impact on ammonium at the end of the growing season. Why might the two ions respond differently to this global change treatment? One possibility is that ammonium transport depends mostly on diffusion, which is temperature-sensitive, whereas nitrate moves largely by mass transport. There may also be a biological effect at work: it is possible that warmer temperatures enhance the activity of ammonifying bacteria, or suppress the activity of nitrifying bacteria.

Neither ion showed a strong response to elevated CO_2, which is not surprising given the variety of previous results reported in this ecosystem. No response whatsoever could be detected in ammonium, either in the ANOVA including all factors or the two-factor ANOVA including only CO_2 and warming responses. According to the two-factor ANOVA, nitrate may have declined slightly in response to elevated CO_2. If true, this could support the microbial immobilization hypothesis proposed by Diaz et al. (1993), but it is not clear why the same effect would not be seen for ammonium.

The same decline in nitrate availability might also be seen if elevated CO_2 promoted plant growth (and therefore plant N uptake), rather than promoting microbial growth. In either case, less N would be accessible to the resin bags. There is little support for this idea, however, because elevated CO_2 did not have a strong effect on plant biomass during the 2001 - 2002 growing season (J. Dukes, unpublished data).

If nitrate and ammonium actually did respond differently to elevated CO_2, one possible explanation is that nitrifying bacteria are more sensitive to environmental changes than are ammonifying bacteria (Foth and Ellis, 1997). Nitrification in this ecosystem may have

decreased slightly under elevated CO_2, while ammonification and other microbial processes remained constant.

Interaction effects

The strongest interaction effect was the interaction between elevated CO_2 and warming, which served to increase ammonium availability and decrease nitrate availability. It is not clear why this should be the case. Perhaps in this ecosystem, both ammonification and denitrification are stimulated by elevated CO_2 (or perhaps nitrification is decreased), and this effect is accentuated by warming. This would explain both the increase in ammonium and the decrease in nitrate.

Harder to explain are the three-way interactions between CO_2, increased precipitation and N deposition (significant for nitrate in the early growing season) and between CO_2, warming, and N deposition (significant for ammonium averaged over the year). The latter interaction seems to have changed direction during the growing season, so even if the effect is meaningful and repeatable, it is probably very complex. It remains to be seen if these patterns repeat in subsequent years.

Future research

A major methodological improvement for future studies would be to use resin bags with a consistent depth and consistent surface area throughout the growing season. It may be best to bury bags at an intermediate depth, such as 8 cm (approximately in the middle of the rooting profile) and leave the bags at that depth throughout the year. Alternatively, if time and resources permit, both a shallow and a deep set of resin bags could be used concurrently.

The resin bags in tubes were often ineffective at picking up ions, especially during the dry season. A better measure of availability might be possible if the bags could be buried in a way that left them more fully in contact with the soil (for example, positioning the end of the tube 1 cm above the bottom of the hole). Although the exposed surface area of the resin bags would be less consistent, collecting measurable numbers of ions is probably a more important consideration. Another solution to this problem would be to leave the resin bags in the ground for several weeks at a time, as is more typical (Stark, 2000).

It would be ideal to measure nutrient availability continuously throughout the year, rather than leaving gaps in the data of several weeks at a time. This would require the use of two sets of resin bags (so that one set could be installed when the other was removed). However, increased temporal resolution should not necessarily take precedence over more replicates for each treatment. Regardless of measurement frequency, it would be advisable to start measurements earlier in the growing season, preferably before the first rains. This would allow better characterization of the nutrient pulse at the beginning of the growing season.

Nutrient availability data could be made more meaningful in combination with other data sets, such as soil moisture, microbial biomass, mycorrhizal colonization, and mineral content of plant tissue (for example, C:N ratio). Also, it is important to consider nutrients beyond nitrate and ammonium, most notably phosphate (see Appendix 6). Because of the low mobility of phosphate, measurement of phosphate in this ecosystem will require either a different kind of ion exchange resin, or a longer time period for which the resin bags are buried.

Before placing too much emphasis on any of the results presented here, it would be highly advisable to repeat this study in a subsequent year and see if similar patterns emerge. Previous results from the Jasper Ridge Global Change Experiment have varied considerably from year to year. This may be partly because the ecosystem is still adjusting to the global change treatments, and may also be due to the considerable year-to-year variation in environmental conditions. It remains to be seen if nitrogen availability in this ecosystem has reached a steady state in response to global change effects.

Conclusions

Our results suggest that the overall effect of the four global change factors we examined (elevated CO_2, warming, increased precipitation and N deposition) was to increase nitrogen availability in the annual grassland at the Jasper Ridge Global Change Experiment. It is misleading, however, to simply say that global change increases nitrogen availability in this ecosystem. We found evidence for interaction between pairs of global change factors (for example, CO_2 and warming) and even tentative evidence for three-way interactions (for

example, CO_2 × warming × N). Furthermore, the effects of global change treatments varied considerably over the course of the growing season, and frequently the two ions under consideration were affected in opposite ways.

It remains to be seen whether these patterns will stay consistent in subsequent years. This study provides only a snapshot of an ecosystem which is probably still out of equilibrium. As plant and microbial communities alter in composition, patterns in nitrogen availability could change considerably or even reverse.

The complexity of these results underscores the need for continuing research into the effects of global change on nutrient availability. Because soil nutrients are a growth-limiting factor for most terrestrial ecosystems, they represent an important mechanism by which global change can affect ecosystem function. Better understanding of soil nutrient dynamics will make it easier to understand, predict, and manage the effects of human-induced global change on natural systems.

Acknowledgements

People

Elsa Cleland invested a great deal of time in this study and should be considered a co-author. She played an integral role every step of the way, from making the resin bags to interpreting the statistics. The project would not have happened without her initiative and hard work.

Chris Field, the author's thesis advisor and one of the Global Change Experiment principal investigators, was instrumental in providing ongoing guidance for questions both great and small.

Rebecca Shaw shared her extensive knowledge of the Global Change Experiment to help us develop our experimental methods and place this project in a broader context.

Sue Thayer laid the groundwork for this project by doing a similar study two years previously. We are indebted to her for access to her data and for contributions to the experimental design.

Doug Turner, along with the facilities of the **Vitousek Lab**, made the laboratory-based part of this study possible. Doug spent many hours running samples and coaxing the aged Alpkem to behave itself.

Lisa Moore provided the below-ground biomass data, and **Jeff Dukes** provided the above-ground biomass data. The whole **Field Lab** worked to collect both data sets.

Erika Zavaleta and others developed a split-plot ANOVA model specifically for the Global Change Experiment, which we used extensively for data analysis.

Nona Chiariello, scientific coordinator of the Global Change Experiment, provided logistical support, meteorological data, and maps and digital photos of the site.

David Vernon earned considerable gratitude from the author for providing volunteer labor, ongoing encouragement, and design of a more efficient way to spin water out of resin bags.

Jodie Kerr, the author's laptop computer, worked diligently at analyzing data and making graphs, as well as formatting the document you are reading right now.

Finally, the author would like to thank her family for moral support and frequent good-natured queries of "Isn't your thesis done yet?" Thanks especially to **Al and Wenetta Childs** and **Carol Childs** for financial assistance, and **Rex Kerr** for technical advice.

Institutions

The **Carnegie Institution of Washington** (Department of Global Ecology), **Jasper Ridge Biological Preserve**, and the **Earth Systems Program** at Stanford University provided resources and connections to make this research possible.

We would also like to extend special appreciation to the **National Science Foundation**, the **Packard Foundation** and the **Morgan Family** for their generous and ongoing financial support of the Jasper Ridge Global Change Experiment.

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