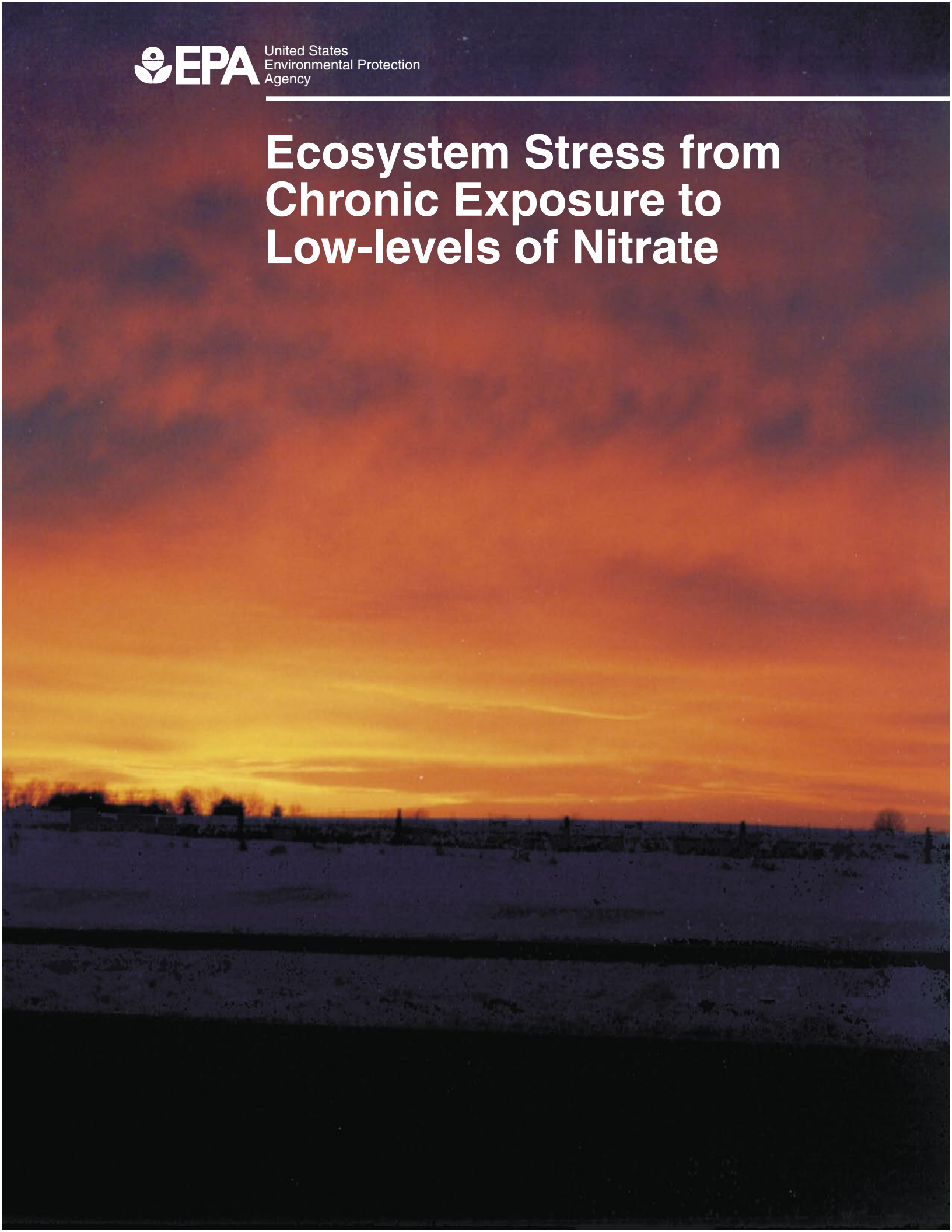
United States
Environmental Protection
Agency

Ecosystem Stress from Chronic Exposure to Low-levels of Nitrate



EPA/600/R-05/087
October 2005

Ecosystem Stress from Chronic Exposure to Low-levels of Nitrate

Eric E. Jorgensen, Scott M. Holub, Paul M. Mayer, & Mary E. Gonsoulin
U.S. Environmental Protection Agency
Office of Research and Development
National Risk Management Research Laboratory
Ada, Oklahoma 74820

Rendahandi G. Silva & Ann E. West
Oak Ridge Institute for Science and Education
Oak Ridge, Tennessee 37831

Susan J. Tunnell, Jay E. Clark, Jennifer L. Parsons, David M. Engle, & Eric C. Hellgren
Oklahoma State University
Stillwater, Oklahoma 74078

Julie D.H. Spears & Clyde E. Butler
Shaw Environmental, Inc.
Ada, Oklahoma 74820

D.M. Leslie, Jr.
United States Geological Survey
Oklahoma Cooperative Fish & Wildlife Research Unit
Stillwater, Oklahoma 74078

Project Officer
Eric E. Jorgensen
Ground Water and Ecosystems Restoration Division
National Risk Management Research Laboratory
Ada, Oklahoma 74820

National Risk Management Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

Notice

The U.S. Environmental Protection Agency through its Office of Research and Development funded, and managed the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

All research projects making conclusions or recommendations based on environmentally related measurements and funded by the U.S. Environmental Protection Agency are required to participate in the Protection Agency Quality Assurance Program. This project was conducted under an approved Quality Assurance Project Plan. The procedures specified in this plan were used without exception. Information on the plan and documentation of the quality assurance activities and results are available from the Principal Investigator.

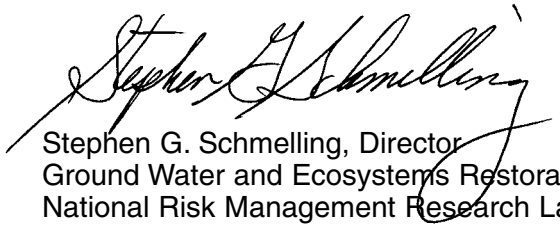
Foreword

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threatens human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients..

In 1998 we initiated an integrated multi-disciplinary study investigating the effects of chronic exposure of ecosystems to low doses of bioavailable nitrogen. We investigated several aspects of ecosystem response to chronic exposure to low doses of bioavailable nitrogen on sixteen 40x40-m study plots in south-central Oklahoma in conjunction with complementary short-term field and laboratory studies. In this nitrogen-limited system, the ability of the soil system to adapt to new nitrogen inputs was compromised after 1 year of exposure. Concentrations of nitrate-N in the soil peaked at 1169% more than expected and averaged 254% greater than expected. Our experiments demonstrate that even the relatively small amounts of bioavailable nitrogen that are deposited in precipitation have the capacity to change multiple aspects of ecosystem nitrogen retention, sequestration, and processing. The changes observed are always deleterious in that they lead to greater concentrations of nitrate-N and thereby make more available for leaching to surface and groundwater. As outputs of nitrogen to the atmosphere can reasonably be expected to increase in the foreseeable decades, it is prudent to identify and develop management options now to both restore ecosystems that are already compromised and to buffer affects to ecosystems that are at risk from new nitrogen inputs.



Stephen G. Schmelling, Director
Ground Water and Ecosystems Restoration Division
National Risk Management Research Laboratory

Abstract

Throughout the eastern United States, from the Front Range of the Rocky Mountains to the Atlantic Ocean, bioavailable nitrogen has been falling in the rain since the industrial revolution. Bioavailable nitrogen is a limiting nutrient throughout this region. While long-term research conclusively demonstrates that exposure of soil ecosystems to large doses of bioavailable nitrogen leads to deleterious environmental impacts (i.e., eutrophication, toxic algae blooms, hypoxia, toxicity, acid rain, global climate change) that can compromise people's health and the economic vigor of communities, the potential effects of chronic exposure to lower doses of bioavailable nitrogen are relatively unknown. However, symptoms of compromised ecosystem function that may be attributable to chronic exposure to bioavailable nitrogen are widespread; many forests routinely leach nitrogen to surface and groundwater and nitrate-N concentration in estuaries perturbs aquatic food-webs and affects fisheries. These observations, among others, support the hypothesis that ecosystem function can be (and has been) deleteriously impacted by chronic exposure to low doses of bioavailable nitrogen. To investigate this, in 1998 we initiated an integrated multi-disciplinary study investigating the effects of chronic exposure of ecosystems to low doses of bioavailable nitrogen. We investigated several aspects of ecosystem response to chronic exposure to low doses of bioavailable nitrogen on sixteen 40x40-m study plots in south-central Oklahoma in conjunction with complementary short-term field and laboratory studies. Plots were manipulated in a factorial arrangement such that 4 plots each received fertilizer only (16.3 kg N ha^{-1} yr^{-1}), herbivory manipulation only (fenced), a combination of fertilizer and herbivory manipulation, or were left as controls. Herbivory population was manipulated by constructing a 2-m tall chain link fence of 2.5-cm wire mesh. In this nitrogen-limited system, the ability of the soil system to adapt to new nitrogen inputs was compromised after 1 year of exposure. Concentrations of nitrate-N in the soil peaked at 1169% more than expected and averaged 254% greater than expected. Plant growth was affected by nitrogen application, wherein biomass increased on fertilized plots and diversity was related to distribution of *Festuca arundinacea*. Microbial activity was naturally limited in this system by carbon availability, but this tendency was exacerbated by additional inputs of nitrogen: further, microbial population response was not qualitatively different in soils that received small nitrogen additions vs. soils that received larger nitrogen additions. The presence of large numbers of small mammals coincided with high concentrations of soil nitrate-N. We estimate that herbivores may be able to re-circulate up to 67% of the bioavailable nitrogen deposited back into the plant and microbial pathways, thereby producing a self reinforcing positive feedback loop leading to ever greater concentrations of soil nitrate-N. This could lead to increased nitrate-N leaching to surface and ground water. The ability of detritus pathways to process nitrogen inputs was compromised after 6 months and this tendency was increased when macroinvertebrate communities were restricted. These experiments demonstrate that even the relatively small amounts of bioavailable nitrogen that are deposited in precipitation have the capacity to change multiple aspects of ecosystem nitrogen retention, sequestration, and processing. The changes observed are always deleterious in that they lead to greater concentrations of nitrate-N and thereby make more available for leaching to surface and groundwater. As outputs of nitrogen to the atmosphere can reasonably be expected to increase in the foreseeable decades, it is prudent to identify and develop management options now to both restore ecosystems that are already compromised and to buffer effects to ecosystems that are at risk from new nitrogen inputs.

Keywords:

Atmospheric Deposition, Bioavailable Nitrogen, Nitrate, Ecosystem Response, Ecosystem Management, Trophic Interactions

Contents

Figures

Tables

Acknowledgments

The U.S. Environmental Protection Agency through its Office of Research and Development funded the research described here both through in-house efforts and through Interagency Agreements with the Biological Resources Division of the U.S. Geological Survey and as administered by the Oklahoma Cooperative Fish and Wildlife Research Unit (Oklahoma State University, Oklahoma Department of Wildlife Conservation, U.S. Geological Survey, and Wildlife Management Institute cooperating) and the U.S. Department of Energy as administered by Oak Ridge Institute for Science and Education (ORISE). ManTech Environmental conducted some of the chemical analyses under contract with the U.S. EPA. It has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Particular thanks go to D.M. Leslie, Jr. (Oklahoma Cooperative Fish and Wildlife Research Unit, USGS), J. Williams, S. Schmelling, and C. Hall (National Risk Management Research Laboratory, U.S. EPA) for facilitating this work. We thank M. Bahm, J. Bahm, C. Bilder, T. Bodine, K. Brazil, A. Burrow, M. Day, C. Deerenberg, B. Faulkner, S. Fuhlendorf, J.Gray, M. Hamilton, P. Hanson, D. Hutchings, J. Jones, S. Kovash, M. Leslie, L. Levesque, J. Lindsey, R. Lochmiller, S. Lyon, K. McBee, L. Mulkey, H. Murray, L. Newby, D. Niyogi, P. Nunn, T. Oppelt, M. Palmer, M. Payton, C. Peterson, D. Pope, H. Purvis, A. Roper, G. Sampson, J. Sheffield, E. Webb, and J. Wilson for their assistance, advice, and counsel.

Introduction

Throughout the eastern United States, from the Front Range of the Rocky Mountains to the Atlantic Ocean, bioavailable nitrogen has been falling in the rain since the industrial revolution (i.e., Smil, 1990; Vitousek *et al*., 1997). In a trend that is expected to continue, these additions have been increasing (Brimblecombe and Stedman, 1982; Galloway *et al*., 1994; U.S. EPA, 1995; Vitousek *et al*., 1997). Because nitrogen is frequently a limiting nutrient for plants and animals, increased quantities of nitrogen in ecosystems alter competitive relationships among terrestrial and aquatic organisms. Nitrogen, particularly nitrate-N, easily moves from terrestrial ecosystems into surface and groundwaters, including lakes, streams, rivers, and estuaries (i.e., Baker, 1992; Kahl *et al*., 1993; Peterjohn *et al*., 1996). As nitrogen concentrates in surface and groundwater sinks, increasingly frequent observations of undesirable effects associated with eutrophication, algae blooms, hypoxia, and toxicity are observed (Kelly *et al*., 1990; Likens, 1992; Glibert and Terlizzi, 1999). Today, acid rain phenomena in North America are largely associated with excess nitrogen (Aber *et al*., 1989; Gilliam *et al*., 1996). Wedin and Tilman (1996) suggested that increasing amounts of nitrogen in the environment may be associated with global warming and climate change (See also Vitousek *et al*., 1997; Shaver *et al*., 2000).

Excess nitrogen is not tightly retained by ecosystems, but is highly mobile (i.e., Vitousek *et al*., 1997). It occurs in ecosystems under a variety of guises (i.e., nitrogen species; NO_3, NH_4, NO_2, DON, TN, etc.), each of which varies in mobility, potential for use by organisms, and expression in site biogeochemistry. Therefore, concern about nitrogen management in ecosystems is focused not only on the amount of nitrogen present, but also its transport and cycling. While the effects of large doses of nitrogen are well documented, it is only recently that attention has been focused toward risks associated with chronic low-level exposure to nitrogen, such as that accompanying atmospheric deposition (Likens, 1992; Jorgensen *et al*., 2002; Jorgensen *et al*., 2003). In order to weigh risks and assess management options, it is important that a thorough understanding of the interactions and transport of nitrogen in terrestrial and aquatic ecosystems and the atmosphere be developed.

The nitrogen cycle is well studied. While many of the cycle's components are important to consider for nitrogen management, there are relatively few that interact closely with atmospherically deposited nitrogen. In this study, we were most interested in those components that are directly affected by nitrogen deposition and their response. Wedin and Tilman (1996) published data demonstrating that exposure to excess bioavailable nitrogen degrades ecosystems in a number of notable ways; 1) retained nitrogen decreases with increasing exposure, 2) biodiversity declines, and 3) plant C/N ratio declines. Of perhaps greater importance is the observation that most of the ecological response measured by Wedin and Tillman (1996) occurs in the first 100 kg N ha^{-1} yr^{-1} of deposition. Elsewhere, others and ourselves have termed this the "ecologically significant dose" (Figure 1) (Jorgensen *et al*., 2003).

Whereas atmospheric deposition in the eastern United States is measured at approximately 10-30 kg N ha^{-1} yr^{-1} (National Atmospheric Deposition Program (NADP-3)/National Trends Network, 2002), it is reasonable to believe ecosystems throughout this region are affected by chronic exposure to excess bioavailable nitrogen. Such effects may be related in part to Perakis and Hedin's (2002) observations concerning relative availability of organic vs. inorganic nitrogen in South America. Ecosystems process deposition in a few ways. Initially, direct deposition to terrestrial systems is processed differently than direct deposition to aquatic systems (note: the research described in this paper does not consider deposition to aquatic systems). Deposition to terrestrial systems may either be processed by biota (i.e., plants and/or microbes), or it may escape to aquatic systems through runoff, percolation, or it may in-part volatilize. Improved nitrogen management will occur where the probability of interaction with biota is high. The probability of interacting with biota is not constant or fixed, because biota may change in response to many environmental conditions. Some of these conditions (i.e., temperature and precipitation) are essentially outside of the scope of management intervention. However, many of the conditions are susceptible to management once their response is better understood.

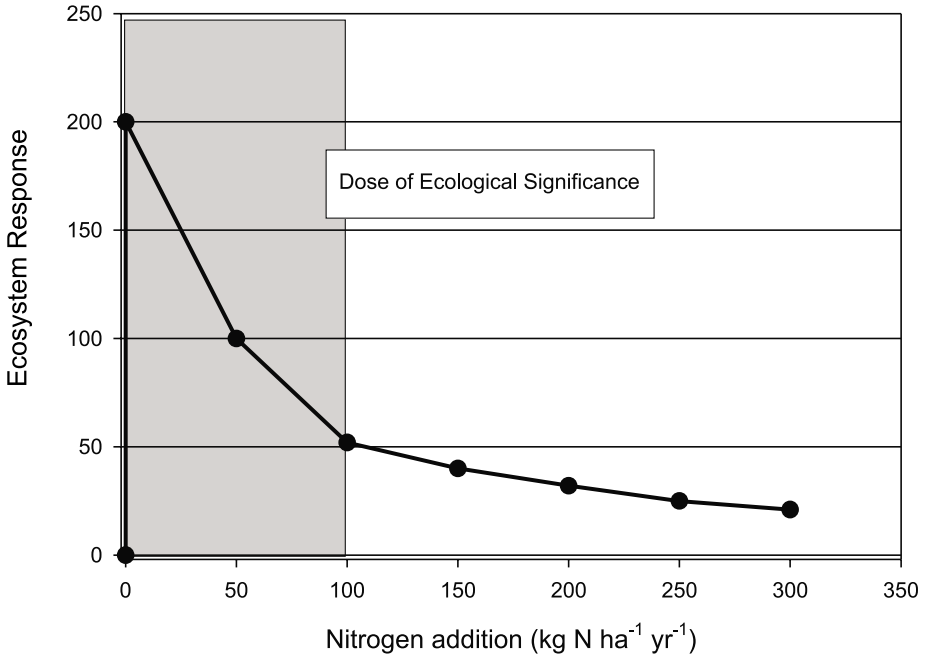


Figure 1. *These data, adapted from Wedin and Tilman (1996), indicate that most of the ecological response associated with nitrogen exposure occurs in the first 100 kg N ha^{-1} yr^{-1} of application. We term this level of exposure the "Dose of Ecological Significance."*

Soil Nitrogen Chemistry

Bioavailable Nitrogen

Ultimately, it is excess bioavailable nitrogen in soil (i.e., bioavailable nitrogen present in amounts greater than can be used by plants and microbes) that leaches to waterbodies and causes the undesirable effects previously identified (Mahli and Nyborg, 1986; Luo *et al*., 2000). Fortunately, terrestrial ecosystems are nitrogen limited; thus they usually use a greater proportion of the bioavailable nitrogen they receive. When terrestrial ecosystems are no longer nitrogen limited, they leach nitrogen to aquatic systems. As terrestrial ecosystems lose their inherent tendency toward nitrogen limitation, they will begin to express elevated concentrations of bioavailable nitrogen in the soil; nitrogen that is immediately available to waterbodies via runoff or leaching (i.e., Kahl *et al.,* 1993; Wedin and Tilman, 1996; Peterjohn *et al*., 1996).

Microbiology; Patterns and Constraints

Soil microbial communities can remove nitrogen from terrestrial ecosystems through denitrification, thereby reducing the potential for contamination (i.e., Mahli and Nyborg, 1986; Luo *et al*., 2000). Denitrification is the biogeochemical process in which bacteria use nitrate instead of oxygen to produce energy during which nitrate is reduced to gaseous nitrogen (NO, N_2O, or N_2 depending on oxygen concentration). Denitrification and the related process of nitrification strongly affect soil N chemistry (Hutchinson and Davidson, 1993; Whitehead, 1995). During nitrification, nitrite (NO_2^-) is formed through bacteria mediated chemical decomposition of intermediates between ammonium (NH_4^+) and nitrate (NO_3^-). Nitrite is an unstable product and may easily convert to N_2O under anaerobic conditions (Wrage *et al*., 2001).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Complete denitrification is regarded as beneficial because of its potential to reduce NO_3^- concentrations in soil. However, partial denitrification products (N_2O and NO) have undesirable environmental effects and are potentially harmful greenhouse gases (Bouwman, 1990; Duxbury and Mosier, 1993).

Denitrification is mainly enabled by bacteria (i.e., Denitrifiers) that are generally heterotrophic; they rely on organic compounds as electron donors. Denitrification in soils has been shown to be limited by nitrogen availability (Groffman *et al*., 1993; Jordan *et al*., 1998) and carbon availability (i.e., Schnabel *et al*., 1996; Ashby *et al*., 1998; Frank and Groffman, 1998). However, information on how these limiting factors fit into a larger ecological context is sparse, incomplete, and complex (Silva *et al*., 2005[b]). Soil denitrification has been shown to be a spatially and temporally variable phenomenon (e.g., Luo *et al*., 2000; Frank and Groffman, 1998; Jordan *et al*., 1998). Further, denitrification can also be constrained by oxygen supply, temperature, soil moisture, soil pore status, soil depth, and pH. Due to the complexity of denitrification,

its importance to agriculture, greenhouse gas emissions, and a widespread desire to better understand soil N chemistry, investigators have studied it throughout the world (i.e., Stanford *et al*., 1975; Westerman and Tucker, 1978; Firestone, 1982; Knowles, 1982; Starr and Gillham, 1993; Weier *et al*., 1993a). These studies, and others, have demonstrated the high degree of apparent variability for soil nitrogen transformation processes. Such variability seriously complicates efforts to develop applied products and management recommendations. Better understanding and characterization of the extent and variability of soil N chemistry will aid development of N management strategies in the future.

Nitrogen Leaching

Nitrogen leaching, in the context of this research, is the movement of inorganic nitrogen (i.e., nitrate-N) from upper soil horizons where processing by either plant or microbial biota occurs into lower soil horizons (essentially below plants' root zones; about 50 cm). Inorganic nitrogen that successfully passes through this region will eventually reach groundwater and be released to surface waters (i.e., Baker, 1992; Kahl *et al*., 1993; Peterjohn *et al*., 1996) where, as already noted, undesirable (and noticeable) consequences occur.

Plant Communities

Old-fields have been used extensively as model ecosystems for the investigation of ecosystem N dynamics and effects (Christensen and MacAller, 1985; Kalisz, 1986; Pastor *et al*., 1987; Robertson *et al*., 1988; Dormaar *et al*., 1990; Gross *et al*., 1995). Soil properties, including nitrogen (Wedin and Tilman, 1990; Knops and Tilman, 2000), soil organic matter (Zedler and Zedler, 1969), phosphorus, potassium, calcium, magnesium, and pH (Kalisz, 1986) change during succession. For example, nitrogen is usually the most limiting nutrient to plant growth during the first 40 to 60 years of old-field succession (Gleeson and Tilman, 1990), and through time, resource limitation can shift from nitrogen to light availability (Tilman, 1988). It is thought that early-successional species are best adapted to low nitrogen availability, whereas late-successional species are associated with more elevated nitrogen levels (Tilman, 1987).

As an ecological effect, nitrogen enrichment seldom occurs in isolation. More frequently, multiple effects occur together. In landscapes where past use has been intensive, as in the eastern United States, current ecological effects are often influenced by the past uses of the land. Reduction or elimination of grazing is frequently used as an early restoration intervention technique, especially in riparian areas. Such landscapes frequently have a history of intentional and/or incidental release and proliferation of non-native species.

In temperate mosaic grasslands, removal of heavy grazing can result in an increase of late-successional warm-season grasses (Freeman, 1998; Engle *et al*., 2000), litter accumulation, and a decrease in abundance of non-dominant grasses and forbs (Weaver, 1968; Knapp *et al*., 1998). Accumulation of litter in the absence of grazing can result in decreasing species richness (Collins 1987; Carson and Peterson 1990; Foster and Gross 1998) which Wedin and Tilman (1996) associated with reduced nitrogen use efficiency. Moreover, introduction or presence of non-native species may alter secondary succession in grasslands following cessation of grazing (Tremmel and Peterson, 1983; Fike and Niering, 1999), further effecting soil N processing.

Tall fescue (*Festuca arundinacea)* is an invasive perennial grass native to Eurasia (Gibson and Newman 2001). Widespread use of tall fescue for forage, turf, and soil conservation purposes began in the 1940s, and fescue gained status as a commonly planted species in the eastern United States (Ball *et al*., 1993; Hoveland, 1993). Although fescue is considered by pastoralists to be a valuable forage species in planted pastures, native ecosystems lacking disturbance may be at risk of fescue invasion resulting in fescue becoming a transformer species (Richardson *et al*., 2000).

Studies of tall fescue have focused on species richness and plant-soil interactions of endophyte-infected fescue *viz* endophyte free fescue (Clay and Holah, 1999; Matthews and Clay, 2001). Our study, on the other hand, provided the opportunity to investigate the overall effect of relatively small amounts of fescue in an old-field ecosystem: an old-field ecosystem released from cattle grazing and experimentally exposed to low-levels of bioavailable nitrogen on an on-going basis.

Primary Consumers - Herbivory

Nutrient enrichment, particularly nitrogen input, affects trophic interactions and nutrient cycling. In irrigated shortgrass prairie, nitrogen supplementation converted experimental plots to "islands of tallgrass" in the shortgrass landscape (Grant *et al*., 1977). Enrichment with nitrogen-rich sludge or fertilizer treatments in old-field communities caused decreases in population density, recruitment, and survival of meadow voles (*Microtus pennsylvanicus;* Hall *et al*., 1991). Primary consumers can have significant top-down effects on the cycling of energy and nutrients through ecosystems, especially grasslands (Gessaman and MacMahon, 1984; McNaughton, 1985). These effects range from physical damage and thrash, deposition of feces and urine to change nutrient status for the plant community composition, seed dispersal and soil impacts (Gessaman and MacMahon, 1984; Heske *et al*., 1994; Silva *et al*., 2005a). In this way, modifications in herbivore assemblages may interact with plant communities to amplify the effects of bioavailable nitrogen (Vitousek, 1994).

Litter Decomposition - Detritivory

Plant litter decomposition is a key process in C and N cycles in terrestrial ecosystems. Litter decomposition is influenced by various factors including litter quality, detritivore diversity, and nutrient availability (Swift *et al.,* 1979; Aber and Melillo, 1991; Schlesinger, 1997). For example, inorganic N inputs may alter microbial decomposition by changing N availability and eliminating microbial nutrient limitation or, conversely, inhibiting decomposition (Carreiro *et al.,* 2000). Detritivore and decomposer diversity can influence decomposition rates (Mikola and Setälä, 1998; Van der Heijden *et al.,* 1998; Naeem *et al.,* 2000; Hobbie and Vitousek, 2000) where the presence of diverse macroinvertebrate functional groups allows more efficient litter processing. Quantifying the influence of factors controlling decomposition is critical to understanding litter dynamics and developing better models of nitrogen flux in ecosystems.

Study Site

This study was conducted in southeastern Oklahoma at the Center for Subsurface and Ecological Assessment Research (CSEAR), operated by U.S. EPA, Robert S. Kerr Environmental Research Center, Ada, Oklahoma, USA. CSEAR is located in an area of interspersed old-field and oak-forest patches characteristic of the Cross Timbers ecotone, historically, a mosaic of mixed grasslands and oak-dominated forest between the southern Great Plains and eastern deciduous forests of Texas, Oklahoma, and Kansas, USA (Hoagland *et al.,* 1999). Cultivation at the CSEAR was abandoned ca. 1950, and cattle grazing was halted in 1998. The soil has been classified as clay loam (Vertic Argiustolls, USDA).

Within a contiguous old-field, sixteen 40 x 40-m plots were established to investigate ecosystem interactions associated with additions of low-level nitrogen and manipulations of herbivore populations. Plots were separated by creating a 5-m mowed pathway. N availability and mammals were manipulated in plots in a randomized factorial experimental design such that 4 plots each received fertilizer only, mammal manipulation only, a combination of fertilizer and mammal manipulation, or neither (control). Plots were fertilized with granular 34% ammonium nitrate at an annual rate of 16.3 kg N ha^{-1} yr^{-1} beginning in February 1999 and every 3 months thereafter for 5 years. Mammal populations were manipulated by a 2-m tall chain-link fence of 2.5-cm mesh that effectively excluded intermediate to large sized mammals such as white-tailed deer (*Odocoileus virginiana*), armadillos (*Dasypus novemcinctus*), rabbits (*Sylvilagus floridana*), coyote (*Canis latrans*), gray fox (*Urocyon cinereoargentious*), and striped skunk (*Mephitus mephtus*) while supporting greater abundance of cotton rats (*Sigmodon hispidus*) and mice (i.e. *Reithrodontomys montanus*) inside fenced plots.

Methods

Soil Nitrogen Chemistry

Bioavailable Nitrogen

Soil extractable nitrogen was measured in the 16 experimental plots three times per quarter for 5 years. Two soil samples (separated by 10-m) were used from each plot. Samples were processed within 24 hours of collection. Samples were hand picked of gross contaminants (i.e., plant material, worms, stones) and homogenized prior to analysis. Two subsamples from each homogenized sample were extracted with 2-M KCl using a soil to extractant ratio of 1:2. Extraction procedures included shaking soil slurry for 1 hour, centrifuging at 1800 rpm for 10 minutes at 15°C (Scharf and Alley, 1988) and filtering using pretreated (with 2-M KCl and deionized water) Whatman 42 filter papers (Sparrow and Masiak, 1987). Extractions were completed within two days of sampling. Filtrates were analyzed for mineral-N using LACHAT QuikChem flow injection analyzer (FIA).

In order to better understand the processes that account for variability in soil nitrogen and litter decomposition (i.e., concentrated excretion spot, fine-scale nitrogen transformation processes, local environmental conditions such as soil moisture and porosity), we complemented studies on our primary old-field plots with related short-term field and laboratory studies using the same soils that were taken close to (but outside of) the already described 0.16-ha experimental plots. Details of the experimental techniques for these experiments are delineated below.

Microbiology; Patterns and Constraints

Microbial Response to Added N .- Topsoil (0-10 cm) was collected from an adjacent old-field and homogenized by air-drying and passed through a 4-mm sieve and mixing. Microcosms were prepared in plastic cups with each microcosm containing approximately 290 grams of homogenized soil. Five treatments including a 1) control with no N applied, 2) 100 kg N ha^{-1}, 3) 200 kg N ha^{-1}, 4) 500 kg N $ha^{-1,}$ and 5) 1000 kg N ha^{-1} were established in randomized design with 3 replicates. Nitrogen was applied as 34% ammonium nitrate. Soil water potential in the microcosms was maintained every alternate day by adding water to bring the microcosms to field capacity. All microcosms were sampled on the following intervals; day 0 (prior to N application) , 14, 28, 64, and 130. Soil moisture in the samples was determined gravimetrically by drying soils to a constant weight at 104-105°C. A 1:2 (soil to reverse osmosis water) soil extract was prepared for pH measurement, followed by analysis by EPA method 150.1. Soil mineral nitrogen was measured using Lachat FIA as described under bioavailable nitrogen section.

Denitrification Assays.- Soil samples were taken from field plots set up for experimental nitrogen and herbivore manipulations (previously described). Denitrification of these samples was examined during February 21 and 23, June 28, and July 19 and 24 of 2000. On dates when these plots were sampled, three soil samples were taken from each plot using segments of PVC pipe (5.5 cm diameter x 7 cm depth). One of the intact soil cores was used for assessment of denitrification rates by placing the pipe with soil into a 1-L jar, and using the acetylene block method (i.e., Groffman *et al.*, 1999). The other two soil samples from each plot were composited, and subsamples were used to measure CO_2 production, soil moisture, soil NH_4^+, and NO_3^- contents.

The main focus of our study was to examine the impact of N on denitrification; however, alteration of nitrogen may change plant composition as well as soil carbon status. Thus, in our study we also included carbon treatment to study its impact in comparison to nitrogen. To measure denitrification potential, both carbon (3-mg C g^{-1} soil) and nitrogen (0.3-mg N g^{-1} soil) were added. Denitrification was measured by placing soils (10-g dw) in serum vials using the acetylene block method. Similarly, carbon dioxide production was also measured by placing soils (10-g dw) in serum vials (160 mL) and monitoring the accumulation of CO_2 in the headspace with a TCD (thermal conductivity detector) gas chromatograph. Gravimetric soil moisture was determined by drying soils to a constant weight at 100°C. Soil ammonium-N, and nitrate-N were determined using a Lachat FIA. Additionally, net nitrification and net nitrogen mineralization were determined by incubating composited soils (20-g dw) in water-tight (130 mL) containers at room temperature and measuring the change in soil nitrate-N and total inorganic-N, respectively.

Gross N Transformation Rates and Soil Water.- Intact soil cores (4-cm diameter x 10-cm depth) collected from 4 randomly selected locations from an adjacent old-field were used to measure gross N mineralization and nitrification rates. Intact soil cores were collected on five occasions (between May and July 2002) at 2, 4, 6, 8, and 12 weeks. ^{15}N dilution technique (Di *et al.,* 2000) was used to measure gross mineralization and nitrification rates. Silva *et al*., (2005b) provided additional detailed descriptions of these methods. Since our N application rate was low (16.3 kg ha^{-1} yr^{-1}), there is a very high probability to collect intact cores (4 cm diameter) with greater variability in fertilized plots; therefore, we did not sample from fertilized plots for this short-term experiment.

Additionally, the effect of various soil moisture contents on N transformation process was examined using sieved old-field soil (with no-fertilizer) placed in lysimeters and compacted to achieve original field bulk density (1.4 g cm^{-3}). Water potentials of 0 kPa, -0.5 kPa, and -1.0 kPa were maintained using a vacuum gauge and a vacuum pump, with water being applied to laboratory soils in concert with precipitation events. Specific water potentials were applied to the soil through a ceramic cup, which was attached to the bottom of each lysimeter. Lysimeters were incubated in the laboratory at a constant temperature of 25°C, and soil collected from lysimeters was analyzed at 2, 4, 6, 8, and 12 weeks to determine the gross mineralization and nitrification rates at different water levels as they occur under field conditions.

Nitrogen Leaching

Nitrate-N, ammonium-N, and total Kjeldahl N (TKN) concentrations were measured in soil water and rain water. Soil water was collected in ceramic cup tension lysimeters installed at approximately 90 cm below the soil surface in each of the plots. Lysimeters were evacuated to 50 centibars and monitored every two weeks to check for water and to re-apply vacuum. Three precipitation collectors consisting of an open funnel at 2 m height connected to a collection vessel were placed across the site. Water, when present, was collected from the lysimeters and the precipitation collectors without filtering. Precipitation samples were occasionally fouled by bird droppings and were discarded. All samples were stored in a freezer to await analysis using a Lachat FIA. Dissolved organic N (DON) was calculated as TKN minus ammonium-N, because the total Kjeldhal digestion method (H_2SO_4 and HgO mixture) excluded reduction of nitrate compounds to ammonium. Total N is the sum of Nitrate-N, ammonium-N, and DON.

Nitrogen fluxes were calculated by multiplying nitrogen concentrations by water flux volume. For rainfall inputs the water-flux volume measured by the on-site meteorological station rain gauge was used to calculate the nitrogen flux. Soil water flux volume was calculated using a simple model that used daily soil matric potential and precipitation data to estimate water volume. On days where the soil matric potential was greater than –33 kPa (field capacity), the previous day's precipitation was assumed to be available for leaching. The total leachable water was summed for each two-week period prior to lysimeter sampling. This model is not precise, but provides a good estimate of the volume of water available for leaching and is not biased toward any treatment over another. It might slightly over-estimate fluxes during heavy rain events because runoff is not subtracted from rainfall inputs.

Plant Communities

We calculated average quadrat (0.1–m^2) canopy cover for each species by plot from 1999 through 2003 and clipped enclosed vegetation for biomass measurement in the same quadrat. We performed redundancy analysis (RDA) (ter Braak & Šmilauer 1998) on the species data with *Festuca* as the explanatory variable to investigate community composition. RDA is an ordination technique used when there is a linear relationship between two variables. We also examined relationships between *Festuca* canopy cover and functional group canopy cover using Pearson's correlation coefficients (r). Plant canopy by species was determined using quadrat sampling in May 1999, August 1999, May 2000, and August 2000. Plant species were identified using twenty five 0.1-m^2 quadrats per plot (Stohlgren *et al*. 1998; Jorgensen and Tunnell 2001). Tunnell (2002) and Tunnell *et al.* (2004) provide additional detailed descriptions of these methods.

Primary Consumers – Herbivory

Population Ecology

We sampled small mammals with Sherman live traps (7.6 × 8.9 × 22.9 cm) for 3 consecutive days at 3–5 week intervals from July 1999 to December 2000. Each plot consisted of 25 traps systematically spaced at 7-m intervals. We released captured animals immediately after marking with a unique identifier. Identification was done without considering animal's sex. We used minimum number known alive (MNKA; Krebs 1966) as an index to abundance for each plot at each sampling period. We made statistical comparisons of MNKA between treatment plots using a 2-way analysis of variance with repeated measures (PROC MIXED, SAS 1990). We fitted a multiple variance model and used the Kenward-Roger approximation to calculate effective degrees of freedom (PROC MIXED, SAS 1990; Kenward and Roger 1997) and used least-squared means separation tests for all significant main effects.

Physiology

To determine nitrogen dynamics and requirements at various life phases, animals underwent a series of feeding trials under laboratory conditions. A captive research colony was formed using wild-caught individuals trapped at various sites in Oklahoma. Not all animals used for feeding trials were wild-caught; some experimental animals were the captive-born progeny of the wild individuals. All free-ranging animals were captured using Sherman live traps (7.6 x 8.9 x 22.9 cm), following standards established by the Animal Care and Use Committee of the American Society of Mammalogists (1998). Research subjects were housed at the Laboratory Animal Resources facility at Oklahoma State University for the duration of the pretrial and experimental periods. Animals were housed individually (except during the breeding phase of the reproduction trial) and kept at 20-25°C under a 12L:12D cycle for the duration of the study. We operated under Animal Care and Use Protocol 723, Oklahoma State University. Mice were housed in 28- x 18- x 13-cm wire-topped plastic cages with corn-cob bedding, and cotton rats were housed in similar cages that were 48 x 25 x 20 cm.

Litter Decomposition - Detritivory

Live and dead grasses and forbs were collected for litter on 18 December 1998 and 18 February 1999 from immediately outside of our experimental plots. Cut litter was turned by hand to form a homogenous mix. A known quantity of dried litter weighing (mean $\pm$ 1 SE) 9.568 g $\pm$ 0.106 was placed inside a bag made from a 0.5 x 0.5 m piece of nylon mesh secured at the top with a locking plastic tie. Coarse (6.35 mm) and fine (0.33 mm) meshes were used to make litter bags. Fine mesh was intended to exclude macro-detritivores such as earthworms (Annelida), soil mites (Arachnida), insects (Insecta), pill bugs (Isopoda), and snails (Gastropoda), whereas coarse mesh was intended to allow access to the litter by all micro and macro-detritivores. A total of 480 litter bags was constructed, half of fine mesh and half of coarse.

Results

Soil Nitrogen Chemistry

Bioavailable Nitrogen

Extractable nitrate-N concentrations never averaged more than 1 mg L^{-1} during the growing season and were always less than 2 mg L^{-1} on control plots during the dormant season. The introduction of an additional 16.3 kg N ha^{-1} yr^{-1} resulted in little or no excess N at Fall 99 (Figure 2). However, during the first dormant season, nitrate-N concentration on fertilized plots was 525% greater than expected (i.e., compared to control plots), and concentrations remained elevated on average throughout the experiment (Figure 2).

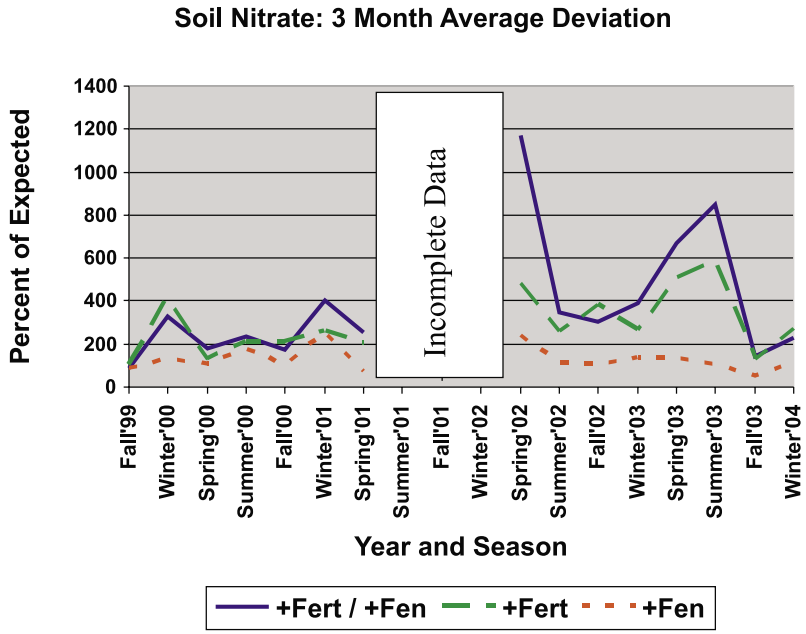


Figure 2. *Percent extractable soil nitrate-N content relative to control treatment. The change from Fall 1999 to Winter 2000 and continuing throughout the experiment is notable because it shows that the ability of soil to adapt to new nutrient inputs is compromised by prior inputs. Further, the magnitude of variation was relatively small during the first year, but increased thereafter, as did the overall variability.*

While most of the elevated soil nitrate-N response is attributable to fertilization, a moderate level of soil nitrate-N was measured in the fenced only treatment. Further, the amount of excess nitrate-N measured on fertilized only and fenced only treatments closely corresponds to the excess nitrate-N measured on fertilizer and fence combined treatment. Therefore, these effects appear to be additive and occur independently. It is noteworthy to add that N measurements on the combined treatment plots during spring exceeded the sum of inputs measured on fenced and fertilized plots alone (Figure 3). This is likely attributable to N cycling from other seasonal sources (e.g., insects) and carryover of N from the preceding winter.

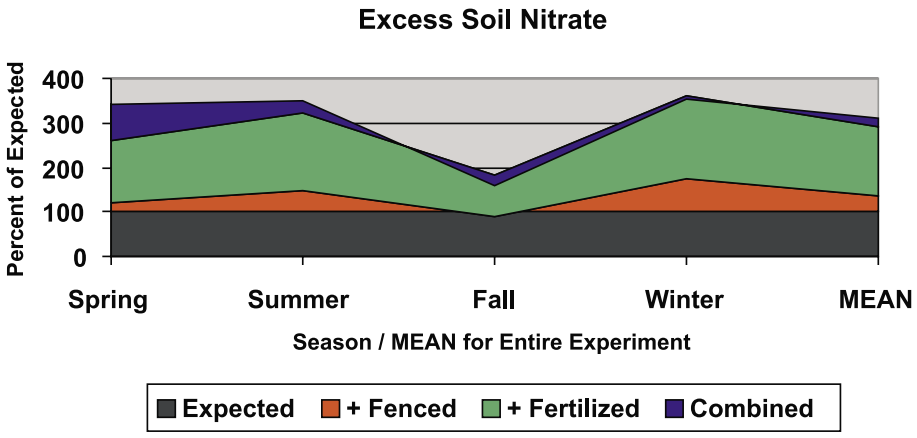


Figure 3. *Both fertilization (+Fertilized) and increased small mammal density (+Fenced) increased soil nitrate-N. The cumulative effects of these appear to be additive (Combined) during most of the year.*

In summary, soil nitrate-N levels averaged over 250% higher on fertilized plots *viz* control plots, and excess nitrate-N was particularly low during fall. The concentrations attributable to fertilization were sufficient to cause observable leaching through the root zone (see Nitrogen Leaching results) while those attributable to increased density of small mammals alone were not. Even though the overall average results from small mammals alone were relatively low, individual excretion patches, latrines, or other local hot-spots can considerably deviate from this trend due to high nitrogen loading as seen in our simulated N application experiment (Figure 4).

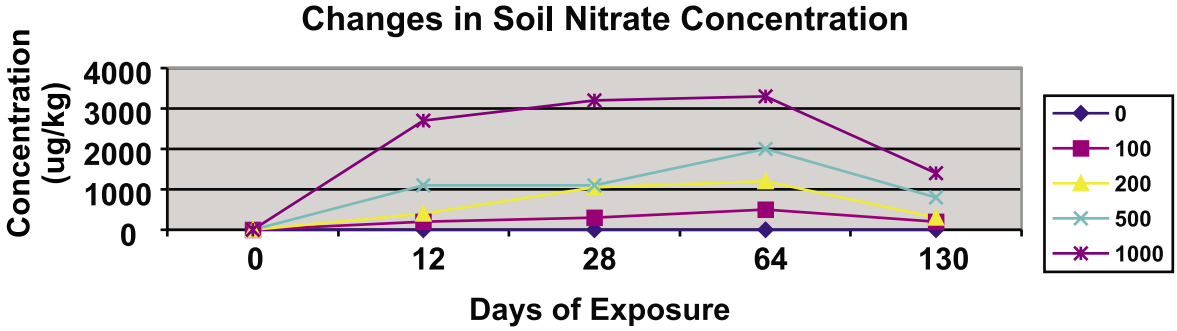


Figure 4. *Soil nitrate chemistry changed quantitatively with nitrogen dose; while the amount of nitrate-N measured in the soil increased with dose, the increase remained proportional to the input. This suggests that microbial responses to nitrogen additions may be relatively fixed.*

Microbiology; Patterns and Constraints

Microbial Response to Added N.- Qualitatively, the biogeochemistry of soils treated with 100, 200, 500, and 1000 kg ha^{-1} of nitrogen responded similarly (Figure 4). Response followed load in the expected fashion, with progressively greater nitrate-N concentrations being present in those microcosms that received greater nitrogen loads. Quantitatively, while the biogeochemistry of the treatments responded differently, the difference was in the expected direction (i.e., greater concentrations observed in those microcosms that received higher loading of nitrogen). However, it is important to note that by the end of the experiment, the observed quantitative difference among the treatments was converging, a point that will be discussed later.

Denitrification Assays.- Even though stimulation of denitrification by nitrogen tended to occur after major precipitation events, denitrification was more often stimulated by carbon than by nitrogen (135 out of 161 composited soil samples). As soils dried and warmed with the onset of summer drought, denitrification became carbon-limited. Denitrification was most stimulated by nitrogen after a rain event in April 2000. At this time, denitrification was also highly stimulated by

carbon. As soils dried and warmed in May and June 2000, denitrification became more exclusively limited by carbon. By July, denitrification activity diminished altogether. Rain events of September and October 2000 renewed denitrification activity, but unlike denitrification after the April rain events, this activity was stimulated only by carbon and not by nitrogen. Anaerobic conditions enhanced denitrification in carbon-amended soils compared to aerobic treatments, but they were not sufficient to stimulate denitrification without added carbon.

Response of denitrification to laboratory additions of nitrogen and carbon was altered by fertilization (Figures 5 and 6). Carbon stimulated denitrification more than nitrogen in fertilized soils, whereas nitrogen stimulated denitrification more in control soils.

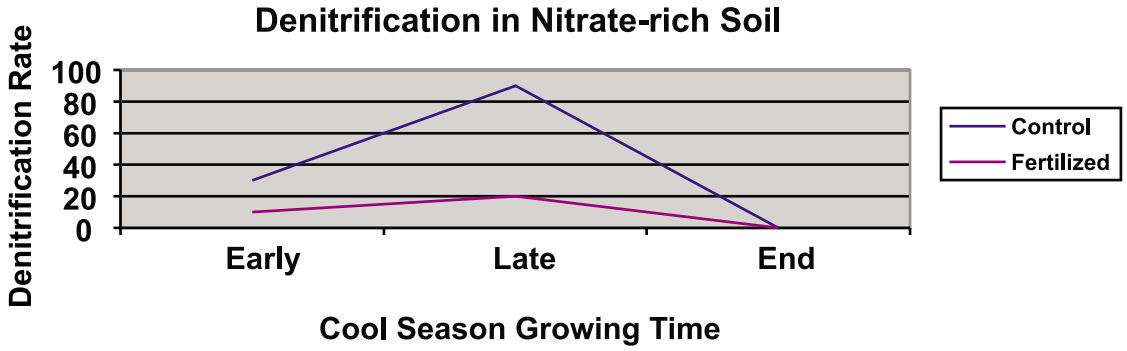


Figure 5. *Denitrification in soils provided with ad libitum amounts of nitrogen was greatest in soils collected from control plots. This means that these soils' ability to respond to new nitrogen inputs (ad libitum nitrogen addition) was reduced by prior exposure to low-level fertilization.*

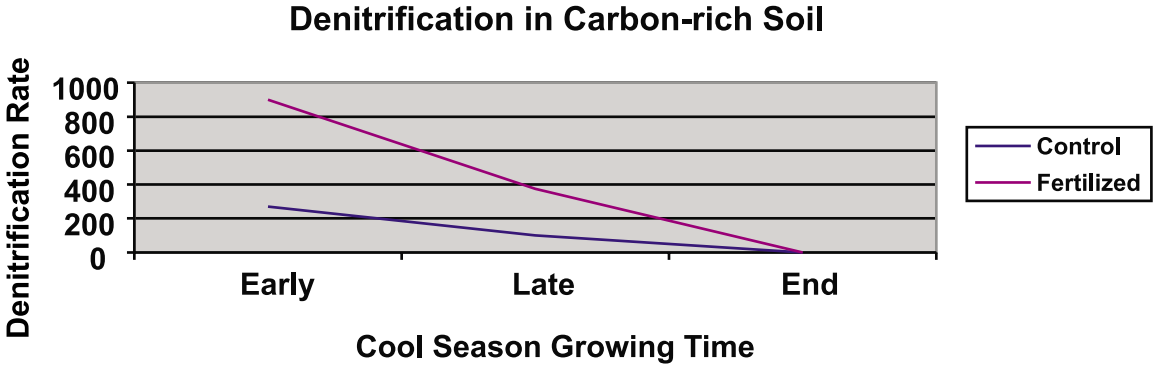


Figure 6. *Denitrification in soils provided with ad libitum amounts of carbon was greatest in soils collected from fertilized plots, especially during wet spring periods. This reveals that these soils' natural tendency for carbon limitation was even further enhanced when exposed to chronic low-level fertilization.*

Gross N Transformation and Mineral N.- Gross N mineralization and NH_4^+ consumption rates changed slightly between sampling times when compared to nitrification and NO_3^- consumption rates. Additionally, both consumption rates (NH_4^+ and NO_3^-) were greater than gross mineralization and nitrification rates (Figure 7); however, differences were fairly constant over the experimental period. Similarly, both ammonium-N (1.5 –2.0 mg kg^{-1}) and nitrate-N (0.8 –1.0 mg kg^{-1}) concentrations did not change over the late-spring to mid-summer period, suggesting that internal N transformation processes in this old-field are fairly consistent under control conditions, and as one process shifts even slightly, others change to counterbalance the system. However, as bioavailable N increases with N additions, the balance of the internal N transformation may alter (i.e., lower N consumption) due to limitations of other nutrients (particularly carbon) leaving excess N in the system.

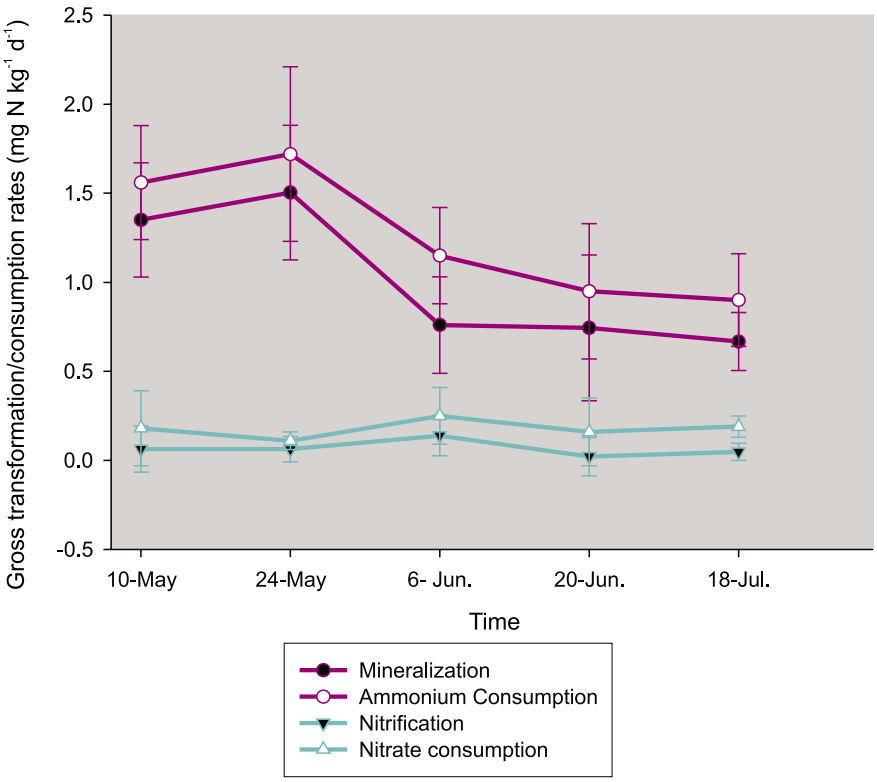


Figure 7. *Gross N mineralization, nitrification, ammonium consumption, and nitrate consumption rates in old-field soil measured under field conditions. Bars indicate the ± standard error of mean.*

Moisture Effects.- Soil N transformation processes were also examined through variation of response in association with water potentials. Average gross NH_4^+ consumption rates were greater than gross nitrogen mineralization rates for a given water potential (Figure 8A), but the differences were constant as observed in intact soil cores under field conditions. Nitrogen transformation rates increased as soil water potential decreased. Greater mineralization and NH_4^+ consumption rates from lower water potentials could be attributable to greater O_2 availability as more soil pores dry with decreasing water potentials. Such differences were not observed in nitrification and NO_3^- consumption rates; however, the magnitude of effect varied during the course of the experiment. This could not be only a result of soil water potentials, but also the effect of soil incubation processes (Figure 8). Our results also reveal that N mineralization is a robust process when compared with nitrification.

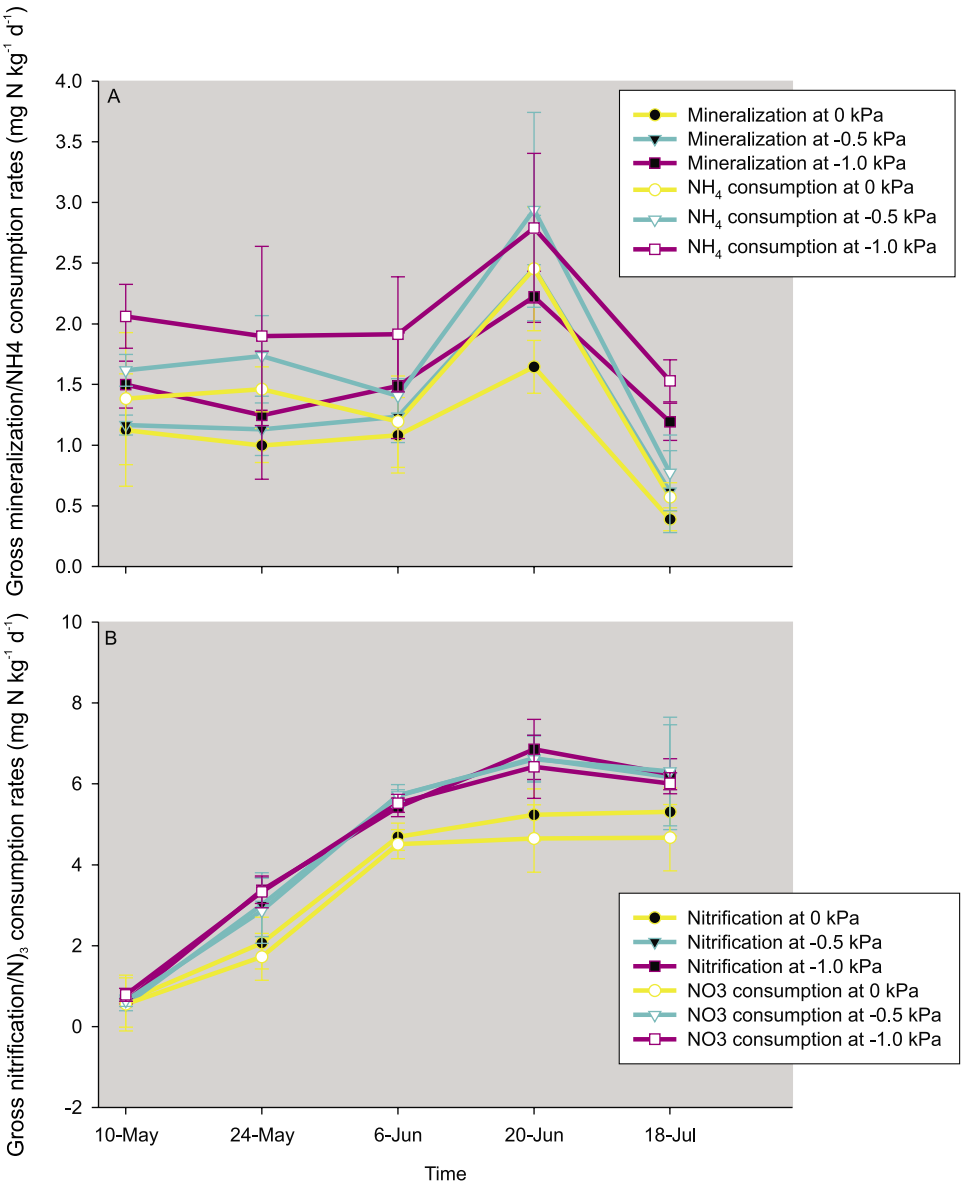


Figure 8. *Gross mineralization and NH_4^+ consumption rates (A) and gross nitrification and NO_3^- consumption rates (B) from old-field soils under three different water potentials measured under laboratory conditions. Vertical bars indicate ± standard error of mean.*

Nitrogen Leaching

Precipitation inputs of nitrogen were distributed fairly equally among nitrate, ammonium, and DON at this site (Table 1). Total nitrogen inputs were 2.5 times greater in the fertilized plots than in the unfertilized, while ammonium-N and nitrate-N were each about 3 times greater in the fertilized plots.

Eighty to ninety percent of the N leaching occurred as nitrate-N in the fertilized plots, while DON was the largest component of total N leaching in unfertilized plots making up about 75% of the total N leaching (Table 2). Fertilization had little effect on the amount of DON leaching with fertilized and unfertilized plots having approximately the same amount of DON leached. Very little N was lost as ammonium in either the fertilized or unfertilized plots. Fencing had no discernable effect on N leaching.

Table 1. Total Nitrogen Inputs (kg N ha^{-1} yr^{-1}) for Water Year 2002-2003

	Control[a]	Fertilized Plots[b]
Nitrate-N	3.7	11.8
Ammonium-N	3.6	11.7
DON[c]	3.8	3.8
Total N	11.0	27.3

Current year's rainfall = 73.1cm (100 cm annual average)
[a]Inputs from rainfall
[b]Fertilizer inputs (16.3 kg N ha^{-1} yr^{-1} as NH_4NO_3) plus rainfall inputs
[c] Dissolved Organic N from total Kjeldahl N minus ammonium N.

Table 2. Nitrogen Leaching Below 90 cm for Water Year 2002-2003 (kg N ha^{-1} yr^{-1})[a]

Treatment	NO_3^- Leaching Flux	NH_4^+ Leaching Flux	DON[b] Leaching Flux	Total N Leaching Flux
Fert x Fence	8.2	0.1	0.7	9.0
Fert Only	12.8	0.3	2.3	15.4
Fence Only	0.5	0.1	2.0	2.6
Control	0.1	0.2	1.0	1.3

Total water leached: 38.7 cm
[a] Calculated from nitrogen concentrations in solutions collected bi-weekly from ceramic cup tension lysimeters multiplied by leachable water (precipitation onto soil at or above field capacity) over the same time period.
[b] Dissolved Organic N from Total Kjeldahl N minus ammonium

Plant Communities

Although no differences in total canopy cover were observed between fenced and control treatments, we observed a slightly positive nitrogen effect on total biomass in later years. Further, even after 5 years of grazing release, biomass continued to increase including in the control plots (Figure 9).

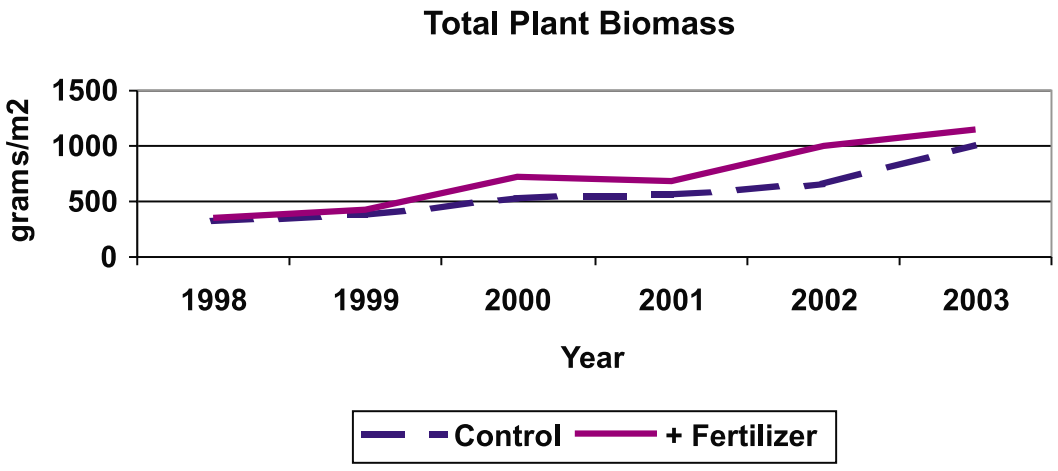


Figure 9. *Plant biomass production in the control and fertilized treatments. There was no measurable difference between control and fenced plots over the experimental period.*

Festuca cover increased nearly five-fold on average across plots from 1999 to 2001 (Table 3), which is reflected along RDA Axis 1. Further, vegetation dynamics in this old-field were explained by increasing abundance of *Festuca*. The change in species composition within plots is visually represented in the ordination diagram in which plots with the greatest amount of *Festuca* (>10% canopy cover) are located in the right half of the diagram (Figure 10).

Table 3. Mean ($\bar{x}$) and Standard Error (±SE) of Species Richness and *Festuca* Canopy Cover in an Oklahoma Old-Field (n=16). There was No Treatment Effect on Species Richness

	Species Richness		Festuca Canopy Cover (%)	
Year	$\bar{x}$	SE	$\bar{x}$	SE
1999	44	±2	2.3	±0.6
2000	37	±1	4.9	±1.0
2001	41	±2	10.9	±2.0

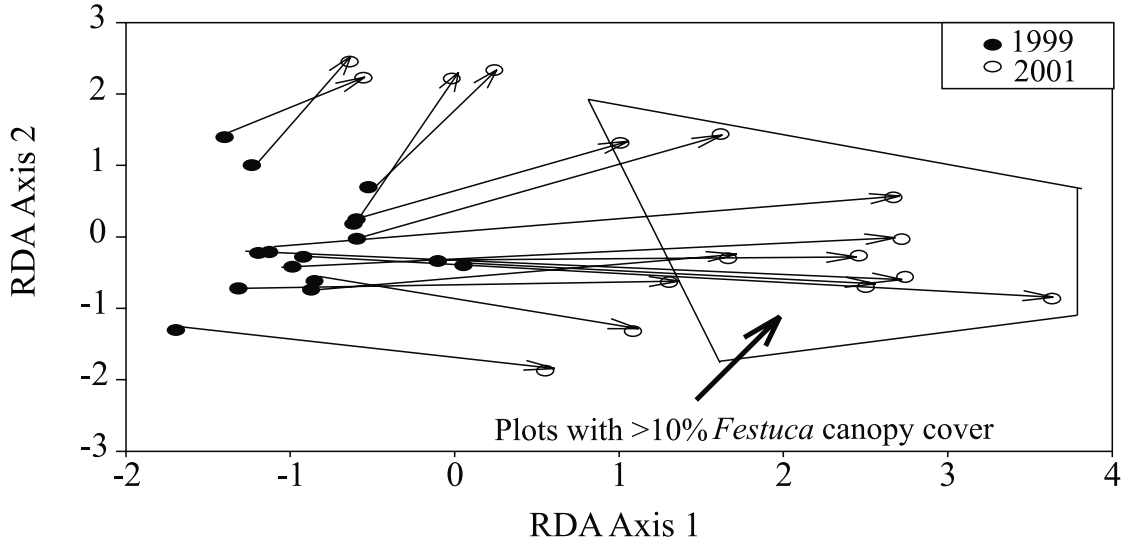


Figure 10. *Site scores for the first two axes of the RDA following in an Oklahoma old-field from 1999 (●) to 2001 (○). RDA axis 1 is represented by increasing Festuca canopy cover and has an eigenvalue of 0.135 (P=0.005).*

Festuca was correlated with the two dominant functional groups, warm-season native grasses and non-legume forbs (Figure 10). *Festuca* canopy cover was correlated negatively with warm-season native grass cover in 2001. Warm-season native grass cover was not correlated with *Festuca* cover in the first two growing seasons when *Festuca* cover was less (Figure 11). However, plots with the greatest warm-season native grass canopy cover had the least amount of *Festuca* cover in 2001, and plots with low warm-season native grass canopy cover had the greatest amount of *Festuca* cover (Figure 11).

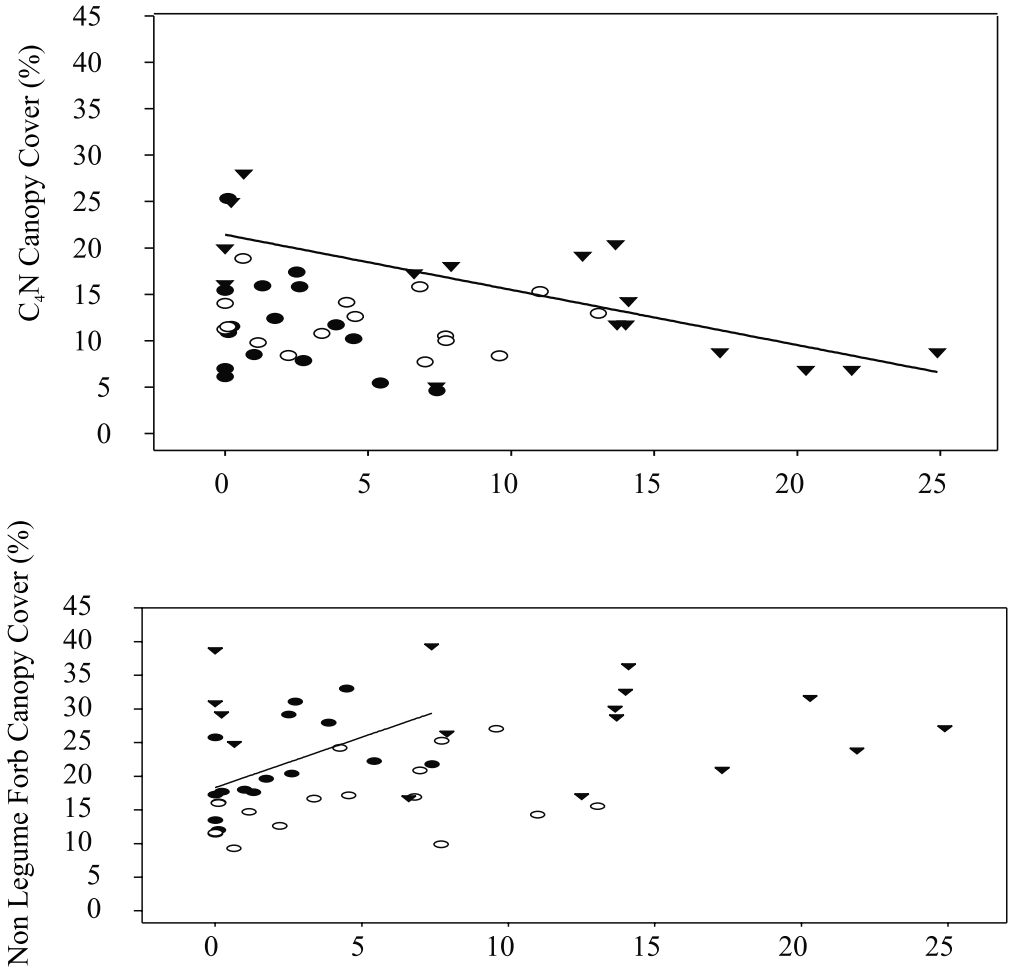


Figure 11. *Correlation between Festuca canopy cover and functional group canopy cover on an Oklahoma old field from 1999 to 2001. a. Correlation between Festuca canopy cover and warm-season native (C_4N) grass canopy cover in 1999 (P = 0.1305), 2000 (P = 0.7644), and 2001 (P = 0.0020, r = -0.25). b. Correlation between Festuca canopy cover and non-legume forb canopy cover in 1999 (P = 0.0335, r = 0.45), 2000 (P = 0.1176), and 2001 (P = 0.4915). ● = 1999, ○ = 2000, and ▼ = 2001.*

The relationship appears to be causal in that, on the average, warm-season native grasses decreased most on plots where *Festuca* increased (Figure 12).

Species richness (change in canopy cover) was negatively related to *Festuca* canopy cover (Figure 12), but species richness was not correlated to litter mass (Figure 13).

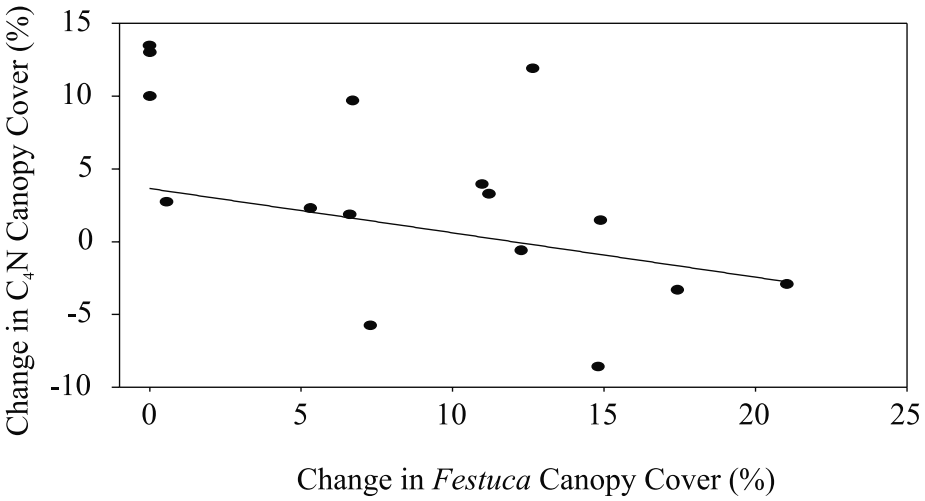


Figure 12. *Plot of the correlation between the change in Festuca canopy cover and the change in warm-season native canopy cover from 1999 to 2001 (P = 0.0410, r = -0.56).*

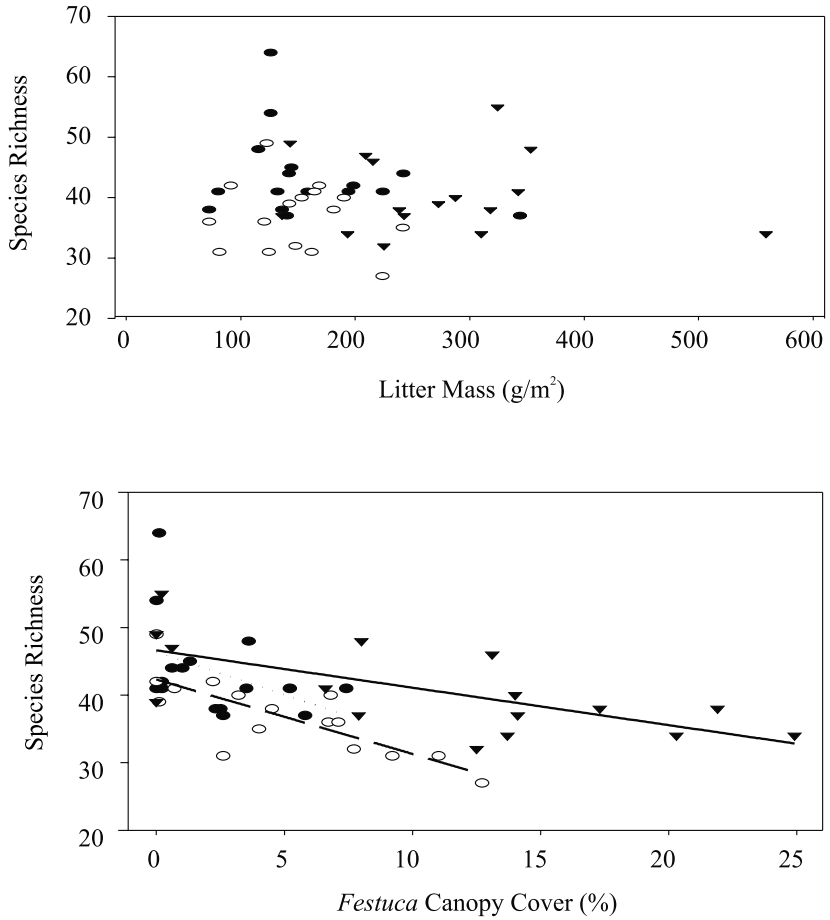


Figure 13. *Plot of species richness as a function of litter mass. Regression models were not significant in 1999 (P = 0.3616), 2000 (P = 0.5560), and 2001 (P = 0.6913). ● = 1999, ○ = 2000, and ▼ = 2001.* **b.** *Plot of species richness as a function of Festuca cover. Regression model in 1999 was not significant (P = 0.1069). Regression model in 2000 was significant (P = 0.0003), with Y = 42.3 – 1.1X where Y = species richness and X = Festuca canopy cover; R^2 = 0.62. Regression model in 2001 was significant (P = 0.0039), with Y = 46.6 – 0.5535X where Y = species richness and X = Festuca canopy cover; R^2 = 0.46. ● = 1999, ○- - - -○ = 2000, and ▼—▼ = 2001.*

Primary Consumers - Herbivory

Population Ecology

Between July 1999 and December 2000, we recorded 7,955 small-mammal captures in 20 sampling periods (i.e., 24,000 potential trap nights). Cotton rats (*Sigmodon hispidus*) accounted for 5,468 (68.8%) captures; two species of harvest mice accounted for 1,971 captures (*Reithrodontomys montanus* N=1229 [15.5%]; *Reithrodontomys fulvescens* N=742 [9.3%]).

We observed 2-way interactions for abundance of cotton rats between the fenced treatment and time ($F_{15, 170} = 1.91$, $P = 0.024$) and between fertilizer and fence treatments ($F_{1, 24.3} = 10.87$, $P = 0.003$). Abundance of cotton rats tended to be higher on fertilizer-fenced plots ($\bar{x} = 18.4$, $SE = 0.8$, $P < 0.001$) compared with other treatments (control: $\bar{x} = 9.8$, $SE = 1.0$; fenced only: $\bar{x} = 11.2$, $SE = 0.8$; fertilized only: $\bar{x} = 9.7$, $SE = 1.0$; Figure 14). Abundance of *R. montanus* tended to be higher on fertilized-only treatment, but lowest on fertilizer-fenced treatment (3-way interaction: nitrogen × fence × time, $F_{15, 171} = 2.22$, $P = 0.007$; Figure 15). We observed no distinct patterns in relation to the treatment plots for abundance estimates of *R. fulvescens* (Figure 16).

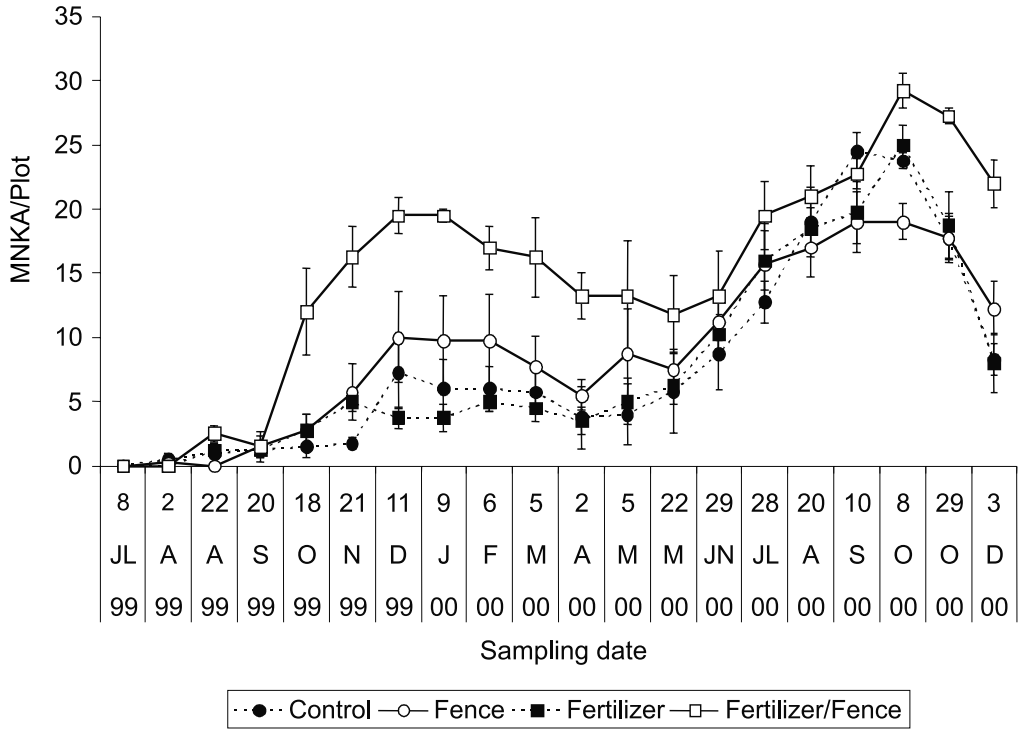


Figure 14. *Estimates and standard errors (±1 SE) of minimum number known alive (MNKA) for Sigmodon hispidus across a landscape manipulated with fertilization and enclosure fencing at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000.*

Physiology

Estimates of mean daily urinary and fecal nitrogen deposition for an average individual *Sigmodon hispidus* were of 100.9 mg day^{-1} (assuming an average weight of 150 g). There are many factors to consider when extrapolating this number to calculate an estimated deposition for each plot. For this presentation, a range of potential outcomes are given that assume constant *Sigmodon hispidus* density for an entire year. Thus, the intent is to estimate a range of outcomes including the most extreme case while providing evidence that may more closely reflect actual conditions.

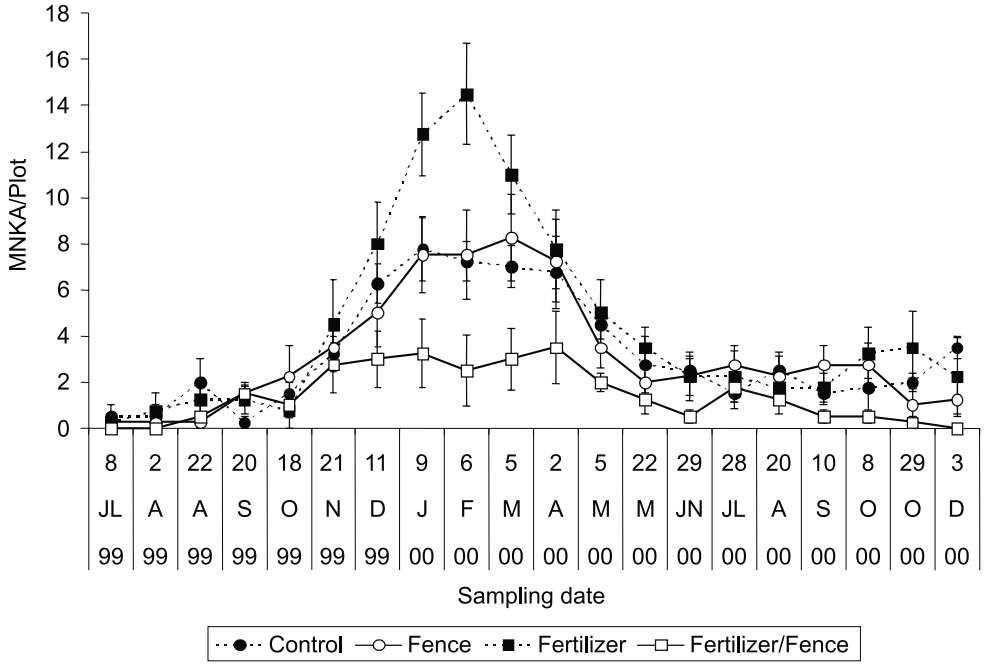


Figure 15. *Estimates and standard errors (±1 SE) of minimum number known alive (MNKA) for Reithrodontomys montanus across a landscape manipulated with fertilization and enclosure fencing at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000.*

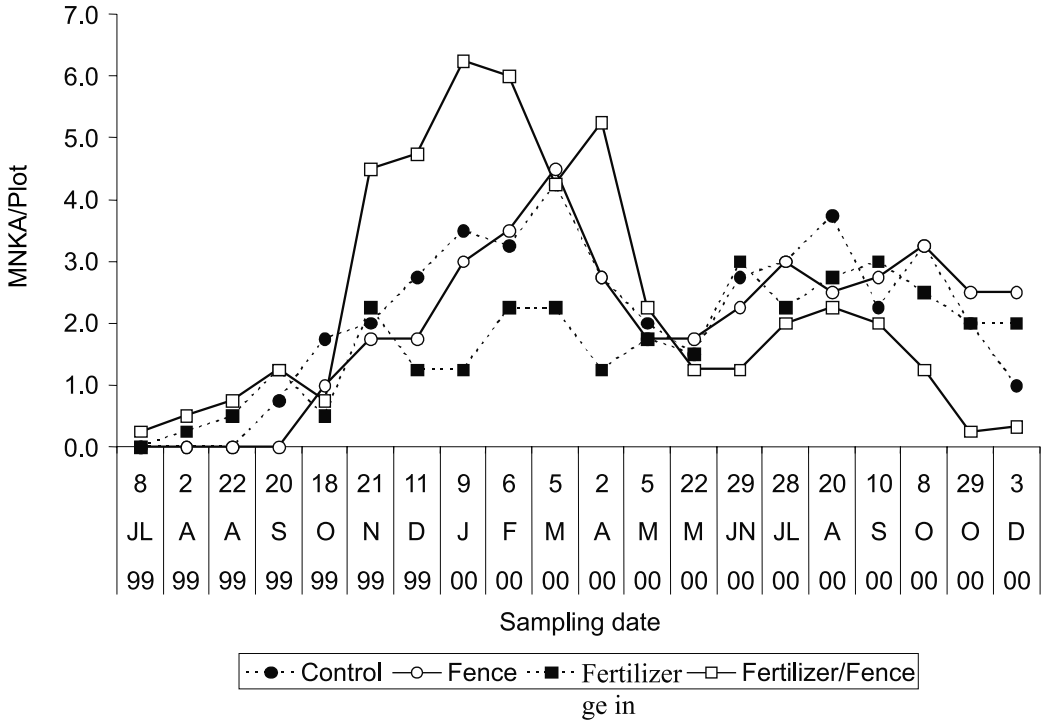


Figure 16. *Estimates of minimum number known alive (MNKA) for Reithrodontomys fulvescens across a landscape manipulated with fertilization and enclosure fencing at the Center for Subsurface and Ecological Assessment Research, Pontotoc County, Oklahoma, 1999–2000. Standard errors were not included because no significant differences (P > 0.05) were detected between treatment combinations.*

In our study, we observed a maximum abundance of *Sigmodon hispidus* of 200 ha^{-1} during October of 2000 (minimum abundance was 0 ha^{-1}) (note: abundance relative to particular experimental treatments has already been given). This means that *Sigmodon hispidus* may have been depositing up to 32.6 kg ha^{-1} yr^{-1} of nitrogen back to the plots as urine and feces (Table 4).

Table 4. Range of Estimates of Potential *Sigmodon hispidus* Urinary and Fecal Nitrogen Deposition under Experimental Conditions Observed in this Experiment and under Conditions Reported Elsewhere in the Literature

Condition/Observation	Abundance (#/ha)	Urine and Fecal Deposition (kg ha^{-1} yr^{-1})
Observations from this experiment		
Maximum abundance	200.0	32.6
+Nitrogen +Fence	115.0	18.8
+Nitrogen – Fence	60.6	9.9
-Nitrogen +Fence	70.0	11.4
-Nitrogen –Fence	61.3	10.0
Nitrogen Mean	87.7	14.3
-Nitrogen Mean	65.6	10.7
Observations from literature		
Jorgensen *et al.* 1994	244.0	39.8
Langley and Shure 1988	119.0	19.4
Schetter *et al.* 1998	111.0	18.1
Doonan and Slade 1995	100.0	16.3
Schetter *et al.* 1998	90.0	14.7
Stafford and Stout 1983	46.9	7.7
Doonan and Slade 1995	39.5	6.4
Fleharty *et al.* 1972	20.6	3.4
Cameron 1977	14.0	2.3

Litter Decomposition - Detritivory

Statistical analysis reveals that litter decomposition was unaffected by either fertilization or exclusion of intermediate and large mammals. All litter showed an increase in nitrogen concentration over time; however, nitrogen in litter placed in fertilized plots displayed a reduced rate of nitrogen loss. Further, it appears that the litter would actually again be accumulating nitrogen relative to its initial concentration after little more than a year of decomposition (Figure 17).

Change of nitrogen loss in litter is not only a function of fertilization. There are several other factors that can influence litter decomposition and subsequently nitrogen loss. Detritivore exclusion altered litter nitrogen dynamics. After an initial loss of nitrogen in both control and exclusion litter, nitrogen began to rapidly accumulate in exclusion litter. After little more than 6 months of decomposition, nitrogen actually began to accumulate in exclusion litter (Figure 18).

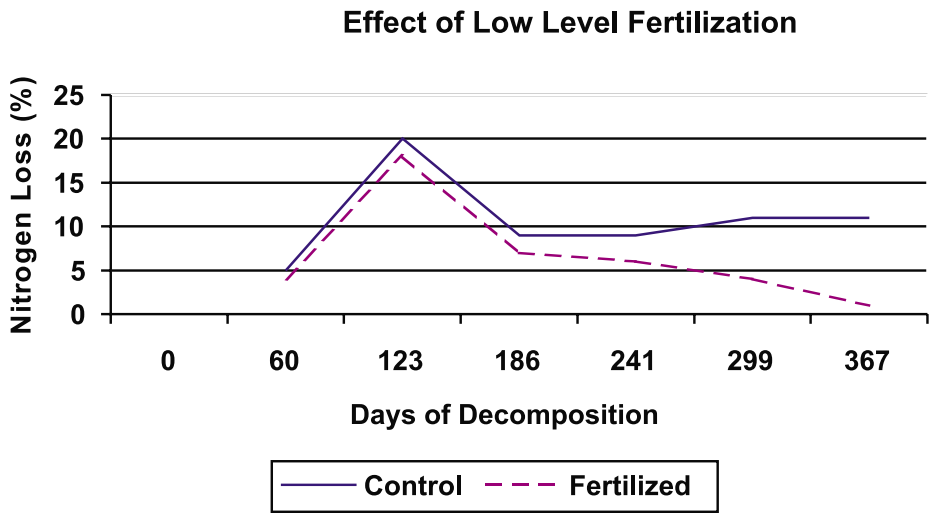


Figure 17. *In litter which was placed in plots that did receive fertilizer amendments, litter nitrogen declined relatively slowly over time. However, in litter which was placed in plots that received fertilizer amendments, nitrogen was lost at a reduced rate.*

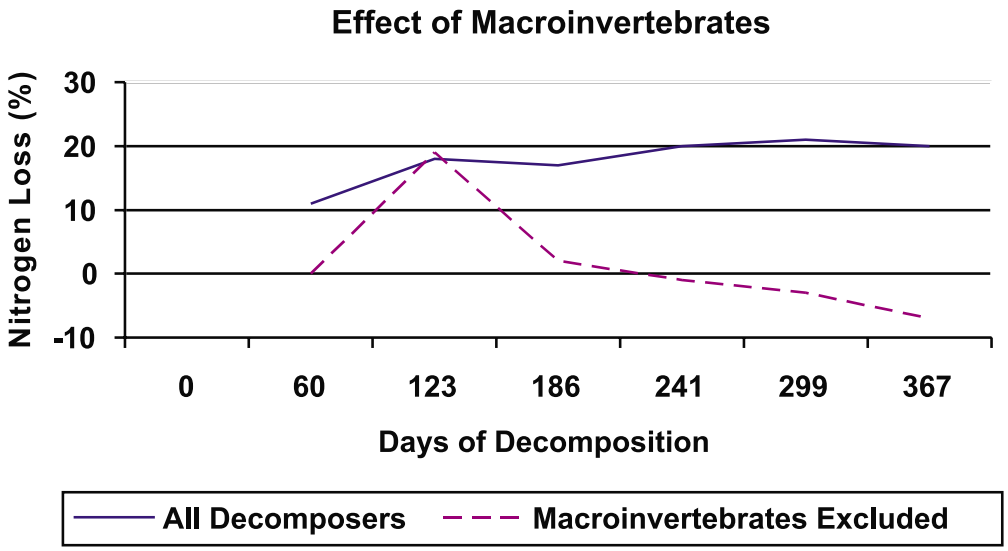


Figure 18. *In litter that allowed access by all decomposers and detritivores, litter nitrogen declined consistently over time. However, in litter where access was denied to macroinvertebrates, nitrogen dynamics were strongly altered. After a little more than 6 months of decomposition under these conditions, nitrogen actually was accumulating in the litter relative even to the litter's initial content of nitrogen.*

Discussion

These results demonstrate that even the relatively small amounts of bioavailable nitrogen that are deposited in precipitation have the capacity to change multiple aspects of ecosystem function. These studies provide insight into the results of Wedin and Tilman (1996), where most ecosystem effects attributable to exposure to excess bioavailable nitrogen occurred in the first 100 kg ha^{-1} yr^{-1} of deposition. In this study, changes among the responses of different trophic levels to low-level nitrogen inputs were always in a direction that favored low nitrogen turnover and/or increased nitrogen flux, both conditions that could contribute to Wedin and Tilman's (1996) observations. These characteristics directly lead to increased levels of exposure to nitrogen and to other trophic levels that will in turn further reduce the ability to process new nitrogen inputs. The changes observed are always potentially deleterious in that they lead to greater concentrations of nitrate-N in soil and thereby make more nitrogen available for leaching to surface and groundwater.

Soil Nitrogen Chemistry

Bioavailable Nitrogen

In this experiment, soil nitrogen chemistry is the main focus of all of the trophic interactions that were investigated. There needed to have been a change in soil nitrogen chemistry if excess nitrate-N were to be available for leaching. While we expected this change to occur, we did not have a clear idea of how long it would take. In fact, it took only a single growing season for changes in the soil's ability to adapt to nitrogen inputs to be evident.

Ultimately, it is excess bioavailable nitrogen in soil that leaches to water-bodies and causes the undesirable effects previously identified (Mahli and Nyborg, 1986; Luo *et al*., 2000). This study demonstrates that even regions of the eastern United States that receive seemingly modest or small amounts of atmospheric nitrogen deposition may have undergone a long-term change to their ability to process further nitrogen inputs. These data are consistent with Perakis and Hedin's (2002) hypothesis that currently observed ecosystem biogeochemistry in much of the Northern Hemisphere may be the product of an historical alteration to biogeochemical cycles that has not yet been identified or understood.

Our data suggest that the inherent capacity of terrestrial ecosystems to process bioavailable nitrogen is alterable by low-level exposures; however, the long-term effects of such exposure remain uncertain.

Microbiology; Patterns and Constraints

Microbial Response to Added N.- We found no indication of a qualitative difference in soil biogeochemical response, expressed as concentration of nitrogenous compounds, among control soils or those dosed with an equivalent of 100, 200, 500, and 1000 kg N ha^{-1} applied as 34% ammonium nitrate (Figure 4). Based upon the findings of Wedin and Tilman (1996), this was somewhat surprising. An important difference between our study and that of Wedin and Tilman (1996) was that our lowest load was an equivalent of 100 kg ha^{-1}. For Wedin and Tilman (1996), ecosystem structure and function were already seriously affected at this load. So, it could be that soil biogeochemical response differs qualitatively with load at low doses (i.e., <100 kg ha^{-1}), but not at higher doses. While our data are silent on this question, the qualitative response we observed for our experimental doses leaves little room for such a response to occur. Therefore, we expect that most of the exponential decay in ecosystem structure and function observed by Wedin and Tilman (1996) is ultimately attributable to plant community dynamics.

Denitrification Assays.- Our results contrast with studies which found denitrification to be N limited (Luo *et al*., 2000; Jordan *et al*., 1998; Groffman *et al*., 1993), but are similar to other studies which found denitrification to be limited by carbon availability (Frank and Groffman, 1998; Luo *et al*., 1998; Schnabel *et al*., 1996). However, after rain events, such as in April 2000, denitrification was sometimes also stimulated by nitrogen, indicating that carbon and nitrogen can transiently co-limit denitrification depending on soil moisture conditions. Similarly, Ashby *et al*. (1998) found that nitrogen was more important to denitrification when soils were wet and carbon was available.

Our data suggest that the ability of microbial consortia to conduct denitrification will be limited and that it will be affected by prior exposure to nitrogen. The ability of microbial consortia to respond to new nitrogen inputs may already be adversely affected throughout many areas of the eastern and central United States. We suggest that integrated ecological

studies, including the consideration of the effects of plants, animals, and upland sites may be essential for constructing predictive models of denitrification throughout a potentially wide area of the eastern and central United States.

Denitrification is less efficient than aerobic utilization of the same carbon compounds, so denitrification rates are highest where soils are saturated and become anoxic. We measured denitrification in upland soils that are generally considered aerobic, perhaps occurring in the anoxic microsites of these soils. Across the central United States, upland soils cover a larger surface area than saturated soils. Therefore, although there is concentrated denitrifying activity in riparian zones, hyporheic zones, and wetlands, denitrification in upland soils may be just as important regionally, especially considering their large spatial extent (Ashby *et al*., 1998).

Nitrogen Leaching

Inorganic N deposition across the United States ranges between near 0 kg N ha^{-1} yr^{-1} in west coast sites to 10 kg N ha^{-1} yr^{-1} in sites in New England. Inorganic N deposition measured at our site was higher (7.3 kg N ha^{-1} yr^{-1}) than the 4.4 kg N ha^{-1} yr^{-1} ten-year average (1993-2002) of three National Atmospheric Deposition Program (NADP) sites in the region (sites OK17, AR27, and TX56). This could be due to undetected other depositions (i.e. bird-related contamination), differences in sampling protocol, or inter-annual variation. The NADP sites do not measure DON, and the results of this study demonstrate that DON is also an important source of N inputs, making up about one third of the total at our site.

In unfertilized plots, only about 10% of rainfall N inputs were detected in leaching water; however, nitrate-N leaching at our site increased as a result of ammonium nitrate additions. About two thirds of the annual addition of fertilizer nitrogen was detected in leachable water as nitrate-N. Some of this nitrogen could have come from previous years' N additions, because we did not monitor nitrate leaching in previous years. The relatively low rainfall during this water year also could have contributed to higher nitrate-N leaching in fertilized plots in two ways: 1) Lower plant growth could have lowered the demand for added N and 2) Drying of the vertic clays at the site may have increased macropore flow by-pass because of the extensive cracking that developed.

Ammonium-N concentrations and flux were very low indicating either strong potential for nitrification or for strong retention of ammonium-N in the cation exchange sites in the soil. Interestingly, DON appeared unaffected by fertilizer addition indicating that inorganic fertilizer does not lead in a direct way to the formation of leachable DON.

Plant Community

Other than increased biomass, we did not observe changes to the plant community that could be explained by additional exposure to the small amounts of bioavailable nitrogen we applied during the first two years. Additionally, following release from grazing, changes to the plant community on this study site were complex, interacting with presence and proliferation of a non-native species, tall fescue. However, changes to the plant community structure remain a virtual certainty with higher N doses (Wedin and Tilman, 1996; Silva *et al*., 2005a).

Festuca increased and altered community composition and short-term succession without decreasing species richness (Table 3), but this was intermittent and did not persist throughout the term of this study. Others have observed that when *Festuca* is the major vegetation component, *Festuca* influences vegetation dynamics by decreasing species richness, (Clay and Holah, 1999) and decreasing litter accumulation (Wieder *et al*., 1983).

Our study was conducted on a seral old-field with small amounts of *Festuca,* whereas other studies have examined vegetation dynamics of *Festuca* monocultures or simple mixtures with the primary focus on endophyte-infected and endophyte-free *Festuca* (Wieder *et al*., 1983; Clay and Holah, 1999; Matthews and Clay, 2001). *Festuca* is an invasive and competitive species that overrides vegetation dynamics in monocultures and simple mixtures, so the possibility this plant might dominate in old-fields remains a legitimate concern for ecosystem management and restoration.

In the absence of an invasive transformer, vegetation change within plots should represent chronosequences of species composition from early- to mid- and late-successional species (Collins and Adams, 1983; Engle *et al*., 2000) consistent with previous observations of similar old-fields following cessation of chronic intense grazing (Engle *et al*., 2000). However, we observed a distinct separation among plots representing a difference in increasing abundance of *Festuca* canopy cover (Figure 12). In the three years following cessation of heavy grazing, changes in species composition of plots were expressed with increasing abundance of *Festuca* rather than increases in late-successional species. That is, increasing *Festuca* canopy cover altered succession dynamics. Increases in warm-season grasses were expected to occupy the major successional pathway in this grassland (Collins and Adams, 1983; Engle *et al*., 2000), but on plots in which *Festuca* cover exceeded 10%, *Festuca* became the driver of succession in place of warm-season native grasses.

These relationships and potential interactions with nitrogen biogeochemistry warrant further investigation for 2 reasons; 1) the time and site specific limitations of our study are insufficient to definitively rule out relationships among nitrogen availability and *Festuca*, 2) because of both the widespread nature of atmospheric nitrogen deposition and *Festuca* distribution, ecosystem effects would be widespread.

Primary Consumers - Herbivory

Population Ecology

Cotton rat density tended to be highest on fertilized-fenced treatment for a considerable time compared to the other treatments. Population density of cotton rats in fertilized only, fenced only treatments was similar to control treatment (Figure 14), but density fluctuated among treatments with significantly greater densities during winter from fertilized-fenced treatments. Thus, we did not observe a fertilizer or fence effect on population densities, but rather a fertilizer-fence interaction. The combination of increased above-ground live mass and protection from predation on fertilized-fenced plots likely accounted for differences in population density of cotton rats among the treatment plots.

Predation has regulatory effects on population densities of small mammals (Desy and Batzli, 1989, Tait and Krebs, 1983). Enclosures that control access by predators have been used to address hypotheses and effects of predation on population characteristics of small mammals (Schnell, 1968; Vaughan and Keith, 1981; Desy and Batzli, 1989). Enclosures at CSEAR were designed primarily to control herbivory by medium and large herbivores with less emphasis on controlling predation. If our observations are in-part attributable to interactions between predation risk and nitrogen availability, then the potential implications for nitrogen management of small or isolated landscapes where herbivore population dynamics are perturbed become quite complicated and worthy of considerable future research.

Physiology

Deposition of urine and feces by *Sigmodon hispidus* may be a significant source of nitrogen for plants and microbes. Further, when it is considered that there are many herbivorous consumer organisms present, the estimates associated with a single species (*albeit* a potentially dominant and abundant one) definitely reflect under-estimates of the extreme case. However, the fact that additional leaching was not observed on fenced-only treatments, coupled with the observation that soil nitrate-N (although elevated) was lower on fenced plots compared to fertilized plots, indicates that this source of input itself is insufficient to drive ecosystem changes during early succession in old-fields.

In this experiment, we are able to present extreme case conditions associated with *Sigmodon hispidus* (Table 4) with considerable certainty. Further, data collection and/or modeling may help us to better estimate actual field depositions of nitrate and exposures to plants and animals associated with herbivory.

Litter Decomposition - Detritivory

This study suggests limits to which ecosystems can process N through the decomposer pathway. Specifically, both the concentration and total amount of N remaining in litter increased after exogenous N addition, indicating that the ecosystem had quickly reached a limit to its N processing capacity (Figure 17). Furthermore, when macro-detritivores were excluded from litter, total N in the litter increased, eventually becoming an apparent sink for the N inputs, implying that a diverse detritivore community is better able to process N even under increased N loads. These observations have 2 potential consequences for ecosystems. First, when N deposition (simulated here by fertilization) increases, the resulting excess N contained in litter may eventually be available for transport through leaching and runoff (i.e., Kahl *et al.,*1993; Peterjohn *et al.,* 1996) potentially adversely impacting ecosystems through eutrophication and acidification (Kelly *et al.,* 1990; Likens, 1992; Carpenter *et al.,* 1998; Glibert and Terlizzi, 1999). Reducing N inputs to the decomposer pathway points logically to source control of fertilizers and atmospheric N, which contribute significantly to the global input of synthesized N currently produced at rates exceeding natural terrestrial N fixation (Galloway and Cowling, 2002). Second, the influence of macro-detritivores on N flux observed here has important implications for systems where detritivore communities may be perturbed through habitat alteration or chemical use. For example, the ecological modification that characterizes urbanized or agricultural systems is likely to adversely affect detritivore communities and their function (Blair, 1999; Paoletti and Hassall, 1999). Direct exposure to chemicals and/or chemical drift may also impact macro-invertebrates (Hershey *et al.,*1998) potentially reducing detritivore diversity and causing indirect effects on litter dynamics and nutrient flux.

Terrestrial ecosystems with impaired ability to process N due to impacted detritivore communities may represent non-point sources of N for ground and surface water. Resource management to reduce risks to ecosystem function (U.S. EPA, 1995) by improving the ability of ecosystems to retain, sequester, and process N may need to address detritivores and their influence on litter biogeochemistry and nutrient flux (Seastedt, 1984; Beare *et al.,* 1992; Griffiths, 1994), especially if global inputs of reactive N increase at current rates (Brimblecombe and Stedman, 1982; Smil, 1990; Vitousek, 1994; Vitousek *et al.,*1997, Galloway *et al*., 2002).

N concentration in litter increased throughout the experiment, but final concentrations did not differ between coarse and fine mesh, a result consistent with studies on *Eucalyptus* litter (Reddy and Venkataiah, 1989); however, total N loss was significantly different between the two mesh types (Figure 18). This paradox may be explained by the conservation of mass in fine mesh bags relative to coarse. Little, if any, additional litter mass was lost in fine mesh bags after the eighth month of exposure, whereas mass was continually lost from coarse mesh bags throughout the experiment; therefore,

mesh effect on total N was a function of reduced mass loss from fine meshed bags rather than from an effect on N concentration. Our results support the suggestion that detritivore diversity and species' characteristics (e.g. size, feeding preference) are important factors in dictating decomposition processes (Beare *et al.,* 1992; Mikola and Setälä, 1998). The significance of macro-detritivore effects on N cycling may be a function of their large size relative to microbes and fungi and thus, their ability to translocate large quantities of mass.

Implications

This study provides evidence for several important phenomena:

1) Soil nitrogen chemistry was altered after one growing season of exposure to low-level nitrogen deposition.
2) The ability of the soil ecosystem to respond to nitrogen inputs can be compromised by previous exposures.
3) Microbial denitrification is primarily carbon-limited during most of the year.
4) The ability of microbes to respond to nitrogen inputs may be largely fixed.
5) Soils that have had prior exposure to low level nitrogen inputs are more severely carbon-limited than soils that have not been so exposed.
6) High abundance of herbivores, in combination with their deposition of urine and feces, can mimic the effect of fertilization and increase exposure of the soil ecosystem to bioavailable nitrogen.
7) Herbivorous consumer communities can likely produce more than inconsequential amounts of bioavailable nitrogen.
8) The nitrogen use efficiency of decomposer and detritivore pathways is deleteriously altered by exposure to excess bioavailable nitrogen.
9) The nitrogen use efficiency of decomposer and detritivore pathways is deleteriously altered by perturbations to those pathways.
10) Changes to ecosystems that are chronically exposed to low doses of bioavailable nitrogen are frequently in a direction that tends to increase the flux of bioavailable nitrogen through the system, thereby increasing the risk of deleterious ecosystem responses.

Ecosystems may be at risk from doses of atmospherically deposited nitrate that is generally considered to be low or modest. As deposition of bioavailable nitrogen from the atmosphere can reasonably be expected to increase in the foreseeable decades, it is prudent to identify and develop management options now to both restore ecosystems that are already compromised and to buffer effects to ecosystems that are at risk from new nitrogen inputs.

We suggest that integrated ecological studies, including the consideration of the effects of plants, animals, and upland sites, may be essential for constructing predictive models of watershed nitrogen risk and management throughout a potentially wide area of the eastern and central United States.

References

Aber, J.D., K.J. Nadelhoffer, P. Steudler, and J.M. Melillo. 1989. Nitrogen saturation in northern forest ecosystems. Bioscience 39:378-386.

Aber, J.D. and J.M. Melillo. 1991. Terrestrial ecosystems. Saunders College Publishing, Orlando, Florida, USA.

Animal Care and Use Committee. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. Journal of Mammalogy 79:1416-1431.

Ashby, J.A., W.B. Bowden, and P.S. Murdoch. 1998. Controls on denitrification in riparian soils in headwater catchments of a hardwood forest in the Catskill Mountains. Soil Biology and Biochemistry 30:853-864.

Baker, L.A. 1992. Introduction to nonpoint source pollution in the United States and prospects for wetland use. Ecological Engineering 1:1-26.

Ball, D.M., J.F. Pedersen, and G.D. Lacefield. 1993. The tall-fescue endophyte. American Scientist 81: 370-379.

Beare, M.H., R.W. Parmelee, P.F. Hendrix, W. Cheng, D.C. Coleman, and D.A. Crossley. 1992. Microbial and faunal interactions and effects on litter nitrogen and decomposition in agroecosystems. Ecological Monographs 62:569-591.

Blair, R.B. 1999. Birds and butterflies along an urban gradient: surrogate taxa for assessing biodiversity? Ecological Applications 9:164-170.

Bouwman, A.F., 1990. Exchange of greenhouse gases between terrestrial ecosystems and the atmosphere. Pages 61-127 *In* Bouwman, A.F. (Ed.), Soils and Greenhouse Effects. John Wiley and Sons Ltd, Chichester.

Brimblecombe, P. and D.H. Stedman. 1982. Historical evidence for a dramatic increase in the nitrate component of acid rain. Nature 298:460-462.

Cameron, G. N. 1977. Experimental species removal: demographic responses by *Sigmodon hispidus* and *Reithrodontomys fulvescens*. Journal of Mammalogy 58:488-506.

Carpenter, S., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorous and nitrogen. Issues in Ecology, No. 3. Ecological Society of America, Washington, D.C., USA.

Carreiro, M.M., R.L. Sinsabaugh, D.A. Repert, and D.F. Parkhurst. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359-2365.

Carson, W.P. and C.J. Peterson. 1990. The role of litter in a old-field community: impact of litter quantity in different seasons on plant species richness and abundance. Oecologia 85: 8-13.

Christensen, N.L. and T. MacAller. 1985. Soil mineral nitrogen transformations during succession in the piedmont of North Carolina. Soil Biology and Biochemistry 17: 675-681.

Clay, K. and J. Holah. 1999. Fungal endophyte symbiosis and plant diversity in successional fields. Science 285:1742-1744.

Collins, S.L. 1987. Interaction of disturbances in tallgrass prairie: a field experiment. Ecology 68: 1243-1250.

Collins, S.L. and D.E. Adams. 1983. Succession in grasslands: thirty-two years of change in a central Oklahoma tallgrass prairie. Vegetation 51:181-190.

Desy, E. A., and G.O. Batzli. 1989. Effects of food availability and predation on prairie vole demography: a field experiment. Ecology 70:411–421.

Doonan, T. J., and N. A. Slade. 1995. Effects of supplemental food on population dynamics of cotton rats, *Sigmodon hispidus*. Ecology 76:814-826.

Dormaar, J.F., S. Smoliak, and W.D. Williams. 1990. Soil chemical properties during succession from abandoned cropland to native range. Journal of Range Management 43:260-265.

Duxbury, J.M. and A.R. Mosier. 1993. Status and issues concerning agricultural emissions of green house gases. Pages 229-258 *In* Kaiser, H.M., Drennen, T.E. (Eds.), Agricultural Dimensions of Global Climate Change. St. Luice Press, Forida, USA.

Engle, D.M., M.W. Palmer, J.S. Crockett, R.L. Mitchell, and R. Stevens. 2000. Influence of late season fire on early successional vegetation of an Oklahoma prairie. Journal of Vegetation Science 1:135-144.

Fike, J. and W.A. Niering. 1999. Four decades of old field vegetation development and the role of *Celastrus orbiculatus* in the northeastern United States. Journal of Vegetation Science 10: 483-492.

Firestone, M.K., 1982. Biological denitrification. Pages 289-326 *In* Stevenson, F.J. (Ed.), Nitrogen in Agricultural Soils. American Society of Agronomy. Madison, WI, USA.

Fleharty, E. D., J. R. Choate, and M. A. Mares. 1972. Fluctuations in population density of the hispid cotton rat: factors influencing a "crash." Bulletin of the Southern California Academy of Sciences 71:132-138.

Foster, B.L. and K.L. Gross. 1998. Species richness in a successional grassland: effects of nitrogen enrichment and plant litter. Ecology 79: 2593-2602.

Frank, D.A. and P.M. Groffman. 1998. Denitrification in a semi-arid grazing ecosystem Oecologia. 117:564-569.

Freeman, C.C. 1998. The flora of Konza Prairie: a historical review and contemporary patterns. Pages 69-80 *In* A.K. Knapp, J.M. Briggs, D.C. Hartnett, and S.L. Collins. (Eds.) Grassland dynamics: long-term ecological research in tallgrass prairie. Oxford University Press, Oxford, United Kingdom.

Galloway, J.N. and E.B. Cowling. 2002. Reactive nitrogen and the world: 200 years of change. Ambio 31:64-71.

Galloway, J.N., H. Levy II, and P.S. Kasibhatla. 1994. Year 2020: consequences of population growth and development on deposition of oxidized nitrogen. Ambio 23:120-123.

Gessaman, J.A., and J.A. MacMahon. 1984. Mammals in ecosystems: their effects on the composition and production of vegetation. Acta Zoologica Fennica 172:11-18.

Gibson, D.J. and J.A. Newman. 2001. *Festuca arundinacea* Schreber (*F. elatior* L. spp. *arundinacea* (Schreber) Hackel. Journal of Ecology 89: 304-324.

Gilliam, F.S., M.B. Adams, and B.M. Yurish. 1996. Ecosystem nutrient responses to chronic nitrogen inputs at Fernow Experimental Forest, West Virginia. Canadian Journal of Forest Research 26:196-205.

Gleeson, S.K. and D. Tilman. 1990. Allocation and the transient dynamics of succession on poor soils. Ecology 71:1144-1155.

Glibert, P.M. and D.E. Terlizzi. 1999. Cooccurrence of elevated urea levels and dinoflagellate blooms in temperate estuarine aquaculture ponds. Applied and Environmental Microbiology 65:5594-5596.

Grant, W.E., N.R. French, and D.M. Swift. 1977. Response of a small mammal community to water and nitrogen treatments in a shortgrass prairie ecosystem. Journal of Mammalogy 58:637–652.

Griffiths, B.S. 1994. Microbial-feeding nematodes and protozoa in soil: Their effects on microbial activity and nitrogen mineralization in decomposition hotspots and the rhizosphere. Plant and Soil 164:25-33.

Groffman, P.M., C.W. Rice, and J.M. Tiedje. 1993. Denitrification in a tallgrass prairie landscape. Ecology 74:855-862.

Groffman, P.M., E.A. Holland, D.D. Myrold, G.P. Robertson, and X. Zou. 1999. In *Denitrification, in Standard Soil Methods for Long Term Ecological Research,* G.P. Robertson, D.C. Coleman, C.S. Bledsoe, and P. Sollins (Eds), Oxford University Press: New York, NY, USA.

Gross, K.L., K.S. Pregitzer, and A.J. Burton. 1995. Spatial variation in nitrogen availability in three successional plant communities. Journal of Ecology 83:357-367.

Hall, A.T., P.E. Woods, and G.W. Barrett. 1991. Population dynamics of the meadow vole (*Microtus pennsylvanicus*) in nutrient-enriched old-field communities. Journal of Mammalogy 72:332–342.

Hershey, A.E., A.R. Lima, G.J. Niemi, and R.. Regal. 1998. Effect of *Bacillus thuringiensis israelensis* (Bti) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. Ecological Applications 8:41-60.

Heske, E.J., J.H. Brown, and S. Mistry. 1994. Long-term experimental study of a Chihuahuan Desert rodent community: 13 years of competition. Ecology 75:438-445.

Hoagland, B.W., I. Butler, F.L. Johnson, and S. Glenn. 1999. The Cross Timbers. In *The Savanna, Barren and Rock Outcrop Communities of North America,* R.C. Anderson, J. Fralish, and J. Baskin (Eds), Cambridge University Press.

Hobbie, S.E. and P.M. Vitousek. 2000. Nutrient limitation of decomposition in Hawaiian forests. Ecology 81:1867-1877.

Hoveland, C.S. 1993. Importance and economic significance of the *Acremonium* endophytes to performance of animals and grass plant. Agriculture, Ecosystems, and Environment 44: 3-12.

Hutchinson, G.L., E.A. Davidson, 1993. Process for Production and Consumption of Gaseous Nitrogen Oxide in Soil. Pages 79-83 *In* L.A. Harpet (Ed.), Agricultural Ecosystem Effects on Trace Gases and Global Climate Change. ASA Special Publication 55ASA, CSSA and SSSA, Madison, Wisconsin, USA.

Jordan, T.E., D.E. Weller, and D.L. Correll. 1998. Denitrification in surface soils of a riparian forest: effects of water, nitrate and sucrose additions. Soil Biology and Biochemistry 30:833-843.

Jorgensen, E.E. 2002. Opportunities in nitrogen management research; improving applications for proven technologies and identifying new tools for managing nitrogen flux and input in ecosystems. Advances in Restoration Science; Number 2. U.S. EPA. EPA/600/S-02/003.

Jorgensen, E.E. and S.J. Tunnell. 2001. The effectiveness of quadrats for measuring vascular plant diversity. Texas Journal of Science 53:365-368.

Jorgensen, E.E., S. Demarais, and W.R. Whitworth. 1994. The effect of box trap design on rodent captures. Southwestern Naturalist. 39:291-294.

Jorgensen, E.E., P.M. Mayer, A.E. West, M.E. Gonsoulin, S.J. Tunnell, J.E. Clark, J.L. Parsons, D.M. Engle, and E.C. Hellgren. 2002. "Early indicators of nitrate stress; effects to ecosystems of chronic exposure to low doses of bioavailable nitrogen." In *Proceedings of the National TMDL Science and Policy Conference, Water Environment Federation*, Phoenix, AZ.

Jorgensen, E.E., S. Holub, R.G. Silva, P.M. Mayer, A.E. West, M.E. Gonsoulin, S.J. Tunnell, J.E. Clark, J.L. Parsons, D.M. Engle, and E.C. Hellgren. 2003. "Sources and estimated load of bioavailable nitrogen attributable to chronic nitrogen exposure and changed ecosystem structure and function." In *Proceedings of the First Interagency Conference on Research in the Watersheds*, Benson, AZ.

Kahl, J.S., S.A. Norton, I.J. Fernandez, K.J. Nadelhoffer, C.T. Driscoll, and J.D. Aber. 1993. Experimental inducement of nitrogen saturation at the watershed scale. Environmental Science and Technology 27:565-568.

Kalisz, P.J. 1986. Soil properties of steep Appalachian old fields. Ecology 67:1011-1023.

Kelly, C.A., J.W.M. Rudd, and D.W. Schindler. 1990. Acidification by nitric acid – future considerations. Water, Air, and Soil Pollution 50:49-61.

Kenward, M.G., and J.H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53:983-998.

Knapp, A.K., J.M. Briggs, J.M. Blair, and C.L. Turner. 1998. Patterns and controls of aboveground net primary production in tallgrass prairie. Pages 193-221 *In* A.K. Knapp, J.M. Briggs, D.C. Hartnett, and S.L. Collins. (eds.) Grassland dynamics: long-term ecological research in tallgrass prairie. Oxford University Press, Oxford, United Kingdom.

Knops, J.M.H. and D. Tilman. 2000. Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. Ecology 81:88-98.

Knowles, R. 1982. Denitrification. Microbiological Review 46:43-70.

Krebs, C.J. 1966. Demographic changes in fluctuating populations of *Microtus californicus*. Ecological Monographs 36:239-273.

Langley, A. K., Jr., and D. J. Shure. 1988. The impact of climatic extremes on cotton rat (*Sigmodon hispidus*) populations. American Midland Naturalist 120:136-143.

Likens, G.E. 1992. The ecosystem approach: its use and abuse. Excellence in Ecology 3. Ecology Institute, Oldendorf/Luhe, Germany. 166pp.

Luo, J., R.W. Tillman, R.E. White, and P.R. Ball. 1998. Variation in denitrification activity with soil depth under pasture. Soil Biology and Biochemistry 30:897-903.

Luo, J., R.W. Tillman, and P.R. Ball. 2000. Nitrogen loss through denitrification in a soil under pasture in New Zealand. Soil Biology and Biochemistry 32:497-509.

Mahli, S.S., and M. Nyborg. 1986. Increase in mineral N in soils during winter and loss of mineral N during early spring in north-central Alberta. Canadian Journal of Soil Science 66:397-409.

Matthews, J.W. and K. Clay. 2001. Influence of fungal endophyte infection on plant-soil feedback and community interactions. Ecology 82:500-509.

McNaughton, S.J. 1985. Ecology of a grazing ecosystem: the Serengeti. Ecological Monographs 55:259-294.

Mikola, J, and H. Setälä. 1998. Relating species diversity to ecosystem functioning: mechanistic backgrounds and experimental approach with a decomposer food web. Oikos 83:180-194.

Naeem, S., D.R. Hahn, and G. Shuurman. 2000. Producer-decomposer co-dependency influences biodiversity effects. Nature 403:762-764.

National Atmospheric Deposition Program (NADP-3)/National Trends Network. 2002. NADP Program Office, Illinois State Water Survey, 2204 Griffith Drive, Champaign, IL 61820.

Paoletti, M. G. and M. Hassall. 1999. Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. Agriculture, Ecosystems, and Environment 74:157-165.

Pastor, J., M.A. Stillwell, and D. Tilman. 1987. Nitrogen mineralization and nitrification in four Minnesota old fields. Oecologia 71:481-485.

Perakis, S.S. and L.O. Hedin. 2002. Nitrogen loss for unpolluted South American forests mainly via dissolved organic compounds. Nature:416-419.

Peterjohn, W.T., M.B. Adams, and F.S. Gilliam. 1996. Symptoms of nitrogen saturation in two central Appalachian hardwood forest ecosystems. Biogeochemistry 35:507-522.

Reddy, M.V. and B. Venkataiah. 1989. Influence of microarthropod abundance and climatic factors on weight loss and mineral nutrient contents of *Eucalyptus* leaf litter during decomposition. Biology and Fertility of Soils 8:319-324**.**

Richardson, D.M., P. Pysek, M. Rejmanek, M.G. Barbour, F.D. Panetta, and C.J. West. 2000. Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6:93-107.

Robertson, G.P., M.A. Huston, F.C. Evans, and J.M. Tiedje. 1988. Spatial variability in a successional plant community: patterns of nitrogen availability. Ecology 69:1517-1524.

SAS Institute Inc. 1990. SAS/STAT User's Guide. Release 6. SAS Institute Inc., Cary, NC.

Scharf, P., and M.M. Alley. 1988. Centrifugation: a solution to the problems posed by ammonium and nitrate contamination of filters in soil extract analysis. Soil Science Society of America Journal 52:1508-1510.

Schetter, T.A., R.L. Lochmiller, D.M. Leslie, Jr., D.M. Engle, and M.E. Payton. 1998. Examination of the nitrogen limitation hypothesis in non-cyclic populations of cotton rats (*Sigmodon hispidus*). Journal of Animal Ecology 67:705-721.

Schlesinger, W.H. 1997. Biogeochemistry: An analysis of global change. Academic Press, San Diego, CA.

Schnabel, R.R., L.F. Cornish, W.L. Stout, and J.A. Shaffer. 1996. Denitrification in a grassed and a wooded, valley and ridge, riparian ecotone. Journal of Environmental Quality. 25:1230-1235.

Schnell, J.H. 1968. The limiting effects of natural predation on experimental cotton rat populations. Journal of Wildlife Management 32:698–711.

Seastedt, T.R. 1984. The role of arthropods in decomposition and mineralization processes. Annual Review of Entomology 29:25-46.

Shaver, G.R., J. Canadell, F.S. Chapin III, J. Gurevitch, J. Harte, G. Henry, P. Ineson, S. Jonasson, J. Melillo, L. Pitelka, and L. Rustad. 2000. Global warming and terrestrial ecosystems: a conceptual framework for analysis. Bioscience 50:871-882.

Silva, R.G., K.C. Cameron, H.J. Di, and E. E. Jorgensen. 2005(a). A lysimeter study to investigate the effect of dairy effluent and urea on cattle urine nitrogen losses, plant uptake and soil retention. Water, Air, and Soil Pollution (In Press).

Silva, R.G., E.E. Jorgensen, S.M. Holub, and M.E. Gonsoulin. 2005(b). Relationship between culturable soil microbial populations and gross nitrogen transformation processes in a clay loam soil across ecosystems. Nutrient Cycling in Agroecosystems (In Press).

Smil, V. 1990. Nitrogen and phosphorus. In *The Earth as Transformed by Human Action; Global and Regional Changes in the Bioshpere over the Past 300 Years,* B.L. Turner II, W.C. Clark, R.W. Kates, J.F. Richards, J.T. Mathews, and W.B. Meyer (Eds). Cambridge University Press, United Kingdom. 713pp.

Sparrow, S.D., and D.T. Musiak. 1987. Errors in analysis for ammonium and nitrate caused by contamination from filter papers. Soil Science Society of America Journal 51:107-110.

Stafford, S.R., and I.J. Stout. 1983. Dispersal of the cotton rat, *Sigmodon hispidus*. Journal of Mammalogy 64:210-217.

Stanford, G., R.A. Vander Pol, and S. Dzienia. 1975. Denitrification rates in relation to total and extractable soil carbon. Soil Science Society of America Proceedings 39:284-289.

Starr, R.C. and R.W. Gillham. 1993. Denitrification and organic carbon availability in two aquifers. Ground Water 31:934-947.

Stohlgren, T.J., K.A. Bull, and Y. Otsuki. 1998. Comparison of rangeland vegetation sampling techniques in the Central Grasslands. Journal of Range Management 51:164-172.

Swift, M.J., O.W. Heal, and J.M. Anderson. 1979. Decomposition in terrestrial ecosystems. Studies in Ecology; Volume 5, University of California Press, Berkeley and Los Angeles. 372 pp.

Tait, M.J., and C.J. Krebs. 1983. Predation, cover, and food manipulations during a spring decline of *Microtus townsendii*. The Journal of Animal Ecology 52:837-848.

ter Braak, C.J.F. and P. Šmilauer. 1998. CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (Version 4). Microcomputer Power, Ithaca, NY.

Tilman, D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. Ecological Monographs 57:189-214.

Tilman, D. 1988. Plant Strategies and the Dynamics and Structure of Plant Communities. Princeton University Press, Princeton, NJ.

Tremmel, D.C. and K.M. Peterson. 1983. Competitive subordination of a Piedmont old field successional dominant by an introduced species. American Journal of Botany 70:1125-1132.

Tunnell, S.J. 2002. Vegetation dynamics following cessation of severe disturbance in an old field. Ph.D. dissertation. Oklahoma State University, Stillwater, OK.

Tunnell, S.J., D.E. Engle, and E.E. Jorgensen. 2004. Old field successional dynamics following cessation of chronic disturbance. Journal of Vegetation Science 15:431-436 15:431-436.

USDA. 1973. Soil survey of Pontotoc County, Oklahoma. USDA Soil Conservation Service.

U.S. EPA. 1995. An SAB report: ecosystem management; imperative for a dynamic world. EPA-SAB-EPEC-95-003.

Van der Heijden, M.G.A., J.N. Klironomos, M. Ursic, P. Moutoglis, R. Streitwolf-Engel, T. Boller, A. Wiemken, and R. Sanders. 1998. Mycorrhizal fungal diversity determines plant diversity, ecosystem variability and productivity. Nature 396:69-72.

Vaughan, M.R. and L.B. Keith. 1981. Demographic response of experimental snowshoe hare populations to overwinter food shortage. Journal of Wildlife Management 45:354–380.

Vitousek, P.M. 1994. Beyond global warming: ecology and global change. Ecology 75:1861–1876.

Vitousek, P.M., J. Aber, R.W. Howarth, G.E. Likens, P.A. Matson, D.W. Schindler, W.H. Schlesinger, and G.D. Tilman. 1997. Human alteration of the global nitrogen cycle: causes and consequences. Issues in Ecology 1:1-16.

Weaver, J.E. 1968. North American Prairie. University of Nebraska Press, Lincoln, NE.

Wedin, D.A. and D. Tilman. 1990. Species effects on nitrogen cycling: a test with perennial grasses. Oecologia 84:433-441.

Wedin, D.A. and D. Tilman. 1996. Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science 274:1720-1723.

Weier, K.L., J.W. Doran, J.F. Power, and D.T. Walters. 1993. Denitrification and dinitrogen/nitrous oxide ratio as affected by soil water, available carbon and nitrate. Soil Science Society of America Journal 57:66-72.

Westerman, R.L. and T.C. Tucker. 1978. Factors affecting denitrification in a Sonoran Desert soil. Soil Science Society of America Journal 42:596-599.

Whitehead, D.C. 1995. Grassland Nitrogen. CAB International, Wellingford, United Kingdom. pp. 405.

Wieder, R.K., J.E. Carrel, J.K. Rapp, and C.L. Kucera. 1983. Decomposition of tall fescue (*Festuca elatior* var. *arundinacea*) and cellulose litter on surface mines and a tallgrass prairie in central Missouri, USA. Journal of Applied Ecology 20:303-321.

Wrage, N., G.L. Velhof, M.L. van Beusichem, and O. Oenema. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology and Biochemistry 33: 1723-1732.

Zedler, J.B. and P.H. Zedler. 1969. Association of species and their relationship to microtopography within old fields. Ecology 50:432-442.

United States
Environmental Protection
Agency

National Risk Management
Research Laboratory
Cincinnati, OH 45268

Official Business
Penalty for Private Use
$300

EPA/600/R-05/087
October 2005

Please make all necessary changes on the below label, detach or copy, and return to the address in the upper left-hand corner.

If you do not wish to receive these reports CHECK HERE ☐; detach, or copy this cover, and return to the address in the upper left-hand corner.

PRESORTED STANDARD
POSTAGE & FEES PAID
EPA
PERMIT No. G-35

Recycled/Recyclable
Printed with vegetable-based ink on paper that contains a minimum of 50% post-consumer fiber content processed chlorine free