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INFLUENCE OF MOISTURE REGIME AND TREE SPECIES ON NITROGEN CYCLING AND DECOMPOSITION DYNAMICS IN DECIDUOUS FORESTS OF MAMMOTH CAVE NATIONAL PARK, KENTUCKY, USA

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ABSTRACT OF THESIS

INFLUENCE OF MOISTURE REGIME AND TREE SPECIES ON NITROGEN CYCLING
AND DECOMPOSITION DYNAMICS IN DECIDUOUS FORESTS OF MAMMOTH CAVE
NATIONAL PARK, KENTUCKY, USA

The study of biogeochemical cycles and their role in ecosystem function has helped to highlight the impacts of human activities on natural processes. However, our understanding of the effects of nitrogen (N) deposition on forested ecosystems remains limited due to the variable controls on N cycling. Soils, microclimate, and vegetation can influence rates and processes of N cycling, singly or in concert at multiple scales. Understanding how these factors influence N cycling across the landscape will help to elucidate the impacts of N deposition. The objectives of this study were to characterize variation in soils, microclimate and vegetation characteristics, and N cycling and decomposition dynamics across the landscape in a region impacted by N deposition. Relationships among these factors were explored to determine the main factors influencing N cycling and decomposition. Strong differences in net N mineralization and nitrification were found between forest stands with contrasting species composition and moisture availability. Nitrate production and leaching were related to sugar maple abundance, and base cation leaching was correlated with nitrate concentrations in soil solutions. Decomposition experiments were installed to examine the effects of substrate quality, microclimate and N availability on decay rates. Nitrogen amendments for the most part did not affect decomposition rates of wood and cellulose, and mass loss rates were correlated with microclimate and forest floor characteristics. In contrast, microclimate did not seem to affect leaf litter decay rates, and the results suggest that the presence of invertebrates may influence mass loss to a greater degree than moisture or litter quality. This work highlights the large degree of variability in N processing across the landscape and suggests that differences in microclimate and species composition may help to predict the impacts of chronic N deposition on N cycling and retention.

KEYWORDS: Nitrogen cycling, moisture regime, decomposition, nitrate leaching, sugar maple

Eric Fabio

September 22, 2006

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NATIONAL PARK, KENTUCKY, USA

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September 22, 2006

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THESIS

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THESIS

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A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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By

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FILES

CHAPTER ONE

Biogeochemical cycling in temperate forests: The impacts of nitrogen deposition and tree species identity

Biogeochemical cycles form the basis of how we understand and model the flow of energy and nutrients through the biosphere. Biotic and abiotic forces act in concert to drive inputs and outputs, and internal cycling within ecosystems. These forces and pathways have intrinsic mechanisms for self-regulation, where imbalances in one lead to compensation in another (Bormann and Likens, 1967). Nitrogen (N) availability limits plant growth in most terrestrial ecosystems, since the majority of N on Earth resides in the atmosphere in a form unavailable to most organisms (N_2 gas), and a relatively small proportion is converted to biologically reactive forms of N, by a small number of prokaryotic microorganisms. However, human activities over the last century have led to a doubling in the amount of N fixed in terrestrial ecosystems (Galloway et al., 1995). The major anthropogenic sources of N fixation are N fertilizer production, fossil fuel combustion, and widespread planting of crops that promote biological N fixation (e.g., legumes and rice) (Vitousek et al., 1997). Such drastic changes to the pools and processes of a nutrient cycle that is so important in the biosphere could have major implications for terrestrial productivity and human life.

For temperate forested ecosystems, long-distance transport of nitrogen oxides released during fossil fuel combustion, and ammonia volatilization from agricultural activities, are important sources of external N. These molecules are deposited onto vegetation through dry deposition or undergo chemical transformation in the atmosphere and fall to the canopy in precipitation as nitric acid and ammonium. These biologically reactive forms of N can quickly enter the biologic cycles of a forest, and chronic N deposition can lead to a situation where N availability exceeds the demand by plants and microbes, a condition referred to as N saturation (Aber et al., 1989). Aber et al. (1989) hypothesized that increases in N deposition would lead to increases in net N mineralization, due to a lowering of the carbon (C):N ratio of soil organic matter, and that nitrification would be stimulated as a result of increased substrate availability. The nitrate ion is a highly mobile form of N due to its negative charge, and thus represents a potential source of N losses from forested ecosystems as it moves through the soil column in

solution and is carried away to surface waters. This flux has the potential to cause eutrophication as this nutrient is added to aquatic ecosystems (Mitchell et al., 2003). Nitrification is an acidifying process, which causes an increase in the hydrogen ion content of soils and increases aluminum mobility. As soil exchange sites are flooded with hydrogen and aluminum ions, the displaced base cations enter the soil solution and are carried away with nitrate ions to surface waters. This increase in hydrogen ion production coupled with the acidic nature of some components of N (and sulfur) deposition, can accelerate base cation leaching from forest soils (Fernandez et al., 2003), leading to nutrient imbalances and declines in productivity (Aber et al., 1998).

The response of a forest stand to chronic N loading will likely be influenced by the species composition of that stand. It has been known for some time that tree species can influence soil chemistry (Zinke, 1962; Boerner, 1984; Kalisz, 1986), and much of the recent work in this area has highlighted specific differences in N cycling associated with particular tree species. For instance, in northwestern Lower Michigan, Zak and Pregitzer (1990) found greater extractable nitrate concentrations and net nitrification rates in stands dominated by sugar maple compared to oak. Studies in single species stands of the northeastern United States have shown that N cycling rates can vary greatly over small spatial scales within forest stands, depending on the species present, with species such as sugar maple and white ash having greater nitrification rates than oak and hemlock (Finzi et al., 1998a; Lovett et al. 2004). These differences are likely due to interspecific variation in litter chemistry. Greater abundances of certain species, in particular sugar maple, has been linked to higher fluxes of nitrate in surface waters draining forested watersheds (Lovett et al., 2000; Mitchell et al., 2003; Christopher et al., 2006), suggesting that tree species influence N retention capacity. With substantial N deposition rates in these forests, continued N loading will likely lower N retention capacity, but the timing of the response may be mediated by species composition. As species composition shifts in response to natural and anthropogenic disturbances, N retention patterns will also likely change.

Litter decay is the primary source of recycled nutrients in forest ecosystems, and there probably exists a positive feedback between litter decay and N availability (Prescott, 2005). Differences in N cycling rates among trees species have been linked to interspecific differences in litter chemistry (Ferrari, 1999) and to the apparent influence different litters have on soil C and N content (Finzi et al., 1998a; Lovett et al., 2002, 2004). Across broad ranges of forest

ecosystem types, the lignin to N ratio of litters can have a strong negative influence over decomposition rates (Scott and Binkley, 1997), but controls at smaller scales are less clear and may depend on environmental factors (Prescott, 2005).

Even greater uncertainty surrounds the effects of added N on litter decay, with studies showing inconsistent effects (Fog, 1988; Knorr et al., 2005). This may be due to differing amounts of recalcitrant C compounds present in litter, such as lignin. For instance, Carreiro et al. (2000) observed an interactive effect of added N on decomposition of different litters, where low-lignin dogwood litter responded with increased decomposition to N, but high-lignin oak litter responded with decreased decomposition. Sinsabaugh et al. (2002) attributed these results to interference of added N with extracellular enzyme production and function. If the effects of added N depend on the identity of the litter present, then chronic N deposition could increase or decrease C storage over time, depending on the dominant vegetation (Waldrop et al., 2004). Understanding spatial variability in environmental factors that affect species composition and N availability will help to uncover the effects of N deposition on decomposition dynamics in forested ecosystems.

The objectives of this study were to (1) examine the relationships between environmental factors, tree species distribution, and spatial variability in N cycling, and (2) to test the effects of substrate quality, microclimate and N availability on short-term decomposition rates at Mammoth Cave National Park, in central Kentucky. The landscape features of this region create strong differences in microclimate, and thus tree species composition, in a manor that is highly predictable. Determining which factors are most influential in determining ecosystem function across the landscape will help us to predict the impacts of N deposition and changes in species composition on a landscape scale.

CHAPTER TWO

Influence of moisture regime and tree species on nitrogen cycling in hardwood forests of Mammoth Cave National Park, Kentucky, USA

Introduction

The impact of atmospheric nitrogen (N) deposition to temperate forests remains an important focus of ecosystem research, as biologically reactive N (i.e. NO_x, NO_3, NH_4) entering these systems can impact ecosystem function in several ways. Impacts include soil acidification and base cation depletion (Fernandez et al., 2003), forest health and productivity (Aber et al., 1998), species diversity (Gilliam, *in press*), and loss of mobile forms of N to aquatic ecosystems (Lovett et al., 2000), leading to potential acidification and eutrophication of aquatic ecosystems (Mitchell et al., 2003). In the eastern United States a growing number of forest stands are showing signs of N saturation, defined as available N exceeding biological demand (Aber et al., 1989). Signs are increased rates of net nitrification, elevated levels of dissolved inorganic N in soil solutions, and increased stream water nitrate and base cation concentrations. However, in many regions where N saturation has been identified, spatial variability in the degree to which onset has occurred is documented both on watershed (Gilliam et al., 2001) and landscape (Lovett et al., 2000; 2002) scales.

Factors relating to climate, topography, soils and vegetation can all exert significant controls on N cycling, either singly or interactively, thus contributing to spatial variability in N cycling at multiple scales. For instance, landscape position (i.e. aspect and slope) can affect N mineralization rates and nitrate leaching through changes in temperature and moisture availability (Morris and Boerner, 1998; Gilliam et al., 2001), but also by creating gradients in forest cover type (Ohrui et al., 1999; Peterjohn et al., 1999). Soil properties such as texture can also affect N mineralization via soil moisture (Aber et al., 1991), or by binding with organic matter in soil aggregates, thereby protecting it from mineralization (Giardina et al., 2001; Denef et al., 2004). Carbon (C) and N content in soils can predict N mineralization and nitrification rates across a range of vegetation types (Booth et al., 2005), but soil organic matter quality is often strongly influenced by the vegetation (Finzi et al, 1998a; Wang and Fernandez, 1999). Furthermore, it has been well documented that tree species can influence soil properties and N cycling (Boettcher and Kalisz, 1990; Zak et al., 1989), largely through differences in litter

quality (Binkley and Giardina, 1998; Lovett et al, 2004). However, the distribution of tree species can be influenced by topograghic variables and soil properties. Therefore, consideration of the possible controls on N cycling within a system must precede determination of the potential for N deposition to impact that system.

In the Ohio River Valley region much of the vegetation is characterized as transitioning between mixed mesophytic forest to the east and oak-hickory to the west (Braun, 1950). The dissected topography of the region drives vegetation patterns by creating substantial changes in microclimate (Muller, 1982), such that mesophytic species like sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*) and yellow-poplar (*Liriodendron tulipifera*) occupy cooler, wetter north- and east-facing aspects and lower slope positions, and oak (*Quercus* spp.) and hickory (*Carya* spp.) are found on drier, south- and west-facing slopes and ridge tops (McEwan and Rhoades, 2005). Variation in microclimate across the landscape creates the potential for spatial variability in N cycling rates, both through changes in soil moisture availability and species composition.

Mammoth Cave National Park (MACA) is an ideal location for examining spatial variability in N cycling resulting from differences in forest vegetation, as the park is situated within this transition zone between the mixed mesophytic forest and oak-hickory assemblage, and tree species distributions are related to landscape position. In addition, geologic substrates of limestone contrast with sandstone and shale, introducing an additional source of spatial variability that could be important for nutrient cycling dynamics. Land management practices in the park are focused on full protection of natural resources, in contrast to most other federally managed lands. Yet despite its protection status, air quality is the second worse in the entire national park system (Ayers et al., 2004), and annual N deposition rates in this region are similar to those in the northeastern US, with total deposition (wet + dry) ranging from 7 to >9 kg ha^{-1}yr^{-1} (EPA-CASTNET, 2005). Continued N loading at these moderately high rates has the potential to alter N cycling patterns in these forests. However, differences in moisture availability and forest cover across the landscape will likely influence the response of these ecosystems to N deposition, because different forest types respond to N additions at different timescales (Wang and Fernandez, 1999; Magill et al., 2004). Understanding this variation in N cycling will help to identify areas that are potential sinks for N and those that have a greater potential for N losses,

which is important when considering regional changes in forest species composition and the release of N to aquatic ecosystems.

The objectives of this research were to (1) characterize N cycling patterns on a landscape scale, across gradients of forest composition and soil type, and (2) determine the major driving factors controlling N cycling in these forests. Seasonal monitoring of N cycling parameters was carried out across two years in forest stands in the park that differ in soil moisture status and tree species composition. We hypothesized that higher moisture status would lead to higher rates of N processing, either directly or by driving differences in species composition. Relationships between N cycling and soil and vegetation characteristics were examined to explain patterns in N cycling across forest stands. This research can help to broaden our understanding of the controls on N cycling, as well as provide land managers with baseline measurements for making informed decisions for managing the effects of atmospheric deposition of N.

Methods

Study site

Mammoth Cave National Park is located in south-central Kentucky, USA. The park was established in 1941, and forests that were previously disturbed by settlement have been allowed to recover during the last 65 years. The park encompasses approximately 21,380 hectares, situated in the transitional zone between the oak-hickory forest region and the western mixed mesophytic region (Braun, 1950). Beneath the surface lies the World's longest cave system. The park is bisected north and south by more than 30 km of the Green River, which is important for its high freshwater mussel and fish diversity (Butler et al., 2003). Long-term mean wet N deposition rates are >6 kg ha^{-1} yr^{-1} (B. Carson, NPS air resources management specialist, *unpublished data*, 2005) and dry deposition measured in 2003 and 2004 represented around 2 kg ha^{-1} yr^{-1} of additional N (EPA-CASTNET, 2006). Mean annual temperature ranges from 7.1 to 20.4°C, and mean annual precipitation is 132 cm (Kentucky Climate Center, 2006). Elevation ranges from approximately 135 to 275 m (US Geological Survey, 1997). The geologic substrate across much of the park is characterized by ridges capped by insoluble shale and sandstone, and valleys underlain by limestone, resulting in large variability in soil nutrient status. Soils derived from shale and sandstone are base poor, Typic Hapludults, whereas soils developing over

limestone have a higher base status and are mostly Typic and Ultic Hapludalfs (Mitchell et al., 1993).

Site selection and sampling plot layout

The dissected topography of the region creates considerable differences in amounts and intensities of solar radiation reaching the forest canopy, this in spite of the relatively large amounts of precipitation received. Therefore, considerable differences in soil moisture contents are found between slopes with northerly aspects and those with southerly. In spring 2003, 16 sites were selected across the park stratified by landscape position into two groups operationally defined as xeric, or low moisture availability, and mesic, or high moisture availability. Eight sites were selected for each moisture regime, where xeric sites had southerly aspects (range: 143 to 212°), and were located on higher slope positions (mean (SE) elevation = 225.8 (8.0) m), compared to mesic sites with northerly aspects (range: 358 to 87°), and lower elevations (mean (SE) = 190.8 (8.8) m). One sampling plot was established at each of the 16 sites, consisting of one 11.3 m radius circle (corrected for slope), with a two m radius circular subplot in the center. Soil samples for various assays were collected around the perimeter of the subplot.

Soil chemical and physical properties

In June 2004, four soil samples were collected from each site to characterize soil chemical and physical properties. First, a 0.073 m^2 wooden frame was placed on the forest floor and the litter layer (Oi horizon) was removed. The remaining organic layers (Oe + Oa) were extracted down to mineral soil and sealed in a plastic bag. A 7-cm-diameter tulip bulb corer was inserted into the mineral soil centered over the excavated forest floor square, and mineral soil was collected to a depth of 10 cm, separated into two depths, 0-5 cm and 5-10 cm, and bagged. Two samples from each layer were composited to yield two replicates per layer, per site. In the laboratory, organic layer samples were passed through a six mm mesh sieve, and mineral soil samples through a two mm sieve, to remove coarse materials. All samples were air-dried and ground using a Spex CertiPrep 8000 Mixer/Mill (Metuchen, NJ) and oven dried (60°C) prior to physical and chemical analyses.

Forest floor mass was calculated as the oven-dry mass of organic layer samples divided by the area of the forest floor sampling frame. Soil texture was determined on subsamples from

upper and lower mineral soil layers collected in June 2004, and expressed as percent sand, silt and clay fractions. Mineral soil bulk density was measured in August 2004 from five soil samples per site, extracted using a hammer-driven corer with removable metal cylinders. Oven-dry (105°C) soil mass was divided by the volume of the metal cylinder to yield bulk density. Gravimetric moisture content was determined on four mineral soil (0-10cm) samples taken per plot on a quarterly basis over two years, followed by drying in an oven at 105°C. To achieve more temporal resolution in soil moisture measurements, additional sampling occurred from January to June 2005 between quarterly sampling periods. At each site a data logger recording ambient air and soil (10 cm depth) temperature was deployed in June 2003 to record data at 30 min intervals over the course of the study.

Total C and N for organic and upper and lower mineral soil layers were determined using a Leco CN 2000 (St. Joseph, Michigan). Available P, Ca, Mg and K were measured using Mehlich III extraction (Mehlich, 1984) followed by determination of elemental concentration using a Varian inductively coupled plasma-mass spectrometer (Palo Alto, CA). Soil pH was measured four times over the course of the study. Mineral soil taken for inorganic N determination (upper-10cm depth--see description below in *Net N mineralization and nitrification rates*) was mixed in a 1:10 ratio of soil to distilled water, shaken and allowed to equilibrate for 1 hr. The slurry was measured for pH using a glass probe pH meter. In March 2004, extractable Al concentrations were determined on KCl extractions from mineral soil samples collected for inorganic N determination, and run on a GBC Avanta atomic absorption spectrophotometer (Hampshire, IL).

Vegetation sampling

In summer 2004, woody vegetation was characterized within the 11.3 m radius circular plot centered over the soil-sampling subplot at each of the 16 sites. All woody stems greater than 2 cm diameter at breast height (DBH; 1.73 m) were identified by species and diameter measured. Slope, elevation and aspect at each site were also recorded.

Litterfall collections

Three litterfall collectors consisting of 0.23 m^2 plastic baskets were installed at each site in summer 2003, and the contents collected once a year after leaf fall from 2003 to 2005. Each

August, litterfall collectors were cleared of any debris that had accumulated since the previous fall collections. Litterfall from each collector was sorted by species, oven dried at 60°C to a constant mass, and weighed. Litterfall mass for each species in each site is the mean from three collectors.

Net N mineralization and nitrification rates

An intact covered core method (Robertson et al., 1999) was used to estimate *in situ* rates of net N mineralization and nitrification in mineral soil. Two five cm diameter PVC tubes were hammered into the mineral soil adjacent to each other to a depth of 10cm. One core was removed immediately and the second core was covered loosely with duct tape to prevent leaching and left in the ground to incubate for 28 days. Once removed, the organic layers were discarded, and the remaining mineral soil was sealed in a plastic bag and kept on ice (usually less than 48 hr) before processing in the laboratory. Four samples were collected at each of the 16 sites during each sampling period. One-month *in situ* incubations were conducted quarterly for two years beginning in May 2003 and ending in June 2005 to capture seasonal trends in N cycling within the two moisture regimes.

In the laboratory, initial and incubated soil samples were passed through a 2mm mesh sieve, and two 10-gram subsamples were weighed and placed in plastic specimen cups. Fifty ml of one M KCl were added to each sample and shaken for one hour, then passed through Whatman no. 40 filter paper. Extracts were collected and analyzed colorimetrically for ammonium (NH_4) and nitrate (NO_3) on a Bran-Luebbe AutoAnalyzer (Norderstedt, Germany). Net N mineralization rates were calculated by subtracting the initial concentrations of NH_4 and NO_3 from the incubated sample concentrations, and are expressed per gram dry weight of soil per day. Net nitrification was calculated as the incubated sample concentration of NO_3 only, minus the initial concentration.

Laboratory incubations were conducted in conjunction with *in situ* incubations in the second year of sampling, from May 2004 to March 2005. Two additional 10-gram subsamples of soil from initial cores were added to plastic specimen cups, covered with perforated lids and placed in an incubator at approximately 20 ^{o}C for one month. Deionized water was added at weekly intervals to keep samples at initial weight, replacing evaporative losses. Extractions and rate calculations were the same as described above for *in situ* samples. For the sampling period

of November to December 2004 no *in situ* incubation was conducted; instead, *in situ* data for this time period were estimated using relationships between previous laboratory and *in situ* rates. Laboratory estimates were always higher than *in situ* N cycling rates and most likely overestimated actual rates. In order to have consistent data on seasonal *in situ* N cycling rates over the course of two years, linear regression models using August to September 2004 *in situ* and laboratory incubation rates were used to predict November to December 2004 *in situ* rates. For N mineralization and nitrification respectively, the following equations were used:

$$\text{\textit{in situ} N mineralization} = 0.382 \times \text{laboratory N mineralization} - 0.057$$
$$(R^2 = 0.65; p = 0.0002);$$

$$\text{\textit{in situ} nitrification} = 0.392 \times \text{laboratory nitrification} - 0.013$$
$$(R^2 = 0.84; p < 0.0001).$$

Soil solution chemistry

Four tension lysimeters, two each at depths of 10 cm and 20 cm, were installed at each site to measure nutrient concentrations in soil solution. Lysimeters were allowed to equilibrate for six months after installation. At each sampling period lysimeters were primed by applying -60 kPa of pressure to the lysimeter column. Soil solutions were evacuated and placed into clean plastic bottles, kept on ice in the field and refrigerated in the lab until filtered and analyzed, usually within 48 hours of collection. Concentrations of NH_4 and NO_3 were determined colorimetrically on a Bran-Luebbe AutoAnalyzer (Norderstedt, Germany). Total dissolved N was determined on filtered samples using a persulfate digestion (Doyle et al., 2004), followed by colorimetric analysis. Dissolved organic N (DON) was calculated as the difference between total N and inorganic N (NH4 + NO3). Calculations occasionally produced negative values, in which case DON was assumed to be zero. Dissolved organic carbon (DOC) was measured on filtered samples using a Shimadzu 5000A TOC analyzer (Columbia, Maryland). Concentrations of Ca, Mg, K and Na in soil solutions were measured on a GBC Avanta atomic absorption spectrophotometer (Hampshire, IL).

Lysimeter collections depended on soil moisture conditions. By late May and throughout the summer months, soil moisture content was too low to produce adequate samples for analyzing soil solution chemistry. Thus, most collections occurred in late fall, winter and spring.

Statistical analyses

Site (n=16) was used as the experimental unit for all statistical analyses. For comparisons between mesic and xeric moisture regimes, two sample t-tests were performed. For comparisons of variables measured across sampling date, factorial ANOVAs were used with moisture regime and sampling date as the main effects. Linear and polynomial regression models were developed to examine patterns in response variables (e.g. N mineralization and nitrification rates) measured across all sixteen sites in relation to potential predictor variables (e.g. soil and vegetation characteristics). All statistical analyses were performed using SAS software, Version 9.1 for Windows (SAS, 2004), and statistical significance was evaluated at α = 0.05.

Results

Soil chemical and physical properties

Mesic and xeric moisture regimes, distinguished by landscape position and species composition, exhibited a number of important differences in soil physical properties (Table 2.1). Mesic sites had significantly (p<0.0001) higher mean soil moisture content compared to xeric sites (30.6 vs. 22.4%, respectively, Table 2.1). Differences in soil moisture between the two moisture regimes were consistent for all sampling periods, even when additional sampling occurred between quarterly sampling periods (Figure 1). Mean annual soil temperature was higher in xeric sites (Table 1). Although not significant, xeric sites tended to have higher sand fractions than mesic sites, which could have contributed to lower moisture status. Forest floor mass in the xeric sites was more than two times that in mesic sites (Table 2.1).

Soil chemical properties also differed in some important respects. In the forest floor, xeric sites had higher concentrations of C and N, but there was no difference in the forest floor C:N ratios (Table 2.1). In contrast, the mineral soil in mesic sites had significantly higher N concentrations, leading to significantly lower C:N ratios. Concentrations of P, Ca, K and Mg in the forest floor tended to be higher in mesic sites, although no significant differences were found.

11

Mineral soil P, Ca, K and Mg concentrations were similar between moisture regimes, and were lower overall compared to forest floor nutrient concentrations. There was more variability in Ca concentrations in xeric sites, resulting in part because two sites occurred on limestone outcrops. Mineral soil pH was significantly (p=0.011) lower in xeric sites, while Al concentrations were marginally significantly (p<0.10) greater, possibly a result of greater sandstone influence.

Overstory and midstory tree species composition

Total basal area of overstory trees (>10 cm DBH) did not differ between mesic and xeric sites (mesic mean (SE) = 30.7 (4.9) m^2 ha^{-1}; xeric mean (SE) = 30.3 (1.9) m^2 ha^{-1}; p=0.94). Overstory tree species composition, however, varied considerably between and among mesic and xeric sites. Sugar maple was often the most dominant species in mesic sites, occurring in seven sites and ranging in relative basal area from 6.8 to 61.7% in those sites. Yellow-poplar occurred in half the mesic sites and was the second most dominant tree species. One site was almost entirely comprised of yellow-poplar (>70%), which was also the site with no sugar maple. American beech occurred in four mesic sites as well, but was usually a minor component (~5% relative basal area), except in one site where it made up almost half of the total basal area. Hickory (*Carya*) species and two oak species, northern red (*Quercus rubra*) and white (*Q. alba*), were also represented on mesic sites (<16% relative basal area).

In contrast, xeric sites were dominated by a suite of oak species, including white, chestnut (*Quercus prinus*), black (*Q. velutina*), post (*Q. stelata*) and scarlet (*Q. coccinea*), and total oak species basal area averaged 60%. White, black and post oak each occurred on more than half the sites, while scarlet oak occurred on only 2 sites. Hickory species occurred on six xeric sites but had only about half the relative basal area compared to mesic sites.

Midstory composition (stems 2-10cm DBH) differed from the overstory. On mesic sites, sugar maple was present on all but the yellow-poplar site, and made up almost 83% of the total basal area in the midstory. Five sites had nearly 100% sugar maple relative basal area in this stratum, and no midstory oaks were recorded at any of the mesic sites.

On xeric sites, only seven oak stems were measured in the midstory, all occurring on two sites. Sugar maple was present on four sites, but was a minor component (<2%). Dogwood (*Cornus florida*) and blackgum (*Nyssa sylvatica*) were the most important tree species in this stratum, occurring on six sites with a combined mean relative basal area of about 40%. One

xeric site that occurred on a limestone outcrop had very high densities of redbud (*Cercis canadensis*) and eastern redcedar (*Juniperus virginiana*).

Litterfall

Mean annual litterfall mass from three years of sampling did not differ between mesic and xeric moisture regimes (mesic mean (SE) = 386 (16.5) g*m^{-2}; xeric mean (SE) = 374 (17.7) g*m^{-2}; p=0.47). When compared by year, mean litterfall mass for all 16 sites in fall of 2005 (434 g*m^{-2}) was significantly (p=0.001) higher than 2003 (348 g*m^{-2}) and 2004 (357 g*m^{-2}), which did not differ (p>0.05).

Significant differences in species composition of litterfall were found as expected from differences in basal area. Sugar maple litterfall was significantly (p<0.0001) greater in mesic sites (33%) compared to xeric sites (2%). Oak species represented a significantly (p<0.0001) smaller proportion of total litterfall in mesic sites (range: 5 to 60%) compared to xeric sites (~70 to 90%). White oak and northern red oak comprised the majority of oak litterfall in mesic sites, while a combination of four to six oak species including chestnut, scarlet, white, black, post, southern red and chinquapin (*Quercus muehlenbergii*) were present in xeric sites. None of these oak species were dominant on all xeric sites. Hickory species represented a significantly (p<0.02) greater proportion of total litterfall in mesic sites compared to xeric sites (13% vs. 6%, respectively). As with basal area, yellow-poplar was the dominant litter type in the one mesic site that contained no sugar maple, and the site with a large proportion of American beech has that species as the dominant litterfall, and only 14% sugar maple. In the other six mesic sites Yellow-poplar, American beech and ash (*Fraxinus* spp.) species were also important components, while in xeric sites blackgum, dogwood and sourwood (*Oxydendrum arboreum*) were important.

Net N mineralization and nitrification rates

In situ N mineralization rates differed significantly between moisture regimes. When averaged across sampling periods for an estimated mean annual rate, N mineralization in mesic sites was more than twice that of xeric sites (0.28 vs. 0.13 µg N g soil^{-1} day^{-1}; p<0.0001). Annual nitrification rates were approximately 10 times higher in mesic sites compared to xeric sites (0.24 vs. 0.023 µg N g soil^{-1} day^{-1}; p<0.0001). Nitrification fraction, or the proportion of N

mineralization that is nitrified, was 10x and 5x greater in mesic sites compared to xeric in years one and two, respectively. When averaged across both years of sampling, nitrification represented >90% of N mineralization rates in mesic sites, compared to ~17% in xeric sites.

Patterns in seasonal and annual variability in N cycling rates were also evident. Virtually no N mineralization and nitrification occurred during the Nov/Dec sampling periods, and the highest rates usually occurred in the May/June sampling periods (Figure 2.2). Rates of N mineralization and nitrification were higher in xeric sites during the Feb/Mar period of year two. Nitrogen mineralization rates during the first year of sampling were higher than the second year (p=0.003), but nitrification in year one and year two were not significantly different (p=0.61).

Laboratory measurements of N mineralization and nitrification rates were higher than *in situ* rates, but the relative magnitude of difference between mesic and xeric site soils was similar (Figures 2.2 and 2.3), except in Feb/Mar of year two, where laboratory measurements showed higher rates in mesic sites than xeric, opposite the pattern observed *in situ*.

Soil solution chemistry

No statistically significant differences were found for concentrations of solutes between 10 and 20 cm depth lysimeters, so measurements from all four lysimeters in a site were averaged together for each sampling date. Mean soil solution NO_3 concentrations were significantly (p<0.0001) greater in mesic sites compared to xeric sites. Nitrate concentrations in xeric sites were slightly above detection limits (~0.01 mg L^{-1}) for most sampling dates, except in June 2004 and January 2005, when nearly all xeric site samples were below detection limits (Figure 2.4). Statistically significant differences were not found between sampling dates (p=0.16); however, NO_3 concentrations in mesic sites tended to be greater in the dormant season than the growing season. No differences where found between moisture regimes for NH_4 concentrations, which represented a minor component of N in soil solutions. Ammonium concentrations in mesic sites were an order of magnitude lower than NO_3, whereas NH_4 and NO_3 concentrations in xeric sites were similar (Figure 2.4). In xeric sites, DON was a greater proportion of total N in soil solution than inorganic N ($NH_4 + NO_3$). In contrast, in mesic sites, NO_3 was the dominant form of N in soil solutions. Mean DOC concentrations were significantly higher in xeric sites (xeric mean (SE) = 22.15 (3.49) mg/L; mesic mean (SE) = 11.44 (1.52) mg/L; p = 0.02). Higher concentrations of DOC occurred during the growing season, compared to the dormant season, opposite the trend

for NO_3. Although concentrations of base cations in soil solutions for mesic sites tended to be higher compared to xeric sites (Figure 2.5), only Mg concentrations were significantly (p=0.01) different between mesic and xeric sites. There were no discernable seasonal patterns in cation concentrations across sampling periods. The two xeric sites located on limestone outcrops had mean soil solution Ca concentrations 2 and 3 times greater than the other six xeric sites and 1.4 and 2.2 times greater than mesic sites.

Factors controlling N cycling

Although strong differences were detected between mesic and xeric site types for N cycling parameters, considerable variation also existed among sites within a moisture regime. We used a number of site characteristics in simple linear regression models to predict variation in net N mineralization and nitrification rates measured in this study. Soil texture was not found to be a good predictor of N mineralization or nitrification rates, as percent sand, silt or clay did not explain a significant amount of variation in mean annual rates for either year of sampling (Table 2.2). Soil pH was a significant predictor of nitrification, explaining nearly one third and one half of the variation in year one and year two, respectively.

Mineral soil C and N concentrations produced mixed results as predictors of net N mineralization and nitrification rates. Mineral soil C was not an important predictor of variation in N cycling in either year, however total soil N explained about half the variation in net N mineralization and nitrification rates across both years. Soil C:N ratios were also significantly correlated with N cycling rates, but C:N ratios had less predictive capability in year one and more in year two compared to total soil N (Table 2.2).

Mean annual soil moisture content explained a large amount of variation in annual N cycling rates among sites at MACA (Table 2.2). Soil moisture content was positively related to net N mineralization and nitrification, but explained slightly more variation in nitrification rates. Also, soil moisture had better predictive power in year two compared to year one.

Litterfall characteristics across the 16 sites were also used as potential explanatory variables. Total litterfall mass (as a measure of stand productivity) did not explain variation in annual N cycling rates. As mentioned above, oak species and sugar maple were dominant components of litterfall, but masses and proportions of these species varied greatly across the sites, presumably in response to moisture availability. When used in regression analyses, oak

and sugar maple, both as mass and proportion of total litterfall, explained a large amount of variation in N cycling rates (Table 2.2). For instance, sugar maple litterfall mass predicted over 80% of the variation in year one N mineralization and nitrification rates, and around 70% for year two. Percent sugar maple litterfall predicted less variation in year one N cycling rates compared to sugar maple litterfall mass, but slightly higher R^2 values were found for year two (Table 2.2). Total oak litterfall mass and percentage oak litterfall within a site were generally less predictive than sugar maple litterfall mass and percentage. This may be due to interspecific differences among oak litterfall, as no single or group of oak species was dominant on all sites. Relationships between sugar maple litterfall and N cycling were positive, in contrast to the negative relationships found between litterfall of oak species and N mineralization and nitrification. No other tree species or genera of litterfall explained a significant amount of variation in N cycling rates.

Factors controlling nitrate and base cation leaching potentials

Because of the strong relationship found between sugar maple litterfall and nitrification rates, and large variation in NO_3 concentrations in soil solution occurring among mesic sites, regression analyses were conducted to determine if similar relationships could be found between tree species composition and soil solution NO_3 concentrations. When averaged across all sampling periods, soil solution NO_3 concentrations varied in response to both the percentage of sugar maple litterfall and the percentage of oak litterfall in a site (Figure 2.6). There was a marginally significant (p=0.06) positive relationship between sugar maple litterfall and soil solution nitrate from mesic sites, but a significant (p=0.04) negative relationship with oak litterfall. It appears that as dominance changes from oak to sugar maple, soil solution nitrate concentrations increase. When used as an independent variable, soil moisture was less predictive of soil solution nitrate concentrations in mesic sites compared to litterfall (Figure 2.6). Relationships between soil solution nitrate and DOC and base cation concentrations were explored using simple linear regression. In mesic sites, Ca and Mg concentrations in soil solutions were positively correlated with both nitrate and DOC concentrations (Figure 2.7), and samples from both the 10 cm- and 20 cm-depths contributed to variation in soil solution Ca and Mg. Positive relationships between DOC and Ca and Mg in soil solutions from mesic sites were also found, but a few samples with high concentrations of DOC from the 10 cm-depth lysimeters

seemed to drive these relationships. In contrast, soil solution concentrations of Ca and Mg from xeric sites were unrelated to nitrate concentrations (Figure 2.8). Differences in geologic substrate among xeric sites contributed to variation in soil solution Ca, such that two sites occurring on limestone outcrops had the highest Ca concentrations. When samples from these these two sites are excluded, DOC explained approximately 30% of the variation in Ca concentrations (Figure 2.8). Soil solution Mg concentrations were unrelated to DOC.

Discussion

Net N mineralization rates reported from stands dominated by sugar maple and oak species in the midwestern (Zak and Pregitzer, 1990) and the northeastern US (Finzi et al., 1998a and Lovett et al., 2004) were greater than those in mesic and xeric sites at MACA (Table 2.3). In oak and hickory dominated stands of southern Indiana and Ohio, Idol et al. (2003) and Morris and Boerner (1998) also found greater N mineralization rates compared to the oak dominated xeric sites at MACA. In contrast, N mineralization rates in MACA xeric sites were greater than those measured on an oak-pine ridgetop in the Cumberland Plateau of eastern KY (Washburn and Arthur, 2003), and comparable to those of Newman et al. (2006), who found no difference in N mineralization rates between mesic and xeric sites in the same region. Trends in nitrification tell an all-together different story, however. At MACA, nitrification in the xeric sites was low and similar to most other oak-dominated sites across the eastern US. Nitrification rates in the mesic sites were high and comparable to those reported for sugar maple dominated stands in the Midwest (Zak and Pregitzer, 1990) and the northeast (Finzi et al., 1998a). But perhaps more importantly, nitrification fraction in mesic sites (0.88) at MACA was higher than all previously mentioned studies, and were only lower than those reported by Gilliam et al. (2001) from Fernow Experimental Forest in the central Appalachians, which included watershed three, where 35 kg N ha^{-1} yr^{-1} (as $(NH_4)_2SO_4$) have been applied since 1989. In addition, spatial patterns in N cycling at MACA are strikingly similar to those reported by Peterjohn et al. (1999) for watershed four at Fernow (untreated), which has been identified by those authors as exhibiting signs of N saturation. Elevated rates of N mineralization and nitrification, as well as NO_3 concentrations in soil solutions, were found to occur on easterly aspects, much like those of mesic sites at MACA, whereas low rates on southerly aspects were very similar to xeric sites in this study. Similar patterns in spatial variability of N cycling between the two studies, as well as high nitrification

17

fractions measured in mesic sites at MACA, are evidence that these sites may be N saturated and thus could become larger sources of N released to streams if current depositional loading is sustained.

Species influence on C and N dynamics

Leaf litter quality can affect rates of decomposition (Melillo et al., 1982, Mudrick et al., 1994), which in turn can lead to spatial variability in soil carbon and nutrient concentrations (Zinke, 1962; Boettcher and Kalisz, 1990; Finzi et al., 1998b), including N mineralization rates (Scott and Binkley, 1997; Finzi et al., 1998a; Ferrari, 1999). For example, litters with higher amounts of recalcitrant C compounds, such as lignin, decompose more slowly (Binkley and Giardina, 1998) and often lead to higher C:N ratios in soils (Finzi et al., 1998a, Ollinger et al., 2002). Sugar maple leaf litter is known to have low C:N and lignin:N ratios (Ferrari, 1999, Lovett and Mitchell, 2004), especially compared to oak litter (Lovett et al., 2004). Sugar maple litterfall from MACA sites was found to have higher N concentrations and lower C:N ratios compared to oak species (Fabio and Arthur, *unpublished data*). Contrasting species composition between mesic and xeric sites at MACA have likely contributed to differences in soil properties, where oak-dominated xeric sites have significantly more forest floor mass and higher C:N ratios in the mineral soil. Conversely, mesic sites, where sugar maple is in higher abundance, have less massive forest floors and lower mineral soil C:N ratios.

These factors, along with soil moisture, seem to be largely responsible for differences in N cycling rates between mesic and xeric sites at MACA. Sugar maple abundance, as measured by litterfall, was strongly and positively correlated with higher net N mineralization, but especially nitrification, while oak species litterfall was negatively correlated with N mineralization and nitrification (Table 2.2). Other factors known to influence N cycling rates, such as soil texture and pH, were not good predictors of N mineralization and nitrification in this study, despite variation across the sites. Soil moisture content is potentially very important in controlling N cycling at MACA, and in this study design moisture and species composition are most certainly confounded. However, species composition, specifically sugar maple abundance, may be more important than soil moisture in controlling N cycling at MACA, particularly nitrification. For instance, not only was sugar maple litterfall highly predictive of net N mineralization and nitrification across all 16 sites, but also the two mesic sites dominated by

American beech and yellow-poplar had 45% lower annual net N mineralization rates, and between 30 and 45% lower annual net nitrification rates compared to the other six mesic sites. This is despite the fact that these two sites have higher soil moisture than all but one other mesic site, and they occurred well within the range of aspects occupied by mesic sites. In addition, rates of N mineralization and nitrification in the beech site were intermediate along the sugar maple to oak gradient observed across all sites, which is a pattern consistent with the findings of Lovett et al. (2004) who examined N cycling rates in single species stands of sugar maple, beech and northern red oak. The high abundance of nearly even-aged yellow-poplar in the other low N cycling mesic site is indicative of recovery from agricultural disturbance seen in this region (Kalisz, 1986). Yellow-poplar stands have been correlated with higher levels of mineralizable N compared to eastern hemlock (*Tsuga canadesis*) stands (Boettcher and Kalisz, 1990) and higher concentrations of other soil nutrients compared to mixed hardwood stands (Kalisz, 1986). Perhaps intermediate litter quality (between sugar maple and oak), altered nutrient status due to disturbance, or both, led to lower N cycling rates in the yellow-poplar site compared to the other six mesic sites. Regardless, when this site is excluded from the regression models using nitrification as the response variable, sugar maple litterfall mass ($g*m^{-2}$) explains about the sample amount of variation ($R^2 = 0.84$) in year one, and 11% more variation ($R^2 = 0.88$) in year two, than does soil moisture. This highly significant relationship between sugar maple litterfall mass and nitrification, coupled with the fact that differences in N cycling among mesic sites cannot be explained by differences in aspect and soil moisture, are evidence that tree species have strong control of N cycling at MACA.

Nitrate and base cation leaching

Sugar maple litterfall was positively correlated with NO_3 concentrations in soil solutions (Figure 2.6), whereas sites dominated by oak species had little to no NO_3 in soil solutions, and the beech and yellow-poplar sites had the lowest levels of NO_3 in soil solutions among mesic sites. These findings suggest some control of N leaching by tree species, most likely regulated through differences in nitrification rates in soils beneath contrasting tree species, a pattern that has been observed in hardwood forests in the Adirondack Mountains of NY (Mitchell et al., 2003). However, some mesic sites in this study that had high sugar maple litterfall inputs did not exhibit high soil solution NO_3 concentrations (Figure 2.6), and it appears that some other

parameter not considered (e.g. plant uptake) also influenced NO_3 concentrations. Nevertheless, elevated NO_3 concentrations in soils associated with high abundance of sugar maple (and low abundance of oak) suggest that higher rates of nitrification lead to higher NO_3 leaching losses. This may be of particular concern at MACA, where in mesic sites a high abundance of sugar maple stems 2-10cm DBH may lead to increased sugar maple dominance as these stands age. Furthermore, a phenomenon of reduced oak regeneration in oak-hickory forests of the lower midwestern region of the US has been identified, where species such as red maple, blackgum, and in some cases sugar maple are increasing in abundance in the under- and midstory canopy layers (Lorimer et al., 1994). Reduced oak regeneration may influence future forest composition at MACA, since only seven oak stems 2-10cm DBH were measured in this study, all which were located in just two sites. Negative relationships between oak abundance and nitrification and NO_3 in soil solutions suggest that oak-dominated stands could be sites of high N retention, and a loss of oak as dominant canopy trees could have implications for N losses in these ecosystems. Differences in N retention on a watershed scale have been attributed to differences in species composition (Lovett et al., 2000), and sugar maple abundance, along with other easily decomposable litters, is likely the cause for elevated stream water NO_3 export (Lovett et al., 2002, Christopher et al., 2006). On average, oak and sugar maple are approximately equal in abundance in mesic sites—31% and 33% of total litterfall, respectively. If species composition shifts dominance toward species with higher quality litter, such as sugar maple, it could have large-scale impacts for N cycling, and N leaching could increase. This could be of particular concern to park managers, as habitats that are likely to exhibit increases in sugar maple abundance (i.e. mesic forests) are in close proximity to aquatic ecosystems and cave openings, which host large biological diversity, including a number of threatened and endangered species (Butler et al., 2003).

In mesic sites at MACA, net NO_3 production, estimated from annual measurements of nitrification across two years of sampling was 75 ($\pm$ 7.4) cmol NO_3 m^{-2} yr^{-1}, which stoichiometrically represents the release of 150 ($\pm$ 14.8) cmol H^+ m^{-2} yr^{-1}. This rate is ten-fold greater than that of xeric sites, and nearly 50 times greater than the mean wet deposition loading of H^+ (3.2 cmol H^+ m^{-2} yr^{-1}) at MACA during 2003 and 2004 (NADP, 2006). These estimates do not include other soil acid-base reactions that could increase (e.g. ammonium uptake) or decrease (e.g. denitrification) proton loading, but clearly nitrification in mesic sites can be a significant

source of H^+. Nitrate in soil solutions from mesic sites was positively correlated with Ca and Mg concentrations in soil solution (Figure 2.7), and has been linked to soil acidification in organic horizons of sugar maple stands in the northeastern US (Fitzhugh et al., 2003). Therefore, soil acidification could be accelerated in forest soils that currently have a high potential to nitrify (i.e. sugar maple stands) or where nitrification rates could increase as a result of encroachment of sugar maple. In addition, NH_4 at MACA accounts for more than 40% of total N deposition and can be readily available for nitrification, especially if plant demand for N becomes saturated. This may become important in the future, as MACA is surrounded by agricultural land use, and no regulations currently exist for the control of NH_4 emissions (EPA-CASTNET, 2006).

Compared to mesic sites, xeric sites exhibited elevated DOC concentrations, which have been positively correlated with concentrations of dissolved organic acids (Dijkstra et al., 2001), and could represent a significant source of acidity in these oak-dominated stands. Soil solution DOC, but not nitrate, was positively correlated with Ca and Mg concentrations in xeric sites (Figure 2.8), suggesting that contrasting biological processes between mesic and xeric sites maybe influencing base cation leaching at MACA. Variability in geologic substrate also influenced the base status of soils in this study, and stands occurring on or near limestone outcrops will most likely be buffered from acidification. However, xeric sites tended to have lower soil pH and base cation concentrations, and higher Al concentrations compared to mesic sites, and thus soil acidification could also be a concern in xeric sites without the influence of limestone. External sources of acidity may also influence soil acidification, as long-term monitoring at MACA has shown no significant declines in acidic deposition.

Conclusions

The dissected nature of the landscape in this region creates important gradients in microclimate and thus moisture status, which in turn controls broad-scale patterns in tree species distribution. Because of differences in geologic substrate, there is a potential for variability in soil texture and nutrients at MACA across the landscape, which have been shown to affect tree species distribution. A lack of significant differences measured in this study for these parameters, but highly significant differences in soil moisture availability, imply that it is the most important factor driving species composition. Sugar maple and oak species were shown to have opposite influences over nitrification and NO_3 in soil solutions, and stands dominated by

sugar maple are exhibiting signs of N saturation. Sites dominated by beech and yellow-poplar had intermediate N cycling rates. Tree species composition in lower canopy levels (i.e. 2-10cm DBH) at MACA suggest that sugar maple abundance may be increasing in mesic sites, and oak regeneration in all sites may be reduced. A shift in species composition toward those with higher quality litter could result in lowered N retention and greater N export to adjacent sensitive ecosystems. With no significant long-term decreases in N deposition observed in this region, and new coal-fired power generating facilities permitted for construction upwind of the park (Mark DePoy, MACA Chief of Science and Resource Management, *personal communication*, 2006) possibly leading to increased rates of N deposition, more stands may become N saturated, especially those where sugar maple may become a larger component of the canopy. Long-term monitoring of not only N cycling but also forest composition will be crucial to understanding the impacts of forest succession and continued N deposition to all ecosystems at MACA.

Table 2.1. Physical and chemical properties of organic (Oe + Oa) and upper mineral soil (0-10 cm) layers from MACA sampling sites.

Soil property	Moisture regime		
	Mesic	Xeric	
	Mean (SE)	Mean (SE)	p value*
Physical			
Forest floor (Oe+Oa) mass (g/m2)	355.1 (87.1)	820.4 (65.9)	0.001
Mineral soil (0-10cm)			
Soil moisture (%)	30.5 (1.2)	22.4 (0.9)	<0.0001
Soil temperature (oC)	13.2 (0.2)	14.1 (0.1)	<0.001
Texture			
Sand (%)	46.3 (2.3)	56.8 (4.9)	0.08
Silt (%)	45.2 (2.0)	34.5 (4.7)	0.06
Clay (%)	8.5 (0.5)	8.8 (1.0)	0.82
Bulk density (g/cm3)	1.2 (0.03)	1.2 (0.1)	0.84
Chemical			
C and N			
Forest floor (Oe+Oa)†			
Total C (%)	19.7 (1.6)	27.2 (0.93)	0.002
Total N (%)	0.93 (0.09)	1.2 (0.04)	0.02
C:N ratio	21.3 (0.5)	22.4 (0.6)	0.21
Mineral soil (0-10cm)			
Total C (%)	2.4 (0.14)	2.3 (0.24)	0.80
Total N (%)	0.2 (0.01)	0.1 (0.01)	0.006
C:N ratio	13.3 (0.3)	19.8 (0.9)	<0.001
Exchangeable nutrients (mg/kg)			
Forest floor (Oe+Oa)			
P	80.5 (13.6)	50.3 (4.3)	0.08
Ca	5940 (694)	5540 (1370)	0.80
K	320 (33.8)	298 (28.4)	0.62
Mg	377 (44.2)	263 (35.9)	0.06
Mineral soil (0-10cm)			
P	19.2 (2.4)	15.1 (0.7)	0.14
Ca	856 (101)	1010 (180)	0.48
K	67.3 (4.0)	70.0 (6.5)	0.73
Mg	52.0 (4.3)	53.1 (5.6)	0.88
NH_4-N	3.2 (0.3)	2.9 (0.3)	0.53
NO_3-N	1.7 (0.3)	0.97 (0.1)	0.03
Mineral soil exch. Al (mg/kg)	23.4 (6.3)	64.4 (21.2)	0.10
Molar Ca:Al ratio	144 (114)	60.2 (40.6)	0.51
pH	4.7 (0.2)	4.2 (0.1)	0.01

* P-values are from two sample t-tests comparing mesic (n=8) and xeric (n=8) sites.
† In mesic sites n=6 and in xeric n=7 for forest floor samples because some sites had no Oe and Oa layers to collect.

Table 2.2. Coefficients of determination (R^2) for simple linear regression models for two years of annual estimates of *in situ* net N mineralization and nitrification against soil and site characteristics.

| | Response variable | | | |
| | Year 1 N mineralization | Year 2 N mineralization | Year 1 nitrification | Year 2 nitrification |
Explanatory variable	R^2	R^2	R^2	R^2
Mineral soil (0-10cm)†				
Sand fraction (%)	0.131	0.17	0.222	0.209
Clay fraction (%)	0.047	0.001	0.009	0.001
Mean pH	0.164	0.161	0.320*	0.465*
Total C conc (g/kg)	0.128	0.149	0.032	0.028
Total N conc (g/kg)	0.491*	0.599**	0.417*	0.543*
C:N ratio	0.383*	0.430*	0.518*	0.732***
Mean soil moisture (%)	0.641**	0.668***	0.765***	0.783***
Litterfall‡				
Sugar maple (g/m2)	0.814***	0.717***	0.839***	0.675***
Oak spp. (g/m2)	0.375*	0.380*	0.474*	0.637**
% sugar maple	0.692***	0.727***	0.690***	0.693***
% oak spp.	0.437*	0.515*	0.619**	0.772***

Note: Asterisks (*) indicate level of statistical significance, where * = $p < 0.05$, ** = $p < 0.001$, *** = $p < 0.0001$.

† Soil texture, pH and C and N concentrations are single means for each of the 16 sites used in both year 1 and year 2 regression models. Mean soil moisture content is the mean of four sample dates per year.

‡ Litterfall collections from 2003 and 2004 were used in regression models for year 1 and year 2 respectively.

Table 2.3a. Comparison of N mineralization and nitrification rates as mg N m^{-2} day^{-1}, and nitrification fractions from sites across the northeast and Midwest USA with varying tree species composition.

Source	Location	Incubation Method	Main effect	Treatments	N min	Nitrif†	Nitrif fraction‡	Dominant tree species §
Current study	South-central KY	Seasonal in situ intact core	Moisture regime	Mesic	33	29	0.88	ACSA, LITU, FAGR, CARYA
				Xeric	16	3	0.19	QUERCUS, NYSY, CARYA
Idol et al., 2003	Southern IN	Growing season in situ intact core	Stand age	31-33 yr-old stand	26	11	0.42	QUERCUS, CARYA
				80-100 yr-old stand	69	23	0.33	QUERCUS, CARYA
Gilliam et al., 2001	Fernow EF, WV	Year-round in situ buried bag	Watershed N additions	Young control	35	31	0.89	PRSE, ACSA, BELE, LITU
				Mature control	34	31	0.91	ACSA, QURU, FAGR
				35 kg N ha^{-1} yr^{-1}	37	39	1.05	PRSE, ACSA, FAGR
Ohrui et al., 1999	Adirondack Mtns, NY	Year-round in situ buried bag	Slope position	Upland hardwood (upper)	29	8	0.28	FAGR, ACSA
				Upland conifer (lower)	22	0.5	0.02	PIRU, TSCA
Peterjohn et al., 1999	Fernow EF, WV	Year-round in situ intact core	Aspect	Easterly	40	35	0.88	ACSA, QUPR, TIAM
				Southerly	23	2	0.09	NYSY, FAGR, OXAR, ACRU, QUERCUS
Finzi et al., 1998	Northwestern CT	Mid-summer in situ intact core	Single species plots	Sugar maple	66	41	0.62	ACSA
				Northern red oak	39	10	0.26	QURU
				American beech	32	7	0.22	FAGR

Note: All studies are reporting net rates from upper mineral soil layers.

† Rounding errors may result in differences between nitrification fraction calculated here and those reported in the above studies.

‡ Dominant tree species data for Gilliam et al., 2001 is from Gilliam et al., 1995. Tree species data for Morris and Boerner, 1998 is from Sutherland et al., 2003 and Hutchinson et al., 1999. All other tree species data are reported in the original source.

Tree species abbreviations are: ACRU, *Acer rubrum*; ACSA, *Acer saccharum*; BELE, *Betula lenta*; CARYA, *Carya spp.*; FAGR, *Fagus grandifolia*; LITU, *Liriodendron tulipifera*; MAAC, *Magnolia acuminate*; NYSY, *Nyssa sylvatica*; OXAR, *Oxydendrum arboretum*; PIRU, *Picea rubens*; PRSE, *Prunus serotina*; QUAL, *Quercus alba*; QUERCUS, *Quercus spp.*; QUPR, *Quercus prinus*; QURU, *Quercus rubra*; QUVE, *Quercus velutina*; TIAM, *Tilia Americana*; TSCA, *Tsuga canadensis*.

Table 2.3b. Comparison of N mineralization and nitrification rates in mg N kg^{-1} day^{-1}, and nitrification fractions from sites across the northeast and Midwest USA with varying tree species composition.

Source	Location	Incubation Method	Main effect	Treatments	N min	Nitrif §	Nitrif fraction †	Dominant tree species ‡
Current study	South-central KY	Summer laboratory incubation	Moisture regime	Mesic	0.97	0.83	0.86	ACSA, LITU, FAGR, CARYA
				Xeric	0.39	0.09	0.23	QUERCUS, NYSY, CARYA
Newman et al., 2006	Cumberland Plateau, KY	Summer in situ intact core	Moisture regime	Mesic	0.43	0.33	0.77	MAAC, LITU, ACSA
				Xeric	0.50	0.02	0.04	QUERCUS, ACRU
Lovett et al., 2004	Catskill Mtns, NY	Mid-summer laboratory incubation	Single species plots	Sugar maple	3.20	2.40	0.75	ACSA
				Northern red oak	2.20	0.50	0.23	QURU
				American beech	3.10	1.80	0.58	FAGR
Morris and Boerner, 1998	Southern OH	May to June laboratory incubations	Moisture regime	Mesic	3.60	0.65	0.18	QUERCUS (51%BA), ACRU, ACSA, LITU
				intermediate	2.75	0.52	0.19	QUERCUS (71%BA), ACRU, CARYA, LITU
				Xeric	1.75	0.10	0.06	QUERCUS (87%BA), ACRU, CARYA
Zak and Pregitzer, 1990	northwestern Lower MI		Stand composition	Sugar maple-basswood	1.17	1.00	0.85	ACSA, TIAM
				Sugar maple-northern red oak	1.05	0.12	0.11	ACSA, QURU
				Black oak- white oak	0.86	0.05	0.06	QUVE, QUAL

Note: All studies are reporting net rates from upper mineral soil layers.

† Nitrification data from Newman et al., 2006 is from Newman, 2002.

‡ Rounding errors may result in differences between nitrification fraction calculated here and those reported in the above studies.

§ Dominant tree species data for Gilliam et al., 2001 is from Gilliam et al., 1995. Tree species data for Morris and Boerner, 1998 is from Sutherland et al., 2003 and Hutchinson et al., 1999. All other tree species data are reported in the original source. Tree species abbreviations are: ACRU, Acer rubrum; ACSA, Acer saccharum; BELE, Betula lenta; CARYA, Carya spp.; FAGR, Fagus grandifolia; LITU, Liriodendron tulipifera; MAAC, Magnolia acuminate; NYSY, Nyssa sylvatica; OXAR, Oxydendrum arboretum; PIRU, Picea rubens; PRSE, Prunus serotina; QUAL, Quercus alba; QUERCUS, Quercus spp.; QUPR, Quercus prinus; QURU, Quercus rubra; QUVE, Quercus velutina; TIAM, Tilia Americana; TSCA, Tsuga canadensis.

Figure 2.1. Mean soil moisture content over time between mesic (n=8) and xeric (n=8) sites at MACA. Error bars represent ± one standard error of the mean. Lines connecting points were drawn to aid interpretation. A factorial ANOVA was used to examine the main effects of moisture regime, sample date (May, Aug, Nov and Feb only) and year on soil moisture content. Significance is represented by * = p<0.05 and *** = p<0.0001.
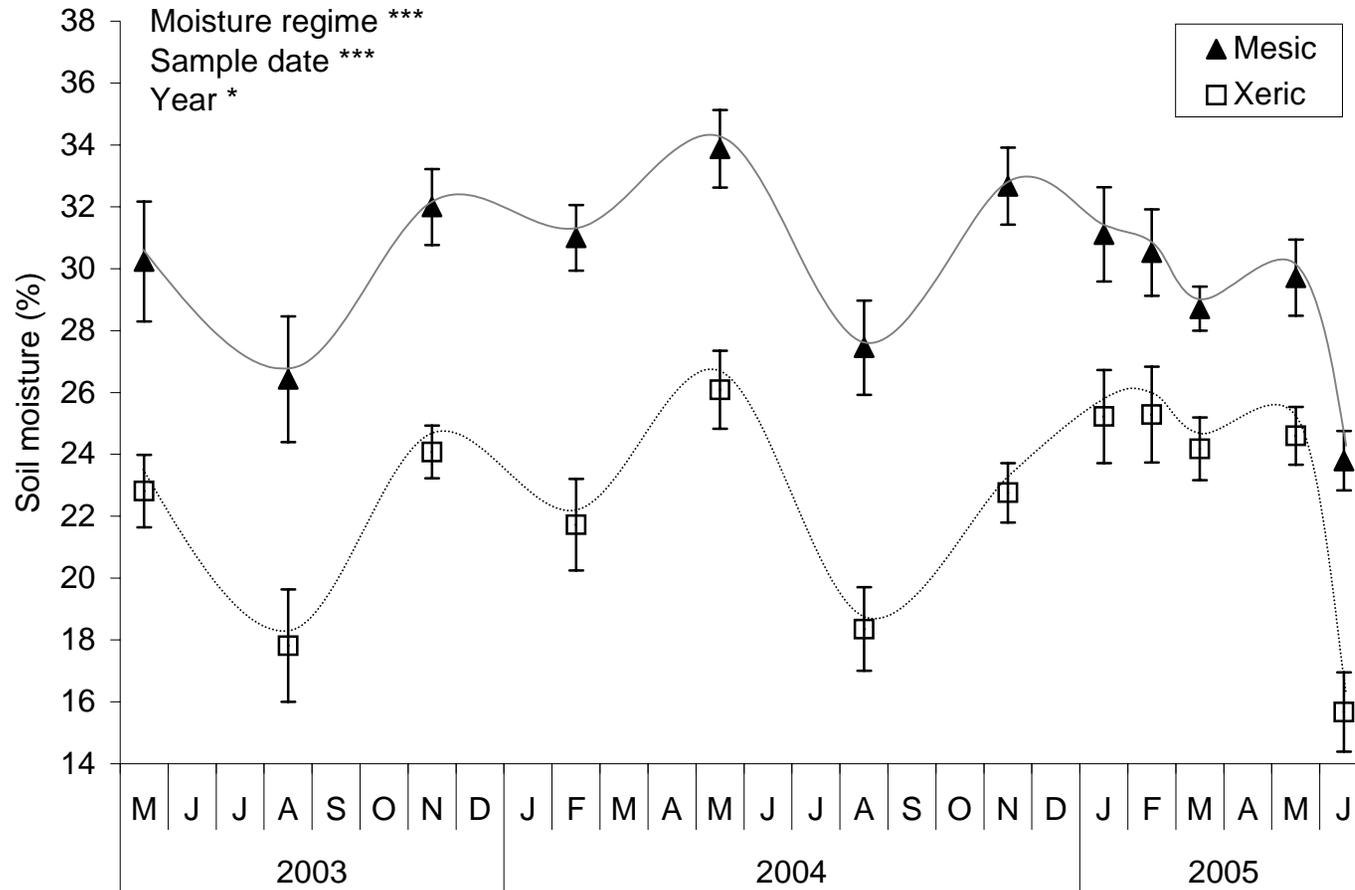
Figure 2.2. Seasonal *in situ* N mineralization (top panel) and nitrification (bottom panel) rates for mesic and xeric moisture regimes at MACA. Error bars represent ± one standard error of the mean.
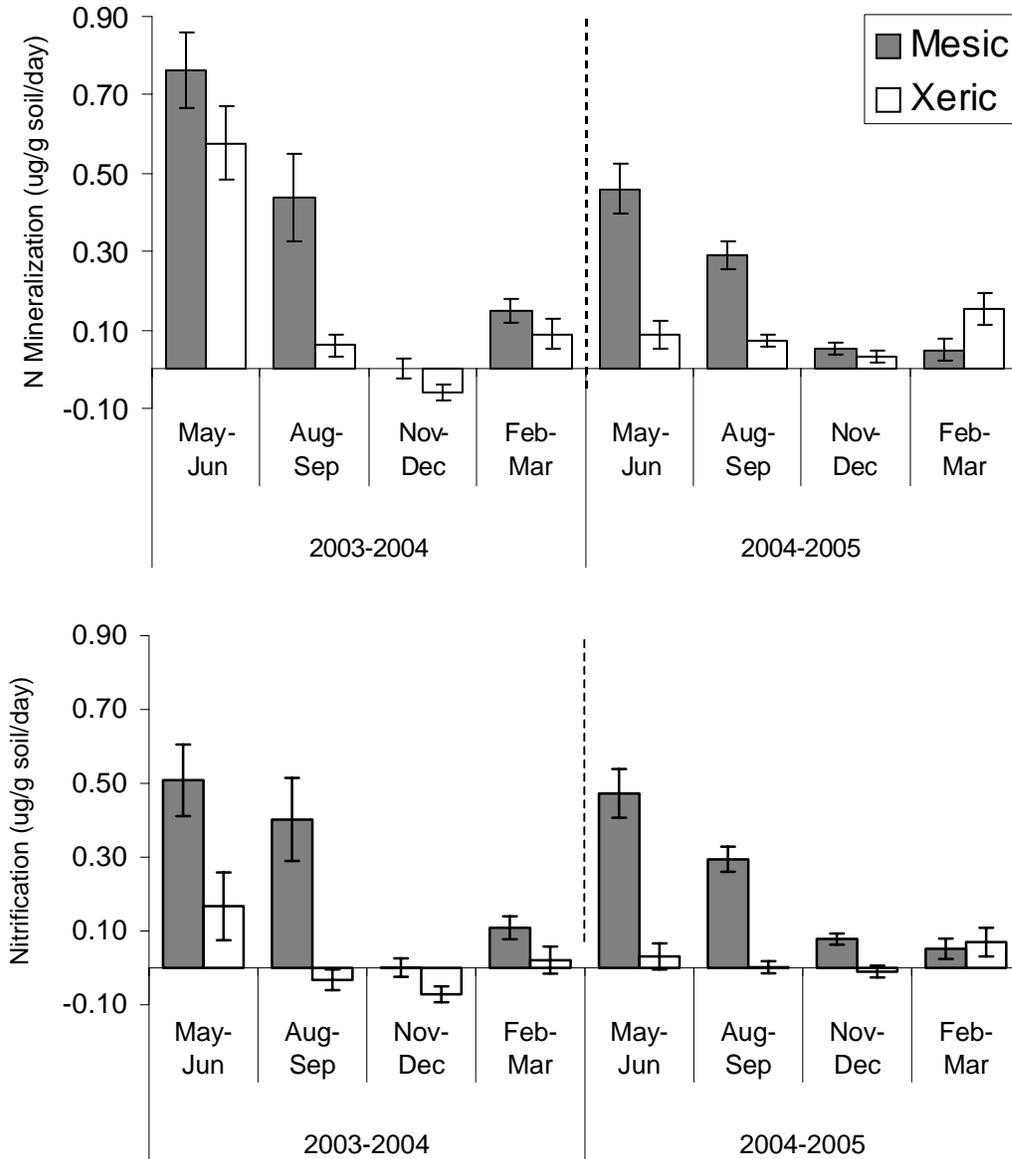
Figure 2.3. Potential net N mineralization (top panel) and nitrification (bottom panel) for mesic and xeric moisture regimes. Measurements were made concurrently with *in situ* incubations during the second year of sampling. Error bars represent ± one standard error of the mean.
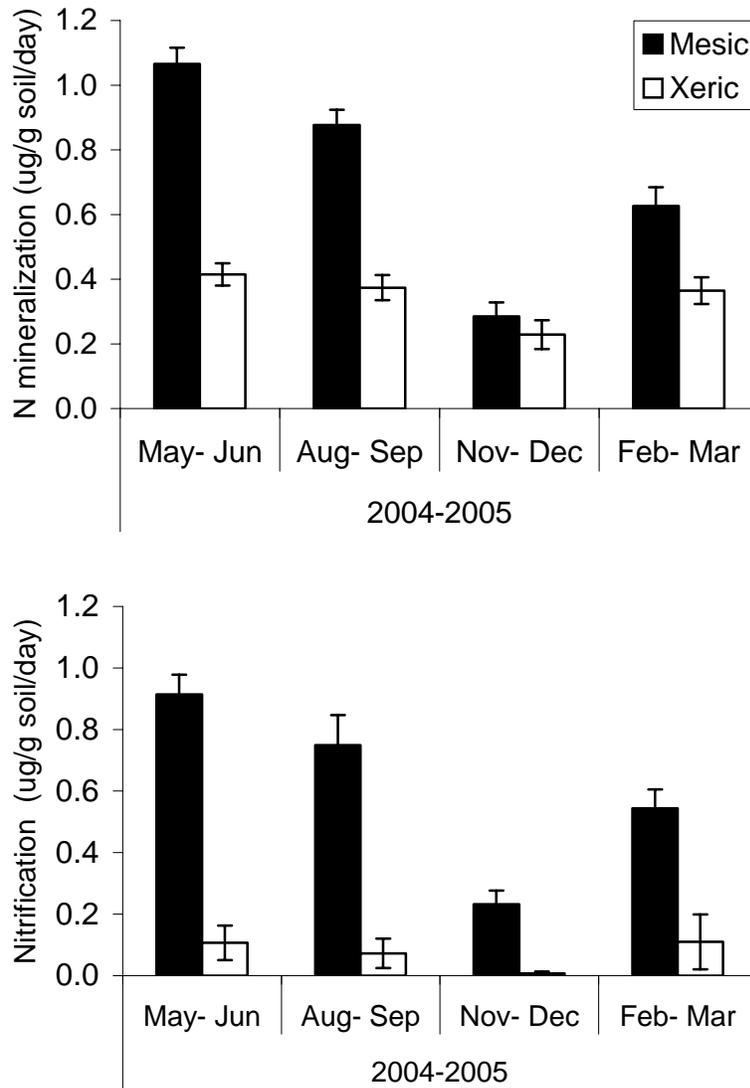
Figure 2.4. Soil solution chemistry for mesic and xeric sites over time from tension lysimeters. Data are mean concentrations for eight plots (four lysimeters each) within moisture regimes. Top panel is nitrate, top-middle panel is ammonium, bottom-middle is dissolved organic N, and bottom panel is dissolved organic carbon (DOC). For the first two sampling dates, November 2003 and March 2004, a laboratory error occurred in the persulate digestion, so DON values were not reported for these dates.
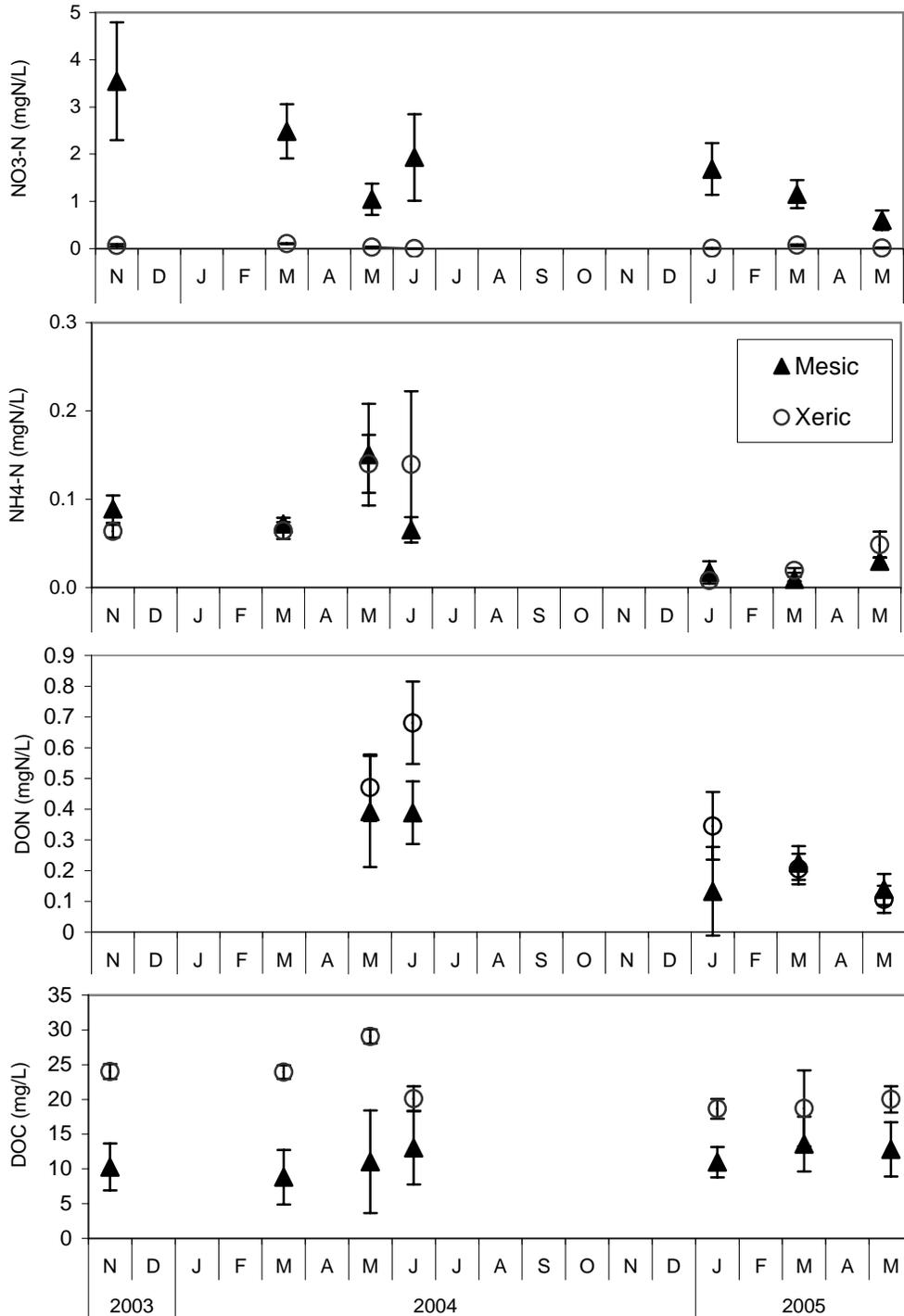
Figure 2.5. Mean soil solution concentrations of base cations (Ca, Mg, K, Na) over time for mesic (closed triangles) and xeric (open circles) sites at Mammoth Cave National Park. Error bars are ± one standard error of the mean.
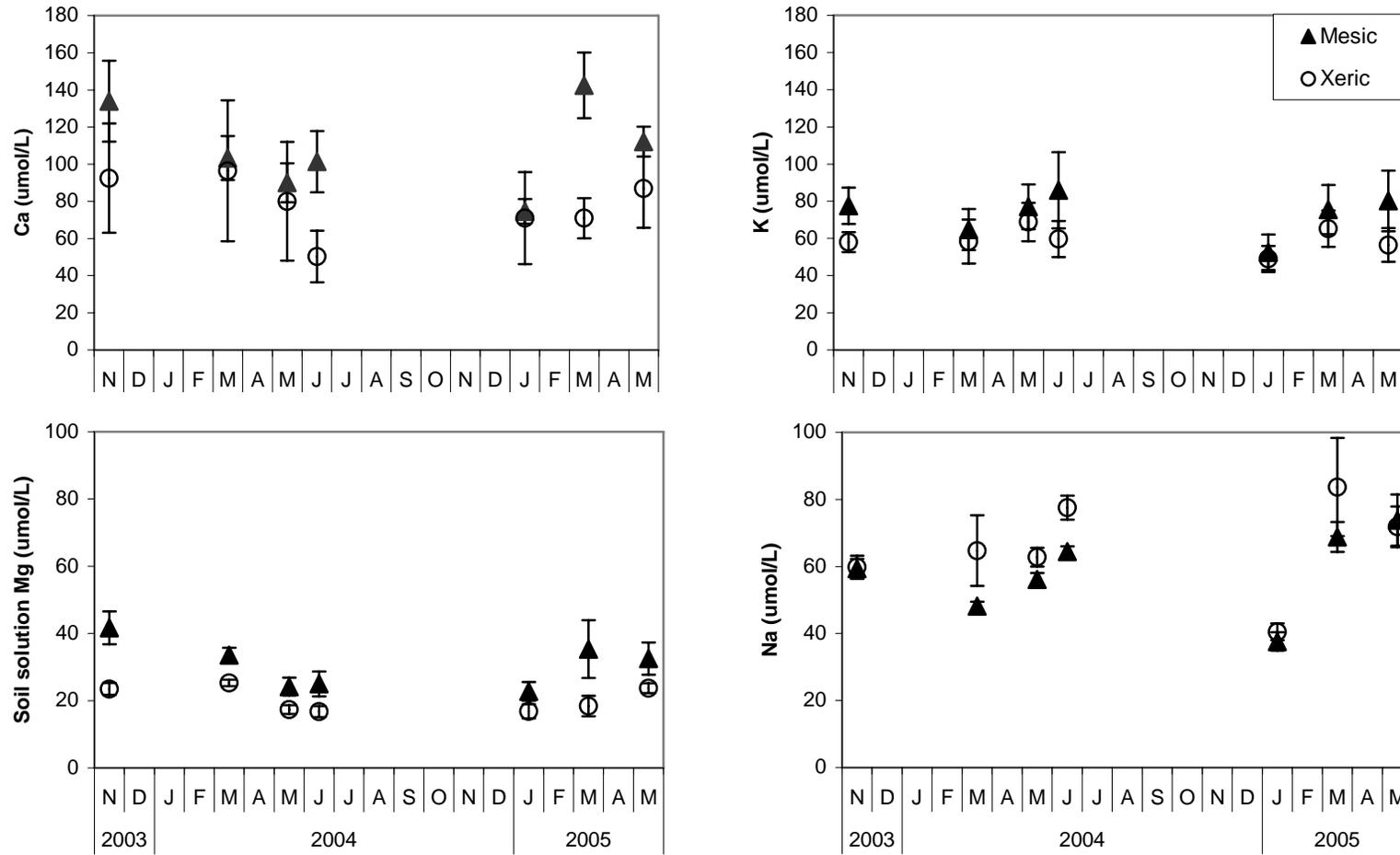
Figure 2.6. Simple linear regression relationships between soil solution nitrate concentrations, and (a) sugar maple litterfall, (b) oak species litterfall, (c) gravimetric soil moisture content and (d) annual net nitrification in mesic sites (closed triangles) at MACA. Xeric sites (open circles) are shown for comparison, but were not used in the regression analyses.
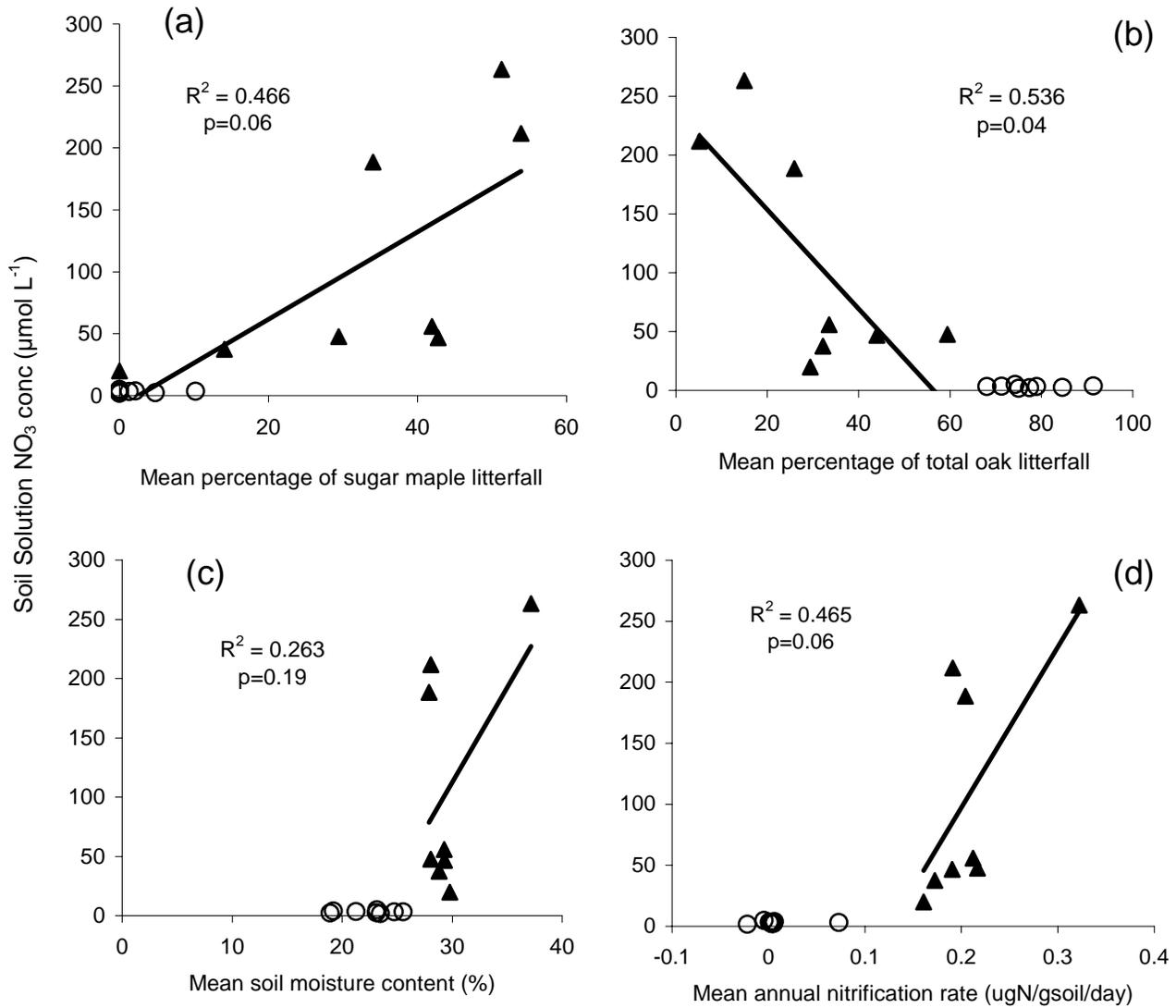
Figure 2.7. Relationships between soil solution (a) Ca and NO3, (b) Ca and DOC, (c) Mg and NO3, and (d) Mg and DOC in mesic sites. Symbols represent sampling points from 10cm- (closed triangles) and 20cm-depth (open triangles), but regression analyses include all points. For panels (a) and (b), n=167, while n=166 for panels (c) and (d).

33
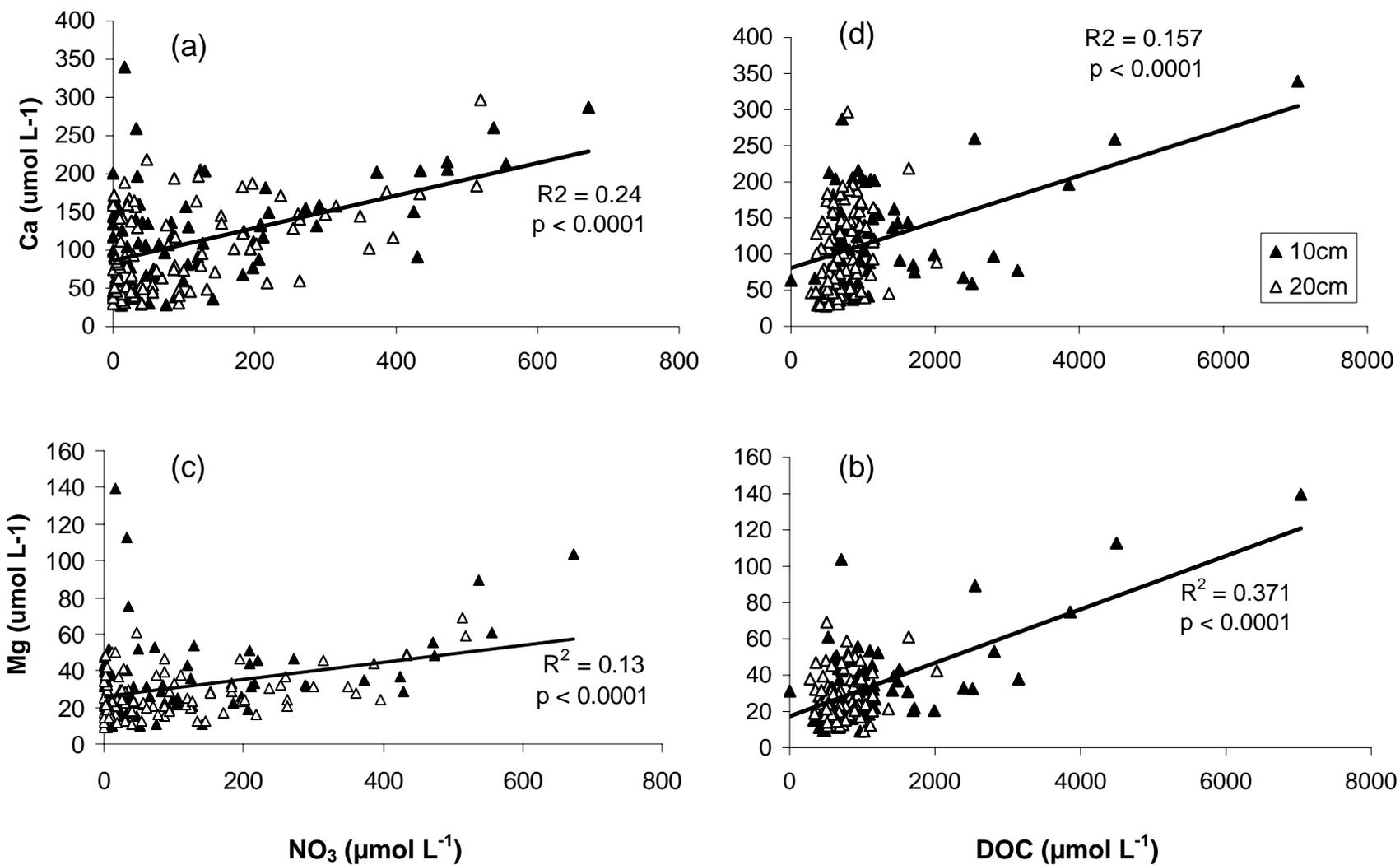
Figure 2.8. Relationships between soil solution (a) Ca and NO3, (b) Ca and DOC (c) Mg and NO3, and (d) Mg and DOC in xeric sites. Sampling points are distinguished by lysimeter depth and presence or absence of limestone influence. In the six non-calcareous sites, 10 cm-depth samples are closed circles (n = 62) and 20 cm samples are open circles (n = 66). In the two calcareous sites, 10 cm samples are plus signs (n = 14) and 20 cm samples are "x" (n = 13). Regression analysis in panel (c) includes samples from non-calcareous only at both depths (n = 128).
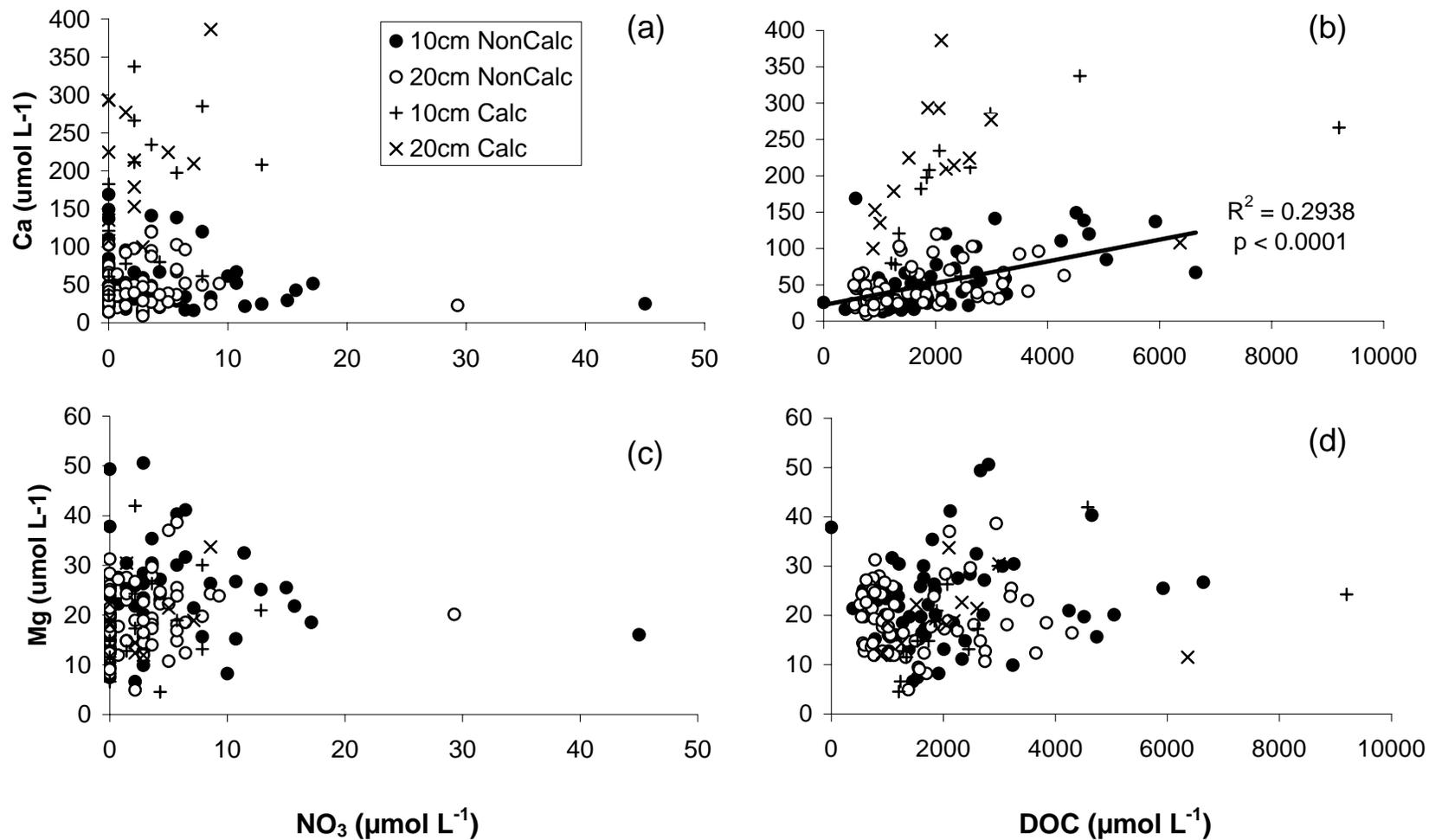
CHAPTER THREE

Short-term decomposition dynamics in forests with contrasting moisture availability and

tree species at Mammoth Cave National Park, Kentucky, USA

Introduction

Decomposition of organic matter on the forest floor is an important component of biogeochemical cycling within temperate forested ecosystems, as most of the nutrients and energy resides in plant litter deposited aboveground. Macroclimate (Meentemeyer, 1978) and microclimate (Mudrick et al., 1994), as well as initial litter quality (Melillo et al., 1982) have been shown to exert significant control on the decomposition process, but understanding which factors are more influential will likely depend on the degree to which they vary across local and regional scales (Prescott, 2006). In the northern temperate regions, topographic variation can create significant changes in microclimate on the local scale, by controlling the amount and intensity of solar radiation (Sartz, 1972). The resulting differences in temperature and moisture can in turn affect decomposition rates (Mudrick et al., 1994; Sariyildiz et al., 2005). In boardleaf forests the eastern United states, decomposition rates are slower in xeric forests (Boerner, 1984), and as a result, organic matter tends to accumulate on the forest floor, while forest floor layers of forests with moister microclimates are often thin (Fabio et al., i*n prep*), and annual leaf litter can be mostly consumed within a growing season. These differences in forest floor thickness could be a result of microclimatic controls on decomposition; however, differences in microclimate across relatively small geographic scales can also affect species composition (Muller, 1982; Newman et al., 2006). Since strong differences in litter quality often occur among dominant tree species in contrasting microclimates, it can be difficult to determine which factors are most influential for determining decomposition rates.

Differences in N availability can also be affected by topographic position (Zak and Pregitzer, 1990). Nitrogen availability may limit the rate of decomposition since the carbon (C) to N ratios of decomposer organisms are lower than those of litter substrates, and the accumulation of N in decomposing litter is a widely observed phenomenon (Melillo et al., 1982). However, studies exploring the effects of N availability on decomposition have had conflicting results. For instance, McClaugherty et al. (1985) found no influence of site N availability on decomposition rates across hardwood and conifer stands in south-central Wisconsin, while

Hobbie (2005) found a significant positive relationship between inorganic N availability in soils and decomposition rates in N-limited sites in central Minnesota. Prescott (1995) found that increasing site available N with both inorganic and organic sources of N had no effect or negative effects on decomposition rates of hardwood and conifer litters. But while evidence that external N additions increase organic matter decomposition rates is inconclusive (Fog, 1988; Knorr et al., 2005), there is some evidence which suggest that litter quality may determine the response to added N (Carreiro et al., 2000; Sinsabaugh et al., 2002; Knorr et al., 2005), with low-lignin litter decay rates responding positively to N, and high-lignin litters responding negatively. Changes in species composition that result in substantial differences in litter quality could therefore affect the response of decomposition rates to increased N deposition, and have broad-scale implications for C storage (Waldrop et al., 2004).

The focus of this study was to examine the effects of microclimate, litter quality and N availability on decomposition of organic matter in forests where these factors vary greatly over small geographic distances. In order to test the effects of site differences in moisture and N availability on decomposition, and to test for the possible effects of simulated increases in N deposition, common substrates with contrasting carbon constituents were decomposed at multiple sites across the landscape. I hypothesized that readily decomposable carbon, cellulose, would be positively affected by N availability, while decomposition of a more recalcitrant carbon source, wood, may be affected differentially between mesic and xeric sites by N additions. Added N would probably negatively impact wood decay in the low-N, xeric sites, and stimulate or cause no change in the high-N, mesic sites.

A reciprocal leaf litter transplant study was also installed to test the effects of litter quality, microclimate and N availability in two forest stands with large differences in species composition, moisture, and N cycling rates. Sugar maple and a mixture of oak species were chosen for their contrasting litter quality, with sugar maple having low lignin content and oak having high lignin content (Ferrari, 1999; Lovett et al., 2004; Washburn and Arthur, 2003). I hypothesized that higher moisture and N availability would lead to greater mass loss rates of both litter types in the mesic site compared to the xeric site. I also hypothesized that the higher quality litter, sugar maple, would decomposer faster than the oak mixture.

Methods

Site description and sampling design

Mammoth Cave National Park is located in south-central Kentucky, USA. The park was established in 1941, and forests that were previously disturbed by settlement have been allowed to recover during the last 65 years. The park encompasses approximately 21,380 hectares of mostly forested land, and is bisected north and south by more than 30 km of the Green River. Mean annual temperature ranges from 7.1 to 20.4^{o}C, and mean annual precipitation is 132 cm (Kentucky Climate Center, 2006). Elevation ranges from approximately 135 to 275 m (US Geological Survey, 1997). Ridges capped by insoluble shale and sandstone, and valleys underlain by limestone characterize the geologic makeup across much of the park. Soils derived from shale and sandstone are base poor, Typic Hapludults, whereas soils developing over limestone have a higher base status and are mostly Typic and Ultic Hapludalfs (Mitchell et al., 1993). Twelve-year mean wet N deposition rates are >6 kg ha-^{1} yr-^{1} (B. Carson, NPS air resources management specialist, *unpublished data*, 2005); recent measurements estimate dry N deposition at 2 kg ha-1 yr-^{1} (EPA-CASTNET, 2006). The park is situated in the transitional zone between the oak-hickory forest region and the western mixed mesophytic region (Braun, 1950). The dissected nature of the landscape results in distinct vegetation patterns driven largely by moisture availability. Components of the oak-hickory assemblage occur on dry, south- and west-facing slopes at higher elevations, while mixed mesophytic species, such as sugar maple (*Acer saccharum*), yellow-poplar (*Liriodendron tulipifera*), and American beech (*Fagus grandifolia*) are found on moister north- and east-facing slopes at lower slope positions (Finney, 1962; Muller, 1982).

In spring 2003, 16 sites were established across MACA stratified by landscape position, producing two groups of sites (n = 8 each) with contrasting moisture availabilities. Low moisture (xeric) sites occurred on aspects ranging from 143 to 212°, and had a mean (SE) elevation of 226 (8.0) m. High moisture (mesic) site aspects ranged from 358 to 87°, and the mean (SE) elevation was 191 (8.8) m. At each site, one 11.3 m radius sampling plot was established to characterize all woody vegetation. A two m radius subplot was established in the center of each vegetation plot for soil and litterfall sampling.

Common substrate decomposition

Two commons substrates were amended with different concentrations of N and incubated at the MACA sites to further test the effects of moisture regime on decomposition rates and also the effects of exogenous N. First, 15 cm x 2 cm, tongue depressors (Solon Manufacturing Company, Skowhegan, ME 04976) made from white birch (*Betula papyrifera*) were used to determine wood decay rates in all 16 sites at MACA. In March 2005, approximately 900 tongue depressors were oven dried at 60°C to a constant mass. A unique code was inscribed on one end and the mass of each tongue depressor was recorded. Tongue depressors were divided into three groups of 300, put into 8 liter plastic buckets and assigned one of three N amendment treatments, 0.5 M NH_4NO_3 (high N) and 0.25 M NH_4NO_3 (low N), and dionized water (control). The tongue depressors were left to soak overnight, after which they were removed and patted dry with cloth towels. The high N and low N treatment groups were reweighed and were found to have gained 1.8 ($\pm$0.04) and 1.76 ($\pm$0.02) g H_2O, respectively. This represented approximately 0.05 g N gained by the high N tongue depressors, while the low N tongue depressors gained an average of 0.025 g of N. A subsample of untreated tongue depressors was found to have mean % C and N of 47.6 and 0.18%, respectively. Based on the weight of N added, the high and low N treatments resulted in approximately 13 and 27 times greater initial N, respectively.

Within two days of soaking, 48 tongue depressors, 18 from each of the three N treatments, were placed in each of the 16 sites. At each site, three tongue depressors from a single N treatment were strung together with nylon string and anchored to the forest floor with a steel pin. Three tongue depressors from each N treatment were placed together, and this grouping was replicated six times around the 2 m radius sampling plot of each of the 16 sites. A total of 864 tongue depressors were deployed (16 sites x 3 N treatments x 3 pickups x 6 replicates). The three pickups occurred at 4, 9 and 15 months, where one tongue depressor of each N treatment from each of the 6 groups was collected, returned to the laboratory, dried in the oven at 60°C, and reweighed to assess mass loss. Since tongue depressors were rigid and homogeneous, no time zero pickup was performed and initial mass loss was assumed to be zero.

A second common substrate, cellulose filter paper, was also deployed in May 2005 to assess the effects of moisture regime and N additions on decomposition. Whatman® no. 40, ashless, 7 cm diameter filter paper disks were weighed and enclosed in 10 cm x 10 cm fiberglass

mesh (1.5 mm x 1.5 mm) bags, labeled with metal tags containing a unique code. Cellulose samples were placed in only 12 sites, six mesic and six xeric. At each of the 12 sites, a 1 m x 2 m plot was installed, positioned five meters to the side of the 2 m radius sampling plot, along the same contour. Cellulose samples were arrayed in six rows of five and anchored to the forest floor using steel pins. One of three N amendment treatments were randomly assigned to each of the 1 m x 2 m plots, such that two plots in each moisture regime received a total of either 0, 1, or 2 g N m^{-2}, applied once a month as an aqueous solution of NH_4NO_3 (or deionized water only in the control) during the first three months of the experiment. Immediately after the first N application, five cellulose samples were collected and brought back to the laboratory. These samples were used to calculate initial moisture content, which was applied to all deployed samples. For this and all subsequent pickup dates, filter paper disks were oven dried at 60°C and weighed to assess mass loss. Six pickups were performed at 0, 0.5, 2, 4.5, 6.5 and 13 months after installation. A total of 360 bags were used (12 sites x 6 pickups x 5 replicates).

Reciprocal leaf litter transplant

Litterfall was collected during November 2003 using three 0.23m^2 plastic litterfall collectors at each of the 16 sites. The contents of each collector were removed, sealed in a plastic bag and returned to the lab, where it was sorted by species and oven dried to a constant mass at 60°C. Oven drying litterfall may have changed its chemical composition and physical structure, but I believe that the samples used in this experiment are valid for determining the relative rates of decomposition between sites with differing moisture availability and species composition. However, decomposition rates derived from this study should not be used as absolute measurements of leaf litterfall decay.

In July 2004, litterbags with dimensions of 20 X 20 cm were constructed with fiberglass mesh screen (mesh size = 1.5 mm x 2 mm). The edges were sealed using a hot glue gun and a metal tag with a unique code was placed in the corner of each bag. Sugar maple litterfall from all sites where it was present was combined into one sample and mixed thoroughly. Oak litterfall, including an approximately even mix of four species black oak (*Quercus velutina*), white oak (*Q. alba*), scarlet oak (*Q. coccinea*), and chestnut oak (*Q. prinus*), was pooled together. A known amount (approximately 10 g) of sugar maple litter was placed in one set of bags, while approximately 2.5 g of each the four oak species litter was placed in another set of

bags. All litterbags were then sealed with glue and taken to the field for incubation. Two sites, one mesic and one xeric, were selected to represent contrasting moisture availability and species composition (Table 3.1). Half of the litterbags of each litterfall type (referred to from here on as "sugar maple" and "oak mixture") were taken to each of the two sites. Sugar maple and oak mixture litterbags were placed on top of the forest floor litter layer in lines perpendicular to the slope on either side of the 2 m radius subplot, and anchored to the ground with steel pins. Five litterbags of each litter type from each site were removed immediately and taken back to the laboratory, dried at 60°C and weighed to determine initial moisture content. A total of 120 litterbags were used in this experiment (2 litter types x 2 sites x 6 pickup dates x 5 replicate bags). Collections were made at 0, 3, 6, 12 and 22. Once collected and returned to the laboratory, contents of the litterbags were oven dried at 60°C, and weighed to determine mass loss.

Statistical analyses

For the wood decay experiment, a nested factorial ANOVA was performed with moisture regime, site (nested in moisture regime), N level, and pickup date as main effects, and mass loss as the response variable. Moisture regime, N level and site were considered fixed effects, and time a random effect. In the cellulose decomposition experiment, since N treatments were not replicated within each site, two separate analyses were run to test the effects of N level and site separately. In the reciprocal litter transplant experiment, a factorial ANOVA was performed, with moisture regime, litter type, and pickup date as main effects, and percent mass remaining as the response variable. All analyses were performed used PROC MIXED in SAS software, Version 9.1 for Windows (SAS, 2004), and statistical significance was evaluated at $\alpha = 0.05$. Linear decay rate constants (k), using linear regression, were developed for the two common substrates in each site, since mass loss over time for these substrates followed a linear pattern and not an exponential one. To examine relationships between common substrate decay rates and site characteristics, Pearson's correlation coeffients were generated using PROC CORR in SAS.

Results

Vegetation and litterfall characteristics

Sugar maple was found in all but one mesic site, and typically made up one third of the total litterfall in these sites. In contrast, sugar maple was found in only 3 xeric sites and always made up less than 10% of the total litterfall. No single oak species was dominant across all xeric sites, but the four species used in this experiment, black oak, white oak, scarlet oak, and chestnut oak, made up the majority of oak litterfall, in these sites. Northern red oak (*Q. rubra*) was usually the dominant oak on mesic sites, but white oak also occurred in these sites. The initial characteristics of both moisture regimes are listed in Table 3.1, along with means from the two sites used in the litter transplant experiment.

Common substrate decomposition

Tongue depressors decomposed significantly ($p<0.0001$) faster in mesic sites compared to xeric. The effect of N amendment level was not significant ($p=0.29$), however. While the interaction between moisture regime and N level was not significant ($p=0.93$), mass loss rates for the high N treatment tended to be greater in xeric sites on all three pickup dates; in mesic sites, a tendency towards higher decomposition for the high N treatment was apparent only for the last pickup in the mesic sites (Figure 3.2).

Cellulose filter disks also decomposed significantly ($p<0.0001$) faster in mesic sites compared to xeric (Figure 3.2). The effect of N amendment level was significant ($p=0.014$), due to a higher mass loss rate in the xeric control treatment, apparent toward the end of the study. The interaction between moisture regime and N level was significant ($p<0.0001$), again due mostly to greater mass loss rate in the control xeric treatment. N additions may have inhibited cellulose decomposition in the xeric sites, while leaving decomposition unaffected in mesic sites, where inherent N availability is high (Table 3.1). However, since N treatments were not replicated within each site, the interaction between moisture regime and N treatment may be due to inherent differences among sites, which could have influenced decomposition.

Decay rates for wood and cellulose filter disks were compared to microclimate, litterfall and N availability among sites to determine if gradients in these variables could help to explain the trends observed in mass loss of common substrates. Wood decomposition was positively correlated with mean soil moisture content, while both wood and cellulose decomposition were

negatively correlated with mean soil temperature (Table 3.2). Wood decomposition was negatively correlated with forest floor mass and oak litterfall mass, and positively correlated with sugar maple litterfall mass. Cellulose decomposition was unrelated to forest floor and litterfall masses, and neither of the two substrates were correlated with total inorganic N or net N mineralization rates.

Reciprocal litter transplant

Interesting temporal patterns in mass loss of leaf litter emerged in the reciprocal transplant experiment. For the first year of decomposition, mass loss rates were greatest for sugar maple in the mesic site, consistent with our hypotheses (Figure 3.3). However, in the xeric site mass loss for both litter types was the same, and the oak mixture in the mesic site decomposed the slowest, suggesting that factors other than moisture and litter quality were important in determining early decomposition rates. The interaction between litter type and time was significant (p<0.0001), and after nearly two years, sugar maple litter decomposition in both sites slowed down relative to initial mass loss rates, whereas oak litter decomposition rates remained fairly constant. By the last pickup, oak litter in the xeric site had the highest mass loss rates, while the other three litter-site combinations had mass loss rates similar to each other, and neither the effect of litter type, nor the effect of moisture regime was significant (p>0.35 for both). On the last pickup, large amounts of insect frass were present in most of the oak mixture bags from the xeric site, but were not apparent in the other litter-moisture regime combinations. Higher invertebrate activity probably led to higher mass loss rates, suggesting that invertebrates may play an important role in the decomposition process at this site.

Discussion

Moisture availability has been shown to positively affect decomposition rates on both regional and local scales (Berg et al., 1993; Austin and Vitousek, 2000; Sariyildiz et al., 2005; Prescott, 2006). Although in general and across broad geographic ranges, warmer temperatures lead to higher decomposition rates (Berg et al., 1993; Prescott, 2006), over small geographic ranges in warm climate regions, higher temperatures can retard decomposition due to a drying-out effect on surface organic matter (Prescott et al., 2000). From the common substrate decomposition experiments it would appear that microclimatic differences between mesic and

xeric sites were important contributors to the observed patterns in mass loss rates, where less solar radiation and consequently moister conditions in the mesic sites led to greater mass loss rates. Austin and Vitousek (2000) found significant positive relationships between moisture and decomposition rates of wooden dowels and tree litter across a large precipitation gradient (500 to 5500 mm) in Hawaii. In this study, wood decomposition was positively correlated with mean soil moisture content, while both wood and cellulose decomposition were negatively correlated with soil temperature (Table 3.2). Forest floor mass and the amount of annual oak litter mass were both negatively correlated with wood decay rates. Since oak litter tends to curl upon drying, the tongue depressors were probably suspended higher on the forest floor of oak-dominated xeric sites, which could have promoted a drying effect. Conversely, forest floors in mesic sites are thin, especially in sites where sugar maple is a dominant component of the litterfall, and tongue depressors in these sites were in closer contact with mineral soil, probably leading to higher moisture content.

The reciprocal litter transplant experiment was used in conjunction with the common substrates to determine the effects of site differences in microclimate and N availability on native litter types. In other reciprocal litter transplant studies examining the effects of microclimate, higher decomposition rates are almost always found under moister conditions. For instance, in a study examining decomposition of leaf litter on north- and south-facing slopes in Northeast Turkey, Sariyildiz et al. (2005) found higher leaf litter mass loss rates on north-facing slopes compared to south-facing slopes. In West Virginia, Mudrick et al. (1994) also found higher leaf litter decomposition on the north-facing slope, which they attributed partly to higher moisture content of the litter. However, the findings of the reciprocal litter transplant experiment in the current study complicate the microclimate hypothesis. After one year of incubation, sugar maple litter in the mesic site had decomposed the fastest, but oak litter in the mesic site decomposed more slowly than either oak or sugar maple litter in the xeric site. And after nearly two years, mass loss of both litter types in the xeric site was the same or greater than litters in the mesic site, with the highest rate of mass loss was found for oak litter in the xeric site. These results suggest that moisture availability alone does not control leaf litter decomposition, at least not after nearly two years of incubation.

Litter quality could also be a factor affecting decomposition rates, and studies reporting on litter transplant studies show that higher quality litter (i.e. lower lignin, higher N content)

usually decomposes faster than lower quality litters, regardless of moisture availability (Sariyildiz et al., 2005; Mudrick et al., 1994). Elliott et al. (1993) tested the effects of forest type and litter quality on decomposition and found that the highest quality litter always decomposed faster than litters of lower quality regardless of the forest type where it was incubated. Surprisingly, though, in the current study after nearly two years of incubation, mass loss of the low-quality litter, oak, was greatest in the low-moisture, xeric site, while mass loss was the same for the other three litter/site combinations. Sugar maple litter incubated in the mesic site did lose more mass (although not statistically significant) than the oak litter in the same site, suggesting that litter incubated in its site of origin decomposes more rapidly than when it is transplanted, a trend also reported by McClaugherty et al. (1985). Perhaps there was a lower capacity for breakdown of recalcitrant organic matter in the mesic site, since oak litter in this site decomposed the slowest after one year of incubation. Greater mass loss in the oak litter samples incubated in the xeric site was enhanced by the presence of detrivorous insects, as evidenced by frass found inside some, but not all of these litterbags. This suggests that in later stages of decomposition, mass loss rates of litters in these systems may be determined less by litter quality or microclimate, and more by the activity of the invertebrate decomposer community.

Another factor considered in this study to potentially affect decomposition rates was N availability. Increased N availability has been shown to have inconsistent effects on rates of organic matter decomposition, with studies showing positive, neutral, and negative effects (Fog, 1988; Hobbie, 2005; Knorr et al., 2005). These inconsistencies have been related to the C and N chemistry of the organic matter. For instance, leaf litters of low quality typically exhibit inhibition of mass loss, while higher quality litters (i.e. less lignin, more N, cellulose and hemicellulose) have exhibited a positive response in mass loss to N additions (Carreiro et al., 2000; Sinsabaugh et al., 2002). By using common substrates with strong differences in carbon chemistry, I hypothesized that decomposition rates of the substrates would be differentially affected by N additions. Added N was expected to slow wood decay in xeric sites, while possibly enhancing it in mesic sites. However, N amendments did not significantly affect wood decay in either mesic or xeric sites, and cellulose decay was unaffected in the mesic sites. Hobbie (2005) found that litters placed in low N sites exhibited positive responses in decomposition to N additions. Nitrogen additions did appear to affect cellulose in low N, xeric sites, but in an opposite manner to most studies in which increased N availability has been shown

44

to stimulate cellulose decomposition (Fog, 1988), through increased production of cellulose enzymes (Carreiro et al., 2000; Saiya-Cork et al., 2002). It is possible that N amendments were not sufficiently high enough to cause an effect, however, decomposition of the common substrates was not significantly correlated with mineral soil total inorganic N, or net N mineralization rates (wood decomposition marginally significant; $p=0.054$), suggesting that N availability did not influence decomposition rates.

Common substrate decomposition was strongly related to microclimate differences between mesic and xeric sites. Nitrogen amendment treatments did not affect decomposition, except for cellulose in xeric sites where it negatively impacted mass loss rates, which was an outcome opposite to that proposed by decomposition theory. Inter-site differences in the decomposer communities or some other factor not considered here were probably responsible for the observed trends. The N amendment treatments used in this study may have been too low to impact decomposition rates, so it is unclear how increases in N deposition over long periods of time will affect decomposition in this system.

The reciprocal transplant experiment somewhat refuted the simple hypothesis of microclimate control on decomposition, and suggests that differences in decomposer communities may be responsible for the trends in mass loss, at least for the first year of decomposition. If species composition is shifted over time away from oak dominance and towards maple dominance, it could cause changes in ecosystem function, such that these forests could have a lowered capacity for recalcitrant organic matter decomposition. After nearly two years of incubation, mass loss rates were similar in all site/litter combinations except the samples that were heavily impacted by invertebrates. Therefore the late stages of decomposition may be independent of litter quality and microclimate, and more influenced by the invertebrate decomposer community.

Table 3.1. Comparison of characteristics between the single mesic and xeric sites chosen for the reciprocal litter transplant experiment, and mean values for characteristics for all mesic and xeric sites (n = 8 for each moisture regime). Asterisks between columns indicate significant differences between mesic and xeric sites, where *=p<0.05, **=p<0.001, and ***=p<0.0001.

	Rec. transplant mesic	Rec. transplant xeric	All mesic		All xeric
Basal area (m^2 ha^{-1})	30.2	33.0	30.7 (4.9)		30.3 (1.9)
% sugar maple	37.9	0.0	27.6 (7.7)	***	1.2 (1.0)
% oak spp	33.1	51.2	24.2 (8.1)	***	60.5 (7.4)
Litterfall mass ($g\ m^{-2}$)	440	403	386 (16.5)		374 (17.7)
% sugar maple	51.3	0.0	30.6 (6.5)	*	2.3 (1.2)
% oak spp	15.0	75.0	33.4 (5.9)	*	77.6 (2.6)
Forest floor mass ($g\ m^{-2}$)	66.9	996	266 (86.3)	*	718 (117.4)
Total C ($mg\ g^{-1}$)	156	262	197 (16.4)	**	272 (9.3)
Total N ($mg\ g^{-1}$)	7.3	11.6	9.3 (0.9)	*	12.2 (0.4)
Mineral soil (0-10cm)					
Moisture content (%)	38.4	24.1	30.5 (1.2)	***	22.4 (0.9)
Temperature (^{o}C)	12.5	14.2	13.2 (0.2)	**	14.1 (0.1)
Total C ($mg\ g^{-1}$)	30.3	19.1	23.7 (1.4)		23.0 (2.4)
Total N ($mg\ g^{-1}$)	2.4	0.9	1.8 (0.1)	**	1.2 (0.1)
Annual N mineralization					
($mgN\ kgsoil^{-1}\ day^{-1}$)	458	79.0	275 (62.2)	***	128 (41.3)
Annual nitrification					
($mgN\ kgsoil^{-1}\ day^{-1}$)	416	2.3	240 (15.4)	***	22.3 (0.7)

Table 3.2. Pearson's correlation coefficients (r) for first order decay rate constants (k) for common substrates in control treatments and various site characteristics. TIN=total inorganic nitrogen.

Common substrate**	Soil moisture (%)	Soil temp (^{o}C)	Forest floor mass (g m^{-2})	Oak litter mass (g m^{-2})	Sugar maple litter mass (g m^{-2})	Soil TIN (ug g^{-1})	Annual N mineralization (ug g^{-1} day^{-1})
Wood	**0.577**	**-0.650**	**-0.609**	**-0.767**	**0.599**	0.406	0.473
p value	*0.02*	*0.01*	*0.01*	*0.00*	*0.01*	*0.12*	*0.06*
Cellulose	0.368	**-0.611**	-0.358	-0.300	0.555	0.327	0.483
p value	*0.24*	*0.03*	*0.25*	*0.34*	*0.06*	*0.30*	*0.11*

*See Chapter 2 for data collection methods
**For wood decay, n=16 sites; for cellulose decay, n=12 sites.

Figure 3.1. Tongue depressor mass loss over time for the three N amendment treatments in mesic and xeric sites. Each bar represents the mean of eight sites. Error bars are ± one standard error of the mean.
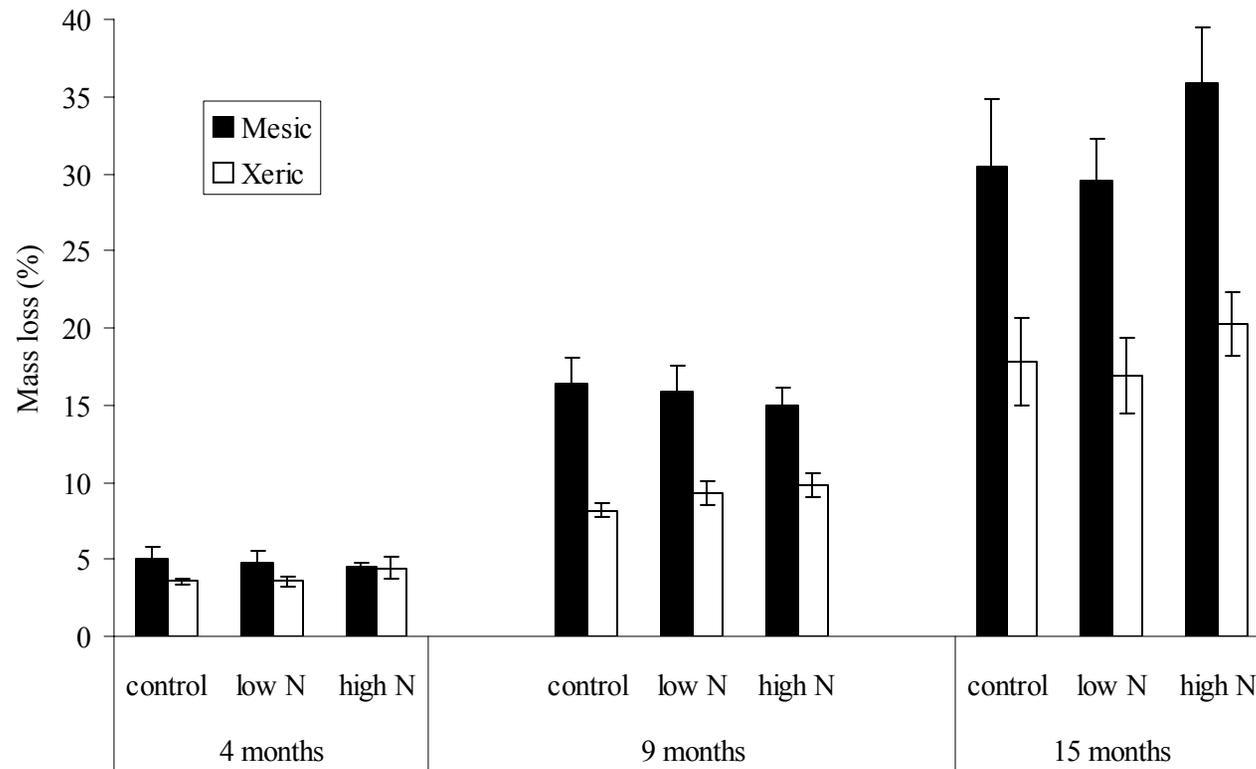
Figure 3.2. Cellulose filter paper mass loss over time for the three N amendment treatments in mesic (a) and xeric (b) sites. Each point represents the mean of two sites, and error bars are ± one standard error of the mean.

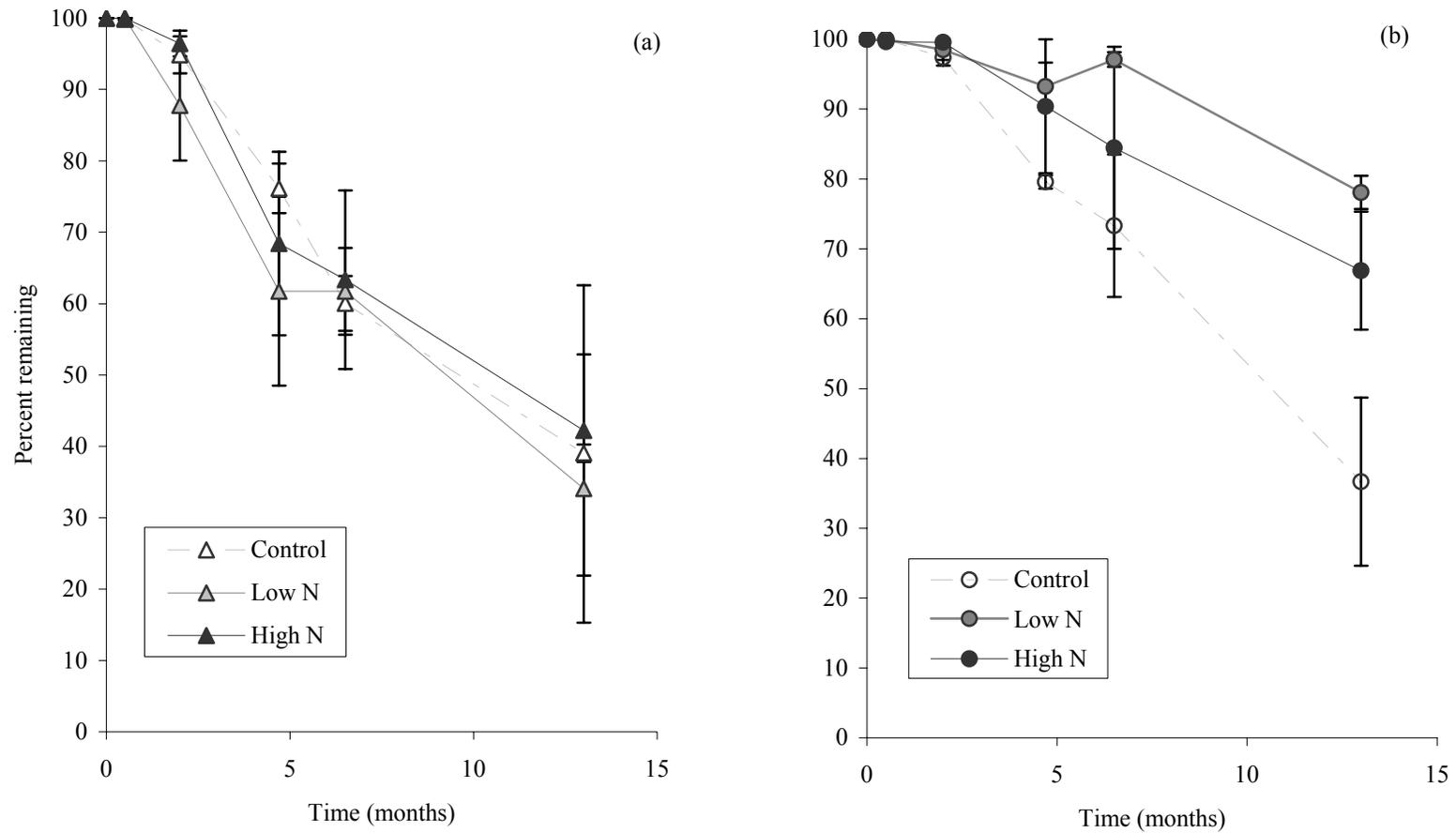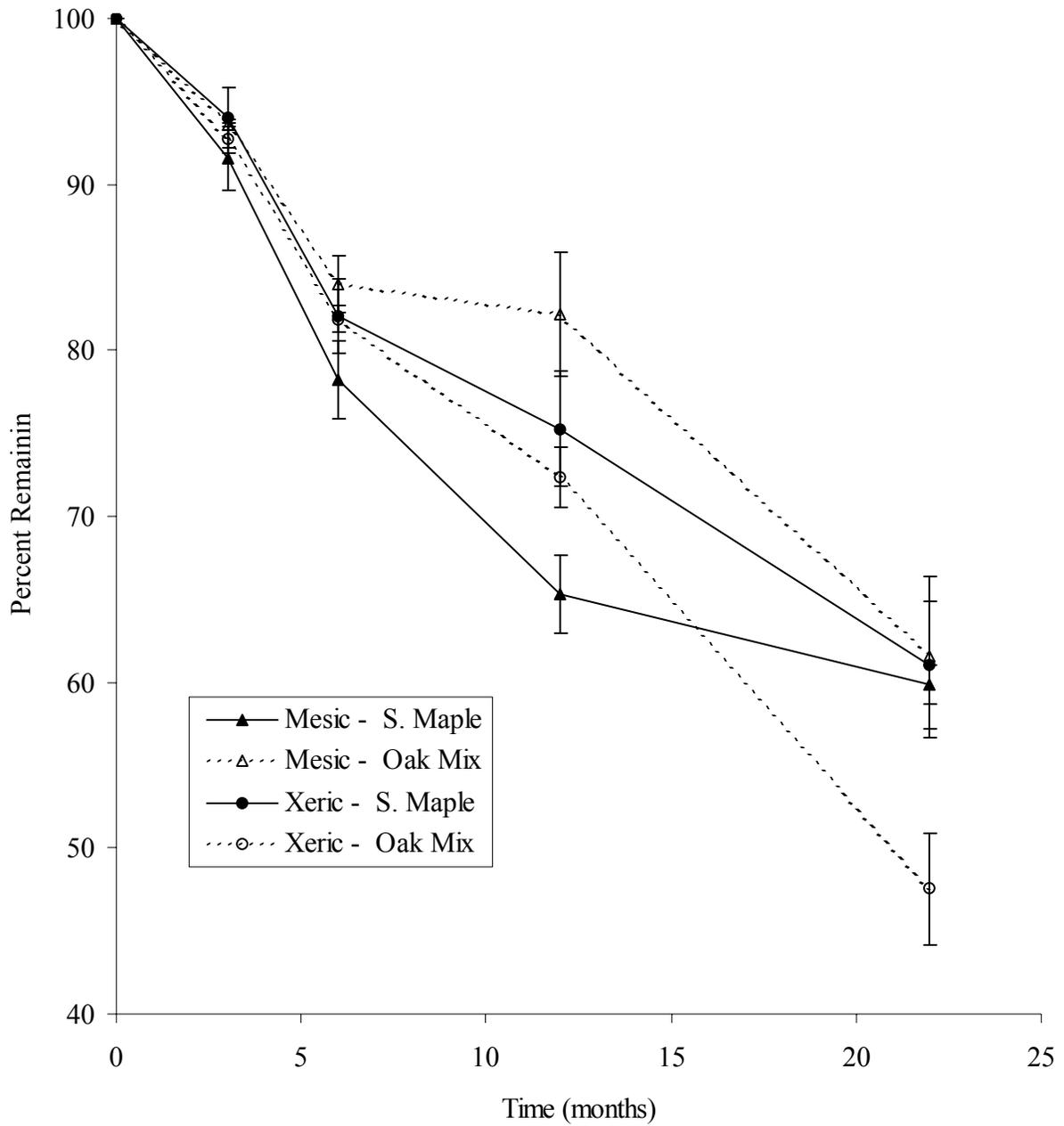Figure 3.3. Mass loss over time for reciprocal litter transplant experiment for the two litter types, sugar maple and oak mixture, in the two sites, mesic and xeric. Each point is the mean of five litterbags, and error bars are ± one standard error of the mean.

CHAPTER FOUR

Concluding thoughts and future directions

Over the last half century, evidence has amassed in the ecological literature that tree species can strongly influence C and N dynamics. The present work appears to support this idea, since stands dominated by sugar maple had thin forest floor layers, lower mineral soil C:N ratios and higher net N mineralization and nitrification rates, compared to oak stands, which seemed to accumulate higher amounts of C on the forest floor, had higher mineral soil C:N ratios and had lower net N mineralization and nitrification rates. Often in these types of investigations, evidence of tree species influence is obtained by selecting single-species plots, or by locating individual trees and measuring soil properties beneath the canopies of the target species. Interestingly, in this study evidence of species influence on N cycling was found in plots with mixed species composition. By measuring the contribution of oak and sugar maple leaf litter in these plots, I was able to predict with some accuracy the rate of net N mineralization and nitrification, as well as nitrate concentrations in soil solutions.

But perhaps more interesting is the fact that soil substrate, or parent material, did not influence N cycling significantly, despite the contrasting characteristics of the two dominant parent material types present at MACA, sandstone/shale and limestone. Soil texture was unrelated to N mineralization or nitrification rates, as were base cation concentrations. Soil pH was significantly correlated with nitrification rates, however pH was probably influenced by the tree species present, since tree species are known to affect soil pH (Finzi et al., 1998b), often through leaf litter deposits.

It may have been the case that parent material influenced N cycling indirectly, by selecting for tree species. Van Breemen et al. (1997) found that the distribution of sugar maple and northern red oak in Connecticut was strongly influenced by soil nutrients, particularly calcium. However, despite the potential for spatial variability in parent material and thus soil characteristics across the park, base cation status among the sites used in this study varied minimally, with the exception of two xeric sites that occurred on or near limestone outcrops. The range of Ca availability across mesic and xeric sites was similar and perhaps sufficiently high so that sugar maple establishment and growth were not limited. Thus, soil nutrients were most likely not important in determining the distribution of sugar maple and oak species in the

present study. Zak et al. (1989) found that N cycling rates varied significantly across a sugar maple to oak species gradient in northwestern Lower Michigan, but differences in soil texture probably drove species composition, with oak occurring on more sandy sites, and sugar maple occurring on sites with higher clay content. At MACA, xeric sites tended to have a higher sand fraction (mean= 57 %, compared to 46 % in mesic sites), which could have led to lower soil moisture, but soil texture was not correlated with mean soil moisture (% sand, $R^2 = 0.24$, p > 0.05; % silt + clay, $R^2 = 0.24$, p > 0.05). Of all the variables measured in these sites, perhaps the most important factor in determining tree species distribution was landscape position, where differences in solar radiation significantly influenced soil moisture availability. Soil moisture in turn controlled the distribution of tree species across the landscape, with xeric sites being dominated by oak species and mesic sites having a large component of sugar maple and significantly less influence from oak species.

This relationship becomes important when considering how to manage for variability in N cycling and N leaching potentials on broader scales. For instance, the relative ease of modeling elevation and aspect on a landscape-scale could help to predict spatial patterns in soil moisture availability, and thus N cycling rates. In addition, technology for remote sensing of forest canopy characteristics has dramatically improved in recent years, allowing for potential opportunities to model C and N dynamics across broad scales (Ollinger et al., 2002). The highly significant relationships found between sugar maple and nitrification and NO_3 in soil solutions could be easily applied in this manner to model the effects of tree species on nutrient cycling across the landscape.

Future work should include a combination of remote sensing of tree species distribution and stream water sampling to determine if the influence of tree species composition on N cycling (and specifically nitrate losses) can be detected on a watershed scale. By selecting watersheds that differ in species composition, i.e. more sugar maple verse more oak, and monitoring N concentrations in stream water over time, it can be determined if the leaching potentials highlighted by this study are contributing to N losses from these systems. In addition, throughfall collectors should be installed within these watersheds to monitor fluctuations in N inputs. If some areas of the park dominated by sugar maple are N saturated, then increases in throughfall inputs should be reflected in the stream water chemistry. Conversely, if oak stands are able to retain excess N, then increases in inputs should not be correspond to increases in

outputs. Since oak stands in this study showed elevated levels of DON in soil solutions relative to those from sugar maples stands, this may represent a significant mechanism of N losses from these stands, and DON levels in stream water should therefore be monitored as well.

Long-term changes in species composition may also have an influence on N cycling dynamics at MACA. Decreased oak regeneration and proliferation of shade tolerant species such as maples has been documented in this region, and vegetation data collected in this study seems to support this phenomenon. The positive, linear relationships between the amount of sugar maple litterfall in a stand and net nitrification rates and nitrate in soil solution suggest that if the abundance of sugar maple increases, nitrate leaching losses could increase. Park managers are currently engaged in the use of prescribed fire as a means of possibly promoting oak regeneration. Monitoring the effects of prescribed fire on tree survival and recruitment will help to evaluate the effectiveness of fire as a tool to promote oak regeneration, and may help to predict the future composition of the forests within the park. Knowing this will help park managers to effectively plan for increased N deposition.

Effects of nitrogen additions and stand composition on potential nitrogen mineralization and nitrification rates

Notes:

1) In October 2005, two sites were selected with stand compositions of approximately 0, 50 and 100% sugar maple, plus 100% red maple, for a total of eight sites. Two existing xeric sites were used to represent the 0% sugar maple stands, and two existing mesic sites were used as the 50% stands. The 100% stands were new locations just prior to the experiment.

2) Six mineral soil samples (0-10 cm depth) were collected in each of the eight sites with a tulip bulb corer. Soil samples within sites were composited in pairs to yield three samples per site (total of 24 samples).

3) Soil samples were sieved and homogenized, and two 10g subsamples from each of the 24 samples were extracted with 50 mL of 1 M KCl, and then analyzed colorimetrically for initial NO_3 and NH_4 concentrations. Additional 10g subsamples were brought to 55% field capacity with distilled water, and randomly assigned one of three N additions treatments, 0.62 mM KNO_3, 0.14 mM $(NH_4)_2SO_4$, or distilled water. A total of six replicate subsamples from each site received 1mL of the treatment solutions.

4) Treated soil subsamples were placed in an incubation chamber at approximately 20°C for 28 days. After the incubation period, soils were extracted with 50 mL of 1 M KCl, and analyzed for NO_3 and NH_4 concentrations. The N addition solutions were also analyzed for NO_3 or NH_4 concentrations.

5) The amount of N added with the treatments was summed with the initial concentrations of NO_3 and NH_4. These values were subtracted from the final concentrations, and divided by the length of the incubation period to yield production rates of NO_3 plus NH_4 (mineralization) and NO_3 only (nitrification).

Figure 1. Potential net N mineralization rates of soils from stands with varying tree species composition. Soils from each stand were treated with distilled water (control), NO_3, or NH_4, and incubated for four weeks. Each bar represents the mean response of two stands to N treatments. Error bars are ± one standard error of the mean. SM = sugar maple, and RM = red maple.
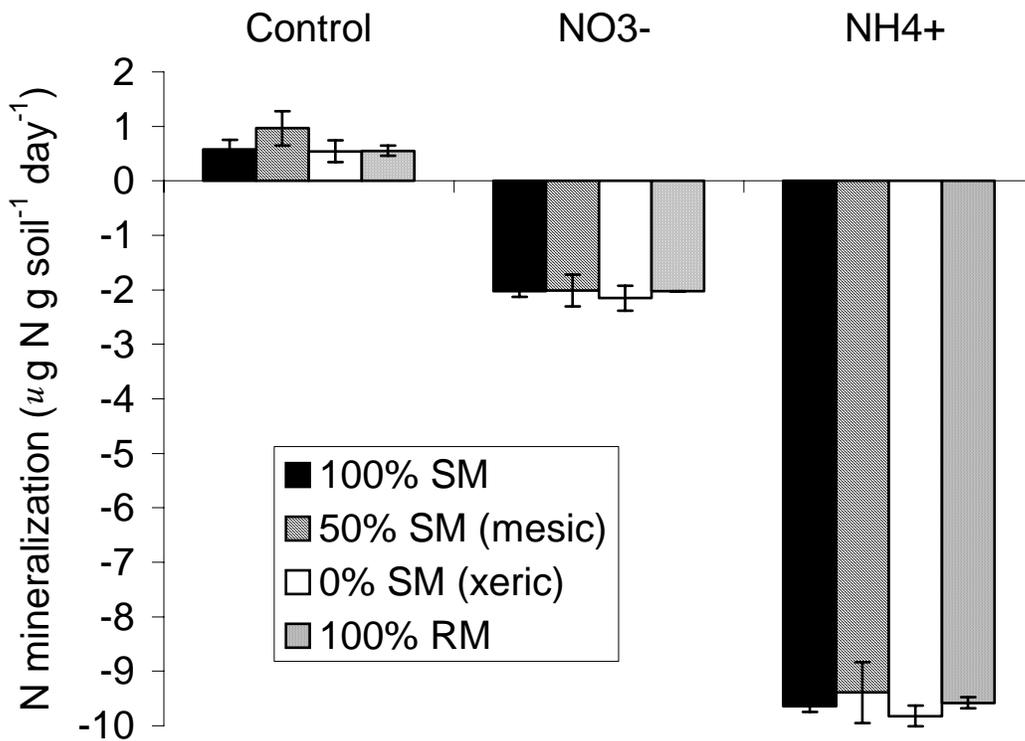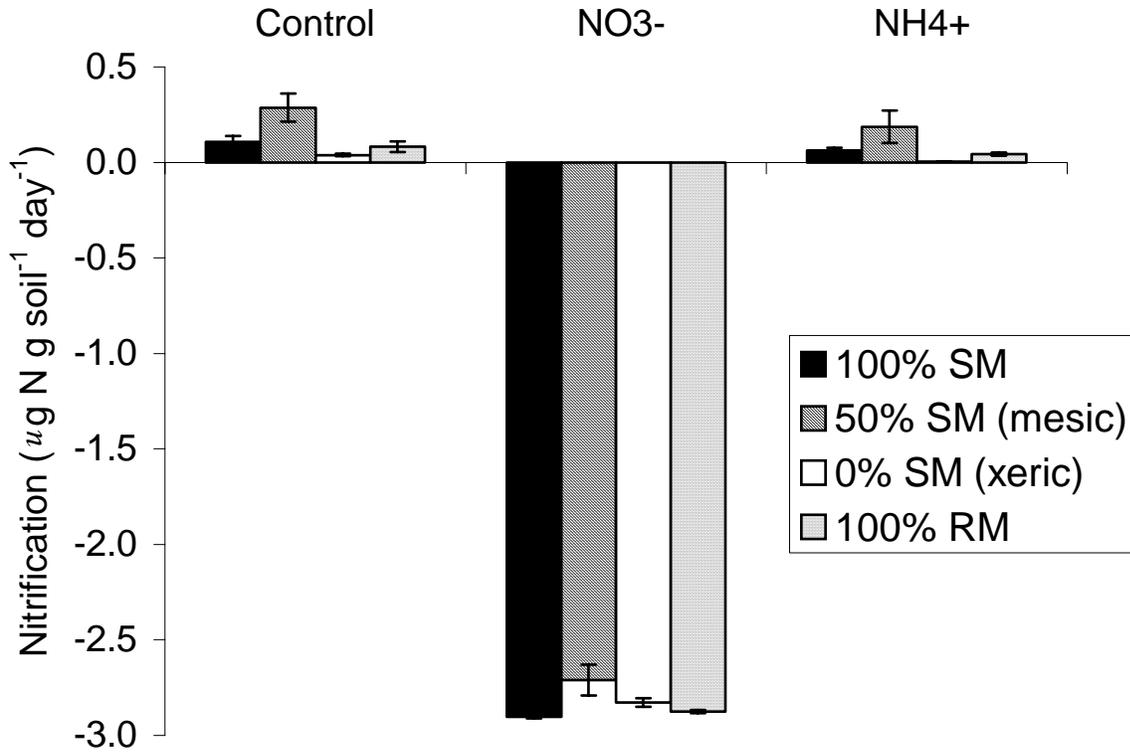
Figure 2. Potential net nitrification rates of soils from stands with varying tree species composition. Soils from each stand were treated with distilled water (control), NO_3, or NH_4, and incubated for four weeks. Each bar represents the mean response of two stands to N treatments. Error bars are ± one standard error of the mean. SM = sugar maple, and RM = red maple.

APPENDIX 2

Effects of in situ N additions on microbial biomass in mesic and xeric sites

Notes:

1) Microbial biomass was assessed on soils from the twelve 1 m x 2 m cellulose decomposition plots. In May 2005, two plots in each moisture regime were treated with aqueous solutions of NH_4NO_3, at dosages of either 0, 1, or 2 g N m^{-2}, representing control, low N, and high N treatments, respectively. Five mineral soil samples (0-10cm depth) were collected three times during the summer of 2005, one month after each of the three N application dates.

2) Microbial biomass was assessed using the chloroform fumigation-incubation technique (Horwath and Paul, 1994). Each soil sample (n = 60 for each of three soil sampling dates) was passed through a 2 mm sieve and homogenized. Two 50 g subsamples from each sample were weighed out into glass vials, placed into glass desiccators, along with 50 mL of ethanol-free chloroform, and fumigated in the dark for 18-24 hr. Vials were then removed, inoculated with fresh soil from the original sample and mixed thoroughly.

3) Each vial was placed in 1 L canning jars, along with two scintillation vials filled with 1 mL of 2 M NaOH. Jars were sealed and placed in an incubation chamber at approximately 20°C for 10 days. The amount of C mineralized over the incubation period was calculated by using the amount of 0.1 M HCl needed to reach the phenolphthalein endpoint. The sum of the two scintillation vials represented the total amount of mineralized C for each sample.

4) I did not subtract a control, and so microbial biomass C estimates reported here are likely higher than those reported elsewhere for similar systems. However, I feel this is a more accurate method, since estimating microbial biomass without subtraction of a control has been shown to be better correlated with other soil C parameters (Franluebbers et al., 1999a, 199b) and is a more stable measure across time, as C mineralization can be highly

sensitive to fluxes of labile C that may not affect microbial population sizes (Franluebbers et al., 199b).

Figure 1. Microbial biomass C estimates from mesic and xeric sites under control, low N, and high N amendment treatments during the summer of 2005. Each bar represents the mean of two sites, and error bars are ± one standard error of the mean.
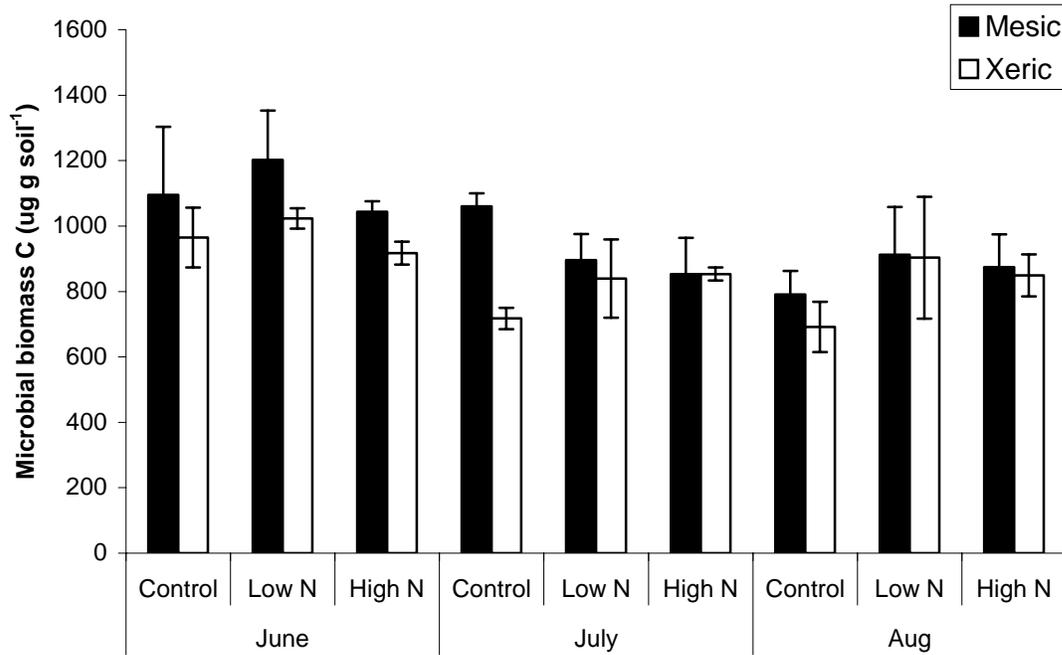
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