

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

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Abstract: Understanding how natural factors interact across the landscape to influence nitrogen (N) cycling is an important focus in temperate forests because of the great inherent variability in these forests. Site-specific attributes, including local topography, soils, and vegetation, can exert important controls on N processes and retention. Seasonal monitoring of N cycling dynamics was carried out for 2 years in deciduous forest stands that differed in soil moisture status and geologic substrate, and thus, in tree species composition to determine the effects of tree species composition, mediated by moisture and soil chemistry, on N cycling. Geologic substrate influenced soil and soil leachate chemistry but did not appear to affect N cycling in the upper 10 cm. Moisture status was strongly correlated with tree species composition, which was significantly related to N cycling parameters. Sugar maple was associated with high net nitrification rates and soil solution NO_3 concentrations, whereas in oak stands nitrification was low and soil solution NO_3 was at or near detection limits. Tree species composition in the understory suggests that sugar maple may be increasing in mesic sites and that oak regeneration in all sites is very limited, and a shift in species composition could result in changes to N retention and export.

Résumé : Il est important de comprendre comment les facteurs naturels interagissent dans le paysage et influencent le recyclage de l'azote (N) dans les forêts tempérées étant donné la grande variabilité inhérente à ces forêts. Les attributs propres à chaque station, incluant la topographie, les sols et la végétation, peuvent exercer un contrôle significatif sur les processus et la rétention de N. Un suivi saisonnier de la dynamique du recyclage de N a été effectué pendant 2 ans dans des peuplements de forêt feuillue avec une humidité du sol et un substrat géologique différents et, par conséquent, différentes compositions en espèces d'arbres. L'objectif consistait à déterminer les effets de la composition en espèces d'arbres sur le recyclage de N, par l'intermédiaire de l'humidité et des caractéristiques chimiques du sol. Le substrat géologique influençait les caractéristiques chimiques du sol et du lessivat dans le sol mais ne semblait pas affecter le recyclage de N dans les 10 premiers centimètres. Le statut hydrique était étroitement corrélé avec la composition en espèces d'arbres, laquelle était significativement reliée aux paramètres du recyclage de N. L'érable à sucre était associée à des taux nets de nitrification et des concentrations de NO_3 dans la solution du sol élevés alors que la nitrification était faible et que la quantité de NO_3 dans la solution du sol était au seuil de détection ou le dépassait à peine dans les peuplements de chênes. La composition en espèces d'arbres dans le sous-bois indique que la proportion d'érable à sucre est probablement en train d'augmenter dans les stations mésiques, que la régénération de chêne est très faible dans toutes les stations et qu'un changement dans la composition en espèces pourrait entraîner des changements dans la rétention et l'exportation de N.

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Introduction

Climate, topography, parent material, and vegetation singly and interactively influence the biogeochemical processes that regulate plant nutrient uptake, turnover, and release at

the local scale. For instance, local topography generates spatial patterns of both forest species composition and soil nitrogen (N) dynamics by creating temperature and moisture gradients (Morris and Boerner 1998; Gilliam et al. 2001). Underlying geology determines the mineralogy and influences the texture and chemistry of soils formed at a given site. Within a forest community, individual tree species can have distinct influences on soil properties and N cycling (Zak et al. 1989; Boettcher and Kalisz 1990), perhaps through differences in the quality of organic matter inputs, including litter, root exudates, and fine root turnover (Scott and Binkley 1997; Lovett et al. 2004). The influence of individual species is also known to vary among distinct substrate types, possibly because of the textural controls on organic matter stabilization (Rhoades et al. 2001; Rhoades 2007). Soil physical and chemical properties influence microbially mediated N turnover by regulating soil temperature, moisture, and acidity (Wagner and Wolf 1999) and by protect-

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ing organic matter in stable soil aggregates (Denef et al. 2004). Understanding how site-specific moisture, geologic substrate, and species composition influence N cycling dynamics across the landscape adds to our knowledge of global biogeochemical cycles and can help predict ecosystem response to changes in species composition or atmospheric N deposition.

The objectives of this research were to (1) characterize the N cycling patterns among forest stands that differed in soil moisture status and geologic substrate, and thus tree species composition and (2) determine the major factors influencing N cycling in these forests. We hypothesized that higher moisture status would lead to higher rates of N processing either directly or by driving differences in species composition. Furthermore, we hypothesized that contrasting geologic substrates would create chemical and physical differences that would influence both soil N cycling and buffering capacity.

Methods

Study area

Mammoth Cave National Park (MCNP), located in south-central Kentucky, USA, afforded the opportunity to evaluate the influence of both landscape position and geologic substrate on forest N cycling. The park was established in 1941 on approximately 21 380 ha, 40% of which was characterized as recovering from logging and agriculture. At present, the majority of the park is forested, composed of oak hickory and western mixed mesophytic forests (Braun 1950). Elevation varies by only 140 m (range: 135–275 m; US Geological Survey 1997), but the region's dissected topography drives vegetation patterns by creating substantial changes in microclimate. For example, mesophytic species like sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.), and yellow-poplar (*Liriodendron tulipifera* L.) occupy cooler, wetter north- and east-facing aspects and lower slope positions, while oak (*Quercus* spp.) and hickory (*Carya* spp.) are found on drier, south- and west-facing slopes and on ridge tops (Muller and McComb 1986; McEwan et al. 2005). In addition, the depositional environment created stratified layers of contrasting geologic substrates composed of limestone and sandstone and (or) shale. The soils derived from limestone substrates of Mississippian age are base cation-rich Alfisols, whereas sandstone and shale substrates of Pennsylvanian age have produced base cation-poor Ultisols. Moist, calcareous soils tend to support a greater abundance of sugar maple, while on drier sites, geology mostly affects the species of oak present.

The long-term mean wet N deposition rates are 6.2 kg·ha⁻¹·year⁻¹ (B. Carson, NPS air resources management specialist, unpublished data) and the dry deposition rates measured between 2003 and 2006 represent around 2 kg·ha⁻¹·year⁻¹ of additional N (EPA-CASTNET 2006). The mean annual temperature ranges from 7.1 to 20.4 °C, and mean annual precipitation is 132 cm (data record 1971–2000; Kentucky Climate Center 2006).

Site selection

Sites were selected using a habitat map that delineates MCNP plant communities based on geologic substrate and

landscape position (Olson et al. 2000). Sites were divided among areas underlain by calcareous (i.e., limestone) and noncalcareous (i.e., sandstone and (or) shale) substrates. We expected that these substrates would provide distinct chemical and physical soil conditions that would contribute to differences in site N cycling and the potential to buffer atmospheric inputs. Landscape categories combined slope and aspect factors and were operationally defined as mesic (high soil moisture) and xeric (low soil moisture). A 2 × 2 factorial treatment structure was used, with geology and landscape position as the main factors, and the treatment combinations being mesic–calcareous ($n = 4$), mesic–acidic ($n = 4$), xeric–calcareous ($n = 3$), and xeric–acidic ($n = 5$). All sites were located on moderate slopes (8° to 25°). Xeric sites were situated on slightly higher slope positions (mean elevation = 226 m), with aspects ranging from 143° to 277°, than mesic sites with lower elevations (mean = 191 m) and more northerly aspects (range: 358° to 87°).

Overstory and midstory tree species composition

In summer 2004, woody stems ≥ 2 cm in diameter at breast height (DBH; 1.4 m) were identified by species and measured for diameter in a circular 400 m² area at each site. Stems with 2–10 cm DBH were classified as “mid-story”, and stems with ≥ 10 cm DBH were classified as “overstory”. Slope, elevation, and aspect at each site were recorded.

Soil chemical and physical properties

At each site, soil samples were collected around the perimeter of a 2 m radius circular subplot centered within the 400 m² vegetation sampling plot. In June 2004, four soil samples were collected from each site to characterize soil chemical and physical properties. Forest floor layers (Oe + Oa horizons) were separated from a 730 cm² sample frame. Mineral soil was then collected using a 7 cm diameter corer; cores were separated into 0–5 and 5–10 cm depths. Reported measurements are the mean values from these two depths. In the laboratory, organic layer samples were passed through a 6 mm mesh sieve, and mineral soil samples through a 2 mm sieve, to remove coarse materials. All samples were air-dried, ground using a Spex CertiPrep 8000 Mixer/Mill (Metuchen, New Jersey), and oven-dried (60 °C) prior to 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 each mineral soil layer and expressed as the percentage of sand, silt, and clay (Sheldrick and Wang 1993). Total carbon (C) and N for organic and mineral soil layers were determined using a Leco CN 2000 (St. Joseph, Michigan). Extractable phosphorus (P), calcium (Ca), magnesium (Mg), and potassium (K) concentrations were determined using a Varian inductively coupled plasma-mass spectrometer (Palo Alto, California) after extraction with Mehlich III reagents (Mehlich 1984).

Mineral soil bulk density was measured in August 2004 on five soil samples per site and 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. Bulk density measurements

were used to estimate areal net N mineralization and nitrification rates.

Four soil samples (0–10 cm) per plot were collected on a quarterly basis over 2 years for determination of gravimetric moisture content. Moisture content was determined on mineral soil by comparing the difference in mass between fresh and oven-dry (105 °C) soil relative to the oven-dry mass. To achieve finer temporal resolution in soil moisture measurements, additional sampling occurred from January to June 2005 between quarterly sampling periods. Data loggers (Onset Corp., Pocasset, Massachusetts) recorded air and soil temperature (10 cm depth) at each site.

Soil pH was measured four times over the course of the study. Mineral soil (0–10 cm) was mixed in a 1:10 ratio of soil to distilled water, shaken, and allowed to equilibrate for 1 h. The slurry pH was measured using a glass probe meter. Extractable aluminum (Al) concentrations of mineral soil were determined in 1 mol/L KCl extract and analyzed by atomic absorption (GBC Avanta, Hampshire, Illinois).

Litterfall collections

Three 0.23 m² plastic litterfall collectors were installed 0.24 m above ground level at each site in summer 2003, and the contents collected after leaf fall from 2003 to 2005. Litterfall was sorted by species, oven-dried at 60 °C to a constant mass, and weighed. Reported litterfall masses for each site are the mean of three collectors.

Net N mineralization and nitrification rates

Covered core incubations (Robertson et al. 1999) were used to estimate in situ net N mineralization and nitrification. Two 5 cm diameter PVC tubes were hammered into the mineral soil adjacent to each other to a depth of 10 cm. One core was removed immediately and the second core was loosely covered to prevent leaching and incubated for 28 days. Once removed, organic layers were discarded, and mineral soil was sealed in a plastic bag and kept on ice (usually less than 48 h) before being processed. Four core pairs were collected from each site during each sampling period. One month incubations were conducted quarterly for 2 years beginning in May 2003 and ending in March 2005 to capture seasonal trends in N cycling.

In the laboratory, soil samples were passed through a 2 mm mesh sieve, and two 10 g subsamples were weighed and placed in plastic cups. Fifty millilitres of 1 mol/L KCl was added to each sample and shaken for 1 h, and then passed through Whatman No. 40 filter paper. Extracts were collected and analyzed colorimetrically for ammonium (NH₄) and nitrate (NO₃) on a Bran-Luebbe auto analyzer (Norderstedt, Germany). Net N mineralization rates were calculated by subtracting the initial concentrations of NH₄ + NO₃ from the incubated sample concentrations. Net nitrification was calculated as the change in nitrate concentrations between the initial and incubated samples (Raison et al. 1987). Negative values were treated as true immobilization and retained within the dataset, rather than set to zero.

Laboratory mineralization assays were conducted in conjunction with in situ incubations for a subset of sample dates. Duplicate 10 g subsamples of soil from initial cores were maintained at 20 °C and field-moist conditions with periodic water replacement for 1 month. Extractions and

rate calculations were the same as described above for in situ samples. From November to December 2004 in situ incubations were not conducted. To have consistent data on seasonal in situ N cycling rates over the course of 2 years, linear regression models based on August to September 2004 in situ and laboratory incubation rates for N mineralization ($R^2 = 0.65$, $P = 0.0002$) and nitrification ($R^2 = 0.84$, $P < 0.0001$) were used to estimate November to December 2004 in situ rates.

Soil solution chemistry

Four tension lysimeters, two each at depths of 10 and 20 cm, were installed at each site to measure nutrient concentrations in soil solution. Prior to sample collection, lysimeters were allowed to equilibrate for 6 months after installation. A soil solution was collected monthly during periods of adequate soil moisture (September through May). At each sampling, lysimeters were primed by applying –60 kPa of tension. A soil solution was evacuated into clean plastic bottles, kept on ice in the field and refrigerated in the laboratory until filtered and analyzed, usually within 48 h. Concentrations of NH₄ and NO₃ were determined colorimetrically as described above. Total dissolved N was determined colorimetrically on filtered samples after persulfate digestion (Cabrera and Beare 1993). Dissolved organic N was calculated as the difference between total N and inorganic N (NH₄ + NO₃). Dissolved organic carbon (DOC) was measured on filtered samples using a Shimadzu 5000A TOC analyzer (Columbia, Maryland). The concentrations of Ca, Mg, K, and sodium (Na) in the soil solutions were measured by atomic absorption.

Statistical analyses

Site ($n = 16$) was used as the experimental unit for statistical analyses. Two-way analysis of variance models were used to assess the main effects and interaction of geologic substrate and moisture regime. Variables were assessed for normality, and data transformations were applied when necessary. Reported means are from nontransformed data. Linear regression was used to examine relations between soil and leachate nutrient concentrations and site conditions. Spearman's rank correlation was used as a nonparametric alternative to linear regression, when normality could not be achieved through data transformations. All statistical analyses were performed using SAS software, Version 9.1 for Windows (SAS Institute Inc. 2004), and statistical significance was evaluated at $\alpha = 0.05$.

Results

Overstory and midstory tree species composition

The total basal area of overstory trees did not differ by moisture regime (mesic = 30.7 m²·ha⁻¹; xeric = 30.3 m²·ha⁻¹; $P = 0.89$) or by geologic substrate (acidic = 32.7 m²·ha⁻¹; calcareous = 27.6 m²·ha⁻¹; $P = 0.38$). Sugar maple was often the dominant species on mesic sites; it occurred on seven sites and represented 7%–62% of total basal area. Yellow-poplar occurred in half of the mesic sites and was the second most dominant species. One site almost entirely comprised yellow-poplar (>70%) but contained no sugar maple. American beech was generally a minor component

Table 1. Comparison of mineral soil physical and chemical properties from the upper 10 cm layer of four moisture regime – geologic substrate classifications.

	Mesic–calcareous*	Mesic–acidic*	Xeric–calcareous*	Xeric–acidic*	Treatment effect <i>P</i> values [†]		
					Moisture	Geology	Interact
Sand (%)	46.8±1.94	45.8±4.46	59.9±2.84	55.0±7.84	0.079	0.62	0.73
Silt (%)	45.0±1.78	45.4±3.95	28.7±2.47	38.1±7.10	0.044	0.37	0.41
Clay (%)	8.28±0.51	8.78±0.79	11.7±0.62	7.07±0.95	0.32	0.028	0.0089
Bulk density (g·cm ⁻³)	1.16±0.04	1.27±0.02	1.15±0.07	1.27±0.06	0.95	0.052	0.88
Moisture content (%)	31.8±2.25	29.3±0.31	22.7±2.28	22.2±0.86	0.0002	0.34	0.53
Temperature (°C)	13.2±0.30	13.1±0.13	13.9±0.21	14.2±0.14	0.0008	0.61	0.31
pH	4.78±0.23	4.58±0.20	4.44±0.08	3.99±0.09	0.017	0.076	0.47
Total C (%)	2.46±0.22	2.29±0.20	2.40±0.61	2.24±0.21	0.86	0.60	0.97
Total N (%)	0.19±0.02	0.17±0.01	0.14±0.04	0.11±0.01	0.011	0.16	0.94
C/N ratio	12.9±0.16	13.8±0.47	17.8±1.15	20.7±0.75	<0.0001	0.018	0.17
Extractable elements (mg·kg⁻¹)							
NO ₃ -N	1.90±0.46	1.56±0.34	0.84±0.05	1.05±0.17	0.027	0.84	0.39
NH ₄ -N	3.01±0.37	3.31±0.42	3.59±0.63	2.48±0.21	0.75	0.31	0.095
TIN	4.92±0.27	4.87±0.19	4.47±0.59	3.53±0.23	0.013	0.14	0.17
P	20.0±3.49	18.4±3.81	14.4±1.61	15.5±0.76	0.15	0.91	0.63
Ca	745±98.1	966±173	1319±363	819±166	0.30	0.49	0.094
K	70.3±4.80	64.3±6.84	75.3±7.54	66.9±9.76	0.65	0.40	0.89
Mg	55.7±5.28	48.4±6.91	62.5±9.25	47.5±6.50	0.68	0.14	0.59
Al	18.8±6.95	27.3±11.4	7.07±3.03	98.7±21.8	0.084	0.0083	0.022

*Values are means ± SE.

[†]Values in bold indicate significant differences within treatment effects.

(~5% of the relative basal area), except in one site where it made up almost half of the total basal area. Mesic–calcareous sites tended to have greater (although not statistically different) relative basal area of sugar maple compared with mesic–acidic sites (34% vs. 21%), but the relative basal area of oak was similar on mesic–acidic and mesic–calcareous sites (26% vs. 19%). On xeric sites, oak species comprised 60% of the total basal area, with no difference occurring between acidic and calcareous substrates. Oak species included white oak (*Quercus alba* L.), chestnut oak (*Quercus prinus* L.), black oak (*Quercus velutina* Lam.), post oak (*Quercus stellata* Wangenh.), and scarlet (*Quercus coccinea* Münchh.). Xeric–acidic sites had a greater proportion of white oak, and xeric–calcareous sites contained more post oak.

Midstory species composition differed from that of the overstory on mesic sites, in that sugar maple made up nearly 100% of the relative basal area on mesic–calcareous sites (including the American beech site) and about half of the relative basal area in this stratum on mesic–acidic sites. Only the mesic site dominated by yellow-poplar in the overstory had no sugar maple in the midstory, with species composition similar to that of xeric sites (see below). No midstory oaks were recorded on any mesic site.

On xeric sites, flowering dogwood (*Cornus florida* L.) and blackgum (*Nyssa sylvatica* Marsh.) were the most important tree species in the midstory, occurring on six sites with a combined mean relative basal area of about 40%. Oak occurred on only two calcareous sites and sugar maple was present on four (two calcareous and two acidic).

Litterfall

The mean annual litterfall did not differ by moisture regime (mesic = 386 g·m⁻²; xeric = 358 g·m⁻²; *P* = 0.17) or by geologic substrate (acidic = 385 g·m⁻²; calcareous = 356 g·m⁻²; *P* = 0.13). Sugar maple litterfall represented a significantly (*P* = 0.0013) greater proportion of total litterfall on mesic sites (33%) compared with xeric sites (2%). Mesic–calcareous sites had about 1.5 times more sugar maple litter than mesic–acidic sites (40% vs. 27%). Oak species represented significantly (*P* < 0.0001) less of the total litterfall in mesic sites (5%–60%) compared with xeric sites (~70%–90%), but no effect was found on oak litterfall mass between geologic substrates. White and northern red oak (*Quercus rubra* L.) comprised the majority of oak litterfall mass over all mesic sites, while a combination of six oak species (chestnut, scarlet, white, black, post, southern red (*Quercus falcata* Michx.), and chinquapin (*Quercus muehlenbergii* Engelm.)) co-occurred on xeric sites.

Soil physical and chemical properties

Soils of mesic sites had a finer texture with marginally less sand and significantly greater silt than that of xeric sites regardless of geologic substrate (Table 1). The acidic sandstone and shale layers had a lower pH (4.3 vs. 4.6; *P* < 0.08) and less clay than limestone substrates (*P* = 0.03). The pH of mesic soils was 0.5 units higher than that of xeric soils (*P* < 0.02).

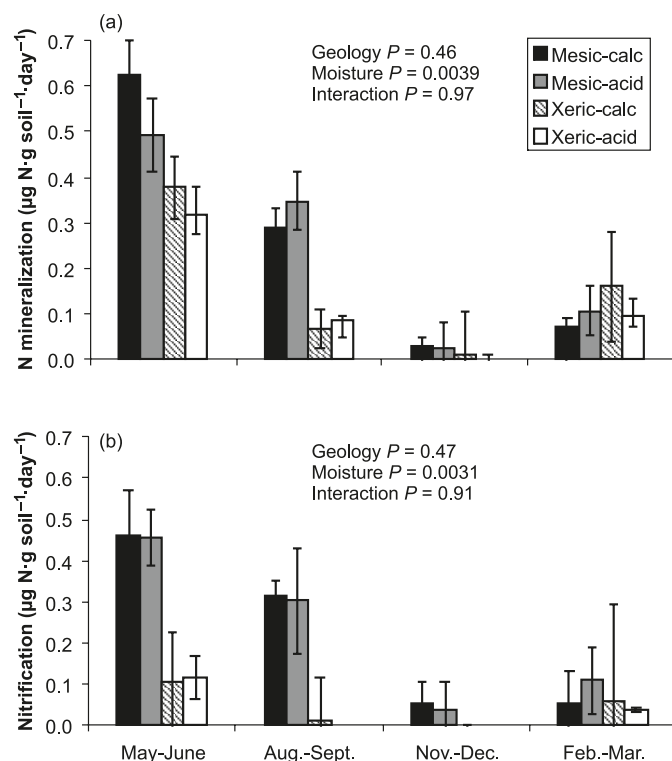
We expected that extractable Ca and Mg would be dramatically higher in limestone-derived soils but found that for the 0–10 cm mineral layer, extractable P and cation con-

Table 2. Comparison of forest floor (Oe + Oa horizon) properties of four moisture regime – geologic substrate classifications.

	Mesic–calcareous*	Mesic–acidic*	Xeric–calcareous*	Xeric–acidic*	Treatment effect <i>P</i> values†		
					Moisture	Geology	Interact
Mass (g·m ⁻²)	167±80.4	365±147	769±104	687±186	0.011	0.71	0.38
Total C (%)	19.9±1.88	19.4±2.55	27.4±0.32	27.0±1.53	0.0047	0.83	0.97
Total N (%)	1.0±0.10	0.92±0.15	1.25±0.06	1.20±0.04	0.019	0.67	0.91
C/N ratio	21.1±0.52	21.5±0.82	21.9±0.99	22.5±0.61	0.28	0.55	0.96
Extractable elements (mg·kg⁻¹)							
P	98.0±15.4	63.0±15.0	57.5±7.97	45.0±2.93	0.09	0.081	0.38
Ca	6730±647	5140±953	9210±796	2780±514	0.94	0.0007	0.014
K	410±41.9	349±69.8	320±59.9	219±31.9	0.081	0.18	0.74
Mg	342±24.8	298±57.5	332±32.7	273±37.9	0.70	0.29	0.87

*Values are means ± SE.

†Values in bold indicate significant differences within treatment effects.

Fig. 1. Seasonal in situ net N mineralization (a) and nitrification (b) rates for four moisture regime – geologic substrate classifications of Mammoth Cave National Park. Errors bars represent ± one standard error of the mean.

centrations did not differ statistically among geologic substrate or moisture classes (Table 1), though a significant interaction indicated that the effect of geology differed between mesic and xeric landscapes. The mesic sites were situated on lower slope positions, where colluvial processes may have masked the signature of the underlying parent material on soil chemistry. In contrast, the xeric sites occurred on higher and flatter (32% vs. 36% slope) landscape positions, and soil chemistry better reflected the parent material differences. Unlike Ca and P, Al in the mineral soil layer reflected our expectations, where concentrations in acidic soils were five times greater than in calcareous soils ($P = 0.01$).

The chemistry of the forest floor also reflected the underlying geology: sites occurring on calcareous substrates had significantly greater Ca concentrations ($P = 0.0007$).

Soil moisture was higher and temperature lower on mesic sites (Table 1). Xeric site forest floor mass was more than twice that in mesic sites and had greater C and N concentrations, but no difference in C/N ratio (Table 2). In contrast, the mineral soil in mesic sites had higher total N concentrations and lower C/N ratios (Table 1). Extractable NO_3^- and total inorganic N were significantly ($P = 0.03$) higher in mesic sites, though NH_4^+ did not differ between moisture regimes. Geology did not have a significant influence on total C and N in organic or mineral soil layers but did influence mineral soil C/N (Table 1).

Net N mineralization and nitrification rates

In situ net N mineralization rates differed significantly between moisture regimes but not between substrates (Fig. 1). The estimated mean annual net N mineralization rate in mesic sites was more than twice that of xeric sites (0.28 vs. $0.13 \mu\text{g N} \cdot \text{g soil}^{-1} \cdot \text{day}^{-1}$; $P = 0.0039$). The annual net nitrification rates were approximately 10 times higher in mesic sites than in xeric sites (0.24 vs. $0.023 \mu\text{g N} \cdot \text{g soil}^{-1} \cdot \text{day}^{-1}$; $P = 0.0031$), and again no influence of geologic substrate was detected (Fig. 1). When averaged across both years of sampling, the net nitrification fraction (the proportion of net N mineralization that was nitrified) represented >90% of the net N mineralization rates in mesic sites compared with ~17% in xeric sites. There was no effect of geologic substrate on nitrification fraction, which was approximately 60% for both acidic and calcareous sites.

Patterns in seasonal and annual variability in N cycling rates were also evident. The highest rates usually occurred in the May–June sampling periods, and virtually no net N mineralization and nitrification occurred during the Nov.–Dec. sampling periods (Fig. 1). Net N mineralization rates during the first year of sampling were higher than the second year, but net nitrification rates in year 1 and year 2 were similar. Rates of net N mineralization and nitrification were higher in xeric sites during the Feb.–Mar. period of year 2.

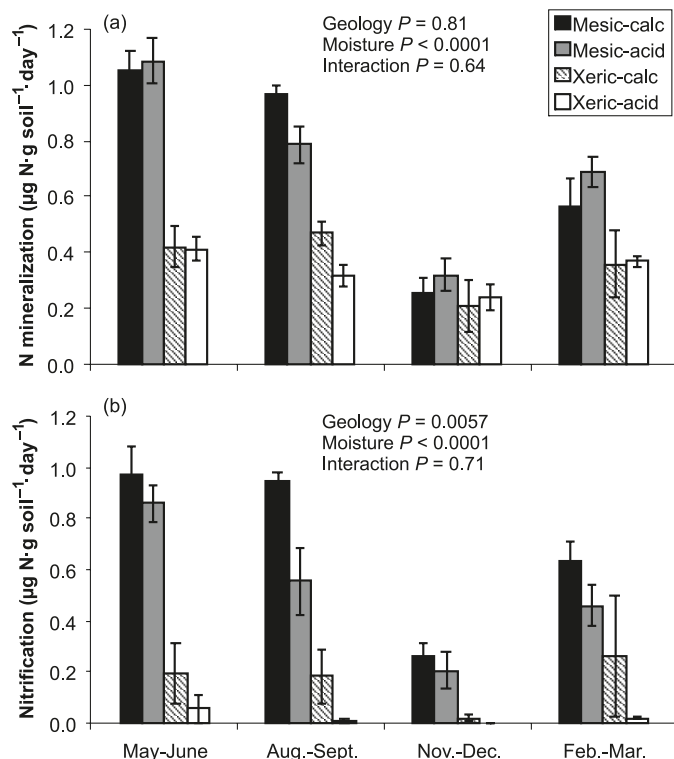
Laboratory measurements of net N mineralization and nitrification rates were higher than in situ rates, but the relative magnitude of differences between mesic and xeric site

Table 3. Mean concentrations for soil solution constituents measured seven times from November 2003 to May 2005.

	Concn. ($\mu\text{mol}\cdot\text{L}^{-1}$)*				Treatment effect <i>P</i> values†		
	Mesic–calcareous	Mesic–acidic	Xeric–calcareous	Xeric–acidic	Moisture	Geology	Interact
NH ₄ -N	5.15±1.35	3.67±0.51	7.32±2.15	3.32±0.17	0.41	0.025	0.26
NO ₃ -N	135±60.1	82.3±35.5	2.94±0.34	3.30±0.54	<0.0001	0.82	0.68
DON-N	19.3±3.74	22.1±6.01	27.2±8.38	24.5±6.34	0.43	0.99	0.67
DOC-C	874±58.6	1030±258	2080±22.5	1700±472	0.0041	0.47	0.32
Ca	114±11.8	103±18.8	159±42.8	39.9±8.46	0.65	0.0064	0.019
K	82.6±19.0	65.9±17.3	53.9±8.37	62.0±12.4	0.37	0.79	0.48
Mg	30.9±3.27	31.0±5.86	18.0±2.27	21.9±1.10	0.0098	0.59	0.62

*Values are means ± SE.

†Values in bold indicate significant differences within treatment effects.

Fig. 2. Potential net N mineralization (a) and nitrification (b) rates for four moisture regime – geologic substrate classifications of Mammoth Cave National Park. Errors bars represent ± one standard error of the mean.

soils was similar (Figs. 1 and 2), except in Feb.–Mar. of year 2, when laboratory measurements showed higher rates in mesic sites than xeric sites, opposite the pattern observed in situ. An effect of geology was not evident for laboratory net N mineralization rates; however, geology was a significant ($P = 0.0057$) factor for laboratory net nitrification rates, with overall rates higher in calcareous sites (Fig. 3).

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. Soil solution

NO₃ concentrations were significantly ($P = 0.012$) greater in mesic sites compared with xeric sites. In mesic sites, NO₃ was the dominant form of soil solution N, while in xeric sites, dissolved organic N was a greater proportion of total N in soil solution than inorganic N (NH₄ + NO₃). Mean DOC concentrations were twice as high in xeric sites (Table 3). Although concentrations of base cations in soil solutions for mesic sites tended to be higher compared with xeric sites, only Mg concentrations were significantly ($P = 0.0098$) different between mesic and xeric sites. Calcareous sites had significantly higher concentrations of NH₄ ($P = 0.025$) and Ca ($P = 0.0064$) in soil solutions (Table 3).

Factors associated with N cycling and base cation leaching

We used a number of site characteristics in simple linear regression models to predict variation in net N mineralization and nitrification rates across all 16 sites. Soil texture and soil exchangeable Ca did not significantly influence net N mineralization or nitrification. Soil H⁺ concentration was a moderately significant ($R^2 = 0.24$) predictor of nitrification rates but not net N mineralization (Fig. 3), and soil P had a small but significant influence on net N mineralization and nitrification rates. Mineral soil C/N ratios and moisture content explained a large portion of variation in net N mineralization and nitrification rates across the 16 sites (Fig. 3). While moisture regime was an important factor influencing the relationships between explanatory and response variables, geologic substrate was not. We used the mass of litterfall by species as a surrogate for species influence on N cycling patterns because these were the dominate genus or species on the sites, and because litter mass closely follows basal area. The mass of litterfall was not significantly correlated with N cycling rates for any species or species groups other than sugar maple and oak. Sugar maple litterfall mass explained approximately equal amounts of the variation in net N mineralization and nitrification rates compared with soil moisture content. Total oak litterfall mass was less predictive of net N mineralization and nitrification than sugar maple litterfall mass, but a negative trend was observed, the opposite of that found for sugar maple (data not shown).

We also used simple linear regression to explore variation in soil solution chemistry across sites. Soil solution NO₃ concentrations were positively correlated with the percentage of sugar maple litterfall ($R^2 = 0.68$, $P < 0.0001$), but significantly declined with increasing abundance of oak litter

Fig. 3. Relationships between site characteristics and N mineralization rates (*a–e*) and nitrification rates (*f–j*) for four moisture regime – geologic substrate classifications.

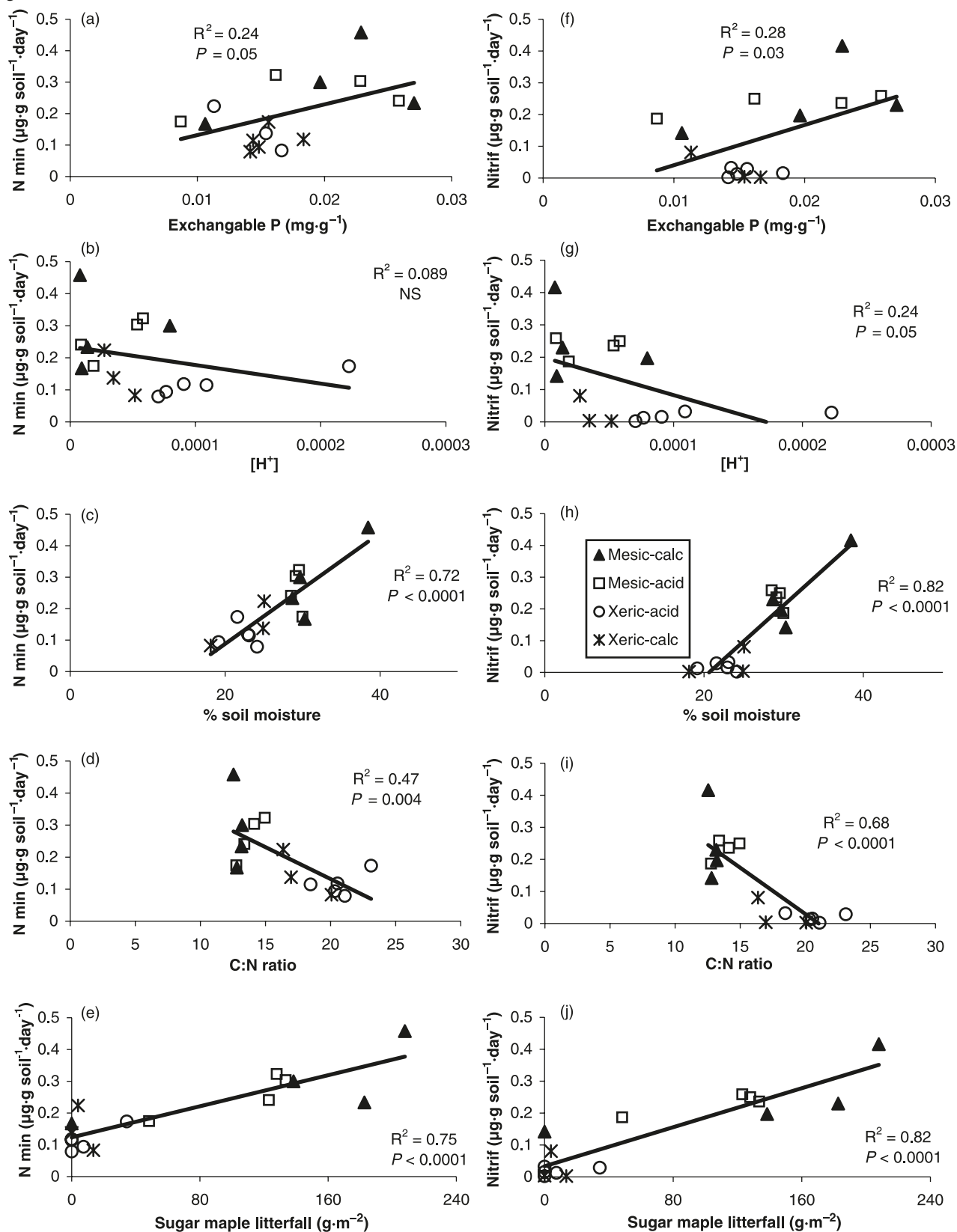


Fig. 4. Relationships between soil solution nitrate concentrations and (a and c) dominant species litterfall, (b) soil moisture content, and (d) annual net nitrification rates.

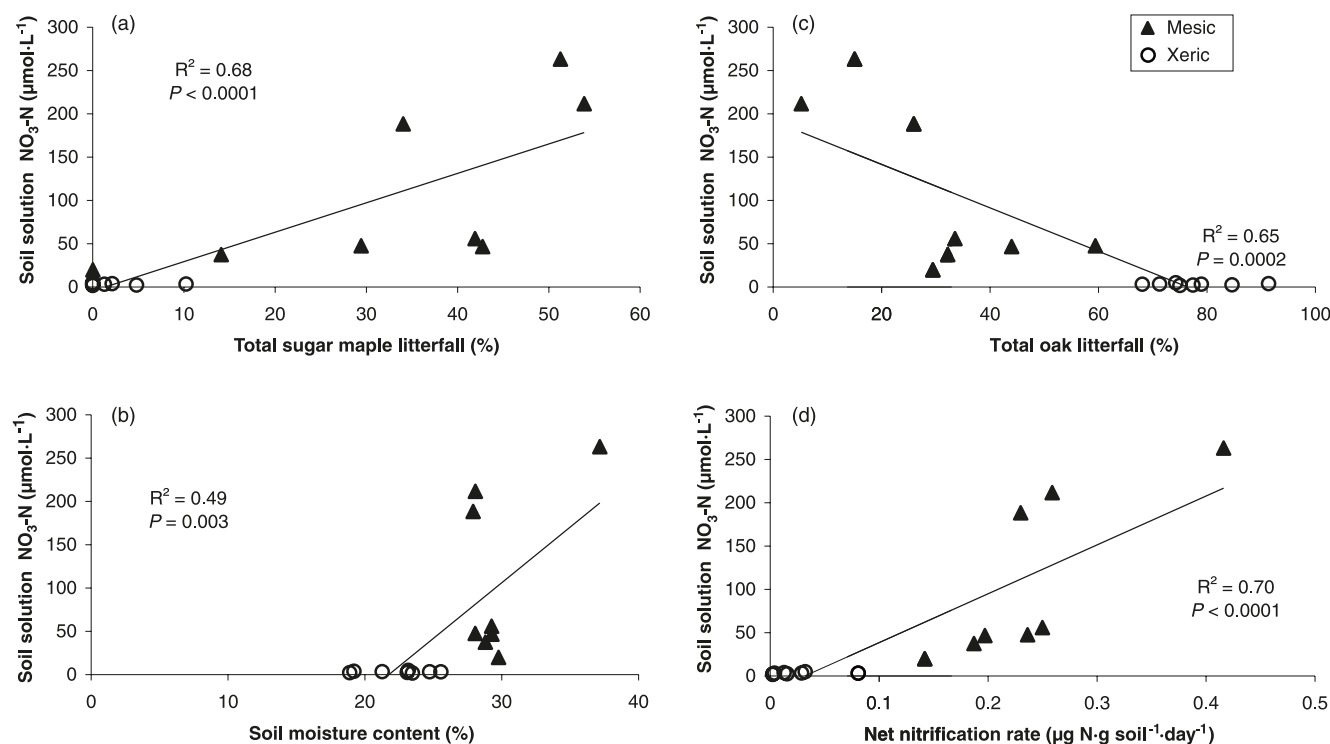
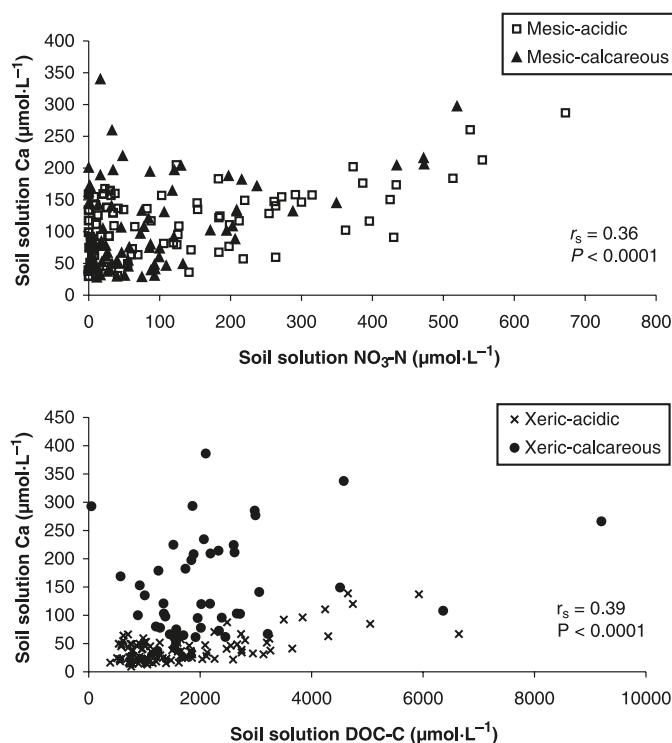


Fig. 5. Relationships between soil solution (a) Ca and $\text{NO}_3\text{-N}$ in mesic sites and between (b) Ca and DOC-C in xeric sites. Spearman's rank correlation coefficients (r_s) were calculated using samples ($n = 167$) from mesic-acidic ($\square$) and mesic-calcareous ($\blacktriangle$) sites combined for panel a, while only samples ($n = 106$) from xeric-acidic sites ($\times$) were used in panel b, since Ca concentrations in xeric-calcareous sites ($\bullet$) were unrelated to DOC-C.



(Fig. 4). Net nitrification rates predicted slightly more variation in soil solution nitrate concentrations compared with litterfall, and no significant relationship was found using soil moisture (Fig. 4).

In mesic sites, soil solution Ca concentrations were positively correlated with NO_3 concentrations regardless of geologic substrate, while in xeric sites occurring on acidic substrates, DOC may have contributed to the variation in soil solution Ca (Fig. 5).

Discussion

We characterized soil properties and processes across two distinct geologic classifications and moisture regimes on a landscape scale. We found that geologic substrate had little detectable influence on soil properties, although by sampling only the upper mineral soil layers, it may be difficult to determine the overall impact of geologic substrate at the sites we studied in MCNP. Nonetheless, soils strongly differed in moisture content and organic matter quality across moisture regimes. The dissected topography of this sedimentary region creates distinct microclimatic conditions that control landscape-scale tree species distribution (McEwan et al. 2005; Fei et al. 2007), confounding the relationship between site soil properties and microbially mediated processes. As reported for other North American forest types (Zak et al. 1989; Van Breemen et al. 1997), the distribution of tree species at MCNP would also be expected to respond to differences in underlying geologic strata. Limestone and acidic sandstone or shale parent materials had little influence over species composition, but moisture regime, mediated strongly by slope aspect, had a much more pronounced influence on forest make-up, which in turn contributed to differences in

Table 4. Comparison of N mineralization and nitrification rates, and nitrification fractions from sites across the Northeast and Midwest

Source	Location	Incubation method	Main effect	Treatments
Current study	South-central Kentucky	Annual (4 seasonal) in situ intact core	Moisture regime	Mesic
Gilliam et al. 2001	Fernow Experimental Forest, West Virginia	Annual (year-round) in situ buried bag	Watershed N additions	Xeric Young control
Ohrui et al. 1999	Adirondack Mountains, New York	Annual (year-round) in situ buried bag	Slope position	Upland hardwood (upper)
Peterjohn et al. 1999	Fernow Experimental Forest, West Virginia	Annual (year-round) in situ intact core		Upland conifer (lower) Aspect easterly
				Southerly
Finzi et al. 1998a	Northwestern Connecticut	Midsummer in situ intact core	Single species plots	Sugar maple
				American beech Northern red oak
Zak and Pregitzer 1990	Northwestern Minnesota	Annual (4 seasonal) in situ buried bag	Stand compo- sition	Sugar maple, basswood
				Sugar maple, northern red oak
				Black oak, white oak

Note: All studies reported net rates from upper mineral soil layers, expressed on an area basis for comparative purposes.

*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 are from Gilliam et al. 1995. All others are reported in original sources.

net N mineralization and nitrification rates and soil solution nitrate concentrations. Thus, this study differs from other studies examining species compositional effects on N cycling (Finzi et al. 1998a, 1998b; Lovett et al. 2004—all from northeastern US) in that forests of the central hardwood region have a much greater tree species diversity and complexity, shaped largely in response to slope aspect and slope position, than northern hardwood forests. Additionally, as noted below, nuanced species composition among sites with similar moisture regimes, coupled with shifts in N cycling, suggests a strong role for species composition in dictating N cycling apart from that mediated by moisture, allowing us to separate these two confounded attributes somewhat (but limited by our relatively small sample size).

The species composition of a site can influence the quality of organic matter deposited on the forest floor through leaf litterfall as well as via other sources of C and nutrients (e.g., root exudates and root turnover). We focused on leaf litter as a potential source of variation leading to differences in net N mineralization because leaf litter quality can affect rates of decomposition (Melillo et al. 1982), which in turn can lead to spatial variability in soil C and nutrient concentrations (Boettcher and Kalisz 1990; Finzi et al. 1998b), and rates of net N mineralization (Scott and Binkley 1997; Ferrari 1999). For example, litters with higher amounts of lignin can contribute higher amounts of recalcitrant organic matter to soils (Binkley and Giardina 1998), leading to higher C/N ratios in soils (Finzi et al. 1998a). Sugar maple leaf litter is known to have low lignin/N ratios (Ferrari 1999; Lovett et al. 2004), especially compared with that of oak litter (Lovett et al. 2004). Contrasting species composition in mesic and xeric sites at MCNP 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 forest floor mass and lower mineral soil C/N ratios.

At MCNP, soil moisture content is potentially very important to N cycling patterns, which are mediated simultaneously via site moisture content and the influence of soil moisture on species composition. As a result, in this study design, moisture and species composition are most certainly confounded. In general, differences in species composition, mediated at least in part through litter quality, likely contributed to differences in N cycling rates between mesic and xeric sites at MCNP. Sugar maple litterfall was strongly and positively correlated with higher net N mineralization, and especially nitrification (Fig. 3), while oak species litterfall was negatively correlated with net N mineralization and nitrification. Other factors known to influence N cycling rates, such as soil texture and pH, were not good predictors of net N mineralization and nitrification in this study, despite variation across the sites (Fig. 3). Instead, species composition, especially sugar maple abundance, may be more important than soil moisture with regard to N cycling at MCNP, particularly net nitrification. As evidence, the two mesic sites dominated by American beech and yellow-poplar had higher soil moisture content but much lower net N mineralization and nitrification rates than the mesic sites dominated by sugar maple. Rates of net 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). The high abundance of nearly even-aged yellow-poplar in the other low N cycling mesic site may be indicative of recovery from agricultural disturbance observed in this region

USA with varying tree species composition.

N mineralization (mg N·m ⁻² ·day ⁻¹)	Nitrification (mg N·m ⁻² ·day ⁻¹)	Nitrification fraction*	Dominant tree species†
33	29	0.88	<i>Acer saccharum</i> , <i>Liriodendron tulipifera</i> , <i>Fagus grandifolia</i> , <i>Carya</i> spp.
16	3	0.19	<i>Quercus</i> spp., <i>Nyssa sylvatica</i> , <i>Carya</i> spp.
35	31	0.89	<i>Prunus serotina</i> , <i>Acer saccharum</i> , <i>Betula lenta</i> , <i>Liriodendron tulipifera</i>
29	8	0.28	<i>Fagus grandifolia</i> , <i>Acer saccharum</i>
22	0.5	0.02	<i>Picea rubrens</i> , <i>Tsuga canadensis</i>
40	35	0.88	<i>Acer saccharum</i> , <i>Quercus prinus</i> , <i>Tilia americana</i>
23	2	0.09	<i>Nyssa sylvatica</i> , <i>Fagus grandifolia</i> , <i>Oxydendrum arboretum</i> , <i>Acer rubrum</i> , <i>Quercus</i> spp.
66	41	0.62	<i>Acer saccharum</i>
32	7	0.22	<i>Fagus grandifolia</i>
39	10	0.26	<i>Quercus rubra</i>
31	26	0.84	<i>Acer saccharum</i> , <i>Tilia americana</i>
28	3	0.11	<i>Acer saccharum</i> , <i>Quercus rubra</i>
23	1	0.04	<i>Quercus velutina</i> , <i>Quercus alba</i>

(Kalisz 1986). Intermediate litter quality (between sugar maple and oak), altered nutrient status due to disturbance, or both, probably led to lower N cycling rates in the yellow-poplar site than in the other six mesic sites. These nuances in tree species composition seem to correspond to changes in N cycling rates among mesic sites.

Nitrate concentrations in soil leachate were elevated in mesic sites compared with xeric sites, which had little to no soil solution NO₃. Further, soil solution NO₃ concentrations were positively correlated with sugar maple litter inputs, while oak litter had a negative correlation (Fig. 4). And among mesic sites, those dominated by beech and yellow-poplar had the lowest levels of NO₃ in soil solutions. These findings suggest the influence of some tree species on N cycling, probably regulated through differences in nitrification rates, a pattern that has been observed in hardwood forests in the Adirondack Mountains of New York (Mitchell et al. 2003) and in the mid-Appalachian region of the eastern USA (Williard et al. 2005). Differences in N retention on a watershed scale have been attributed to differences in species composition (Lovett et al. 2000), and the abundance of sugar maple, along with other easily decomposable litters, is likely the cause for elevated stream water NO₃ export in those systems (Lovett et al. 2002; Christopher et al. 2006). Although we did not measure stream water NO₃ export, we can infer that high sugar maple abundance in mesic sites in close proximity to riparian areas could result in a substantial release of N, and future investigations should be directed at quantifying the contribution of tree species to stream water N in central hardwood forests. Nitrate in soil solutions from mesic sites was also positively correlated with Ca and Mg concentrations in soil solution (Fig. 5) and has been linked to soil acidification in organic horizons of sugar maple

stands in the northeastern US (Fitzhugh et al. 2003). 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 contrast, xeric sites exhibited elevated DOC in soil solutions, which has been positively correlated with concentrations of dissolved organic acids (Dijkstra et al. 2001). If DOC can serve as a proxy for organic acids, then a high concentration of DOC could represent a mechanism for base cation leaching in these oak-dominated stands, since soil solution DOC was positively correlated with Ca concentrations in xeric acidic sites. The relationships between soil solution NO₃ and base cations in mesic sites, but between DOC and Ca in xeric acidic sites, suggest that base cation leaching within moisture regimes could be influenced by contrasting biological processes, perhaps mediated through differences in tree species.

To highlight the differences between mesic and xeric sites in the context of species influence and atmospheric N deposition, we have compared our results with those of other studies conducted in hardwood and mixed hardwood-conifer forests of the eastern and midwestern US (Table 4). Net nitrification rates in the xeric sites at MCNP were low and similar to other oak-dominated sites, whereas net nitrification rates in mesic sites were high and comparable to those reported for sugar-maple-dominated stands in the midwestern (Zak and Pregitzer 1990) and northeastern US (Finzi et al. 1998a). But perhaps more importantly, nitrification fraction in mesic sites (0.88) at MCNP was quite high and similar to that reported by Gilliam et al. (2001) from Fernow Experimental Forest in the central Appalachians, which included Watershed Three, where 35 kg N·ha⁻¹·year⁻¹ (as

(NH₄)₂SO₄) has been experimentally applied since 1989. In addition, spatial patterns in N cycling at MCNP 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. At Watershed Four, elevated rates of N mineralization, nitrification, and NO₃ concentrations in soil solutions were found to occur on easterly aspects, much like those of mesic sites at MCNP, whereas low rates on southerly aspects were very similar to xeric sites in this study. Similarities in spatial variability of N cycling between the two studies, as well as high nitrification fractions measured in mesic sites at MCNP, are evidence that these sites could become significant sources of N released to streams if current depositional loading is sustained.

Management implications

Across the landscape at MCNP, slope aspect created strong differences in solar radiation and, consequently, in soil moisture status. Soil moisture in turn controlled the distribution of tree species, with xeric sites being dominated by oak species and mesic sites having a large component of sugar maple and less oak. This relationship becomes important, since forest dynamics may create positive feedbacks on species influence on N cycling. For instance, 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 mid-story canopy layers (Lorimer et al. 1994). This is supported in our study by the lack of oak recruitment in the midstory across both mesic and xeric sites. The loss of oak species as dominant canopy trees could have implications for N retention in these ecosystems, as evidenced by the inverse relationships between oak abundance and net nitrification and NO₃ in soil solutions.

Of further concern are the habitats at MCNP with the highest sugar maple abundance (i.e., mesic forests), which are in close proximity to aquatic ecosystems and cave openings, both of which host large biological diversity, including a number of threatened and endangered species. The abundance of understory sugar maple on mesic sites suggests that it will become dominant as these stands age, even in sites that are not currently dominated by sugar maple in the overstory stratum. Long-term monitoring of N dynamics, as well as forest composition, will be crucial to understanding the impacts of forest succession and continued N deposition to this region.

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