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Soil Nitrification and Mineralization Rates Along an Elevation Gradient in the Great Smoky Mountains National Park

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To the Graduate Council:

I am submitting herewith a thesis written by Christopher James Rolison entitled "Soil Nitrification and Mineralization Rates Along an Elevation Gradient in the Great Smoky Mountains National Park." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Environmental Engineering.

John S. Schwartz, Major Professor

We have read this thesis and recommend its acceptance:

Qiang He, Amy M. Johnson

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Soil Nitrification and Mineralization Rates Along an Elevation Gradient in the Great Smoky Mountains National Park

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Christopher James Rolison
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Abstract

The Great Smoky Mountains National Park (GRSM) is an area sensitive to acid deposition. Although reports indicate there have been reductions of acid deposition in the eastern United States, water quality in streams has not recovered to perceived natural levels. Coupled soil biogeochemical processes of nitrification and nitrogen mineralization can acidify soil water and play a key role in the fate of nitrogen-based acid deposition and observed stream acidification. Characterizing nitrogen decomposition rates at different elevations improves our understanding of the potential effects of acid deposition and soil interactions with acid ions. Soil chemical properties and potential reaction rates for nitrification and mineralization among 36 sites in three GRSM watersheds were characterized by 28-day laboratory incubation experiments. In addition, relationships were identified by comparing soil chemistry to watershed characteristics including site location, soil characteristics, and geomorphic factors. Nitrification rates ranged between 1 and 177 µeq kg^{-1} dry soil day^{-1}, and mineralization rates ranged between 2 and 339 µeq kg^{-1} dry soil day^{-1}. For the three watersheds combined, mineralization and nitrification rates were significantly correlated with elevation. Mineralization was increasing at a rate of 0.1578 / 0.0816 µeq kg^{-1} dry soil day^{-1} m^{-1} in the A and B/C soil horizons, and nitrification at 0.1269 / 0.0425 µeq kg^{-1} dry soil day^{-1} m^{-1}, in the A and B/C soil horizons. For individual watersheds, Cosby and the West Prong of the Little Pigeon shared this significant positive correlation while the Noland Divide watershed did not because sample sites were only located at higher elevations. Soil horizon class played a key role in controlling the nitrogen cycle processes, where the A soil horizon was found to be more dependent on total organic nitrogen, and the B/C soil horizon was more dependent on organic matter. Nitrification and mineralization rates were not correlated with site slope, organic matter to total organic nitrogen ratio, and A soil horizon depth. The study results illustrate that nitrification and mineralization play a significant part of the soil biogeochemical process that govern episodic stream acidification response in the GRSM.

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INTRODUCTION

The Great Smoky Mountains National Park (GRSM) receives high rates of nitrogen deposition; in fact deposition has been increasing in recent decades in many world regions prone to stream acidification (Nodvin et al., 1995; Likens et al., 1996; Jeffries et al., 2003; NADP, 2006; Helliwell et al., 2007). Nitrogen is widely available in soil ecosystems, but only a small amount of nitrogen is accessible in mineral form, mainly ammonium and nitrate (Paster et al., 1984). Common inputs of nitrogen include wet and dry deposition and nitrogen fixation by microbes. Sustained elevated levels of atmospheric nitrogen deposition lead to nitrate leaching through increased nitrification rates (Knoepp et al., 1997). It has been observed in the Appalachian region that there is a positive correlation between wet deposition of nitrogen and nitrogen concentrations within a forested soil ecosystem (Lawrence et al., 2000).

Soil is a key medium regulating nitrogen deposition through many complex reactions, such as to nitrogen mineralization and nitrification reactions (Bonito et al., 2003). These reactions are carried out by microorganisms, where they generate protons leading to soil and water acidification. These reactions are known as the dominant reactions in the nitrogen cycle and control the effects of nitrogen deposition on aquatic environments (Vitousek et al., 1997). In the GRSM some watersheds have reached stage II nitrogen saturation, a condition where excessive nitrogen from forest soil systems is released into surface waters (Nodvin et al., 1995; van Miegroet et al., 2001). Leaching of nitrate from the soil is a current problem causing episodic stream acidification during stormflow (Deyton et al. 2009; Cai et al. 2010). It appears stream acidification from multiple acid sources is a responsible for brook trout expiration (Neff, 2009). Because the nitrogen cycle is a dominant biogeochemical processe in forest ecosystems, understanding process rates as a function of various environmental conditions in the GRSM will aid park resources managers with the protection of the Park’s streams. Environmental conditions include elevation, geomorphic basin factors (i.e., basin slope), vegetation, and soil chemical characteristics.

Elevation plays a role affecting nitrogen mineralization and nitrification rates in mountainous regions due to nitrogen deposition rates and seasonal temperature changes. For example, in the Coweeta watershed of southern Appalachian region, study sites in high elevation areas were found to have higher mineralization and nitrification rates in comparison to lower elevation areas (Bonito et al., 2003; Knoepp and Vose, 2007). Differences in mineralization and nitrification rates along an elevation can also be attributed to the elevation change of nitrogen deposition loads. Atmospheric deposition of nitrogen, both in dry and wet depositions, has been shown to increase with elevation (Lawrence et al., 1999). The GRSM also has been shown to have increased nitrogen deposition with elevation (Weathers et al., 2003). Microorganisms that decompose organic nitrogen are present in temperate soil environments and are temperature-sensitive. Low temperatures in high elevation areas can hinder these organisms from mineralizing nitrogen (Bonito et al., 2003). Elevation and temperature are inversely correlated which is one reason why nitrogen cycle rates within these temperate soils often correlate with differences in elevation. Inverse effects of lower temperatures and higher acid deposition in high elevation areas, in addition to other soil and geomorphic basin factors, suggest that measurement of nitrification and mineralization rates would be useful to characterize and investigate their influence on stream acidification.

Nitrification and mineralization rates can be affected by vegetation and soil type, soil organic matter content (OM), soil total organic nitrogen (TON), and soil organic carbon to nitrogen ratio (C:N). Vegetation type plays a role in nitrification and mineralization rates because of leaf litter inputs to the soil. Leaf litter is a key source of organic carbon in soils and varies annually. Variance on availability of organic carbon can hinder microbial nitrogen mineralization (Gosz et al., 1976; Monk and Day, 1985). Soil type also has an effect on mineralization and nitrification rates due to biotic properties of different soils (Knoepp and Vose, 2007). Garter et al. (1990) found soils within the same watershed that had similar nitrogen uptake rates by vegetation exhibited different mineralization and nitrification rates per soil type, as a function of soil organic carbon and nitrogen content. Carbon flux provided by local vegetation affects the C:N ratio (carbon to nitrogen ratio) in the soils, where low C:N ratios favor mineralization (Paul and Clark, 1989) and high ratios favor nitrogen retention. Microorganisms that release inorganic nitrogen are limited by soil carbon content rather than nitrogen levels at low C:N ratios. Higher levels of the C:N ratio favor nitrogen retention by decomposers and a reduced availability of nitrogen within the soil. Low availability of nitrogen within the soil leads to high nitrogen use efficiency (NUE) (Vitousek, 1982). The higher the NUE, the less nitrates are released into the groundwater and into the atmosphere via gaseous compounds, such as nitrous acid, and nitric acid, so the higher the C:N the more likely soil leaching of nutrients will occur. Characteriztion of nitrification and mineralization rates as a function of controlling environmental factors aids in better understanding the biogeochemical processes related to soil nitrogen, and their influence on stream acidification.

The objectives of this study were to: 1) characterize nitrification and mineralization rates along an elevation gradient in the GRSM, and 2) identify relationships between nitrification and mineralization rates and watershed characteristics including soil type, vegetation type, and basin geomorphic factors. The study design consisted of 36 samples sites among three GRSM watersheds. Results of this study provide basic information on dominant biogemochemical proceses assocated with stream acidification, and they will also be useful for PnET modeling of these processes in the prediction of long-term trends for different deposition scenarios.

BACKGROUND

Acid Deposition

Acid deposition sources occur from chemical reactions associated with coal-fired power plants and vehicle emissions of sulfur oxides (sulfate deposition) and nitrogen oxides (nitrogen deposition), which are deposited on the earth's surface. Acid deposition is also known as the "acid rain", where rain water can have a pH below 4.5. The chemical products that are created within the atmosphere are deposited two ways onto the earth's surface; those two ways are dry and wet deposition. Dry deposition describes processes by which pollutant and pollutant constituents reach the earth's surface that do not happen in the atmosphere where wet deposition occurs. Wet deposition encompasses all process by which atmospheric pollutants and constituents are transported to earth's surface via one of the many forms of precipitation (rain, fog, snow, sleet). Acids in deposition react with surface waters, in the soil, and on manmade structures.

Nitrogen Cycle

The nitrogen cycle is a dominant process of the soil chemistry; where the nitrogen cycle defines the processes by which nitrogen is converted into various chemical forms. The processes of the nitrogen cycle include fixation, mineralization, nitrification, and denitrification (Fig. 1). Inputs of nitrogen into the ecosystem include sources from human activity. The combustion of fossil fuels, and nitrogen based fertilizers are two main constituent sources of nitrogen into the nitrogen cycle.

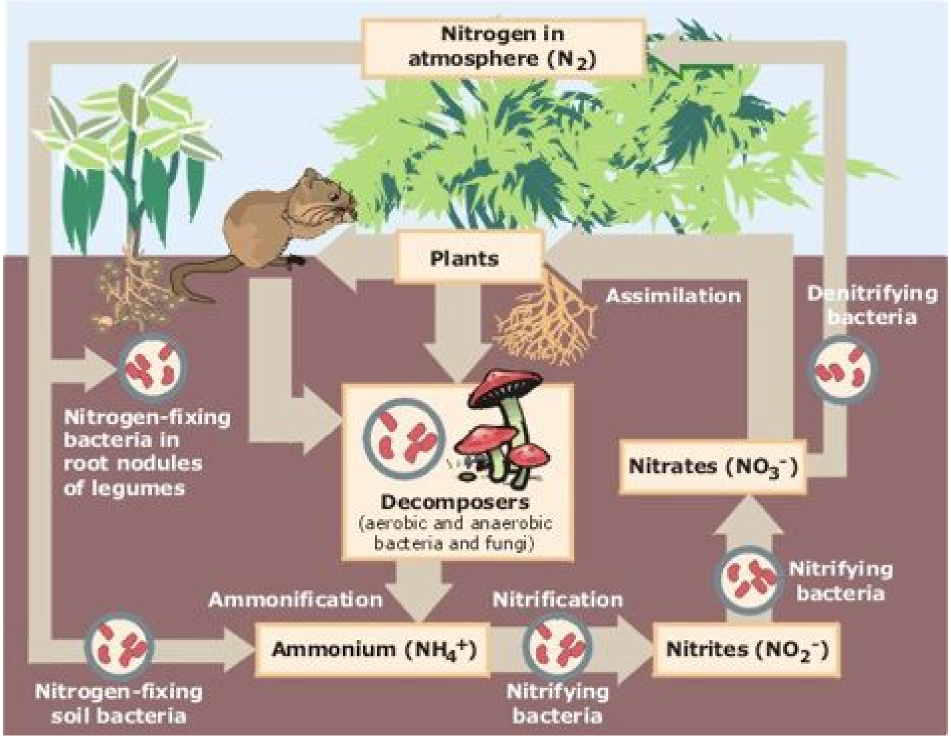

Figure 1. The Nitrogen Cycle (EPA, 2011).

Nitrogen fixation is the process of converting atmospheric nitrogen (N_2) into ammonia (NH_3). Fixation is occurs from either biological or abiotic processes, such as microorganism, or combustion. Biological nitrogen fixation is when atmospheric nitrogen is converted by enzymes called nitrogenase (Postgate, 1998).

The reaction for biological nitrogen fixation:

$$N_2 + 8\,H^+ + 8\,e^- \rightarrow 2\,NH_3 + H_2$$

The mineralization reaction is a process that decomposes organic nitrogen and converts it into inorganic ammonium by bacteria and fungi. Ammonium supplied by mineralization reaction, and other sources like direct atmospheric deposition was oxidized by ammonia oxidizing microorganisms to become nitrate by a process called nitrification. As a general rule, decomposition processes are affected by the source supply, the abundance of microorganism and the environmental condition. Organic nitrogen is the direct energy source for mineralization microbiological reactions, and also indirect NH_3 source for nitrification.

The mineralization reactions:

$$CO(NH_2)_2 + 3\ HOH \text{ -------> } 2NH_4^+ + HCO_3^- + OH^-$$

Nitrification is the process of converting ammonium to nitrate. The process is carried out by nitrifying bacteria. The two step process of nitrification starts off with the oxidation of ammonium (NH_4^+) in nitrites (NO_2^-). The second level is the oxidation of nitrites into nitrates (NO_3^-) (Smil, 2000).

The nitrification reactions:

$$NH_4^+ + 1/2\ O_2 \text{ -------> } NH_2OH + H^+$$

$$NH_2OH + O_2 \text{ ---------> } NO_2^- + HOH + H^+$$

$$NO_2^- + 1/2\ O_2 \text{ -------> } NO_3^- +$$

Denitrification is the process of microorganism reducing nitrate into nitrogen (N_2). This process reduces the oxidized states of nitrogen in response to electron donors within the organic matter and completes the nitrogen cycle by returning nitrogen back into the atmosphere.

The reactions for denitrification:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO + N_2O \rightarrow N_2\ (g)$$

$$2\ NO_3^- + 10\ e^- + 12\ H^+ \rightarrow N_2 + 6\ H_2O$$

In an acidified watershed, nitrogen mineralization and nitrification reactions are particularly of interest because these two reactions can change soil and water acidity.

Soil Type

Soils are classified by 12 soil orders. In the GRSM, Inceptisols and Ultisols are the dominant soil orders. Inceptisols are soils that exhibit minimal horizon development and are found across a wide range of ecological settings. Inceptisols are often found in areas with fairly steep slope and young geomorphic surface, where most Inceptisols are found in mountainous are and are used for forestry, reaction, and watersheds. Ultisols are strongly leached acid forest soils with low native fertility. Ultisols are primarily found in humid, temperate, and tropical areas, typically on older stable landscapes. Ultisols exhibit intense weathering as primary minerals of Ca, Mg, and K have been leached form the soil. In GRSM, the Inceptisols were found from low to high elevations whereas Ultisols were only found at low elevations (McDaniel, 2011).

Vegetation Type

In the GRSM, overstory vegetation can be separated into three classes, including coniferous, deciduous, and mix class vegetation. Coniferous are gymnosperms, they are cone-bearing seed plants with vascular tissue; all extant conifers are woody plants, the great majority being trees with just a few being shrubs. Typical examples of conifers include cedars, Douglas-firs, cypresses, firs, junipers, kauris, larches, pines, hemlocks, redwoods, spruces, and yews (Campbell, 2005). Deciduous are vegetation that include trees, shrubs and herbaceous perennials that lose all of their leaves for part of the year, or abscission. In some cases leaf loss coincides with winter - namely in temperate or polar climates. In other parts of the world, including tropical, subtropical, and arid regions, plants lose their leaves during the dry season or other seasons, depending on variations in rainfall. The mix vegetation class is one where deciduous or coniferous were not the main type of vegetation.

METHODS

Site Description

Within the GRSM of Tennessee and North Carolina, USA, three watersheds were chosen for study. They included the West Prong of the Little Pigeon (WPLP), Cosby Creek, and Noland Divide watersheds (Fig. 1). Based on information from Neff et al. (2010), WPLP and Cosby watersheds were chosen because of their unique characterization of stream water quality, where Cosby is a highly acidified watershed and WPLP was not. WPLP was influenced by the addition of calcium from dolomite applications to roads during severe winter conditions, buffering stream acid conditions. In Cosby and WPLP watersheds the samples were collected from an elevation gradient from about 450 m to 2000 m. The Noland Divide watershed was chosen in order to compare data with existing data from Cai et al. (2011) at high-elevation watershed sites.

Of the total 36 soil sample sites chosen for study, 16 were located in the WPLP watershed, 6 in the Noland Divide watershed and 14 in the Cosby Creek watershed. During the field sampling and laboratory pre-treatment processes, samples from two sites in B/C horizon were lost (Cosby C2, and Noland NK4). Characterization of each sample site includes site location, elevation, slope, A horizon depth, soil type, and vegetation type (Table 1). Coordinates and elevations for each of the sampling locations were measured in the field with a Garmin-Etrex GPS unit. Elevations ranged between 476 m and 1918 m. Slopes of each site were obtained by creating a GIS shapefile of the sampling locations to overlay on a digital slope map. Site slopes ranged between 2.5% and 39.6%. In total, the sampled soil sites were covered by 21 vegetation and 14 soil types. For statistical analysis, these 21 vegetation types were grouped into three classes: coniferous, deciduous, and mix class vegetation. The 14 different soil types studied can be categorized into two soil orders; they were Inceptisols (34 sites) and Ultisols (2 sites).

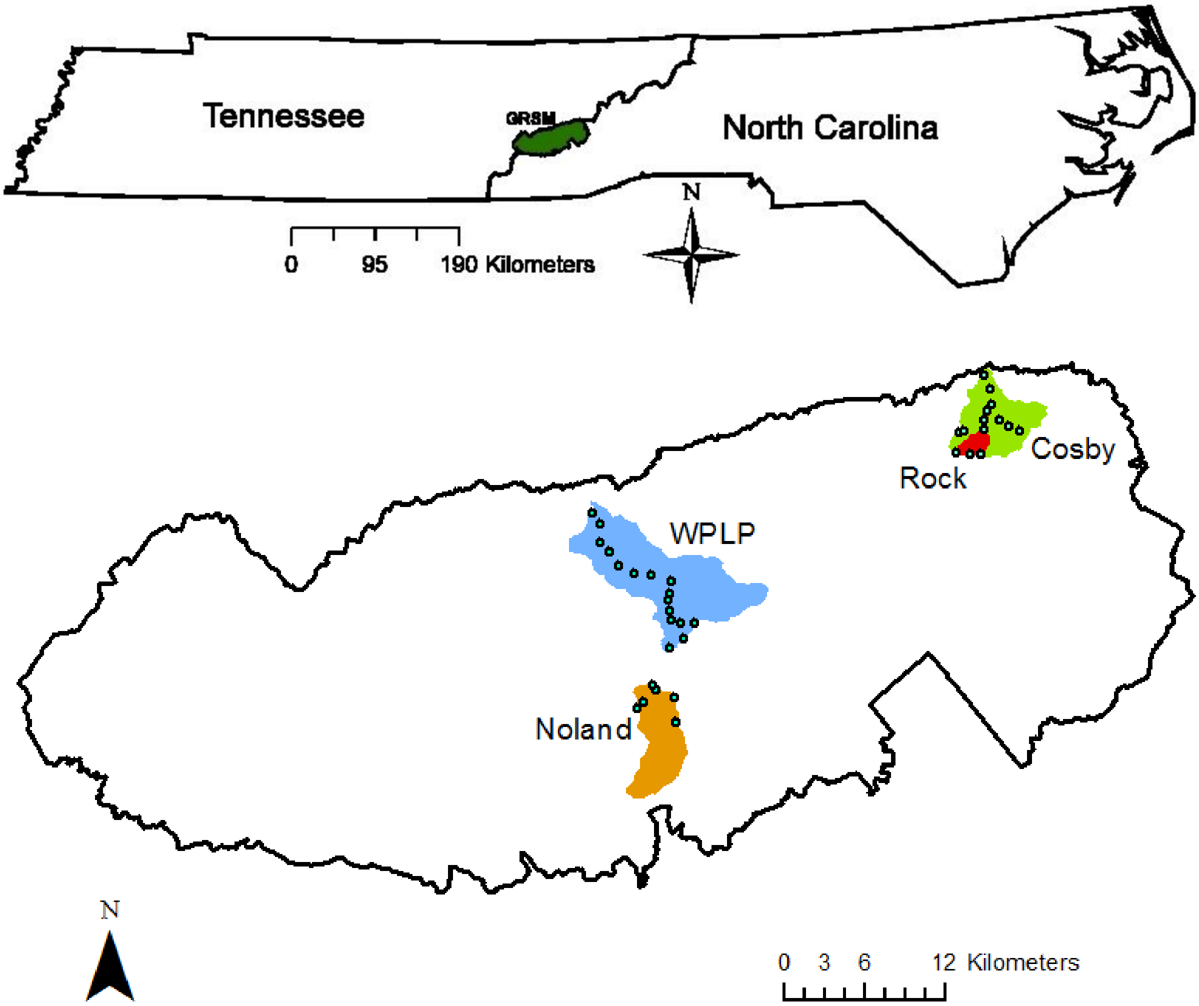

Figure 2. GSRM Watersheds Surveyed

See Appendix 1 for detailed maps of site locations.

Table 1. Characterization of soil samples sites by location, elevation, slope, A-horizon depth, soil type, and vegetative cover.

Watershed	Site No#	West Longitude (degree)	North Latitude (degree)	Elevation (m)	Average Slope (%)	A-Horizon Sample Depth (cm)	Soil Type	Vegetation type
WPLP	R1	83.4690	35.5910	1807	7	5	BpD	S(F)
	R2	83.4560	35.5980	1738	10	20	BpD	NHxB/S
	R3	86.4470	35.6090	1604	30	8	LrF	NHxB
	R4	86.4560	35.6107	1492	16	6.5	BpF	S/NHxB
	R5	86.4670	35.6110	1384	21	12	HrF	NHxB
	R6	83.4700	35.6160	1341	35	5	BpF	NHxR
	R7	83.4710	35.6240	1218	20	5	DtF	NHxR
	R8	84.4690	35.6290	1131	13	6	SsE	S/NHxB
	R9	83.4660	35.6380	1132	40	15	DtF	CHxR
	R10	83.4849	35.6417	1033	21	9	dDtF	HxL
	R11	83.4991	35.6418	861.2	31	9	RxF	OmH
	R12	83.5128	35.6470	787	17	24	SsE	HxL
	R13	83.5190	35.6560	725	23	12	JbE	OmH
	R14	83.5262	35.6616	616	11	8	JbE	CHx
	R15	83.5276	35.6736	526	8	3	SoF	OmH
	R16	83.5320	35.9800	476	14	10	SoF	OmHr
Noland	NK1	83.4637	35.5591	1622	21	8	OwF	S/NHxB
	NK2	83.4800	35.5643	1688	22	10	OwF	NHxB/S
	NK3	83.4818	35.5670	1806	20	10	BpF	RD
	NK4	83.0494	35.5597	1918	21	20	BpF	S-NHxB
	101S	83.4940	35.5520	1548	24	16	BpF	NHxE
	102S	83.4620	35.5430	1420	14	20	SoF	MOr
Cosby	C1	83.2385	35.7272	1740	35	6	LrF	S
	C2	83.2272	35.7258	1656	26	1	BpF	Sb
	C3	83.2164	35.7265	1527	23	17	BpF	S
	C4	83.2150	35.7440	883	20	30	SsE	OmHr
	C5	83.2150	35.7470	838.4	9	25	SsD	OmHL
	C6	83.2129	35.7560	719	7	23	SsC	OmHL
	C7	83.2104	35.7602	666	6	25	SsC	P
	C8	83.1870	35.7420	1123.5	35	1	DtF	OzHf
	C9	83.1966	35.7447	933	28	10	DtF	OzHf
	C10	83.2040	35.7500	774.2	3	30	SsC	HxL
	C11	83.2370	35.7407	1420	31	10	BpF	NHx
	C12	83.2330	35.7415	1299	25	11	BpF	NHx
	C13	83.2113	35.7707	573	8	15	SsC	OmHL
	C14	83.2169	35.7804	525	3	15	Dg	MALc

See Appendix 2 and 3 for soil and vegetation characteristics.

Soil Sample Collections

Soil samples were collected during March 16-18, 2011. In order to avoid effects of antecedent precipitation, at least two dry days were required prior to sampling. Because Cai et al. (2011) showed significant differences in soil chemistry between the A horizon and the B/C horizon, but little difference between the B and C horizons, samples were taken from two separate depths, one comprising of the A horizon and one the B/C horizon.

At each site three to five soil cores from both horizons were taken within a close proximity by a random sampling approach. A total of 70 excavated soil cores were taken. All samples were considered to be disturbed soil, procured using a stainless-steel hand auger with a diameter of approximately 8 cm and length of 18 cm. The depth of the A horizon and the total sample depth were measured prior to removing soil from the auger. The depth of core samples taken from the A horizon ranged from 3 to 30 cm, with an average depth of 13.5 cm.

Variations in sample depths were due to the occasional presence of a tree root or rock. All transitional soil was removed from the sample. The soil cores from the same layer were thoroughly combined into an A horizon composite sample and a B/C horizon composite sample for each site. All soil samples were immediately transported to the laboratory and stored in sealed plastic bags at a temperature of 4°C until time of analysis.

Laboratory Analysis of Soil

Only soil with a diameter of less than 2-mm was used for chemical analysis and three laboratory replicates were run for all measurements as a quality assurance/quality control measure.

Moisture Content

Moisture content was determined by drying wet weight samples at 105°C, and then subtracting a final dry weight from the initial wet weight. The weight difference divided by the wet soil weight to obtain moisture content as units of percentage. Immediately before each measurement of chemical content in soil, moisture content was tested in order to convert the chemical content to the unit of per kilogram dry soil (Hart et al. 1994).

Exchangeable ammonium and nitrate

Ammonium and nitrate were measured by extracted using 0.5 mM KCI by adding 50 ml KCl to 5 g soil samples. The samples were shaken by a reciprocal shaker at a speed of 200 rpm for 3 hours, centrifuged at 5000 rpm for 5 minutes, filtered through a 0.4 µm membrane, the filtered solution analyzed on a Dionex ion chromatograph (IC) measurement of ammonium and nitrate (Cronan and Schofield 1990; Stams and Marnette 1990).

Organic Matter Content

To determine organic matter content by the loss-on-ignition method, 1-3 g soil samples in aluminum cups were dried for 24 hrs at 105°C and weighed, and then dried at 400°C for 16 hrs and weighed. The organic matter content by taking the W_{105} subtracting the W_{400} and dividing by W_{105}.

$$\mathrm{LOI}\ \% = \frac{W_{105} - W_{400}}{W_{105}} \times 100$$

Mineralization and Nitrification Incubations

To determine net N mineralization and nitrification rates, soil samples were incubated for 7, 14, and 28 days, for each incubation period there was a different soil sample set up for this analysis. At the beginning and at the end of each incubation period, exchangeable ammonium and nitrate content in soil was measured by using the 0.5 mM KCl extraction method. The net mineralization rate was calculated as the change in total inorganic N ($NH_4^+ + NO_3^-$) during the incubation period divided by the number of days incubated. The net nitrification rate was determined by the change in nitrate concentration divided by the incubation period (Persson and Wirén 1995).

Total Kjeldahl Nitrogen and Total Organic Nitrogen

To determine total Kjeldahl nitrogen (TKN) and then total organic nitrogen (TON) of the soil, a slurry of 0.4 g of wet soil, 1.5 g K_2SO_4, 0.125 g $CuSO_4$ and 3.5 ml concentrated H_2SO_4 were placed in a digester tube and dried for 2 hours at 160°C to evaporate all water, then digested on an aluminum block digester for 2 hours at 390°C to convert all organic nitrogen to ammonium. The digested samples were allowed to cool and dionized water (DI) water was added to 50 mL, samples were then allowed to settle overnight. After that, ammonium concentration was measured using an Automated Ion Analyzer. Total organic nitrogen was calculated as:

$$\mathrm{TON} = \mathrm{TKN} - \mathrm{Exchangeable\ NH_4^+}$$

See Appendix 4 for further explanation of laboratory methods.

Data Analysis

Tukey-Kramer HDS was used for means separation and significant differences were based on a significance level (*P*-value) of less than or equal to 0.05. Single linear regression was used to identify any correlation between geomorphic factors and soil chemical properties and to evaluate the change slope of mineralization and nitrification rates with these properties. All data analysis was performed using JMP 9 software.

See Appendix 4 for full data sets for this study.

Date from soil chemical properties measured and calculated included organic matter content via the loss-on-ignition method (OM), exchangeable nitrate (NO_3^-), exchangeable ammonium (NH_4^+), mineralization rate, nitrification rate, total Kjeldahl nitrogen (TKN), and total organic nitrogen (TON) (Appendix 5). In order to evaluate the effects of watershed characterizes in study sites, soil mineralization and nitrification rates were tested on elevation, slope, vegetation type, soil type, soil contents of organic matter and organic nitrogen using Tukey test, ANOVA and single linear regression correlation.

Nitrification and mineralization rates for the WPLP and the Cosby watersheds were mapped in ArcMap 10 using a standard kriging method to interpolate nitrification and mineralization rates of measured rates across the entire watershed spatially.

RESULTS

Soil Chemistry

The A soil horizon was generally larger in organic matter, organic nitrogen, ammonium and nitrate than in the B/C horizon for all three studied watersheds (Table 2). Soil organic matter content ranged between 17% and 25% in the A horizon, and less than 10% in the B horizon. Organic nitrogen accounted for about 2% of organic matter in both A and B/C horizons. Compared to the content of organic nitrogen, the inorganic forms of nitrogen (nitrate and ammonium), where total exchangeable nitrate and ammonium content was found to be less than 0.5% of the total organic nitrogen. Soils in the studied watersheds had similar levels of exchangeable nitrate and ammonium with averages in the A horizon between 500 and 1000 µeq kg^{-1} respectively, and in the B/C horizon between 150 and 400 µeq kg^{-1} respectively. In the Cosby watershed, soil was slightly greater in ammonium compared with nitrate, whereas in Noland Divide and WPLP watersheds nitrate was slightly greater than ammonium, but these differences were not significant.

Nitrification and Mineralization Rates

Despite differences in location and watershed characteristics, potential mineralization and nitrification rates in the three studied watersheds were generally similar to each other per incubation period (Table 2). In the A soil horizon, mineralization ranged from 278 µeq kg^{-1} day^{-1} to 196 µeq kg^{-1} day^{-1}, whereas in the B/C soil horizon rates ranged from 107 µeq kg^{-1} day^{-1} to around 50 µeq kg^{-1} day^{-1}. In the A soil horizon, nitrification rates ranged from about 145 µeq kg^{-1} day^{-1}, and to 93 µeq kg^{-1} day^{-1}. In the B/C horizon, nitrification rates ranged from about 47 µeq kg^{-1} day^{-1} initially, and reduced to less than 19 µeq kg^{-1} day^{-1}. Figures 3 A,B,C show the trend in mineralization rates in all three watersheds, while Figures 4 A,B,C show the trend in nitrification rates.

Table 2. Average soil content of organic matter, total organic nitrogen, ratio of organic matter to organic nitrogen, exchangeable ammonium and exchangeable nitrate, and potential nitrification and mineralization rates among the three study watersheds during the first incubation period.

Watershed	Soil Layer	OM (%)	TON (%)	OM/TON	Nitrate (µq/kg dry soil)	Ammonium (µq/kg dry soil)	Mineralization Rates*	Nitrification Rates*
Cosby	A	24.9	0.5	50.4	737	972	278.1	93.3
	B	9.1	0.2	42	202	306	72	18.3
Noland	A	17.3	0.5	46.4	752	510	196.1	122
	B	7.4	0.2	50.2	353	293	107	47.4
WPLP	A	20.9	0.5	45.9	851	549	278.9	145.7
	B	6.8	0.1	80.9	212	169	50.1	26.9

***Nitrification and Mineralization rates: µeq kg^{-1} dry soil day^{-1}**

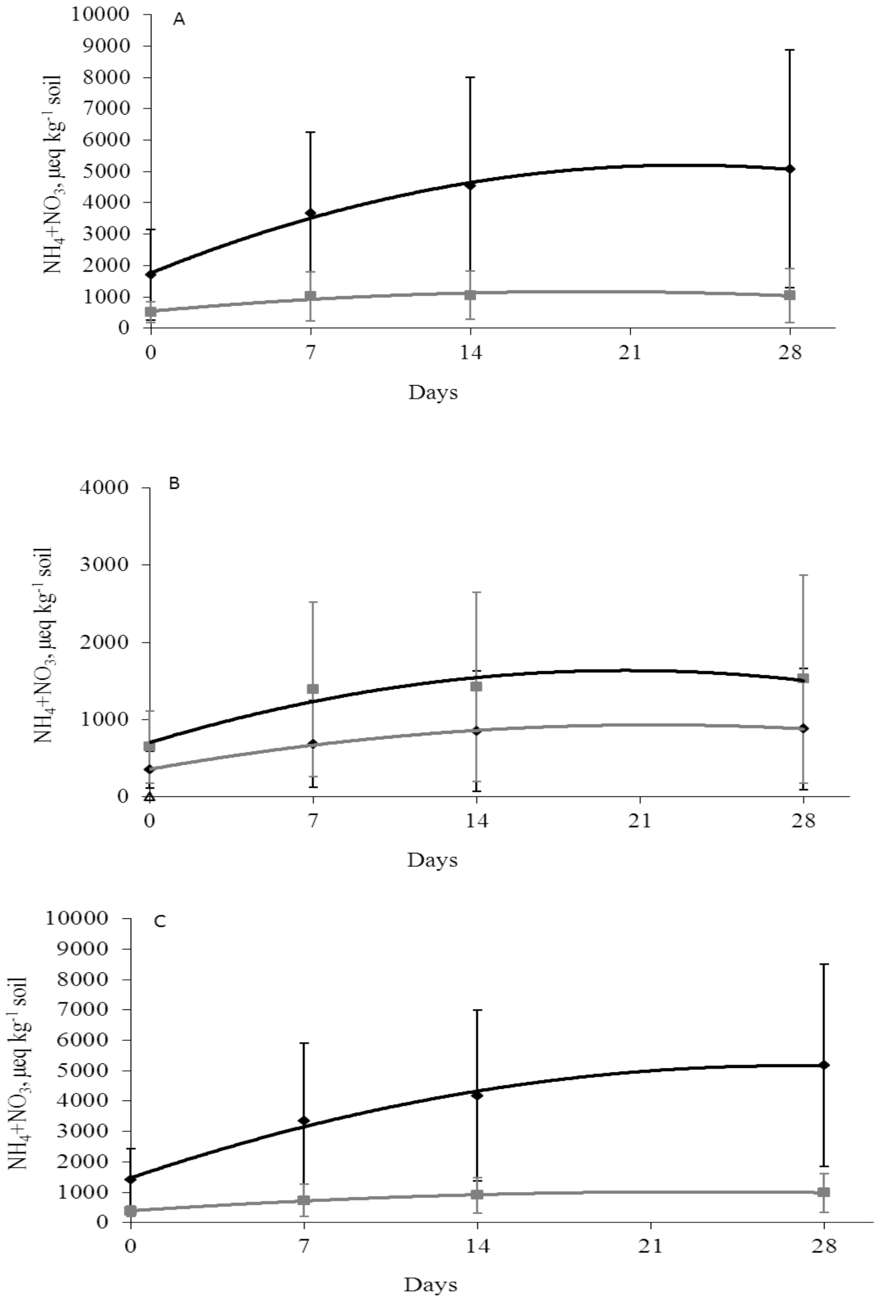

Figure 3. Representing mineralization rates, the change of inorganic nitrogen during 28-day incubation experiment for Cosby watershed (A), Noland Divide Watershed (B) and WPLP watershed (C). Dark line is the trend of data for A horizon, grey line is a fit for the data in B/C horizon.

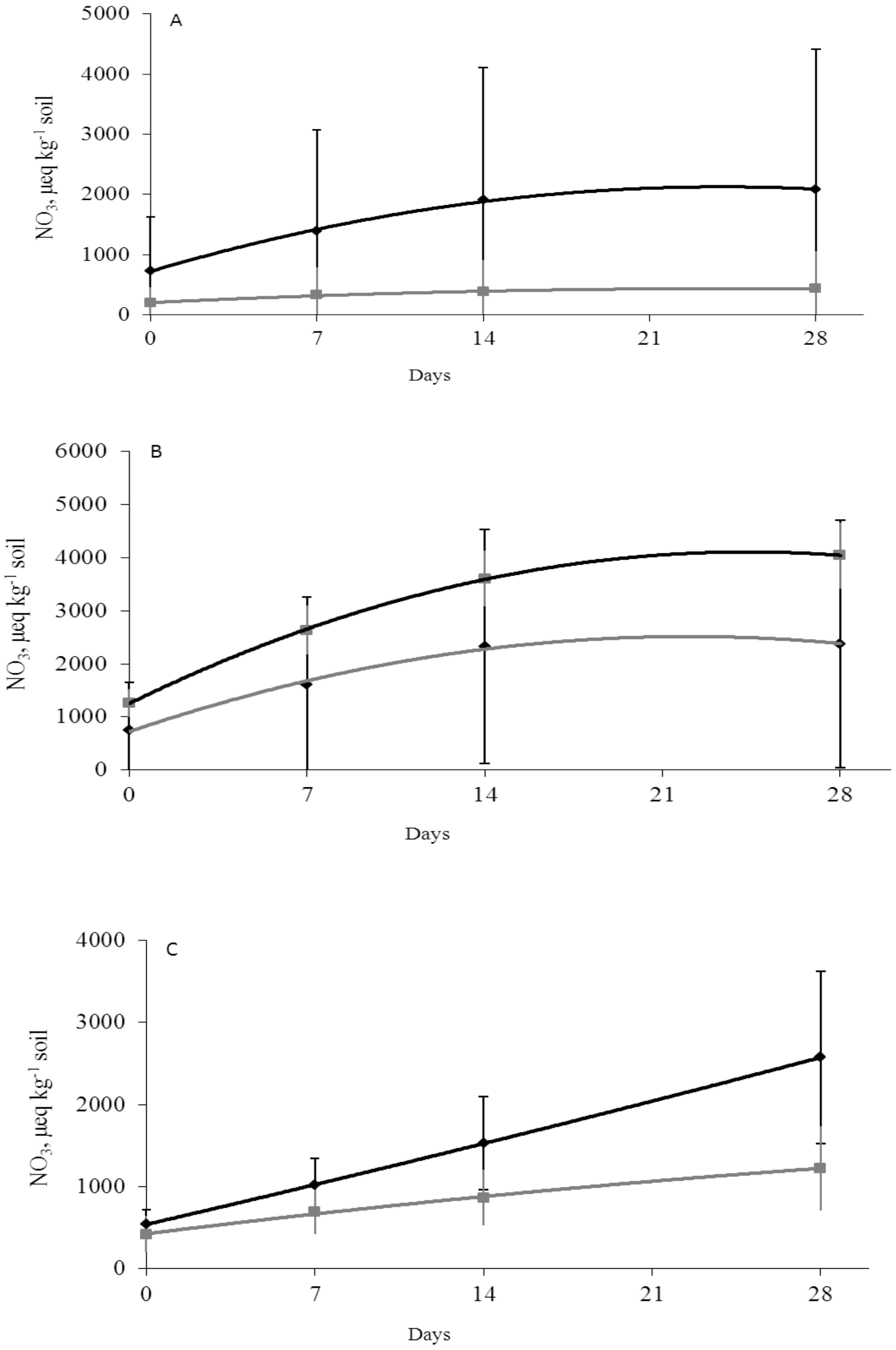

Figure 4. Representing nitrification rates, the change of total inorganic nitrogen during 28-day incubation experiment for Cosby watershed (A), Noland Divide Watershed (B) and WPLP watershed (C). Dark line is the trend of data for A horizon, grey line is a fit for the data in B/C horizon.

Statistical Means Comparison by Basin Factors

Comparison by Elevation

Nitrification showed a significant difference due to elevation in both soil horizons and per nitrification incubation period (Table 3). Of the total 36 study sites, average nitrification rates increased notably from 48 to 177 µeq kg^{-1} dry soil day^{-1} in A horizon and from 10 to 50 µeq kg^{-1} dry soil day^{-1} in B/C horizon when elevation changed from less than 500 m to above 1500 m (Table 3). Differences of nitrification rates per horizon depth were pronounced in the shallow soil layers and tended to have higher rates regardless of the change of elevation.

Mineralization rates also showed significant differences with regards to elevation in the A horizon (Table 3). The average mineralization rates for the A soil horizon were 161-339 µeq kg^{-1} dry soil day^{-1}. In the B/C soil horizon, mineralization rates ranged from 39-104 µeq kg^{-1} dry soil day^{-1}. At the same elevations, A horizon soils mineralized organic nitrogen much at greater rates then the B/C soil horizon.

Table 3: Mean 7-day net soil nitrogen mineralization and nitrification rates (µeq kg^{-1} dry soil day^{-1}) for A and B/C horizons affected by elevation change. Statistically significant figures are in bold.

Horizon	**Obs.**	**Elevation Range (m)**	**Mineralization Rate***		**Nitrification Rate***	
A.	11	>1500	**AB**	339	**A**	177
	11	1000-1500	**A**	269	**AB**	159
	14	<1000	**B**	161	**B**	48
B/C	9	>1500	**A**	104	**A**	50
	11	1000-1500	**AB**	62	**AB**	29
	14	<1000	**B**	39	**B**	10

***Nitrification and Mineralization rates: µeq kg-1 dry soil day-1**

Means Comparison by Slope

Based on this analysis, slope did not appear to influence soil chemistry in the GRSM (Table 4).

Table 4. Mean 7-day net soil nitrogen mineralization and nitrification rates (µeq kg^{-1} dry soil day^{-1}) for A and B/C horizons affected by slope.

Horizon	**Obs.**	**Slope (%)**	**Mineralization Rate***	**Nitrification Rate***
A.	16	<20	207.8	91.5
	20	>20	310.4	145.3
B/C	16	<20	46	22.8
	18	>20	85.4	30

***Nitrification and Mineralization rates: µeq kg-1 dry soil day-1**

Means Comparison by Vegetation Type

In this study, a total 21 types of forests occurred among the 36 study sites (Table 6), and these vegetation types were categorized in three different categories (conifer, deciduous, and mixed). Because of the relatively few number of observations per vegetation type, the comparison of mineralization and nitrification rates by vegetation types lacked statistical power although the mean rates were presented here within.

Table 5. Mean 7-day net soil nitrogen mineralization and nitrification rates (μeq kg-1 dry soil day^{-1}) for A and B/C horizons at first 7 days, second 7 days and last 14 days affected by vegetation type. Statistically significant figures are in bold.

Horizon	**Obs.**	**Vegetation Type**	**Mineralization Rate***		**Nitrification Rate***	
A.	22	Deciduous	A	320	A	202
	4	Conifer	A	296	A	163
	10	Mix	A	234	A	88
B/C	22	Deciduous	A	78	A	41
	4	Conifer	A	71	A	23
	8	Mix	A	62	A	18

***Nitrification and Mineralization rates: μeq kg-1 dry soil day-1**

Means Comparison by Soil Type

Similar to the mean comparison by vegetation, 14 types of soil types were categorized by their soil order. The two orders present were Inceptisol and Ultisol (Table 6). Nitrification and mineralization rates in the A horizon were significantly different between these two soil orders..

Table 6. Mean 7-day net soil nitrogen mineralization and nitrification rates (μeq kg^{-1} dry soil day^{-1}) for A and B/C horizons affected by soil type. Statistically significant figures are in bold.

Horizon	**Obs.**	**Soil Type**	**Mineralization Rate***	**Nitrification Rate***
A.	34	Inceptisols	**272**	**125.7**
	2	Ultisols	**142.9**	**48.1**
B/C	32	Inceptisols	67.6	27.5
	2	Ultisols	54.7	12.9

***Nitrification and Mineralization rates: μeq kg-1 dry soil day-1**

Statistical Correlation by Basin Factors

Correlation Analysis of Elevation vs Nitrification and Mineralization Rates

Nitrification rates were highly correlated with elevation (*P* values <0.05; n = 36), such that rates that were all over a 95% chance probability of correlation in both soil horizons (Table 7). Nitrification rates in the A horizon increased at 0.1269 µeq kg^{-1} dry soil day^{-1} m^{-1}, and in B/C horizon increased at 0.0425 µeq kg^{-1} dry soil day^{-1} m^{-1}. Mineralization rates were also significantly correlated with elevation (Table 7). Nitrification rates in the A horizon increased at 0.1578 µeq kg^{-1} dry soil day^{-1} m^{-1}, and in B/C horizon increased at 0.0816 µeq kg^{-1} dry soil day^{-1} m^{-1}.

Further analysis by each watershed showed significant correlations between nitrification rates and elevation in Cosby for the A soil horizon, and in WPLP for the B soil horizon (Table 7). Significant correlations between mineralization rates and elevation were exhibited in both WPLP and Cosby for the A soil horizon, but only Cosby for the B soil horizon.

Soil content of organic matter was positively correlated with elevation in B/C soil horizon (Table 7). In contrast, soil organic nitrogen was not different by elevation in the B/C horizon, but generally increased with elevation at the trend slope of 0.0002 % m^{-1}. The increase in organic matter percentage over a meter of elevation change in the A soil horizon.

Table 7. Change of mineralization and nitrification rates, organic matter, and total organic nitrogen by elevation. The unit of trend slope is µeq kg^{-1} dry soil day^{-1} m^{-1}. Trend slopes with *P*-value below 0.05 were considered statistically significant (figures in bold).

Soil Layer	**Water-Shed**	**Elevation vs**	**Mineralization Rate***	**Nitrification Rate***	**Slope (%)**	**OM (%)**	**TON**
A	**All**	Slope	0.1578	0.1269	0.009	0.009	0.0002
		P	**0.031**	**0.007**	**0.01**	0.08	**0.038**
		obs: **36**					
	Noland	Slope	0.0122	0.1769	0.007		-0.0003
		P	0.974	0.634	0.487		0.666
		obs: **6**					
	WPLP	Slope	0.2705	0.1533	0.002		0.0003
		P	**0.046**	0.06	0.789		**0.03**
		obs: **16**					
	Cosby	Slope	0.2622	0.1772	0.024		0.0003
		P	**0.022**	**0.016**	**0**		0.091
		obs: **14**					
B/C	**All**	Slope	0.0816	0.0425	0.01	0.003	0.0001
		P	**0.002**	**0.002**	**0.02**	**0.04**	0.07
		obs: **34**					
	Noland	Slope	0.4237	0.2245	0.011	0.009	
		P	0.2408	0.1866	0.4714	0.4119	
		obs: **5**					
	WPLP	Slope	0.0529	0.0373	0.002	0.002	
		P	0.119	**0.05**	0.789	0.259	
		obs: **16**					
	Cosby	Slope	0.1208	0.0389	0.026	0.008	
		P	**0.008**	0.11	**0**	**0.01**	
		obs: **13**					

***Nitrification and Mineralization rates: µeq kg-1 dry soil day-1**

Correlation Analysis of Slope vs Nitrification and Mineralization Rates

The nitrification and mineralization rates were not statistically correlated with site slope as *P* values were all greater than 0.05, indicating a less than a 95% chance probability of correlation with all the watersheds (Table 8).

Table 8. Change of mineralization and nitrification rates by slope. The unit of slope is µeq kg-1 dry soil day^{-1} m^{-1}. Slopes with *P*-value below 0.05 are statistically significant (figures in bold).

Soil Layer	**Water-Shed**	**Slope vs**	**Mineralization Rate***	**Nitrification Rate***
A	**All**	Slope	5.227	3.035
		P	0.11	0.16
		obs: **36**		
B/C	**All**	Slope	2.191	0.731
		P	0.06	0.23
		obs: **34**		

***Nitrification and mineralization rates: µeq kg-1 dry soil day^{-1} m^{-1}**

Correlation Analysis of OM vs Nitrification and Mineralization Rates

Nitrification rates were highly correlated in the B/C horizon, where *P* values were less than or equal to 0.05 (Table 9). Further analysis showed a correlation with the Noland Divide watershed in the A horizon, and Noland Divide and WPLP in the B/C horizon. Noland Divide nitrification rates increased at rates of 21.96 µeq kg^{-1} dry soil day^{-1} %$^{-1}$ (the change in rate per change in % OM) the A horizon, and 16.69 µeq kg^{-1} dry soil day^{-1} %$^{-1}$ in the B/C horizon. WPLP nitrification rate increased 8.68 µeq kg^{-1} dry soil day^{-1} %$^{-1}$ in the B/C horizon.

The mineralization rate was highly correlated with OM, p values were less than 0.05 for both soil horizons (Table 9). In the A horizon the mineralization rate increased at 8.64 µeq kg^{-1} dry soil day^{-1} %$^{-1}$. In the B/C horizon the mineralization rate increased at 13.83 µeq kg-1 dry soil day^{-1} %$^{-1}$. Further analysis of each watershed showed that Noland Divide in the B/C horizon and Cobsy in both soil horizons showed significant trends for OM and mineralization rates. Cosby showed increases in mineralization rates of 11.32 µeq kg^{-1} dry soil day^{-1} %$^{-1}$ in the A horizon, and an increase of 13.36 µeq kg-1 dry soil day^{-1} %$^{-1}$ in the B/C horizon. Noland Divide showed an increase in mineralization rates in the B/C horizon showed an increase of 33.89 µeq kg-1 dry soil day^{-1} %$^{-1}$.

Table 9: Change of mineralization and nitrification rates by organic matter (OM). The unit of trend slope is µeq kg^{-1} dry soil day^{-1} %$^{-1}$. Trend slopes with *P*-values less than or equal to 0.05 were statistically significant (figures in bold).

Soil Layer	**Water-Shed**	**OM vs**	**Mineralization Rate***	**Nitrification Rate***
A	**All**	Slope	8.64	2.55
		P	**0**	0.1
		obs: **36**		
	Noland	Slope	20.4	21.96
		P	0.06	**0.03**
		obs: **6**		
	WPLP	Slope	5.43	2.91
		P	0.13	0.2
		obs: **16**		
	Cosby	Slope	11.32	1.98
		P	**0**	0.42
		obs: **14**		
B/C	**All**	Slope	13.83	5.03
		P	**0**	**0**
		obs: **34**		
	Noland	Slope	33.89	16.69
		P	**0.02**	**0.01**
		obs: **5**		
	WPLP	Slope	8.54	8.68
		P	0.08	**0**
		obs: **16**		
	Cosby	Slope	13.36	3.54
		P	**0**	0.09
		obs: **13**		

***Nitrification and mineralization rates: µeq kg^{-1} dry soil day^{-1} %$^{-1}$**

Correlation Analysis of TON vs Nitrification and Mineralization Rates

Statistical correlations between nitrification rates and total organic nitrogen (TON) were highly correlated in both soil horizons (Table 10). Nitrification rates were 260.7 µeq kg^{-1} dry soil day^{-1} %$^{-1}$ in the A horizon, and 109.4 µeq kg-1 dry soil day^{-1} %$^{-1}$ in the B/C horizon. Per analysis of each watershed, WPLP watershed was observed with correlations in both the A and B/C soil horizons.

Statistical correlations between mineralization rates and TON were highly correlated with both soil horizons. Mineralization rates were 598.2 µeq kg^{-1} dry soil day^{-1} %$^{-1}$ in the A soil horizon, and 377.9 µeq kg^{-1} dry soil day^{-1} %$^{-1}$ in the B/C soil horizon. WPLP and Cosby watersheds showed a significant difference in the A horizon with mineralization rates, but only the Cosby watersheds showed a correlation within the B/C soil horizon. For the A soil horizon, WPLP had slope of 728.6 µeq kg^{-1} dry soil day^{-1} %$^{-1}$, while Cosby had slope of 887.2 µeq kg-1 dry soil day^{-1} %$^{-1}$. Within the B/C horizon, Cosby had an increase of mineralization of 353.9 µeq kg-1 dry soil day^{-1} %$^{-1}$.

Table 10. Change of mineralization and nitrification rates by TON. The unit of trend slope is µeq kg-1 dry soil day^{-1} %$^{-1}$. Trend slopes with *P*-value less than or equal to 0.05 were statistically significant (figures in bold).

Soil Layer	**Water-Shed**	**TON vs**	**Mineralization Rate***	**Nitrification Rate***
A	**All**	Slope	598.2	260.7
		P	**0**	**0**
		obs: **36**		
	Noland	Slope	232.2	153
		P	0.34	0.55
		obs: **6**		
	WPLP	Slope	728.6	357.6
		P	**0**	**0**
		obs: **16**		
	Cosby	Slope	887.2	221.9
		P	**0**	0.1
		obs: **14**		
B/C	**All**	Slope	377.9	109.4
		P	**0**	**0.03**
		obs: **34**		
	Noland	Slope	822.6	362.8
		P	0.08	0.14
		obs: **5**		
	WPLP	Slope	194.1	222.9
		P	0.15	**0.01**
		obs: **16**		
	Cosby	Slope	353.9	67.8
		P	**0.01**	0.36
		obs: **13**		

***Nitrification and Mineralization rates: µeq kg-1 dry soil day^{-1} %$^{-1}$**

Correlation Analysis of OM/TON vs Nitrification and Mineralization Rates

Nitrification and mineralization rates were not statistically correlated with the ratio of OM/TON as p values were all greater than 0.05, less than a 95% chance probability of correlation with all the watersheds (Table 11).

Table 11. Change of mineralization and nitrification rates by OM/TON. The unit of trend slope is μeq kg-1 dry soil day^{-1} m^{-1}. Trend slopes with *P*-value less than or equal to 0.05 were statistically significant (figures in bold).

Soil Layer	**Water-Shed**	**OM/TON vs**	**Mineralization Rate***	**Nitrification Rate***
A	**All**	Slope P obs: **36**	-0.84 0.63	-1.54 0.26
B/C	**All**	Slope P obs: **34**	-0.87 0.11	-0.29 0.34

***Nitrification and Mineralization rates: μeq kg-1 dry soil day^{-1} m^{-1}**

Correlation Analysis of A Horizon Depth vs Nitrification and Mineralization Rates

Nitrification and mineralization rates were not statistically correlated with the A horizon depth as p values were all greater than 0.05, less than a 95% chance probability of correlation with all the watersheds (Table 12).

Table 12. Change of mineralization and nitrification rates by A horizon depth. The unit of trend slope is µeq kg-1 dry soil day^{-1} cm^{-1}. Trend slopes with *P*-value less than or equal to 0.05 were statistically significant (figures in bold).

Soil Layer	**Water-Shed**	**A horizon Depth vs**	**Mineralization Rate***	**Nitrification Rate***
A	**All**	Slope	-5.51	-4.79
		P	0.19	0.08
		obs: **36**		
B/C	**All**	Slope	-2	-1.38
		P	0.19	0.08
		obs: **34**		

***Nitrification and Mineralization rates: µeq kg-1 dry soil day^{-1} cm^{-1}**

Maps of Nitrification and Mineralization Rates in WPLP and Cosby Watersheds

The WPLP watershed showed a reduction of both nitrification and mineralization rates from high to low elevations along the elevation gradient (Figures 5 and 6). The Cosby watershed shared the same trend of the A horizon showing a change in nitrification and mineralization rates during all three incubation periods (0-7, 7-14, 14-28 days) whereas the B/C horizon were about the same rates for all three periods (Figures 7 and 8). Cosby showed a relationship with a high nitrification rate and elevation, as the lower right corner of the map shows low nitrification rate, but high elevation. The rates map for Noland watershed was not generated because we only had data for six sites with limited range in an elevation gradient.

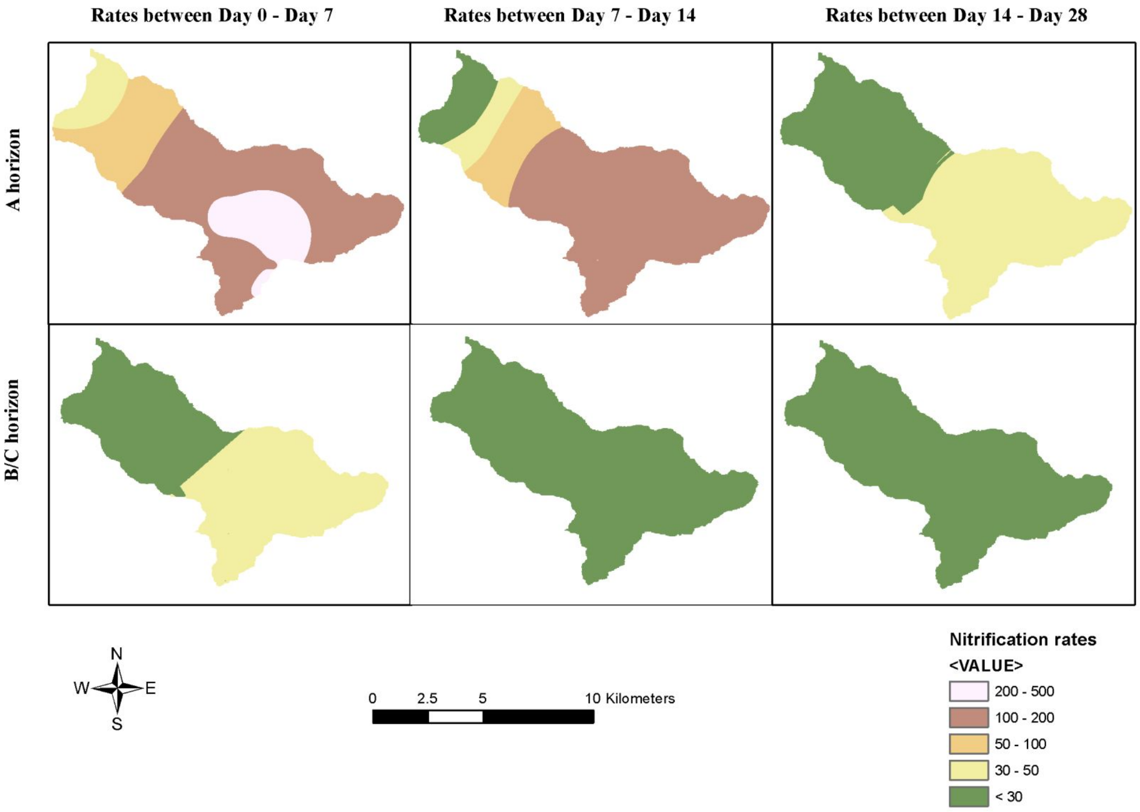

Figure 5. Potential nitrification rates at the unit of µeq kg^{-1} dry soil day^{-1} in the WPLP watershed for A and B/C soil horizons during three incubation periods. The watershed elevation reduces in the direction from lower right to upper left in charts. These maps were interpolated by using measured nitrification rates of 16 sites in this watershed through kriging method in ArcMap 10.

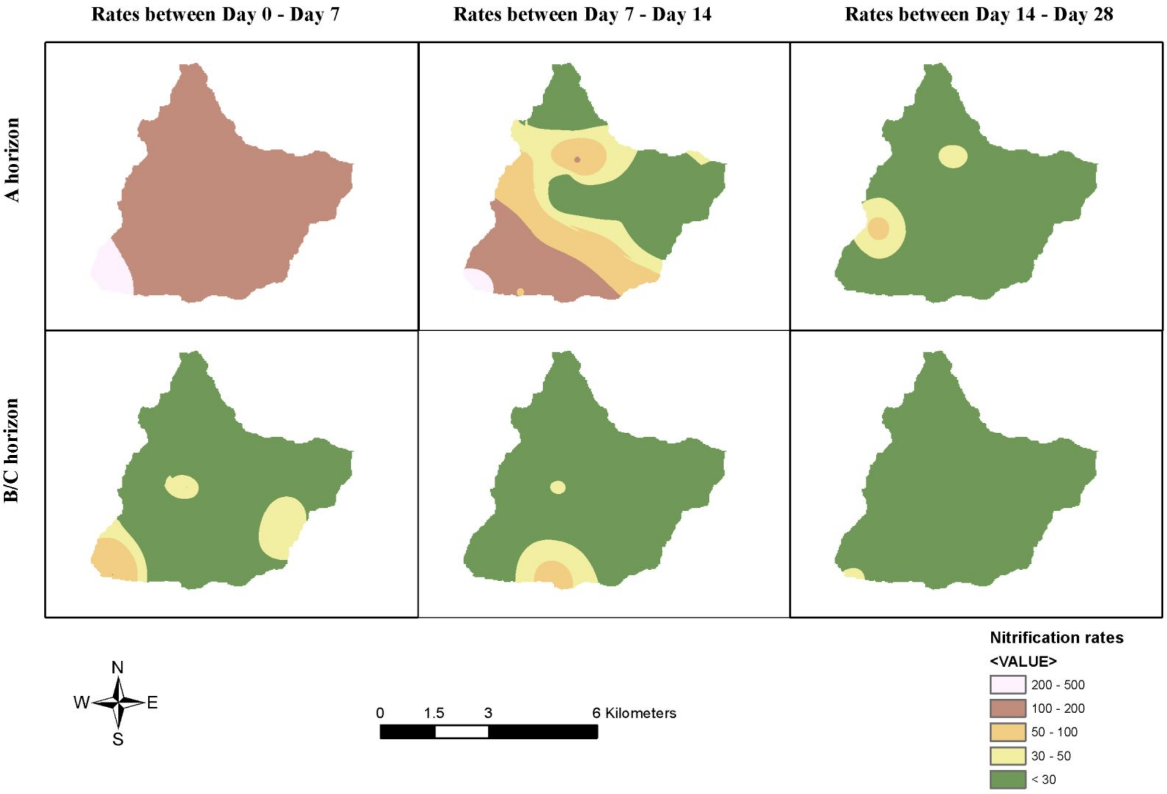

Figure 6. Potential nitrification rates at the unit of µeq kg^{-1} dry soil day^{-1} in the Cobsy watershed for A and B/C soil horizons during three incubation periods. The watershed elevation reduces in the direction from lower to upper in charts. These maps were interpolated by using measured nitrification rates of 14 sites in this watershed through kriging method in ArcMap 10.

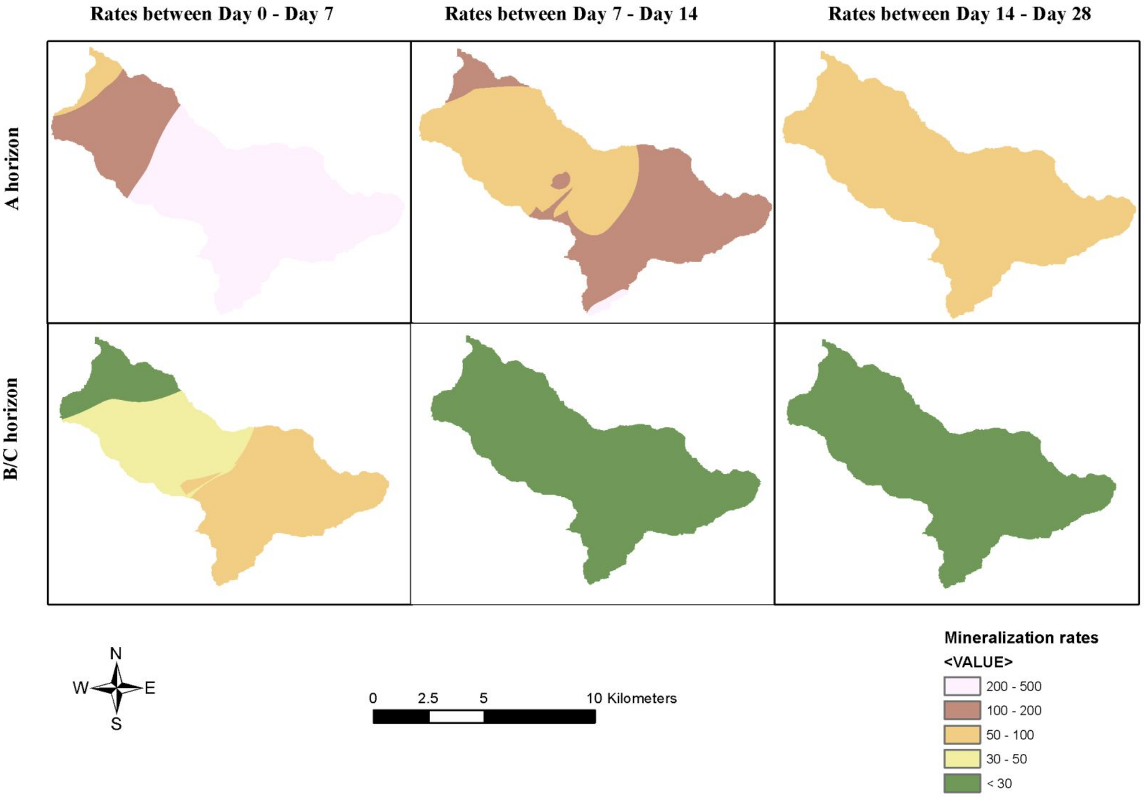

Figure 7. Potential mineralization rates at the unit of µeq kg^{-1} dry soil day^{-1} in the WPLP watershed for A and B/C soil horizons during three incubation periods. The watershed elevation reduces in the direction from lower to upper in charts. These maps were interpolated by using measured nitrification rates of 14 sites in this watershed through kriging method in ArcMap 10.

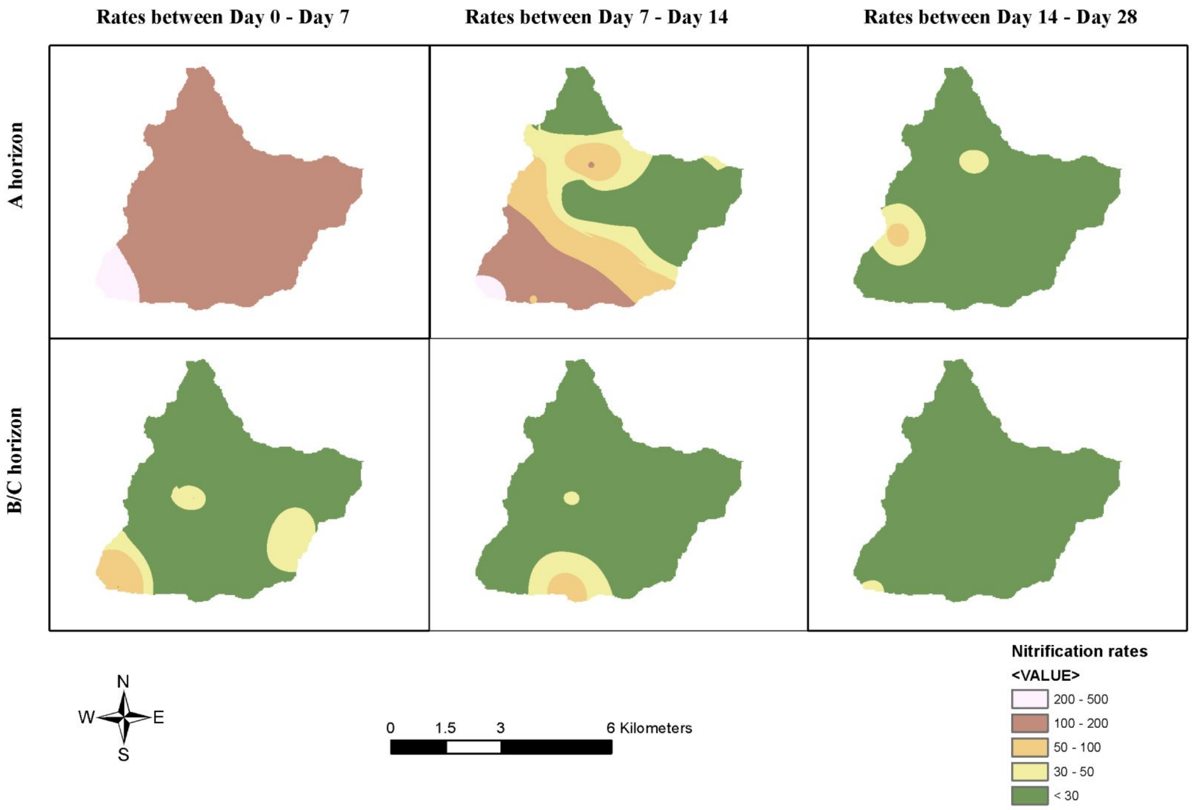

Figure 8. Potential mineralization rates at the unit of µeq kg^{-1} dry soil day^{-1} in the Cosby watershed for A and B/C soil horizons during three incubation periods. The watershed elevation reduces in the direction from lower to upper in charts. These maps were interpolated by using measured nitrification rates of 14 sites in this watershed through kriging method in ArcMap 10.

DISCUSSION

Elevation Gradient

In the GRSM, elevation plays an important role in regulating the nitrogen cycle by affecting nitrification and mineralization rates. Nitrification and mineralization rates increased with elevation, and these increases in rates were observed in both A and B/C soil horizons. However, rates in the A horizon were pronounced in contrast to the B/C horizon. OM and TON showed a positive correlation between the nitrification and mineralization rates. This showed that both OM and TON can limit organic matter decomposition reactions associated with the nitrogen cycle. Other environmental conditions, such as vegetation type, soil type, site slope and soil depth appear to minimally influence microorganism activity associated with the nitrogen cycle, at least not as significantly as elevation and soil horizon differences. In the GRSM, both A and B/C horizon were rich in organic nitrogen as TON content was about 200 times greater than soil inorganic nitrogen content as $NH_3 + NO_{3.}$

Elevational differences in mineralization and nitrification rates have been shown in previous studies around the GRSM. In the Coweeta Hydrologic Laboratory, a southern Appalachian watershed, Garten and Van Miegroet (1994) reported that above 700 m elevation mineralization and nitrification rates were greater than below 700 m, around 1.0 µg N g dry soil^{-1}below 700 m and about 5.3 µg N g dry soil^{-1} above 700 m elevation. In this GRSM study the same pattern was observed except the elevation threshold was found to be significant between elevations above 1500 m and below 1000 m.

Among the 36 study sites, organic nitrogen content significantly differed by elevation in the A soil horizon, and it was also significantly different in the B/C horizon ($P< 0.1$). Positive correlations between organic nitrogen content and mineralization and nitrification rates were found by simple regression models. The increase of reaction rates and soil organic nitrogen with elevation implied that the elevation trend for mineralization and nitrification rates may be caused by the increase of organic nitrogen content along the elevation. Nitrogen deposition levels have been correlated to elevation with significantly higher loads in higher elevation (Weathers et al., 2006). The excess nitrogen provided by acidic deposition appears to increase both nitrification and mineralization rates.

Environmental conditions like temperature may also affect the mineralization of nitrogen. Microorganisms responsible for mineralization and nitrification are poikilotherms, in which temperature and soil moisture are main regulators for their nitrogen constitute transformations. Temperature is inversely correlated with elevation change, but an increase of elevation is directly correlated with precipitation, which makes it difficult to access which factor is limiting these microorganisms (Drury et al., 1991; Bonito et al., 2003). Knoepp and Swank(1998) in a study of elevation and vegetation in the Appalachian Mountains found a counter intuitive result to the predicted assumption of temperature/elevation versus nitrification rate, where nitrification increased with elevation. Fernandez et al. (2003) in a study of nitrification and mineralization rates along a climate gradient in Maine showed that temperature was negatively correlated with mineralization rates, an indication that higher elevation sites would have higher nitrification rates. Precipitation and soil moisture are positively correlated with mineralization rates, and due to increases in precipitation with elevation this plays a role in governing nitrification and mineralization along an elevation gradient (Knoepp and Swank, 1998).

During March 2011 in the GRSM, the survey month in this study, the temperature was recorded as between -8.3 to 15 ^{o}C at the Mt. Leconte weather station weather station around 1940 m, and it was -3 to 22.5 ^{o}C at the Gatlinburg weather station located at 560 m in the GRSM (NPS 2011, NWS 2011). The temperature range difference inferred that the general temperature differences from the lowest to the highest sites were around 5 to 7^{o}C. This temperature difference may not affect the biologic activities dramatically. Knoepp and Swank (1998) reported that annual temperature fluctuations do not necessarily directly affect mineralization and nitrification rates.

Watershed Differences

Of this study's three watersheds, Cosby and WPLP watersheds were significantly affected by elevation with nitrification and mineralization rates. Cosby and WPLP were similar in mineralization and nitrification rates for both A and B/C soil horizons. At the upper headwater areas of WPLP, application of dolomite chat to the road during severe winter weather added calcium and some magnesium into adjacent soil compared with other areas in the watershed. It appears the addition of these base cations to the soil does not affect the mineralization and nitrification rates.

The observed insignificant trend of nitrification rates by elevation in the Noland Divide watershed was probably due to the elevation range of sampled soil, the number of samples taken, and/or weather. The higher elevation samples were collected with snow still covering the ground, and this could cause nitrification and mineralization rates to differ compared to samples taken with non-snow cover. Research by Knoepp and Swank (1998) suggest this may be a valid interpretation. Only 6 samples were taken in the Noland Divide watershed, which is eight less than the Cosby watershed (14 samples), and ten less than the WPLP watershed (16 samples). The lower number of samples may have not provided sufficient statistical power to detect a significant trend. The elevation range covered in the Noland Divide watershed was 386.0 m, while Cosby was 598.5 m, and WPLP was 1,331.0 m.

In order to extend the elevation range in the Noland Divide watershed, results from this study were compared to Cai et al. (2011), which conducted a similar study on four sites from 2008 to 2009. These four sites were located from 1700 m to 1900 m in Noland Divide, and included samples for four seasons to get annual average mineralization and nitrification rates. In Cai et al. (2011), annual average mineralization rates were around 93 and 40 µeq kg^{-1} day^{-1} for the A and B/C horizons, respectively. Sample mean nitrification rates decreased approximately 65 and 30 µeq kg^{-1} day^{-1} between the A and B/C soil horizons. Compared to this previous study the rates were smaller than the current study, but on the same order.

Soil A and B/C Horizons

The A and B/C soil horizons have significantly different chemical properties. The A soil horizon generally contained partially decomposed organic matter, which allows for excess carbon and nitrogen within the soil ecosystem, whereas the B/C layer was mostly composed of mineral soil. The B/C soil horizon showed significant correlations in each watershed with organic matter ($P = 0.04$). Organic matter was also significantly correlated with elevation in the

B/C soil horizon, whereas the total organic nitrogen only showed correlations with elevations in the A horizon. Lack of excess organic matter can reduce chemical and biological activity, reduced inputs for microorganism (Brady and Weil, 2008), and the measured levels of organic content in this study's samples support this claim. In this study, mineralization and nitrification rates were highly correlated with organic carbon content in both A and B/C soil horizons, implying that organic carbon is a limiting factor to the decomposition of organic nitrogen in soil. Mineralization rates were highly correlated with elevation, but the B/C horizon had a lower p-value of 0.002 compared to the A horizon of 0.031. This result also supports the idea that mineralization rates in the B/C layer are a better indicator of the significant of nitrification at different elevations. Higher nitrification and mineralization rates in the A horizon occur because of its higher organic matter, which usually contains a surplus of organic nitrogen (Sullivan, 2007).

In comparing the mean chemical and physical properties between the A and B/C soil horizons, the A soil horizon was consistently greater than the B horizon for organic matter, total organic nitrogen, nitrification rates, and mineralization rates. Comparatively, the organic matter and total organic nitrogen were three times greater, the nitrification rate ranged from 4 to 6 times greater, and the mineralization rate ranged from 3 to 12 times greater in the A horizon compared to the B/C horizon. The total organic matter/total organic nitrogen was approximately 1 to 1 between the A and B/C horizon.

Vegetation, Soil Type and Site Slopes

Significant correlations were not observed for nitrification and mineralization rates, and vegetation types, slope classes, and soil types. In this study, soils were sampled among 21 different vegetation types. Grouped into 3 different vegetation classes, they were not found statistically significant. This result may be due to the means comparison statistical test where there was a limited number of observations per vegetation type. Elevation and slope have been correlated with vegetation type in other studies, and typically in higher elevation locations the slope is steeper (Palmer et al., 2004). In the three study watersheds the slope typically increased with elevation, and soils were found with higher nitrate levels in higher elevations areas. However, nitrification rates and slope were not significantly correlated. Soil type did show a significance in both mineralization and nitrification rates in only the A horizon. Inceptisols showed a much higher mean than Ultisols (272 vs 143 µeq kg-1 dry soil day-1 for mineralization, and 126 vs 48 µeq kg-1 dry soil day-1 in nitrification rates). Inceptisols were found at every high elevation site, whereas Ultisols are all found at low elevation sites. The difference of these two soil types was due more to the influence of elevation and rates than the actual difference in soils.

Summary

Nitrification and mineralization rates within three GRSM watersheds were directly correlated to elevation within a range of 450 m to 2000 m. Microorganisms, temperature, and soil moisture have correlated nitrification and mineralization rates along elevation gradients in other studies (Knoepp and Swank 1998). While relationships between microbial-based chemical reactions and air temperature predicted negative correlations with nitrification rates and elevation, there are geomorphic factors that can influence these rates too. For example, soil moisture was positively correlated with nitrification rates and elevation (Knoepp and Swank 1998). The Cosby and WPLP watersheds both showed significant relationships between nitrification and mineralization rates, and elevation, while the Noland Divide watershed did not due apparently due to insufficient sample data along an elevation gradient. Correlation and means comparison statistical analyses showed that nitrification and mineralization rates were significantly different by soil horizon along the elevation gradient. The A and B/C horizons also showed significant relationships with organic matter and total organic nitrogen in the soil, influencing for both nitrification and mineralization rates. The B/C horizon was correlated to organic matter because the A horizon had an excess of organic constituents that control nitrification and mineralization rates, while the B/C horizon was limited with organic content. Vegetation type, soil type, and site ground slope did not show any significant difference with nitrification and mineralization rates as it appears there was not enough variety of each vegetation and soil type to perform statistical testing with enough power. Nitrification rates along an elevation gradient within the GRSM are influenced by many complex interactions and biogeochemical processes in the soils. Studying nitrification and mineralization rates per elevation can help us better understand observed seasonal and watershed variances in stream acidification responses and their potential impact on aquatic ecosystems.

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APPENDIX

Appendix 1. Site Location Figures

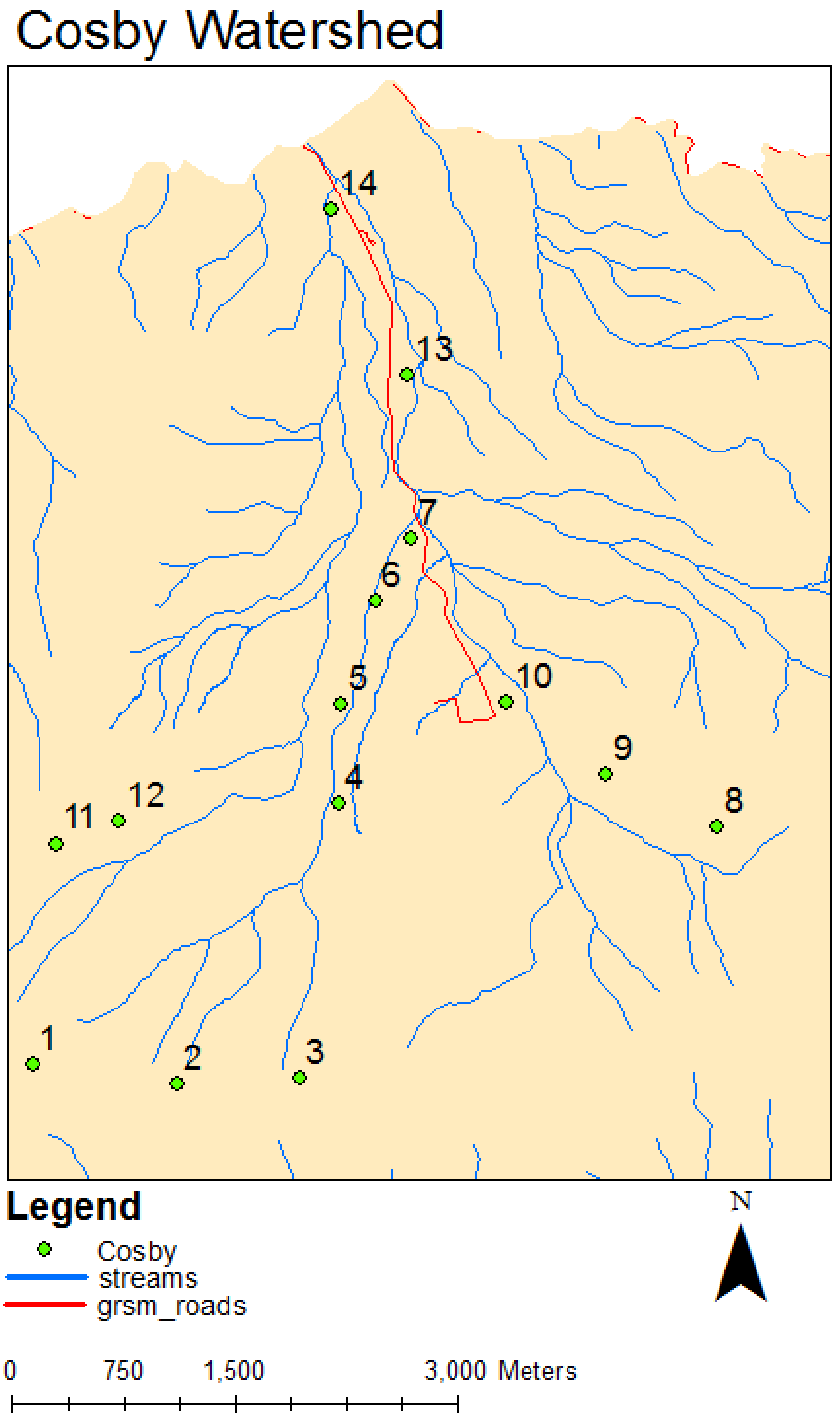

Figure Appendix 3.1. Cosby watershed made in ARCMAP 10.

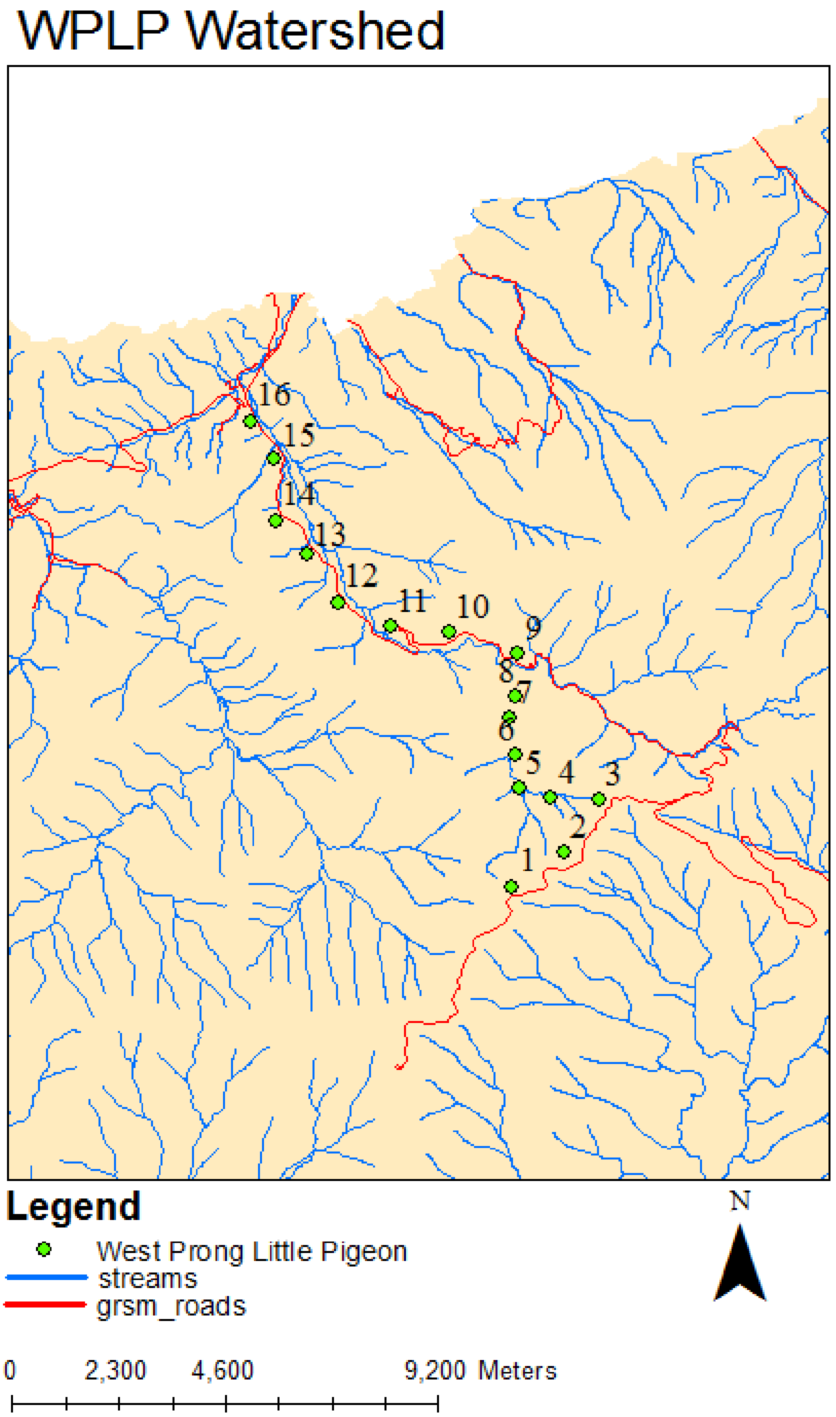

Figure Appendix 3.2. WPLP watershed made in ARCMAP 10.

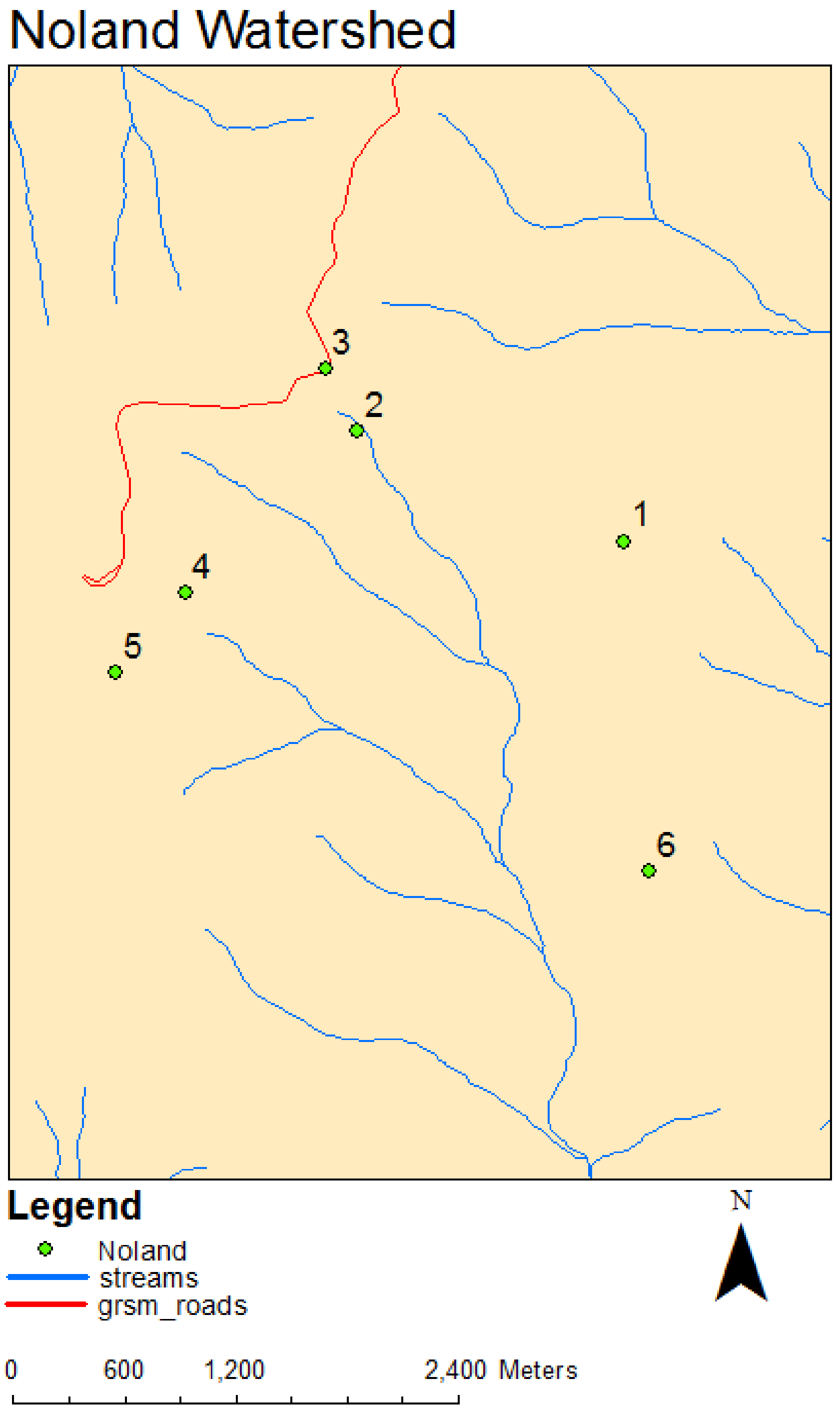

Figure Appendix 3.3. Noland watershed made in ARCMAP 10.

Appendix 2. Soil Symbol Legend for GRSM

Symbol	Soil Type & Description
BpD	Breakneck-Pullback complex, 15 to 30 percent slopes, very rocky
BpF	Breakneck-Pullback complex, 30 to 95 percent slopes, very rocky
Dg	Dellwood-Smokemont complex, 0 to 5 percent slopes, frequently flooded
DtF	Ditney-Unicoi complex, 30 to 95 percent slopes, very rocky
HrF	Heintooga-Rubble land complex, 50 to 95 percent slopes, extremely bouldery
JbE	Junaluska-Brasstown complex, 30 to 50 percent slopes, stony
LrF	Luftee-Anakeesta complex, 30 to 95 percent slopes, very rocky
OwF	Oconaluftee-Guyot-Cataloochie complex, 50 to 95 percent slopes, stony, windswept
RxF	Rubble land-Spivey complex, 50 to 95 percent slopes, extremely bouldery
SoF	Soco-Stecoah complex, 30 to 95 percent slopes, stony
SsC	Spivey-Santeetlah-Nowhere complex, 8 to 15 percent slopes, very stony
SsD	Spivey-Santeetlah complex, 15 to 30 percent slopes, very stony
SsE	Spivey-Santeetlah complex, 30 to 50 percent slopes, very stony

Appendix 3. Vegetation Symbol Legend for GRSM

Symbol	Soil Type & Description
CHx	Southern Appalachian Cove Hardwood Forests (2000-4000/4500 ft.)
CHxR	Southern Appalachian Cove Hardwoods, Rich Type (with Hemlock)
HxL	Tuliptree-Red Maple-Sweet Birch -(Black Locust),
MALc	American Hornbeam Thicket
MOr	Montane Northern Red Oak (3500-5000 ft.)
NHx	Southern Appalachian Northern Hardwoods (4000-5500/6000 ft.)
NHxB	S. Appalachian Northern Hardwoods, Yellow Birch Type
NHxB/S	S. Appalachian Northern Hardwoods, Yellow Birch Type (4800-6000 ft.)
NHxE	Exposed, Disturbed Northern Hardwood Woodland /(Spruce)
NHxR	Southern Appalachian Northern Hardwoods, Rich Type (3500-5500 ft.)
OmH	Submesic to Mesic Oak/Hardwoods (1000-3500/4000 ft.)
OmHL	Red Oak-Red Maple Type, Liriodendron co-dominant
OmHR	Spivey-Santeetlah complex, 30 to 50 percent slopes, very stony
OzHf	Chestnut Oak-Red Maple/Sourwood/Herbaceous Forest (2000-3000 ft.)
P	Pine
RD	Rhododendron
S	Red Spruce
S(F)	Red Spruce - Fraser Fir
S/NHxB	Red Spruce - Birch- (Northern Hardwood) / Shrub/ Herbaceous (4500-6000 ft.)
Sb	Red Spruce/Southern Mountain Cranberry-Low Shrub/Herbaceous (5400-6200 ft.)

Appendix 4. Extended Lab Methods

Only soil with a diameter less than 2-mm was used for chemical analysis and three laboratory replicates were run for all measurements as a quality assurance/quality control measure.

Mineralization and Nitrification Incubations

To determine net N mineralization and nitrification rates, a 50 g soil sample containing approximately 30% moisture was placed in a plastic cup, covered with aluminum foil which was perforated for ventilation, and incubated in the dark at ~22oC for 7, 14, and 28 days. At the end of each incubation period, a 5-g subsample of the incubated soil taken was extracted with 50mL of 0.5 mM KCl, filtered, and chilled until analysis of NH4+ and NO3- by IC (Jefts et al., 2004). A pre-incubation sample was also analyzed to represent time zero.

The net mineralization rate was calculated as the change in total inorganic N (NH4+ + NO3-) during the incubation period divided by the number of days incubated. The net nitrification rate was determined by the change in nitrate concentration divided by the incubation period (Persson and Wirén, 1995).

Moisture Content

Moisture content was determined gravimetrically (Hart et al. 1994). Since the composite samples were air-dried at 4°C prior to measurement, these values are not an accurate measure of in-situ moisture content, but rather a means of converting chemical property values into units of kilograms of dry soil. Approximately 5 g of wet soil was placed in an aluminum cup and weighed. The cup containing the soil was then placed in a 105°C oven and allowed to dry to a constant weight. Moisture content on a dry weight basis was then calculated by subtracting the final dry weight from the initial wet weight.

Exchangeable ammonium and nitrate

Ammonium and Nitrate were extracted using KCI through the following steps (Cronan and Schofield 1990; Stams and Marnette 1990). 5g Soil was transferred to 50ml plastic tubes, and to each tube 50ml of .5 mM KCL was added then sealed with a tube cap. The soil was shaken vigorously for 3 hours, and then the soil slurry was centrifuged at 5000 rpm for 5 minutes. The soil slurry was then filtered through a 0.4 µm membrane. The filtered water samples are refrigerated for IC measurement.

Organic Matter Content

The loss-on-ignition (LOI) method was used to determine the soil organic matter content. The organic matter content is assumed to equal the LOI in most surface soils (Nelson & Sommers 1996). Empty aluminum cups were placed in a muffle furnace at 400°C for 2 hours, cooled and weighed to 0.1 mg. Soil was air-dried at room temperature overnight and sieved to a diameter less than 0.4 mm. Approximately 1-3 g of soil was then placed in the cups and heated to 105°C for 24 hr. Upon removing samples from the oven, they were cooled in a desiccator containing $CaCl_2$ and weighed to 0.1 mg. The oven-dried sample weight, W_{105}, could then be calculated by subtracting the weight of the cup. Next the samples were ignited in muffle furnace at 400°C for 16 hr. Afterwards, the cups and the ignited samples were again cooled in a desiccator over $CaCl_2$ and weighted to 0.1 mg. The ignited sample weight, W_{400}, could then be calculated by subtracting the weight of the cup. The organic matter content was then calculated by the following equation,

$$\mathrm{LOI}\,\% = \frac{W_{105} - W_{400}}{W_{105}} \times 100$$

Total Kjeldahl Nitrogen and Total Organic Nitrogen

The results generated by the Total Kjeldahl Nitrogen (TKN) method are used to calculate the total organic nitrogen (TON) (Bremner 1996). The TKN method consists of a two-step procedure that involves the digestion of a sample to convert organic N to NH_4^+-N and the determination of NH_4^+-N in the digest. H_2SO_4 is used to promote oxidation of organic matter and conversion of organic N to NH_4^+-N. Catalysts such as Hg, Cu, or Se increase the rate of oxidation of organic matter by H_2SO_4, while K_2SO_4 or Na_2SO_4 increase the temperature of digestion. In a glass digester tube, slurry was formed by mixing 0.4 g of wet soil, 1.5 g K_2SO_4, 0.125 g $CuSO_4$ and 3.5 ml concentrated H_2SO_4. A few boiling stones were also added to each tube. The slurry was then dried in a Lachat Instruments Block Digestor BD-46 at 160°C for 2 hours and then digested at 390°C for an additional 2 hours (Hach 2005). Deionized water was added, after the tubes partially cooled, bringing the solution volume to 50 ml. A clear supernate was achieved by allowing the tubes to sit upright in a fume hood for several hours. The concentration of ammonium in the supernate, which actually represents the N-NH_4^+ and organic N which has been reduced to ammonium, known as the TKN concentration, was measured using an Automated Ion Analyzer. Therefore, TKN is equal to the sum of the total organic nitrogen and the ammonium, and once values were determined for the exchangeable ammonium, measured by a different method discussed later, total organic nitrogen was calculated using the equation,

$$\text{TON} = \text{TKN} - \text{Exchangeable NH}_4^+$$

Note the average TKN and average exchangeable NH_4^+ from the three replicates was used in the calculation.

Appendix 5. Site Nitrification, Mineralization, OM, TON, and OM/TON data.

Soil Layer /	Site	OM	Nitrification Rate µeq/Kg dry soil			Mineralization Rate µeq/Kg dry soil			TON	OM/
Watershed	#	(%)	0-7	7-14	14-28	0-7	7-14	14-28	(%)	TON
A Cosby	C1	28.4	372.6	251.9	5.8	376.1	378.9	63.5	0.73	39.047
	C2	42.1	88	62.5	11	531.9	279.4	-6.3	0.35	119.59
	C3	31.9	159.3	211.8	-14	265.8	349.6	22.5	0.74	43.251
	C4	16.8	68.3	57.6	0.6	152.9	28.8	58.9	0.17	96.6
	C5	15.6	-0.2	-0.3	0.7	89.5	30.7	30.8	0.32	48.5
	C6	18.8	4.6	11.8	-1.5	130.1	-5	42.8	0.52	36.4
	C7	25.9	201.6	134.3	50.5	486.7	133.8	70.1	0.76	34
	C8	21.5	9.6	-2.3	6.9	213.9	-22.4	33.2	0.68	31.7
	C9	24.5	106.1	31	8.9	303.2	-50.9	50.6	0.45	54.3
	C10	10.5	5.7	7.3	0.8	75.5	20.4	17.4	0.36	29.3
	C11	25.9	258.4	122.5	39.8	405.7	176.4	7.4	0.72	36.1
	C12	62	33.1	144.8	69.9	607.8	319.3	145.4	1.01	61.7
	C13	14.1	2.2	-0.7	-0.1	171	75.9	36.5	0.42	33.7
	C14	10.9	-3.2	1.3	-0.1	83.1	49	-26.6	0.26	41.8
Noland	NK1	22.2	291.7	151.7	-11.1	301.6	265.4	-30.4	0.35	62.9
	NK2	20.6	190.4	271.1	-1.6	386.2	194.6	102	0.8	25.7
	NK3	21.1	218.2	162.5	25.4	193.9	279.4	81.3	0.59	36.1
	NK4	9.4	31	30.4	6.2	71.2	-10.9	9.8	0.21	45.2
	N101	15	-3.8	6	-1.4	139.5	76.1	-5.7	0.18	84.9
	N102	15.6	4.6	1.1	0.1	84.2	29.3	28.8	0.67	23.3
WPLP	R1	14.2	74	81.9	111.9	57	263.1	149.4	0.42	33.5
	R2	56.6	359.7	265.6	58.8	856.5	320.5	93.2	1.32	42.8
	R3	18.1	167	176.4	-1.5	363.9	175.9	59.7	0.51	35.8
	R4	18.9	247.8	115.1	-12.7	392.1	91.9	-5.6	0.47	40.1
	R5	19.9	19.8	30.1	-2.5	109.7	89	33.9	0.39	51
	R6	9	101.8	110.8	16.9	237.1	89.9	88.8	0.28	31.8
	R7	20.3	456.2	143.6	133.6	642.3	12.5	238.2	0.59	34.3
	R8	15	285.5	80.1	61.2	320.8	115.8	107.7	0.46	32.3
	R9	63.1	105.2	95.6	78.8	388.8	30.5	103.5	0.51	124.9
	R10	18	225.5	206.5	22.8	327.5	169.1	43.2	0.61	29.6
	R11	21.9	158.5	200	30.1	268.7	157.4	103.6	0.56	38.8
	R12	11	33.8	12.3	5	120.7	-8.8	38.7	0.14	79.6
	R13	12.2	66.6	52.9	3.3	113.5	87.7	14.3	0.32	38.5
	R14	9	29.6	22.4	-0.2	172.3	15.2	49.4	0.26	34.5
	R15	17.6	0.1	-2.5	0.9	153.2	36.4	-2	0.33	53.8
	R16	9	0.3	2.7	-2.3	-61.3	254.1	19.9	0.27	33.5

Soil Layer / Watershed	Site	OM	Nitrification Rate µeq/Kg dry soil			Mineralization Rate µeq/Kg dry soil			TON	OM/
	#	(%)	0-7	7-14	14-28	0-7	40738	14-28	(%)	TON
B/C Cosby	C1	15.7	101.4	-1.4	34	219.9	-51.8	57.8	0.43	36.5
	C3	10.3	-21.5	75.7	5.1	43.3	64.5	14.3	0.47	22.1
	C4	6.2	4.9	-0.5	1.1	15.1	6.2	-1.1	0.2	31.1
	C5	10.1	51.8	49.6	2.3	90	36.2	1.8	0.19	54
	C6	4.8	-1.3	2.1	-2.9	32.8	1.9	-15.5	0.11	44.3
	C7	6.2	-0.6	-0.3	1.2	34.3	-9.3	-0.2	0.21	29
	C8	9.3	48.5	-47.3	0.6	61	-6.6	1.3	0.24	39.3
	C9	17.9	8.6	1.7	1.4	158.2	-84.6	14.8	0.38	47.6
	C10	5.3	0.4	-0.4	1.2	4.2	6	2.2	0.14	37
	C11	13.2	31.4	31.8	1.7	113.3	86.6	-22.1	0.32	41.5
	C12	10.8	3.6	2.4	-0.1	134.1	5.9	-53.6	0.28	38.3
	C13	3.9	5.2	-4.2	0.9	16.8	4.4	-4.2	0.07	52.3
	C14	4.1	4.9	-4.4	0.7	12.8	17.5	-4.9	0.06	72.8
Noland	NK1	11.1	95.1	50.4	5.3	197.6	37.2	-0.1	0.17	64.3
	NK2	8.8	84.3	77.5	-2.9	202.5	20	30.7	0.32	27.2
	NK3	7.3	60.8	-8.9	8	115.7	-42.6	6.2	0.15	48.4
	N101	4.7	2.6	-3.1	0.5	16.9	-6.6	3.3	0.09	53.6
	N102	5.1	-5.6	2	0.7	2.1	14.6	-4.2	0.09	57.4
WPLP	R1	3.3	-6.6	1.8	1.2	-12.1	0.2	0.2	0.03	121.5
	R2	14.2	99	59.2	6.9	210.6	9.3	0.7	0.37	38.1
	R3	5.8	34.6	35.8	2.6	50.8	50.5	5.8	0.02	269.4
	R4	7.7	93.1	35.8	7	98.3	75.2	11.7	0.16	47.7
	R5	7.5	7.1	-0.4	0.3	16.9	14.5	-5	0.13	56.9
	R6	6	29.6	30	2.3	56.6	26.3	15.3	0.18	32.8
	R7	9.5	56	68	8.1	96.2	47	22.6	0.2	47.6
	R8	8.6	42.6	21.2	-1.1	53.4	24.7	1.1	0.14	62.8
	R9	2.9	2.9	-0.7	-0.9	30.8	8.2	-0.8	0.03	91.4
	R10	8.5	8.7	3.6	0.3	18.2	10.3	7.9	0.09	95.5
	R11	4.3	21.6	16.1	1.5	69	12.9	18	0.24	18
	R12	6.2	15.1	7.2	-1.3	15.3	8.8	2.2	0.17	35.8
	R13	7.5	20.8	23.2	1.2	36.1	36.2	3.4	0.21	35
	R14	6.5	5	3.1	1.4	73.2	-14.6	6.6	0.03	237.8
	R15	5.1	1.1	-0.7	4.4	-26.9	72.2	-0.4	0.08	60.8
	R16	4.6	-0.6	0.4	0.5	14.6	13.7	3	0.11	43.4

VITA

Christopher James Rolison was born the younger of two children to Corite J.Rolison III and Jane E. Rolison on January 30, 1987 in Juliet Illinois. He attended Stuart Elementary School in Cleveland, TN from 1992-1999, followed by Cleveland Middle School from 1999-2001, and then Cleveland High School from 2001-2005. He then went on to attend The University of Tennessee where he received a bachelor’s degree in Civil and Environmental Engineering, emphasis in Water Resources and Environmental. During his undergraduate career at The University of Tennessee he was initiated into Chi Epsilon Honor Society for Civil Engineers, participated in the on campus chapter of the American Society of Civil Engineers. He also worked as a co-op student for two general contracting firms in Chattanooga. The contracting firms concentrated in business and industrial construction. During the last two years of his undergraduate degree he worked as a lab assistant within the Environmental Engineering Department at The University of Tennessee. Following his graduation he studied abroad for a summer concentrating on sustainability within 9 different countries. After this summer educational experience he received a graduate research assistantship at the University of Tennessee. During graduate school his main responsibilities was to carry out soil chemistry and water quality research in the Great Smoky Mountains National Park. Chris will graduate this coming December with his graduate degree in Environmental Engineering with an emphasis in water resources from The University of Tennessee.