NITROGEN CYCLING, GREENHOUSE GAS EMISSIONS AND LAND-USE CHANGE IN THE SOUTHERN APPALACHIAN MOUNTAINS

by

PETER BAAS

(Under the Direction of Jacqueline E. Mohan)

ABSTRACT

The nitrogen cycle is one of the most complex and spatially heterogeneous elemental cycles crucial for life on earth. The southern Appalachian Mountains are currently experiencing unprecedented increases in anthropogenic residential land use, which is projected to intensify over the next few decades. The future is also expected to bring greater nitrogen deposition and net primary productivity. Therefore, it is imperative that we develop a strong understanding of how ecosystems in this region will respond to projected environmental stressors. In order to address the high spatial variability of soil nitrogen cycling processes, I have developed a novel extrapolation approach based on geophysical tools to decrease the uncertainty around process estimates. Forest growth is mainly nitrogen limited in the temperate zone; however, the limitations of soil microbial activity and growth are far less clear. I found soil microbial respiration to be driven solely by carbon while nitrogen removal (i.e. denitrification) and retention was solely controlled by nitrate. These results suggest that a world of increased nitrogen and carbon availability will result in lower soil carbon sequestration and higher nitrogen removal (potentially in the form of the potent greenhouse gas N_2O). In order to address the dominant pathways of nitrogen removal and retention, I assessed gross nitrogen cycling rates

using ^{15}N isotopic techniques. I showed nitrogen removal by nitrifier denitrification and denitrification to be prevalent in soils of all forest types. Nitrogen retention by dissimilatory nitrate reduction to ammonium (DRNA) is important in some forest soils. Riparian soils are crucial in mitigating terrestrial pollution from reaching the stream. Therefore, I assessed gross riparian nitrogen cycling processes and greenhouse gas emissions under the three dominant land use types in the region (i.e. forested, agricultural and residential). Soils in residential development had low rates of nitrogen cycling and high rates of CH_4 emissions. This implies that future riparian zones will have lower nitrogen retention and removal capacity while reducing the riparian CH_4 sink. In conclusion, southern Appalachian nitrogen cycling will likely be characterized by higher leaching into streams, greater gaseous efflux, and lower soil carbon sequestration.

INDEX WORDS: Nitrogen cycling, denitrification, mineralization, nitrification, dissimilatory nitrate reduction to ammonium, respiration, microbial limitation, land use change, riparian zone, forest, agriculture, biogeochemistry.

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PETER BAAS

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PETER BAAS

Major Professor:	Jacqueline E. Mohan
Committee:	Jennifer D. Knoepp Daniel Markewitz Matthew D. Wallenstein Nina Wurzburger

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
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DEDICATION

To my parents for showing me the wonders of the natural world and for stimulating my fascination in understanding the world around me.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The nitrogen cycle is one of the most dynamic and complex cycles on earth, limiting primary productivity in much of the world's ecosystems (Vitousek & Howarth, 1991). The nitrogen limited nature of plant growth makes microbial decomposition of organic matter crucial for regeneration of plant available nutrients. Nitrogen possesses nine forms corresponding to different oxidative states. Dinitrogen gas (N_2) is by far most abundant in the atmosphere but this form is inaccessible to most plants (Robertson & Groffman). Biological nitrogen fixation is the dominant natural pathway in which nitrogen enters the soil ecosystem. However, industrial nitrogen fixation, for the purpose of nitrogen fertilizer production, now rivals biological nitrogen fixation as a soil input of N and is expected to surpass biological fixation in the next few decades (Millennium Ecosystem Assessment, 2005). The fact that total nitrogen fixation, both natural and industrial, has already outpaced rates of removal processes, such as denitrification, nitrogen now is becoming a major pollutant (Galloway *et al.*, 2003).

Nitrogen mineralization is one of the most critical processes in the nitrogen cycle, converting organic nitrogen into ammonium, a readily available nutrient for plant growth. Microbial nitrogen mineralization is controlled by the quality or C/N ratio of the organic matter deposited into the soil (Chapin III *et al.*, 2011). If the C:N ratio is < 25:1, inorganic nitrogen is released by mineralization and organic nitrogen with wider ratios result in microbial immobilization (Robertson & Groffman, 2007). This makes the microbial community a direct competitor with plants for available inorganic nitrogen. Conversely, some studies have suggested

that the mechanisms driving this relationship are largely due to the density and activity of the microbial community not necessarily the C:N ratio (Bengtsson *et al.*, 2003).

Nitrification is the process that sequentially oxidizes ammonia into NO_2^- and NO_3^-. This process is very important because the anion NO_3^- is much more mobile in soil than NH_4^+ cation (Johnson & Cole, 1980) with the exception of sequioxide rich tropical soils that can have high anion exchange capacity (Kinjo *et al.*, 1971). With most soils having a net negative charge, positively charged ions such as ammonium can associate with cation-exchange sites related to soil organic matter, clay, and other mineral surfaces (Robertson & Groffman, 2007). Many studies have shown that disturbance results in increased nitrate leaching for a large part due to reduced vegetative nitrogen uptake (Likens *et al.*, 1970, Swank, 1988, Vitousek & Melillo, 1979). In addition, nitrogen deposition in access of ecosystem uptake capabilities can result in nitrogen saturation (Aber *et al.*, 1998, Aber *et al.*, 1989). The vast majority of nitrification occurs autotrophically, derived from NH_4^+ oxidation (De Boer & Kowalchuk, 2001, Robertson & Groffman, 2007), although heterotrophic nitrification (not coupled to cellular growth) has been observed in some ecosystems (Pedersen *et al.*, 1999, Schimel *et al.*, 1984). Autotrophic nitrification derives its carbon from CO_2 or carbonates rather than organic matter and is characterized by a two-step process. The first step involves ammonia oxidation with oxygen to produce NO_2^-, H^+ and water. It is catalyzed sequentially by ammonia mono-oxygenase and NH_2OH oxidoreductase. The second step is NO_2^- oxidation to NO_3^- which also produces H^+ and is catalyzed by nitrite oxidoreductase (Robertson & Groffman, 2007). The production of H^+ during nitrification can result in soil acidification in ecosystems with high nitrification rates (Van Miegroet & Cole, 1984). The process of nitrification can be conducted by a wide range of microbes mainly from the Betaproteobacteria (Teske *et al.*, 1994) and some placed in the

Gammaproteobacteria (*Nitrosococcus*) (Purkhold *et al.*, 2000). Archaea have also been shown to be capable of ammonia oxidation (Konneke *et al.*, 2005) though their relative importance in soils is unclear (Norman & Barrett, 2014). The presence of oxygen is a prerequisite for the obligate aerobic nitrifiers with NH_4^+ availability being the most important controlling variable (Robertson & Groffman, 2007). Following kinetic principles, the response of nitrification is temperature dependent. Additionally, soil moisture and pH are often found to control nitrification rates (Knoepp & Vose, 2007, Robertson & Vitousek, 1981); however, the causal mechanisms between pH and nitrification are poorly understood as high nitrification rates have been found in acidic soils (pH < 4.5) (De Boer & Kowalchuk, 2001).

Nitrate reduction involves a wide variety of processes including denitrification (Groffman, 2012), anammox (Jetten *et al.*, 1999, Mulder *et al.*, 1995), assimilatory nitrate reduction to ammonium (ANRA) (Sias & Ingraham, 1979) and dissimilatory nitrate reduction to ammonium (DNRA) (Tiedje, 1988). These processes all use nitrate instead of oxygen as an electron acceptor. Denitrification and anammox result in the conversion of inorganic nitrogen into gaseous nitrogen (N_2, N_2O and NO) while DNRA results in nitrogen retention (Burgin & Hamilton, 2007). Denitrification can be conducted by a wide range of organisms ranging over 50 genera (Zumft, 1992) although generally only 3-6 genera are present in soils (Tiedje, 1988). Historically, denitrification was thought to only occur under waterlogged conditions; however, acetylene block and more recently stable isotopic methods have shown denitrification to be important in more well-drained or aerated soil including agricultural, forest and grassland soils (Groffman, 2012, Groffman *et al.*, 2006). Non-respiratory denitrification also results in conversion into gaseous nitrogen (mainly N_2O) with much of this activity currently attributed to nitrifiers (Robertson, 1987). Denitrification processes are catalyzed by nitrate reductase, nitrite

reductase, nitric oxide reductase and nitrous oxide reductase (Zumft, 1997). Denitrification is a facultative anaerobic process while DNRA and anammox have been considered to be obligate anaerobic processes with little tolerance of oxygen (Dalsgaard & Thamdrup, 2002, Tiedje, 1988, Tiedje *et al.*, 1984). However, recent research has shown DNRA (Fazzolari *et al.*, 1998, Morley & Baggs, 2010) and anammox (Schmidt *et al.*, 2002, Strous *et al.*, 1997) to be more resilient under oxygenated conditions. There is even evidence that DNRA is more resilient to oxygen exposure than the denitrification process (Pett-Ridge *et al.*, 2006, Schmidt *et al.*, 2011). While much is known about the ecology of denitrification much less is known about the ecology of DNRA. This is in large part due to the historical assumption that DNRA is extremely oxygen intolerant and, thus, would only occur under continuously waterlogged conditions (Tiedje, 1988). The current state of the literature has found $C:NO_3^-$ ratios to be one of the most important drivers of the relative magnitude of denitrification versus DNRA (Burgin & Hamilton, 2007). Recent research has found DNRA to be important in tropical forests (Silver *et al.*, 2001) and in a wide range of other ecosystem types (Morley & Baggs, 2010, Yang, 2010). DNRA is catalyzed by either membrane-bound nitrate reductase, similar to denitrification, or by periplasmic nitrate reductase with nitrite subsequently being reduced to ammonium by the NrfA nitrite reductase (Baggs & Philippot, 2010) which can be N_2O-genic (Jackson *et al.*, 1991). We know even less about the prevalence of anammox in non-wetland or aquatic ecosystems. The anammox process has been shown to be inhibited by various simple carbon compounds (Jetten *et al.*, 1999), however, significant rates have been observed in tropical soils coupled to iron oxidation (Feammox) (Yang *et al.*, 2012) and agricultural soils (Long *et al.*, 2013). Anammox rates in a temperate forest have been found to contribute no more than 5% to the total nitrate reduction (Davies *et al.*, unpublished). In order to unravel how gross nitrogen cycling relates to previously

determined net rates (Knoepp *et al.*, 2008, Knoepp & Swank, 1998, Mclean, 2011) and potential assays (Groffman *et al.*, 1991), I conducted ^{15}N tracer assays in addition to potential assays along a elevation and vegetation gradient (***Chapter 4***) including assessment of potential environmental drivers of the different processes.

The rates of microbial processes in the nitrogen cycle have great consequences for the movement of nitrogen through the landscape as well as for soil fertility and for atmospheric composition. However, currently large uncertainties surround extrapolation efforts of assessed soil processes (Groffman *et al.*, 2009a, McClain *et al.*, 2003). Therefore, in ***Chapter 2***, I developed a novel approach to improve the prediction of nitrogen cycling heterogeneity on a plot scale (~ha) using geophysical tools.

Increased nitrogen inputs (i.e. fertilization and N deposition) (Webster *et al.*, 2012) as well as disturbances can increase nitrate leaching into streams and rivers (Likens *et al.*, 1970, Swank, 1988, Vitousek & Melillo, 1979) and create a cascading effect of nitrogen pollution (Galloway *et al.*, 2003) from affecting drink water quality to coastal eutrophication (Driscoll *et al.*, 2003, Erisman *et al.*, 2013). In addition, increased nitrogen availability can exacerbate climate change via the emission of the potent greenhouse gas N_2O which is 300 times as potent as CO_2 (Denman *et al.*, 2007).

Currently, the southern Appalachian Mountains are experiencing unprecedented increases in suburban development and projections estimate the rate of development to only increase in future decades (Gragson & Bolstad, 2006, Kirk *et al.*, 2012). In addition, nitrogen deposition rates are expected to increase over the next half a century (Anderson *et al.*, 2006, Galloway *et al.*, 2008) increasing the availability of reactive inorganic nitrogen. It is crucial that we develop a better understanding of nitrogen cycling and its associated effect on water quality and climate

change in this rapidly changing region. The current paradigm states that higher nutrient containing (e.g. < C:N ratios) organic matter results in greater decomposition rates (Chapin III *et al.*, 2011). This suggests that microbes are nutrient limited and rates would increase under projected increases in land use and nitrogen deposition. On the other hand, increased atmospheric CO_2 could result in greater net primary production and consequently carbon subsidies to the soil (Phillips *et al.*, 2011). Current research, however, provides no consistent and sometimes contradictory findings on whether microbial activity in soils is controlled by carbon or nutrient availability (Allen & Schlesinger, 2004, Allison & Vitousek, 2005, Hollender *et al.*, 2003). With projected increases in carbon and nitrogen availability, rates of nitrate reduction can increase for both denitrification and dissimilatory nitrate reduction to ammonium (DNRA). Both processes are, for a large part, controlled by carbon and nitrate availability with DNRA being more dominant with higher $C:NO_3^-$ ratios (Burgin & Hamilton, 2007). To assess the effect of greater carbon and nitrogen availability on forest soil removal or retention capacity, I determined how carbon and/or nutrient availability control soil respiration and nitrate reduction in the dominant forest types of the southern Appalachian Mountains.

Riparian zones can function as efficient buffers between the aquatic and terrestrial biome (Gregory *et al.*, 1991, Naiman & Decamps, 1997) mitigating the effects of nitrogen non-point source pollution. Estimates of the riparian zone to retain nitrogen pollution varies between 7-89% (Dosskey, 2001, Peterjohn & Correll, 1984, Vought *et al.*, 1994) and even small buffers have been found successful at maintaining stream water quality after disturbance events in the southern Appalachian Mountains (Knoepp & Clinton, 2009). However, riparian zones can also be hotspots of N_2O emissions (Groffman *et al.*, 2000, Hefting *et al.*, 2003) and conversion to grass or agriculture dominated ecosystems can result in increases in CH_4 (Boeckx *et al.*, 1997,

Steudler *et al.*, 1989) and CO_2 efflux (Raich & Tufekciogul, 2000). To assess how projected land use change would affect riparian greenhouse gas emission and nitrogen cycling, in ***Chapter 5***, I assessed greenhouse gas emissions (N_2O, CO_2 and CH_4) and nitrogen cycling along a land use gradient in the region.

In conclusion, my dissertation focuses on improving our ability to assess nitrogen cycling heterogeneity and identifying the main drivers of forest microbial activity. Finally, my research elucidated the relevance of anaerobic processes and how projected land use change might affect riparian nitrogen dynamics and greenhouse gas emissions.

CHAPTER 2

ASSESSING HETEROGENEITY IN SOIL NITROGEN CYCLING: A PLOT SCALE APPROACH [1]

[1] Baas, P., D. Markewitz, J.D. Knoepp and J.E. Mohan. 2014. *Soil Science Society of America Journal*.

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Abstract

The high level of spatial and temporal heterogeneity in soil nitrogen cycling processes hinders our ability to develop an ecosystem-wide understanding of this cycle. This study examines how incorporating an intensive assessment of spatial variability for soil moisture, carbon, nutrients, and soil texture can better explain ecosystem nitrogen cycling on the plot scale. Five sites distributed over a regionally representative vegetation and elevation gradient at the Coweeta Hydrologic Laboratory in the southern Appalachian Mountains were sampled five times between November 2010 and March 2012. We used electromagnetic induction (EMI) to survey for soil moisture, soil texture and near infrared reflectance spectroscopy (NIRS) to estimate extractable NH_4^+, total C, and total N concentrations. Laboratory assays of nitrification and denitrification potential rates were used as an index for nitrogen cycling dynamics. Multivariate regression analysis indicated NIRS and EMI survey data explained 30-90% of the variability in potential nitrification rates ($p<0.01$) and 16-70% of variability in potential denitrification rates ($p<0.01$). Two extrapolation approaches were used to calculate the mean and the variability of potential rates: 1) stratified selection of collected samples based on EMI and NIRS predictors and 2) random selection of collected samples. The mean for potential nitrification rates based on EMI and NIRS stratification yielded similar (oak-pine and mixed oak) and greater (northern hardwood and cover hardwood) rates whereas potential denitrification rates were greater in all sites for the stratified based estimates. This study demonstrated that the application of geophysical tools may enhance our ecosystem level understanding of the nitrogen cycle.

Introduction

Soil nitrogen cycling processes are heterogeneous in both space and time. Process rates can dramatically change over the range of centimeters to meters and minutes to days, hindering our ability to predict and model these dynamics (Groffman *et al.*, 2009a, McClain *et al.*, 2003). The environmental factors (i.e. soil moisture, pH, inorganic nitrogen concentrations, organic nitrogen and available carbon) controlling nitrogen cycling processes such as nitrogen mineralization (Knoepp *et al.*, 2008, Pastor *et al.*, 1984, Pastor & Post, 1986), nitrification (Breuer *et al.*, 2002, Nielsen & Revsbech, 1998, Sahrawat, 2008, Ste-Marie & Paré, 1999) and denitrification (Burgin *et al.*, 2010, Firestone *et al.*, 1980, Groffman & Tiedje, 1991) are well documented. However, the complexity and heterogeneity of these environmental factors create hotspots and hot moments with accelerated N transformation rates (Burgin *et al.*, 2010, Johnson *et al.*, 2010, Parkin *et al.*, 1987, Schimel & Bennett, 2004) that are challenging to quantify and model (Groffman *et al.*, 2009a, McClain *et al.*, 2003).

Hydrologic models in combination with soil biogeochemical models such as the NGAS Century (Parton *et al.*, 1996) simulate N_2 and N_2O emissions from nitrification and denitrification using large watershed scale patterns of soil moisture (Band *et al.*, 2001, Swank & Crossley Jr, 1988, Tague, 2009, Tague *et al.*, 2010), but lack utility on the plot scale (~ha) (Tague *et al.*, 2010). Approaches capable of capturing fine-scale heterogeneity are needed to improve our ability to model and scale nitrogen cycling rates and fluxes (Groffman *et al.*, 2009a, Tague *et al.*, 2010).

Geophysical techniques such as electromagnetic induction (EMI) provide a quantitative means to assess fine scale soil texture and moisture variation through the measurement of soil conductivity (Abdu *et al.*, 2008, Robinson *et al.*, 2008a). Soil conductivity is mainly controlled

by volumetric water content, clay content, temperature and salinity (Everett, 2005, Friedman, 2005). In agricultural and marine systems, research has shown that conductivity is controlled by nutrient concentrations and salinity (Corwin & Lesch, 2005, Sheets *et al.*, 1994, Zhu *et al.*, 2010), whereas in forested systems soil moisture and soil texture are the controlling factors (Sheets & Hendrickx, 1995, Triantafilis & Lesch, 2005). Therefore, EMI approaches have been used for soil textural (Triantafilis & Lesch, 2005, Weller *et al.*, 2007) and moisture (Reedy & Scanlon, 2003, Robinson *et al.*, 2008a, Sheets & Hendrickx, 1995) assessments, but far less commonly to estimate biogeochemical patterns and processes (Cockx *et al.*, 2005). Unlike traditional soil surveys methods, EMI rapidly captures thousands of measurements per hectare. Combining the high spatial intensity soil geophysical data from EMI with field portable spectrophotometric sensors such as near infrared reflectance spectroscopy (NIRS), which provides soil chemical attributes, allows mapping of both physical and chemical attributes. EMI and NIRS map layers have been used extensively in precision agriculture to predict and optimize crop yields (Van Vuuren *et al.*, 2006, Zhang *et al.*, 2002).

The southern Appalachian Mountains have been described as the "water tower" of the Southeast (Gragson & Bolstad, 2006) making stream and river water quality important for the regional water supply. Nitrate concentrations in particular increase with anthropogenic disturbance and have been found to be a key player for regional water quality (Knoepp & Clinton, 2009). Nitrate is the mobile form of inorganic nitrogen in soils often susceptible to leaching (Aber *et al.*, 1998, Aber *et al.*, 1989). Therefore, this study focused on the process that produces nitrate (i.e. nitrification) and the process that removes it from the ecosystem by converting it into a gaseous form (i.e. denitrification) and, thus, preventing leaching. Previous studies have identified soil moisture, ammonium concentrations, C:N ratios and pH to be main

drivers of nitrification rates (Donaldson & Henderson, 1990, Knoepp & Vose, 2007, Robertson & Vitousek, 1981, Sahrawat, 2008) whereas denitrification rates are generally thought to be controlled by soil moisture, nitrate concentrations and organic carbon availability (Groffman, 2012, Tiedje *et al.*, 1984). In addition, wetting and drying cycles have been found to stimulate mineralization, nitrification (Cabrera, 1993, Fierer & Schimel, 2002) and denitrification (Groffman & Tiedje, 1988) making variability in soil moisture an important factor controlling process rates both instantaneous and via distal controls on the community composition (Groffman *et al.*, 1988, Wallenstein *et al.*, 2006).

In this study we investigated the potential of high spatial resolution EMI and NIRS measurements to estimate variability of potential nitrification (pNTR) and potential denitrification (pDNF) rates in five forest types along a gradient in elevation, vegetation, nitrogen and water availability in the southern Appalachian Mountains.

We hypothesized that patterns of spatial variability in soil conductivity, a proxy for soil moisture, will relate to patterns of process rates for nitrification and denitrification. In addition, we hypothesized that greater total carbon and inorganic nitrogen concentrations would result in higher pDNF rates, whereas pNTR would be positively correlated to inorganic nitrogen concentrations. We expect spatial autocorrelation to indicate pNTR to be less heterogeneous (large range) than pDNF (small range) because redox conditions that drive pDNF are likely more variable across plots. We used spatial modeling techniques to predict pNTR and pDNF providing insights concerning where we could expect high and low process rates. This approach could dramatically improve both sample collection efficiency as well as the accuracy of predicted N cycling rates over a larger spatial scale.

Methods

Study site

The study was conducted at the Coweeta Hydrologic Laboratory, a 2,180 ha USDA Forest Service experimental forest in the southern Appalachian Mountains in western North Carolina. This area receives an average of 1800 ± 34 (low elevation) to 2400 ± 44 mm (high elevation) of precipitation a year (Coweeta Long Term Ecological Research Database, 1936-2011). The highest average air temperatures are between June and August (20 °C) and the lowest average air temperatures are between December and January (5 °C). The growing season starts in May and ends in September (Swift & Cunningham, 1988).

We examined five sites (80x80 m) that represent the major vegetation types within the Coweeta basin. All five sites are located in reference watersheds that have been undisturbed since 1929 (Knoepp & Swank, 1998). Watershed 18 is a low elevation watershed (13 ha) and includes xeric oak-pine (OP), cove hardwood (CH) and low elevation mesic mixed oak (MO-low) forest community types. Watershed 27 is a high elevation watershed (39 ha) and includes high elevation mesic mixed oak (MO-high) and northern hardwood (NH) forest community types. Table 1 provides detailed information regarding the elevation, slope, dominant vegetation and soils for each site.

Soil sampling and incubations

We determined potential nitrification (pNTR) and potential denitrification (pDNF) on fresh soil samples collected in March 2011, July 2011, November 2011, and March 2012. Using a stainless steel soil push tube, cores (15 cm mineral soil) were collected from randomly selected different locations (3-6) within each of the five sites (OP, CH, MO-low, MO-high and NH). For

potential nitrification N_{total} = 67 (N_{OP} = 12; N_{CH} = 16; $N_{MO\text{-}low}$ = 16; $N_{MO\text{-}high}$ = 7; N_{NH} = 16) and for potential denitrification N_{total} = 87 (N_{OP} = 15; N_{CH} = 17; $N_{MO\text{-}low}$ = 16; $N_{MO\text{-}high}$ = 20; N_{NH} = 19). The location of each sample was obtained using an Archer GPS (Juniper Systems Inc., Logan, Utah). We divided core samples into forest floor and 0-15 cm mineral soil (pNTR) or forest floor and 0-5, 5-10, and 10-15 cm mineral soil (pDNF) and stored samples in reclosable plastic bags at 4°C. Each soil sample was sieved (< 2 mm) and homogenized. Gravimetric soil moisture was determined by oven drying a 10 g subsample of each soil at 105 °C to constant weight. Potential nitrification assays were determined on field moist soils within 72 h of collection and pDNF assays were conducted on field moist soils stored at 4 °C for less than 2 weeks.

We used the amended slurry incubation method for pNTR determinations on both soil and forest floor (N_{total} = 67) samples (Bodelier *et al.*, 1996). Five grams of sieved soil or forest floor was placed in 37-ml serum vials with 15 ml of media (0.33 g L^{-1} $(NH_4)_2SO_4$ in DI water buffered with 0.14 g L^{-1} K_2HPO_4 and 0.027 g L^{-1} KH_2PO_4) (pH = 7.5). Each vial was wrapped in aluminum foil with Al foil cap to prevent evaporation and UV light inhibition of ammonia-oxidizers. After the addition of the media, vials were shaken at 10 relative centrifugal force (rcf) at 25 °C; 2-ml sub-samples were collected after 0.5, 2, 6-8 and 24 h of incubation using a cut-off pipette tip. Sub-samples were centrifuged for 10 minutes at 11,000 rcf and the supernatant was immediately frozen at -20 °C until analysis for NO_2^- + NO_3^- using colorimetric methods (Bendschneider, 1952).

Potential denitrification rates were determined using the acetylene block method (Groffman *et al.*, 1999) on both soil and forest floor samples (N_{total} = 87). Five grams of sieved soil (2 g for forest floor samples) was added to 37-ml serum vials. Serum vials were purged with

He for 1 minute to displace oxygen from the vial, and then five ml of incubation media was added to the serum vials. Media consisted of dextrose (1 mM) and sodium nitrate (1 mM) in DI water purged for 30 minutes with He gas. Assays were initiated by replacing 4 ml of headspace with 99% pure acetylene (10% v/v). Samples were incubated at 20ºC while shaking (5 rcf) for 6 hours. Gas subsamples were taken 0.5, 2 and 6 h after initiation of the incubation and stored in 3-ml gas vials until analysis for N_2O on a GC-ECD (Shimadzu Corporation GC-14A, Kyoto, Japan) with a 10-port Valco valve (Valco Instruments, Houston, Texas) to prevent acetylene from saturating the detector.

Rates of pNTR and pDNF were determined via regression analysis of changes in solution NO_3^- or N_2O concentrations over time (rates were accepted if $r^2 > 0.8$). All rate data were calculated per gram dry soil as ng N g_{soil}^{-1} h^{-1}. Using the total weight of dry soil or forest floor from each core sub-sections a rate was calculated for the whole 0-15 cm core (ng N g_{soil}^{-1} h^{-1}).

In June 2011, geo-referenced soil texture analyses were done on each of the five sites (OP, CH, MO-low, MO-high, NH) (N = 10 per site) on 0-20 cm soil samples collected from randomly distributed locations throughout each 80 x 80 m site. The sample location was also determined using an Archer GPS. Soil texture analysis was done using the hydrometer method (Robertson *et al.*, 1999). In short, soils were oven dried at 105 °C to a constant weight and sieved to < 2 mm, all roots and rocks were removed and weighted. Forty grams of sieved soil was added to 175-ml plastic bottles with 100 ml 5% hexametaphosphate and shaken overnight. The next day the solution was washed through a 53 µm sieve with DI water. The particles captured on the sieve were collected and oven dried (105 °C) overnight (this constitutes the sand fraction). The suspension that passed through the sieve was placed in a 1 L volumetric cylinder filled to 1 L. The suspension was stirred and a few drops of amyl alcohol were used to reduce foaming.

Hydrometer readings were taken at 1.5 h and 24 h. Silt and clay fractions were calculated according to Robertson et al (1999).

Surveying

EMI surveys were conducted in November 2010, March 2011, July 2011, November 2011 and March 2012. EMI measurements were collected for one hour per site yielding approximately 500 measurements. We used a DUALEM-2S (DUALEM Inc., Milton, Ontario, Canada) electromagnetic induction (EMI) sensor carried at a height of one meter above the ground connected to an Archer GPS data logger. The EMI recorded bulk soil conductivity (ECa) for both the horizontal co-planar coils (ECa-3m; theoretical cumulative signal of 70% at 3 m depth) and the perpendicular coils (ECa-1m; theoretical cumulative signal of 70% at 1 m depth) resulting in two measurements per sample location (http://www.dualem.com) (Beamish, 2011). Temperature varies widely among sites and between seasons and has a significant effect on conductivity readings. Therefore, all EMI conductivity measurements were standardized to the equivalent value at 25 °C (EC25) (Reedy & Scanlon, 2003). Spatial analysis of EMI data was done using ArcGIS 10.0. In each site, predicted conductivity measurements were produced by ordinary kriging (Isaaks & Srivastava, 1989, Johnston *et al.*, 2001). Subsequently, predicted values were extracted at soil sampling locations of process rates or soil texture outlined earlier. For every pixel (~ 1 m^2) of the kriged maps the standard error (SE), lowest value (low), highest value (high) and the Δ (i.e. max – min) was calculated across all sampling dates. Data was checked for normality using normal QQ plots. Ordinary kriging can accommodate non-normal distribution as long as spatial autocorrelation structure is not masked by extreme values (Isaaks & Srivastava, 1989). Therefore, in the case of non-normal distribution after natural log

transformation, data was checked for extreme value outliers and points were removed if needed before kriging analysis.

We used an ASD FieldSpec 3 NIRS (Analytical Spectral Devices Inc., Boulder, CO) to assess total soil C and N (Chang & Laird, 2002) and KCl extractable NH_4^+ (Janik *et al.*, 1998). We used the FieldSpec NIRS which provides reflectance data with one nanometer resolution between 350-2500 nanometer wavelengths. All NIRS data was first transformed to the first derivative before analysis. For calibration purposes, forty six sieved (< 2 mm) oven-dried catalog samples previously analyzed for total C and N with a Elementar Flash EA 1112 NC analyzer (Thermo Scientific, Waltham, MA) were used. Carbon and nitrogen contents were converted to an aerial measure (carbon: kg C m^{-2}; nitrogen: g N m^{-2}) using bulk density measures determined on each of the collected core sections (g cm^{-3}) (N_{total} = 79). Calibration between NIRS and KCl extractable $NH_4^+$was established on March 2011 and 2012 samples. Twenty-five percent (N = 12 for each sampling time) of the samples collected on those dates were immediately extracted with 2M KCl on all mineral soil depths (0-5, 5-10 and 10-15 cm); supernatant NH_4^+ concentrations were analyzed according to colorimetric methods using an Alpkem 300 Series Autoanalyzer (OI Analytical, College Station, TX, USA). Calibration models were developed by running full factorial cross-validation multivariate analyses using the Unscrambler software and produced predicted values for total C, total N and NH_4^+ concentrations.

Model development and statistical analyses

Model development took place in three steps. First, a model was developed to assess correlations between soil physical properties (soil moisture and soil texture) and EMI (ECa-1m-low, ECa-1m-high, ECa-3m-low, ECa-3m-high, SE ECa-1m, SE ECa-3m, ΔECa-1m and ΔECa-

3m). To determine significant correlations between soil physical properties and conductivity data, stepwise multivariate standard least squares regression analysis was utilized and the best model was selected by using the corrected Akaike's Information Criteria. We used the same stepwise regression analysis to predict pNTR and pDNF rates based on EMI and NIRS variables (total C, total N, $NH_4^+{}_{0\text{-}5cm}$, $NH_4^+{}_{5\text{-}10cm}$, $NH_4^+{}_{10\text{-}15cm}$). All data were natural log transformed to acquire normal distribution and regression residuals were evaluated for the absence of heteroscedasticity. Validation was done by randomly separating the dataset into a calibration (70%) and validation (30%) dataset. Model parameterization was done using only the calibration dataset (training dataset) whereas the predicted process rates were confirmed using the validation dataset by regressing predicted against observed values.

The second step was to extract predicted EMI and NIRS values for each of the sampling locations. The extracted data were natural log transformed and checked for normality using QQ plots in ArcGIS. Subsequently, the parameters selected through the stepwise regression analysis described above were used to estimate process rates across the plots by simple ordinary kriging in ArcGIS 10.0 (Johnston *et al.*, 2001), assigning a predicted potential process rate to each pixel. To assess spatial autocorrelation (major range) and spatial structure (nugget:sill ratio) of the predicted potential process rates, the range, sill, nugget and nugget:sill ratio were determined using a stable semivariogram fit (Isaaks & Srivastava, 1989, Johnston *et al.*, 2001). A maximum lag distance of 75.4 m ($^2/_3$ of maximum pairwise distance between sampling points) was considered to prevent interpretations over a larger area than the plot size (Webster & Oliver, 2005). Each standard semivariogram was also tested for directionality, or anisotropy (Isaaks & Srivastava, 1989). The root mean squared error never decreased by more the 5% when adding anisotropy into the model and, therefore, isotropy was assumed in all models.

The third step was to compare two extrapolation approaches, one based on random sampling and one based on stratified sampling determined by NIRS and EMI layers. First, we determined the mean, standard error (SE) and coefficient of variation (CV) of eight randomly selected samples of which potential rates were measured for each site (i.e. OP, CH, MO-low, MO-high and NH) spanning all sampling times (i.e. March 2011, July 2011, November 2011 and March 2012). Second, for the stratified approach, we determined the quartiles in data distribution for (EMI and NIRS) predicted process rates for each site spanning all sampling times. We then used the predicted process rates to randomly select two actually measured samples from all four strata as determined by the quartile analysis (N = 8). Process rates predicted by NH_4^+ concentrations were based only on March 2011 and 2012 (N = 4; one selection per strata).

To assess significant differences in soil characteristics and process rates among different sites and watersheds, we conducted one-way ANOVA analyses combined with Tukey pairwise comparisons ($p<0.05$ unless specified differently). All data were tested for normality and natural log transformed if needed to acquire normality. All statistical analyses were conducted in JMP 9.

Results

Soil conductivity

Soil conductivity estimated with EMI was not significantly different among sites. Significant differences were found among sampling dates for ECa-3m conductivity with values measured in November 2011 being greater than March 2011 ($F_{2,55} = 3.2$; $p= 0.0473$). ECa-1m conductivity was greatest in March 2012, exceeding both November and March 2011 ($F_{2,54} = 4.9$; $p = 0.055$).

Soil properties and EMI

Soil physical properties varied significantly among sites (**Table 2.1**). The percent coarse fraction (> 2 mm) was significantly greater at the CH site compared to all other sites except the OP ($F_{4,42}$ = 10.6; $p < 0.001$). Percent sand was greater in OP than all other sites and lowest in NH ($F_{4.42}$ = 26.0; $p < 0.001$). Percent clay was greatest in WS 27 (MO-high and NH) compared to WS 18 (OP, CH and MO-low) and lowest in OP ($F_{4,42}$ = 21.6; $p < 0.001$), whereas percent silt was greater in NH compared to CH and OP ($F_{4,42}$ = 10.8; $p < 0.001$).

Conductivity measures at specific dates were unable to predict soil physical properties (soil texture and soil moisture). However, when pooling all data (i.e. ΔECa-3m, SE ECa-1m and ECa-3m-high) significant correlations were detected. Overall, ΔECa-3m (i.e. max – min) conductivity proved to be the best predictor of soil texture (i.e. coarse fraction and percent clay; **Figure 2.1**; **Table 2.2**). Since particle size differed greatly between WS 18 and WS 27 (**Table 2.2**) watershed was also a significant predictor in the regression analysis ($p<0.001$), therefore, the stepwise regression analyses for watershed 18 and 27 were conducted separately. No significant relationships were found between pooled conductivity measures and the coarse fraction when separated by watershed. SE ECa-1m (i.e. standard error of the mean ECa-1m conductivity) was the best predictor for percent sand and silt in WS 18 (**Figure 2.2**) whereas ECa-3m-high (i.e. highest ECa-3m conductivity for a specific location) was the best predictor of percent sand and silt in WS 27 (**Table 2.2**). In WS 27, SE ECa-1m was correlated strongly with soil moisture (**Figure 2.3**). Analysis of data within individual forest types showed that the best predictor of the soil coarse fraction was ECa-3m-high in OP and MO (2^{nd} order polynomial; r^2 = 0.44; $p < 0.01$; peak at 1.2 mS and 20% coarse fraction; 95% confidence interval: 0.74 – 1.67 mS and 14 – 18%

coarse fraction), and CH (r^2 = 0.81; p<0.001; 95% confidence interval: 0.51 – 3.24 mS and 21 – 29% coarse fraction). No significant correlations with the coarse fraction were found in NH.

Nitrogen cycling and spatial data

Calibration models with NIRS were used to predict soil NH_4^+ concentrations, and total carbon and nitrogen (**Table 2.3**). Potential nitrification (pNTR) was predicted by a multivariate regression model including ECa-3m-high and NIRS estimated $NH_4^+{}_{5\text{-}10cm}$ concentrations (**Table 2.4**). For NH the best model included only NIRS based $NH_4^+{}_{5\text{-}10cm}$ concentration (**Figure 2.4**; **Table 2.4**). The other four sites were included in a single model, and pNTR was best predicted by ECa-3m-high alone (**Table 2.4**). Overall, pDNF rates were best predicted by NIRS based total carbon (**Table 2.5**). Watershed proved to be a significant predictor in a multivariate regression with SE ECa-1m (p < 0.0001) and, therefore, the analysis was conducted by watershed. SE ECa-1m proved to have the best predictive power in WS 27 for pDNF rates (**Figure 2.4**; **Table 2.5**) while total carbon provided the best prediction in WS 18 (**Table 2.5**).

Spatial autocorrelation of the predicted potential nitrification and denitrification rates as indicated by semivariogram analysis showed the major range and spatial dependence was often larger than the longest considered distance between points (75.4 m) (**Table 2.6**). Major ranges smaller than the plot sizes were found for pNTR (OP, MO-low and MO-high) and pDNF (all but CH). Spatial structure was strong for all pNTR models (nugget/sill < 0.3) whereas the pDNF models showed weak spatial structure (nugget/sill > 0.3) for WS 18 (OP, CH and MO-low) and strong spatial structure for WS 27 (MO-high and NH) (**Table 2.6**).

Additionally, we compared random to stratified methods (using EMI and NIRS data) of calculating a site specific mean rate, SE and CV (**Table 2.7**). Comparing the mean rates based on

random or stratified selection showed greater estimates with the stratified approach for pNTR in CH (17 ± 11%) and in NH (32 ± 14%). No large differences could be observed for pNTR in OP and MO sites. However, for pDNF measurements random selection resulted consistently in lower estimates than the stratified approach (OP: 5 ± 3%; CH: 193 ± 128%; MO-low: 14 ± 3%; MO-high: 24 ± 6%; NH: 41 ± 18%). Estimates of SE and CV were generally equal or higher in the stratified approach compared to the random selection approach.

Discussion

The objective of our study was to investigate the utility of high-resolution geophysical methods, which estimate soil water content and soil texture, in assessing plot scale nitrogen cycling heterogeneity in southern Appalachian forests. Our results indicate that a combination of NIRS and EMI techniques are capable of predicting a significant portion of the within and between site variability of pNTR and pDNF activity. Soil type (i.e. soil particles size distribution) had a profound effect on relationships found between process rates and predicting variables (e.g. conductivity, ammonium, carbon).

To understand the mechanisms behind the relationship between soil conductivity and pNTR and pDNF, we first needed to disentangle the relationship between soil conductivity and soil abiotic properties. Soil conductivity is simultaneously controlled by multiple factors, including soil moisture, soil texture and soil ionic concentrations (Everett, 2005). We found that the abiotic factors controlling soil conductivity were highly watershed dependent. Soil texture alone correlated strongly with conductivity in WS 18 (SE ECa-3m), whereas in WS 27 soil moisture and texture were best correlated to SE ECa-1m and ECa-3m-high, respectively. Although relationships between conductivity and abiotic factors such as soil moisture and texture

have been confirmed in studies of agricultural systems (Hedley *et al.*, 2004, Robinson *et al.*, 2008b, Zhu *et al.*, 2010) and managed forest ecosystems (Doolittle *et al.*, 1994, Huth & Poulton, 2007, McBride *et al.*, 1990), rarely are they assessed in soils as heterogeneous as mountain forest soils (Zhu & Lin, 2010). For example, percent clay was found to correlate with conductivity measures in a semiarid rangeland ecosystem (Abdu *et al.*, 2008), but no such relationship was found in heterogeneous mountain ecosystems (Zhu et al., 2010). Zhu et al (2010) suggested that the presence of higher clay content soils on dry slopes confounded the clay-conductivity correlation, which could potentially be the case in our study as well.

Conductivity for any given sampling date did not correlate strongly with abiotic factors assessed (i.e. soil moisture and soil texture); however, the variance in conductivity measurements among dates (SE and ΔECa-3m) and ECa-3m-high were good predictors of abiotic factors. The use of variance measures (i.e. Δ and SE) and maximum values observed (EC-high) is not common but they have proven to be successful variables in more heterogeneous systems (Vachaud *et al.*, 1985, Zhu *et al.*, 2010). Several reasons can be suggested for the value of variance measures. First, soil texture always influences soil conductivity, while soil moisture increases in importance during wetter times. The difference between soil conductivity measured during dry and wet conditions has been demonstrated to be the most effective predictor for hotspots in soil moisture variation in a semi-arid rangeland ecosystem (Robinson *et al.*, 2008b). We found a similar result for WS 27 where soil moisture was correlated to SE ECa-1m. WS 27 had greater precipitation inputs in combination with finer textured soils that potentially resulted in higher soil moisture retention as has been found before (Bonito *et al.*, 2003). Alternatively, increases in soil moisture could increase the contribution of soil texture to the overall conductivity signal. In other words, increased soil moisture could act as a conductor and, thus,

allow spatial variance in soil texture to be more apparent in the soil conductivity measures. For example, Zhu et al (2010) found soil texture mapping to be most successful with EMI surveys after rain events. Similarly, we found that ECa-3m-high correlated with soil texture only in the wetter WS 27 (Swift & Cunningham, 1988) and SE ECa-1m correlated with soil texture in WS 18. Thus, generally the finer textured and wetter soils in WS 27 resulted in a significant correlation between soil moisture and conductivity (SE ECa-1m) in addition to soil texture and conductivity (ECa-3m-high).

Potential nitrification rates would be expected to be greatest at near field capacity soil moisture (Strong *et al.*, 1999), high ammonium availability (Donaldson & Henderson, 1990), high pH (Donaldson & Henderson, 1990, Knoepp & Vose, 2007), low C:N ratios (Knoepp & Vose, 2007) and high oxygen and low CO_2 concentrations (Keeney *et al.*, 1985, Sahrawat, 2008). In forested ecosystems soil ammonium concentrations are considered the main limiting factor of nitrification rates (Montagnini *et al.*, 1989, Ste-Marie & Paré, 1999), which is the case only for NH in this study. For all other sites, an increased ECa-3m-high conductivity, correlated to soil texture, showed a negative relationship with pNTR rates. Higher ECa-3m-high conductivity is related to greater percent sand and lower percent silt and, thus, greater gas diffusion rates. Since oxygen availability is one of the main controllers of nitrification (Keeney *et al.*, 1985, Sahrawat, 2008), a higher sand content would allow for higher nitrification rates as has been observed by Strong et al (1999). Alternatively, these results could suggest that the fine particles (i.e. silt and clay) protect ammonium from oxidation and, thus, reduce available ammonium for microbial uptake. This was confirmed by Strong et al. (1999) for soils with higher soil moisture content, similar to NH, while soils exposed to frequent drying and rewetting events did not show a similar level of physical protection by finer particle sizes.

Overall, pDNF was best predicted by total soil C as found in previous studies (Groffman & Tiedje, 1991, Luo *et al.*, 1999, Myrold & Tiedje, 1985). However, we found pDNF in WS 27 to be best correlated with conductivity (SE ECa-1m), which likely reflects the strong correlation with soil moisture in WS 27. No improvement in model prediction was accomplished by including total carbon and, thus, carbon does not appear to be a limiting factor for pDNF in this watershed. This is similar to the results of other studies in northern hardwood forests that found denitrification rates were limited by soil moisture and nitrate concentrations rather than organic carbon due to the high organic carbon availability at these sites (Groffman, 2012, Groffman & Tiedje, 1988, Melillo *et al.*, 1983).

Plot scale potential nitrification and potential denitrification rates determined by random sampling compared to EMI and NIRS stratified random sampling showed no significant differences for pNTR in sites with low rates (i.e. OP and MO) whereas rates were greater for CH and NH. Scaling potential denitrification rates to the plot level, however, showed that stratified sampling would result in greater mean rates for all forest types. Stratified sampling generally increased the variability (SE and CV) of the assessment, indicating that the results from random sampling under-represented hotspot areas in the landscape and, thus, underestimates overall site N transformation rates. In line with our hypothesis that pNTR would be less heterogeneous than pDNF, the autocorrelation analysis using semivariograms showed a larger range for MO (MO-low and high) and NH while showing a similar range for OP and CH. Selecting larger plot sizes might have enhanced spatial structure and increased model predictive strength in the sites with a larger range than the plot size (pNTR: CH and NH; pDNF: CH).

This data suggests that on the plot scale (ha) assessing heterogeneity is most important in cove and northern hardwood systems potentially underestimating rates by as much as 200%. The

importance of soil heterogeneity have been shown by previous studies for soil nutrient concentrations (Johnson *et al.*, 2010, Johnson *et al.*, 2011) and processes (Groffman & Tiedje, 1989, Harms & Grimm, 2008, Vidon *et al.*, 2010), but rarely with the high resolution needed for plot level assessment. We found no strong significant correlations between pNTR and pDNF. This uncoupled nature could be the result of low C:NH_4^+ ratios in OP, CH and MO (Chiu *et al.*, 2007) while in NH it could be due to lower oxygen concentrations as indicated by higher soil moisture (Focht & Verstraete, 1977) and lower sand content (Strong *et al.*, 1999).

This study showed that improved precision in extrapolating biogeochemical data to an ecologically relevant scale through the use of geophysical approaches that provide high resolution spatial data. Including spatially dependent data increases the representative estimates and reduces sampling redundancy for nitrogen cycling processes. However, site specific calibration to the biotic processes of interest is generally required. These approaches will enable us to assess the spatial variability of biogeochemical cycling and improve the extrapolation by stratified sampling methods. Combining geophysical and stratified sampling allowed us to address more specific questions regarding the regulation of nitrogen cycling processes. Approaches similar to the one utilized in this study are needed over multiple spatial scales to better parameterize the biogeochemical models of the future.

Table 2.1: Selected site characteristics. Data compiled from Coweeta Long Term Ecological Research Program records. See website (www.coweeta.uga.edu) for additional information. Modified from Knoepp et al (1998).

Site	**Oak-Pine (OP)**	**Cove Hardwood (CH)**	**Mixed Oak-low (MO-low)**	**Mixed Oak-high (MO-high)**	**Northern Hardwood (NH)**
Geographic coordinates	83° 26' N 35° 3' W	83° 26' N 35° 2' W	83° 26' N 35° 2' W	83° 27' N 35° 2' W	**83° 27' N 35° 1' W**
Elevation (m)	788	801	860	1094	**1389**
Aspect (degrees)	180	340	15	75	**20**
Slope (degrees)	34	21	34	33	**33**
Vegetation	oak-pine	cove hardwood	mixed Oak	mixed oak	**northern hardwood**
Dominant Species	*Pinus rigada* *Quercus coccinea* *Quercus prinus* *Carya spp.* *Kalmia latifola*	*Liriodendron tulipifera* *Quercus prinus* *Carya spp.*	*Quercus prinus* *Carya spp.* *Quercus rubra* *Rhododendron maximum*	*Quercus prinus* *Quercus rubra* *Carya spp.* *Rhododendron maximum*	***Betula allegheniensis*** ***Quercus rubra*** ***Betula lenta*** ***Tilia heterophylla***
Moisture Regime	xeric	mesic	mesic	mesic	**mesic**
Soil Series	Evard/Cowee Chandler Edneyville/Chestnut	Saunook Tuckaseegee	Trimont	Chandler	**Plott**
Soil Texture	Fine-loamy Coarse-loamy Coarse-loamy	Fine-loamy Fine-loamy	Fine-loamy	Coarse-loamy	**Coarse-loamy**
Soil Subgroup	**Typic Hapludults** **Typic Dystrochrepts**	**Humic Hapludults** **Typic Dystrochrepts**	**Humic Hapludults**	**Typic Dystrochrept**	**Typic Haplumbrepts**

Table 2.2: Soil properties by forest type for the coarse fraction, sand fraction, silt fraction and clay fraction (0-20 cm) collected in June 2011. The values for sand, silt and clay are only considering the < 2 mm fraction. Different letters indicate significant differences ($p<0.05$). The C and N data on <2mm bulk soil were determined using NIRS. Forest types are oak-pine (OP), cove hardwood (CH), mixed oak low elevation (MO-low), mixed oak high elevation (MO-high) and northern hardwood (NH).

Site	N	Coarse ($g\ kg^{-1}$)	Sand ($g\ kg^{-1}$)	Silt ($g\ kg^{-1}$)	Clay ($g\ kg^{-1}$)	C ($kg\ C\ m^{-2}$)	N ($kg\ N\ m^{-2}$)
OP	10	180 ± 20^{ab}	820 ± 20^{a}	110 ± 20^{c}	70 ± 10^{c}	3.9 ± 0.3^{ab}	0.22 ± 0.01^{b}
CH	10	240 ± 20^{a}	720 ± 20^{ab}	170 ± 20^{b}	110 ± 10^{bc}	4.5 ± 0.3^{ab}	0.25 ± 0.01^{ab}
MO-low	10	140 ± 20^{bc}	700 ± 20^{b}	170 ± 10^{b}	130 ± 10^{b}	4.3 ± 0.3^{ab}	0.25 ± 0.01^{ab}
MO-high	10	150 ± 20^{bc}	680 ± 40^{b}	170 ± 20^{bc}	200 ± 10^{a}	3.3 ± 0.4^{b}	0.21 ± 0.01^{b}
NH	10	100 ± 10^{c}	550 ± 10^{c}	250 ± 10^{a}	200 ± 10^{a}	5.0 ± 0.3^{a}	0.27 ± 0.01^{a}

Table 2.3: Regressions between soil conductivity and soil particle abundance. All data were natural log transformed to assure normal distribution for statistical analysis. The values indicate the r^2 for $p < 0.05$ ("§" indicates $p<0.1$) and the '+' or '-' indicate the direction of the linear relationship. N = 50 (WS18 N = 30; WS27 N = 20). Watershed 18 (WS 18) includes oak-pine (OP), cove hardwood (CH) and mixed oak low elevation (MO-low). Watershed 27 includes mixed oak high elevation (MO-high) and northern hardwood (NH). ΔECa-3m indicates the difference between the minimum and maximum value, SE ECa-1m the standard error and ECa-3m-high the highest value measured.

	Particle Size (g kg^{-1})								
	----------Sand----------			----------Silt----------			**----------Clay----------**		
WS	ΔECa-3m	SE ECa-1m	ECa-3m-high	ΔECa-3m	SE ECa-1m	ECa-3m-high	ΔECa-3m	SE ECa-1m	**ECa-3m-high**
All	0.12(+)	-	-	0.20 -)	0.12(-)	-	0.28(-)	0.20(-)	-
WS18	0.25(+)	0.45(+)	-	0.29(-)	0.53(-)	-	0.13(-)§	0.19(-)	-
WS27	-	**0.22(-)§**	**0.33(+)**	-	**0.24(+)§**	**0.30(-)**	-	-	-

Table 2.4: P-values and coefficients of determination for calibration models between near-infrared reflectance spectra (NIRS) and percent soil C and N, and extractable NH_4^+. Calibration models were developed on mineral soil (0-15 cm) for soil C and N and on both mineral soil and forest floor for NH_4^+.. The validation statistics are representative of a regression analysis between observed and predicted values. All data were natural log transformed to assure normal distribution for statistical analysis.

Parameter	**Entire dataset**			**Calibration dataset**			**Validation dataset**		
	p	r^2	N	p	r^2	N	p	r^2	**N**
C mineral soil (g kg^{-1})	<0.001	0.997	46	<0.001	0.90	30	0.003	0.65	**16**
N mineral soil (g kg^{-1})	<0.001	0.998	46	<0.001	0.89	30	0.024	0.52	**16**
Extr-NH_4^+ (mg N kg^{-1})	**<0.001**	**0.99**	**24**	†	†	†	†	†	†

†dataset was too small to separate into calibration and validation models

Table 2.5: Regression analyses for potential nitrification (pNTR). All data were natural log transformed to accomplish normal distribution for statistical analysis. The r^2 values indicate the coefficients of determinations. The '+' or '-' indicates the direction of the relationship. * indicates $p< 0.05$; ** indicates $p<0.01$. RMSE indicates the root mean square error and N the total number of values used in model ($NH_4^+{}_{5\text{-}10cm}$ for March 2011 and 2012 only and 4 missing values for SE ECa-3m). The validation statistics are representative of a regression analysis between observed and predicted values. ECa-3m-high indicates the highest conductivity value measured and $NH_4^+{}_{5\text{-}10}$ the concentration at 5-10 cm. The forest types include oak-pine (OP), cove hardwood (CH), mixed oak (both low and high elevation) and northern hardwood (NH).

	Entire dataset			**Calibration dataset**			**Validation dataset**			**Parameters**
	RMSE	r^2	N	RMSE	r^2	N	RMSE	r^2	N	
All sites	0.16	0.31 **	31	-	-	24	-	-	9	High ECa-3m (-) & $NH_4^+{}_{5\text{-}10cm}$ (+)
OP, CH & MO	0.01	0.30 **	49	0.005	0.29 **	36	0.004	0.62	13	High ECa-3m (-)
NH	0.09	0.90 **	9	0.07	0.94 **	5	0.08	0.95	4	$NH_4^+{}_{5\text{-}10cm}$ (+)

Table 2.6: Regression analyses for potential denitrification (pDNF). All data were natural log transformed to accomplish normal distribution for statistical analysis. The r^2 values indicate the coefficients of determinations. The '+' or '-' indicates the direction of the relationship. * indicates $p<0.05$; ** indicates $p<0.01$. RMSE indicates the root mean square error and N the total number of values used in model (4 missing samples for SE ECa-1m). The validation statistics are representative of a regression analysis between observed and predicted values. SE ECa-1m indicates the standard error of the values measured and C indicates the carbon content. The forest types include oak-pine (OP), cove hardwood (CH), mixed oak low elevation (MO-low), mixed oak high elevation (MO-high) and northern hardwood (NH).

	Entire dataset			**Calibration dataset**			**Validation dataset**			**Parameters**
	RMSE	r^2	N	RMSE	r^2	N	RMSE	r^2	N	
All sites	1.37	0.15 **	75	1.30	0.11 *	52	1.47	0.32 **	23	C (+)
OP, CH & MO-low	0.97	0.16 **	41	0.91	0.13 §	29	1.03	0.41 *	12	C (+)
OP	0.64	0.41 *	12	†	†	†	†	†	†	C (+)
CH	1.06	0.26 *	16	†	†	†	†	†	†	C (+)
MO-low	-	-	13	†	†	†	†	†	†	C (+)
NH & MO-high (WS27)	0.98	0.70 **	36	0.98	0.64 **	25	0.91	0.84 **	11	SE ECa-1m (+)

†dataset was too small to separate into calibration and validation models

Table 2.7: Semivariogram parameters fitted using a stable fit model with predicted process rates for potential nitrification and denitrification. The forest types include oak-pine (OP), cove hardwood (CH), mixed oak low elevation (MO-low), mixed oak high elevation (MO-high) and northern hardwood (NH).

Nitrogen Cycling Processes								
	----------pNTR----------				----------**pDNF**----------			
Site	Major Range (m)	Full sill	Nugget	Nugget/ Sill	Major Range (m)	Full sill	Nugget	**Nugget/ Sill**
OP	41	$1.1*10^{-5}$	0	0	43	0.15	0.11	**0.73**
CH	>75.4	$3.0*10^{-5}$	$3.4*10^{-6}$	0.11	>75.4	0.077	0.058	**0.76**
MO-low	69	$8.8*10^{-6}$	0	0	29	0.10	0.031	**0.31**
MO-high	64	$2.1*10^{-5}$	$2.1*10^{-6}$	0.10	0.18	0.015	$1.5*10^{-5}$	**$1.0*10^{-3}$**
NH	**>75.4**	**0.25**	**0.049**	**0.20**	**37**	**54**	**0**	**0**

Table 2.8: Comparison of the effectiveness of extrapolation for potential nitrification (pNTR) and potential denitrification (pDNF) as assessed by random selection and by stratified selection (determined by quartiles) based EMI and NIRS data (n=8 for both approaches, except pNTR 427 and pNTR 527 N=4). The rates are in ng N g_{soil}^{-1} h^{-1}, SE indicates the standard error and CV indicates the coefficient of variation. The forest types include oak-pine (OP), cove hardwood (CH), mixed oak low elevation (MO-low), mixed oak high elevation (MO-high) and northern hardwood (NH).

	Potential Nitrification – *Random*			**Potential Nitrification – *Stratified Random***			**Potential Denitrification – *Random***			**Potential Denitrification – *Stratified Random***		
	Rate	SE	CV	Rate	SE	CV	Rate	SE	CV	Rate	SE	CV
OP	-0.01	0.01	394	0.02	0.03	386	200.7	81.5	115	210.7	81.6	110
CH	0.06	0.03	127	0.07	0.03	117	426.2	114.4	76	1247.7	754.3	171
MO-low	-0.02	0.01	124	0.001	0.01	2169	168.9	34.0	57	193.1	37.6	55
MO-high	-0.02	0.03	225	-0.02	0.03	274	161.4	29.3	51	200.0	34.8	49
NH	2.2	0.6	53	2.9	1.0	66	3874.4	892.5	65	5456.6	1969.8	102

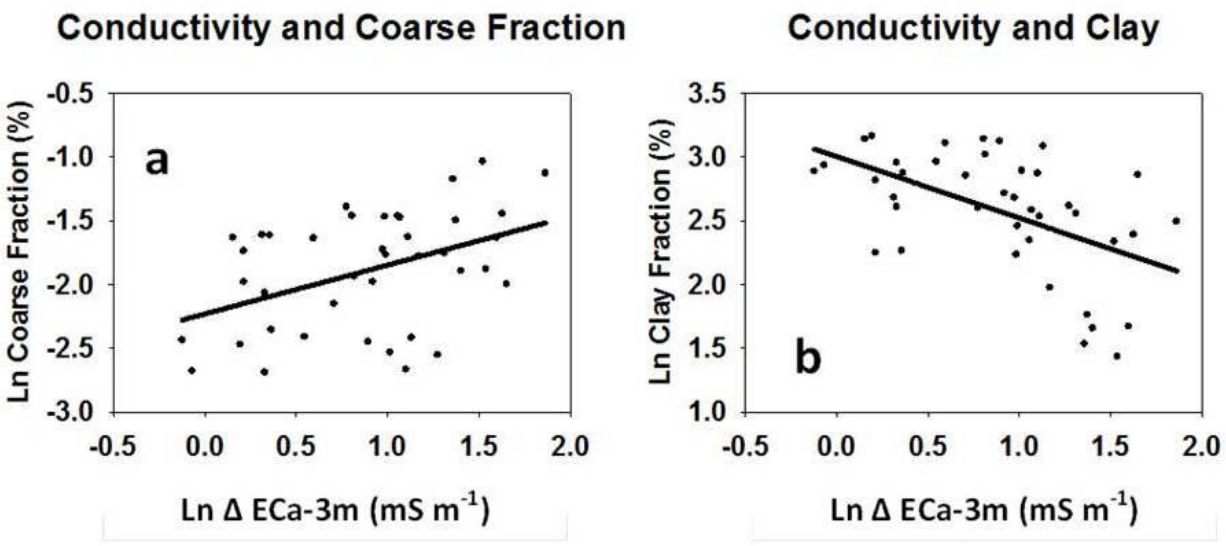

Figure 2.1: Regression between ΔECa-3m conductivity and coarse soil fraction (a) and clay content (b) for all five sites (0-20 cm). Both regression a ($p = 0.0055$; $r^2 = 0.19$) and b ($p = 0.0004$; $r^2 = 0.28$) were significant. All data were natural log transformed.

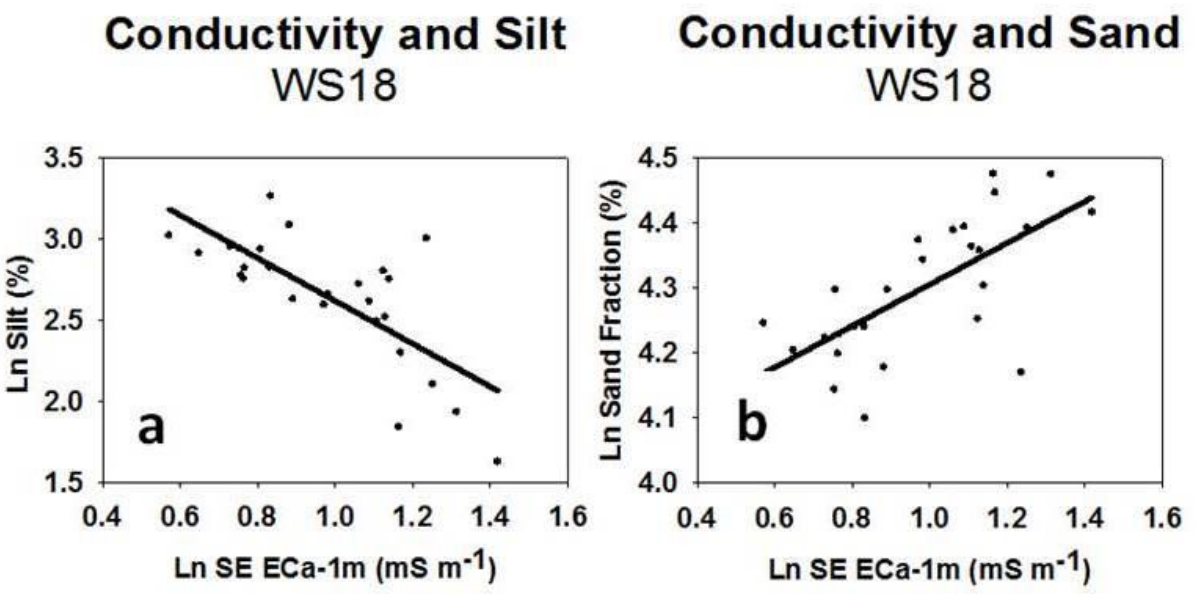

Figure 2.2: The silt (a) and sand (b) fraction (0-20 cm) regressed with SE ECa-1m conductivity for watershed 18. Both regressions a ($p < 0.0001$; $r^2 = 0.53$) and b ($p = 0.0003$; $r^2 = 0.45$) were significant. All data were natural log transformed.

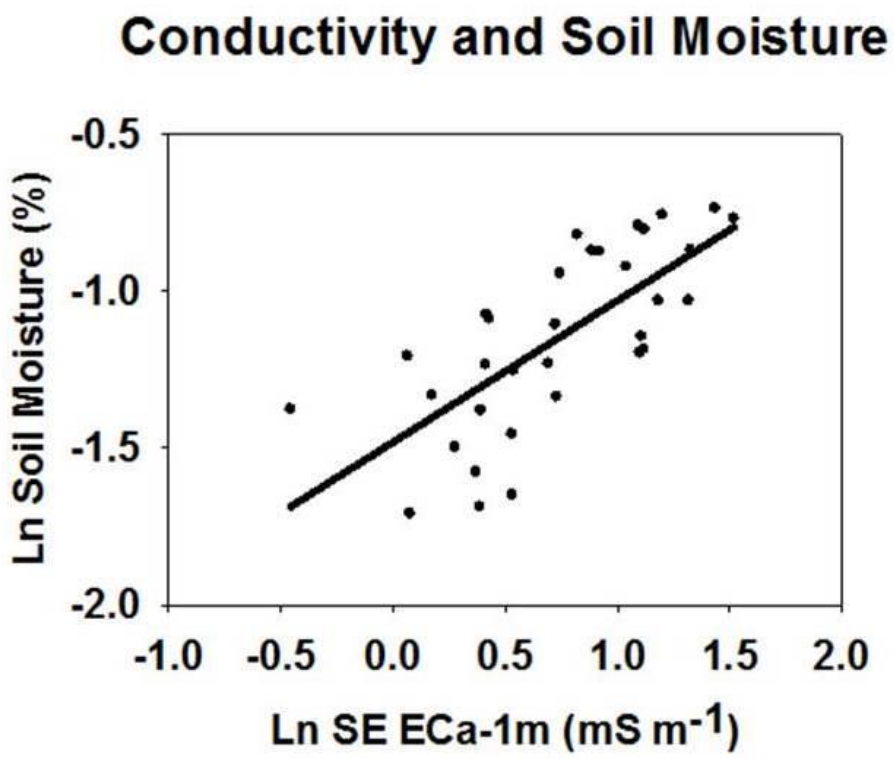

Figure 2.3: Fractional soil moisture (0-20 cm) regressed with the standard error of ECa-1m conductivity for WS 27 ($p < 0.0001$; $r^2 = 0.56$). All data were natural log transformed.

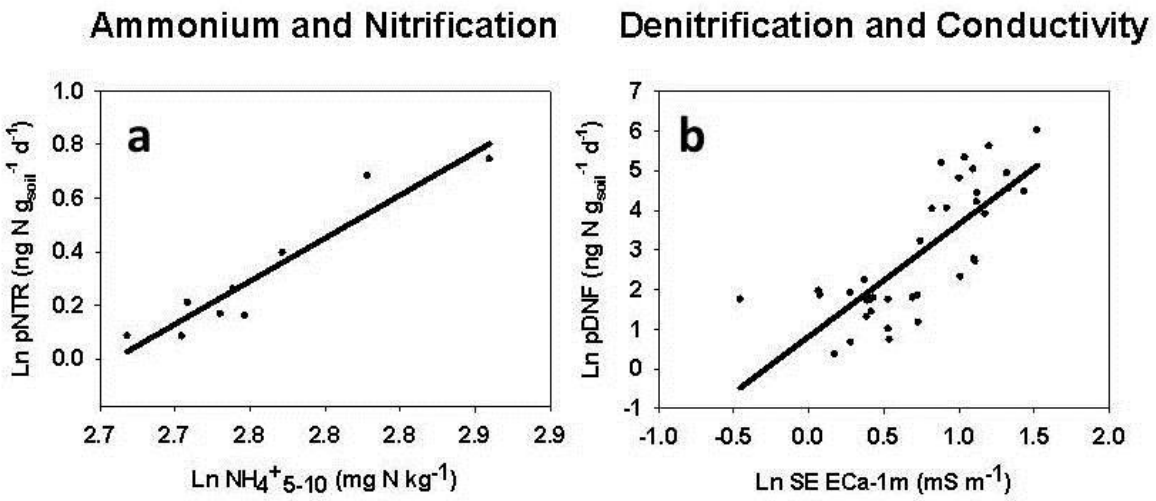

Figure 2.4: Potential nitrification rates (pNTR) regressed with the NH_4^+ concentrations at 5-10 cm soil depth in NH (a) ($p < 0.001$; $r^2 = 0.90$) and potential denitrification rates (pDNF) regressed with the standard error of ECa-1m conductivity in WS 27 (b) ($p < 0.001$; $r^2 = 0.70$). All data were natural log transformed.

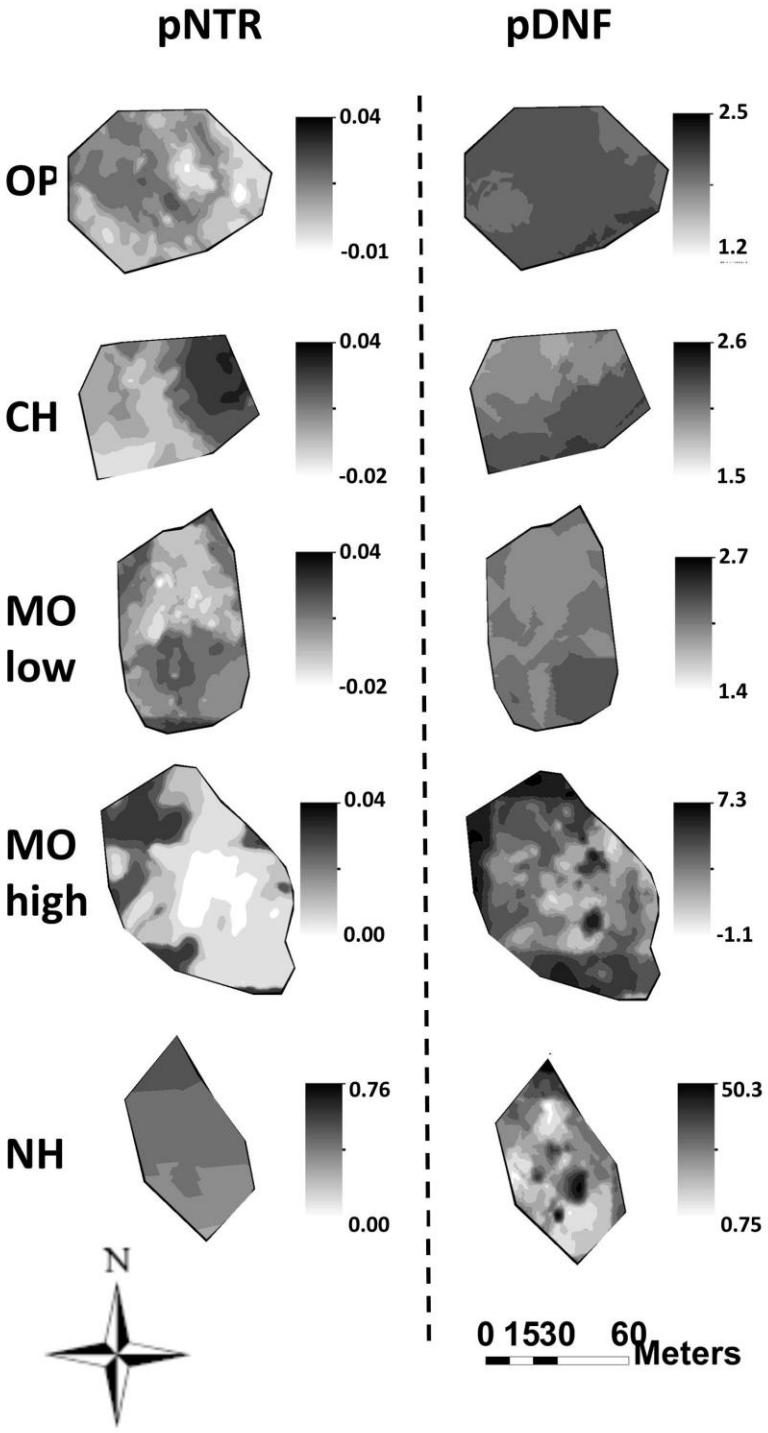

Figure 2.5: Predicted potential nitrification (pNTR) (left) and potential denitrification (pDNF) (right) based on EMI and NIRS data. The numbers represent the natural log of predicted process rates (ng N g_{soil}^{-1} d^{-1}) as produced by ordinary kriging. The contour gradient indicates geometric intervals. Potential nitrification was predicted using ECa-3m-high (OP, CH, MO-low and MO-high) and $NH_4^+{}_{5\text{-}10}$ concentrations (NH). Potential denitrification was predicted using C (g C m^{-2}) (OP, CH, MO-low) and SE ECa-1m conductivity (MO-high and NH). For predictive models see table 5 and 6.

CHAPTER 3

ENHANCING OUR UNDERSTANDING OF FOREST SOIL MICROBIAL CARBON AND NUTRIENT (N, P) LIMITATIONS FOR RESPIRATION AND NITRATE REDUCTION [2]

[2] Baas, P., Rebecca Risser, J.D. Knoepp and J.E. Mohan. To be submitted to *Soil Biology and Biochemistry*.

Abstract

Developing a mechanistic understanding of how soil microbial activity is affected by carbon and nutrient limitations is pivotal given current and projected increases in atmospheric carbon dioxide and nitrogen deposition. In order to explore the nutrient and carbon limitations on soil respiration, nitrate removal and retention processes, soils were collected along an elevation gradient in the southern Appalachian Mountains consisting of oak-pine, mixed-oak, cove hardwood and northern hardwood forest types. Respiration rates were examined in response to *ex-situ* amendments with dextrose, trehalose, mannitol, phosphate and nitrate. Denitrification (nitrogen loss) and nitrate reduction to ammonium (nitrogen retention) responses were also determined under *ex situ* laboratory conditions for soils amended with varying carbon and nitrate concentrations. Soil respiration was limited by carbon with the strongest response to dextrose and trehalose but there was no significant effect of nutrient amendments. Nitrate reduction through denitrification or reduction to ammonium was controlled solely by nitrate concentrations. Forest floor respiration rates were generally greater with carbon amendments in oak-pine and mixed-oak forest soils. Northern hardwood forest soils had the greatest response to carbon amendments in 0-10 cm mineral soil. Denitrification was more sensitive to nitrate amendments than was nitrate reduction to ammonium. This study suggests that a future world of greater C and N availability will result in greater respiration rates and that gaseous losses (denitrification) will increase more rapidly than internal N recycling (nitrate reduction to ammonium).

Introduction

Many soil microbial processes in temperate forests are thought to be nitrogen (N) limited (Vitousek & Howarth, 1991). The N mining hypothesis predicts an N- poor environment would lead to enhanced decomposition rates (Craine *et al.*, 2007), while the basic stoichiometric theory suggests N-poor conditions reduce decomposition (Hessen *et al.*, 2004). Field N fertilization experiments have found soil carbon (C) to decrease (Mack *et al.*, 2004) and increase (Aber *et al.*, 1995, De Vries *et al.*, 2006, Sutton *et al.*, 2008) after treatment. Further, a lack of a response in microbial respiration to nutrient amendments (Allen & Schlesinger, 2004, Allison & Vitousek, 2005, Hollender *et al.*, 2003) questions whether nutrient availability is the mechanism driving the correlation between decomposition and soil organic matter stoichiometry.

In a future world with greater C and nutrient availability, unraveling the microbial response is crucial for our understanding of ecosystem functioning. C availability may increase via greater plant inputs to the soil due to increased productivity associated with greater atmospheric carbon dioxide (CO_2) concentrations (Schlesinger & Lichter, 2001). N availability is expected to increase in many forest systems, either via atmospheric deposition (Galloway *et al.*, 2008, Knoepp *et al.*, 2008) or agricultural and urban run-off (Webster *et al.*, 2012), potentially relieving microbial nutrient limitation. Determining how microbes respond to predicted increases in C and nutrient availability will elucidate whether the microbial response mitigates or exacerbates environmental issues such as pollution and climate change.

Increased availability of C and N could result in either higher C:N ratios or smaller C:N ratios, depending on how the microbial community responds. Increased heterotrophic respiration would reduce soil C amounts and, thus, decrease C:N ratios. Conversely, enhanced nitrate reduction to ammonium (NRA), via dissimilatory reduction to ammonium (DNRA) (Tiedje,

1988) or assimilatory nitrate reduction to ammonium (ANRA), (Sias & Ingraham, 1979) would result in increased retention of ecosystem N (Templer *et al.*, 2008), thus, decreasing C:N ratios. Finally, priming of soil microbial processes by labile C inputs in the form of root exudates can also significantly affect decomposition (Kuzyakov & Domanski, 2000). Phillips *et al.* (2011) found higher atmospheric CO_2 concentrations led to increased rates soil organic matter (SOM) decomposition as a result of the priming of old SOM. Greater soil C availability can result in greater N mining, to maintain a microbial stoichiometric balance this can enhance decomposition (Drake *et al.*, 2013, Phillips *et al.*, 2011). Labile C availability is largely controlled by plant exudation and fungal decomposition. The main labile fungal C inputs into the soil occur as trehalose (storage sugars) and mannitol (Boer *et al.*, 2005). The magnitude of the synergistic priming effect forms another mechanism controlling soil C dynamics. The southern Appalachian Mountains are currently enduring and expected to experience greater N inputs in the future (Galloway *et al.*, 2008). This region plays a pivotal role in providing abundant high-quality fresh water to the southeastern US region (Gragson & Bolstad, 2006). Understanding what is limiting soil microbial activity in the southern Appalachian Mountains will determine how changes in nutrient and C inputs will affect soil C sequestration and nutrient dynamics in this region.

This study aimed to elucidate whether microbial respiration and nitrate reduction in the different forest ecosystems of the southern Appalachian Mountains are generally nutrient or C limited. We hypothesized that respiration would show the greatest increase with N and phosphorus (P) amendments by relieving nutrient limitation for enzyme production. Respiration would also increase with P amendments in higher elevation sites that have greater N deposition. We expected nitrate reduction pathways to be limited by C in mixed-oak and pine oak ecosystems that have lower soil carbon, while we expected nitrate to be limiting in cove and

northern hardwood forest ecosystems that have higher soil C. In addition, we expected nitrate reduction to ammonium pathways would dominate in the high carbon sites.

Methods

Site description

This study was conducted in the Coweeta Hydrological Laboratory USDA experiment forest associated with the Coweeta Long Term Ecological Research site in the western North Carolina Appalachian Mountains. The region is characterized by average temperatures ranging from 5°C (December – January) to 20°C (June – August), and average annual precipitation of 1900 mm with the growing season stretching from May through September (Swift & Cunningham, 1988).

The study was conducted at five forest sites, undisturbed since 1929, along an elevation gradient representing the main forest types present in the southern Appalachian Mountains (Turner *et al.*, 2003). The sites include a xeric oak-pine stand (OP), cove hardwood stand (CH), two mixed oak stands (MO) and a northern hardwood stand (**Table 3.1**).

Soil sampling and preparation

Soils were sampled in June 2011 for laboratory estimates of respiration and in July 2011 for estimates of potential denitrification and potential NRA. In June 2011, three replicates of forest floor samples were collected from random locations in each stand. After removal of the forest floor twenty cm of mineral soil was collected with a 3-cm diameter core. The mineral soil was divided into 0-10 cm and 10-20 cm depth increments. In July 2011, forest floor samples and 0-5 cm (previous research has shown these depths to show the greatest rates) of mineral soil were

collected to assess carbon and nitrate controls of potential denitrification and potential NRA. All soils were kept at 4°C until the start of the experiments and the experiments were started within two weeks of sample collection.

Respiration experiment

Estimates of soil respiration were determined by placing ten grams of field moist soil in a 50-ml centrifuge tube retrofitted with a cap containing a septum for gas collection. Gravimetric water content was determined earlier on two grams of field moist soil by drying the soil to a constant weight at 105°C (Robertson *et al.*, 1999). Soils were kept at 50% water content which is within the range of the most favorable conditions for microbial respiration (Bradford *et al.*, 2010, Paul *et al.*, 2001). The soil water content was kept constant by weighing the tube twice daily and correcting by adding deionized water. After allowing the soils to equilibrate at 50% soil water content for two days, soils were amended with solution containing dextrose (40 mg C g^{-1} dry soil), trehalose (40 mg C g^{-1} dry soil), mannitol (40 mg C g^{-1} dry soil), $KHPO_4$ (5 µg P g^{-1} dry soil), KNO_3 (15 µg N g^{-1} dry soil) or deionized water as a control. This resulted in a sample size of 270 (5 sites, 3 depths, 3 replicates, 6 treatments). Previous research has shown 40 mg C g^{-1} dry soil to consistently overcome substrate limitation and, therefore, prevent confounding effects of varying levels of substrate limitation between different soils (Bradford *et al.*, 2010, Davidson *et al.*, 2006). The nitrate and phosphate amendments represent an increase of at least an order of magnitude in concentrations of available nitrate (2 M KCl extract) (Knoepp *et al.*, 2008, Knoepp & Swank, 1998, Mclean, 2011) and phosphorus (labile plus reducible Fe-P) (Mclean, 2011) found in the field and, thus, represented a significant increase in available nutrient concentrations. Immediately after the amendment the centrifuge tube was purged with CO_2-free

air for 30 seconds before being capped. Head space subsamples (1 ml) were taken 2, 6 and 24 h after the start of the incubation and immediately analyzed on a LICOR 7000 infrared gas analyzer (LICOR, Lincoln, NE, USA) set up for small volume injections. Concentrations of the samples were determined by comparing peaks to a 1990 ppm CO_2 standard (Air Liquide America Speciality Gases LLC, Plumsteadville, PA, USA). Respiration rates were determined by linear regression of CO_2 concentrations over time.

Potential nitrate reduction experiment

Potential nitrate reduction assays were conducted following a similar set-up to the acetylene block method (Baas *et al.,* In press, Groffman *et al.,* 1999). Five grams of field moist soil were added to 37 ml serum vials purged with He for 30 seconds to ensure anoxic conditions and subsequently capped using a septum and crimp seal. Next, five ml of media were added to each of the vials (inserting an additional needle to prevent over-pressurizing) and 4 ml of headspace was replaced with acetylene (10% v/v). The media consisted of a fully factorial set up with three NO_3^- (0, 100 and 1000 µM) and three dextrose (0, 100, 1000 µM) concentrations resulting in nine treatment combinations per site and depth with 2-6 replicates (N_{total} = 246). The vials were shaken at 150 rpm throughout the incubation. Head space subsamples for N_2O analysis were collected after 30 min, 2 h and 6 h. For NH_4^+ concentrations, 2 M KCl extractions were conducted before the start of the incubation and immediately after the 6 h incubation. N_2O was analyzed using a GC-ECD (Shimadzu Inc., Tokyo, Japan) and NH_4^+ concentrations were determined colorimetrically (USEPA, 1983b). Rates of potential denitrification were conducted by regression analysis and potential NRA rates (only determined on the following combinations NO_3^-/Dextrose: 1000/1000, 100/1000, 0/1000, 1000/100, 1000/0 and 0/0) were estimated by

dividing the increase in NH_4^+ concentrations by total time incubated (6 h). Anaerobic N-mineralization, anaerobic nitrification, DNRA and ANRA all contribute to the overall NRA rate determined in this study.

Statistics

To test for site and treatment effects on respiration rates we conducted standard least squares analysis of variance (ANOVA) at each of the soil depths (forest floor, 0-10 cm and 10-20 cm) and site. For denitrification rates we conducted ANOVA analyses at each depth and site to test for differences between nitrate amendments with different dextrose amendments and for differences between dextrose treatments with difference nitrate amendments. For pNRA rates we conducted a similar analysis only combining all sites in the analysis. In addition, for both potential denitrification and pNRA, multivariate regressions using continuous data (nmol amended g_{soil}^{-1}) were conducted to assess interaction effects of dextrose and nitrate. Log transformations were conducted if data were not normally distributed and rank transformations were used if data was non-lognormal. All analyses were conducted in JMP 11.0 (SAS Institute Inc., Cary, North Carolina).

Results

Soil respiration

Soil respiration rates showed different responses among sites and soil depths (**Figure 3.1**). In the forest floor horizon, significant treatment effects were observed for oak-pine ($F_{5,12}$ = 6.4,, p = 0.004), mixed-oak high ($F_{5,12}$ = 14.7, p< 0.0001) and northern hardwood ($F_{5,12}$ = 47.8, p < 0.0001). For oak-pine the trehalose and dextrose treatment had significantly greater rates

compared to the N ($p < 0.05$), P ($p < 0.05$) and the control treatment ($p < 0.07$). In the forest floor, the higher elevation sites (mixed-oak high and northern hardwood) showed all carbon amendment to result in greater respiration rates than the control and N and P treatments ($p < 0.05$). No significant differences in forest floor respiration rates were detected between treatments in the cove hardwood and mixed-oak low site.

Different patterns were observed in the 0-10 cm mineral layer with treatments rarely being significantly greater than the control. Significant treatment effects were observed for oak-pine ($F_{5,12} = 6.5$, $p = 0.004$), cove hardwood ($F_{5,12} = 9.6$, $p = 0.0007$) and northern hardwood ($F_{5,12} = 6.5$, $p = 0.004$). Oak-pine showed significantly greater rates for the dextrose treatment compared to the N and P treatment ($p < 0.05$) with a trend of trehalose and mannitol having greater rates than the N treatment ($p < 0.06$). Cove hardwood sites showed significantly greater rates for all carbon amendments compared to the P treatment ($p < 0.05$) with the trehalose treatment being greater than the N treatment ($p = 0.03$). Northern hardwood respiration rates were greater for all carbon amendments than the N treatment ($p < 0.05$). In the 10-20 cm mineral layer we found significant treatment effects for cove hardwood ($F_{5,12} = 4.3$, $p =0.02$), mixed-oak low ($F_{5,12} = 13.6$, $p =0.0001$) and northern hardwood ($F_{5,11} = 16.1$, $p < 0.0001$). Tukey pairwise comparisons found no significant differences in cove hardwood and all carbon amendments had greater rates than the control and both nutrient treatments in mixed-oak low ($p < 0.05$) and northern hardwood ($p < 0.001$).

Nitrate reduction assays

Potential denitrification rates showed few significant treatment differences. In the forest floor horizon, oak-pine showed a significantly effect of the nitrate treatment when dextrose was

amended at 100 mM ($F_{2,2}$ = 28.6, p = 0.03) and at 1000 mM (F2,7 = 5.9, p = 0.03). With the 100 mM dextrose amendment 100 and 1000 mM nitrate amendments resulted in greater denitrification rates than the 0 mM amendment ($p < 0.05$) while with the 1000 mM nitrate amendment resulted in significantly greater rates than the 0 mM amendment ($p < 0.05$). Cove hardwood mineral soils showed significant nitrate treatment effects with a dextrose amendment of 100 mM ($F_{2,3}$ = 13.5, p = 0.03) with 100 and 1000 mM nitrate amendments resulting in greater rates than the 0 mM treatment ($p < 0.05$). Mixed-oak low showed a significant nitrate treatment effect when no dextrose was amended ($F_{2,7}$ = 6.6, p = 0.02) with denitrification rates being significantly greater for the 1000 mM nitrate amendment compared to the 0 mM amendment. Mixed-oak high mineral soil showed a significant treatment effect no dextrose was amended ($F_{2,7}$ = 5.4, p = 0.04), however, pairwise comparisons only resulted a trend of the 100 (p = 0.07) and 1000 mM (p = 0.10) nitrate amendment being greater than the 0 mM treatment. Northern hardwood soils never showed a significant treatment effect. When testing for the effect of dextrose amendments at different nitrate amendments, no significant differences were observed for any site and soil depth.

pNRA rates did not show significant nitrate treatment effects with any dextrose amendment. However, dextrose treatment effects were observed for the mineral soil when amended with 1000 mM nitrate ($F_{2,13}$ = 4.6, p = 0.03) with the 1000 mM dextrose amendment yielding lower rates than the 100 mM dextrose amendment (p = 0.03).

Regression analyses between potential denitrification with nitrate concentrations (μg N g_{soil}^{-1}) at a specific nitrate treatment (using the experimental variation in nitrate amendment per g soil) combining all sites found a significant relationship in the mineral soil at 100 and 1000 mM nitrate amendment. Within those amendment the sensitivity (i.e. slope) for nitrate decreased by

twenty fold from the 100 mM to the 1000 mM amendment. When doing the same analysis for dextrose, a similar pattern was observed when comparing the sensitivity at 100 mM and 1000 mM dextrose amendments where we saw a tenfold decrease in sensitivity. Regression analysis for each site between concentrations of nitrate (μg N g_{soil}^{-1}) amended showed forest floor potential denitrification to be significantly positively related to nitrate concentrations for mixed-oak high ($p = 0.04$, $r^2 = 0.69$) and negatively related to northern hardwood ($p = 0.03$, $r^2 = 0.74$) when amended with 100 mM nitrate. When amended with 1000 mM nitrate, a positive relationship was observed between nitrate concentrations and potential denitrification in mixed-oak low ($p = 0.05$, $r^2 = 0.40$). Potential denitrification rates in mineral soils showed a significant positive relationship in mixed oak low ($p = 0.03$, $r^2 = 0.72$) when amended with 100 mM nitrate while when amended with a 1000 mM nitrate a positive relationship was found for oak-pine ($p = 0.03$, $r^2 = 0.65$), cove hardwood ($p = 0.001$, $r^2 = 0.75$) and mixed-oak low ($p = 0.01$, $r^2 = 0.60$). Regression analysis between potential denitrification and dextrose concentrations (μg C g_{soil}^{-1}) for each dextrose amendment showed forest floor denitrification rates to be significantly negatively related to dextrose concentrations when amended with 100 mM dextrose in cove hardwood ($p = 0.01$, $r^2 = 0.82$) while no significant relationships were found when amended with 1000 mM dextrose. Potential denitrification rates were positively related to dextrose concentrations when amended with 100 mM dextrose for oak-pine ($p = 0.006$, $r^2 = 0.99$) and mixed-oak low ($p = 0.05$, $r^2 = 0.76$) while amending with 1000 mM dextrose resulted in significant positive relationships for oak-pine ($p = 0.002$, $r^2 = 0.78$) and cove hardwood ($p = 0.002$, $r^2 = 0.73$).

Regression analyses between potential denitrification and NRA showed a significant interaction (**Table 3.2**) and significant interactions were found for each of the different forest

types with the exception of northern hardwood. The ANCOVA analysis showed that the slope for oak-pine is significantly lower compared to the slope for cove and northern hardwood. This indicates a lower proportion of NRA relative to denitrification in northern hardwood.

Discussion

This study assessed how future forest soils might respond to increased C and nutrient availability. Contrary to our hypothesis, we found soil respiration to be limited by labile C with no indications of nutrient limitations, hence confirming the nutrient mining hypothesis. We hypothesized nitrate reduction to be limited by nitrate in forest ecosystems high in soil C while C would be limiting in a carbon poor forest ecosystem. However, we found both denitrification and NRA to be limited by nitrate in all forest ecosystems. We found greater nitrate sensitivity of denitrification compared to NRA suggesting that denitrification might be mitigating some of the projected increases in inorganic N.

Responses of microbial activity have shown contradictory responses to nutrient amendments with different effects between forest floor and mineral soil (Allen & Schlesinger, 2004) or only exhibiting nutrient limitation under C amended conditions (Allison *et al.*, 2008, Allison & Vitousek, 2005). This study showed microbial respiration to be mainly controlled by the availability of labile C. Heterotrophic soil respiration increased the most with dextrose and trehalose amendments. Trehalose is a fungal-derived sugar, so these results suggest an associated fungal-bacterial coupling within the microbial community of these forest ecosystems (Boer *et al.*, 2005).

Contrary to our hypothesis, nutrient additions did not stimulate soil respiration. This response indicates a shift towards more microbial biomass accrual (Schimel & Weintraub,

2003). However, while experimental studies have generally found microbial biomass N to increase with fertilization (Allen & Schlesinger, 2004), other studies have found no effect on total microbial biomass (Allison *et al.*, 2008, Allison & Vitousek, 2005). Allison *et al.* (2008) hypothesized that the lack of N fertilization response in their study might have been due to secondary P limitation which findings by Allen and Schlesinger (2004) corroborate for a pine ecosystem. In our study we see no clear indication of P limitation except for slightly higher respiration rates in 0-10 cm soil of the northern hardwood forest type with P addition. The lack of a nutrient response indicates that heterotrophic respiration is limited by C substrates.

Increased C subsidies to the soil are expected to result in little increases in nitrate reduction since denitrification and NRA were found to be controlled by nitrate availability. Previous research has found that texture is an important predictor of denitrification rates (Groffman & Tiedje, 1989), however, we found no evidence of this in the current study based on previously determined soil textures (Baas *et al.*, In press). A lack of sensitivity to carbon is to be expected in forest types with high C concentrations like northern hardwood (Vitousek *et al.*, 1982) but contrary to our hypothesis, we found forest types with lower C to exhibit N limitation as well. The size of the C pool does not necessarily reflect C availability since a high turnover rate of a small but labile carbon pool is possible. The high forest floor respiration rates in oak-pine in addition to the tight nitrogen cycle found in this ecosystem (Bonito *et al.*, 2003) support the hypothesis that labile C availability might be higher than the total C pools suggest. In addition, N retention can be hypothesized to decrease with greater N deposition due to larger relative increases in denitrification compared to NRA. It is important to note that denitrification under greater nitrate concentrations often results in a greater proportion of denitrification being emitted as the potent greenhouse gas N_2O with potential implication in exacerbating climate

change (Denman *et al.*, 2007, Weier *et al.*, 1993). In the current experimental approach it is impossible to distinguish between NRA and increased net N mineralization and, therefore, we can only hypothesize about the mechanisms driving the relative contribution of both processes contributing to these results. N mineralization rates have been found to increase under anoxic conditions (Powers, 1990) which likely accounted for a significant part of the total NRA rate. Additionally, N mineralization could have increased due to reduced NH_4^+ immobilization as has been found before with nitrate amendments (Recous *et al.*, 1990) potentially accounting for some of the treatment effects. In addition, since acetylene and low O_2 inhibits nitrification (Walter *et al.*, 1979), it is possible some of the NH_4^+ accumulation can be attributed to reduced nitrification during the anoxic slurry incubations. Nitrate immobilization decreases with greater N availability due to stoichiometric principles (Manzoni *et al.*, 2010) and an increase in the easier to assimilate NH_4^+ concentrations will result in lower assimilatory nitrate reduction (Tiedje, 1988). NRA, on the other hand, would become a more likely pathway if inorganic N (in the form of nitrate) becomes available in access of microbial stoichiometric requirements (Tiedje, 1988). This data suggest that, independent of increases in soil C inputs and plant uptake, greater N deposition scenarios might results in increased N gaseous and leaching losses from the ecosystem.

We found respiration was mainly controlled by labile C with no discernable indications of nutrient limitation. Further, nitrate reduction pathways were controlled by nitrate, independent of carbon concentrations, and denitrification proved to be relatively more sensitive to increases in nitrate concentrations than NRA. Additionally, gaseous losses via denitrification will be greatest in northern and cove hardwood ecosystems while pine-oak and mixed-oak will show less N mitigation capacity and increased N leaching.

Table 3.1: Site characteristics compiled from the Coweeta Long Term Ecological Research Program (www.coweeta.uga.edu). Some data modified from Knoepp et al (1998).

Site	Oak-Pine	Cove Hardwood	Mixed Oak-low	Mixed Oak-high	Northern Hardwood
Geographic coordinates	83° 26' N 35° 3' W	83° 26' N 35° 2' W	83° 26' N 35° 2' W	83° 27' N 35° 2' W	83° 27' N 35° 1' W
Elevation (m)	788	801	860	1094	1389
Aspect (degrees)	180	340	15	75	20
Slope (degrees)	34	21	34	33	33
Dominant Species	*Pinus rigida* *Quercus coccinea* *Quercus prinus* *Carya spp.* *Kalmia latifola*	*Liriodendron tulipifera* *Quercus prinus* *Carya spp.*	*Quercus prinus* *Carya spp.* *Quercus rubra* *Rhododendron maximum*	*Quercus prinus* *Quercus rubra* *Carya spp.* *Rhododendron maximum*	*Betula allegheniensis* *Quercus rubra* *Betula lenta* *Tilia heterophylla*
Moisture Regime	xeric	mesic	mesic	mesic	mesic
Soil Series	Evard/Cowee Chandler Edneyville/Chestnut	Saunook Tuckaseegee	Trimont	Chandler	Plott
Soil Texture	Fine-loamy Coarse-loamy Coarse-loamy	Fine-loamy Fine-loamy	Fine-loamy	Coarse-loamy	Coarse-loamy
Soil Subgroup	Typic Hapludults Typic Dystrochrepts	Humic Hapludults Typic Dystrochrepts	Humic Hapludults	Typic Dystrochrept	Typic Haplumbrepts
Total Carbon†	3.1 ± 0.3	6.4 ± 0.3	3.3 ± 0.1	3.9 ± 0.2	10.1 ± 0.5
Total Nitrogen†	0.09 ± 0.01	0.36 ± 0.02	0.15 ± 0.01	0.14 ± 0.01	0.69 ± 0.04

†Data from Coweeta database for 1995 (www.coweeta.uga.edu)

Table 3.2: Regression analysis between potential denitrification (pDNF) and potential nitrate reduction to ammonium (pNRA) rates. pNRA = slope * pDNF. OP = oak-pine; CH = cove hardwood; MO-L = mixed oak low elevation; MO-H = mixed oak high elevation; NH = northern hardwood. Data is log transformed before analyses to acquire normal distribution.

Forest Type	**Slope**	**r^2**	**N**
Overall	0.68	0.11**	64
OP	0.77	0.78**	9
CH	1.23	0.20*	27
MO-L	1.09	0.60**	10
MO-H	ns	ns	9
NH	ns	ns	9

*$p < 0.01$
**$p < 0.05$

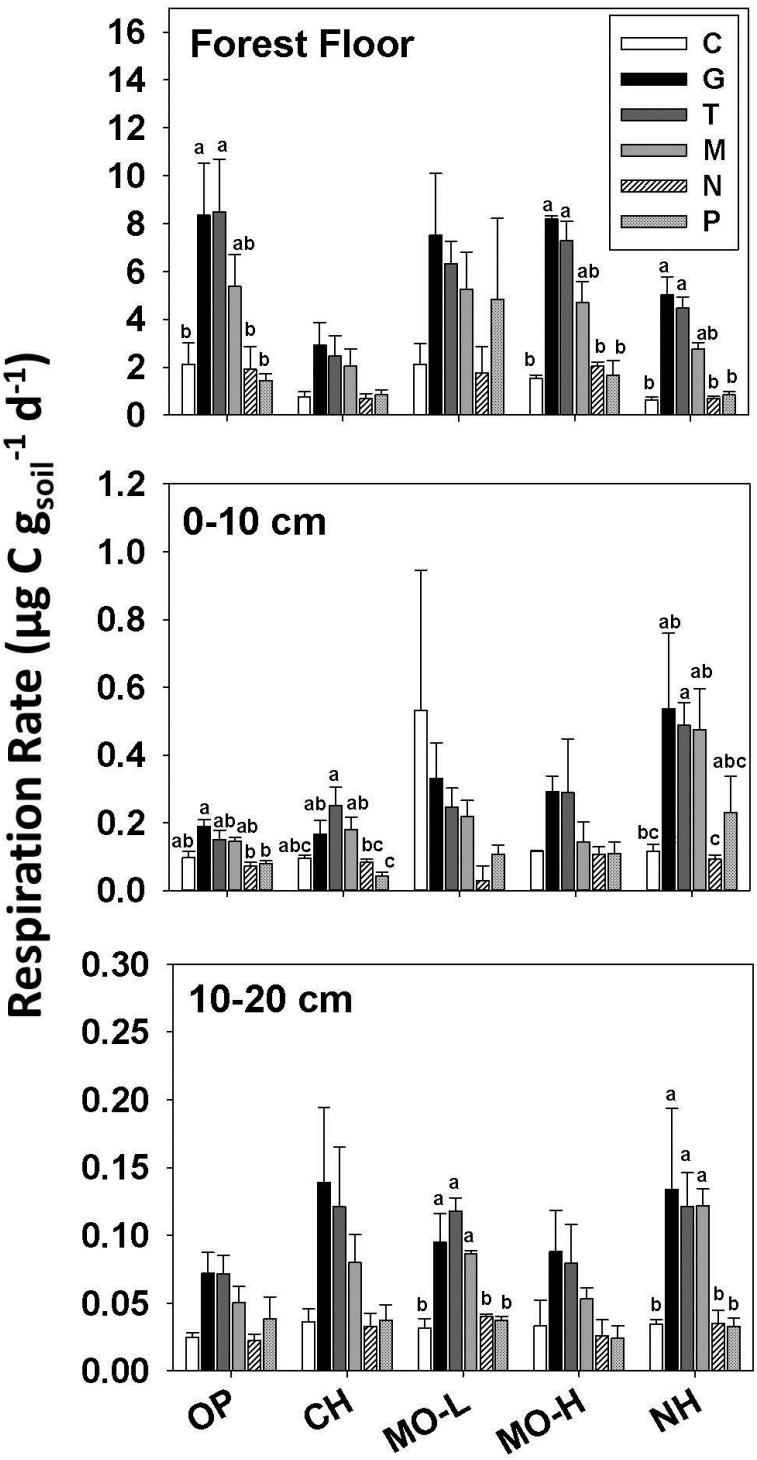

Figure 3.1: Soil respiration rates for forest floor (top, left), 0-10 cm (top, right) and 0-20 cm mineral soil (bottom, left). Soils were amended with mannitol (M), trehalose (T), dextrose (D), phosphate (P) or nitrate (N) and included a DI water amendment control (C). The bars indicate means and the errors represent the standard error of the mean (N=3). Different capitalized letters indicate significant differences between treatments and non-capitalized letters indicate significant differences between forest types within treatments.

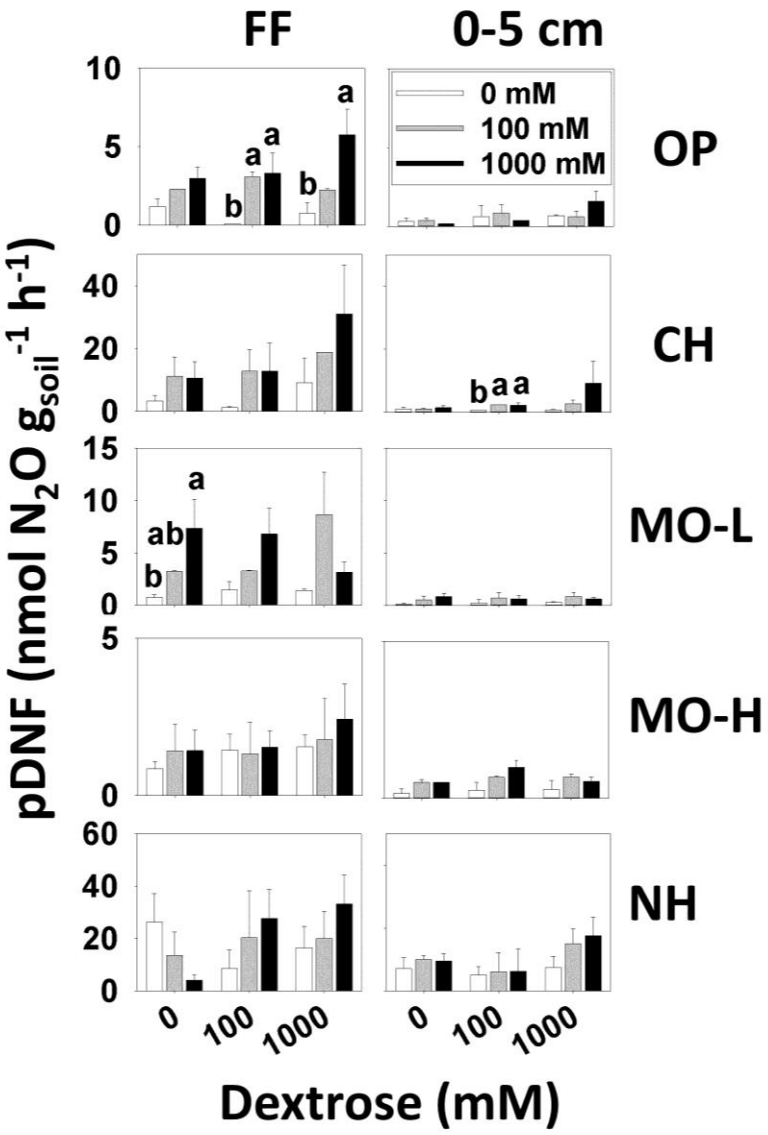

Figure 3.2: Potential denitrification rates amended with different stoichiometric ratios of NO_3^- to dextrose. Soil samples were amended with three levels (0, 100, 1000 mM) of dextrose and NO_3^- with a total of nine treatment combinations. The bars indicate means and the errors represent the standard error of the mean (N=3). Different letters indicate significant differences between nitrate treatments

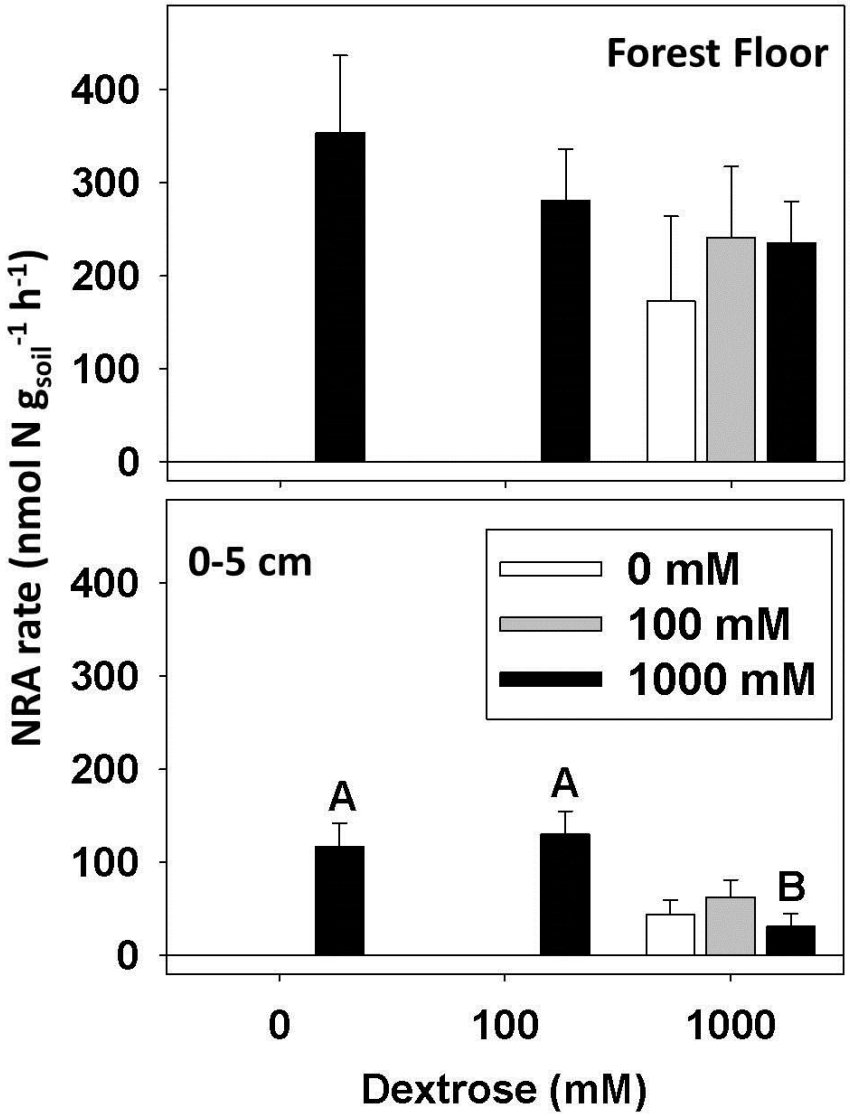

Figure 3.3: Potential nitrate reduction to ammonium (NRA) rates with soil slurry solution amended with varying concentrations (0, 100 and 1000 µM) of nitrate and dextrose. The bars indicate means and the errors represent the standard error of the mean (N = 3-6). Different letters indicate significant differences between dextrose treatments.

CHAPTER 4

NITRIFIER DENITRIFICATION IS THE MAIN PATHWAY OF GASEOUS LOSSES IN SOUTHERN APPALACHIAN FORESTS [3]

[3] Baas, P., J.D. Knoepp and J.E. Mohan. To be submitted to *Plant and Soil*.

Abstract

Understanding the specific processes dominating nitrogen (N) cycling in different forest types is crucial in predicting how forest soils might respond to future increases in N deposition and net primary production. Recent literature suggests that anaerobic processes such as denitrification, which results in gaseous loss of N, and dissimilatory nitrate reduction to ammonium (DNRA), which results in ecosystem N retention, are more common in non-wetland soils than previously thought. Along a vegetation and elevation gradient in the Coweeta Hydrologic Laboratory basin, we conducted a lab incubation assessment for gross N cycling process rates (N-mineralization, nitrification, denitrification, dissimilatory reduction to ammonium (DNRA) and immobilization rates) and potential nitrification and denitrification rates. Overall, high elevation northern hardwood proved to have the greatest N mineralization and nitrification rates while oak-pine forest ecosystems had the lowest. Ecosystem retention, defined as the proportion of nitrification that is reduced by DNRA, was highest in the mixed-oak forest ecosystems. Gross denitrification was prevalent in all forest types. Gaseous losses via denitrification were greatest in oak-pine while gaseous losses via nitrifier denitrification were greatest in northern hardwood. All processes, except denitrification, showed a correlation with soil moisture, total carbon and inorganic N concentrations. These results suggest that increased available soil carbon would result in the greatest increases in gaseous nitrogen emissions from mixed-oak and northern hardwood forest soils. The greatest increase in gaseous N loss with increased N inputs can be expected in oak-pine forest types. Finally, the greater microbial immobilization capacity in cove and northern hardwood suggests these forest types will be most resistant to N leaching.

Introduction

Forest nitrogen cycling responses to changes in nitrogen (N) deposition, warming induced increases in N mineralization and primary production will be dependent on the dominance of specific transformations resulting in either ecosystem removal or retention. Net N mineralization and nitrification rates are often used as indicators of plant available N (Keeney, 1980) and are generally correlated to forest productivity (Reich *et al.*, 1997). In the southern Appalachian Mountains, net N mineralization and nitrification rates are lower in low elevation oak-pine and mid elevation mixed-oak forest community types, relative to cove hardwood and high elevation northern hardwood forest communities (Knoepp *et al.*, 2008, Knoepp & Swank, 1998). The quality of litter inputs have been found to drive soil N cycling (Knoepp & Vose, 2007) while at individual sites interactions between soil temperature and moisture modulate the response on N cycling processes. A recent modeling effort by Bouwman *et al.* (2013) estimated soil denitrification (N_2 and N_2O) rates to be a great as 25% of all global N inputs (~90 Tg N y^{-1}). The extent of gaseous N losses from forest soils, however, remains largely unclear (Groffman *et al.*, 2009a, Seitzinger *et al.*, 2006).

Attempts to balance N budgets have revealed a tight N cycle in oak-pine systems while high elevation northern hardwood ecosystems exhibit leaky N cycling, with high transformation rates and a large pool of unaccounted for mineralized N (Bonito *et al.*, 2003). This finding raises the question of whether additional N cycling processes might be important in these ecosystems and could provide additional insight into the fate of mineralized nitrogen.

Future projections of N deposition and land use change suggest that N availability will increase dramatically over the next few decades (Fowler *et al.*, 2013, Kirk *et al.*, 2012, Webster *et al.*, 2012). Additionally, indications that northern hardwood ecosystems are in a state of near

nitrogen saturation have already been observed (Aber *et al.*, 1989, Knoepp *et al.*, 2008). Climate change feedbacks need to also be considered since ecosystems with high N availability can also significantly increase the emission of N_2O, a potent greenhouse gas (Butterbach-Bahl *et al.*, 2013, Hefting *et al.*, 2003). N_2O can be produced by a wide range of processes including nitrification, nitrifier denitrification, denitrification and DNRA (Baggs & Philippot, 2010). In order to predict how the different forest types of this region will respond to increases in N inputs, we need to understand which pathways dominate this region's N cycle, and what environmental conditions control their activity. Therefore, a secondary objective of this study is to investigate potential drivers of individual N transformations in the dominant forest types.

In order to mechanistically understand how the N cycle functions, determinations of a process have to exclude any other competing processes. Nitrogen cycling dynamics have often been assessed by net transformation rates; however this approach has several limitations. First, net rate assessments represent the results of multiple processes happening simultaneously (**Figure 4.1**). The nitrogen cycle involves many oxidation and reduction steps that contribute to the depletion or accumulation of various N pools (**Figure 4.1**). For example NH_4^+ is produced both by the ammonification of organic N (N mineralization) (Schlesinger & Bernhardt, 2013) as well as by the less-studied dissimilatory nitrate reduction to ammonium (DNRA) (Tiedje, 1988) and assimilatory nitrate reduction to ammonium (ANRA) (Cole, 1988). Second, correlation between gross and net rate assays is common are usually poor, making mechanistic conclusions based on net rates unreliable (Davidson *et al.*, 1992, Verchot *et al.*, 2001). Nitrate reduction pathways such as denitrification and DNRA were historically considered less important in well-aerated, non-wetland soils (Tiedje, 1988). However, isotopic tracer methods have determined that rates of denitrification (Pett-Ridge *et al.*, 2006) and DNRA (Morley & Baggs, 2010, Pett-

Ridge *et al.*, 2006, Yang, 2010) in non-wetland soils can be substantial. This counters the paradigm that these processes are marginal in forest soils that are not periodically flooded and we have only begun to elucidate the importance of nitrate reduction in oxic soils such as those in the southern Appalachian Mountains. These patterns are likely driven by spatial hotspots such as anoxic microsites (Parkin, 1987) with the potential to result in ecosystem scale process rates (Baas *et al.*, In press, Groffman *et al.*, 2009a, McClain *et al.*, 2003).

The loss of N via denitrification or retention of N by DNRA in soils is largely controlled by total carbon, carbon to nitrate ratios, reduced sulfur availability and reduced iron availability (Burgin & Hamilton, 2007). Due to a lack of reduced iron and sulfur in Appalachian soils, carbon and nitrate availability are likely the most important drivers.

We used a combination of gross and potential nitrogen cycling assays to determine the importance of specific processes in the dominant forest types of the southern Appalachians. We assessed seasonal patterns of potential nitrification and potential denitrification and did a one-time assessment of gross nitrogen cycling transformation rates. We also examined the relationship with a wide range of potentially controlling variables to determine drivers of processes. We hypothesized northern hardwood to exhibit the greatest transformation rates and that gaseous losses would explain a large proportion of unaccounted for N. Due to high carbon availability we hypothesized DNRA to be greatest in northern hardwood while, due to low nitrification rates, its retention capacity would be greater in the oak-pine, mixed-oak and cove hardwood forest types.

Methods

Site description

This study was conducted in the USDA experimental forest at the Coweeta Hydrologic Laboratory associated with the Coweeta Long Term Ecological Research Site in the southern Appalachian Mountains of western North Carolina. The average temperatures in the region range from 20°C mid-summer (June-August) to 5°C mid-winter (December-January) with an annual average precipitation of 1900 mm. The growing season extends from May through September (Swift & Cunningham, 1988).

The study was conducted at five 80x80 m plots established along an elevation and vegetation gradient. All forest stands investigated have been undisturbed since 1929. The sites included a xeric mixed oak-pine (Typic Hapludult and Typic Dystrochrept), a mesic cove hardwood (Humic Hapludult and Typic Haplumbrept), two mesic mixed oak (Humic Hapludult, Typic Dystrochrept) and a mesic northern hardwood (Typic Haplumbrept) forest stands representing respectively 17, 20, 55 and 8% of the land cover in the southern Appalachian Mountains (Turner *et al.*, 2003). **Table 4.1** contains more detailed climatic, vegetation and soils information.

Sample collection

Soil cores (0-15 cm) for estimates of seasonal nitrogen cycling dynamics using potential nitrification and potential denitrification assays were collected using a stainless steel soil push tube corer at each site (n = 3-6). Samples were collected in November 2010, March 2011, June 2011, November 2011 and March 2012. Potential denitrification (pDNF) cores were separated into top 0-5 (including forest floor), 5-10 and 5-15 cm depth (November 2010 and March 2011),

and forest floor, 0-5, 5-10 and 10-15 cm (June 2011 – March 2012). Potential nitrification (pNTR) soil cores were separated into 0-5 (including forest floor) and 5-15 cm (November 2010 and March 2011) or forest floor and 0-15 cm (June 2011 – March 2012). Soil samples for potential nitrification were stored at ambient conditions for no more than 72 h before conducting the assays. Soil samples for potential denitrification were stored at 4°C for no more than 2 weeks before conducting the assays.

Soil cores (0-10 cm) for assessing gross nitrogen cycling rates using a ^{15}N stable isotope approaches were collected in May 2012 using a stainless steel push tube corer from 5 randomly selected locations per plot (mixed-oak only from the low elevation site). Samples were stored at ambient conditions and the experiment was conducted within 48 h of collection.

Nitrogen cycling assays: Gross & Potential

Gross nitrogen transformation rates were conducted using laboratory ^{15}N tracer approaches, providing estimates of N-mineralization, nitrification, denitrification and DNRA (Davidson *et al.*, 1991, Silver *et al.*, 2001). All sieved soil from one site was consolidated and thirty grams of soil was extracted using 100 ml 2M KCl to determine site specific initial mineral nitrogen content and isotopic natural abundance. The soil was subsequently divided into two batches. One batch was labelled with 3.8 ± 2.3 % $K^{15}NO_3$ (98%) (oak-pine: $4.1*10^{-5}$ mg N kg_{soil}^{-1}; cove hardwood: $3.4*10^{-5}$ mg N $kg_{soil}l^{-1}$; mixed-oak low: $2.6*10^{-5}$ mg N kg_{soil}^{-1}; northern hardwood: $2.5*10^{-2}$ mg N kg_{soil}^{-1}) and the batch second with 14.6 ± 8.0 % $(^{15}NH_4)_2SO_4$ (98%) (oak-pine: 0.16 mg N kg_{soil}^{-1}; cove hardwood: 0.21 mg N kg_{soil}^{-1}; mixed-oak low: 0.06 mg N kg_{soil}^{-1}; northern hardwood: 0.33 mg N kg_{soil}^{-1}). By keeping label enrichment lower than 20%, fertilization artifacts were kept at a minimum. From each isotopically labelled batch, fifty grams

of soil was added to six 1 L Mason jars per site, 2 times 3 replicates, and were incubated under ambient atmospheric and soil moisture conditions at 20°C. The Mason jar lids were adapted with a septum for gas sampling purposes. Two 9 ml gas samples were collected for isotopic analysis ($^{15}N2O$ and $^{15}N_2$) and gas concentrations (N_2 and N_2O) analysis after 4 and 24 h for $^{15}NO_3^-$ labelled soils and after 24 h for the $^{15}NH_4^+$ labelled soils. Gas samples were stored in Labco Exetainer (Labco Limited Inc., Ceredigion, United Kingdom) until analysis. After gas sampling, the incubated soils were assessed for mineral ^{15}N and microbial biomass ^{15}N content and concentrations. Twenty grams were immediately extracted with 100 ml of 2 M KCl for determining NH_4^+ and NO_3^- concentrations and ^{15}N content of the NO_3^- and NH_4^+. Samples were composited among replicates for microbial biomass ^{15}N content. Ten grams were immediately extracted with 80 ml of 0.5 M K_2SO_4. Another ten grams were fumigated with ethanol free chloroform for 5 days and subsequently extracted with 0.5 M K_2SO_4. The difference between the non-fumigated and fumigated extractions was used to determine the microbial biomass, total N content and ^{15}N content.

Potential nitrification and denitrification rates were determined based on amended slurry approaches (Baas *et al.*, In press, Groffman *et al.*, 2006). In short, for potential nitrification assays, five grams of soil samples were amended with 15 ml of media (2.5 mM $(NH_4)_2SO_4$ and 0.80mM K_2HPO_4 and 0.20 mM KH_2PO_4). Slurries were incubated on a shaker at 25°C to maintain oxic conditions. Sub-samples were collected at 0.5, 2, 6-8 and 24 h for NO_x analysis using colorimetric methods (USEPA, 1983a). Potential denitrification rates were determined by the acetylene block method (Groffman *et al.*, 1999). Five grams of soil (2 g for forest floor) were amended with 5 ml of He purged (30 min) media (1 mM dextrose & 1 mM nitrate). Ten percent of the headspace volume was displaced with acetylene. Slurries were shaken at 150 rpm for no

more than 6 h at 20°C to minimize the effect of *de novo* enzyme production. Subsamples were collected at 0, 2 and 6 h for N_2O analysis using a GC-ECD (Shimadzu Inc., Tokyo, Japan).

C lability assessments

Carbon lability was determined by potential C mineralization incubations under both anoxic (C_{min}-anoxic) and oxic (C_{min}-oxic) conditions via a modified potential respiration approach (Bradford *et al.*, 2010). Five grams of field moist soil from each site were weighed into six 50 ml centrifuge tube retrofitted for gas sampling. Subsequently, soils were adjusted with deionized water to 50% water holding capacity. Half of the tubes (N=3) were purged with He for 1 minute to assure anoxic conditions and half (N=3) were purged with CO_2-free air for 1 minute to represent ambient oxygen conditions. Soils were incubated at 25°C and gas samples (1 ml) were taken after ½, 4 and 24 h after capping the centrifuge tubes. Gas samples were immediately analyzed for CO_2 content using a LICOR 7000 setup for small volume injections. Potential respiration rates in µg C g_{soil}^{-1} d^{-1} were determined by regression analysis.

Analytical procedures

Concentrations for NH_4^+, NO_3^- + NO_2^- were determined colorimetrically (USEPA, 1983a, USEPA, 1983b) using an AlpKem model 3590 Autoanalyzer. Soil total carbon was determined using near infrared reflectance spectroscopy (NIRS) techniques as described in Baas et al. (in press). Microbial biomass was determined based on dissolved organic carbon (DOC) analysis using a total carbon analyzer (Shimadzu TOC-V_{CPH} TNM). Microbial biomass nitrogen (MBN) was determined by persulfate digestions and colorimetric analysis of NO_3^- (Cabrera & Beare, 1993). Soil extracts were prepared for isotopic analysis using the diffusion method (Brooks *et*

al., 1989, Herman *et al.*, 1995). Extract nitrogen isotopic ratios were determined using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of California Davis Isotope Facility. N_2O concentrations were determined using a gas chromatograph coupled to an electron capture detector (^{63}Ni) (Shimadzu GC-14A). Gaseous isotopic ratios (N_2O and N_2) were determined using a ThermoFinnigan GasBench with a PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Precon-IRMS) (Bremen, Germany) at the University of California Davis Isotope Facility. Potential nitrification and denitrification rates were calculated in $\mu g\ kg_{drysoil}^{-1}\ h^{-1}$ and in $mg\ N\ m^{-2}\ d^{-1}$ using previously determined bulk densities (Baas *et al.*, In press). Gross nitrogen cycling rates were reported as $\mu g\ N\ g_{soil}^{-1}\ d^{-1}$. Gross N-mineralization, nitrification, NH_4^+ and NO_3^- consumption rates were calculated based on the isotope pool dilution (Davidson *et al.*, 1991, Kirkham & Bartholomew, 1954). NH_4^+ Immobilization rates were determined by subtracting nitrification rates and gaseous losses from the NH_4^+ consumption rate. NO_3^- immobilization rates were determined by subtracting gaseous losses and DNRA from the NO_3^- consumption rate. Gross DNRA was calculated based on the $^{15}NO_3^-$ labeled incubations by using the difference in NH_4^+ (24 h incubation), corrected for the mean residence time (MRT) and divided by the average NO_3^- pool atom% ^{15}N excess (Pett-Ridge *et al.*, 2006, Silver *et al.*, 2001). N_2 and N_2O production rates were determined from both the $^{15}NH_4^+$ labeled (nitrifier denitrification) and $^{15}NO_3^-$ labeled soils (denitrification). $^{15}N_2$ and $^{15}N_2O$ fluxes over the whole 24 h incubation were determined and divided by the average $^{15}NO_3^-$ or average NH_4^+ atom% ^{15}N excess to estimate total N_2 and N_2O production (Pett-Ridge *et al.*, 2006). After 24 h the $^{15}NO_3^-$ labeled soil had a very low level of enrichment for NH, thus, N_2 fluxes in NH from $^{15}NO_3^-$ labeled incubations were determined by

the revised isotope pairing technique (r-IPT) with the r_{14} value determined by ratio of produced $^{45}N_2O$ and $^{46}N_2O$ as described in Trimmer *et al.* (2006). To represent the flux data the same from all forest types, the r-IPT determined rate for NH was corrected with the mean ratio of the direct flux assessment and the r-IPT assessment for the other three sites (ratio direct : r-ITP = 0.19) between the direct flux approach and the r-IPT. Detectable gaseous $^{15}N_2$ and $^{15}N_2O$ enrichment was only consistently detectable (atom% = 0.0003) after the 24 h incubation period and, therefore, denitrification rate estimates were based on the 24 h incubation period. Microbial biomass was determined by the difference between fumigated and non-fumigated DOC with the efficiency correction factor of 0.45 (Beck *et al.*, 1997). MBN was determined by the difference in nitrogen content between fumigated and non-fumigated samples with the efficiency correction factor 0.54 (Brookes *et al.*, 1985).

Statistical procedures

We tested whether depth integrated potential nitrogen cycling differed between forest type using a mixed model approach with sample time and forest type as fixed effects while sampling location was treated as a random effect. If a significant interaction between time and forest type was detected, differences between forest types were analyzed at each separate time point and differences between sample times was analyzed for each different forest type. Depth specific differences between forest types were analyzed by one-way analysis of variance (ANOVA) and Tukey posthoc tests. We also tested whether gross N-cycling, C-mineralization rates and microbial biomass (C & N) differed between forest types using one-way ANOVA. Microbial biomass assessments were statistical analyzed by pooling all three timepoints. Data was log transformed if needed to meet ANOVA assumption of normal distribution. If log

transformations did not result in normal distribution, non-parametric Wilcoxon tests were conducted. All variability error was represented by the standard error of the mean unless specified differently. Regression analysis was conducted to investigate relationships between gross nitrogen cycling processes and MB, MB-N, C-mineralization rates and initial NO_3^- and NH_4^+ concentrations. Statistical analyses were conducted using JMP 11.0 (SAS Institute Inc., Cary, NC). Significance was determined as $p < 0.05$ unless stated otherwise.

Results

Potential Nitrogen Cycling

Forest type and sampling date effects showed significant effects in whole core (0-15 cm mineral soil plus forest floor) for potential nitrification (**Figure 4.3; Table A1**) and potential denitrification (**Figure 4.4; Table A2**). Potential nitrification showed a significant interaction between sample time and site, thus, one-way ANOVA's were conducted separately by both sample time and site. Potential nitrification was significantly higher in June 2011 compared to March 2011 and November 2010 for cove hardwood ($F_{4,16} = 5.5$, $p < 0.05$) while March 2011 and 2012 were significantly greater than November 2010 for northern hardwood ($F_{4,14} = 5.6$, $p < 0.05$). Further, November 2010 showed greater potential nitrification rates than all other sample times in mixed-oak low ($F_{4,16} = 6.8$, $p < 0.05$) while March 2011 and 2012 were greater than November 2010 in mixed-oak high ($F_{3,7} = 9.3$, $p < 0.05$). Potential nitrification rates were consistently greater in northern hardwood compared to all other forest types in March 2011 ($F_{4,19} = 16.3$, $p < 0.0001$), June 2011 ($F_{3,12} = 23.8$, $p < 0.001$), November 2011 ($F_{4,9} = 6.8$, $p < 0.05$). In November 2010 mixed-oak high potential nitrification rates were greater than all other forest types ($F_{4,21} = 99.0$, $p < 0.0001$) and in March 2012 northern hardwood showed a trend of greater

rates than mixed-oak high in nitrification rates ($F_{3,6} = 4.0$, $p = 0.08$). Mixed model analyses for potential denitrification showed significant sample time ($F_{4,86} = 57.4$, $p < 0.0001$) and forest type ($F_{4,86} = 10.3$, $p < 0.0001$) effects with the interaction not being significant. Northern hardwood was found to be significantly greater than all other forest types and cove hardwood was greater than oak-pine and both mixed-oak forest types ($p < 0.001$). Potential denitrification rates in June 2011 were significantly greater than both November 2010 and 2011 ($p < 0.001$) as well as March 2011 being greater than November 2010 ($p = 0.0003$).

Depth specific rates for potential nitrification rates (**Table A1**) showed northern hardwood to be greatest for the forest floor in all sample times assessed (June 2011, November 2011 and March 2012) while deeper soil (0-15 or 5-15 cm) showed northern hardwood to be significantly greatest in March 2011, November 2011 and March 2012. No significant differences were observed in November 2011 and 0-5 cm plus forest floor in March 2011 was significantly greater than all other forest types. Depth specific potential denitrification rates showed northern hardwood to be greatest for all depths in November 2010, March 2011 and June 2011 (except for forest floor; **Table A2).** Forest floor potential denitrification rates were greater in northern hardwood than mixed-oak high and oak-pine in November 2011 and greater than mixed-oak (low and high) and oak-pine in March 2012. In addition, cove hardwood potential denitrification rates were greater than mixed-oak high in June 2011 and greater than oak-pine in March 2012.

Gross Nitrogen Cycling

Gross nitrogen transformation rates were determined for nitrogen mineralization, nitrification, DNRA, denitrification, nitrifier denitrification and N_2O production (with either

NH_4^+ or NO_3^- as source) rates in May 2012 (**Table 4.2; Figure 4.2**). Gross nitrogen mineralization rates (ranging from 0.55 to 7.8 µg g_{soil}^{-1} d^{-1}) were significantly greater in northern hardwood compared to mixed-oak and oak-pine ($F_{3,8}$ = 7.2, p = 0.0116) and gross nitrification rates were greater in northern hardwood than any other forest type. No differences could be determined in net mineralization and net nitrification in the May 2012 lab incubation (data not shown). Denitrification rates were showed no significant differences between forest types while nitrifier denitrification rates were significantly greater in northern hardwood compared to cove hardwood and oak-pine ($F_{3,8}$ = 19.2, p < 0.01). In addition nitrifier denitrification rates were greater in mixed-oak low compared to oak-pine ($F_{3,8}$ = 19.2, p = 0.03). Denitrification was greater than nitrifier denitrification in oak-pine ($F_{1,4}$ = 33.7, p = 0.0044) while nitrifier denitrification was greater in northern hardwood ($F_{1,4}$ = 9.3, p = 0.04). No significant differences were observed between nitrifier denitrification and denitrification in mixed-oak low and cove hardwood. Denitrification (1.4 ± 0.15 µg g_{soil}^{-1} d^{-1}) and nitrifier denitrification (1.5 ± 0.22 µg g_{soil}^{-1} d^{-1}) rates were greater than DNRA rates (0.03 ± 0.01 µg g_{soil}^{-1} d^{-1}) for all forest types (p < 0.05) and DNRA rates were responsible for 0.6 ± 0.0% (oak-pine), 2.0 ± 1.5% (cove hardwood), 1.7 ± 1.9% (mixed-oak low) and 5.3 ± 3.5% (northern hardwood) of total nitrate reduction. DNRA rates were significantly greater in northern hardwood compared to oak-pine ($F_{3,8}$ = 19.2, p = 0.0005). Denitrification rates were similar compared to nitrifier denitrification rates in cove hardwood and mixed-oak while denitrification rates were greater (oak-pine) and lower (northern hardwood) than nitrifier denitrification rates. N_2O production rates were greater from the NO_3^- than from NH_4^+ for oak pine (p = 0.06) and cove hardwood (p = 0.05). No significant differences were observed between the N_2O production rates from either NH_4^+ or NO_3^- for mixed oak low and northern hardwood. The total fraction of nitrification being reduced by DNRA was

significantly greater in mixed-oak low compared to northern hardwood ($F_{3,7}$ = 3.8, p = 0.07; oak-pine: 9%; cove hardwood: 16%; mixed-oak low: 20%; northern hardwood: 2%) while the total fraction of the nitrification rates reduced by denitrification was significantly lower in northern hardwood compared to oak-pine (p = 0.04) and showed a trend of being lower than cove hardwood and mixed-oak low (p < 0.10). In all forest types more NO_3^- was reduced than nitrified except for northern hardwood (oak-pine: 1500%; cove hardwood: 800%; mixed-oak: 1200%; northern hardwood: 37%).

Microbial biomass, C-mineralization and correlation with gross rates

Microbial biomass was not significantly different among forest types; however the nitrogen content of the microbial biomass was significantly lower in oak-pine compared to the other forest types ($F_{3,12}$ = 12.3, p = 0.0006; **Table 4.3**). The potential C-mineralization rates (**Table 4.3**) showed significant forest type ($F_{3,14}$ = 11.7, p = 0.0001) and redox treatment (oxic or anoxic; $F_{1,14}$ = 33.3, p < 0.0001) with no interaction effects. Potential mineralization rates were significantly greater under oxic conditions than anoxic (p < 0.0001). Further, C-mineralization rates were greater in northern hardwood than cove hardwood and oak-pine (p < 0.001) with a trend of mixed-oak low being lower than northern hardwood (p = 0.08).

A wide variety of variables correlated significantly with gross N-mineralization including soil moisture, MB, MB-N, NH_4^+, NO_3^- and total C (**Table 4.4**). Due to the high gross N mineralization rates found in northern hardwood we also tested relationships when excluding northern hardwood to ensure relationships were not driven by site differences. Analyses excluding northern hardwood showed similar relationships with the exception of MB-N no longer being significant. Gross nitrification rates correlated with the same variable as N

mineralization although not at the same level of significance. However, when excluding northern hardwood, the analysis showed no significant correlations. DNRA rates were most highly correlated with soil moisture, total carbon, extractable NH_4^+ and microbial biomass (**Table 4.4**), whether northern hardwood was excluded or not, with total carbon and soil moisture being strongly correlated with each other ($p < 0.001$; $r^2 = 0.99$). In addition, microbial biomass stoichiometry (C:N) correlated significantly with DNRA. When excluding northern hardwood, nitrifier denitrification was significantly correlated to all variables assessed except for MB C:N and C:NO_3^- ratios. Northern hardwood showed similar correlations between MB-N and C_{min}-oxic in addition to C:NO_3^-. Denitrification, on the other hand, showed no significant correlations to any of the explanatory variables (**Table 4.4**) regardless of the inclusion or exclusion of northern hardwood. N_2O production from NH_4^+ showed significant correlations with all explanatory variables except C_{min} (**Table 4.4**). In contrast, N_2O from NO_3^- showed significant correlations only with C-mineralization variables. No significant correlations with N_2O production from NO_3^- were observed for the analysis excluding northern hardwood while N_2O from NH_4^+ showed marginally significant correlations with C_{min} variables. Potential denitrification rates were significantly related to gross N-mineralization (rho = 0.94; $p < 0.01$), nitrification (rho = 0.60; $p = 0.051$), nitrifier denitrification (rho = 0.72, $p < 0.01$) and DNRA rates (rho = 0.69; $p = 0.013$; **Table 4.5**). Gross nitrification rates showed a positive significant correlation with N mineralization and nitrifier denitrification production while showing a significant negative correlation with denitrification (**Table 4.5**). Further, N mineralization was significantly correlated to DNRA and nitrifier denitrification.

Discussion

This study focused on elucidating how N transformations differ among forest types and what factors drive these processes in the face of future increases in N deposition and soil carbon subsidies. Our results suggest that denitrification and nitrifier denitrification are more important than previously thought. Gross N mineralization and nitrification were greatest in northern hardwood and gross denitrification rates were most dominant in oak-pine. DNRA rates were relatively low; however, in mixed-oak it represented a significant pathway of nitrogen retention.

The patterns of gross N mineralization and nitrification in this study are similar to those patterns previously determined net transformation rates (Knoepp *et al.*, 2008, Knoepp & Swank, 1998, Mclean, 2011). Similarly, gross mineralization and nitrification rates were significantly greatest in northern hardwood followed by cove hardwood. Potential nitrification rates showed a similar pattern with rates in northern hardwood being significantly greater. The proportion of mineralized product that was subsequently nitrified was similar in cove hardwood (net = 11%; gross = 9%), mixed-oak (net = 14%; gross = 10%) and northern hardwood (net = 50%; gross = 50%) while the fraction is substantially greater for gross rates in oak-pine (net = 9%; gross = 20%) (Knoepp & Swank, 1998). This suggests that mechanisms based on the measurement of net mineralization and nitrification rates are poor predictors of nitrogen cycling dynamics in oak-pine, and we should be cautious when using net rates for developing mechanistic models of the nitrogen cycle in this forest type.

Nitrate reduction to N_2 via denitrification or nitrifier denitrification proved to be a critical process in each forest type. Gross denitrification was highest in oak-pine. Greater denitrification rates in this forest type might explain the discrepancy between the ratio of net and gross mineralization and nitrification rates (Knoepp & Swank, 1998). Gross denitrification rates

consistently ranged between 8 to 15 fold higher rates than gross nitrification rates in all forest types except northern hardwood. This could be a result of the low nitrate concentrations generally present in these forest types (Knoepp *et al.*, 2008, Mclean, 2011), and therefore, our experimental procedure may have resulted in a fertilization effect on the denitrification process. The high nitrifier denitrification rates could have also been produced via anammox (Brandes *et al.*, 2007, Poth & Focht, 1985). However, potential gross anammox and denitrification activities has been assessed for a mixed-oak forest type in the Coweeta basin, showing anammox contributing at the most 5.7% of nitrate reduction to N_2 (Davies *et al.*, unpublished). Therefore, it seems unlikely that anammox is a major pathway in the southern Appalachian soils and that high nitrifier denitrification is more likely the dominant pathway explaining the N_2 fluxes from NH_4^+. It is currently not clear whether nitrifier denitrification *in situ* sequentially reduces NO_3^- all the way to N_2 (Wrage *et al.*, 2001), thus, making a coupling between nitrifier denitrifiers and denitrifiers the most likely reduction pathway for the N_2 fluxes from NH_4^+ observed in this study. In addition, the correlation between potential denitrification and nitrifier denitrification further suggests the importance of coupled nitrifier denitrification and denitrification in these ecosystems.

Similar to the nitrifier denitrification rates, potential denitrification rates were greatest in northern hardwood. Mixed-oak, however, while showing high nitrifier denitrification rates, did not show high potential denitrification rates. Perhaps nitrifier denitrification is not coupled to denitrification in this ecosystem and, instead, completely reduces to N_2 (Wrage *et al.*, 2001). The lack of a relationship between potential denitrification and gross denitrification rates indicates that nitrifier denitrification might be the rate limiting step in the proposed hypothesis of coupled nitrifier denitrification-denitrification. We also observed seasonal patterns of potential

denitrification rates with generally the highest rates in the late winter (March) for northern hardwood and mixed-oak, likely due to lower soil moisture (Groffman & Tiedje, 1989) in the summer and fall (Swift & Cunningham, 1988).

Inorganic nitrogen retention, indicated by the ratio of gross DNRA to gross nitrification, has been found to be important in moist tropical forests (Silver *et al.*, 2001, Silver *et al.*, 2005), but rarely in temperate ecosystems. However, the results from this study and from Yang (2010) suggest a more important role for DNRA in temperate ecosystems. Although it is possible that remineralization of $^{15}NO_3^-$ was responsible for the DNRA rates observed, our results suggest this is unlikely as we observed no changes in biomass ^{15}N content during the incubation and microbes preferentially uptake NH_4^+ over NO_3^- (Vitousek & Matson, 1988). The preference for NH_4^+ was confirmed in this study for cove hardwood and northern hardwood by higher NH_4^+ immobilization rates than NO_3^- immobilization rates.

The total carbon to nitrate ratio has often been shown to determine the relative dominance of denitrification versus DNRA (Burgin & Hamilton, 2007). We found strong evidence that the $C:NO_3^-$ ratio controls nitrifier denitrification with wider ratios resulting in a greater rate in all except the northern hardwood forest type. We also see some trends for DNRA to be greater with wider ratios but no significant correlations for denitrification. These data suggest that both carbon and nitrate availability are not limiting N_2 production in northern hardwood while the stoichiometry of C and NO_3^- is very important in all other forest types. It also provides additional evidence suggesting that northern hardwood is progressing towards N saturation (Aber *et al.*, 1998). Likely, the presence of oxygen is the main limiting factor of northern hardwood nitrifier denitrification (Dundee & Hopkins, 2001, Goreau *et al.*, 1980) and denitrification (Burgin & Groffman, 2012). These findings support previous research showing potential denitrification not

to be limited by nitrate in northern hardwood while limited by nitrate in all other forest types (Baas *et al.*, In prep-b).

Research conducted by Brumme *et al.* (1999) and Groffman *et al.* (2009b) in forest ecosystems suggests that the greatest potential for future N_2O fluxes is in forest systems with soil high in organic matter. The magnitude of N_2O fluxes would be expected to increase with greater access of inorganic N substrate. We found a positive correlation between nitrifier N_2O production and NH_4^+ concentrations, however, only when excluding northern hardwood. High carbon lability resulted in lower nitrifier N_2O production in oak-pine, cove hardwood and mixed-oak while resulting in greater N_2O production from denitrifier N_2O production. This suggests that denitrifiers increase their proportional N_2O:N_2 production ratio when labile carbon is more abundant. Since denitrifier N_2O production in oak-pine, cove hardwood and mixed-oak is generally greater than nitrifier N_2O production this indicates higher carbon subsidies to the soil to could result in greater N_2O emissions with little indication of a strong effect of inorganic nitrogen on N_2O emissions. Northern hardwood soils with similar nitrifier and denitrifier N_2O production rates, however, will see an increase in denitrifier N_2O with more carbon in addition to an increase in denitrifier N_2O with more N inputs. Therefore, northern hardwood soils, similar to other studies (Brumme *et al.*, 1999, Groffman *et al.*, 2009b), have the greatest potential of increased N_2O emissions in the future.

We found gross N cycling to vary among forest types with N mineralization and nitrification exhibiting similar patterns to previously determined net rates. This study showed that nitrate reduction pathways are more common in these soils than previously thought with denitrification and nitrifier denitrification pathways capable of substantial N_2 production. N_2O production was found to be inconsequential and DNRA was relevant in mixed-oak only. The

relevance of N_2O emissions in the future remains unclear. Soil moisture, nutrient and carbon concentrations were all found to potentially control N mineralization and nitrification, nitrifier denitrification and DNRA rates.

Table 4.1: Selected site characteristics. Data compiled from Coweeta Long Term Ecological Research Program records. See website (www.coweeta.uga.edu) for additional information. Modified from Knoepp et al (1998).

Site	Oak-Pine	Cove Hardwood	Mixed Oak-low	Mixed Oak-high	Northern Hardwood
Geographic coordinates	83° 26' N 35° 3' W	83° 26' N 35° 2' W	83° 26' N 35° 2' W	83° 27' N 35° 2' W	**83° 27' N 35° 1' W**
Elevation (m)	788	801	860	1094	**1389**
Aspect (degrees)	180	340	15	75	**20**
Slope (degrees)	34	21	34	33	**33**
Vegetation	oak-pine	cove hardwood	mixed Oak	mixed oak	**northern hardwood**
Dominant Species	*Pinus rigada* *Quercus coccinea* *Quercus prinus* *Carya spp.* *Kalmia latifola*	*Liriodendron tulipifera* *Quercus prinus* *Carya spp.*	*Quercus prinus* *Carya spp.* *Quercus rubra* *Rhododendron maximum*	*Quercus prinus* *Quercus rubra* *Carya spp.* *Rhododendron maximum*	***Betula allegheniensis*** ***Quercus rubra*** ***Betula lenta*** ***Tilia heterophylla***
Moisture Regime	Xeric	mesic	mesic	mesic	**mesic**
Soil Series	Evard/Cowee Chandler Edneyville/Chestnut	Saunook Tuckaseegee	Trimont	Chandler	**Plott**
Soil Texture	Fine-loamy Coarse-loamy Coarse-loamy	Fine-loamy Fine-loamy	Fine-loamy	Coarse-loamy	**Coarse-loamy**
Soil Subgroup	**Typic Hapludults** **Typic Dystrochrepts**	**Humic Hapludults** **Typic Dystrochrepts**	**Humic Hapludults**	**Typic Dystrochrept**	**Typic Haplumbrepts**

Table 4.2: Gross nitrogen cycling in May 2012. All units are in μg N g_{soil}^{-1} h^{-1} with N between parentheses. OP = oak-pine; CH = cove hardwood; MO = mixed oak; NH = northern hardwood. Error is indicated by standard error of the mean. Different letters indicate significant differences.

Forest Type	Gross Mineralization	Gross Nitrification	Gross DNRA	Gross Denitrification	Gross Nitrifier-Denitrification	NH_4^+ immobilization	NO_3^- Immobilization	Gross N_2O $^{15}NO_3^-$ (*10^{-3})	Gross N_2O† $^{15}NH_4^+$ (*10^{-3})
OP	0.55 ± 0.33 (3)^{b}	0.11 ± 0.04 (3)^{b}	0.01 ± 0.00 (3)^{b}	1.67 ± 0.12 (3)^{a}	0.75 ± 0.10 (3)^{b}	-0.10 ± 0.40 (3)^{ab}	-0.15 ± 0.09 (3)	0.2 ± 0.1 (3)	0.0 ± 0.0 (3)^{b}
CH	2.15 ± 0.39 (3)^{ab}	0.19 ± 0.08 (3)^{b}	0.03 ± 0.02 (3)^{ab}	1.50 ± 0.53 (3)^{ab}	0.97 ± 0.05 (3)^{b}	1.16 ± 0.25 (3)^{a}	-0.12 ± 0.06 (3)	0.2 ± 0.0 (3)	0.0 ± 0.0 (3)^{b}
MO	0.95 ± 0.41 (3)^{b}	0.10 ± 0.07 (3)^{b}	0.02 ± 0.02 (3)^{ab}	1.17 ± 0.27 (3)^{b}	1.71 ± 0.22 (3)^{a}	-0.91 ± 0.29 (3)^{b}	-0.02 ± 0.24 (3)	0.4 ± 0.1 (3)	0.0 ± 0.00 (1)
NH	7.85 ± 1.34 (3)^{a}	3.38 ± 1.07 (2)^{a}	0.07 ± 0.03 (3)^{a}	1.24 ± 0.32 (3)^{ab}	2.5 ± 0.27 (3)^{a}	3.13 ± 1.83 (2)^{a}	0.28 ± 0.71 (2)	3.8 ± 1.9 (3)	1.8± 0.42 (3)^{a}

† $p < 0.1$

Table 4.3: May 2012 assessments of nutrient concentrations (after 4 hours of incubation), initial microbial biomass (MB), initial biomass N (MB-N) and C mineralization rates (C_{min}). OP = oak-pine; CH = cove hardwood; MO = mixed oak; NH = northern hardwood. Error is indicated by standard error of the mean. Different letters indicate significant differences. N is indicated in parentheses.

Forest Type	NH_4^+ (μg N g^{-1})	NO_3^- (μg N g^{-1})	MB (μg C g^{-1})	MB-N (μg N g^{-1})	C_{min} oxic (μg C g^{-1} d^{-1})	C_{min} anoxic (μg C g^{-1} d^{-1})
OP	0.79 ± 0.03 (3) c†	1.26 ± 0.43 (3) b	287 ± 69 (3)	127 ± 16 (4) b	239 ± 106 (3) b	155 ± 38 (3) b
CH	1.31 ± 0.04 (3) b†	1.01 ± 0.06 (3) b	499 ± 164 (3)	224 ± 17 (3) a	262 ± 26 (3) b	117 ± 12 (3) b
MO	0.77 ± 0.00 (3) c†	0.67 ± 0.05 (3) b	538 ± 45 (3)	215 ± 20 (4) a	455 ± 41 (3) ab	196 ± 8 (3) ab
NH	5.6 ± 0.23 (3) a†	5.4 ± 0.44 (3) a	634 ± 57 (3)	286 ± 22 (4) a	632 ± 33 (2) a	297 ± 30 (2) a

†$p < 0.1$

Table 4.4: Spearman's correlation coefficients between gross nitrogen cycling rates and edaphic characteristics (i.e. nutrient concentrations (NO_3^- and NH_4^+), microbial biomass (MB), microbial biomass N (MB-N), carbon mineralization rates (C-min) incubated under oxic or anoxic conditions and percent soil moisture). OP = oak-pine; CH = cove hardwood; MO = mixed oak; NH = northern hardwood. DNRA = dissimilatory nitrate reduction to ammonium; C-min = carbon mineralization; pDNF = potential denitrification. Correlations in bold show significant relationships ($p < 0.05$).

Explanatory variable	**N-mineralization**		**Nitrification**		**DNRA**		**N_2-NH_4^+**		**Denitrification**		**N_2O-NH_4^+**		**N_2O-NO_3^-**	
	All	Excl NH	All	Excl NH	All	Excl NH	All	Excl NH	All	Excl NH	All	Excl NH	All	Excl NH
Soil moisture	0.91**	0.79*	0.55†	0.16	0.82**	0.74*	0.73**	0.37	-0.32	-0.32	0.66*	0.00	0.17	-0.05
C-min oxic	0.27	-0.32	-0.02	-0.40	0.55†	0.17	0.82**	0.68*	-0.11	-0.15	0.18	-0.71†	0.75**	0.57
C-min anoxic	0.28	-0.32	0.03	-0.33	0.39	-0.10	0.66*	0.38	-0.24	-0.23	0.22	-0.68†	0.65*	0.37
MB	0.91**	0.79*	0.55†	0.16	0.82**	0.74*	0.73**	0.37	-0.32	-0.32	0.66*	0.00	0.17	-0.05
MB-N	0.65*	0.16	0.38	0.16	0.73**	0.53	0.95**	0.90**	-0.39	-0.47	0.66*	0.00	0.35	0.37
MB C:N	-0.26	0.79*	-0.38	0.16	-0.17	0.74*	-0.43	0.37	-0.02	-0.32	-0.66*	0.00	-0.22	-0.05
NO_3^- initial	0.92**	0.82**	0.63*	0.27	0.74**	0.55	0.58*	-0.09	-0.22	-0.09	0.67*	0.00	0.10	-0.27
NH_4^+ initial	0.91**	0.79*	0.54†	0.16	0.82**	0.73*	0.73**	0.37	-0.32	-0.32	0.66*	0.00	0.17	-0.05
Total C	0.91**	0.79*	0.55†	0.16	0.82**	0.74*	0.73**	0.37	-0.32	-0.32	0.66*	0.00	0.17	-0.05
C:NO_3^-	-0.52†	0.16	-0.55†	0.16	-0.26	0.53	-0.22	0.90**	-0.09	-0.47	-0.66*	0.00	-0.04	0.37
pDNF	0.94**	0.87**	0.60†	0.27	0.69*	0.53	0.72**	0.35	-0.42	-0.42	0.64*	0.04	0.20	0.18

**$p < 0.01$, *$p < 0.05$, †$p < 0.1$

Table 4.5: Pearson's correlation coefficients between gross nitrogen cycling processes. DNRA = dissimilatory nitrate reduction to ammonium. Correlations in bold show significant relationships ($p < 0.05$).

	N-mineralization	**Nitrification**	**DNRA**	**N_2-NH_4^+**	**Denitrification**	**N_2O-NH_4^+‡**	**N_2O-NO_3^-‡**
N-mineralization		0.70*	0.61*	0.64*	-0.36	0.60†	0.10
Nitrification			0.47†	0.67*	-0.63*	0.72*	-0.06
DNRA				0.54†	-0.34	0.62†	0.54†
N_2-NH4					-0.42	0.59†	0.28
Denitrification						-0.36	-0.13
N_2O-NH_4^+							0.19
N_2O-NO_3^-							

**p < 0.01, *p < 0.05, †p < 0.1

‡Spearman correlation coefficient (rho) used due to non-normal distribution N_2O fluxes.

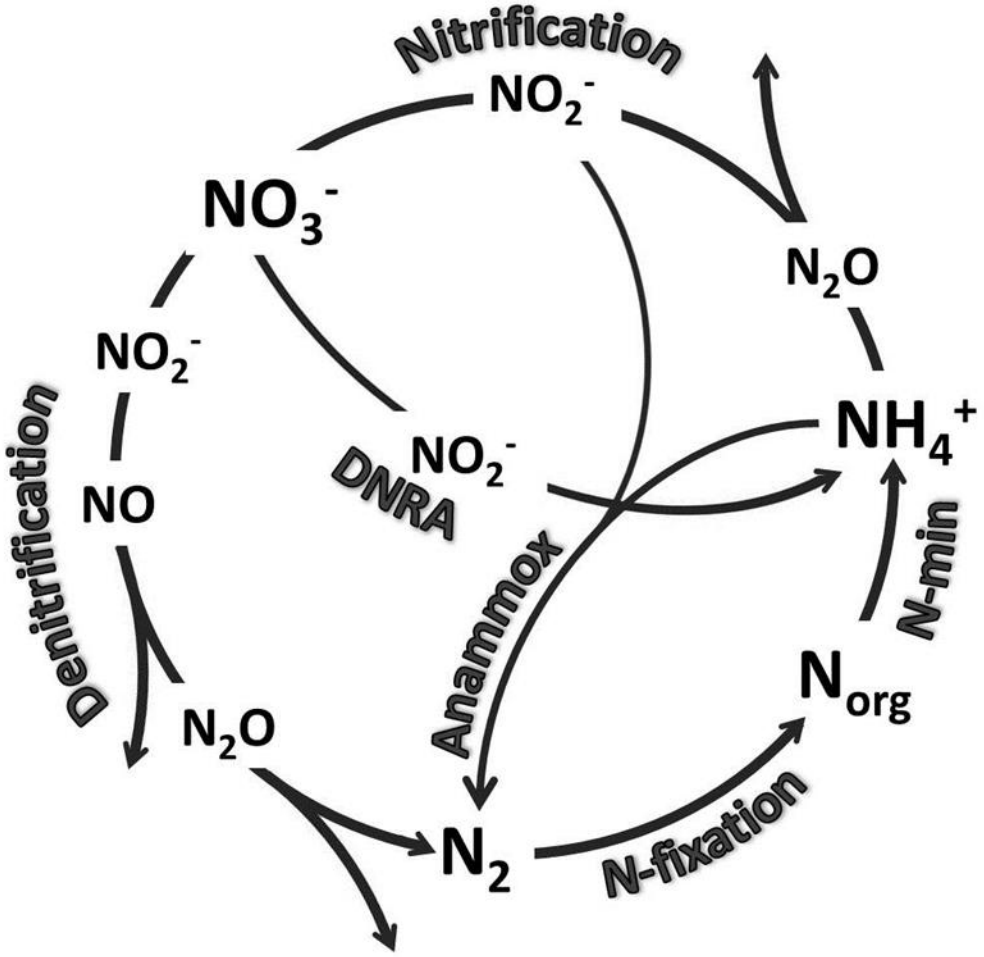

Figure 4.1: Conceptual representation of the nitrogen cycle. Arrows indicate the direction of transformations. N-min = nitrogen mineralization. Adapted from Brandes *et al.* 2007.

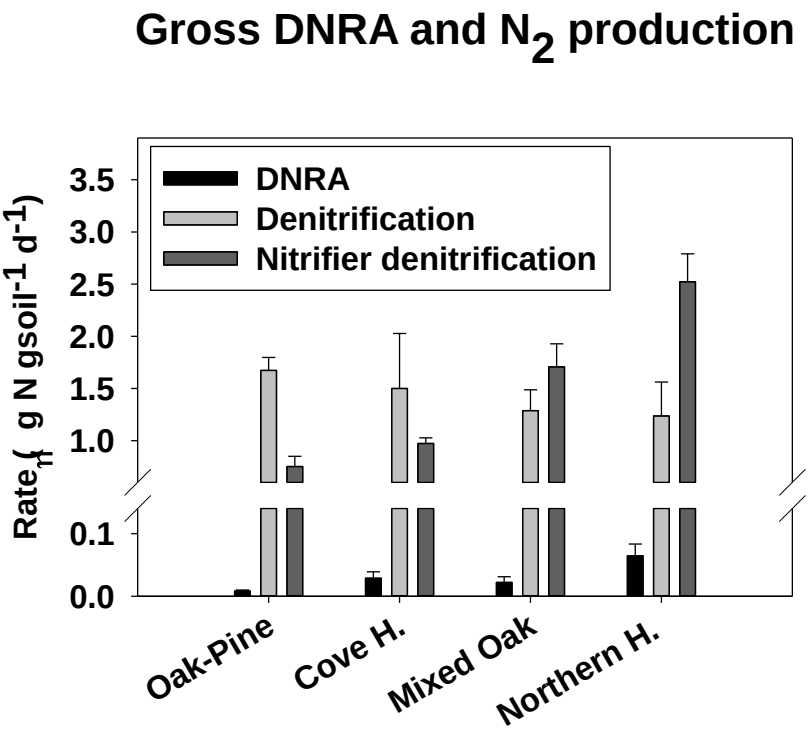

Figure 4.2: Dissimilatory nitrate reduction to ammonium (DNRA) and N_2 production rates (nitrifier denitrification and denitrification).

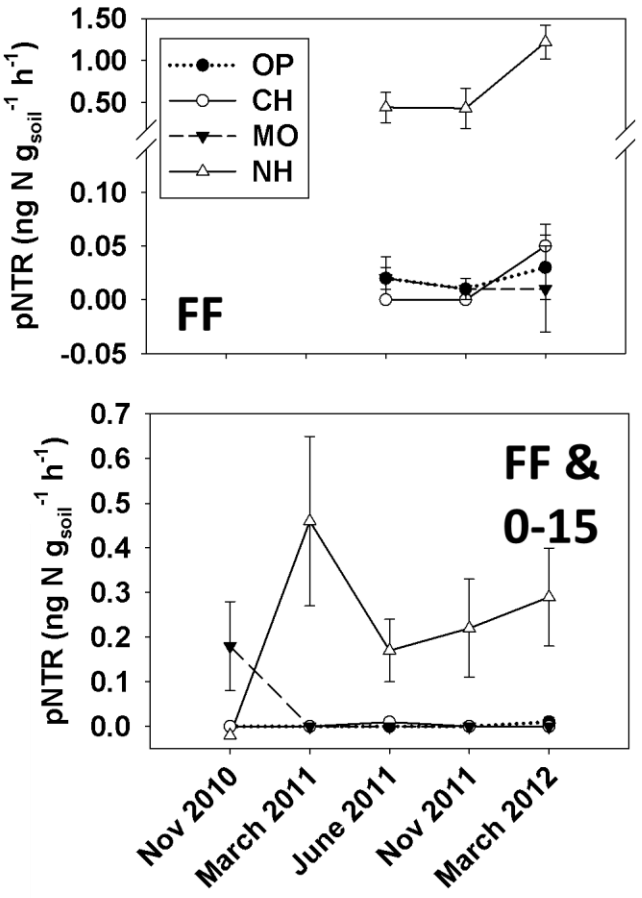

Figure 4.3: Potential nitrification (pNTR) rates for different forest types. FF = forest floor; FF & 0-15 = bulk density integrated rates over entire sampling depth.

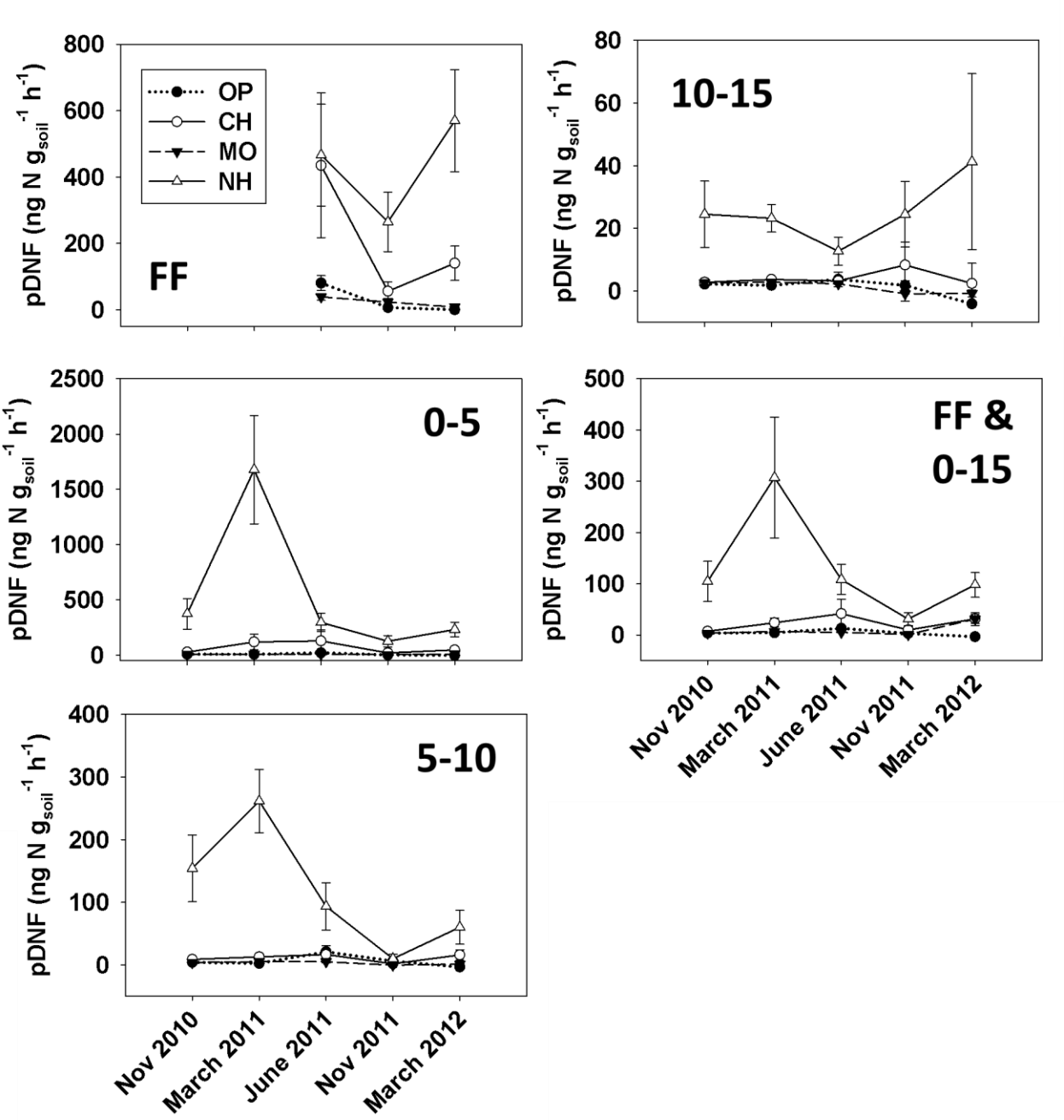

Figure 4.4: Potential denitrification (pDNF) rates for different forest types. FF = forest floor; 0-5, 5-10 and 10-15 = depth mineral soil; FF & 0-15 = bulk density integrated rates over entire sampling depth.

CHAPTER 5

LAND-USE DRIVEN PATTERNS IN RIPARIAN NITROGEN CYCLING AND GREENHOUSE GAS EMISSIONS [4]

[4] Baas, P., J.D. Knoepp, D. Markewitz and J.E. Mohan. To be submitted to *Global Change Biology*.

Abstract

Over the last few decades the southern Appalachian Mountains have experienced a surge in residential development. How this has affected this ecosystem, also referred to as the "water tower of the southeast", is unclear. Riparian zones play a crucial role in mitigating the movement of nitrogen pollutants from terrestrial to the aquatic ecosystems. In this study we focused on assessing riparian nitrogen cycling differences between the different types of land-use. We hypothesized that with increasing inputs of inorganic nitrogen, nitrogen cycling rates will increase and nitrogen retention will be reduced, thereby increasing nitrate leaching and the emission of the potent greenhouse gas N_2O. We assessed differences in nitrogen cycling processes among land use types by measuring potential nitrification, potential denitrification, and *in situ* greenhouse gas fluxes (N_2O, CO_2 and CH_4) among sites representing agricultural development, residential development and forested reference conditions. We found N_2O and CO_2 efflux to be greatest under agricultural land use. Both residential and agricultural land-use exhibited CH_4 efflux while forested ecosystems showed CH_4 uptake. Under projected residential development of forested riparian ecosystems, our data suggests that in the future this region will become less of a CH_4-sink with greater nitrogen leaching into our streams. However, the overall greenhouse gas budget suggests a small decrease in global warming potential (-0.9%) due to the slight decrease in agricultural land cover.

Introduction

Anthropogenic land use in the southern Appalachian Mountains has intensified over the last few decades and is expected to continue over subsequent decades (Gragson & Bolstad, 2006, Kirk *et al.*, 2012, Wear & Bolstad, 1998). Traditional human development in this region has generally consisted of small scale agriculture and residential development (Gragson & Bolstad, 2006). However, current predictions expect 75% of the new development to be of a (sub-)urban nature (Kirk *et al.*, 2012). Furthermore, by 2030 it is expected that 67% of all new development is to be on previously forested land (Kirk *et al.*, 2012). Riparian zones including those in the Appalachian Mountains are particularly sensitive to changes in land use (Turner *et al.*, 2003). Indeed, agricultural and (sub-)urban land use has been shown to increase stream nitrate concentrations substantially compared to forested streams (Kaushal *et al.*, 2008, Webster *et al.*, 2012). In forested conditions, the main source of stream nitrate is from terrestrial soil microbial processes. In contrast, nitrates from waste water leaching dominate streams in developed areas (Kaushal *et al.*, 2011). Riparian degradation is critical in the southern Appalachian Mountains (Webster *et al.*, 2012), and is one of the major causes of decreasing water quality in the US as a whole (Faustini *et al.*, 2009).

Riparian zones are uniquely situated on the interface between the aquatic and the terrestrial biome for intercepting nutrients, sediments, and water as they move through the riparian zone into the stream (Gregory *et al.*, 1991, Naiman & Decamps, 1997). Thus, riparian ecosystems modulate the cascading effect of nutrients downstream of agricultural areas and residential development. Riparian ecosystems can retain up to 89% of the nitrogen loading from upland anthropogenic activities (Dosskey, 2001, Peterjohn & Correll, 1984, Vought *et al.*, 1994). Previous research has shown that forested riparian zones in the southern Appalachian

Mountains have high rates of nitrogen (N) cycling and can effectively mitigate nutrient influx associated with upslope disturbances (Knoepp & Clinton, 2009).

Higher nitrate loading results in enhanced riparian N cycling (Kaushal *et al.*, 2008); however, this often coincides with greater emissions of the potent greenhouse gas and stratospheric ozone-depleting compound, nitrous oxide (N_2O) (Groffman *et al.*, 1998, Hefting *et al.*, 2003, Ravishankara *et al.*, 2009). Recent models estimate that 0.9 Tg N_2O-N yr^{-1} of the total N_2O emission of 16 Tg N_2O-N yr^{-1} are from riparian zones, thus indicating riparian zones as hotspots for N_2O emissions (Bouwman *et al.*, 2013, Groffman *et al.*, 2000). Further, currently no known substantial global N_2O sink exists (<2% of net emissions) (Schlesinger, 2013). Previous work on riparian zones in the southern Appalachian Mountains disturbed for cattle grazing showed that N_2O emissions could be as high as 24.5 kg N_2O-N ha^{-1} yr^{-1} (Walker *et al.*, 2002), and restoration of the vegetative riparian zone decreased emissions by 75% (Walker *et al.*, 2009). Further, restoration shifted the main source of N_2O from nitrification to denitrification (Walker *et al.*, 2009). In addition, restoration showed suggestions of a potential role for dissimilatory nitrate reduction to ammonium (DNRA) in restored riparian zones (Walker *et al.*, 2002), an important mechanism for nutrient retention in some forest systems (Silver *et al.*, 2005). However, the consequences of rapid land use change in the southern Appalachian Mountains on riparian nutrient retention and greenhouse gas emissions remain unclear.

Current models suggest the CO_2 sink might be less than generally thought when including C-N interactions in N limited non-tropics, indicating a potential 70% underestimation of net CO_2 emissions (Jain *et al.*, 2013). Since grasslands often have a greater CO_2 soil efflux than forests (Raich & Tufekciogul, 2000), conversion to more grass dominated riparian systems (i.e. pasture or lawn) could increase soil respiration. Furthermore, CH_4 soil oxidation rates

generally decrease when land is converted to agriculture (Boeckx *et al.*, 1997, Powlson *et al.*, 1997) and fertilization reduces the CH_4 uptake potential (Steudler *et al.*, 1989), thus increasing CH_4 emission rates. Widespread lawn fertilizer use in residential developments could shift riparian soils from a sink to a source of CH_4 (Law *et al.*, 2004).

Many factors influence CO_2, CH_4 and N_2O emissions. CO_2 emissions are well studied, with soil temperature and moisture being the main controlling factors (Davidson *et al.*, 2000, Raich & Potter, 1995). Models of CH_4 and N_2O emissions are more complicated. Both are controlled by anaerobic processes and are the net result of both reduction and oxidation reactions (Tiedje *et al.*, 1984), thus, largely controlled by the presence of anoxia or anoxic microsites (Parkin, 1987). N_2O emissions are generally found to increase with soil moisture. Soil moisture is reported to stimulate CH_4 emission, yet CH_4 uptake rates have also been found to be stimulated (Castro *et al.*, 1994) and inhibited (Le Mer & Roger, 2001) by greater soil moisture with maximal CH_4 uptake rates at around 15% (w/w) (Boeckx & Van Cleemput, 1996). In general, aeration due to soil texture and bulk density is often controlling N_2O and CH_4 fluxes on the long term, while soil temperature, moisture and substrate availability determine short term responses (Werner *et al.*, 2007).

The objective of the current study is to assess the N retention and associated greenhouse gas emission rates with different riparian land uses in addition to elucidating potential drivers of N cycling and greenhouse gas emissions. We hypothesized that higher inorganic N inputs from agricultural and residential activities would result in greater N_2O emissions relative to forested ecosystems. High labile carbon substrate availability in agricultural and residential ecosystems due to greater grass productivity and cattle or sewage inputs would result in a decreased CH_4

uptake and potentially increased CH_4 emissions rates, greater CO_2 emissions and greater N cycling. DNRA, however, would be more dominant in forested ecosystems.

Methods

Site Description

The study was conducted in Macon County, North Carolina, in the Blue Ridge physiographic province in the southern Appalachian Mountains. This region receives an average of 1300 mm of precipitation a year (NOAA, 1950-2011). The highest temperatures are between May and September (20 °C) and the lowest temperatures are between December and February (5 °C). The growing season starts in May and ends in September (Swift & Cunningham, 1988). Historically, this region has been dominated by logging and agricultural activities with the majority of the region clear-cut in the early 1900's (Gragson & Bolstad, 2006). Small scale agricultural activities still exist but logging activities have been significantly reduced since the 1960's (Gragson & Bolstad, 2006). Currently, residential land use is becoming increasingly more predominant (Kirk *et al.*, 2012).

We examined 8 sites along a range of forested cover (N=3), agricultural usage (N=3) and residential development (N=2). At each site the sampling area of the riparian zone spanned a distance of twenty meters perpendicular to the stream. Two of the forested sites were on predominantly classified as a fine loamy, parasesquic, mesic Typic Hapludult soils (Evard-Cowee soil series; altitude: 818 and 815 m) while the third was mainly a fine-loamy, mixed, superactive, mesic Humic Hapludult (Saunook soil series; altitude 811 m). The agricultural sites ranged from soils predominantly classified as a fine-loamy, mixed, superactive, mesic Humic Hapludult (Saunook soil series; altitude: 664 m; hay field), a mix between fine loamy,

parasesquic, mesic Typic Hapludult (53%; Evard-Cowee soil series)and a fine-loamy, mixed, superactive, mesic Humic Hapludult (47%; Saunook soil series; altitude: 661 m; partially cattle grazed with no access to stream), and the third site was classified as a mix of a coarse-loamy, mixed, superactive, mesic Oxyaquic Humudept (60%; Reddies fine sandy loam soil series) and a fine-loamy, mixed, superactive, mesic Typic Hapludult (27%, Saunook soil series; altitude: 761 m; cattle grazed with access to stream). The residential sites were mainly classified as a fine-loamy, mixed, superactive, mesic Humic Hapludult (Saunook soil series; altitude: 661 m; tree dominated vegetation) and a mix of fine-loamy, isotic, mesic Typic Humudept (59%; Tuckasegee-Cullasaja soil series) and fine loamy, parasesquic, mesic Typic Hapludult (41%; Evard-Cowee soil series; altitude: 720 m; mix of lawn and tree vegetation).

Experimental design

Measurements for greenhouse gas emissions were taken eight times from May 2012 to May 2013 at five locations at each site. Three locations were randomly selected within 3 meters from the stream and 2 locations were placed along the range of 3-20 meters from the stream. At the location of greenhouse gas measurement, soil cores were collected for potential N cycling rates and soil abiotic characteristics in May 2012, July 2012, November 2012, March 2013 and May 2013. In addition, gross N cycling rates were assessed using isotopic tracer techniques (describe below) in May 2012 on the agricultural and forested sites. Soil bulk density was determined in April 2013 for all sites.

Soil preparation and climate data

We measured soil moisture and temperature at 5 cm depth at every sampling time and location using a Hydrosense sensor and soil thermometer, respectively. Soil samples (0-15 cm) were collected for nitrogen cycling assays and edaphic characteristics using a 5 cm diameter stainless steel soil push tube from three randomly determined locations per site. We divided core samples into forest floor (if present), 0-5, 5-10, and 10-15 cm mineral soil and stored samples in sealed plastic bags. Each soil sample was individually sieved and homogenized to 2 mm. Gravimetric soil moisture was determined by oven drying >2 grams of soil to constant weight at 105°C. We determined potential nitrification (pNTR) and potential denitrification (pDNF) rates at every sample time and extractable NO_3^- and NH_4^+ concentrations (November 2012 and March 2013 only) on fresh sieved soils. Soils were air-dried (2 weeks) befor soil pH in water (2:1) was determined as described in Robertson *et al.* (1999). Soil bulk density (g soil cm^{-3}) of the surface 0-15 cm was determined on samples (< 2mm) collected separately in April 2013 using a 4.3 cm diameter PVC pipe corer. C and N concentrations were determined on the soil bulk density soils using an Elementar Flash EA 1112 NC analyzer (Therm Scientific). Carbon and N content was determined by near infrared reflectance spectroscopy (NIRS) using predictive models developed by Baas *et al.* (In press) similar to Chang and Laird (2002) for an ASD FieldSpec 3 and the Unscrambler software (Camo Software Inc., Woodbridge, NY, USA). The FieldSpec NIRS provides 1 nm resolution reflectance data between 350-2500 nanometer wavelengths. All NIRS data were first-derivative transformed before analysis. Patterns of site specific precipitation were collected using rain gauges according to Laseter *et al.* (2012). Historic precipitation data were retrieved from the NOAA climate database for Macon County, NC (NOAA, 1950-2011).

Greenhouse gas emissions

We measured net soil CO_2, CH_4 and N_2O fluxes between 9:00 and 16:00 h using static chamber PVC flux chambers with an inner diameter of 15.1 cm and a height of 8 cm. The PVC collars were installed one hour before flux determination to a soil depth of 10 cm. Lids were adapted with septa for gas sampling; nine ml gas samples were taken using a ten cc plastic syringe at 1, 5, 10 and 30 minutes after placing the lid on the collar. Gas samples were analyzed for CO_2 using a LICOR-7000 (LICOR Inc., Lincoln, NE, USA) and for N_2O and CH_4 using a Shimadzu GC-ECD and GC-FID (Shimadzu Co., Kyoto, Japan), respectively. Fluxes were determined using linear regression analysis. Rates are presented as mg N m^{-2} d^{-1} (N_2O), mg C m^{-2} d^{-1} (CH_4) and g C m^{-2} d^{-1} (CO_2). We assumed the flux measurement to be representative for the whole day (Bremer *et al.*, 1998) and calculated the annual flux as the product between the daily flux and the sum of half the days before and half the days after measuring dates. We also calculated changes in CO_2 equivalent fluxes ($CO_{2\text{-eq}}$) over a 20 year timespan with a global warming potential of 86 for CH_4 and 268 for N_2O (Myhre, 2013) for Macon County, North Carolina based on projected land use changes from 2010-2030 (Kirk, 2009). We calculated the overall Macon County riparian CO_2, CH_4, N_2O and $CO_{2\text{-eq}}$ emissions. Riparian zones were defined as a buffer of 20 meters along streams and their area was estimated using the buffer tool in ArcGIS 10.0 on the Coweeta LTER stream GIS data (Coweeta Long Term Ecological Research Database, 1936-2011).

Nitrogen cycling assays

We determined pNTR and pDNF on fresh soil samples using oxic and anoxic incubation techniques, respectively. Potential nitrification assays were determined within 72h of collecting

and pDNF assays were conducted on soils stored (4°C) for less than 2 weeks at field moisture conditions. Gross N cycling assays were conducted using stable isotope tracer techniques on mineral soil cores (0-10 cm) collected in May 2012.

We used the amended slurry incubation method to determine pNTR (Baas *et al.*, In press, Bodelier *et al.*, 1996). Five grams of sieved soil (< 2 mm) was placed in 37 ml serum vials with 15 ml of media (0.33 g L^{-1} $(NH_4)_2SO_4$ and buffered with 0.14 g L^{-1} K_2HPO_4 and 0.027 g L^{-1} KH_2PO_4 in DI water). Each serum vial was wrapped in aluminum foil and had an Al foil cap to prevent evaporation and UV light inhibition of ammonia-oxidizing bacteria. After the addition of the media, vials were shaken at 10 relative centrifugal force (rcf) at 25°C; 2 ml sub-samples were collected after 0.5, 2, 6-8 and 24 h of incubation using a cut-off pipette tip (to facilitate pipetting a slurry). Samples were centrifuged for 10 minutes at 11,000 rcf and the supernatant was immediately frozen at -20°C until thawing for NO_2^- + NO_3^- analysis sing colorimetric methods (Bendschneider, 1952). Potential denitrification rates were determined using the acetylene block method (Groffman *et al.*, 1999). Five grams of sieved soil (one gram for forest floor) was added to 37 ml serum vials. Serum vials were purged with He for 1 minute to displace oxygen from the vial, and then five ml of incubation media was added to the serum vials. Media consisted of dextrose (1 mM) and sodium nitrate (1 mM) in DI water purged for 30 minutes with He. Assays were initiated by replacing 4 ml of headspace with 99% pure acetylene (10% v/v). Samples were incubated at 20ºC while shaking (150 rpm) for 3 hours. Gas subsamples were taken after 0.5 and 3h, and stored in 3.5 ml vacutainers (Labco) until analysis for N_2O on a GC-ECD (Shimadzu Inc., Tokyo, Japan) with a 10-port Valco valve (preventing acetylene from saturating the detector). Rates of pNTR and pDNF were determined via regression analysis of changes in solution NO_3^- or N_2O concentrations over time and are presented per soil dry weight.

Gross N cycling rates were determined as described in Baas *et al.* (In prep-a) and Silver *et al.* (2001) using both the isotope dilution technique and direct tracer techniques. Fifty grams of sieved soils (2 mm) from agricultural and forested sites were added to Mason jars. Half of the sample jars per site were amended with less than 20% enrichment of 98% pure $(^{15}NH4)_2SO4$ (9.5 ± 8.3%) and the other half with $K^{15}NO_3$ (2.7 ± 2.5%). The incubation was conducted under oxic conditions at room temperature (20°C). At three time points: before amendment, after 4 h and after 24 h, soils were sub-sampled for nutrient concentrations and isotopic compositions were collected in addition to gas samples for analyses of $^{15}N_2$ and $^{15}N_2O$. Nutrient concentrations were determined on 100 ml 2 M KCl extractions and samples for nutrient isotopic composition was collected using the diffusion technique (Brooks *et al.*, 1989). Microbial biomass N was determined using the fumigation approach (Cabrera & Beare, 1993) coupled with a persulfate digestion and the diffusion technique for determination of the isotopic composition (Templer *et al.*, 2008). Nutrient concentrations were determined according to colorimetric approaches (USEPA, 1983a, USEPA, 1983b) and the ^{15}N content was determined using a EA-IRMS (Sercon Ltd., Cheshire, UK). Gaseous concentrations for N_2O were determined using a GC14A-ECD (Shimadzu Inc., Tokyo, Japan). N_2 concentrations and sample N_2O and N_2 isotopic composition was determined using a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced with a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany). N mineralization, nitrification, NH_4^+ consumption and NO_3^- consumption rates were estimated using the isotope pool dilution technique (Davidson *et al.*, 1991, Kirkham & Bartholomew, 1954). Dissimilatory nitrate reduction to ammonium (DNRA) and nitrifier denitrification ($NH_4^+ \rightarrow N_2$) and denitrification rates ($NO_3^- \rightarrow N_2$) were determined according to Silver *et al.* (2001). NH_4^+ immobilization rates were estimated by subtracting the nitrification

rates and gaseous loss rates from the NH_4^+ consumption rates. NO_3^- immobilization rates were determined by subtracting the DNRA and denitrification rates from the NO_3^- consumption rates.

Carbon Composition

We estimated the available C content of mineral soils, as lignin, cellulose, dextrose, trehalose and mannitol using a NIRS in July 2012, November 2012, March 2013 and May 2013. This required us to create a NIRS calibration model for mineral soil (0-15 cm) from each of the eight sites. First, soils were combusted (500°C for 24 h) to remove all organic matter and mixed with random concentrations of different carbon compounds (0-5% w/w range) in the form of lignin (Lignin, alkali Sigma-Aldrich 370959, batch #: 0801288), cellulose, dextrose, trehalose and mannitol. Mixtures were scanned by an ASD FieldSpec 3 described above and transformed to the first-derivative before statistical analysis. The samples were divided into a calibration (70%) and validation (30%) dataset. Using The Unscrambler software partial least squares best cross-validated models were developed with the training dataset for each of the specific carbon compounds. The models were validated by regression analysis using the validation dataset. In addition, we validated this technique by using traditional extraction and analysis approaches on a subset of air-dried field collection samples (N=10) for lignin using Pyrolysis-GC-MS, cellulose by phenol and acid extractions followed by colorimetric analysis (Dubois *et al.*, 1956) and sugar (glucose, trehalose and mannitol) analyses by High pH Anion Exchange Chromatography at the Complex Carbohydrate Research Center at the University of Georgia.

Statistics and extrapolation

Greenhouse gas fluxes, potential N cycling rates and soil characteristics measured over time were all tested for differences among land use types using a mixed model repeated measures approach (Restricted Maximum Likelihood (REML); Fixed: time and land use type; Random: site) and Tukey posthoc analyses. If a date and land use type interaction was determined, land use type was tested for each time point using one-way ANOVA and Tukey posthoc analyses. In addition, the analysis was also conducted including only the agricultural sites with cattle present. Differences in gross nitrogen cycling rates were analyzed by Student's t-tests. If data were not log-normally distributed, rank transformation was conducted to allow for parametric analyses (Conover & Iman, 1981). Several parameters proved non-normal after log transformation; therefore, we used Spearman correlation analysis on non-transformed data to determine the strongest predictors for greenhouse gas fluxes and N cycling rates. All statistical analyses were conducted in JMP 11 (SAS Institute Inc., Cary, NC) and significance differences are indicative of $p < 0.05$ unless otherwise stated.

Results

Edaphic characteristics

Soil temperature (**Figure 1)** was consistently greater in agricultural sites (15.4 ± 0.55°C) compared to forested (12.4 ± 0.45°C) and residential (13.5 ± 0.67°C) sites ($F = 3.5$, $p = 0.02$). A sample time and land use type interaction was observed ($F_{14,271} = 580.0$, $p < 0.001$), thus, the temperature data was analyzed separate per sampling time. Agricultural sites were significantly warmer than residential and forested land use in July 2012 ($F_{7,30} = 25.4$, $p < 0.001$), September 2012 ($F_{7,29} = 8.2$, $p < 0.001$), November 2012 ($F_{7,32} = 4.3$, $p < 0.05$), February 2013 ($F_{7,26} = 20.2$,

$p < 0.001$), March 2013 ($F_{7,32} = 46.0$, $p < 0.01$) and May 2013 ($F_{7,32} = 32.2$, $p < 0.001$). In addition, residential sites were warmer than forested and agricultural sites in May 2012 ($F_{6,27} = 9.2$, $p < 0.002$) and agricultural sites were greater than forested sites ($F_{6,27} = 9.2$, $p = 0.001$). Further, soil temperature was greater in residential development compared to forested sites in July 2012 ($F_{7,30} = 25.4$, $p < 0.001$), March 2013 ($F_{7,32} = 46.0$, $p < 0.001$) and May 2013 ($F_{7,32} = 32.2$, $p = 0.006$). Mixed model analysis of soil moisture data (**Figure 5.1)** showed no significant land use effect while sample times were significantly different ($F_{7,260} = 26.1$, $p < 0.001$) with a near significant interaction observed ($F_{14,260} = 1.7$, $0 = 0.062$). July 2012 (14.9 ± 2.1%) and September 2012 (14.8 ± 1.8%) were significantly drier than all other sample times (May 2013: 30.4 ± 1.8%; December 2012: 29.4 ± 2.1%; May 2012: 30.9 ± 3.2%; March 2013: 25.7 ± 2.0%; February 2013: 25.9 ± 2.3%; November 2011: 19.7 ± 1.7%). Since we observed a near significant interaction between sample time and land use type ($p = 0.06$) we also analyzed the data for land use effects at each of the individual sample times. Soil moisture was greater in agricultural sites than residential and forested sites for July 2012 ($F_{7,27} = 4.0$, $p < 0.05$) , September 2012 ($F_{7,29} = 3.9$, $p < 0.05$) and February 2013 ($F_{7,24} = 4.7$, $p < 0.05$) in addition to a trend in May 2012 ($F_{6,21} = 2.2$, $p < 0.08$) and December 2012 ($F_{7,29} = 4.9$, $p < 0.06$) . No differences between land use types were observed in November 2012, March 2013 and May 2013. Soil pH, total N concentrations and bulk density varied among land use types; however, total carbon concentrations did not vary significantly (**Table 5.1**). The pH showed a significant land use type ($F_{2,5} = 15.7$, $p = 0.007$) and sample time effect ($F_{3,128} = 4.3$, $p = 0.006$) with no significant interaction. The pH was greater in agricultural compared to residential and forested land use types ($p < 0.05$) and the pH was greater in May 2013 than July 2012 ($p < 0.05$). Total N concentrations were greater in agricultural and in residential sites than in forested sites ($F_{35,60} =$

1.8, p = 0.020). Total soil carbon concentrations ranged from 18.4 to 31.9 mg C g_{soil}^{-1} and did not differ significantly between land use types. Soil NH_4^+ concentrations showed a trend of a land use effect ($F_{2,4}$ = 4.9, p = 0.081) and a significant sample time effect ($F_{1,15}$ = 4.7, p = 0.046) with no significant interaction effect. Agricultural (7.6 ± 3.4 mg N kg_{soil}^{-1}) and residential (3.1 ± 0.70 mg N kg_{soil}^{-1}) NH_4^+ concentrations were greater compared to forested (2.0 ± 0.57 mg N kg_{soil}^{-1}) sites (p < 0.1) with greater concentrations in March 2013 (2.2 ± 1.02 mg N kg_{soil}^{-1}) than November 2012 (4.9 ± 1.7 mg N kg_{soil}^{-1}; p = 0.046). Soil NO_3^- concentrations showed a significant land use ($F_{2,6}$ = 11.5, p =0.01) and sample time effect ($F_{1,14}$ = 6.8, p =0.02) in addition to a near significant interaction effect ($F_{3,6}$ = 3.6, p =0.056). The NO_3^- concentrations were significantly greater in agricultural (5.9 ± 2.3 mg N kg_{soil}^{-1}) sites compared to both forested (0.04 ± 0.03 mg N kg_{soil}^{-1}) and residential (0.50 ± 0.43 mg N kg_{soil}^{-1}) sites (p < 0.05) and greater concentrations in March 2013 (2.7 ± 1.3 mg N kg_{soil}^{-1}) than November 2012 (0.74 ± 0.53 mg N kg_{soil}^{-1}; p = 0.02). Finally, the bulk density was greater in agricultural sites than forested and residential sites ($F_{7,92}$ = 5.4, p < 0.001).

Greenhouse gas emissions and land use

The cumulative fluxes from May 2012 to May 2013 are shown in **Table 5.2**. CO_2 fluxes were significantly greater in agricultural land use compared to residential and forested land use ($F_{2,5}$ = 22.1, p = 0.003), while CH_4 ($F_{2,5}$ = 1.2, p = 0.39) and N_2O fluxes ($F_{2,5}$ = 0.4, p = 0.71) showed no significant differences in cumulative flux. However, when excluding the agricultural plot without cattle from the analysis, N_2O fluxes were significantly greater in agricultural land use than in forested sites ($F_{2,4}$ = 13.4, p = 0.017) and showed a trend of being greater than residential land use ($F_{2,4}$ = 13.4, p = 0.07). Mixed model repeated measures analysis of CO_2

fluxes (**Figure 2**) showed a significant time and land use type interaction effect ($F_{14,258}$ = 2.0, p = 0.017), therefore, we tested land use effects at each sample time. Agricultural land use was greater in CO_2 flux than both residential and forested land use in May 2012 ($F_{5,21}$ = 3.0, p < 0.05), July 2012 ($F_{7,27}$ = 7.4, p < 0.001), March 2013 ($F_{7,32}$ = 7.4, p < 0.001) and May 2013 ($F_{7,32}$ = 11.9, p < 0.05) and forested sites only in November 2012 ($F_{7,31}$ = 2.5, p = 0.036), February 2013 ($F_{7,25}$ = 4.1, p = 0.003). Additionally, CO_2 flux in residential site soils was also greater than forested sites in May 2013 ($F_{7,32}$ = 11.9, p = 0.026). Mixed model analysis of N_2O fluxes showed no significant land use effect while significant differences were observed between sampling dates ($F_{7,124}$ = 3.1, p = 0.005) with May 2012 and July 2012 being greater than March 2013 (p < 0.05). No significant interaction effect between land use type and sample time was found. When excluding the agricultural sites without cattle from the mixed model analysis, N_2O flux did show a trend of land use effects ($F_{2,6}$ = 5.5, p = 0.046) with agricultural land use being greater than forested (p = 0.07) and residential (p = 0.09). Excluding the non-cattle sites still resulted in a significant sample time effect ($F_{7,108}$ = 3.1, p = 0.005) with July 2012 being greater than March 2013 (p = 0.006) and a trend for May 2012 being greater than March 2013 (p = 0.08). Mixed model analysis of CH_4 showed no significant time or land use effects whether the agricultural site without cattle was included or excluded from the analysis. However, when conducting the analyses per sample time, residential land use was significantly greater than forested sites in November 2012 ($F_{7,30}$ = 2.3, p = 0.050). When comparing all the sites individually, the forested residential site (Saunook soil series; 9.2 ± 6.2 mg C m^{-2} d^{-1}) was greater than one of the forested sites (Saunook soil series; -7.7 ± 2.6; $F_{7,30}$ = 2.3, p = 0.050).

Estimates of annual greenhouse gas emissions based on predicted land use showed that soil CH_4 fluxes will increase while both CO_2 and N_2O fluxes will decrease (**Figure 5.3**). Overall

CO_2 equivalent emissions would decrease by 0.9% between 2010 and 2030. Reductions in CO_2 and N_2O emissions from the decreasing area of agricultural land use accounted for most of the reductions in overall greenhouse gases while increased CH_4 emissions due to an increasing area of residential land use at the expense of the CH_4 consuming forested ecosystems represent the main increase in greenhouse gas emissions. CO_2 emissions account for 100% (Forest), 98% (Res) and 97% (Ag) of the overall global warming potential of riparian zone greenhouse gas emissions.

Nitrogen cycling

Mixed model analyses showed a significant interaction between sample time and land use for potential nitrification ($F_{8,105} = 2.5$, $p = 0.0161$) while potential denitrification rates showed no significant interaction with significant sample time ($F_{4,169} = 18.5$, $p < 0.001$) and land use ($F_{2,5} = 67.0$, $p < 0.001$) effects (**Figure 5.4; Table B1 & 2**). Potential nitrification rates ranged from -0.02 to 0.46 ng N g_{soil}^{-1} h^{-1} and were greater in agricultural sites compared to forested and residential sites in May 2012 ($F_{6,14} = 5.1$, $p < 0.05$), November 2012 ($F_{7,16} = 13.9$, $p < 0.001$), March 2013 ($F_{7,16} = 7.4$, $p < 0.001$) and May 2013 ($F_{7,24} = 4.8$, $p < 0.001$) ; sample date did not differ significantly. Potential denitrification rates ranged from -4.3 to 307.3 ng N g_{soil}^{-1} h^{-1} and were significantly greatest in agricultural sites ($p < 0.001$); residential sites were also greater than forests ($p = 0.050$). July 2012 showed the significantly greatest potential denitrification rates while May 2013 showed the lowest ($p < 0.05$).

Gross N cycling (**Table 5.3**) showed significantly greater rates of N mineralization in agricultural sites compared to forested sites ($F_{4,5} = 8.2$, $p = 0.02$) . Gross nitrification, similar to N mineralization, showed greater rates in agricultural sites than forested sites ($F_{4,5} = 6.7$, $p =$

0.03) . Gross DNRA, the transformation of NO_3^- to NH_4^+, did not differ between agricultural sites and forested sites, however, agricultural sites differed significantly within agricultural land use; sites used for hay production only (without cattle; 0.21 ± 0.04 µg N g_{soil}^{-1} d^{-1}) were significantly greater ($F_{4,9}$ = 10.0, $p < 0.05$) in DNRA rates compared agricultural sites with cattle (-0.05 ± 0.03 µg N g_{soil}^{-1} d^{-1}) and forested sites (0.07 ± 0.03 µg N g_{soil}^{-1} d^{-1}). In addition, forested sites were significantly greater in DNRA rates than agricultural cattle sites ($p = 0.036$). Further, the percent of gross nitrification reduced by DNRA was significantly greater ($F_{4,5}$ = 6.1, $p = 0.037$) in forested sites (250 ± 234%) compared to agricultural sites (16 ± 17%). Gross denitrification rates were not significantly different between agricultural and forested sites ($F_{4,5}$ = 5.6, $p = 0.16$) while nitrifier denitrification (from NH_4^+) was significantly greater in agricultural compared to forested sites ($F_{4,5}$ = 233.5, $p < 0.001$). Both gross N_2O from NH_4^+ ($F_{4,5}$ = 5.7, $p = 0.042$) and from NO_3^- ($F_{4,5}$ = 10.2, $p = 0.013$) were significantly greater in agricultural than forested sites although two-three orders of magnitude lower than denitrification rates. However, N_2:N_2O ratios from NO_3^- were significantly greater in agricultural sites (302 ± 188 µg N g_{soil}^{-1} d^{-1}) compared to forested sites (2978 ± 452 µg N g_{soil}^{-1} d^{-1}) ($F_{4,6}$ = 14.3, $p = 0.003$). Further, N_2:N_2O ratios from the NH_4^+ label (Forest: $1.562*10^5$ ± 2.630; Ag: 8505 ± 2474) were significantly greater than ratios from NO_3^- ($F_{9,16}$ = 5.1, $p < 0.001$).

Carbon Composition

Our ability to measure specific C compound concentrations using NIRS varied with C type (**Table 5.4)**. We were able to validate all the different carbon compound concentrations in the mixed samples with our internal validation dataset, explaining between 48 – 88% of the variation. However, external validation using traditional extraction methods proved less

successful. We found significant relationships for dextrose and trehalose while mannitol and cellulose were not significantly related to the NIRS predicted carbon concentrations. Lignin concentrations were found be below detection limit and, thus, no external validation of the NIRS method was possible.

Specific carbon compound concentrations varied by both sample times and land-use types (**Figure 5.5**). Dextrose showed an interaction effect between sample date and land use ($F_{6,140} = 6.4$, $p < 0.001$). Dextrose concentrations were greater in agricultural and residential sites than forested sites in November 2012 ($F_{7,31} = 4.6$, $p = 0.001$) while in May 2013 forested land use was greater in dextrose concentrations than agricultural land use ($F_{7,32} = 9.5$, $p < 0.001$). Mannitol ($F = 0.6$, $p = 0.58$) and trehalose ($F = 0.2$, $p = 0.81$) concentrations showed no significant land use effects. Mannitol concentrations showed a significant sample time effect ($F_{3,140} = 8.1$, $p < 0.0001$) with a non-significant interaction with land use type. July 2012 was found to be greater in mannitol concentrations than November 2012, March and May 2013 ($p < 0.001$). Trehalose concentrations also showed a significant sample time effect ($F_{3,140} = 4.1$, $p = 0.008$) with no significant interaction effects. May 2013 proved significantly greater than July 2012 and March 2013 in trehalose concentrations ($p < 0.05$). Cellulose showed no significant land use effect but a strong sample time effect ($F_{3,140} = 30.2$, $p < 0.0001$) with a non-significant interaction effect. May 2013 was greater in cellulose concentrations than July 2012, November 2012, and March 2013 ($p < 0.0001$). Cellulose concentrations in November 2012 were also significantly greater than in July 2012 ($p < 0.001$). Lignin concentrations showed a near significant land use type effect ($F_{2,5} = 5.4$, $p = 0.056$) and a significant sample time effect ($F_{3,140} = 17.3$, $p < 0.0001$) with

the interaction effect not being significant. Residential sites were greater than agricultural sites ($p = 0.053$) and May 2013 was greater than July 2012, November 2012 and March 2013 ($p < 0.0001$).

Drivers of fluxes and processes

Overall, Spearman correlation analysis (**Table 5.5**) showed soil temperature to be strongly positively correlated to CO_2 fluxes and showed significant positive correlations with both CH_4 and N_2O fluxes. The positive correlations for CO_2 were significant for all land use types while they were only significant with N_2O fluxes for agricultural land use. Soil moisture was significantly negatively correlated to CO_2 flux and positively to CH_4 flux. When separating the analysis per land- use type, CH_4 fluxes correlated significantly positively to soil moisture. No relationships, either overall or per land use type, between soil moisture and N_2O flux were found. pH was significantly positively related to the CO_2 and N_2O flux while no relationships with CH_4 flux were observed. Cumulative precipitation over the previous 48 hours showed a significant positive correlation with N_2O fluxes while no relationship was found with either CO_2 or CH_4 fluxes. Cumulative precipitation over the previous 24 hours showed a significantly negatively correlation with CO_2 flux. Potential nitrification rates showed a significant negative relationship with CO_2 flux for agricultural ecosystems while showing a significant positive relationship with N_2O flux overall. Potential denitrification rates were significantly positively correlated with CO_2, CH_4 and N_2O fluxes with the strongest relationship found for CO_2 fluxes. The soil dextrose concentrations did not yield any significant correlations, although several trends ($p < 0.1$) were observed for CO_2 (negative) and CH_4 (positive for agricultural) fluxes. Soil mannitol concentrations also did not yield significant correlations, only showing a trend with CO_2

(positive) flux in agricultural land use. Trehalose concentrations showed a significant correlation with CH_4 flux in residential ecosystems while no other significant correlations or trends were observed. Cellulose concentrations proved to correlate significantly negative with CO_2 flux in forested and residential ecosystems. N_2O flux showed a trend for a positive relationship with cellulose concentrations. Lignin concentrations showed no significant correlations and only showed a positive trend with CO_2 flux in agricultural land use.

Correlation analysis between potential nitrification or denitrification and edaphic variables showed the strongest correlation to be with pH (pNTR: $\rho = 0.39$; pDNF: $\rho = 0.58$). Temperature exhibited a significant correlation with potential denitrification ($\rho = 0.33$; $p < 0.01$) while no relationship could be detected with potential nitrification. Soil moisture showed no significant relationships with either potential nitrification or potential denitrification, although, a trend was observed with potential denitrification ($\rho = 0.14$; $p < 0.1$). Cumulative precipitation over the previous 48 hours also yield significant correlations for both potential nitrification ($\rho = 0.22$) and potential denitrification ($\rho = 0.18$). The specific carbon concentrations showed a significant correlation between trehalose and potential denitrification ($\rho = 0.33$; $p < 0.01$) while potential nitrification was negative correlated with lignin concentrations ($\rho = -0.22$; $p < 0.05$). Additionally, a trend was observed between potential denitrification and lignin concentrations ($\rho = -0.15$; $p < 0.1$).

Discussion

Land-use change may promote or reduce N cycling and greenhouse gas emissions in riparian ecosystems across the southern Appalachians. Our study found N cycling rates and CO_2 and N_2O emissions to be significantly greater in agricultural land use compared to forest and

residential land use, thus, suggesting greater removal and retention capacities in agricultural systems. N cycling rates were generally greater with agricultural land use compared to residential and forested ecosystems. Further, denitrification played a greater role than previously thought in riparian ecosystems in the southern Appalachians. Importantly, our results suggest that residential development of forested riparian ecosystems would shift the ecosystem from a sink to a source of CH_4.

We did not observe an effect of residential development on potential nitrification rates. Increasing densities of human development have been shown to increase stream nitrate concentrations (Hatt *et al.*, 2004, Poor & McDonnell, 2007, Webster *et al.*, 2012), thus reduce water quality. In addition, changes in the hydrological cycle often result in drier soils, lowering the N retention capacity (Groffman *et al.*, 2003) through greater rates of N cycling and N_2O emissions (Gift *et al.*, 2010, Groffman *et al.*, 2002, Roach & Grimm, 2011). However, similar soil moisture and greater total N concentrations in residential soils compared to forested soils suggest that residential riparian development is capable of some N retention. Riparian zones are often found to be crucial in removing excess inorganic N along the aquatic-terrestrial interface (Hefting *et al.*, 2003, Knoepp & Clinton, 2009, Ullah & Zinati, 2006). However, the low potential denitrification rates indicate that residential riparian soils in the current study are poor at removing excess N from the ecosystem, thus, explaining the large increases in stream nitrate concentrations with residential development in this region (Webster *et al.*, 2012).These results coupled with projections of regional land use change suggest that CO_2 and N_2O emissions will decrease by 2030 due to a decrease in the total area of agricultural land use. However, CH_4 emissions are expected to increase with increasing residential development. The replacement of forest with residential development may effectively shifts riparian zones from a CH_4 sink into a

source. CO_2 emissions will account for the majority of the global warming potential of emissions; thus, reductions in agricultural land use will reduce total global warming potential of greenhouse gas emissions. It is important to note that the projected forest clearing of an estimated 700 ha of riparian forest from 2010-2030 for Macon County alone would result in the reduction of an approximate 105,000 Mg of sequestered carbon based on the loss of above ground biomass alone (Bolstad & Vose, 2005)[1]. The magnitude of that amount of C loss is equivalent to more than a tenfold reduction of total riparian $CO_{2\text{-eq}}$ greenhouse gas emissions projected in the current study between 2010 and 2030 (8,000 Mg C-equivalence). Therefore, the removal of vegetation associated with land use might play a more important role than riparian emissions in the total regional greenhouse gas budget.

Emissions of CO_2 were similar to rates found in previous studies in the region for cove forested ecosystems (Bolstad & Vose, 2005) although much higher rates of 2.0 to 2.8 kg C m^{-2} y^{-1} have been reported for forests in the region (Vose & Bolstad, 2007). However, rates for cove agricultural systems were almost three fold more in the current study than observed in previous research by Bolstad and Vose (2005). CO_2 fluxes have been found to be lower due to lower soil moisture in agriculture (Davidson *et al.*, 2000) yet also higher due to greater root density in agriculture (Kellman *et al.*, 2007). Even though we observed greater root densities in the forests (data not shown), the higher soil moistures and input of labile carbon from grasses in agricultural sites appear to have resulted in higher CO_2 fluxes. As shown in numerous previous studies CO_2 emissions are well correlated with temperature and soil moisture (Davidson *et al.*, 2012, Lloyd & Taylor, 1994) which is confirmed for temperature in the current study. We did not see a strong

[1]Macon County covers a total area of 133,000 ha (Kirk, 2009). Assuming riparian forests clearing occurs at the same rate as projected for overall forest ecosystems, 700 ha riparian forest would be cleared between 2010 and 2030.

correlation with soil moisture in our study, however, the relatively high soil moistures in this region (Knoepp & Swank, 2002) potentially alleviated any moisture limitation. Cumulative precipitation over the previous 24 h, however, showed a negative correlation with CO_2 flux. It is likely that reduced O_2 diffusion resulted in lower CO_2 fluxes (Davidson *et al.*, 1998). The negative correlation between cellulose content and CO_2 flux in forested and residential ecosystems likely indicates that higher cellulose concentrations represent higher substrate recalcitrance (Plante & Parton, 2007). The reduction in CO_2 flux with a lower pH in this study has been found in many previous studies and represents higher maintenance costs for microbes under more acidic conditions (Anderson & Domsch, 1993).

Riparian ecosystems in the southern Appalachian Mountains can be considered hotspots of CH_4 uptake while under either agricultural or residential development shift into a CH_4 source. We found CH_4 production rates in the residential and agriculture ecosystems to show similar rates found in loam grassland soils (Boeckx *et al.*, 1997) and CH_4 uptake rates in forested ecosystems are on par with the high uptake rates often found in tropical forests (190 - 700 mg C m^{-2} y^{-1}) (Verchot *et al.*, 2000). Goldman *et al.* (1995) found soil CH_4 consumption to decrease by 30% in urban forests compared to rural or suburban forests suggesting residential development can reduce the soil CH_4 uptake capacity. When combining all land use types, CH_4 efflux showed a positive correlation with temperature and soil moisture while, specifically in residential development, soil moisture and trehalose concentrations showed significant correlations. The overall positive correlation with soil moisture and temperature found in this study is comparable to previous studies (Verchot *et al.*, 2000, Werner *et al.*, 2007) as well as increased rates with the availability of labile carbon (Hedin *et al.*, 1998). However, we are not aware of any other studies suggesting trehalose to be a preferred substrate for methanogenesis. Alternatively, it is also

possible that soils with high trehalose content stimulated respiration and, thus, the creation of anoxic microsites were conducive to methanogenesis (Von Fischer & Hedin, 2007).

Biologically relevant N_2O fluxes were only observed in the agricultural ecosystems where fluxes were comparable to emissions measured in riparian zones (Groffman *et al.*, 1998, Hefting *et al.*, 2003, Weller *et al.*, 1994) and agricultural ecosystems (Matson *et al.*, 1998), although not as high as the 2,000 mg N m^{-2} y^{-1} found for high nitrate loaded forested riparian buffers (Hefting *et al.*, 2003). Similarly to other studies, N_2O production was positively correlated with temperature (Betlach & Tiedje, 1981, Smith *et al.*, 1998). The correlation between pH and N_2O overall is most likely largely an artifact from the higher pH in the agricultural sites since a trend of an inverted pattern was observed in the agricultural sites which conforms to higher N_2O:N_2 ratios under acidic conditions (Van den Heuvel *et al.*, 2011). Higher precipitation over the previous 48 h appeared to relate to N_2O emissions and could indicate a lag effect in the microbial response to increases in soil moisture (Geyer *et al.*, 1992, Rabot *et al.*, Sexstone *et al.*, 1985). Both overall and with agricultural land use potential denitrification proved the best predictor for N_2O emissions and, therefore, denitrification is the most likely pathway for N_2O production.

As shown by the CO_2 and N_2O emissions, agricultural land use has a disproportionately high impact on the greenhouse gas budgets and potential climate change feedbacks. The high inputs of labile carbon via grasses and manure resulted in gross N mineralization and nitrification rates 5-33 times higher than in forested ecosystems. In addition, gaseous losses predominantly occurred via nitrifier denitrification ($NH_4^+ \rightarrow N_2$) in agricultural ecosystems. Even though gross N_2O production rates in the oxic lab incubation proved to be fairly low, it is likely a result of a reduction in anoxic microsites due to the break up if soil structure and macroaggregates (Parkin,

1987). The ratio of N_2O:N_2 emissions from denitrification were tenfold greater in agricultural ecosystems, thus, supporting the finding of greater N_2O emissions in the agricultural sites. We hypothesized DRNA to be greater in forested conditions due to the high C:NO_3^- ratios (Burgin & Hamilton, 2007), however, DNRA proved to be greatest in agricultural ecosystems used for hay production. The proportion of gross nitrification that was reduced via DNRA was significantly greater in forested (78%) ecosystems than agriculture systems (1%) suggesting an important role for DNRA as a N retention mechanism in forested riparian ecosystems. Indeed, Walker et al (2002) found indications for the occurrence of DNRA in restored previously agricultural riparian zones based on NH_4^+:NO_3^- ratios (Schipper *et al.*, 1994). Our results indicated that DNRA has the greatest rates in riparian zones with no cattle presence while rates in forested systems are more inconsistent. The lower nitrification rates in forested ecosystems suggest that as a mechanism of N retention DNRA might be more important in forested riparian zones which is consistent to previous findings for forest ecosystems in this region (Baas *et al.*, In prep-a). Without any plant competition for NH_4^+ and NO_3^- and under well homogenized conditions (i.e. high diffusion), the gross rates assessed in this study can represent an overestimation of field activities in addition to the fact that the low ^{15}N amendments could have somewhat stimulated the DNRA and denitrification rates. However, amendments were always kept under 20% of ambient conditions to reduce a fertilization effect (Silver *et al.*, 2001). Additionally, the break-up of anoxic microsites due to soil sieving would have resulted in an underestimation of nitrate reduction pathways (i.e. DNRA and denitrification). Similar to previous studies (Baas *et al.*, In prep-a, Pett-Ridge *et al.*, 2006, Yang, 2010), even well oxygenated soils are capable of anaerobic processes such as denitrification and DNRA with denitrification being capable of reducing

significant proportions of the total mineralization and nitrification in both forested and agricultural ecosystems.

Conclusions

Agricultural land use supports the largest fluxes of greenhouse gasses in the southern Appalachian Mountains' riparian zones. Recent and projected increases in residential development show little indication of contributing to total riparian greenhouse gas emissions. However, the transition of forest into residential land use, can results in a transition from a CH_4 sink into a source, and it is unclear how this trend will continue under more intense (sub-)urban development. While conversion of riparian land to agricultural and residential development both lead to increased nitrate pollution, residential land use shows substantially less N removal capacity by denitrification and a lower retention capacity than agricultural ecosystems. Therefore, residential development will likely result in relatively more stream nitrate pollution than agricultural land use.

Table 5.1: Soil properties for bulk density, pH and total carbon (C) and nitrogen (N) in the top 10 cm of mineral soil. For = forest; Res = residential; Ag = pasture; BD = bulk density. The letters indicate significant differences between land use types ($p < 0.05$)

	BD (g/cm^3)	**pH**	**C (mg/g)**	**N (mg/g)**
For	0.65 ± 0.06 **b**	5.0 ± 0.40 **b**	26.1 ± 1.3	1.1 ± 0.1 **c**
Res	0.69 ± 0.05 **b**	5.2 ± 0.51 **b**	27.2 ± 1.4	1.6 ± 0.1 **b**
Ag	1.08 ± 0.08 **a**	5.9 ± 0.42 **a**	24.6 ± 1.3	2.1 ± 0.1 **a**

Table 5.2: Cumulative fluxes for CO_2, CH_4 and N_2O for the three land use types. For = forest; Res = residential; Ag = pasture. The values indicate the mean and the standard error. The letters indicate significant differences between land use types ($p < 0.05$) (both all Ag and Ag with cattle only were compared to other land uses).

	CO_2 (kg C m^{-2} y^{-1})	**CH_4 (mg C m^{-2} ½y^{-1})†‡**	**N_2O (mg N m^{-2} y^{-1})**
For	1.3 ± 0.1 **b**	-460 ± 230 **b**	-12 ± 11 **b**
Res	1.2 ± 0.1 **b**	279 ± 333 **a**	35 ± 0.1 **ab**
Ag /	3.6 ± 0.6 **a** /	122 ± 230 **a** /	428 ± 316 **ab** /
Ag_{cattle}	2.9 ± 0.5 **a**	253 ± 35 **a**	665 ± 362 **a**

†$p < 0.1$

‡CH_4 flux determined from November 2012 through May 2013.

Table 5.3: Gross N cycling rates. Letters indicate significant differences ($p < 0.05$). Units are in µg N g_{soil}^{-1} d^{-1}.

Type	Gross Mineralization	Gross Nitrification	Gross DNRA	Gross Denitrification	Gross N_2 $^{15}NH_4^+$	NH_4^+ immobilization	NO_3^- Immobilization	Gross N_2O $^{15}NO_3^-$ (*10^{-3})	Gross N_2O $^{15}NH_4^+$ (*10^{-3})†
For	0.92 ± 0.25[b]	0.09 ± 0.03[b]	0.07 ± 0.03	0.79 ± 0.18	0.54 ± 0.14[b]	-0.21 ± 0.44	-0.68 ± 0.18	0.3 ± 0.0[b]	0.0 ± 0.0[b]
Ag	5.11 ± 0.81[a]	2.95 ± 0.83[a]	0.03 ± 0.05	0.96 ± 0.16	2.47 ± 0.52[a]	-1.12 ± 1.01	1.09 ± 0.95	27.4 ± 12.8[a]	1.1 ± 0.4[a]

†$p < 0.1$

Table 5.4: Near infrared reflectance spectroscopy (NIRS) prediction calibration and validation statistics. External validation was done on field air-dried soils.

	Calibration dataset		**Validation dataset**		**External validation**	
	p	r^2 (N)	p	r^2 (N)	p	r^2 (N)
Dextrose	<0.001	0.95 (31)	0.008	0.66 (9)	0.0565	0.38 (10)
Mannitol	<0.001	1.00 (31)	0.038	0.48 (9)	0.12	0.49 (6)ǂ
Trehalose	<0.001	0.53 (31)	<0.001	0.82 (9)	0.0298	0.51 (9)
Cellulose	<0.001	1.00 (31)	0.002	0.75 (9)	0.68	0.03 (9)
Lignin	<0.001	0.69 (31)	0.015	0.60 (9)	bdl	bdl
Total C-mix†	<0.001	0.90 (31)	<0.001	0.88 (9)	N/A	N/A
Total C	<0.001	0.77 (65)	0.002	0.39 (22)	N/A	N/A

† determined on combusted soil mixed with C-compounds

ǂ Saturation after 0.35 µg C g_{soil}^{-1}. Samples over threshold not included in validation

Table 5.5: Spearman rank correlations. The values indicate the correlation coefficient ρ (rho). Significant effects are shown in bold. pNTR = potential nitrification rates 0-15 cm mineral soil; pDNF = potential denitrification rates 0-15 cm mineral soil.

	CO_2				**CH_4**				**N_2O**			
	All	For	Res	Ag	All	For	Res	Ag	All	For	Res	Ag
Temperature	**0.63****	**0.60****	**0.43****	**0.71****	**0.16***	0.08	0.13	0.02	**0.19***	0.01	0.12	**0.21***
Soil Moisture	0.06	**-0.24***	-0.08	-0.06	**0.20****	0.00	**0.38***	-0.09	0.03	0.06	-0.17	-0.20†
pH	**0.33****	-0.08	0.24	-0.24†	0.16	0.14	-0.06	-0.06	**0.24****	0.10	0.11	-0.14
Precip 48 h	-0.04	-0.15	-0.06	-0.14	0.10	0.17	-0.14	0.04	**0.14***	0.02	0.10	0.09
Precip 24 h	**-0.14***	-0.18†	-0.20	-0.18†	0.06	0.15	-0.19	0.17	0.07	0.04	0.00	0.01
pNTR	0.18	-0.09	0.13	**-0.31***	0.18	0.11	-0.33	0.05	**0.22***	0.01	0.25	0.04
pDNF	**0.48****	0.24†	0.12	0.15	**0.28****	-0.03	0.18	0.15	**0.30****	-0.08	-0.14	**0.27***
Dextrose	-0.16†	0.16	-0.31†	-0.20	0.09	0.02	0.00	0.31†	-0.07	-0.09	-0.17	0.04
Mannitol	0.13	0.15	0.20	0.23†	0.07	-0.02	0.29	0.25	-0.02	-0.19	0.10	0.16
Trehalose	-0.06	-0.04	-0.06	0.01	0.14	0.10	**0.47***	0.00	0.04	0.11	0.21	-0.08
Cellulose	-0.05	**-0.32***	**-0.36***	0.19	-0.18	-0.04	-0.28	-0.15	0.16†	0.17	0.11	0.04
Lignin	0.06	0.18	0.06	0.22†	0.05	0.09	0.27	-0.09	-0.05	-0.08	0.15	-0.11

*p < 0.05
**p < 0.01
†p < 0.1

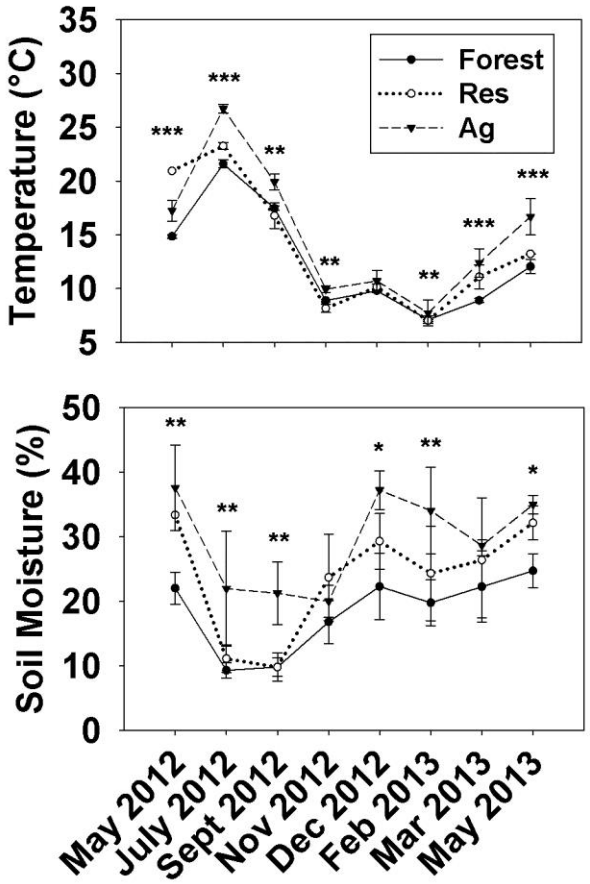

Figure 5.1: Riparian soil moisture and soil temperature. The points indicate the mean and the error bars indicate the standard error of the mean. * indicates Ag is significantly greater than Forest; ** indicates Ag is significantly greater than Forest and Res; *** indicates all land use types are significantly different from each other ($p < 0.05$). Forest = forested riparian zones; Res = residential development; Ag = agricultural development.

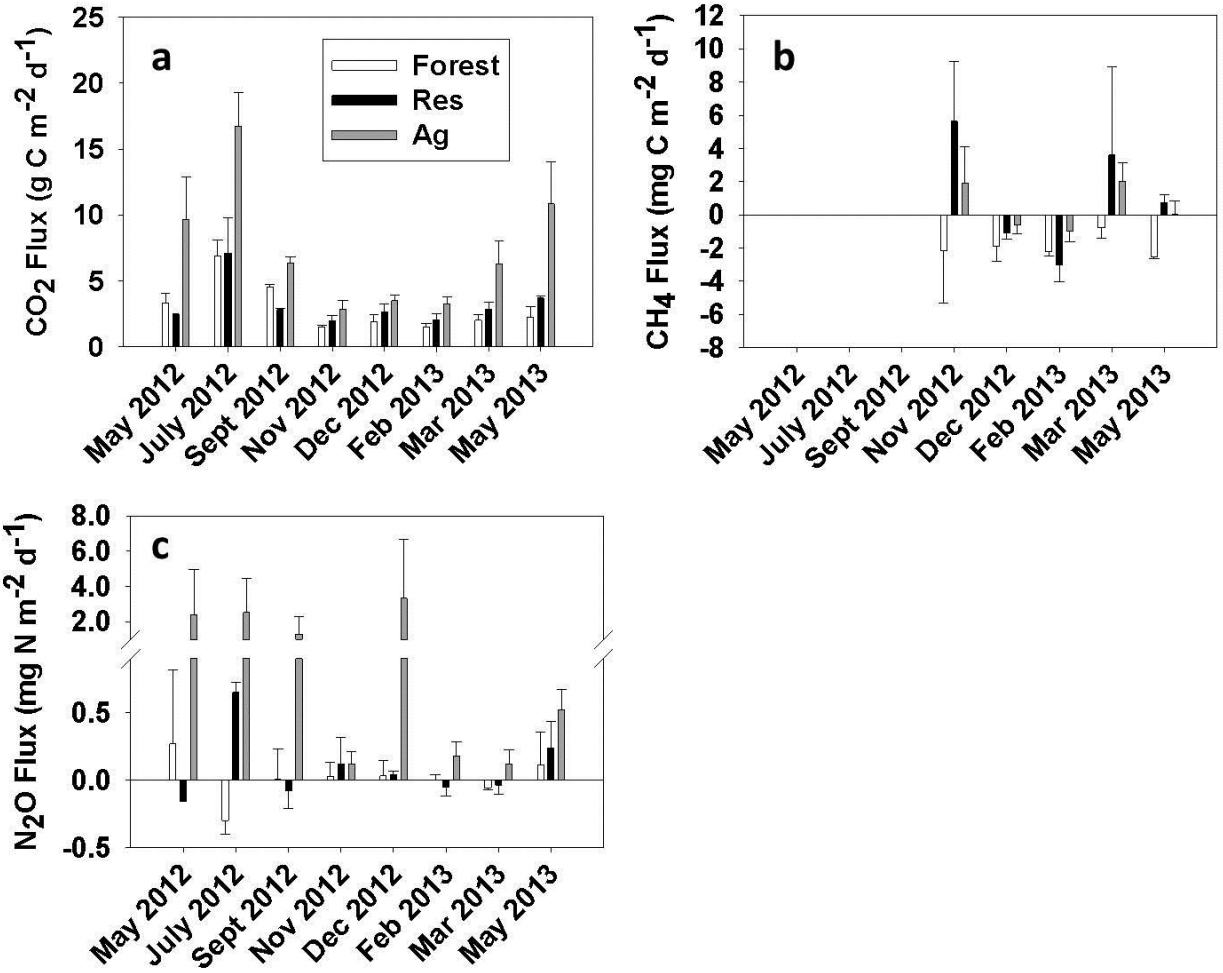

Figure 5.2: CO_2, CH_4 and N_2O fluxes. The bars indicate the mean and the error bars indicate the standard error of the mean. Forest = forested riparian zones; Res = residential development; Ag = agricultural development.

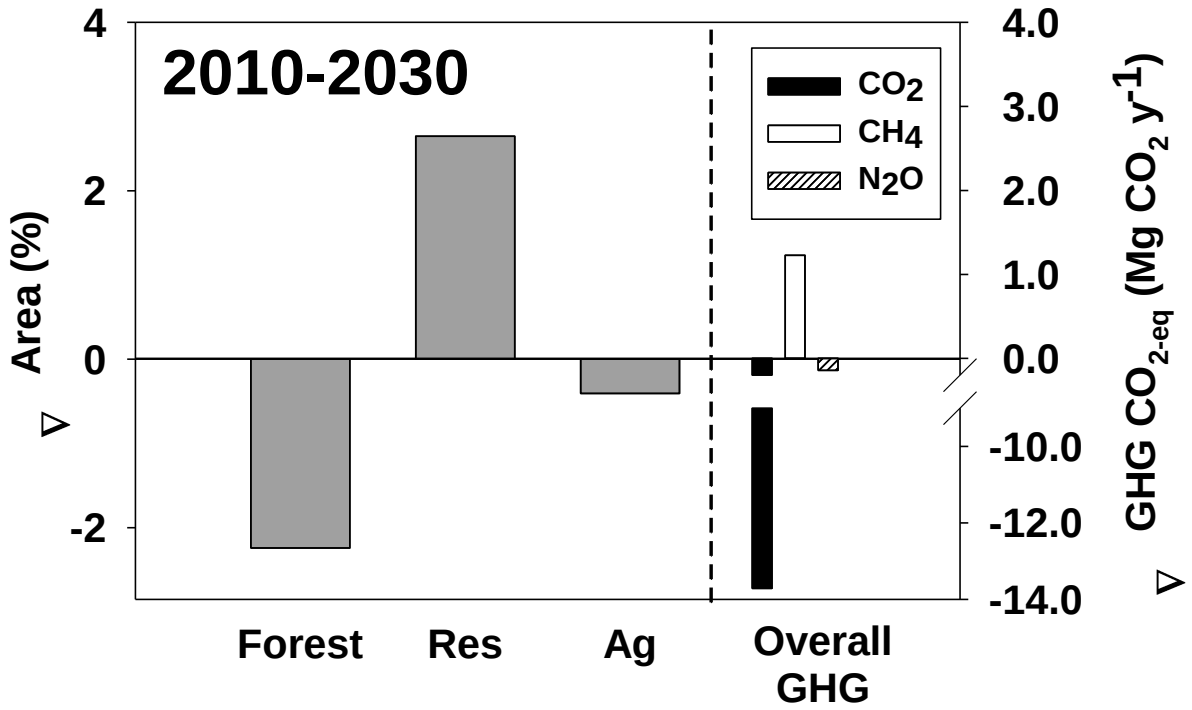

Figure 5.3: Projected land use changes from 2010-2030 from current use based on Kirk (2009) and estimated regional riparian CO_2, CH_4 and N_2O fluxes for the Macon County, NC. Greenhouse gas emissions (GHG) for all of Macon County's riparian zones are presented in CO_2-equivalent fluxes. The bars indicate the estimated change in area or GHG emission rate. Forest = forested riparian zones; Res = residential development; Ag = agricultural development.

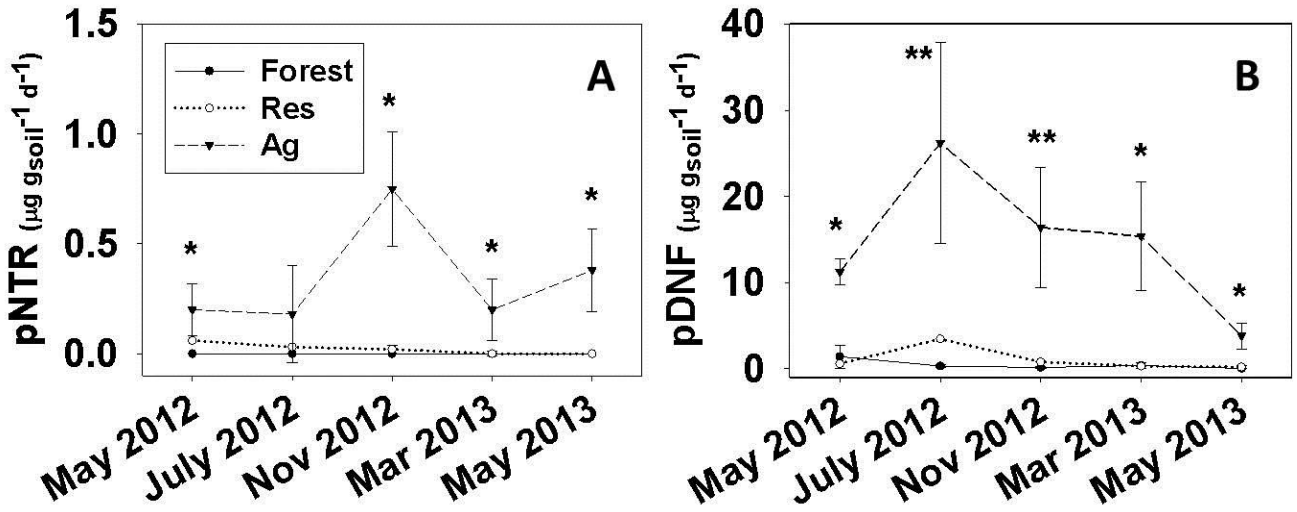

Figure 5.4: Potential nitrification (A) and potential denitrification (B) rates for the different sampling times. The bars indicate the mean and the error bars indicate the standard error of the mean. Forest = forested riparian zones; Res = residential development; Ag = agricultural development. * indicates Ag being significantly different from Forest and ** indicates Ag being significantly different than both Forest and Res ($p < 0.05$).

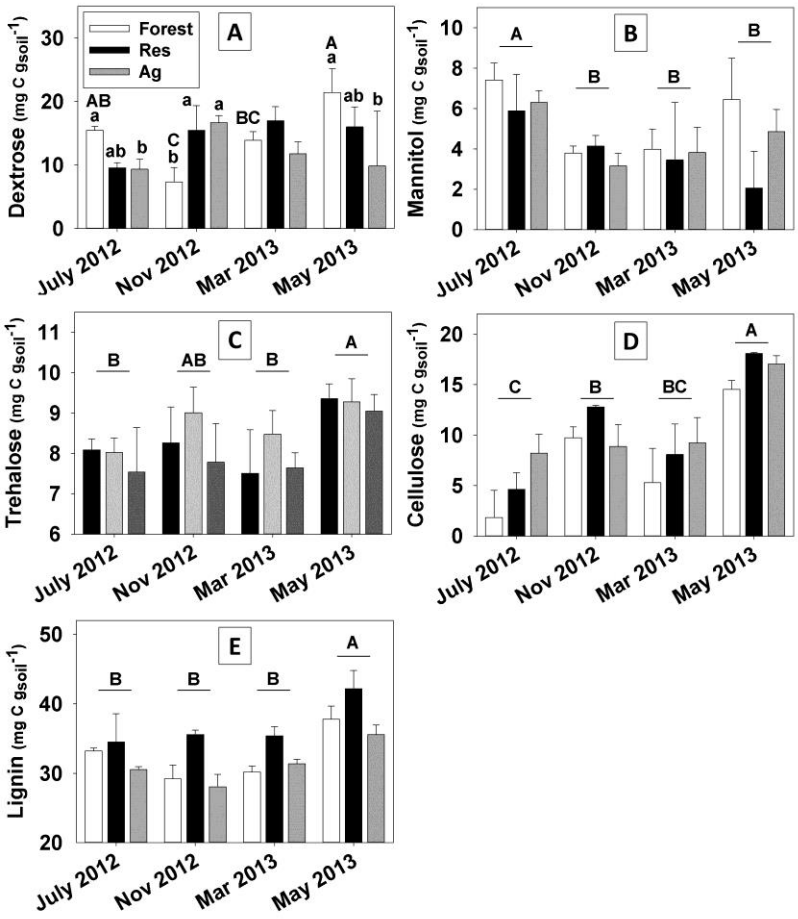

Figure 5.5: Concentration of dextrose (A), mannitol (B), trehalose (C), cellulose (D) and lignin (E) based on near infrared reflectance spectroscopy (NIRS) models. The bars indicate the mean and the error bars indicate the standard error of the mean. Different lower case letters indicate significant differences between land uses and different capitalized letters indicate differences between sample dates ($p < 0.05$). Forest = forested riparian zones; Res = residential development; Ag = agricultural development.

CHAPTER 6

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The overarching goal of this dissertation was to develop an understanding of nitrogen cycling in the southern Appalachian Mountains with respect to projected increases in land use, N deposition and soil carbon subsidies. The fact that this work was conducted within the Coweeta Long Term Ecological Research site provided an excellent framework for developing novel questions building on the already acquired knowledge. Previous research has shown how different forest types distributed over an elevation gradient have distinct nitrogen dynamics and that vegetation type, reflecting substrate quality, is a likely driver of transformations rates (Knoepp & Swank, 1998, Mclean, 2011). In addition, nitrogen deposition, ranging from 9.5 – 12.4 kg N ha^{-1} y^{-1}, has been found to be greater in high elevation northern hardwood forests which may be approaching nitrogen saturation (Knoepp *et al.*, 2008). Previous research has also shown the importance of nitrate leaching with disturbance events (Swank, 1988) and that vegetated riparian buffers can mitigate nitrate losses during logging events (Knoepp & Clinton, 2009). Additional riparian work has shown the importance of N_2O emissions in riparian zones of agricultural land use (Walker *et al.*, 2002). Walker et al. (2002) also showed that restoring riparian zones mitigates N_2O emissions. N_2 losses have been found to be small in upland soils while riparian denitrification is hypothesized to result in large N_2 losses although this was methodologically complicated to determine in the 1980's (Davidson & Swank, 1986). Efforts to create nitrogen budgets for different forest types showed that lower elevation forests are likely nitrogen limited. In addition, higher elevation forests indicated nitrogen saturation and had a

large amount of unaccounted for nitrogen (Bonito *et al.*, 2003). Therefore, a main motivation of this dissertation was to determine if anaerobic processes (that result in either gaseous loss or retention) can explain some of the unaccounted for nitrogen.

The wide range of oxidative states and controls on the processes controlling nitrogen cycling make it one of the most complex nutrient cycles (Robertson & Groffman, 2007). Large variability can occur on a spatial scale and change orders of magnitude over the range from mm to meters making extrapolating results subject to large amount of error (Groffman *et al.*, 2009a, McClain *et al.*, 2003). To address this issue, I developed a novel approach based on geophysical techniques to improve our ability to reduce variability in our estimates of nitrogen cycling processes (***Chapter 2***). By using high resolution soil conductivity and near infrared reflectance spectroscopy (for NH_4^+, total carbon and total nitrogen estimates), I was able to provide more accurate estimates of potential nitrification and denitrification activities on the hectare scale. This approach allowed for more spatial explicit questions regarding hotspot areas of coupled nitrification-denitrification. Future research can be improved by this approach in providing robust knowledge of soils within a site before sampling, thus, ensuring that hotspots and coldspots are not excluded during the sampling phase. The high resolution data could then be used as a spatial covariate to improve plot scale predictions of nitrogen cycling and reduce variability. This would ensure that the findings are more representative of the real world.

Forest ecosystems have been found to be mainly nitrogen limited throughout the temperate zone (Vitousek & Farrington, 1997). What is less clear is whether the same is true for microbial activity and growth. This is particularly relevant in the face of projected increases in nitrogen deposition and carbon subsidies to the soil (Mclean, 2011, Phillips *et al.*, 2011, Schlesinger & Lichter, 2001). The current state of the literature has two competing hypotheses.

The first is based on the traditional stoichiometric model which assumes microbial growth and activity to be N limited and, thus, would expect nitrogen amendments to result in increased respiration rates (Hessen *et al.*, 2004). The nitrogen mining hypothesis states that nitrogen limited conditions will increase decomposition resulting in greater "mining" of organic matter for nitrogen (Craine *et al.*, 2007). In ***Chapter 3*** I focused on determining the factors limiting microbial activity in the different forest types of the southern Appalachian Mountains. I found soil respiration to be primary limited by labile carbon while nitrate reduction pathways were controlled by nitrate concentrations with denitrification exhibiting the greatest increase in activity. These results suggest that in a world of greater carbon and nitrogen inputs to forest soils, soil respiration will increase and reduce carbon stocks. Gaseous N losses are going to be more important in northern hardwood and mixed-oak forests while oak-pine forests will see an increased N leaching. High immobilization rates combined with moderate gaseous losses will result in high retention in cove hardwood forests.

In the ***fourth chapter*** I investigated the importance of the different processes in forest soil N cycling. I found gross transformation rates of nitrogen mineralization and nitrification to largely confirm findings from previously conducted studies on net mineralization and nitrification. However, the potential for gaseous losses to N_2 via denitrification or nitrifier denitrification are much greater than previously thought in non-wetland soils (Tiedje, 1988). In addition, in lower elevation sites with low nitrification rates and lower carbon availability (e.g. mixed-oak), DNRA can result in a significant retention of ecosystem nitrogen. Potential rates of nitrification and denitrification were generally greater in the higher elevation northern hardwood forest and the majority of activity occurred in the top 5 cm of mineral soil. Overall nitrogen mitigation capacity was found to be greatest in northern and cove hardwood forests based on

microbial immobilization rates. However, both pine-oak and mixed-oak forest types showed remarkable capacity to transform inorganic nitrogen into a N_2. The proposed mechanism is that denitrification dominates pine-oak N_2 production rates, while nitrifier denitrification is most important in mixed-oak forests with nitrifier denitrification rates generally being greater than denitrification. Overall, the results from Chapter 3 and 4 suggest that in a future world with greater carbon and nitrogen availability, forests of the southern Appalachian will either increase retention by greater immobilization and presumably growth (northern and cove hardwood) or increase nitrogen leaching and gaseous losses (pine-oak, mixed-oak and northern hardwood).

Chapter 5 aimed to elucidate the effect of land-use change on the dynamics of nitrogen cycling and greenhouse gas emissions on riparian soils. Riparian zones in agricultural areas can mitigate stream nitrogen pollution (Groffman *et al.*, 2000, Hefting *et al.*, 2003, Pinay *et al.*, 1993). Urban and suburban development can reduce and increase nitrogen retention, respectively (Groffman *et al.*, 2002, Groffman *et al.*, 2004). I found gross mineralization and nitrification rates to be substantially greater with agricultural land use compared to residential or forested areas. Denitrification rates, however, were similar in the different ecosystems. In addition, N_2O emissions, likely from the greater nitrifier denitrification, are much greater in agriculture than residential or forested ecosystems. CH_4 emissions are similar in residential and agricultural riparian ecosystems while forest systems oxidize significant amount of methane. With the large projected increase in residential land use at the expense of forested ecosystems, we can expect the southern Appalachian Mountains to become a less substantial CH_4 sink or even a CH_4 source over time. Over the next 40 years, the effects of land use change on greenhouse gas emissions are expected to be minimal. It is important to note that this hinges, for a large part, on the small projected decrease of the agricultural riparian areas.

In conclusion, this research has developed an approach to address nitrogen cycling heterogeneity and showed that the forests of the future will likely store less soil C and have greater N loss via leaching and gaseous losses. Further, this dissertation provided evidence of anaerobic processes in non-wetland soils and has shown that future increases in riparian greenhouse gas emissions from residential land use are likely to be mitigated by reduced agricultural land use.

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APPENDIX A

SUPPORTING INFORMATION CHAPTER 4

Table A1: Potential nitrification rates per depth horizon sampled. All units are in ng N g_{soil}^{-1} h^{-1} with N between parentheses. Error is indicated by standard error of the mean. Different letters indicate significant differences.

	Site	Nov-2010†	March-2011†	June-2011‡	Nov-2011‡	March-2012
Forest floor	OP	n/a	n/a	0.02 ± 0.02 (4)b	0.01 ± 0.01 (3)ab	0.03 ± 0.03 (3)b
	CH	n/a	n/a	0.00 ± 0.00 (5)b	0.00 ± 0.00 (3)b	0.05 ± 0.02 (3)b
	MO	n/a	n/a	0.02 ± 0.01 (7)b	0.01 ± 0.01 (6)b	0.01 ± 0.04 (6)b
	NH	n/a	n/a	0.44 ± 0.18 (4)a	0.43 ± 0.24 (4)a	1.22 ± 0.20 (3)a
0-5 cm	OP	-0.03 ± 0.02 (6)ab	0.01 ± 0.01 (3)b	n/a	n/a	n/a
	CH	-0.01 ± 0.01 (4)ab	-0.01 ± 0.01 (5)b	n/a	n/a	n/a
	MO	0.40 ± 0.24 (10)a	-0.01 ± 0.01 (8)b	n/a	n/a	n/a
	NH	-0.06 ± 0.03 (4)b	2.4 ± 0.77 (6)a	n/a	n/a	n/a
5-15 or 0-15 cm	OP	0.00 ± 0.00 (3)	0.00 ± 0§ (1)b	0.00 ± 0§ (1)	0.00 ± 0.00 (3)b	-0.01 ± 0.02 (3)b
	CH	0.00 ± 0.00 (3)	0.00 ± 0§ (1)b	0.01 ± 0§ (1)	0.00 ± 0.00 (3)b	0.00 ± 0.01 (3)b
	MO	0.15 ± 0.11 (5)	-0.01 ± 0.01 (6)b	0.00 ± 0§ (1)	0.00 ± 0.00 (6)b	-0.01 ± 0.01 (6)b
	NH	-0.02 ± 0.01 (2)	0.71 ± 0.26 (3)a	0.13 ± 0.11 (3)	0.29 ± 0.12 (4)a	0.28 ± 0.11 (3)a
Depth integrated	OP	0.00 ± 0.00 (5)	0.00 ± 0.00 (3)b	0.00 ± 0.00 (4)b	0.00 ± 0.00 (3)b	0.01 ± 0.01 (2)b
	CH	0.00 ± 0.00 (4)	0.00 ± 0.00 (5)b	0.01 ± 0.00 (5)b	0.00 ± 0.00 (3)b	0.00 ± 0.01 (3)b
	MO	0.18 ± 0.10 (8)	0.00 ± 0.00 (7)b	0.00 ± 0.00 (5)b	0.00 ± 0.00 (6)b	0.00 ± 0.00 (5)b
	NH	-0.02 ± 0.00 (3)	0.46 ± 0.19 (6)a	0.17 ± 0.07 (4)a	0.22 ± 0.11 (3)a	0.29 ± 0.11 (3)a

†0-5 cm mineral soil plus forest floor.
‡Cores were separated in forest floor and 0-15 cm mineral soil.
§No replicates available

Table A2: Potential denitrification rates per depth horizon sampled. All units are in ng N g_{soil}^{-1} h^{-1} with N between parentheses. Error is indicated by standard error of the mean. Different letters indicate significant differences.

	Site	Nov-2010†	March-2011†	June-2011‡	Nov-2011‡	March-2012‡
Forest floor	OP	n/a	n/a	80.8 ± 22.5 (6)ab	6.6 ± 3.7 (3)b	0.4 ± 10.7 (5)b
	CH	n/a	n/a	435.1 ± 218.11 (6)a	56.0 ± 27.9 (6)ab	140.5 ± 51.6 (10)a
	MO	n/a	n/a	39.0 ± 10.0 (12)b	23.2 ± 11.7 (6)b	8.3 ± 5.1 (10)b
	NH	n/a	n/a	466.1 ± 153.7 (6)a	264.7 ± 89.2 (4)a	569.6 ± 153.7 (5)a
0-5 cm	OP	8.7 ± 2.4 (6)b	6.9 ± 3.0 (6)c	21.2 ± 7.6 (6)bc	2.1 ± 0.9 (3)b	-2.6 ± 0.7 (5)b
	CH	27.7 ± 7.5 (6)b	119.5 ± 70.1 (6)b	128.7 ± 98.9 (6)b	20.3 ± 14.0 (3)b	47.3 ± 21.7 (5)b
	MO	8.1 ± 1.8 (10)b	8.6 ± 1.8 (12)c	9.0 ± 1.5 (12)c	5.0 ± 2.1 (6)b	9.9 ± 14.5 (10)b
	NH	374.2 ± 138.0 (4)a	1678.1 ± 490.2 (6)b	296.5 ± 82.0 (5)a	126.2 ± 51.6 (4)a	231.7 ± 67.0 (5)a
5-10 cm	OP	4.2 ± 1.1 (6)b	2.5 ± 0.5 (6)b	21.0 ± 9.9 (6)b	6.1 ± 4.1 (3)	-3.2 ± 0.1 (5)c
	CH	9.0 ± 3.4 (6)b	12.9 ± 5.0 (5)b	16.7 ± 8.6 (6)b	1.9 ± 0.8 (3)	16.0 ± 8.3 (5)b
	MO	3.9 ± 0.5 (10)b	5.3 ± 1.6 (12)b	5.1 ± 1.5 (12)b	-0.3 ± 0.4 (6)	1.2 ± 1.2 (10)bc
	NH	154.2 ± 53.5 (4)a	261.7 ± 50.6 (6)a	93.6 ± 37.6 (5)a	10.0 ± 7.1 (4)	60.2 ± 27.0 (5)a
10-15 cm	OP	2.2 ± 0.8 (6)b	1.8 ± 0.1 (6)b	3.7 ± 2.3 (6)	1.8 ± 1.4 (3)ab	-4.1 ± 0.8 (5)b
	CH	2.8 ± 1.1 (5)b	3.7 ± 0.9 (6)b	3.2 ± 0.9 (6)	8.3 ± 7.3 (3)ab	2.4 ± 6.5 (5)b
	MO	2.6 ± 0.5 (10)b	2.8 ± 0.7 (12)b	2.3 ± 1.0 (12)	-0.9 ± 2.4 (6)b	-0.8 ± 1.1 (10)b
	NH	24.5 ± 10.6 (4)a	23.2 ± 4.4 (6)a	12.7 ± 4.5 (5)	24.5 ± 10.5 (4)a	41.3 ± 28.1 (5)a
Depth integrated	OP	3.8 ± 0.6 (6)b	4.4 ± 1.0 (6)c	12.9 ± 3.9 (6)bc	3.0 ± 0.8 (3)	-3.4 ± 0.7 (5)c
	CH	7.3 ± 2.1 (6)b	23.9 ± 8.9 (5)b	41.7 ± 28.0 (6)b	10.0 ± 7.4 (3)	31.4 ± 9.1 (3)b
	MO	3.7 ± 0.5 (10)b	6.6 ± 3.8 (12)bc	5.1 ± 0.8 (12)c	1.0 ± 1.5 (6)	2.5 ± 1.3 (10)b
	NH	104.5 ± 39.2 (4)a	307.3 ± 117.7 (6)a	108.2 ± 29.4 (5)a	31.1 ± 12.4 (3)	97.9 ± 24.1 (5)a

†0-5 cm mineral soil plus forest floor.

‡Cores were separated in forest floor, 0-5, 5-10 and 10-15 cm mineral soil.

APPENDIX B

SUPPORTING INFORMATION CHAPTER 5

Table B1: Nitrogen cycling May 2012. Letters indicate significant differences ($p < 0.05$; N = 3). Min = N-mineralization; NTR = nitrification; DNRA = dissimilatory nitrate reduction to ammonium; immob = immobilization; Ag-hay = Ag with no cattle; Ag-low = Ag with low density cattle; Ag-high = Ag with high density cattle.

Type	**Gross Min**	**Net Min**	**Gross NTR**	**Net NTR**	**Gross DNRA**	**Gross DNF**	**Gross N_2 $^{15}NH_4^+$**	**NH_4^+ immob**	**NO_3^- immob**	**Gross N_2O $^{15}NO_3^-$ ($*10^{-3}$)**	**Gross N_2O $^{15}NH_4^+$ ($*10^{-5}$)†**
Forest 1	0.60 ± 0.03 **c**	0.66 ± 0.57	0.09 ± 0.04 **b**	0.01 ± 0.01	0.00 ± 0.00 **bc**	0.57 ± 0.18	0.24 ± 0.02 **c**	-0.60 ± 0.56	-0.63 ± 0.21 **b**	0 ± 0 **c**	0 ± 0 **b**
Forest 2	1.24 ± 0.47 **bc**	-0.20 ± 0.08	0.09 ± 0.09 **b**	0.24 ± 0.24	0.11 ± 0.03 **ab**	1.00 ± 0.29	0.84 ± 0.06 **bc**	0.37 ± 0.65	-0.73 ± 0.39 **b**	0 ± 0 **c**	2 ± 2 **ab**
Ag-hay	3.32 ± 0.50 **ab**	-0.62 ± 0.60	0.39 ± 0.23 **ab**	0.29 ± 0.08	0.21 ± 0.04 **a**	1.31 ± 0.16	2.86 ± 0.39 **ab**	0.69 ± 0.19	-1.47 ± 0.20 **b**	47 ± 27 **a**	108 ± 58 **a**
Ag-low	7.60 ± 0.66 **a**	-0.84 ± 0.26	3.64 ± 0.90 **a**	0.94 ± 0.03	-0.02 ± 0.02 **bc**	0.45 ± 0.13	3.68 ± 0.99 **a**	-4.67 ± 0.55	2.36 ± 0.83 **a**	1 ± 0 **bc**	77 ± 34 **a**
Ag-high	4.40 ± 1.50 **ab**	-0.93 ± 0.33	5.04 ± 0.72 **a**	0.30 ± 0.75	-0.08 ± 0.06 **c**	1.18 ± 0.10	0.87 ± 0.15 **bc**	-0.58 ± 1.82	3.64 ± 0.08 **a**	34 ± 26 **ab**	150 ± 130 **a**

Table B2: Potential nitrification and denitrification for mineral soil (0-15 cm). Letters indicate significant differences ($p < 0.05$), N is given between parentheses and the error term represents the standard error.

		pNTR ($\mu g\ N\ g_{soil}^{-1}\ d^{-1}$)	pDNF ($\mu g\ N\ g_{soil}^{-1}\ d^{-1}$)
May 2012	For	0.00 ± 0.00 (3) **b**	1.40 ± 1.35 (3) **b**
	Res	0.06 ± 0 (1) **b**	0.56 ± 0 (1) **b**
	Ag	0.20 ± 0.12 (3) **a**	11.22 ± 1.48 (3) **a**
July 2012	For	0.00 ± 0.00 (3)	0.31 ± 0.13 (3) **c**
	Res	0.03 ± 0 (1)	3.44 ± 0 (1) **b**
	Ag	0.18 ± 0.22 (3)	26.2 ± 11.66 (3) **a**
Nov 2012	For	0.00 ± 0.00 (3) **b**	0.14 ± 0.10 (3) **b**
	Res	0.02 ± 0.02 (2) **b**	0.78 ± 0.01 (2) **ab**
	Ag	0.75 ± 0.26 (3) **a**	16.4 ± 7.0 (3) **a**
March 2013	For	0.00 ± 0.00 (3) **b**	0.37 ± 0.32 (3) **b**
	Res	0.00 ± 0.01 (2) **b**	0.29 ± 0.02 (2) **ab**
	Ag	0.20 ± 0.14 (3) **a**	15.36 ± 6.30 (3) **a**
May 2013	For	0.00 ± 0.00 (2) **b**	0.03 ± 0.01 (2) **b**
	Res	0.00 ± 0.00 (2) **b**	0.18 ± 0.15 (2) **b**
	Ag	0.38 ± 0.19 (3) **a**	3.81 ± 1.51 (3)**a**

Table B3: Potential nitrification and denitrification for the forest floor. Letters indicate significant differences ($p < 0.05$), N is given between parentheses and the error term represents the standard error.

		pNTR ($\mu g\ N\ g_{soil}^{-1}\ d^{-1}$)	**pDNF ($\mu g\ N\ g_{soil}^{-1}\ d^{-1}$)**
May 2012	For	-0.03 ± 0.03 (3)	0.70 ± 0.32 (3)
	Res	0.79 ± 0 (1)	46.25 ± 0 (1)
July 2012	For	0.00 ± 0.01 (2)	0.83 ± 0.11 (3)
	Res	0.00 ± 0 (1)	0.31 ± 0 (1)
Nov 2012	For	0.01 ± 0.01 (3)	0.36 ± 0.12 (3)
	Res	N/A	N/A
March 2013	For	0.01 ± 0.00 (3)	0.04 ± 0.26 (3) **b**
	Res	0.07 ± 0.06 (2)	4.96 ± 4.01 (2) **a**
May 2013	For	0.01 ± 0.01 (3)	0.07 ± 0.07 (3)
	Res	-0.02 ± 0.02 (2)	0.09 ± 0.09 (2)