NITROGEN CYCLING IN THE NORTHERN HARDWOOD FOREST: SOIL, PLANT, AND ATMOSPHERIC PROCESSES

DISSERTATION

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By

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ABSTRACT

Nitrogen (N) is as important to forest ecosystem processes as water, sunlight, and carbon (C), and is often the mineral nutrient most strongly limiting plant growth (*NPP*) and microbial activity. In N-limited temperate forests, most of the N required for tree growth is internally recycled between soil and plant N pools through decomposition of organic detritus, or N-mineralization (N_{min}). However, within the last 50-100 years, humans have significantly altered the global N cycle, leading to increased atmospheric N inputs to forests. Fossil fuel combustion, fertilizer- and manure-intensive agriculture, and biomass burning all mobilize reactive N into the atmosphere, where it can be transported thousands of kilometers before being deposited to ecosystems. Decades of debate and research on N inputs to forest ecosystems have indicated that atmospheric N deposition (N_{dep}) may increase *NPP*. But, the temporal scales of forest process responses to N_{dep} are not satisfactorily understood, and depend on how atmospheric N inputs are partitioned between soils and plants.

For my dissertation research, I studied soil, plant, and atmospheric aspects of the N cycle in a regionally representative northern hardwood forest. Field measurements of soil and atmospheric N supply processes, and the N requirement of *NPP* (N_{req}) at the University of

Michigan Biological Station (UMBS) formed the foundation for my work. I tested field observations of N_{dep} and forest canopy retention of N deposition (N_{cr}) in a mesocosm experiment, using ^{15}N as a label to observe how N_{dep} and N_{cr} partitioned into plant and soil N pools. I also investigated the effects of this partitioning on tree seedling growth and physiology in a greenhouse study. The final element of my dissertation research was a meta-analysis of the effects of N addition on forest soil chemistry and N cycling.

From my field data collection, I estimated that forest *NPP* from 1999-2005 required approximately 51 kg N ha^{-1} yr^{-1}, most of which was used for fine root and leaf production (62% and 31%, respectively). On an annual basis, N_{min} supplied 87% of N_{req}, while N_{dep} contributed an additional 13%. Forest canopy retention of N_{dep} provided ≤4% of the forest's annual *NPP* N requirement. Data from my ^{15}N labelling experiment suggested that very little (<10%) of N_{cr} was actually incorporated into trees via foliar uptake, and that the majority of N_{dep} (>85%) was rapidly assimilated into soil N pools. These results suggested that N_{dep} could not have significantly increased forest *NPP* at UMBS over the time scale of my studies. My greenhouse experiment corroborated this conclusion, as there was no significant increase in photosynthesis or growth among tree seedlings exposed to N_{dep} at regional ambient rates. However, N_{dep} to forest ecosystems has been occurring for 50-100 years in industrialized regions, and most of the N inputs have been incorporated into soil organic matter (SOM). Research across temperate forests has suggested that forests exposed to large N inputs over time exhibit decreased soil C/N ratios, which are associated with faster N_{min} rates. Using meta-analysis, I verified this

pattern in the literature, and discovered novel relationships between forest soil properties and their responses to N inputs. My results demonstrated a long-term, quantitative relationship between N_{dep} and N_{min}, and suggest that NPP may increase in temperate forests affected by N_{dep}.

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FIELDS OF STUDY

Major Field: Evolution, Ecology, and Organismal Biology

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CHAPTER 1

INTRODUCTION TO FOREST N CYCLING

THE ROLE OF N IN TREE GROWTH AND FUNCTION

Trees require N to build and maintain the biochemical machinery necessary for photosynthesis, nutrient assimilation, protein synthesis, and reproduction. Tree tissues with the highest N concentrations are devoted to the most energy-intensive physiological processes. Green leaves have high N concentrations (1-3% by mass) because photosynthetic pigments and proteins are very rich in N (Reich et al, 1999). Fine roots (<2 mm diameter) also have relatively high N concentrations (0.5-2%) because of N investment in proteins and enzymes responsible for nutrient assimilation (Burton et al, 2002). Most wood is dead at functional maturity and performs transport, structure, and storage duties—processes which require virtually no N. The N concentration of bulk wood is therefore low (<0.2%). The presence of N in DNA and RNA means that trees require N for reproduction, and also protein synthesis, which is required for growth and tissue maintenance (Chapin, 1980).

Trees obtain most of their N through fine root uptake of inorganic N compounds, (NH_4^+ and NO_3^-). Root uptake of organic N compounds, such as amino acids, may play a minor role in the N nutrition of temperate forest trees (Raison et al, 1987). A significant fraction of the N entering roots first passes through mycorrhizal fungi, which engage in symbiosis with trees. In relatively infertile northern forests, NH_4^+ dominates the inorganic fraction of soil N, and is the principal form of N taken up by fine roots (Aber et al, 1989; Hart et al, 1994). Once inside, NH_4^+ is quickly assimilated into amino-N compounds via the glutamine synthetase/glutamate synthase enzymatic pathway (Miller and Cramer, 2005). This process is highly energy (C)-intensive, and this is the basis for the short lifetime (~2 yr) of fine roots. As a fine root grows into and exploits an N-rich microsite in the soil, it depletes the N available from that microsite. When the tree is no longer receiving an adequate N return on its C investment, C allocation ceases to the fine root, which subsequently dies (Burton et al, 2000). This is the process of fine root turnover, which is made doubly expensive by the fact that trees are unable to recover the N compounds built into the fine root before it dies and sloughs off (Gordon and Jackson, 2000). Fine roots are nutritionally and energetically expensive, but they are responsible for virtually all water and mineral acquisition. Larger coarse roots (>2 mm diameter) are longer-lived, have lower N concentrations, and are involved in anchorage and storage (Pregitzer et al, 1997; Pregitzer et al, 2002). Coarse roots also conduct water and minerals to the stem via xylem, which ultimately delivers these vital compounds to the canopy.

In the canopy, green leaves use N-rich proteins and pigments for photosynthesis—the process that provides the C necessary for a tree's growth and maintenance. As a leaf's lifetime progresses, its N concentration and photosynthetic capacity decline concurrently (Ellsworth and Reich 1992, 1993). By the time an average hardwood leaf falls in autumn, its N concentration is only 40-60% of its early summer green condition (Duchesne et al, 2001). This happens due to retranslocation, the process by which N is removed from a senescing leaf and transported through phloem into wood or roots for storage (Chapin and Kedrowski, 1983).

Trees sacrifice up to 25% of their N content every year through the loss of their most N-rich tissues: leaves and fine roots. Given the physiological imperative of acquiring N, and the inevitability of losing a significant fraction of it annually, it is no surprise that tree growth in most forests is N-limited. But, trees are not the only forest organisms that require N. Microbes residing in the soil also depend on N to maintain their physiological processes, and it is their activity that keeps the N cycle moving.

SOIL N POOLS AND PROCESSES

Over 90% of the N in a typical temperate forest resides in the soil, and a significant fraction of this is not actively involved in cycling at any given time (Bormann et al, 1977). In fact, most of the N in forest soil is in organic form, locked within plant/microbial detritus (or soil organic matter, SOM). Inorganic N is released from organic compounds through N-mineralization (N_{min}), which occurs as soil

microorganisms break down organic matter (Hart et al, 1994). Heterotrophic microbes respire C as an energy source, and also take up some of the N they have mineralized. Net N-mineralization therefore refers to the amount of NH_4^+ left in the soil after microbial immobilization has removed some of it. In more fertile forest soils, NH_4^+ can build up in the soil to sufficient levels that nitrification can occur. Most nitrifiers are bacteria that use chemoautotrophy to generate energy through the oxidation of NH_4^+ to NO_3^- (the process of nitrification), and are poor competitors for NH_4^+ (Vitousek et al, 1982). Nitrification is important precursor to ecosystem N export, because most N losses from forest soil are due to NO_3^- leaching, which occurs when this highly soluble anion is exported to surface- or groundwater through soil solution (Aber et al, 1995)

Mycorrhizae are another class of microorganisms involved in the soil N cycle, and they play a major role in forest nutrition. Mycorrhizae are fungi that colonize tree roots and enter into symbioses with their hosts, receiving fixed C (photosynthate) from the tree in return for N (and also phosphorous). It was long believed that mycorrhizae were only conduits of inorganic N from the soil into tree roots, but recent research indicates that some ectomycorrhizal fungi are as capable of breaking down organic detritus and mineralizing N as free-living, heterotrophic soil fungi (Chalot and Brun, 1998; Read and Perez-Moreno, 2003).

ATMOSPHERIC N DEPOSITION

Anthropogenic N additions to Earth's atmosphere are one aspect of humans' far-reaching impacts on global biogeochemical cycles (Galloway et al, 2003). Human activities such as agricultural operations, biomass burning, and fossil fuel combustion convert organic or diazo-N (N_2) into oxidized and reduced N compounds, which can travel through the atmosphere over distances as great as 2500 km (Irwin and Williams, 1988). Agricultural activities, such as feedlot operations and field application of anhydrous NH_3 are important pathways of NH_3 volatilization to the atmosphere (Krupa, 2003). In the atmosphere, NH_3 is hydrated to NH_4^+ and often binds to anions to form salts (such as $(NH_4)_2SO_4$). Biomass burning volatilizes NH_3 as well, but, like fossil fuel combustion, it also generates oxidized N compounds such as NO and NO_2. Oxidized N compounds are formed by thermal decomposition of N_2 in the presence of oxygen, and comprise the principal source of NO_3^- in wet deposition (Jacob, 1999). Oxidized N compounds are also subject to dry deposition in numerous forms. Atmospheric deposition also includes organic N compounds, which originate as biogenic molecules such as amino acids, or man-made chemicals such as organic nitrates (peroxyacetyl nitrate is one example; Neff et al, 2002a).

Atmospheric N deposition (N_{dep}) occurs in all phases, includes a huge number of chemical compounds, and covers vast areas of the northern hemisphere. Forests within several hundred kilometers (and downwind) of major industrial or agricultural areas are subject to the highest rates of deposition, which may supply over half of the amount of N

needed for growth every year (30-60 kg N ha^{-1} yr^{-1}; Emmett et al, 1998). The spatial extent of exceedingly high N deposition rates such as these is relatively small, but forests across extensive areas of eastern North America and western Europe receive 10-30% of their growth N requirements from N_{dep} every year (Lovett and Lindberg, 1993; Friedland, 1991). This is a major change from pre-industrial times, but the consequences of N deposition for forest ecosystems are still not completely understood.

ECOSYSTEM PARTITIONING AND IMPACTS OF N DEPOSITION

Atmospheric N deposition affects many ecosystem processes and properties. One of the first ecosystem responses to N_{dep} is increased vegetation N concentration (Aber et al, 1989). Vegetation eventually senesces, dies, and moves into the soil as detritus. If the detritus is similarly enriched in N, there may be effects on soil chemistry and N cycling (Fog, 1988). Chronic N_{dep} also leads to soil acidification, which hastens the loss of nutrients in soil solution (Vitousek et al, 1997) and interferes with microbial production of enzymes involved in decomposition (Carreiro et al, 2000), both of which further affect soil chemistry and nutrient cycling. If N_{dep} continues for a sufficient time, it can lead to N saturation—a state in which N supply exceeds forest N demand, leading to tree senescence and mortality, and N is exported from the system to groundwater, aquatic ecosystems, and the atmosphere (Aber et al, 1998). Nitrogen can be a limiting nutrient or an oversupplied, toxic liability within ecosystems; whether its addition to N-limited forests increases growth has long been debated.

Some studies have suggested that N_{dep} may increase forest net primary productivity (*NPP*; Marcos and Lancho, 2002; Sievering et al., 2000; Sievering et al, 2007). Establishing the validity of these results is very important, because increased forest *NPP* means that plants are removing more CO_2 from the atmosphere, which mitigates the greenhouse effect. It has been estimated that N_{dep} may increase annual *NPP* in North American forests by up to 15% (Lovett and Lindberg, 1993). However, findings based on ^{15}N redistributions demonstrate that, over the time span of 10-20 years, most N_{dep} moves into soils, and not trees (Nadelhoffer et al, 1999b). Other research suggests N partitioned into soil pools may remain there for decades before entering the actively cycling fraction of soil N (Currie et al, 2004). These findings suggest that atmospheric N inputs have no immediate effect on forest *NPP*.

Forest canopies often retain 50-70% of N_{dep} (Lindberg et al., 1983; Lovett and Lindberg, 1993; Morris et al., 2003; Tomaszewski et al., 2003), suggesting that foliar N uptake might allow trees immediate access to a significant fraction of N inputs to forests. If N retained by the canopy (N_{cr}) is subsequently absorbed by leaves, this could increase leaf N concentrations, and photosynthesis and *NPP* in turn. Estimates of this increase suggest N_{cr} may increase *NPP* by 10-100% higher in coniferous forests of the eastern United States due to N_{cr} (Sievering et al, 2000)

Research on N_{dep} and *NPP* at diverse sites in North America and Europe has generated diverse conclusions. The objectives of my dissertation work were to address several

issues related to N_{dep} and *NPP* within one regionally representative forest site. First, I quantified the amount of N needed for forest *NPP*, as well as the contributions of soil and atmospheric processes to that N requirement. Second, I measured trees' ability to take up atmospheric N inputs through their leaves, and through their roots. As part of a related experiment, I also measured trees' physiological and growth responses to N inputs. For my final project, I analyzed the literature for general patterns underlying temperate forest soil responses to N inputs.

CHAPTER 2

THE CONTRIBUTION OF ATMOSPHERIC DEPOSITION TO NET PRIMARY PRODUCTIVITY IN A NORTHERN HARDWOOD FOREST

ABSTRACT

Net primary productivity (*NPP*) in north-temperate forests is an important part of the global carbon (C) cycle, particularly since these forests are recovering C stocks lost during historic disturbance. Because *NPP* in temperate forests is N-limited, N inputs from atmospheric deposition N_{dep} may increase forest *NPP*. We developed an N budget for a regionally representative northern hardwood forest, in order to 1) quantify the rates of forest N supply by N_{dep}, canopy retention of N_{dep} (N_{cr}), and soil net N-mineralization (N_{min}), 2) measure the N requirements of the individual biomass components of *NPP*, including leaves, above- and belowground wood, and fine roots, and 3) calculate the fraction of *NPP* that could be attributed to atmospheric N inputs. Soil net N-mineralization supplied 44.3 kg N ha^{-1} yr^{-1} (87% of the *NPP* N requirement), while atmospheric N inputs (N_{dep}, N_{cr}) supplied 6.5 kg N ha^{-1} yr^{-1}, or 13% of the *NPP* N requirement. Of the 6.5 kg atmospheric N ha^{-1} yr^{-1} input to the site, 2.2 kg N ha^{-1} was retained by the canopy, suggesting that up to 4% of the annual *NPP* N requirement could

be met through canopy N uptake. Fine root (62%) and leaf (31%) N requirements dominated the *NPP* N requirement, which was 50.7 kg N ha^{-1} yr^{-1}. Our forest N supply and *NPP* N requirement estimates matched, suggesting that the annual 13% contribution of N_{dep} and N_{cr} may provide enough N to account for an additional year's worth of *NPP* every 7-8 years.

INTRODUCTION

Humans have significantly increased the amount of reactive nitrogen (N) in the atmosphere within the past 60-70 years, primarily through fossil fuel combustion, industrial N_2 fixation, and intensive agricultural operations (Galloway et al, 2003). These activities have increased atmospheric N deposition (N_{dep}) to terrestrial ecosystems, in which net primary productivity (*NPP*) is often N-limited (Vitousek et al, 1997). Global carbon (C) budget calculations have implicated a 'missing C sink' (Schimel, 1995), and north-temperate forest *NPP* is a component of this phenomenon (Goodale et al, 2002). However, the factors responsible for C storage in northern forests are not fully understood. Temperate forests across a significant area of the northeastern United States are recovering C lost during logging and fires that occurred 100-200 years ago (Goodale and Aber, 2001; Gough et al, 2007), and receive atmospheric N inputs of 5-27 kg N ha^{-1} yr^{-1} (Lovett and Lindberg, 1993). Atmospheric N deposition of this magnitude can increase in the C sink strength of forests in this region, in part through increased *NPP* (Magill et al, 2000; Pregitzer et al, in press).

There has been longstanding disagreement over the role of N_{dep} in forest N growth. Some studies suggest that N_{dep} can enhance *NPP* through canopy (Sievering et al., 2000) or root uptake (Marcos and Lancho, 2002). Lovett and Lindberg (1993) suggested that North American forest ecosystems could derive as much as 15% of their annual *NPP* N requirements from N_{dep}, and forest sites receiving N addition show increased wood production (Magill et al, 2000; Pregitzer et al, in press). However, a synthesis of ^{15}N tracer addition studies to forest floors in North America and Europe concluded that N deposition does not significantly affect forest growth, since soils, and not trees, are the primary sinks for tracer additions (Nadelhoffer et al, 1999b). Atmospheric N incorporated into stable soil N pools may remain immobilized there for years or even decades (Currie et al., 2004). However, N_{dep} assimilated into rapid-turnover soil N pools may re-enter the actively cycling soil N fraction relatively quickly and be available for plant uptake (Compton and Boone, 2002).

Canopy retention of atmospheric N (N_{cr}) may provide trees with immediate access to atmospheric N inputs that would otherwise be assimilated into soil N pools. Forest canopies throughout eastern North America retain N_{dep}, and if foliar assimilation of canopy-retained N occurs, this pathway may increase the amount of N available for *NPP*. Forest canopies typically do not retain organic N compounds, but they do retain 1 to 12 kg inorganic N ha^{-1} yr^{-1}, or 50-70% of N_{dep} (Lindberg et al., 1983; Lovett and Lindberg, 1993; Morris et al., 2003; Tomaszewski et al., 2003). If N_{cr} is coupled directly with *NPP*,

then ecosystem-scale stoichiometry suggests that N_{cr} rates measured at northeastern U.S. forest sites could increase C storage by 285-2950 kg C ha^{-1} yr^{-1} (Sievering et al., 2000).

Accurate predictions of C storage in north-temperate forests require complete accounting of forest growth, and the N supply processes that affect it. However, we are not aware of any study of a north-temperate forest that has integrated measurements of N supply by N_{cr}, N_{dep}, and soil net N-mineralization (N_{min}) with estimates of *NPP* and the *NPP* N requirement of different components of forest biomass. Studies of this type have been conducted in forests of the southern United States (Bonito et al, 2003; Finzi et al, 2002; Johnson et al, 2004), and others have measured most of the above C and N cycling processes at northern sites (Friedland et al, 1991; Sievering et al, 2000). Our study develops a more complete picture of N cycling in a well-studied, regionally representative northern hardwood forest, by quantifying soil N supply, plant N demand for different components of *NPP*, and atmospheric N inputs to the forest floor (N_{dep}) and canopy (N_{cr}). Our principal objectives with this study were to 1) quantify the rates of forest N supply by N_{dep}, N_{cr}, and N_{min}, 2) measure the N requirements of the individual biomass components of *NPP*, including leaves, above- and belowground wood, and fine roots, and 3) calculate the extent to which *NPP* could be subsidized by atmospheric N inputs.

MATERIALS AND METHODS

Study site

This study was conducted at the University of Michigan Biological Station (UMBS) in northern Lower Michigan, USA (45°35.5'N 84°43'W). The study area encompasses 105 ha of forest on a level to gently sloping high outwash plain derived from glacial drift. A permanent plot (1.1 ha) is situated in a representative stand at the center of the 105 ha study area, and smaller circular plots (0.1 ha, 60 total) are established along transects 1 km in length, which radiate out from the 1.1 ha plot. Soils at the site are excessively drained, sandy, mixed frigid Entic Haplorthods consisting of 92% sand, 7% silt and 1% clay. Mean annual temperature (1942-2003) is 5.5° C and mean annual precipitation is 817 mm.

Bigtooth aspen (*Populus grandidentata*) dominates the secondary successional mixed northern hardwood forest within the study area. Other canopy species include red maple (*Acer rubrum*), red oak (*Quercus rubra*), paper birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*), sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and white pine (*Pinus strobus*). The understory is dominated by red maple, red oak, juneberry (*Amelanchier* spp.), American beech, and white pine, while bracken fern (*Pteridium aquilinum*) and blueberry (*Vaccinium angustifolium*) are the most abundant ground flora. Canopy height is approximately 22 m, and dominant tree age averages 85 yr. The forest at the study site is typical of the northern Great Lakes region,

where aspen-dominated mixed hardwoods replaced the old-growth forests of white pine, red pine (*Pinus resinosa*) and eastern hemlock (*Tsuga canadensis*) following clearcutting and wildfires in the late 19th and early 20th centuries (Gough et al., 2007).

Environmental parameters

We measured soil temperature (T_s) and soil moisture (W_s) continuously to determine the influence of these factors on N_{min}. Soil temperature at 7.5 cm depth was measured with three type E thermocouples arrayed within the central 1.1 ha study plot. Four probes (Campbell CS616, Campbell Scientific, Logan, UT, USA) deployed within the 1.1 ha plot measured volumetric soil moisture to 30 cm depth.

Forest N supply

We determined the quantity of N available for *NPP* by measuring N_{min}, N_{dep}, and N_{cr}. Soil net N-mineralization was measured in 2005-2006; N_{dep} and N_{cr} data were drawn from direct measurements at the UMBS forest in 2004, as well as archived data from the National Atmospheric Deposition Program (NADP, 2000-2004).

Net N-mineralization

Soil net N-mineralization was measured using an *in situ* core/ion exchange resin technique (ISC/IER; Brye et al., 2002). Previous work at the site established a gradient in aboveground *NPP* across the 0.1 ha permanent plots (Gough at al., 2007), so we sampled 20 plots along the gradient to capture landscape-level variation in aboveground *NPP* and

N_{min}. We sampled N_{min} five times over 1 yr using open-topped, 5 cm diameter PVC tubes as corers/incubators. Incubation periods ranged from 30-45 d (May-October) to 196 d (October-May). On each sampling date, one soil core from each plot was removed to a depth of 25 cm (including O_e, O_a, A, E, and B horizons) for determination of initial [NH_4-N] and [NO_3-N]. An adjacent core was taken to a depth of 30 cm, and the bottom 5 cm of soil was removed and replaced with a nylon pack of Dowex Monosphere UPW mixed-bed ion exchange resin (Dow Chemical, Midland, MI, USA) held in the bottom of the tube by wire mesh. The tube was then replaced in the soil to incubate. In the lab, initial and incubated soil cores were weighed, passed through a 2 mm sieve, and 10 g of sieved soil were extracted in 50 mL of 2M KCl overnight. Ion exchange resins were also extracted in 2M KCl. Gravimetric soil moisture and bulk density were determined for each soil core, and filtered KCl extracts were analyzed on a Bran Luebbe autoanalyzer by the phenate (NH_4^+) and cadmium reduction (NO_3^-) methods (standard methods 350.1 and 353.2; US EPA, 1983). Soil and ion exchange resin N concentrations in KCl extracts (mg N L^{-1}) were scaled to a land area basis (kg N ha^{-1}) using soil bulk density and incubation tube area. Within each incubation period, mean N_{min} was the difference between final and initial [NH_4-N] and [NO_3-N], averaged across all 20 cores, and the standard error was computed to quantify across-plot variation. To determine cumulative annual N_{min}, we summed mean N_{min} values from each of the 5 incubation periods, and we summed the within-period standard errors to estimate the cumulative uncertainty of our repeated plot-level measurements.

Atmospheric N deposition and canopy N retention

We quantified two types of N_{dep}: 1) wet inorganic N deposition (N_{wd}, kg N ha^{-1} yr^{-1}), measured at UMBS by the NADP since 1979, and 2) bulk N deposition (wet + dry deposition; N_{bd}, kg N ha^{-1}), which we measured from April to November 2004. Both types of N_{dep} were collected in an open field ~2 km east of the 1.1 ha plot.

The NADP samples N_{wd} with an electronically controlled collector, which opens automatically when precipitation is detected and closes when precipitation stops. The collector is a high-density polyethylene (HDPE) bucket mounted on a platform ~2 m above the ground. Precipitation is removed from the collector every week and sent on ice to the National Trends Network Central Analytical Laboratory (Champaign, IL, USA), where $[NH_4^+]$ is determined by flow injection analysis, and $[NO_3^-]$ is measured by ion chromatography (Rothert et al., 2002). These data are available from the NADP website (http://nadp.sws.uiuc.edu). We retrieved 5 yrs (2000-2004) of weekly precipitation volume, $[NH_4^+]$, and $[NO_3^-]$ data from the NADP website, and aggregated weekly data into intervals that matched our five N_{min} incubation periods from 2005-2006. Within each interval, we calculated mean N_{wd} across all 5 yrs of data. We also calculated the standard error of N_{wd} within each interval across the 5 yrs of data to quantify interannual variation in N_{wd}. We summed the 5 yr mean N_{wd} values from each of the 5 periods to determine mean annual N_{wd}, and summed the standard errors to calculate the cumulative uncertainty of our measurements.

We sampled N_{bd} (wet + dry deposition) at weeklong intervals in one continuously open collector adjacent to the NADP collector in the open field. The collector consisted of a 10 cm diameter HDPE funnel inserted into a rubber stopper in the neck of a 1 L, narrow-mouthed HDPE bottle. The collector was mounted ~1 m above the ground, and was removed and replaced with a clean collector every week. Upon collection, N_{bd} samples were frozen until the end of each sampling period (2-4 wk), composited into a volume-weighted aggregate sample, filtered (0.45 μm), and analyzed for NH_4-N, NO_3-N, and bulk organic N concentrations on a BranLuebbe autoanalyzer. Ammonium-N and NO_3-N concentrations were determined by the phenate and cadmium reduction methods, and organic N was calculated as the difference between total N (persulfate oxidation method, EPA 351.2) and summed inorganic N (US EPA, 1983). Within each sampling period, concentrations were scaled to fluxes on an area basis (kg N ha^{-1}) using precipitation volume and collector area. Logistical constraints precluded wintertime collection of N_{bd}, and since we did not replicate N_{bd} collection during other years, we chose to use N_{wd} as our principal measure of annual N_{dep}. Bulk N deposition data from our 7-mo (April-November) collection period were used for 1) a conservative estimate of the contribution of organic N deposition to annual N_{dep}, and 2) for calculating N_{cr}.

We measured N_{cr} by collecting canopy throughfall concurrently with N_{bd}. Canopy throughfall collectors (20 total) were located at random, fixed points within the 1.1 ha plot. We chose the 1.1 ha plot based on logistical considerations, and also because the plot was representative of the study area as a whole in terms of overstory composition,

leaf area index (LAI), and site productivity. Collector design, collection frequency, and analysis methods for canopy throughfall samples followed N_{bd} sampling protocol. Canopy N retention was calculated for each collector within each 2-4 wk sampling period as the difference between N_{bd} and throughfall N fluxes. Within each sampling period, we averaged N_{cr} across all 20 collectors and computed the standard error of the mean to estimate spatial variation. We summed N_{cr} from the individual sampling periods over the 7 mo period of collection to determine cumulative N_{cr}, and summed the standard errors to calculate the uncertainty of cumulative N_{cr}.

Forest N requirements

We used ecosystem-scale stoichiometry to calculate the N requirement of annual forest *NPP*, by multiplying production rates (kg ha^{-1} yr^{-1}) with tissue %N for leaves, above- and belowground wood, and fine roots. Biomass production rates were measured in the 1.1 ha and 30, 0.1 ha permanent plots at the UMBS forest from 1999-2005, and tissue chemistry ([C] and [N], g kg^{-1}) was measured on a Perkin-Elmer CHN analyzer for each biomass N pool.

Leaves

Leaf litterfall (kg ha^{-1} yr^{-1}) was estimated from 50, 0.26 m^2 litter baskets within 31 permanent plots. Leaf litter was sorted by species, oven dried, and weighed to determine species-specific annual leaf litterfall. Leaf litter chemistry was measured for 7-18 samples per species over 3 years of leaf litterfall collection (total n=77). Mean annual

leaf litterfall was determined by averaging leaf litterfall across all plots and years. Since we averaged leaf litterfall across space and time, the standard error of mean annual leaf litterfall encapsulates both spatial and temporal variation. Nitrogen retranslocated from senescing leaves is stored in perennial tissues and can be used for the subsequent year's leaf production (Chapin et al., 1990), so we set the quantity of N lost in leaf litterfall equal to the annual leaf N requirement. We calculated annual leaf N requirements for bigtooth aspen, red maple, and red oak, which together account for ~80% of canopy leaf area; all other species were pooled. The mean annual leaf N requirement (N_{lf}, kg N ha^{-1} yr^{-1}) was estimated as the product of mean annual leaf litterfall and leaf litter [N]. The standard error of the mean annual leaf N requirement was calculated by summing the mean-weighted standard errors of leaf litterfall and leaf litter [N].

Wood

We estimated above- and belowground wood production by calculating the annual incremental increases in standing wood mass. We used allometric regression models to quantify the standing crop of aboveground wood mass for >700 trees (≥10 cm dbh) in 61 plots (Gough et al., 2007). Belowground wood mass was calculated from aboveground wood mass according to a regression model from Cairns et al. (1997). Wood production (kg ha^{-1} yr^{-1}) was the annual change in wood mass. Aboveground wood at the UMBS forest has similar [N] across species, and its chemical composition was treated as the across-species mean of sapwood from 59 tree cores collected in 2000. Belowground

wood [N] was determined from coarse root (>2 mm diameter) samples extracted from 4, 60 cm soil cores. The mean and standard error of the annual wood N requirement (N_w) were calculated according to the same techniques described above for N_{lf}.

Fine roots

Fine root turnover (*FRT*) was estimated from 1999-2005 by expanding on results reported for our site by Gough et al. (2007). Those authors derived three independent estimates of *FRT* from site-specific and published relationships between *FRT* and several other parameters. The first of the three methods was a site-specific regression model relating mean annual soil temperature to *FRT,* as determined from cohort analyses of minirhizotron images ($FRT = 1.13 + 3.5T_s$; $r^2 = 0.47$; $P < 0.001$). The second method was a published empirical model (Aber et al., 1985) predicting *FRT* from available soil N ($FRT = 0.789 - 0.0191N_{avail} + 0.000211N_{avail}^2$; $r^2 = 0.95$; $P < 0.01$). The final estimate of *FRT* was based on the mass balance approach of Raich and Nadelhoffer (1989):

$$FRT = [R_h - (M_{ll} + M_{wd})] / M_{fr} \quad (1)$$

This model assumes a steady state of soil organic matter, so that annual heterotrophic respiration, R_h, is equivalent to annual detritus inputs, which consist of fine roots (M_{fr}), leaf litter (M_{ll}), and woody debris (M_{wd}). For each year (1999-2005), we averaged *FRT* across all three methods, and calculated annual fine root litter production (kg ha^{-1} yr^{-1}) as the product of mean *FRT* and the standing crop of fine root mass, which was determined

from 30, 80 cm deep soil cores. We then averaged across all 7 yr to estimate mean annual fine root litter production. The standard error was calculated from the sample of n=7 annual fine root litter production values. Fine root chemistry was analyzed for roots <2 mm diameter extracted from 4, 60 cm deep soil cores, and the annual fine root N requirement (N_{fr}) was assumed to be equal to the annual N losses in fine root litter production, as trees are unable to recover N from senescing fine roots (Gordon and Jackson, 2000).

RESULTS

Soil temperature and W_s throughout the period of N_{min} measurement (2005-2006) were representative of typical conditions at our site, remaining within the 95% confidence intervals of 1999-2005 daily means (Figure 2.1). During 7-yrs, T_s peaked at 18.6 to 21.6 °C, between day of year (DOY) 183 and 250. Across years, W_s exhibited a gradually declining trend throughout the growing season, followed by an increase during autumn and a return to stable, high levels during the winter and early spring.

Net N-mineralization rates varied seasonally, with higher rates during the growing season than during the non-growing season (Figure 2.2). Daily N_{min} was 0.25-0.31 kg N ha^{-1} d^{-1} from May to September 2005, but fell to 0.03-0.10 kg N ha^{-1} d^{-1}during the period from September 2005 to May 2006. While the categorical differences between growing and non-growing season N_{min} rates were significantly different (ANOVA, $p<.0001$), we did not detect significant linear relationships between T_s or W_s and N_{min} (regression, NS). We

did not observe net nitrification during any of the *in situ* incubation periods. The rate of N_{wd} was stable but relatively low compared to N_{min}. Wet inorganic N deposition occurred at a rate of 0.01 kg N ha^{-1} d^{-1}.

Over the seven-month period of measurement, bulk N deposition to the UMBS forest occurred at a consistent rate of 0.03 kg N ha^{-1} d^{-1}. Ammonium-N was the most abundant compound in N_{bd}, accounting for 44% of the total, while NO_3-N and organic N were 29% and 27% of total N_{bd}, respectively (Figure 2.3). The forest canopy retained 41% of bulk-deposited N, 61% of which was NH_4-N. Organic N compounds were 28% of total N_{cr}; only 1% of N_{cr} was NO_3-N. Canopy retention of bulk-deposited N occurred at a rate of less than 0.01 kg N ha^{-1} d^{-1}.

Net primary productivity at the UMBS forest averaged 12300 kg ha^{-1} yr^{-1}, with an N requirement of 50.7 kg N ha^{-1} yr^{-1} (Table 2.1). Fine root production was the biggest single component of *NPP*, accounting for 42% of the total. Aboveground wood production was 29% of total *NPP*, followed by leaves (21%). Fine roots and leaf litter shared a high N concentration (6 g kg^{-1}), as well as high production rates. Accordingly, fine roots and leaves were the two largest shares in the annual forest N requirement (62% and 31% of the total, respectively). Bigtooth aspen, the dominant tree species at the site, had the highest annual leaf litterfall (29% of total), leaf litter N concentration (8 g kg^{-1}), and leaf N requirement of all species. Red maple had the second-highest leaf N requirement, with relatively high litterfall (27% of total), but lower leaf litter N

concentration (5 g kg^{-1}). In spite of high annual production, wood was only 7% of the annual forest *NPP* N requirement because of low tissue N content. Nitrogen supply by N-mineralization, N_{dep}, and N_{cr} matched exactly the annual *NPP* N requirement at the UMBS forest, suggesting that the UMBS forest N requirement may be met entirely by these three sources (Figure 2.4). Individually, N_{min} supplied 87% of the annual *NPP* N requirement, followed by N_{dep} (8.5%) and N_{cr} (4%).

DISCUSSION

The UMBS forest *NPP* N requirement (50.7 kg ha^{-1} yr^{-1}) is similar to values reported for other north-temperate forest sites. An aspen-maple site in Wisconsin had a vegetation N uptake rate of 59 kg N ha^{-1} yr^{-1} (Pastor and Bockheim, 1984), and other hardwood stands in Wisconsin had N requirements of 90-140 kg N ha^{-1} yr^{-1} (Nadelhoffer et al, 1985). A high-elevation spruce-fir-birch forest in New York had an N requirement of 53.6 kg N ha^{-1} yr^{-1} (Friedland et al, 1991). At these northern forest sites, net atmospheric inputs to the forest floor (after accounting for soil solution losses) were 5-12 kg N ha^{-1} yr^{-1}, which represented 6-22% of their stand-level *NPP* N requirements. At UMBS, the input of 6.5 kg N ha^{-1} yr^{-1} (combined N_{dep} and N_{cr}) represents 13% of the *NPP* N requirement—a significant fraction that equates to an additional year's worth of *NPP* every 7-8 years.

Most of the atmospheric N inputs to the UMBS forest originated from N_{wd}. At 3.6 kg N ha^{-1} yr^{-1}, N_{wd} is in the low-to-median range of deposition compared to other sites throughout the eastern United States (Lovett, 1994; NADP, 2006). Total N_{dep}, which also

includes wet organic N deposition, and dry deposition of organic and inorganic N compounds, is undoubtedly higher than N_{wd} alone. Our estimate of N_{dep} (4.3 kg N ha^{-1} yr^{-1}) probably is conservative, since it includes only 7 mo of organic N deposition collections, and does not include dry deposition, which contributes an additional 1-2 kg N ha^{-1} yr^{-1} to forests in this region (Baumgardner et al., 2002).

Atmospheric N inputs to the UMBS forest also occurred through N_{cr}, principally in the form of NH_4-N and organic N, originating in N_{bd}. Canopy NH_4-N retention has been documented across forests throughout the eastern United States (Lindberg et al., 1983; Lovett and Lindberg, 1993), while organic N typically is not retained by forest canopies (Piirainen et al., 1998; Tomaszewski et al., 2003; Morris et al., 2003). Many forest canopies retain NO_3-N; the lack of canopy NO_3-N retention at our site may be due to washoff of dry deposition (Lovett, 1994). The lack of canopy NO_3-N retention also may result from a lack of foliar nitrate reductase in the dominant tree species present at the UMBS forest. Downs et al. (1993) demonstrated that the presence of NH_4-N in soils inhibited leaf-level induction of nitrate reductase in red maple and white pine. At our study site, where NH_4-N is present in the soil and NO_3-N is absent, trees may not have the physiological capacity for canopy-level assimilation of atmospheric NO_3-N.

It is likely that some of the 6.5 kg ha^{-1} yr^{-1} of atmospheric N deposited at our study site has no immediate, direct effects on *NPP* by the dominant trees. Unmeasured quantities of N_{cr} may be assimilated by epiphythic microbes or lichens (Houle et al, 1999; Morris et

al, 2003), emitted back to the atmosphere (Harrison and Kitto, 1994) or washed off to the forest floor before foliar assimilation (Lovett, 1994). In addition, SOM is a tremendously important sink for inorganic N inputs to the forest floor (Buchmann, 1996; Currie et al, 2004), so immobilization of some N_{dep} in soil pools is likely. Over time, however, a significant fraction of SOM breaks down, and the N immobilized within it is released into extractable (plant-available) N pools (Compton and Boone, 2002). Accurate measurements of N turnover rates within forest SOM pools are therefore very important for understanding how quickly atmospheric N inputs become available for plant uptake.

Soil net N-mineralization was the principal N supply process at the UMBS forest, generating 44.3 kg N ha^{-1} yr^{-1}. This rate is lower than most of the N_{min} rates reported for other mature, second-growth forests of the Upper Great Lakes region (25-105 kg N ha^{-1} yr^{-1}; Nadelhoffer et al., 1985; Reich at al., 1997; White et al, 2004). Daily N_{min} rates at UMBS were three- to ten-fold greater during the growing season than in the non-growing season, which probably reflects the positive impact of T_s on N_{min} (Zak et al., 1999). However, other factors, such as seasonality of C substrate quality or microbial population dynamics, may be responsible for seasonal variation in N_{min} rates (Scott and Binkley, 1997; Edmonds et al., 1998; Vogt et al., 1981).

In this study, we assumed that N_{min} measured over the course of one year was an accurate estimate of plant-available soil N supply. However, *in situ* soil net N-mineralization may not be the most appropriate measure of plant-available soil N in certain systems,

particularly those where organic N uptake or mycorrhizal associations play a significant role in plant N acquisition (Schimel and Bennett, 2004). These mechanisms have been documented in other northern hardwood forests (Finzi and Berthrong, 2005; Fahey and Hughes, 1994; Fahey et al., 2005), and if they are significant at our study site, we may have underestimated soil N availability. Different methods are also known to influence N_{min} rates (Binkley et al., 1986), which may be the reason for the discrepancy between our measurements and others at UMBS (11 kg N ha^{-1} yr^{-1}, Curtis et al, 2002; ~25 kg N ha^{-1} yr^{-1}, White et al, 2004). Although we measured N_{min} for only one year, interannual variation in the climatic drivers of microbial activity probably did not influence the rate of soil N supply during *in situ* incubations. Soil microbial activity changes with T_s and W_s (Curtis et al., 2005; Hobbie, 1996; Liski et al., 2003; Raich and Schlesinger, 1992), and the consistency of these environmental parameters in 2005-2006 relative to other years suggests that our N_{min} estimate is representative of typical conditions.

Net primary productivity at the UMBS forest (12300 kg ha^{-1}) is comparable to other northern hardwood forests in the Upper Great Lakes region. Studies in Wisconsin have estimated *NPP* at 8020-12500 kg ha^{-1} for aspen-dominated forests, 9300-13300 kg ha^{-1} for maples and mixed hardwoods, and 15000-17000 kg ha^{-1} for oaks (Ahl et al., 2004; Nadelhoffer et al., 1985; Pastor and Bockheim, 1984). At our site, fine root and leaf production accounted for over 60% of *NPP*, and over 90% of the *NPP* N requirement. The large N requirement of these two biomass components is due to their high N concentration, itself a consequence of the metabolic demands of resource acquisition

(Burton et al., 2002; Reich et al, 1999). Wood was a minor sink for N because of its low N concentration. Some studies have estimated C storage increases due to N_{dep} based upon ecosystem stoichiometry. This approach assumes that tree C/N ratios remain unchanged with N_{dep}, and that for each unit of atmospheric N assimilated, there is a corresponding production increase in wood (Magnani et al, 2007) or total aboveground biomass (Sievering et al, 2000). Our measurements indicate that stand-level N allocation favors fine roots and leaves, both of which have much lower C/N than wood or total aboveground biomass. Nitrogen limitation theory predicts that trees in N-limited north-temperate forests would first allocate N inputs to leaves and fine roots, as these tissues are actively involved in acquiring resources used for further C fixation and growth (Chapin, 1980). Increased wood production could only be an indirect consequence of N_{dep}, because increased wood growth depends on prior N allocation to resource-acquiring, low C/N tissues. Therefore, C storage increases in north-temperate forests due to N_{dep} probably are lower than current published estimates based on ecosystem stoichiometry.

Uncertainty in our *NPP* and *NPP* N requirement calculations is driven primarily by our ability to accurately estimate fine root production. Fine root production was the biggest single component of *NPP*, and the *NPP* N requirement, and as such exerts substantial leverage on our N budget. We quantified fine root litter production by measuring *FRT* with three independent methods, because doing so allows us to constrain our estimate of fine root litter production with greater confidence. The three methods exhibited good

agreement, with a mean coefficient of variation (across all years and methods) of 28%. The respiration mass balance approach (Eq. (1); Raich and Nadelhoffer, 1993) was consistently higher than the other two methods, and was responsible for nearly all of the between-method variation. However, this method is an upper-bound estimate of total belowground *NPP* (Clark et al., 2001), so overestimating *FRT* relative to the other two methods is expected.

Conclusion

The immediate impacts of N_{dep} on *NPP* may be limited somewhat by the immobilizing activity of SOM, but significant anthropogenic N inputs to the atmosphere have been increasing the N capital of this ecosystem for half a century (Galloway et al, 2003). Increased rates of soil N cycling (N_{min}) frequently have been observed under chronic, elevated N inputs (Gundersen et al, 1998; Magill et al, 2000), and the positive relationship between N_{min} and *NPP* (Reich et al, 1997) suggests that faster N_{min} rates resulting from N_{dep} are likely to contribute to productivity increases in northern forests. Based on our ecosystem-scale work, it appears likely that some of the *NPP* observed in this forest can be attributed to atmospheric N inputs.

Tissue	ΔM (kg ha^{-1} yr^{-1})	C content (%C)	ΔC (kg ha^{-1} yr^{-1})	C/N	N content (%)	N_{req} (kg ha^{-1} yr^{-1})
Leaf litter[a]						
Pogr	777 (96.6, 31)	50.2 (0.21, 7)	390 (50.1, 31)	68 (3.0, 7)	0.8 (0.03, 7)	6.2 (0.81, 31)
Acru	701 (70.2, 31)	48.0 (0.25, 14)	336 (35.4, 31)	93 (5.8, 14)	0.5 (0.03, 14)	3.5 (0..41, 31)
Quru	335 (62.3, 31)	48.8 (0.31, 18)	164 (31.4, 31)	69 (1.9, 18)	0.7 (0.02, 18)	2.3 (0.44, 31)
Other	829 (106.9, 31)	49.3 (.03, 38)	409 (52.9, 31)	86 (4.0, 38)	0.6 (0.03, 38)	5.0 (0..69, 31)
Leaf litter total	2640 (107.0, 31)	49.2 (0.17, 77)	1300 (44.1, 31)	81 (3.9, 77)	0.6 (0.03, 77)	15.8 (1.43, 31)
Aboveground wood	3530 (126.9, 61)	48.7 (0.25, 59)	1720 (70.6, 31)	1203 (160.2, 59)	0.05 (0.006, 59)	1.8 (0.28, 31)
Fine root	5210 (55, 7)	46.7 (0.80, 4)	2430 (67, 7)	77 (7.8, 4)	0.6 (0.05, 4)	31.3 (2.94, 7)
Belowground wood	896 (32.2, 61)	46.9 (0.27, 4)	420 (17.5, 31)	198 (18.4, 4)	0.2 (0.02, 4)	1.8 (0.24, 31)
Total	12300 (321, 7)	--	5870 (199, 7)	--	--	50.7 (4.89, 7)

[a] Leaf litter species codes are: Pogr- *P. grandidentata,* Acru- *A. rubrum,* Quru- *Q. rubra*, and Other- all others.

Table 2.1. Mass production (ΔM), C production (ΔC), tissue chemistry, and N requirements (N_{req}) of forest growth at UMBS. Annual leaf ΔC and N_{req} were calculated from leaf litterfall ΔM and tissue chemistry. Fine root ΔC and N_{req} were determined from annual fine root litterfall and live fine root tissue chemistry. Above- and belowground wood ΔC and N_{req} were calculated using ΔM and tissue chemistry for sapwood and coarse roots, respectively. All values are expressed as mean (SE, n).

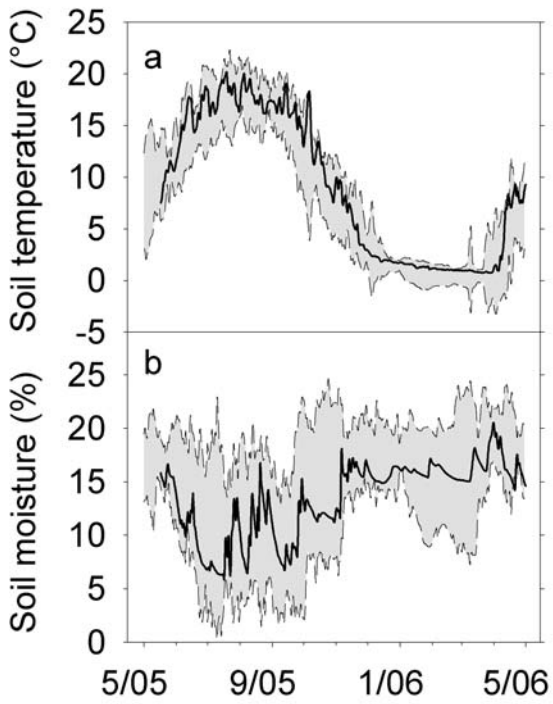

Figure 2.1. Soil temperature (A, 7.5 cm depth) and soil moisture (B, 0-30 cm) in the 1.1 ha study plot. Daily averages in 2005-2006 (solid lines) are compared to the 95% confidence intervals (CI) from measurements in 1999-2005 (shaded areas).

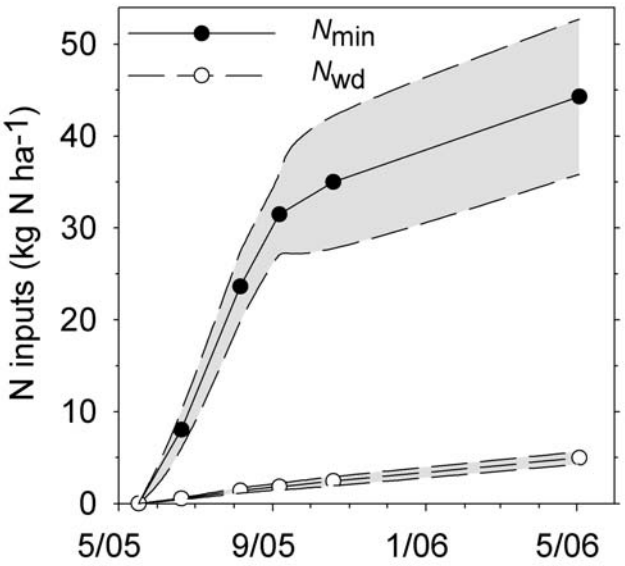

Figure 2.2. Cumulative supply of inorganic N by soil net N-mineralization (N_{min}, closed symbols) and wet deposition (N_{wd}, open symbols). Shaded areas indicate cumulative standard errors of N supply functions.

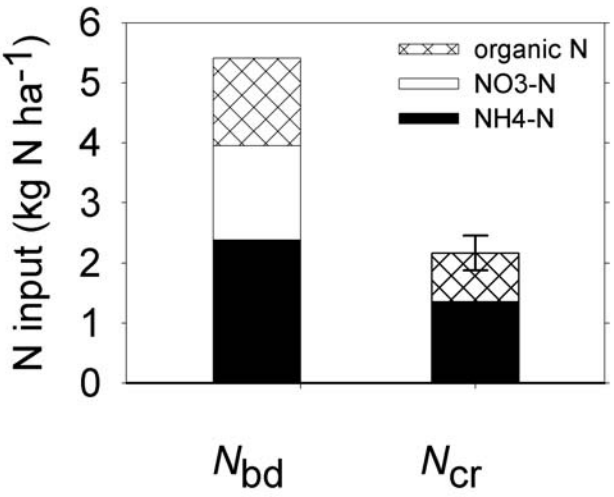

Figure 2.3. Nitrogen inputs to a northern hardwood forest from bulk deposition (N_{bd}) and canopy N retention (N_{cr}, ±SE) April-November 2004. Vertical bars on the left indicate the quantity of organic N, NH_4-N, and NO_3-N deposited to the forest canopy by N_{bd}. Vertical bars on the right show the amount of N_{bd} retained by the canopy.

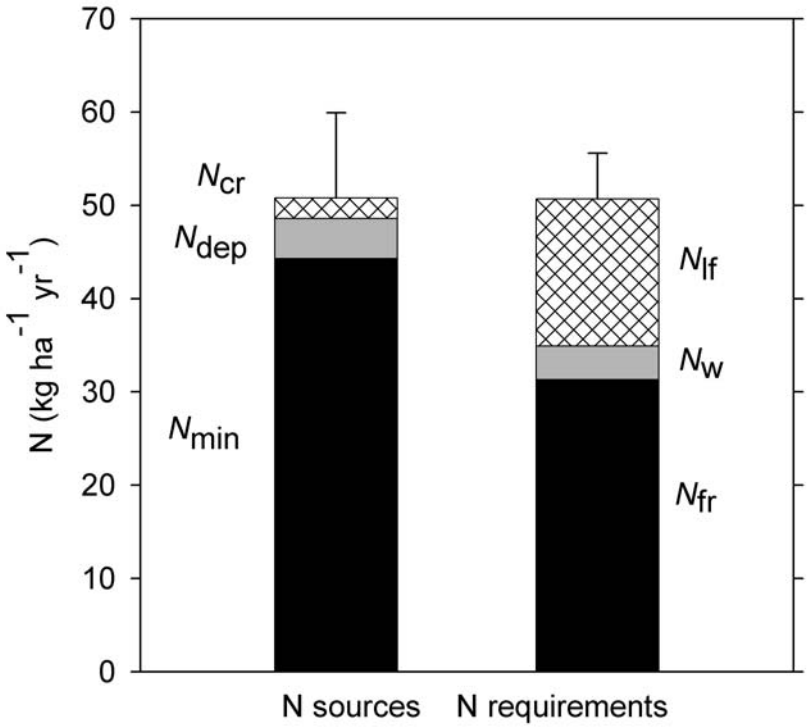

Figure 2.4. Nitrogen sources and requirements for the UMBS forest. Soil net N-mineralization (N_{min}) supplied 44.3 kg N ha^{-1} yr^{-1}, while atmospheric N deposition (N_{dep}) and canopy retention (N_{cr}) supplied an additional 6.5 kg N ha^{-1} yr^{-1}. The forest N requirement of 50.7 kg N ha^{-1} yr^{-1} was dominated by leaf (N_{lf}) and fine root (N_{fr}) N requirements; the wood N requirement (N_w) was small. Error bars represent the summed standard errors of individual N sources and requirements.

CHAPTER 3

FOLIAR AND ROOT UPTAKE OF ^{15}N-LABELED WET DEPOSITION IN FOREST MESOCOSMS

ABSTRACT

Atmospheric N deposition (N_{dep}) increases the supply of nitrogen (N) to north-temperate forest ecosystems, where net primary productivity (*NPP*) is often N-limited. In many forests, the canopy retains 50-70% of N_{dep}, suggesting that trees may access atmospheric N inputs through foliar uptake. The potential for this N uptake pathway to occur is very important, because ^{15}N additions to forest floors demonstrate that soil pools immobilize most N inputs before roots can access them. We conducted a replicated, controlled field experiment using mesocosms to quantify foliar and root uptake of N_{dep} independently, as well as the distribution of N_{dep} within forest soil. We also measured biomass and leaf gas exchange parameters within one growing season for greenhouse seedlings subjected to N_{dep} at rates similar to those observed at a nearby forest site. On average, trees in the mesocosm study were able to take up 10% of the N applied to their canopies, and 14% of the N applied to the forest floor beneath them. Up to 29% of the ^{15}N recovered in *Pinus strobus* biomass was derived from foliar uptake, while *Populus tremuloides* seedlings did

not acquire more than 2% of their biomass ^{15}N content through foliar uptake. Tracer recovery in soil pools was typically >85%, and was a linear function of soil organic matter concentration. Greenhouse seedlings exhibited no significant changes in biomass, N concentration, or leaf gas exchange in response to foliar or soil N addition. Together, the results of these two experiments demonstrate that, over the short term, N_{dep} probably does not change photosynthesis, biomass chemistry or production at similar forest sites.

INTRODUCTION

Human alteration of the global nitrogen (N) cycle has increased the rate of atmospheric N deposition (N_{dep}) to forests, where N usually is the mineral nutrient that most severely limits net primary productivity (*NPP*; Galloway et al, 2003). Tree uptake of N_{dep} may increase forest *NPP* and carbon (C) storage, but decades of research have not completely resolved this issue. In general, ^{15}N tracer studies indicate relatively little root ^{15}N uptake following forest floor label additions (Buchmann et al, 1996; Perakis and Hedin, 2001), suggesting that there is little opportunity for immediate *NPP* responses to N_{dep}. Alternatively, some ecosystem-scale research suggests that canopy retention of N deposition (N_{cr}) can increase plant access to atmospheric N through foliar uptake (Sievering et al, 2000; Tomaszewski et al, 2003).

Forest floor ^{15}N additions reported in the literature demonstrate that soils are the principal sinks for N_{dep} within forests (Micks et al, 2004; Zogg et al, 2000). In a review of ^{15}N additions to forest floors in Europe and North America, tracer recoveries in soil pools

averaged 70%, with tree biomass assimilating 20% of the ^{15}N added (Nadelhoffer, 1999). Most of the ^{15}N recovered in soil is thought to be immobilized in soil organic matter (SOM), which can retain N inputs for timescales of less than one year to many decades, depending on its degree of stabilization. Nitrogen initially incorporated into labile SOM pools may rapidly cycle into the plant-available fraction of soil N (Compton and Boone, 2002; Neff et al, 2002b), while N moving into recalcitrant SOM may remain immobilized there for decades or longer, remaining unavailable for plant uptake and of little consequence to forest *NPP* (Currie et al, 2004).

While trees are unable to access some of the N_{dep} intercepted by forest soils, their ability to retain atmospheric N within their canopies opens the possibility for an alternate N nutrition pathway. Across a variety of forest types, N_{cr} frequently has been measured at up to 50-70% of N_{dep} (Lindberg et al., 1983; Morris et al., 2003; Pirainnen et al, 1998). If the efficiency of foliar N uptake is significant, trees may have immediate access to a substantial fraction of N_{dep}, and this might lead to increased *NPP*. In the context of forest *NPP* N requirements, it has been suggested that canopy N uptake could supply 10-20% of forests' annual N requirements (Lovett and Lindberg, 1993), and be responsible for 10-100% increases in annual forest C storage (Sievering et al., 2000).

Experimental N additions to forests often lead to increased foliar [N], demonstrating that leaves are an important sink for N incorporated into biomass (Aber et al, 1995, Magill et al, 2000; Nasholm, 1994). Given the positive relationship between leaf [N] and

photosynthesis (Reich et al, 1997), the observed increase in leaf [N] suggests that N inputs may increase CO_2 assimilation, and possibly *NPP*. However, while leaf [N] responses to N deposition are fairly consistent, photosynthesis and growth responses are not, and span the gradient of changes from positive, to negative, to none at all (Bauer et al, 2004; Crabtree and Bazzaz, 1993; Gough et al, 2004; Pearson and Soares, 1998). The consequences of N deposition on photosynthesis and growth may depend on the form and quantity of N added, as well as the N status of the ecosystem (Aber and Driscoll, 1997; Magill et al, 1997).

We are aware of only one experiment that has quantified tree uptake of atmospheric N by both root and foliar pathways (Lumme, 1993). Leaf ^{15}N application studies have demonstrated foliar N uptake, but they have largely have been conducted on conifers in greenhouses (e.g, Macklon et al., 1996; Wilson and Tiley, 1998; Boyce et al., 1996; Chavez-Aguilar et al., 2006). Root N uptake into forest biomass mostly has been measured in stand-level ^{15}N addition experiments (Nadelhoffer et al, 2004; Tietema et al, 1998), but precise resolution of ^{15}N distribution within this experimental framework can be complicated by spatial heterogeneity. The primary objective of this experiment was to quantify N uptake by roots and leaves of trees exposed to ^{15}N-labelled N_{dep} under tightly controlled field conditions. Additional goals were to: 1) quantify N retention within soil pools, 2) determine whether tree physiological parameters exhibited short-term responses

to regionally representative N_{dep} rates, and 3) assess the implications of ^{15}N partitioning into soils and biomass for *NPP* and the N budget of a northern hardwood forest (Nave et al, in preparation).

MATERIALS AND METHODS

Study site

This experiment was conducted at the University of Michigan Biological Station (UMBS), in northern lower Michigan, USA (45°35'N 83°42'W). Mean annual temperature (1942-2003) at UMBS is 5.5 C, and mean annual precipitation is 817 mm. Soils at UMBS are excessively drained, sandy, mixed frigid Entic Haplorthods consisting of 92% sand, 7% silt and 1% clay. Forests at the study site are aspen-dominated mixed hardwoods, which replaced the old-growth forests of white pine (*Pinus strobus*), red pine (*Pinus resinosa*) and eastern hemlock (*Tsuga canadensis*) following clearcutting and wildfires in the late 19th and early 20th centuries. The most common species in the upland forests of the area are aspens (*Populus grandidentata* and *P. tremuloides*), red oak (*Quercus rubra*), red maple (*Acer rubrum*), paper birch (*Betula papyrifera*), and white pine (Gough et al., 2007).

Mesocosm ^{15}N experiment

The mesocosm ^{15}N-labelling experiment was designed to trace the movement of atmospheric N deposited in a single, simulated precipitation event through forest soil and biomass N pools. The experiment was conducted on an array of 60 mesocosms arranged

in a 250 m^2 grid in an open field. Each mesocosm consisted of a 72 L plastic tub buried level to its top, with a drain in the bottom. Soil profiles within the mesocosms were similar to local forest soil, which was harvested and homogenized by horizon for use in mesocosm construction. Organic horizon (homogenized O_i and O_e) depths in mesocosm soil profiles were 2-3 cm, upper mineral soil layers (homogenized O_a, A, and E) were 10-12 cm, and B horizons were ~20 cm deep. Commercially grown seedlings of *P. tremuloides* and *P. strobus* were planted in the mesocosms in September 2004 and May 2005, respectively. *Populus tremuloides* were one-year-old seedlings of aspen parkland provenance, and *P. strobus* were 3-0 nursery grown seedlings of Great Lakes provenance. The trees were watered until their root systems established, and again during dry periods in July and August 2005.

Each mesocosm was randomly assigned to one of three treatments: control (no label addition), soil ^{15}N application, or foliar ^{15}N application, so that the movement of ^{15}N into tree biomass could be assessed for root- and foliar-uptake pathways independently. The ^{15}N was delivered as an aqueous $^{15}NH_4Cl$ solution (98 atom% ^{15}N) at a concentration of 1.5 mg N l^{-1}, which is approximately three times the average concentration of NH_4-N in rain at the site (Nave, unpublished data). Each soil-treated mesocosm received 1 L ^{15}N solution, which was uniformly and directly applied to the soil surface with a watering can, and spray bottles were used to saturate the canopies of seedlings in the foliar-treated mesocosms with a known volume of ^{15}N solution. Each foliar-treated seedling had a funnel sealed around the base of its stem to capture throughfall, and we calculated the

mass of ^{15}N applied to each tree from difference between initial and throughfall solution volumes. We labeled all treated mesocosms on 8 July 2005, and four dry days elapsed before the canopies of foliar-treated mesocosms were washed, with dropcloths in place, to prevent soil contamination by residual ^{15}N on leaf surfaces. We determined that four days was an adequate time period for foliar uptake based on our measurements of greenhouse-grown seedlings that showed no significant increases in foliar ^{15}N between leaves harvested 1, 2, and 4 days following label application

We harvested mesocosms 10 days, 1 month, and 4 months after label application. At each harvest, trees were clipped at ground level and separated into leaf and aboveground wood components for each species. Next, organic horizons were removed, and each mesocosm was inverted onto a sieve table (6 mm hardware mesh) for hand-separation of roots from the mineral soil. Mineral soil was thoroughly homogenized and then subsampled, and coarse (>2 mm) and fine roots (<2 mm) of both tree species were separated after being washed to remove organic matter and mineral soil. We also collected and froze soil solution samples every 2 wk throughout the duration of the experiment, with passive solution collectors attached to the undersides of 6 mesocosms (2 per treatment). At the end of the experiment, soil solution samples were composited into volume-weighted aggregate samples and extracted with Dowex Monosphere UPW mixed-bed ion exchange resin (Dow Chemical, Midland, MI, USA). Ion exchange resins, biomass, and organic and mineral soil samples were oven-dried, weighed, ground, and subsampled for %N and ^{15}N analysis on a Costech ECS-4010 CHN analyzer and a ThermoFinnigan Delta^{Plus} XL

isotope ratio mass spectrometer at UMBS. Organic horizons from harvest three were not analyzed. After analyzing O-horizons from harvests one and two, we passed all O-horizon samples through a 2 mm sieve to separate the litter and humus/topsoil fractions of the O horizon samples. These pools were then measured separately for %N and ^{15}N content. We measured soil organic matter (SOM) content by loss on ignition (LOI) at 550˚ C until combusted samples had reached constant mass.

Greenhouse N-addition experiment

The greenhouse N-addition experiment was conducted to examine the effects of low-level N deposition on tree seedling physiology and biomass, and to validate the results of the mesocosm ^{15}N experiment. *Populus tremuloides* and *P. strobus* seedlings used for the greenhouse experiment were of the same age and provenance as those from mesocosms the previous year. Each seedling was planted in May 2006 in an 11 L pot, using homogenized soil (O_a, A, E) left over from mesocosm construction as the planting medium. Trees of both species were fully leafed out by early June, and the seedlings were allowed to establish their root systems before N additions began on 31 July. The seedlings were arranged in a randomized block design, with 5 replicates of each species per treatment (control, foliar N addition, and soil N addition). We applied NH_4Cl in aqueous solution five times between 31 July and 24 August, delivering mean cumulative N additions of 4.0 kg N ha^{-1} and 1.7 kg N ha^{-1} for soil- and foliar-treated seedlings, respectively. At UMBS, bulk NH_4–N deposition and canopy retention of atmospheric

NH_4–N deposition are 2.4 and 1.3 kg N ha^{-1} yr^{-1}, respectively (Nave et al., in preparation). Nitrogen application methods in the greenhouse were the same as those used for the mesocosm ^{15}N experiment.

We measured leaf gas exchange parameters once before N application (in late July), and subsequently three more times before seedlings were destructively harvested for biomass measurements in early October. We used a LI-COR 6400 portable photosynthesis system (LI-COR, Lincoln, NE USA) to measure leaf gas exchange on all 30 seedlings. Chamber conditions were held constant at 1500 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), 380 µmol mol^{-1} [CO_2], 25 °C temperature, and > 65% humidity. For each *P. tremuloides* seedling, we measured gas exchange on the youngest fully expanded leaf, while *P. strobus* measurements were made on excised fascicles immediately following detachment. We used the equation published by Ginn et al. (1991) to determine fascicle leaf area, since several physiological parameters were expressed on an area basis. On all seedlings, we recorded light-saturated photosynthesis (A_{sat}, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mmol m^{-2} s^{-1}) and intercellular [CO_2] (C_i, µmol mol^{-1}).

Data analysis

We used isotope mass balance equations from Nadelhoffer and Fry (1994) to quantify ^{15}N partitioning within each N pool sampled from the mesocosm ^{15}N experiment. For biomass N pools, which had relatively small and non-constant mass over the duration of the experiment, the equation takes the form:

(1) $^{15}N_{mass} = (N_s) * [(atm\%^{15}N_s - atm\%^{15}N_c) / (atm\%^{15}N_l - atm\%^{15}N_c)]$

Where $^{15}N_{mass}$ is the mass of ^{15}N in the sample derived from the label, and N_s is the total N mass of the labeled sample. $Atm\%^{15}N_s$ and $atm\%^{15}N_c$ are the isotope signatures of the labeled and control (unlabeled) samples, and $atm\%^{15}N_l$ is the isotope signature of the label. For soil N pools, which had constant mass that was very large relative to the quantity of ^{15}N added, the mass balance equation is as follows:

(2) $^{15}N_{mass} = (N_c) * [(atm\%^{15}N_s - atm\%^{15}N_c) / (atm\%^{15}N_l - atm\%^{15}N_s)]$

Where N_c is the total N mass of the control sample.

After checking parametric assumptions and performing transformations as necessary, we used the general linear model (GLM) function in MINITAB with Tukey post-hoc comparisons to identify significant differences in response parameters. For seasonal gas exchange parameters, we used the repeated measures GLM function in SAS. For all analyses, we set $p<.10$ as the *a priori* confidence level for accepting test results as statistically significant.

RESULTS

The small quantity of ^{15}N tracer added to mesocosms had no effect on soil N concentrations or pool sizes, but it did result in significant changes in the ^{15}N-enrichment of soil N pools (Table 3.1). Soil isotope signatures and ^{15}N recoveries were not significantly different between control and foliar-treated mesocosms, but soil-treated mesocosms had significantly higher $\delta^{15}N$ and ^{15}N recovery than both the control and foliar treatments. More ^{15}N was recovered in the litter/humus/topsoil fraction of soil (50% of added ^{15}N) than in the E/B horizon mineral soil (38%). Fifty-five percent of the ^{15}N recovered in the litter/humus/topsoil fraction was in the litter layer, which was by mass ~55% of the litter/humus/topsoil N pool. Soil organic matter content explained 79% of the variation in ^{15}N recovery per unit soil mass among litter/humus/topsoil and mineral soil samples (Figure 3.1). Recovery rates were 0.37-2.48 ug ^{15}N g^{-1} in litter/humus/topsoil samples and 2-3 orders of magnitude lower for mineral soils.

Recovery of ^{15}N in plant biomass averaged 10-15% of the amount applied for foliar and soil treatments. By mass, most (71-99%) of the ^{15}N recovered in tree biomass was assimilated by root uptake (Table 3.2). Foliar N uptake was relatively more important for *P. strobus* than for *P. tremuloides*, at least in terms of ^{15}N assimilation into leaves, wood, and coarse roots. In these three biomass pools, 12-29% of the ^{15}N derived from label was incorporated via foliar uptake. The absolute quantity of ^{15}N taken up by leaves was not significantly different between species, but within the soil treatment, *P. tremuloides* took

up significantly more ^{15}N than *P. strobus* (215.7 vs. 22.2 ug ^{15}N seedling^{-1}). Altogether, the mass of ^{15}N recovered in biomass (average: 244 μg ^{15}N mesocosm^{-1}) was significantly less than soil N pools, which typically was >1200 μg ^{15}N mesocosm^{-1}.

Tree seedlings exhibited variable patterns of within-plant ^{15}N allocation. Green leaves were more enriched in ^{15}N than other tissues, but there was no difference in the proportion of ^{15}N allocated to leaves between foliar- and soil-treated seedlings of either species (Figure 3.2). For *P. strobus*, wood was a significantly larger sink for ^{15}N taken up by leaves (32-45% across harvests) than for ^{15}N taken up by roots (8-14%). For both species, the percentage of ^{15}N taken up by fine roots and remaining within them was greater than the percentage of foliar-assimilated ^{15}N allocated to fine roots. There was a strong seasonal trend in the partitioning of ^{15}N among biomass pools for *P. tremuloides*, as leaf ^{15}N was retranslocated into wood and coarse roots prior to leaf abscission.

In the greenhouse experiment, inorganic N additions had no significant effects on leaf-level gas exchange parameters for either species. Across the time-series of observations, mean A_{sat} rates were consistently higher for fertilized seedlings than unfertilized controls of both species, though the difference was never significant (Figure 3.3). Other physiological parameters, including g_s, C_i, and seedling biomass and N concentrations, were not significantly different between treatment groups for either species.

DISCUSSION

The ^{15}N added to mesocosms showed limited movement into plant biomass following both soil and foliar application. Trees assimilated ca. 10% of the label applied to their canopies, suggesting that actual canopy uptake is significantly less than N_{cr} measured in the field at UMBS (1.3 kg N ha^{-1} yr^{-1} for NH_4–N; Nave et al, in preparation). On average, mesocosms had lower leaf area index (LAI) than the nearby forest site (2.4 vs. 3.5 m^2 m^{-2}), so their canopies retained a smaller quantity of N relative to the amount that was deposited to the forest floor than at the forest site. However, we detected no relationship between mesocosm leaf area and ^{15}N recovery in biomass within the foliar treatment (regression, $p>.10$), suggesting that the low leaf area of our mesocosms did not bias our results towards underestimating the importance of N_{cr}. Furthermore, the low efficiency of foliar uptake (10%) indicates that, even if a greater proportion of N_{dep} had been retained by mesocosm canopies, overall N movement into leaves would have remained quite low.

Mesocosm trees were able to access more of the N deposited to the soil beneath them than to their leaves. However, root uptake efficiency of N deposited to soil (~14%) was not much higher than foliar uptake efficiency—soil pools clearly were more important sinks for ^{15}N additions than biomass. Most of the ^{15}N added in the soil treatment remained in the O-horizon, instead of moving into mineral soil. This probably was a consequence of abiotic NH_4^+ immobilization in SOM (Aber et al, 1998; Johnson et al, 2000), which was present in greater concentrations in O-horizon samples than in mineral

soils. The higher [SOM] of O-horizon material indicates that a greater proportion of its total mass was involved in N retention than in mineral soil, and ^{15}N recovery was correspondingly higher.

Because we added tracer to leaves and soils independently, we were able to assess the relative contributions of foliar and root N uptake of N_{dep} to tree N nutrition. In all cases, trees acquired more of the ^{15}N recovered in their tissues by root uptake of soil-deposited N. The only instances of foliar N uptake contributing substantially to tissue ^{15}N content were observed in *P. strobus* seedlings, which acquired, on average, 14% of their leaf ^{15}N, 29% of their wood ^{15}N, and 12% of their coarse root ^{15}N from foliar N uptake. *Populus tremuloides* seedlings were far more effective at removing N from the soil, exhibiting root-derived ^{15}N recoveries nearly 10 times greater than those observed for *P. strobus*. This likely was a consequence of different growth rates of the two species—the change in average *P. tremuloides* seedling biomass between harvests 1 and 3 was over 300%, while *P. strobus* seedling biomass increased by an average of 50% over the same interval.

Seedlings of both species allocated most of the ^{15}N they took up to leaves, which is congruent with field studies that show increased foliar [N] with N addition (Aber et al, 1995, Magill et al, 2000; Naesholm, 1994). Allocation of N to leaves may reflect the importance of N-rich pigments, enzymes, and other compounds to photosynthesis (Reich et al, 1997). However, it is possible that relative N allocation to leaves of the magnitude observed in these seedlings (40-60% of assimilated ^{15}N) is an artifact of ontogeny, as

exponential increases in leaf area are common for young trees, but not observed in mature forests (Norby et al, 1999). Fine roots generally were more enriched in ^{15}N than coarse roots, a pattern that reflects the N cost of high metabolism and respiration in fine roots (Burton et al, 2002). An exception to this pattern did occur, though, when ^{15}N recovery was higher in coarse than fine roots of *P. tremuloides* at harvest 3, reflecting N retranslocation from leaves prior to abcission. Nitrogen resorption from senescing leaves also enriched the ^{15}N content of *P. tremuloides* wood at this time. For the most part, patterns of within-plant ^{15}N allocation were proportionally similar for foliar- and root uptake pathways. *Pinus strobus* seedlings departed somewhat from this trend, exhibiting spatially conservative partitioning that tended to keep N taken up by leaves in aboveground biomass, while N taken up by roots remained belowground. It is possible, though, that the relatively high quantity of ^{15}N recovered in wood of foliar-treated *P. strobus* was not due to redistribution of N from foliar uptake, but rather more efficient N uptake by twigs than leaves. In coniferous trees, bark may be less of a barrier to NH_4^+ uptake by diffusion than the needles, which have waxy cuticles that limit ionic diffusion (Wilson, 1992).

There were no detectable treatment effects of N_{dep} on biomass, N concentrations, or leaf-level gas exchange for seedlings of either species in the greenhouse. Mesocosm data suggest that seedlings assimilated into their leaves <10% of the N supplied to their roots, which was ~25 mg N tree^{-1} in the greenhouse study. Greenhouse seedlings, which were of the same size and provenance as those from the mesocosms, had 100-300 mg of total leaf

N, and the assimilation of ~2.5 mg N into a pool two orders of magnitude larger was not sufficiently large to induce a change in photosynthesis. If mesocosm seedlings are representative of the nearby UMBS forest site, their 10% root uptake efficiency suggests that stand-level annual atmospheric NH_4-N uptake is <1 kg N ha^{-1} yr^{-1} at the forest site (Nave et al, in preparation). At most, this amount to 3% of growing season canopy N content, suggesting that the atmospheric NH_4-N deposited to forests in this region has no short-term effect on canopy photosynthesis. It should be noted, however, that roots in the mature forest have over ten times the biomass density (~13000 vs. 1000 kg ha^{-1}) as in the mesocosms, and withdraw nearly three times as much N from similar soil each year (51 vs. ~18 kg N ha^{-1} yr^{-1}; see Nave et al, in preparation, for biomass N uptake calculations). Therefore, while mature tree roots at the forest site have lower N uptake per unit root mass, their more complete colonization of the soil may allow them to take up a greater proportion of N inputs than observed for seedlings in the mesocosms.

The patterns of ^{15}N distribution in mesocosms showed that trees were a much smaller short-term sink for N inputs than soils. Growth and physiological parameters of greenhouse seedlings were unaltered by N_{dep} treatments, which is in accordance with the results of the mesocosm experiment. Taken together, these experiments suggest that N_{dep} does not have any significant, short-term effects on forest biomass chemistry or productivity at UMBS, or at similar forest sites. Future research that quantifies the residence time of N inputs within the soil pools that initially retain them will be important for understanding the long-term effects of N_{dep} on forest growth.

Pool	Treatment	[N] ($g\ kg^{-1}$)	Pool size ($g\ N\ mesocosm^{-1}$)	$\delta^{15}N$ (‰)	^{15}N recovery ($\mu g\ ^{15}N\ mesocosm^{-1}$)
Litter	CTRL	7.5 (0.8, 6)	2.0 (0.33, 4)	-2.3 (0.51, 5)	-0.2 (2.39, 4)
Litter	SOIL	8.0 (0.3, 12)	1.6 (0.13, 12)	40.8 (2.14, 12)***†	393.0 (19.32, 12)***
Litter	LEAF	--	--	--	--
Humus/topsoil	CTRL	1.5 (0.2, 3)	0.7 (0.17, 3)	-0.8 (0.15, 3)	0.0 (0.39, 3)
Humus/topsoil	SOIL	1.8 (0.2, 12)	0.8 (0.22, 12)	48.5 (2.81, 12)***	211.4 (12.00, 12)***
Humus/topsoil	LEAF	--	--	--	
Litter/ humus/ topsoil	CTRL	5.7 (0.6, 12)	3.2 (0.35, 12)	-1.8 (0.19, 12)	0.0 (2.86, 12)
Litter/ humus/ topsoil	SOIL	5.9 (0.4, 13)	3.4 (0.39, 13)	44.6 (2.29, 12)***	703.1 (34.65, 12)***
Litter/ humus/ topsoil	LEAF	5.9 (0.4, 13)	3.4 (0.32, 15)	-1.7 (0.17, 13)	1.8 (2.36, 13)
E/B mineral soil	CTRL	0.33 (0.03, 9)	20.8 (1.66, 9)	3.0 (0.24, 9)	-3.4 (16.93, 9)
E/B mineral soil	SOIL	0.39 (0.02, 16)	23.9 (1.09, 16)	9.4 (0.56, 14)***	536.0 (23.43, 14)***
E/B mineral soil	LEAF	0.36 (0.03, 8)	22.6 (2.06, 8)	3.2 (0.24, 8)	21.1 (23.00, 8)

†Asterisks denote significant differences between treatments within a pool; ***p<.001, **p<.05, *p<.10

Table 3.1. Concentrations, masses, and isotope signatures of N in soil pools over 4 months following label addition. Pulse-labeling rates averaged 1400 and 62 μg ^{15}N per mesocosm for soil and leaf applications, respectively. Litter, humus, and topsoil were sampled 10 days and 1 month after label application; E/B mineral soils were sampled 10 days, 1 month, and 4 months after label application. Values are means, with standard errors and sample sizes in parentheses.

^{15}N recovery					ANOVA			
Species	Pool	Soil treatment	Leaf treatment	% from foliar uptake	Term	df	MS	*F*
Populus tremuloides	Green leaves	124.1 (28.48)	2.0 (0.62)	2	Treatment	1	685.6	461.7***
	Wood	46.1 (8.29)	0.7 (0.13)	2	Species	1	58.2	39.2***
	Coarse roots	22.1 (5.03)	0.2 (0.11)	1	Pool	3	37.7	25.4***
	Fine roots	23.4 (4.36)	0.3 (0.12)	1	T x S	1	84.3	56.7***
					T x P	3	4.2	2.8**
Pinus strobus	Green leaves	9.5 (2.71)	1.6 (0.37)	14	S x P	3	5.1	3.5**
	Wood	2.7 (0.63)	1.1 (0.27)	29	T x S x P	3	3.2	2.2*
	Coarse roots	1.5 (0.25)	0.2 (0.04)	12	Error	278	1.5	
	Fine roots	8.5 (1.3)	0.4 (0.10)	4				
Total	Biomass	237.9 (51.05)	6.5 (1.76)	3				

Table 3.2. Recovery of ^{15}N in tree seedling biomass pools over 4 months following label addition, by treatment and species. Values are mean recoveries over 3 harvests, expressed in μg ^{15}N seedling^{-1}. The standard error is in parentheses, with n=20 seedlings of each species. Also displayed is the per cent of seedling biomass ^{15}N derived from foliar uptake. The ANOVA table shows significance of terms in ln-transformed ^{15}N recoveries (see Table 1 for p-value definitions).

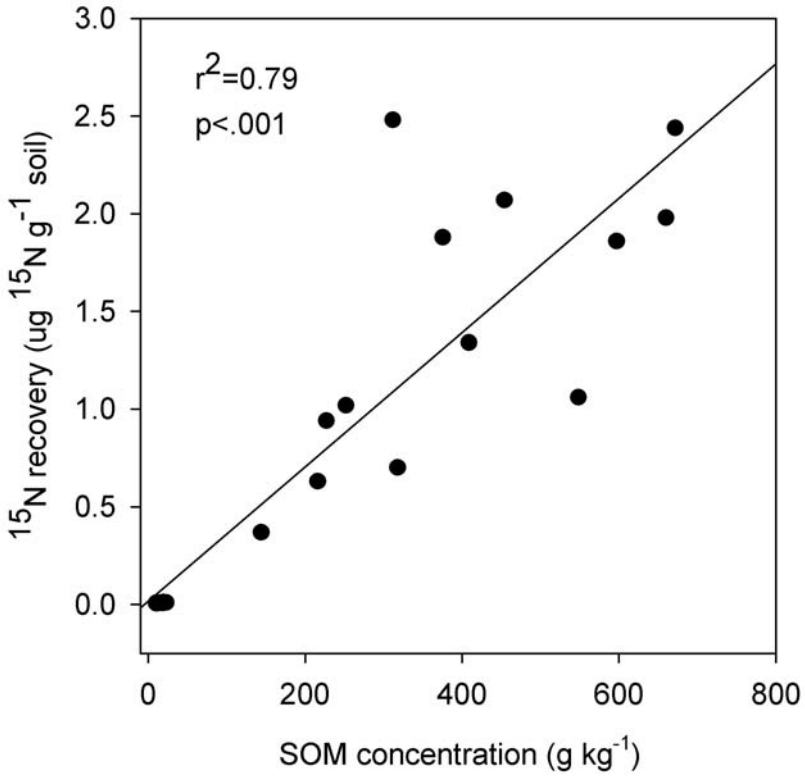

Figure 3.1. Relationship between soil organic matter content (g kg^{-1}) and ^{15}N recovery per unit soil mass, including mineral and litter/humus/topsoil fractions. Mineral soil samples had [SOM] of 10-23 g kg^{-1}; [SOM] values of 140 g kg^{-1} and greater correspond to litter/humus/topsoil fractions.

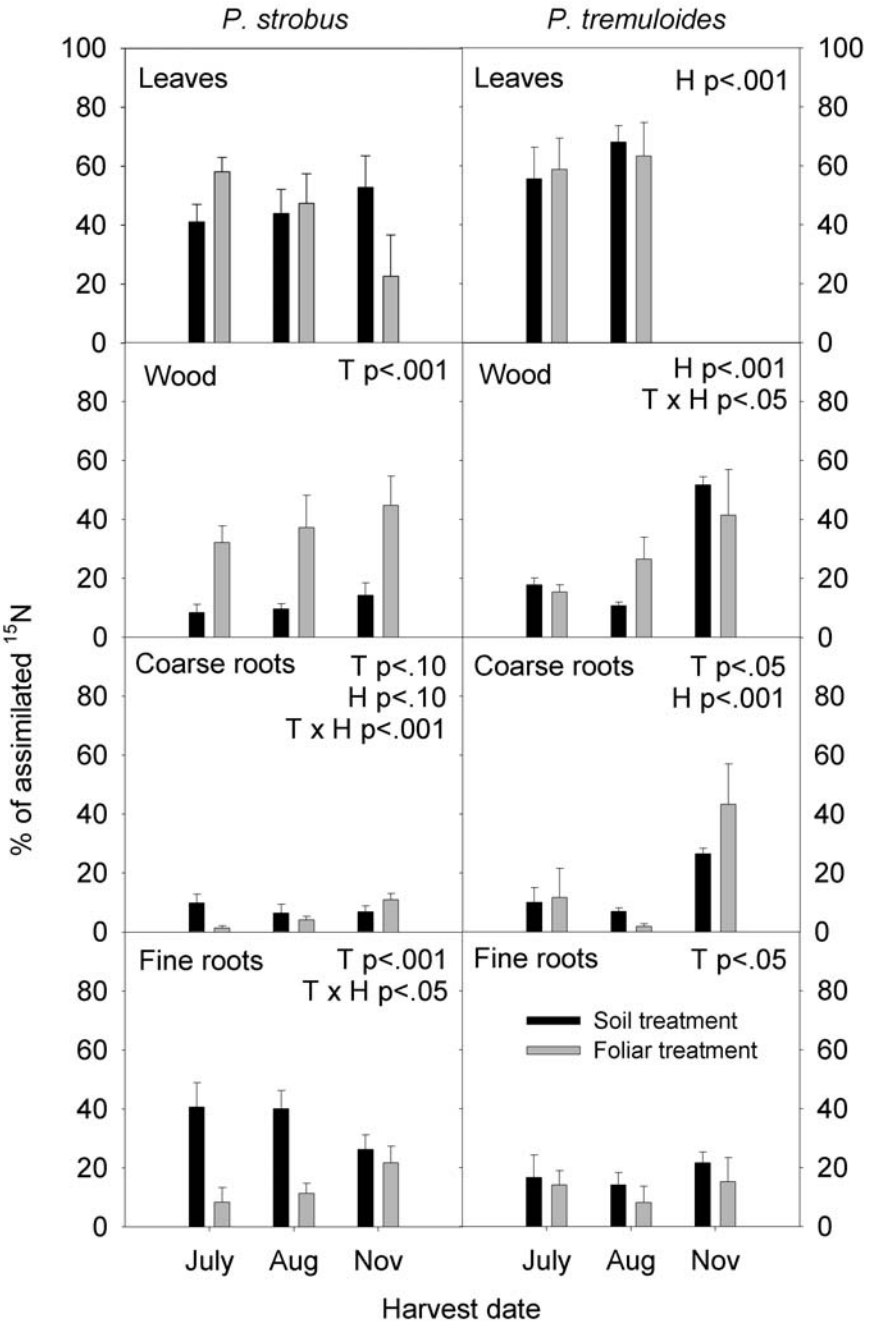

Figure 3.2. Proportion of tree-assimilated ^{15}N in green leaves, wood, and coarse and fine roots for *P. strobus* and *P. tremuloides* seedlings. Bars are means (+SE; n=5-7 seedlings per harvest); p-values denote significance of ANOVA tests within each species/biomass pool combination, where T=treatment and H=harvest.

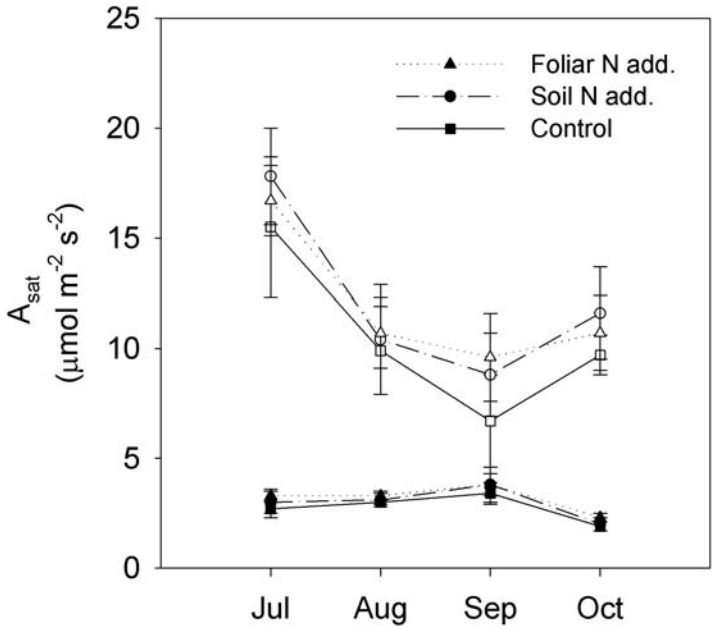

Figure 3.3. Seasonal progression of light-saturated photosynthesis for *P. tremuloides* (open symbols) and *P. strobus* (closed symbols) seedlings exposed to wet inorganic N deposition under greenhouse conditions. Nitrogen applications (5 total) during August 2006 averaged 1.7 and 4.0 kg N ha^{-1} for foliar- and soil- treated seedlings, respectively.

CHAPTER 4

NITROGEN INPUTS CHANGE FOREST SOIL CHEMISTRY AND N CYCLING: A META-ANALYSIS

ABSTRACT

North-temperate forests exhibit variable responses to experimental nitrogen (N) addition. Soils are the principal sinks for N inputs, and changes in their chemistry and N-transforming processes may affect major ecosystem processes, including forest growth and N retention. This meta-analysis was undertaken to test for general patterns in forest soil responses to N inputs, to determine whether such changes might impact forest growth, and to identify possible causes of variation between experimental systems. Analysis of soil C/N ratios and net N-mineralization (N_{min}) rates from 23 north-temperate forest sites throughout Europe and North America revealed several significant soil responses to experimental N addition, primarily in O-horizons. In general, C/N ratios in mineral soil layers did not respond to N inputs, while O-horizons showed a significant 6% decrease in treated relative to control plots. However, closer inspection of underlying data structure revealed that both mineral and organic soil layers with C/N ratios <24 had C/N increases averaging 3% following N addition, whereas soils with initial C/N ≥24

exhibited C/N declines that were stronger for higher C/N ratios. Organic and mineral soil horizons subjected to N addition exhibited 96% and 8% increases in N_{min} relative to controls. The total amount of N added to a system was a more significant predictor of the magnitude of change in O-horizon C/N and N_{min} than the duration or type of N amendments, climatic parameters, soil type, or forest composition. Results indicate a period of elevated N_{min} in N-amended forest soils that may significantly increase soil N availability for several decades following the initiation of N inputs, with potential consequences for forest growth.

INTRODUCTION

Inputs of anthropogenic N from the atmosphere to forest soils have been occurring across vast areas of the north-temperate forest since human activities began to significantly influence the global N cycle half a century ago (Galloway et al, 2003). In these forests, soils often hold 90% or more of the ecosystem nitrogen (N) content (Bormann et al, 1977), and nitrogen deposition to them is allocated in similar proportion. This pattern of internal redistribution of N inputs to soils has been verified by short-term (year-to-decadal-scale) ^{15}N-addition studies, in which the ^{15}N excess of soils represents 70-90% of total tracer recovery (Buchmann et al, 1996; Nadelhoffer et al, 1999a; Perakis and Hedin, 2001). Scientific interest in forest growth responses to N inputs has remained high, but the primary role of soils in ecosystem N retention suggests that they probably bear a larger fingerprint of anthropogenic changes in the global N cycle.

Research at many sites throughout the north-temperate forest has examined correlations between N inputs, soil N retention, and soil N supply processes (Aber et al, 2003; Emmett et al, 1998; Goodale et al, 2000). In a large-scale review of sites along an N deposition gradient in the northeastern United States, lower soil C/N ratios were observed at sites with greater atmospheric N inputs (Aber et al, 2003). This pattern probably is due to abiotic N immobilization, which is an important mechanism of soil N retention involving the physical and chemical incorporation of N into soil organic matter (SOM; Aber et al, 1998; Johnson et al, 2000). Over time, saturation of SOM with N inputs may decrease the soil C/N ratio, which can enhance microbial decomposition and soil net N-mineralization (N_{min}; Finzi et al, 1998; Janssen, 1996). However, other research suggests that the effects of decreasing C/N on microbial activity vary between SOM pools, with fast-turnover pools decomposing more rapidly and slow pools becoming more recalcitrant, as C/N declines (Berg and Matzner, 1997). There is also evidence that N additions can increase or decrease decomposition and N_{min} by mechanisms that do not necessarily involve changes in substrate C/N ratios (Hagedorn et al, 2003; Neff et al, 2002b).

On a case-by-case basis, experimental N additions to forests have led to highly variable soil responses. Following N addition, soil C/N ratios have increased in some systems (Magill et al, 2000) and decreased in others (McNulty and Boggs, 2005). Proportional changes in soil C/N ratios typically are rather modest, ranging from 5-10% in most systems, regardless of the amount, type, and duration of N addition. Soil net

N-mineralization rates are much more responsive to N amendment, and may involve deviations of 30-300% from pretreatment values. Changes in N_{min} have been positive at some sites and negative at others (Kjonaas, 1998; Zak et al, 2006). In fact, all pairwise combinations of increased and decreased soil C/N and N_{min} have been reported in the literature, yet no attempt has been made to synthesize results from experimental N addition studies in search of general patterns across study systems. The present analysis is intended to fill this gap in the synthesis of N deposition-forest soil research. The principal objectives of this study were: 1) to quantify changes in soil chemistry and N cycling (in terms of C/N ratios and N_{min} rates) in response to N inputs, and 2) to determine whether these changes support the hypothesis that N deposition may enhance forest growth through increased soil N supply.

MATERIALS AND METHODS

I conducted a meta-analysis of N addition studies in north-temperate forests to investigate soil responses to elevated N inputs. I searched the peer-reviewed literature using keyword searches within all years cataloged in the ISI Web of Science, and accessed additional data through professional contacts. I also checked references of N enrichment papers, including both original research and review articles. Total literature search returns numbered 811 papers, but 657 of these were clearly not relevant to this analysis based on their titles and abstracts. After inspecting the full text of the remaining 154 papers, I found relevant soil data in only 24 sources, including forest fertilization trials and chronic N amendment studies. I downloaded these relevant publications in .pdf format, while

managing the hundreds of citations returned by ISI with EndNote bibliographic software (Thomson ResearchSoft, Philadelphia, PA). During data extraction, I organized meta-data (predictor variables) and response data (dependent variables) from each source in a master database (MS Excel), and used MetaWin (Sinauer Associates, Sunderland, MA) for analysis. Table 4.1 outlines the scope of the studies included in this analysis, as well as the meta- and response data extracted from each source. To be included in the analysis, each study had to exhibit a temperate climate, and report control and treatment values for at least one response of interest.

Table 4.1. Definition of components of review questions.

Population of interest

Temperate forest soils[1] that have been subjected to N amendments

Treatments

Inorganic or labile organic N amendments (NH_4-N, NO_3-N, urea)

Meta-data

time since amendments initiated; annual amendment rate; cumulative amendment mass; ambient N_{dep}, forest type[2]; soil layer[3]; soil texture/type[4]; soil sample depth and bulk density; mean annual temperature and precipitation; soil pH

Responses of interest

Means, standard deviations, and sample sizes of O-horizon and mineral soil C/N and net N-mineralization for control vs. N-amended soils

[1] defined by Köppen (1931) as having 4-8 months of mean air temperature >10˚ C

[2] hardwood, conifer, or mixed

[3] organic horizon or mineral soil

[4] texture: organic, coarse, or fine; type: soil order

To test for statistically significant soil responses to N amendment, I analyzed studies in four groups: 1) all studies reporting mean C/N values for control and N-amended soils; 2) only studies reporting mean C/N values *and* associated measures of variance and sample size; 3) all studies reporting mean N_{min} values; and 4) only studies reporting mean N_{min} values *and* measures of variance and sample size. The test statistic most commonly used to assess significance in meta-analysis of continuously varying ecological data is the ln-transformed response ratio *R*, which is calculated as:

$$\ln(R) = \ln (\bar{X}^E / \bar{X}^C) \qquad (1)$$

Where $\bar{X}^E$ is the mean value of experimental (N-amended) observations, and $\bar{X}^C$ is the mean value of control (unamended) observations for a study, for either C/N or N_{min}. Because it is unitless, *R* is a standardized metric that allows comparison of data between studies reporting responses in different units (Hedges et al, 1999), and can be conceptualized as the proportional change in a parameter relative to its control value. Estimates of the errors and sample sizes associated with each $\bar{X}^E$ and $\bar{X}^C$ are desirable, as these statistics are required to calculate the variance of ln(*R*). When the variance of ln(*R*) is available for each study, the power of the analysis is enhanced, because 1) patterns of within- and between-group variation can be correlated with variation in meta-data according to parametric assumptions, and 2) a weighted meta-analysis can be used, which gives more weight to studies with more precise estimates of ln(*R*). This is accomplished by weighting each study by the inverse of its variance, and stands in contrast to an

unweighted meta-analysis, which assigns equal weight to all studies. Implicit in this comparison is the assumption that response ratios with smaller variances are closer to their 'true' underlying values.

I performed unweighted meta-analysis of groups 1 and 3, and weighted meta-analysis of groups 2 and 4, and compared the results of these analyses to determine whether error-reporting and non error-reporting studies differed in their estimates of ln(R) for soil C/N and N_{min}. Analyses indicated that estimates of ln(R) were not substantially different, so I pursued further analysis and investigations of underlying variation within groups 2 and 4. Before analyses, I eliminated R values that were negative, zero, or beyond 2σ from their group means in all four groups of studies. Outlying R values such as these were not present among soil C/N responses, which were relatively modest. However, removing outliers from the N-mineralization database decreased the sample size of error-reporting studies used in the final, weighted analysis from 68 to 61. Most of these extreme N-mineralization responses were the result of shifts between net N-mineralization and immobilization with treatment, which I assumed to be unrepresentative of the larger distribution of responses.

RESULTS

The C/N ratios of forest soils as a whole (control range: 13-39; treatment range: 13-37) exhibited a statistically significant 3-5% decrease with N amendment. While O-horizon C/N decreased by 5-8% (95% confidence interval), mineral soils were more variable,

with the 95% confidence interval of *R* including 1.0 (categorical meta-analysis; Table 4.2). Soil net N-mineralization rates increased significantly in response to N addition, with proportional increases over control values of 96% and 8% for O-horizons and mineral soils, respectively. The cumulative amount of N added to forest soil explained 64% and 53% of the heterogeneity in C/N and N_{min} response ratios (continuous meta-analysis; Table 4.2). Linear regression produced results similar to the continuous meta-analysis, and overall, both tests suggested that N addition induced O-horizon C/N to decrease, but had no overall effect on mineral soil and O-horizon + mineral soil C/N (Figure 4.1). However, closer inspection revealed that both organic and mineral horizons with initial C/N ratios less than 24 exhibited increased C/N ratios (up to10%) with N addition (Figure 4.2). Above the C/N threshold value of 24, organic and mineral horizons with higher pretreatment C/N ratios exhibited greater declines in C/N following N amendment. Organic horizon N_{min} rates demonstrated a sudden, dramatic increase (up to 4-fold) with the onset of N addition, but the elevation of N_{min} rates declined with further N inputs, eventually falling back to pretreatment levels (Figure 4.3).

Due to limitations of sample size, it was not possible to identify mechanisms of variation in soil responses to N addition beyond the soil layer (O-horizon vs. mineral soil), the cumulative quantity of N added, and the control plot C/N ratio. Attempts to partition within-group heterogeneity further according to climatic, soil and other meta-data made certain study sites highly influential and confounding, so I abandoned interpretation of further refinements to the data structure.

DISCUSSION

The most significant effects of N inputs on soil chemistry and N cycling were concentrated in the O-horizon. Unlike mineral soils, which were mostly unchanged, O-horizon C/N decreased significantly in response to N addition. The divergent behavior of these two soil layers probably is related to differences in their inherent capacities for N immobilization in SOM. Undisturbed forest O-horizons typically have higher SOM concentrations than the mineral soils beneath them (Johnson et al, 2000), hence a greater proportion of their mass is involved in immobilizing N than mineral soils. However, the response of organic and mineral soil layers to N addition depended on their initial C/N ratios. In forests with already low soil C/N (<24), N additions increased C/N, probably due to NO_3^- leaching, soil C accrual, or both. Soil C/N ratios of 20-25 have been cited in the literature as a threshold range beneath which net nitrification increases substantially (Aber et al, 2003; Emmett et al, 1998). Increased net nitrification may indicate progression towards N saturation, and can lead to significant N losses by NO_3^- leaching. Alternatively, C/N ratios can increase due to soil C accumulation, which can occur when chronic N deposition inhibits microbial enzyme production and decomposition of soil C (Pregitzer et al, in press). Further analysis of soil responses to N addition will establish the degree to which soil C storage is responsible for increased C/N below this ecologically significant threshold.

In a pattern similar to soil C/N ratios, shifts in N_{min} were different for organic and mineral soil layers. The lack of a statistically significant C/N decrease in mineral soils as a whole

corresponded with a modest increase in N_{min} (8%) in response to N inputs, while O-horizon N_{min} rates nearly doubled following treatment. Because N_{min} rates depend upon C substrate quality (in terms of C/N) for forest litter and organic matter (Finzi et al, 1998; Janssen, 1996), the difference in N_{min} responses between O-horizons and mineral soils can be explained by their changes in C/N. Abiotic N immobilization within the O-horizon decreases its C/N ratio, rendering some of its C substrates more labile, and increasing microbial decomposition and N_{min}. Mineral soils contain less C substrate per unit mass, so N_{min} responses in this layer are less sensitive to N inputs.

Correlations between O-horizon C/N and N_{min} and continuously varying meta-data were stronger when response ratios were meta-regressed against the cumulative amendment mass (kg N ha^{-1}) than against the annual amendment rate (kg N ha^{-1} yr^{-1}), or the years elapsed since N additions began. However, O-horizon C/N and N_{min} responses behaved differently along the axis of increasing cumulative N inputs. Organic horizon C/N ratios continued to decline linearly as amendments increased up to 3000 kg N ha^{-1}, while evidence of increased N_{min} disappeared by the time 410 kg N ha^{-1} had been added. The lack of a sustained N_{min} increase, in spite of steadily decreasing O-horizon C/N, may indicate declines in microbial population size or activity (Kirk and Farrell, 1987; Waldrop et al, 2004). Another possibility is that N additions enhance decomposition within rapid-turnover SOM (decadal or shorter time scales), while further stabilizing the recalcitrant SOM pool (Fog, 1988; Neff et al, 2002). In forest O-horizons, enhanced N_{min} within the former SOM pool may deplete its supply of C substrate, causing N_{min} to

decline back towards pretreatment levels. Concurrently, the overall O-horizon C/N ratio would continue to decline as the recalcitrant SOM fraction immobilized further N inputs, with no increase in N_{min}.

Although elevated N_{min} does not appear to be sustained beyond cumulative N inputs of ~400 kg N ha^{-1}, accelerated N_{min} during the period leading up to this threshold ensures that soil N availability is significantly elevated for some time. Atmospheric N deposition has been increasing rapidly since the 1960s (Galloway et al, 2003), and current annual inputs are ~4 kg N ha^{-1} yr^{-1} in the least-impacted regions of the north-temperate forest (Aber et al, 2003). Forests of industrialized regions are characterized by significantly higher deposition rates (15-50 kg N ha^{-1} yr^{-1}; Wright and van Breemen, 1995). In many regions, atmospheric inputs of the order of several hundred kg N ha^{-1} may have been deposited to forests within the last 30-60 years, and could today represent a significant fraction of the O-horizon N pool, which is in the range of 600-1200 kg N ha^{-1} for most north-temperate forests (Nave et al, in preparation). But, by the time advanced ecological research of forest soil and tree growth responses to N inputs began in the late 20th century, cumulative N inputs had pushed many forests into the stage at which further N inputs have an ever-diminishing impact on N_{min}. Considering the strong correlation between N_{min} and net primary productivity in northern forests (Reich et al, 1997), this suggests that forest growth increases due to N deposition may have been greater in the past than in the present time.

The historic impacts of N deposition on forest growth may be significant, but current research indicates that N deposition still may be responsible for observed variation forest growth and C storage (De Vries et al, 2006). Spatial and temporal variation in forest N status and climatic conditions, and interactions between them are responsible for heterogeneity in N cycling at scales from the individual tree, to the stand, to the region (Boerner and Koslowsky, 1989; Fitzhugh et al, 2003; Groffman et al, 1993; Prescott, 2002). This suggests that unique combinations of climate, soil microbial dynamics, and plant N uptake processes at all spatial scales may affect the fate of atmospheric N inputs to forests. In most systems thus far studied, N inputs elicit more significant responses from soils than vegetation. The apparent lack of significant forest growth responses to N addition may in part reflect limitations of design, because most forest N enrichment studies are performed at the stand level, and are located in human-dominated areas that already have relatively high atmospheric deposition rates (in this study: 8-10 kg N ha^{-1} yr^{-1}, and frequently higher). Research in oligotrophic forests that improves the spatial resolution of forest N cycling from stands to individual trees, advances the understanding of stabilization and mobilization of SOM and N, and integrates these two areas will elucidate the consequences of the human fingerprint on soil chemistry and N cycling.

Categorical results					Continuous results				
Parameter	Pool	n	*R*	95% CI	Parameter	Model	df	Q	*P*
C/N	O-horizon	30	0.94	(0.92, 0.95)	O-horizon	Regression	1	181.1	<0.001
	Mineral soil	17	0.98	(0.95, 1.01)	C/N	Residual	28	102.1	<0.001
N_{min}	O-horizon	34	1.96	(1.79, 2.14)	O-horizon	Regression	1	136.9	<0.001
	Mineral soil	27	1.08	(1.03, 1.14)	N_{min}	Residual	32	122.4	<0.001

Table 4.2. Summary of results from categorical and continuous meta-analyses of forest soil responses to N amendment. Categorical results include only error-reporting studies and present the number of responses, mean response ratios, and 95% confidence intervals of C/N and N_{min} sampling distributions. Continuous results show significance of meta-regression models relating cumulative N amendment quantity to ln(R) among error-reporting studies. Heterogeneity (Q) is a measure of variance explained by each model term.

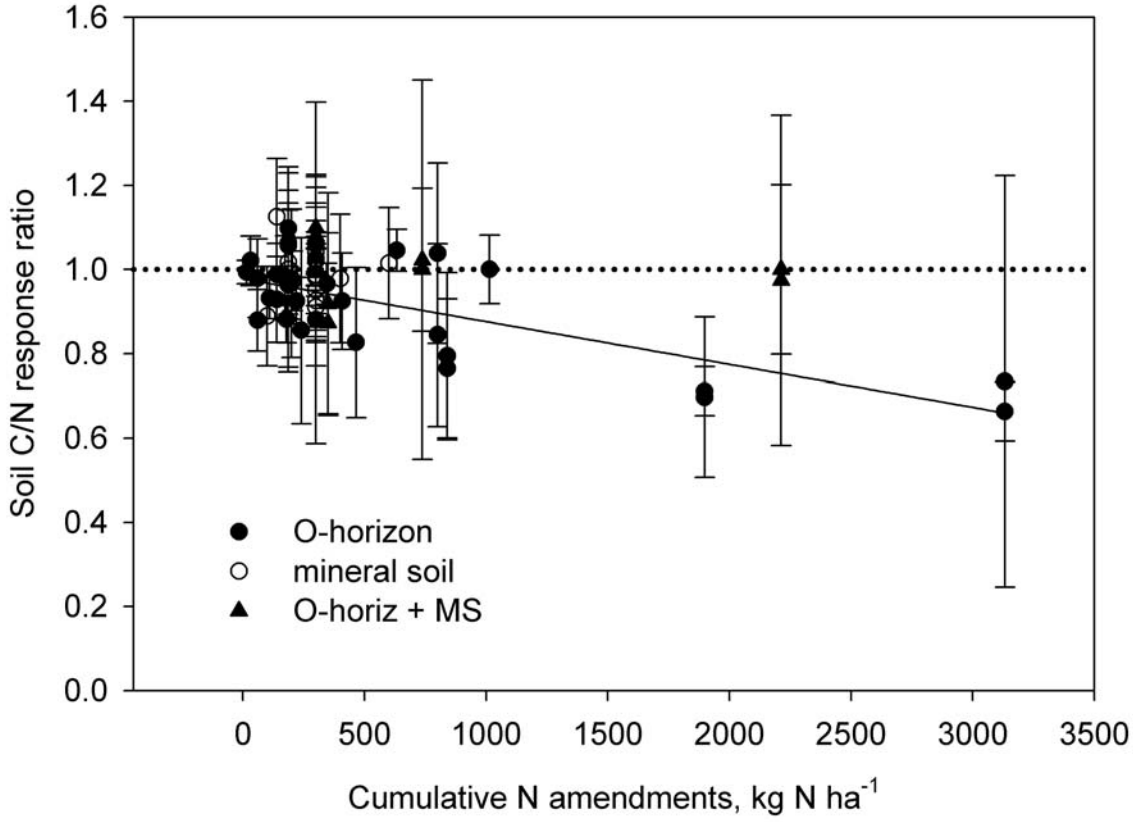

Figure 4.1. Proportional change in soil C/N with N amendment, by horizon. Error bars are individual study 95% confidence intervals. Cumulative N amendment mass explained 52% of the variation in O-horizon responses (regression, $P<.0001$, solid line), but had no relationship with mineral soil or O-horizon + mineral soil pools. A response ratio of 1.0 (no change in soil C/N) is indicated by the dotted reference line.

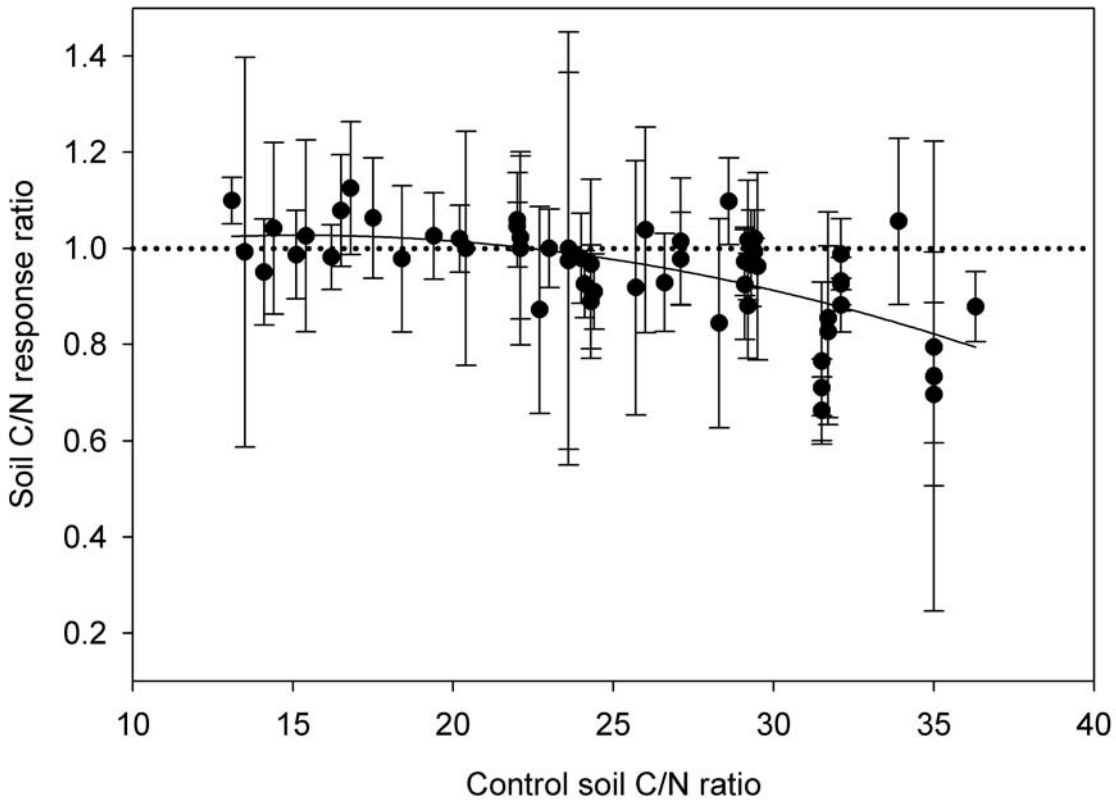

Figure 4.2. Change in soil C/N ratio with N deposition, as influenced by initial soil C/N. For soils with C/N <24, N amendment caused a 3% increase in C/N. At and above C/N values of 24, soils with higher C/N ratios exhibit stronger decreases in C/N with N amendment. Best-fit curve is a quadratic regression model with r^2=.42, P<.0001. Dotted reference line designates a response ratio of 1.0 (no change in soil C/N), and error bars are individual study 95% confidence intervals.

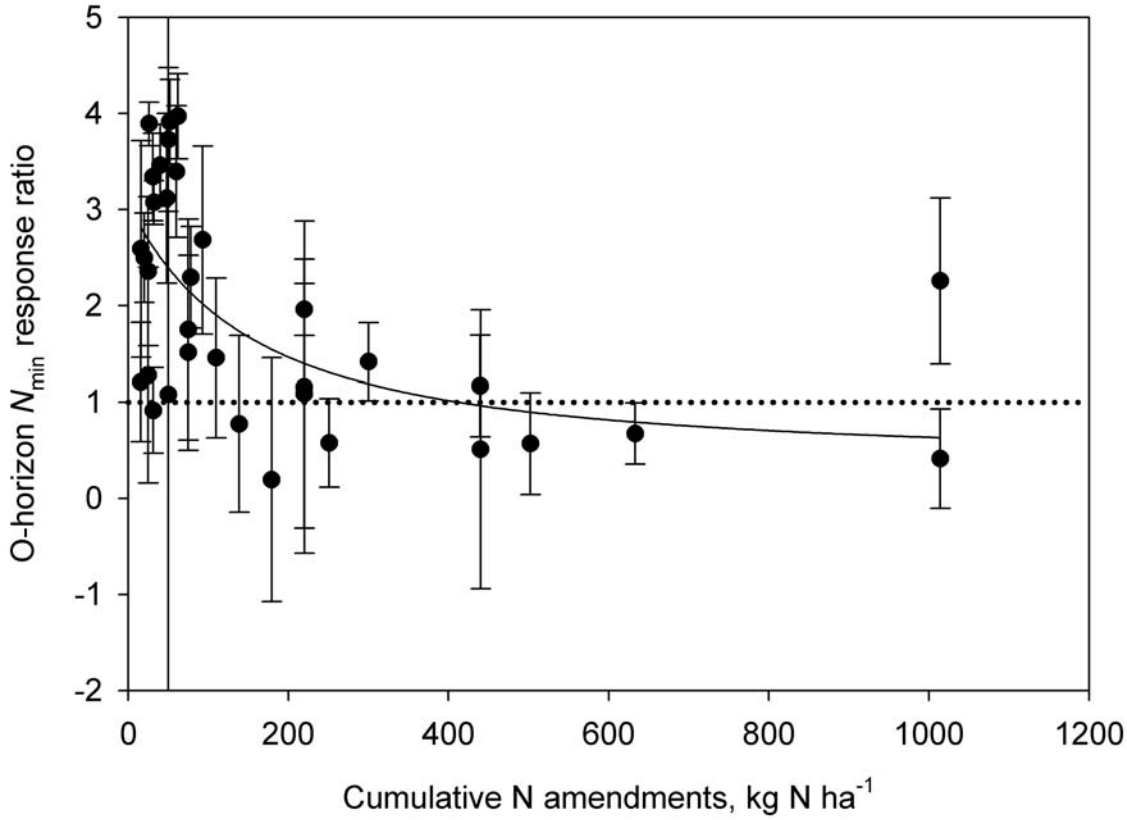

Figure 4.3. Proportional change in O-horizon N_{min} with N deposition. The best-fit curve is an exponential decay model with r^2=.42, P<.001. According to the model, O-horizon N_{min} returns to pre-amendment rates after 410 kg N ha^{-1} have been added. The dotted reference line designates a response ratio of 1.0 (no change), and error bars are individual study 95% confidence intervals.

CHAPTER 5

SYNTHESIS: FOREST PARTITIONING OF N DEPOSITION, AND ITS EFFECTS ON N CYCLING AND NET PRIMARY PRODUCTIVITY

Atmospheric N deposition is an important source of N to the N-limited UMBS forest. Evidence of N limitation at the site includes its N_{min} rate (44 kg N ha^{-1} yr^{-1}), which is lower than the rates in most mature hardwood and mixed forests of the Upper Great Lakes region (range: 25-135 kg N ha^{-1} yr^{-1}; Nadelhoffer et al., 1985; Reich at al., 1997; White et al, 2004). Also, N_{min} measurements revealed no net nitrification, and ion exchange resins deployed in N_{min} incubation tubes and beneath ^{15}N-labelled mesocosms showed no evidence of N leaching losses from the top 25 cm of the soil profile (O, A/E, and B-horizons). Clearly, the UMBS forest is N-limited, all N inputs are retained, and N_{dep} will continue to increase the N capital of the system for a long time. The proportion of the N inputs accessed by plants will determine the impact of N_{dep} on *NPP*.

The UMBS forest requires less N for *NPP* (51 kg N ha^{-1} yr^{-1}) than most other Upper Great Lakes forests, which use 47-143 kg N ha^{-1} yr^{-1} (Pastor and Bockheim, 1984; Nadelhoffer et al, 1985). When the N use efficiency (NUE) of *NPP* is calculated, the

value for UMBS (241 kg biomass kg N^{-1}) is higher than the sites in Pastor and Bockheim (1984) and Nadelhoffer et al (1985), which average 147. Across all of these forests, NUE decreases as *NPP* N requirements increase (Figure 5.1). This trend may indicate that vegetation N uptake and *NPP* become decoupled with increased N supply, such that increasing the supply of N for *NPP* leads to a decrease in NUE, rather than an increase in *NPP*. Conversely, the seminal work of Vitousek (1982) suggests that forests with lower *NPP* N requirements inherently have higher N use efficiency. This suggests that, because of its high NUE, *NPP* at the UMBS forest would be exceptionally responsive to N_{dep}, which adds 15-20% to the amount supplied by N_{min} every year.

While the N budget study demonstrates that N inputs are a significant proportion of the *NPP* N requirement, the results of the mesocosm experiment suggest that immediate *NPP* responses to N_{dep} are unlikely. The low foliar N uptake efficiency in mesocosm seedlings (10% of applied ^{15}N) indicates that the UMBS forest canopy probably assimilated <0.2 kg NH_4-N ha^{-1} of the 1.3 kg NH_4-N ha^{-1} retained during seven months of N_{bd} and throughfall collections in 2004. Root access to $^{15}NH_4$-N inputs to mesocosms was also limited (14%). Assuming that roots have similar uptake efficiency for the other N compounds present in N_{dep}, this corresponds to annual stand-level uptake of N_{dep} of approximately 0.8 kg N ha^{-1} yr^{-1}. Unfortunately, it is not possible to generalize the results of the mesocosm experiment to the mature forest with complete confidence, owing to the impacts of ontogeny on tree physiology. Older trees/stands usually have slower relative growth rates (*RGR*; Walters et al, 1993), lower tissue N concentration (Grulke and

Retzlaff, 2001), lower leaf gas exchange rates (Yoder et al, 1994), and greater fine root density (Norby et al, 1999) than young trees, but not all of these ontogenetic differences are equally relevant to the results of my experiments. All, however, are worth considering. First, stand-level *RGR* at UMBS was less than 0.1 (calculated from Curtis et al (2002) as the ratio of *NPP* to the standing crop of forest biomass), while total seedling *RGR* in mesocosms averaged 1.2 (the change in average mesocosm biomass between harvests 1 and 3, divided by average mesocosm biomass at harvest 1). In mesocosms, N concentrations in wood and coarse roots (6 g kg^{-1} for each), and also fine roots (1.1%), were greater than in the mature forest ([N] of 0.5, 2, and 6 g kg^{-1}, respectively). Data on light-saturated photosynthesis of mature *Populus* trees at UMBS are sparse, but suggest that mature trees may have higher photosynthesis than seedlings (21 vs.17 μmol CO^2 m^{-2} s^{-2}). This difference, if significant, may reflect hydraulic limitation of photosynthesis in greenhouse and mesocosm seedlings, which had significantly lower fine root density than the mature forest (1 vs. 13 Mg ha^{-1}). This final disparity between the two study systems probably overrides all others mentioned above, because greater fine root density means that plants are able to take up more N (Holmes et al, 2003; Norby et al, 1999; Pregitzer et al, 2000). Compared to the mesocosms, the greater fine root density of the mature forest allows its trees to remove more N (and water) from the soil, and suggests that the proportion of N inputs accessed by roots may be significantly higher than predicted from the mesocosm experiment. As an approximation, average mesocosm biomass increased by 2200 kg ha^{-1} between harvests 1 and 3, and the weighted average N concentration across all components of this biomass was 8 g kg^{-1}. This corresponds to seasonal N

uptake into biomass of ~18 kg N ha^{-1}, whereas the mature forest withdraws nearly three times as much N from similar soil to meet its annual *NPP* N requirement. Mathematically 'correcting' for this difference estimates that atmospheric N uptake by roots at the UMBS forest is not 0.8, but rather 2.2 kg N ha^{-1} yr^{-1}. While this is difference is significant in relative terms, 2.2 kg N ha^{-1} yr^{-1} still is less than 5% of the forest *NPP* N requirement. Retention of the balance of atmospheric N inputs in soil pools was clearly demonstrated in the mesocosm experiment, and suggests that the most significant ecosystem responses to N_{dep} occur in the soil.

In the United States, anthropogenic N inputs to the atmosphere began a dramatic increase around 1950, and tripled between 1961 and 1997 (Galloway et al, 2003). Given the timing of this increase in atmospheric N, significant N_{dep} probably has been occurring at UMBS for 50-60 years. If average N_{dep} over this time period is estimated at 2-4 kg N ha^{-1} yr^{-1}, cumulative N inputs today have reached 130-270 kg N ha^{-1}. This amount is 10-20% of the total soil N pool (1500 kg N ha^{-1}), and possibly more than the entire O-horizon, which contains 145 kg N ha^{-1}. Atmospheric N retention in SOM during the last fifty years probably has reduced soil C/N ratios UMBS, and will continue to do so in the future. The decrease in soil C/N probably has increased rates of decomposition and N_{min} within fast-turnover SOM, and the best-fit curve in Figure 4.3 predicts that although N_{min} was higher when N_{dep} began its dramatic increase 50 years ago, the rate of soil N supply will remain elevated until an additional 140-280 kg N^{-1} ha^{-1} have been deposited to the system.

Forest *NPP* is positively correlated with N_{min}, and this relationship suggests that increased decomposition and N_{min} within fast-turnover SOM could increase forest growth. But, unless the NH_4^+ produced during N_{min} is somehow different than the NH_4^+ deposited from the atmosphere, there is no reason to expect that mineralized N would be distributed differently than atmospheric N among ecosystem N pools (Figure 5.2). Free NH_4^+ represents less than 1% of the N in an N-limited forest ecosystem (Bormann et al, 1977), because there are numerous, competing biotic and abiotic sinks removing NH_4^+ from the soil as quickly as it appears. The most important of these sinks appears to be SOM, which has major N immobilizing potential over timescales from hours to decades (Nadelhoffer et al, 2004; Perakis and Hedin, 2001). The tremendous N sink strength of SOM suggests that vegetation uptake is not likely to be a significant atmospheric N retention mechanism on decadal- or shorter timescales, and that *NPP* probably does not respond to N inputs during this time frame. Investigating longer-term (successional) patterns of N redistribution between soils and vegetation may reveal mechanisms that allow forest biomass to access soil N stocks that have been augmented over time by N_{dep}.

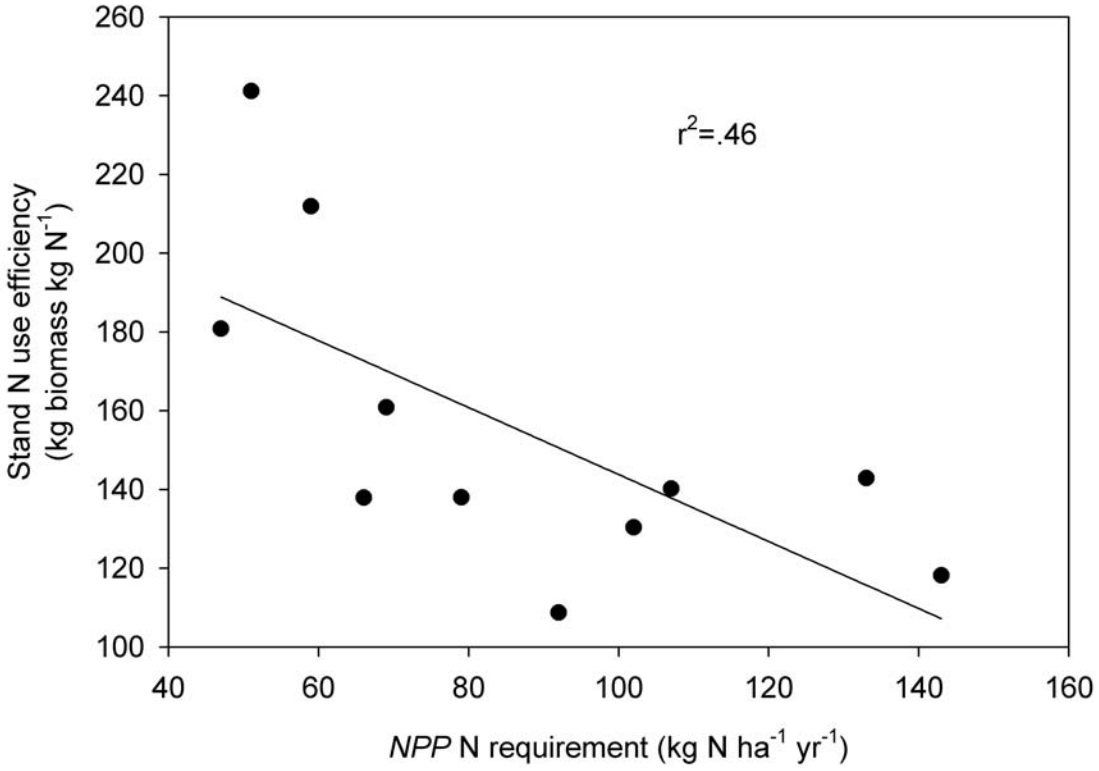

Figure 5.1. Forest N use efficiency (kg biomass kg N^{-1}) of *NPP* as a function of the *NPP* N requirement. Data points are hardwood and mixed hardwood-conifer forests in Wisconsin, and UMBS. The UMBS forest has the highest N use efficiency.

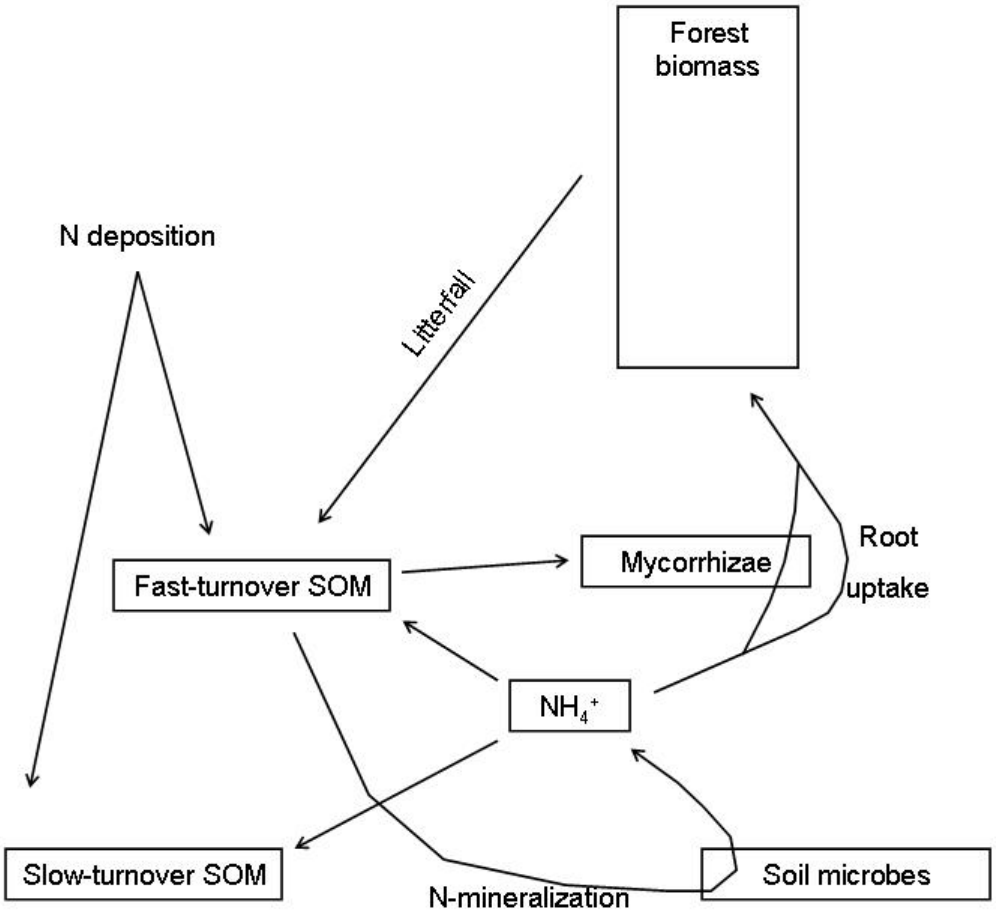

Figure 5.2. Conceptual diagram of N pools and fluxes influencing the distribution of atmospheric N inputs within a forest ecosystem. Pools containing N are in boxes, arrows represent N transfer processes between pools. Atmospheric N retention in soil pools changes soil chemistry, organic matter turnover, and N-mineralization rates. Short-term (decadal-scale) availability of N inputs to plants is low because of retention by SOM, but long-term increases in soil N stocks and cycling rates may lead to greater plant N uptake.

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