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SOIL NITROGEN TRANSFORMATIONS AND RETENTION DURING A

DECIDUOUS TO CONIFEROUS SUCCESSIONAL TRANSITION

A

DISSERTATION

Presented to the Faculty

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DOCTOR OF PHILOSOPHY

By

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SOIL NITROGEN TRANSFORMATIONS AND RETENTION DURING A

DECIDUOUS TO CONIFEROUS SUCCESSIONAL TRANSITION

By

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ABSTRACT

The mineralization, retention and movement of soil nitrogen (N) was investigated in forest types which encompass one of the most dramatic plant successional transitions in the boreal forest – the shift from mid-succession stands of balsam poplar (*Populus balsamifera*) to late-succession stands of white spruce (*Picea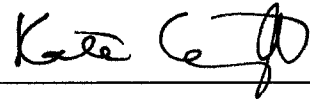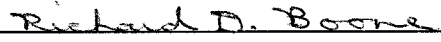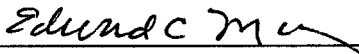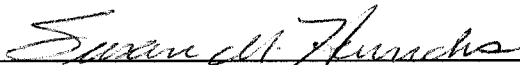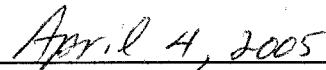 glauca*). Nitrogen is an essential nutrient that often limits plant productivity in the boreal forest. However, N uptake by plants is constrained by the activity of soil microbes which break down organic molecules and release N to plants (e.g., as amino acids, ammonium and nitrate). The availability of labile carbon (C) is generally thought to limit soil microbes; however, it has been hypothesized that soil microbes in stands of balsam poplar are actually N limited. Balsam poplar trees also have large N requirements; thus, the overall demand for N is considerable in these stands and biological N retention should be high. In contrast, lower primary productivity and more recalcitrant soil organic matter in white spruce stands should result in comparatively less immobilization and less retention of N in this stand type.

Experimental N additions resulted in the acceleration of net N mineralization and nitrate leaching in both stand types, probably because biological N demand was rapidly satiated. In balsam poplar soil, net nitrification was greatly stimulated by N additions, but in white spruce soil only ammonification was stimulated, indicating that different mechanisms control N transformations in these stands. Nitrogen additions did not affect soil microbial biomass in either stand. Results from a laboratory soil incubation study

indicate that soil organic matter in late succession stands was more labile and the mineralization of C and N were significantly more temperature sensitive than in mid succession. Thus, climatic warming may result in the release of a larger proportion of soil C and N from late succession stands. A study examining soil solution N concentrations and movement also showed that the Tanana River is a source of nitrate to the active layer during the growing season in both mid- and late succession stands.

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I reserve my biggest thanks to my wife, Allison Bidlack, who was always there for me and gave me encouragement throughout this difficult process. Thanks!

DEDICATION

For Carl

Chapter 1

An Introduction to Successional Control Over Soil C and N Transformations

Nitrogen (N) is a fundamental element within protein molecules and therefore is needed by all forms of life on Earth. As N_2 gas, N constitutes the largest fraction (> 78%) of gas within the Earth's atmosphere. Thus, it is somewhat paradoxical that N is also the element which limits plant growth in a wide range of terrestrial biomes (Vitousek & Field 2001). Plant N limitation is thought to be particularly prevalent in high-latitude ecosystems such as the boreal forest (Nasholm *et al.* 1998; Persson & Nasholm 2001) due to the spatial and temporal extent (well into the growing season) of cold soil. Cold soil conditions limit the rate of microbially-mediated organic matter depolymerization (breakdown) and restrict the release of useable forms of N (amino acids, NH_4, NO_3) to plants (Figure 1.1; Klingensmith & Van Cleve 1993; Schmidt *et al.* 1999; Jonasson *et al.* 2001; Hobbie *et al.* 2002; Schimel & Bennett 2004).

While the boreal forest is characterized by a short growing season and cold conditions, it is currently experiencing the initial effects of a warming climate (Houghton *et al.* 1996; Serreze *et al.* 2000; Hassol 2004; Overland *et al.* 2004). The warming of this cold-dominated region could induce a substantial increase in the mineralization of large stores of soil organic matter, which, in turn, could promote further atmospheric warming. However, because C fixation by boreal plants is largely thought to be N limited, an intimate knowledge of the controls over soil N transformations is fundamental to forecasting how warming will influence the net balance of C within the boreal forest

(Hobbie *et al.* 2002). For example, how sensitive is the heterotrophic utilization of soil organic matter to increases in temperature in the boreal forest? Does successional stage have a large influence on the quality of soil organic matter and therefore the rate of N mineralization? To what extent could microbial N demand control N losses if mineralization rates were to increase? The research presented within this dissertation addresses these questions while also investigating biologically-driven modifications to soil N and C transformations brought about by a natural alteration of the dominant plant community over time (plant succession).

Plant succession in the boreal forest can have a dramatic influence on ecosystem N and carbon (C) cycling (Flanagan & Van Cleve 1983; Fox & Van Cleve 1983; Van Cleve et al. 1996). A prime example of successional control over biogeochemical processes can be seen on the floodplain ecosystems of Alaska's interior (Figure 1.2; Viereck 1989) where there is a major modification to the soil chemical and physical environment as mid-succession stands of balsam poplar (*Populus balsamifera*) succumb to dominance by white spruce (*Picea glauca*) during the advent of late succession (Viereck et al. 1983; Van Cleve et al. 1991). As balsam poplar is shaded out by white spruce there is a decrease in the amount of leaf litterfall, which enables moss to establish in the understory (Viereck *et al.* 1993a). A progressively thicker layer of moss acts as insulation and inhibits soil warming during the summer months such that surface horizons remain frozen throughout an ever-increasing portion of the growing season. Eventually the soil becomes permanently frozen year-round (permafrost) with only a shallow active layer that is unfrozen during the growing season. Plant litter produced

during late succession contains high ratios of lignin:N and C:N relative to earlier successional stages and is generally thought to be more recalcitrant to microbial breakdown (Van Cleve & Viereck 1981; Flanagan & Van Cleve 1983). Thus, the successional transition from a deciduous to coniferous-dominated landscape in the boreal forest is a fundamental turning point in which plant species composition mediates declines in soil temperature, organic matter decomposition, and rates of nutrient cycling.

In this research I used a combination of *in situ* N fertilizer additions, field and laboratory soil incubation experiments, and chemical analyses of soil and water samples to test several predictions regarding succession-induced changes to soil N and C transformations during the transition from mid to late succession. Specific hypotheses and predictions will be given in detail within the subsequent chapters; however, an overarching assumption when this research began was that the demand for N by plants and soil microbes would be higher in mid-succession stands of balsam poplar than in late-succession stands of white spruce. Higher N demand in balsam poplar stands, I reasoned, should limit leaching losses of biologically-available N forms and allow for the microbial immobilization of N added during experimental additions.

In the first study (Chapter 2) experimental N additions (100 kg N·ha^{-1}·yr^{-1} as NH_4NO_3) were used to investigate microbial N demand in balsam poplar and white spruce soil. I predicted that soil microbes in balsam poplar stands would readily accommodate N additions through N immobilization and increased microbial biomass with only small changes to soil N transformations or evidence of N leaching losses. In contrast I predicted that N additions in white spruce stands would result in the leaching of

biologically-available forms of dissolved N below the main rooting zone and would cause an immediate increase in net N transformation rates because microbial demand for N should be satisfied rapidly. To examine these predictions, intact buried soil cores were incubated in control and fertilized plots of balsam poplar and white spruce. The *in situ* soil incubations took place each month (or over winter) for two years (1999 –2001). From these soil cores, net N mineralization rates, microbial biomass C and N and nitrate leaching to deeper soil layers were measured.

In the second study (Chapter 3), arrays of tension lysimeters were installed within and below the main rooting zone in balsam poplar and white spruce stands in order to investigate the leaching of dissolved organic N (DON) and inorganic N (DIN) below the main rooting zone during the 2000-2001 growing seasons. I predicted that the ratio of DON:DIN in the soil solution below the rooting zone would be much higher in balsam poplar stands than in white spruce stands. This prediction was, again, based on higher relative N demand in balsam poplar stands which should have resulted in a greater utilization of the biologically-available DIN. I also reasoned that much of the DON likely consists of recalcitrant molecules that, for the most part, are not available for utilization by plants or microbes. Thus, I assumed that losses of DON were not under a high degree of biological control. Major ions in the soil solution and river water also were measured in this study in order to investigate the possibility that soil solution moves into the soil active layer during the growing season.

In the third and final study (Chapter 4), the temperature sensitivity of soil C and N mineralization was investigated in soil collected from mid and late succession stands.

The goals of this study were to: (1) test the hypothesis that soil organic matter becomes more recalcitrant during late succession; (2) investigate the temperature sensitivity of organic N and C mineralization due to predicted climatic warming; and (3) determine whether the lower rates of net N mineralization generally observed in white spruce soil are due to lower gross N mineralization or greater microbial immobilization. Organic soil from balsam poplar and white spruce stands was incubated at four temperatures (5, 10, 15 and 20°C) in the laboratory for over 300 days. Gross N mineralization and microbial biomass were measured during the first month of the incubation while net N mineralization was measured periodically for 182 days. The rate and cumulative amount of C mineralization was measured for the entire study.

Study Sites

This research was conducted within or adjacent to the Bonanza Creek Long Term Ecological Research Site (BNZ-LTER), approximately 30 km south of Fairbanks, Alaska USA (64°45′ N, 148°18′ W; Figure 1.3). Balsam poplar sites (LTER sites BP1, BP2 and BP3) contained trees 80-100 years old with a substantial understory of rose (*Rosa acicularis*) and N-fixing thinleaf alder (*Alnus tenuifolia*). White spruce sites (LTER sites FP4A, FP4B and FP4C) generally consisted of trees 200+ years in age and an understory of alder (*A. crispa* and *A. tenuifolia*) and rose; however, alder was not present at the FP4C site. White spruce soils were covered by a carpet of moss approximately 10-15 cm thick (*Hylocomium splendens* and *Pleurozium schreberi*). All research sites were on islands within the active portion of the Tanana River floodplain, and all soil profiles

contain multiple buried organic horizons as a result of past flooding events. The buried organic horizon closest to the soil surface was likely the result of a massive flood in 1967. Frozen soil in balsam poplar sites was gone by the end of July but persisted throughout the entire growing season in white spruce sites (Brenner et al. *In Press*). Soil temperature was generally highest around the second week in August in both stand types with maximum values ranging from 10-14°C at 5 cm to ~5°C at 20 cm (LTER unpublished – See Reference section). Select soil characteristics for these stands can be found in Table 2.1, and a complete overview of the climate, soil and vegetation of these stands can be found in Viereck *et al.* (1993a; 1993b).

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Figure 1.1. A contemporary view of soil N transformations from Schimel & Bennett (2004). Here, microbial depolymerization of soil organic matter controls successive transformation processes such as N mineralization and the availability of amino acids, NH_4^+ and NO_3^- for plants. Used with permission from the Ecological Society of America, 2004.

Bare Silt Bar

Mature
Balsam
Poplar
80-100 yrs.

Mature
White
Spruce
200-300 yrs.

Permafrost

TIME
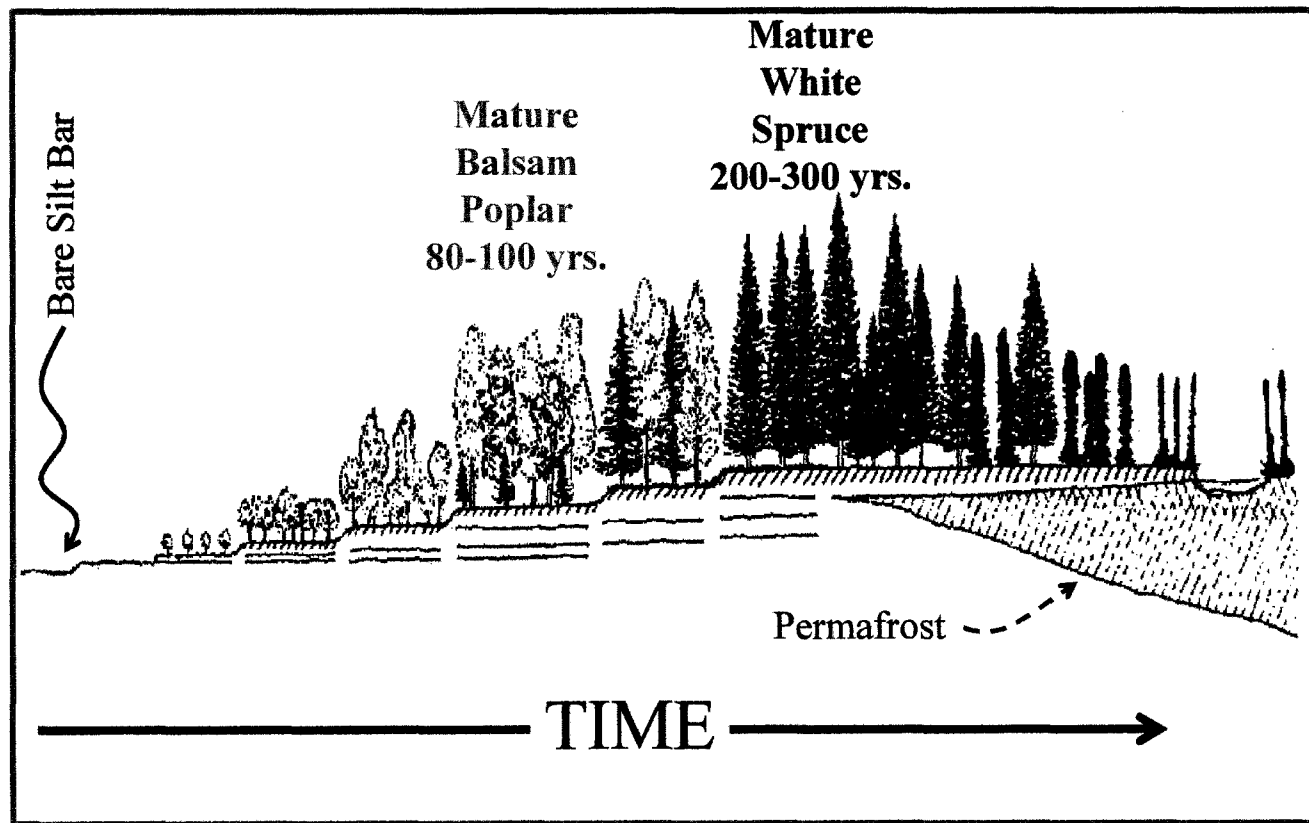
Figure 1.2. A boreal forest primary successional sequence along rivers in interior Alaska. Modified from Viereck (1989).

11
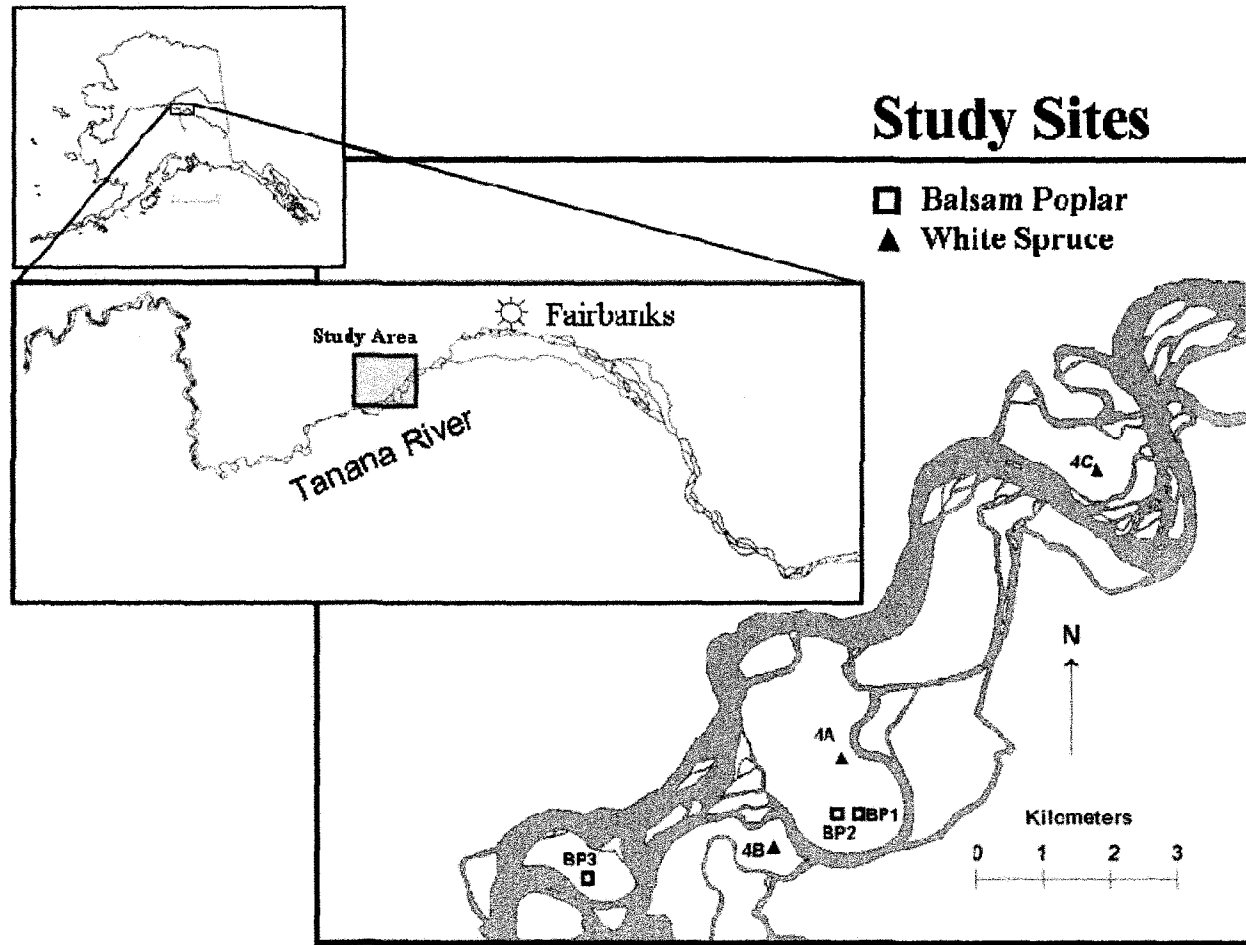
Figure 1.3. The location of balsam poplar (squares) and white spruce (triangles) study sites. All study sites were located along the Tanana River in interior Alaska.

Chapter 2

Nitrogen Additions to Pristine, High-Latitude, Forest Ecosystems: Consequences for Soil

Nitrogen Transformations and Retention in Mid and Late Succession[1]

Abstract

We hypothesized that differences in microbial and plant N demand in balsam poplar and white spruce stands would control *in situ* net N transformation and retention following N additions. Throughout the study, N fertilizer (NH_4NO_3) was added in three increments during the growing season, giving an annual N addition of 100 $kg \cdot ha^{-1} \cdot yr^{-1}$. In balsam poplar, fertilization induced a large (~285%) increase in annual net nitrification but tended to reduce net ammonification. In white spruce, fertilization generally stimulated net N mineralization (via higher net ammonification) while net nitrification increased only slightly or remained unchanged. For 0-20 cm soil cores of both stand types, fertilization rapidly increased extractable DIN pools; however, the absolute amount of this increase was significantly larger in white spruce than in balsam poplar. In both stands, extractable NO_3^--N in 20-30 cm mineral cores increased within the first year following N additions, indicating that leaching of NO_3^--N was fairly rapid. Fertilization
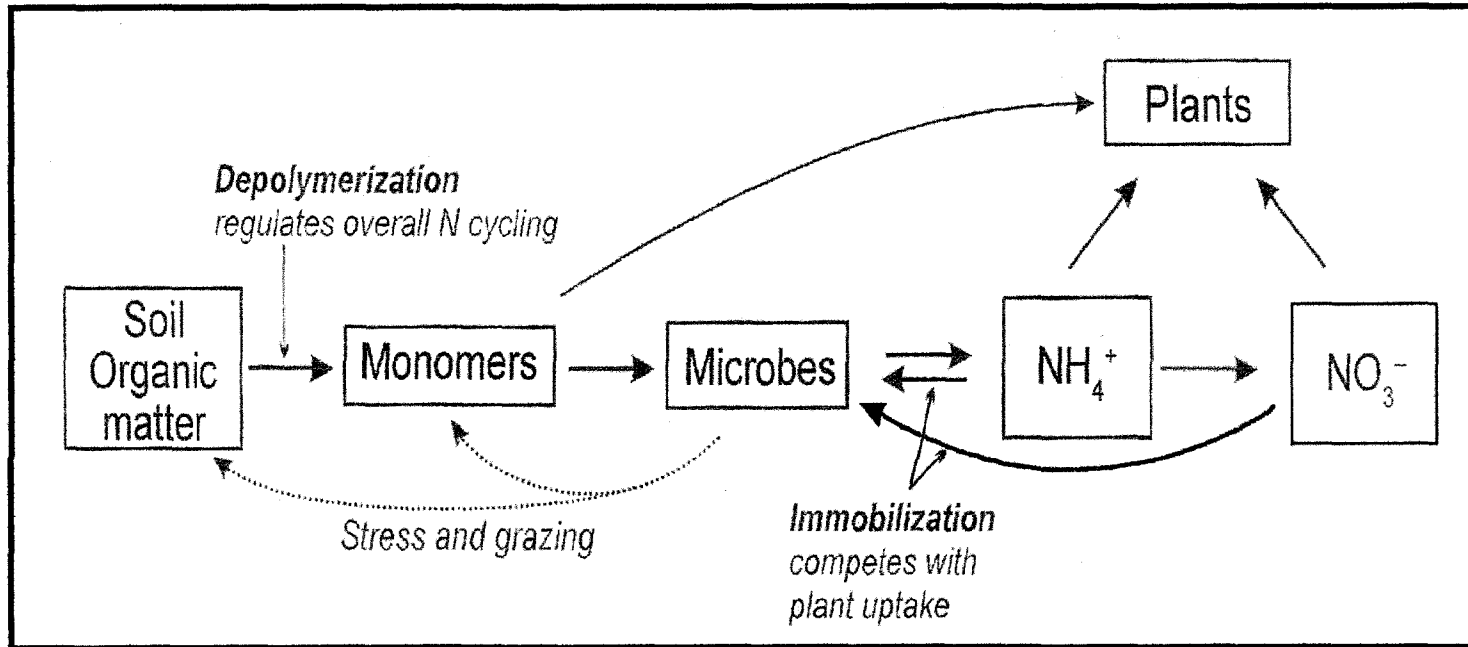
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[1]Brenner, R.E., Boone, R.D. and Ruess, R.W. (in press). Nitrogen Additions to Pristine, High-Latitude, Forest Ecosystems: Consequences for Soil Nitrogen Transformations and Retention in Mid and Late Succession. *Biogeochemistry*.

did not significantly alter microbial biomass N or C. After four years of fertilizer additions there were slight but insignificant changes in fine root C:N and % N. The immediate alteration of N transformation rates and extractable DIN pools, notably the higher NO_3^--N at the 20-30cm depth, may indicate that this ecosystem is sensitive to atmospheric N deposition. However, we also speculate that plants and microbes in this ecosystem, in which the extractable DIN pool is dominated by NH_4^+ (NH_4^+-N: NO_3^--N = 18-30), might be poorly adapted or physiologically unable to assimilate significant quantities of NO_3^-.

Introduction

Prompted by concern for the large increase in human-derived nitrogen (N) inputs into terrestrial ecosystems during the past several decades (Galloway et al. 1995; Vitousek et al. 1997; Asman et al. 1998; Fowler et al. 1998; Mosier et al. 2001), a number of studies in recent years have examined the consequences of experimental N additions to soil nutrient and carbon (C) cycling in temperate and boreal forest ecosystems (Aber et al. 1998; Wright & Rasmussen 1998). These experiments have provided insights into factors regulating nutrient cycling and retention in forest soils and have helped increase our knowledge of the consequences of human-alterations to the global N and C cycles. These studies have shown that forest soils often respond to such additions with increased leaching of nitrate (Tietema et al. 1997; de Schrijver et al. 2000) and base cations (Adams et al. 2000; Hruska et al. 2001), soil acidification (Fenn et al. 1998; Bergholm & Majdi

2001), and increases in N transformation rates, notably net nitrification (Aber et al. 1995; Gundersen et al. 1998; Tietema 1998; Andersson et al. 2001).

Nearly all studies which have simulated N deposition in temperate and sub-arctic forest ecosystems, or which have examined increasing ambient N deposition, have taken place in the northeastern US and Europe where the deposition of N, sulfur and acid rain have been substantial for several decades and also where humans otherwise have altered the landscape for centuries and sometimes longer. Thus, there is the potential that forest research sites experimentally amended with N were adversely affected by one or more anthropogenic disturbances prior to the start of the N additions (Emmett et al. 1998a). Additionally, some N-amended sites are on previously agricultural landscapes that were once fertilized with inorganic and/or manure N. Therefore, despite controls on the amounts of N applied and, to a lesser degree on land-use history (Aber & Driscoll 1997), it has not always been clear to what extent plants and soils responded to the experimental N additions alone versus those plus the combination of long-term atmospheric pollutants, prior fertilization, and land-use change.

Forests in interior Alaska are part of a pristine landscape in a region which has been negligibly influenced by logging or other human disturbances (Van Cleve & Viereck 1981) and which has very low background levels of ambient wet N deposition averaging approximately 0.21 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ (NADP unpublished). This rate is similar to that reported by Perez et al. (1998) for an unpolluted temperate forest in southern Chile (0.1 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$) but is substantially smaller than values reported for forests of western Europe and Scandinavia (2.6-59 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$; wet + dry) (Emmett et al. 1998a; Wright

& Rasmussen 1998), and the northeastern US (5-19 kg $N \cdot ha^{-1} \cdot yr^{-1}$; wet + dry) (Ollinger et al. 1993; Likens & Bormann 1995; Magill et al. 2000) where most experimental N-deposition studies have taken place. Low ambient N deposition and a historic lack of disturbance from pollution or land clearing make Alaska's interior forests an ideal location to investigate the consequences of N additions to soil nutrient cycling in high-latitude forest ecosystems.

In this study we examined the influence of experimental N additions to deciduous and coniferous forest stands which represent mid and late stages of a primary successional sequence along the Tanana River floodplain in interior Alaska. Primary succession in the floodplain ecosystem is initiated by the deposition of glacially-derived silt loam alluvium, on which the early succession plant communities (e.g., willow (*Salix spp.*) and thin-leaf alder (*Alnus tenuifolia*)) are established. The continued addition of mineral alluvium during flooding events builds terraces several meters above the river on which successive plant communities develop. During early succession the N cycle is relatively open, with large amounts of soil N accretion (up to 164 kg $N \cdot ha^{-1} \cdot yr^{-1}$) due to N_2-fixation by thin-leaf alder (Van Cleve 1971; Klingensmith & Van Cleve 1993a). Although, N inputs into this system have been shown to rapidly enter a stable organic matter pool and are not immediately available for microbial processing (Kaye et al. 2003). Balsam poplar (*Populus balsamifera* L.) replaces thin-leaf alder to begin the mid-successional period 20-30 years after initial alluvium deposition, though alder remains a significant component of the understory. Soil N cycling slows during this stage with much reduced rates of N_2-fixation (avg. 38 kg $N \cdot ha^{-1} \cdot yr^{-1}$; Uliassi & Ruess 2002) and soil

N accumulation, and vegetation that is thought to be increasingly N limited (Van Cleve & Viereck 1981; Flanagan & Van Cleve 1983). These deciduous stands are eventually dominated by white spruce (*Picea glauca* (Moench) Voss) after approximately 150 years (Van Cleve et al. 1996).

The transition from balsam poplar to mature white spruce is characterized by significant changes in nutrient cycling processes that are induced by changes in vegetation, notably the formation of a substantial moss layer (Van Cleve et al. 1991; Viereck et al. 1993a). Accompanying this transition there is a general decline in average soil temperatures, primary productivity, soil organic matter decomposability (Flanagan & Van Cleve 1983; Van Cleve et al. 1991) and net soil N transformation rates (Klingensmith & Van Cleve 1993b; Van Cleve et al. 1993b). While primary productivity of both balsam poplar and white spruce may be limited by soil N or P availability, soil microbial communities in white spruce stands are likely limited by labile C due to inputs of recalcitrant litter with a high lignin:N ratio (Flanagan & Van Cleve 1983). In contrast, the soil of balsam poplar stands contains a large pool of labile C from low-molecular weight phenolics, and heterotrophic microbes in this stand type have been theorized to be N limited (Clein & Schimel 1995; Schimel et al. 1998). Thus, within the span of 50-150 years there may be a shift from N-limited to C-limited soil microbes corresponding to changes in aboveground plant community structure.

The objective of this study was to examine the initial effects of approximately 3.5 years of experimental N additions (100 kg $N \cdot ha^{-1} \cdot yr^{-1}$) to stands of balsam poplar and white spruce in order to determine how major differences in plant species composition

and associated soil properties affect soil N transformations and retention. Specifically, we hypothesized that soil microbes in late-successional stands of white spruce (C limited) would be ineffective at immobilizing added N, and would rapidly exhibit characteristics of "N saturation" (Aber et al. 1989; Aber et al. 1998), including an increase in the pool size of nitrate at depth, an increase in net mineralization and net nitrification, and a decrease in soil pH. In contrast, we hypothesized that stands of balsam poplar, in which soil heterotrophs are thought to be N limited and plants have higher rates of net primary productivity (higher N demand), would readily accommodate N additions through N immobilization and increased microbial biomass with lesser changes to soil N transformations or evidence of N leaching losses than in white spruce.

Methods

Study Sites

This study took place within the Bonanza Creek Long Term Ecological Research Site (BNZ-LTER) located along the Tanana River in interior Alaska, approximately 30 km south of Fairbanks (64°45′ N, 148°18′ W). Annual precipitation in this region is very low, averaging only 269 mm, and is exceeded by potential evapotranspiration of 466 mm. The mean annual air temperature is -3.7 °C with extremes ranging from -50 °C in winter to 35 °C in summer (Viereck et al. 1993b). Our research sites were in stands of balsam poplar and white spruce located on terraces >3 m above average river height on islands within the active portion of the floodplain. All sites had frozen soil throughout a significant portion of the growing season; however, balsam poplar sites became ice-free

by early August while white spruce sites generally contained frozen soil until at least early October (Figure 2.1). The soils in these sites are classified as Typic Cryofluvents (Viereck et al. 1983; Van Cleve et al. 1993a) and consist of silt with occasional pockets of sand. Mineral soils on the floodplain are alkaline (pH > 7) due to the high concentrations of $CaCO_3$ from the weathering of carbonate rock by glaciers in the Alaska Range (Marion et al. 1993a; Marion et al. 1993b). As a result of flooding events all sites contained multiple buried organic layers, although the number, depth and thickness of these varies among sites.

Balsam poplar sites (BP1, BP2 and BP3) consisted of mature, uneven stands with some individuals exceeding 100 years of age and a dense understory dominated by thin-leaf alder, rose (*Rosa acicularis*) and intermittent white spruce. White spruce sites (4A, 4B and 4C) consisted of both mature and senescing stands 200+ years in age with an understory of thin-leaf alder, rose and feather mosses (*Hylocomium splendens* and *Pleurozium schreberi*). Alder (*Alnus crispa* and *A. tenuifolia*) was a much smaller component of the understory in white spruce sites than in balsam poplar and was nearly absent at the 4C site. Table 2.1 lists above- and belowground biomass and productivity for these sites. A complete description of plant and soil characteristics for the floodplain successional sequences can be found in Viereck et al. (1993) and on the Bonanza Creek LTER website (LTER unpublished – See References Section).

N-fertilized (30 × 30 m) and control plots (30 × 30 m) were established at each site (n=3 sites per successional stage) when fertilizer additions began during the summer of 1998. Ammonium nitrate (NH_4NO_3) as dry pellets was applied by hand spreader to

fertilized plots in three equal portions over the course of the growing season (June-August) at a rate of 100 kg N ha^{-1} yr^{-1}. Fertilizer was applied each summer during this study and was ongoing as of 2003.

Net Nitrogen Transformations

Net rates of nitrogen (N) mineralization, nitrification and dissolved organic N production were assessed *in situ* from August 1999 to August of 2001 (except for September of 1999) with a modified intact-core incubation technique (Raison et al. 1987). In our view it was not possible or practical to separate organic and mineral horizons without causing a major disturbance to the physical characteristics of the soil due to the high degree of integration between forest-floor, buried organic horizons and mineral soil. Thus, pool sizes, net transformation rates and other dependent variables measured in this study were obtained from intact soil cores consisting of both organic and mineral soil and are presented on an area basis.

At the beginning of each incubation period five pairs of soil cores at the 0-20 cm depth were randomly collected from each plot with a 5.8 cm ID steel hand corer fitted with a 0.5 mm thick plastic sleeve. Cores started at approximately the interface of the Oi and Oa layers and included highly decomposed leaf litter in balsam poplar plots and dead moss in white spruce plots. One core from each pair was immediately brought to the laboratory for processing. The other core, collected within a sleeve pre-drilled with approximately 30, 0.5 cm- diameter holes, was placed in a 0.025 mm (1 mil) plastic bag within a fiberglass "mosquito mesh" bag, then re-inserted into the soil and covered with

surface litter. This procedure was designed to keep cores intact and allow the free exchange of air while keeping soil moisture constant. Cores remained in the soil for approximately 30 days during the growing season (June to October) but were incubated from October to late-May during the winter. All cores were kept cool during transport to Fairbanks where they were sieved (to 5.6 mm) and homogenized within 24-48 hrs of collection. A 10 g sample of homogenized field-moist soil from each core was extracted with 75 mL of 0.5M K_2SO_4 for 24 hrs before vacuum-filtration through a 1-μm pore diameter glass fiber filter. Subsamples from each core were taken for determination of gravimetric water content. Four times during the study (July 1999, July 2000, August 2000 and September 2000) we also incubated soil from the 20-30 cm depth of the soil profile *in situ* in order to examine net N transformations and extractable N within the deeper mineral soil.

Soil extracts were analyzed for ammonium and nitrate + nitrite with an API 300 segmented flow autoanalyzer (Astoria-Pacific Inc., Clackamas, Oregon, USA). Dissolved organic nitrogen (DON) in extracts was determined by digestion with a buffered potassium persulfate solution (Cabrera & Beare 1993) followed by nitrate analysis.

Net mineral N production was calculated as the total change in extractable NH_4^+-N + NO_3^--N per incubation period while net production of ammonium (ammonification) and nitrate (nitrification) were separately determined from the change in NH_4^+-N or NO_3^--N, respectively, during the incubation period. Net DON production was calculated as the net change in DON per incubation period. Annual net N transformations were calculated

for the complete 1-yr period (June 2000-June 2001) and the 2-yr period from August 1999-August 2001 (excluding September-October 1999) by summing N production across incubation periods within each plot and – in the case of the 2-yr data set – adjusting for the total number of incubation days. Mean annual net N transformation rates from individual plots were then used as replicates in the split-plot ANOVA (see *Statistical Analysis* below).

Microbial Biomass

Microbial biomass in soil samples taken from initial cores in June of 2001 was determined using chloroform fumigation-extraction (CFE) (Horwath & Paul 1994). Fumigated and non-fumigated extracts were digested with potassium persulfate as described for DON except that the digestion took place in serum vials fit with rubber septa which were crimp-capped. Solutions containing 0 to 150 mg $C \cdot L^{-1}$ phenylalanine were used as internal digestion standards. Phenylalanine was chosen as a standard because it contains an aromatic ring and was thought to provide a good comparison to the types of complex molecules (e.g., humics and phenolics) found in soil solution.

Following digestion, serum vials were cooled to room temperature and the pressure inside each vial was measured using a pressure transducer (Soil Measurement Systems, Tucson, Arizona, USA). A 10-15 cc headspace sample was then drawn into a syringe and immediately analyzed for CO_2 using a LICOR 6200 (LICOR, Lincoln, Nebraska, USA) modified with a syringe-injection system. In order to determine an approximate digestion efficiency of samples, the predicted amount of CO_2 in the

headspace of the phenylalanine standards was calculated using a pressure-volume equation and compared to linear curves of the actual CO_2 in the headspace of the standards measured with the LICOR. The digestion efficiency of the phenylalanine standards was determined to be >90%, and the amount of dissolved C in the samples was subsequently determined using the linear regressions from these standards. Digestion efficiency was based on C rather than N because it is 10 or more times more prevalent than N in the extraction solution and requires the largest portion of oxidizing power from the potassium persulfate. After headspace sampling, the solution in each serum vial was removed and analyzed for nitrate as for soil extracts. We did not use a conversion factor to correct for extraction efficiency of C (K_{ec}) or N (K_{en}). A conversion factor is dependent upon soil properties (e.g., organic matter content) and is likely highly variable among floodplain forests due to stand and plot-level variation in buried organic horizons and, perhaps, associated differences in microbial community composition.

Soil and Fine-Root C, N and pH

Total soil C and N were determined for subsamples of homogenized soil cores collected throughout the course of the study. Total C and N of live fine-roots (<0.5 mm), removed from soil cores collected in August of 2001, were determined using a LECO CNS 2000 autoanalyzer (LECO, St. Joseph, Michigan, USA). Soil pH from 0-20 cm soil was determined on field-fresh samples collected in October of 2000 (Robertson et al. 1999). All pools of C and N in the soil were first calculated per g 105°C oven-dry soil and then converted to an area basis using plot-level estimates of bulk density.

Statistical Analysis

Comparisons of N transformation rates and pool sizes between individual incubation periods were done with a restrictive maximum likelihood (REML) technique (Littell et al. 2002) using PROC MIXED in SAS (SAS 1999). Models with appropriate covariance structures (first-order autoregressive, unstructured and Toeplitz) were compared, and the model with the lowest Akaike Information Criterion (AIC) value was used for further analyses. Standard errors and degrees of freedom were obtained using a Kenward and Roger correction (Littell et al. 2002).

Pool size and N transformation rate data from individual soil cores collected within each of the 12 research plots were averaged so that each plot represented a single replicate. Data that were not collected over several time periods (e.g., fine-roots, microbial C and N, soil pH) or that were obtained by averaging values across time (e.g., DIN pools and yearly N transformation rates) were analyzed using a split-plot ANOVA design with the GLM module of Statistica (Statsoft 2003) or with PROC MIXED in SAS. Stand type was the between-subject (whole plot) factor, and treatment the within-subject (split-plot) factor. Significant effects for planned tests were further analyzed using paired contrasts, and Satterthwaite's approximation was implemented when exact F-tests were not possible. Both linear and non-linear regressions were used to explore relationships between microbial C and N and soluble N pools. For all analyses the homogeneity of variance assumption was tested using Levene's test. When there were significant deviations from this assumption, data were square-root transformed (Zar 1999), and an

additional analysis was performed. Data presented in tables and figures are means ± 1 standard error (S.E.) from untransformed data. Significance for all tests was established at the $P \leq 0.05$ level; however, we classify as "marginally significant" values ≤ 0.10.

Results

Extractable N Pools

Fertilization significantly increased 0-20 cm extractable DIN pools within the first year (1999) following N additions (Figure 2.2) ($F_{1,4}=8.8$; $p=0.04$); however, there was not a consistent increase in soil DIN over time for either stand type. When averaged across all sampling periods, pools of extractable NH_4^+-N and NO_3^--N (mg $N \cdot m^{-2}$) were significantly larger in fertilized plots compared to control plots for both stand types (Table 2.2). Although pool sizes of extractable NH_4^+-N and NO_3^--N were similar for the fertilized plots of both stand types, there was a significant and much larger absolute increase in DIN following fertilization in white spruce compared to balsam poplar ($F_{1,4}=14.7$, $p=0.02$), due primarily to an increase in NH_4^+-N (Figure 2.2, Table 2.2). During July of 2001, the last measurement period, there was a spike in NO_3^--N in fertilized white spruce which was significantly larger (Figure 2.2b; $F_{1,4}=15.4$; $p=0.02$) than extractable NO_3^--N pools during all other periods. For 0-20 cm soil control plots, extractable NH_4^+-N made up the vast majority of DIN in both stands types; however, the pool size of NH_4^+-N was over twice as large ($F_{1,4.7}=8.3$; $p=0.04$) in control plots of balsam poplar compared to white spruce. Pools of extractable NO_3^--N in control plots were quite low (<0.04 g $N \cdot m^{-2}$) and not significantly different between stand types (Table 2.2a; $F_{1,7.98}=0.03$; $p=0.86$).

Dissolved organic nitrogen (DON) made up the largest pool of soluble N (mg N·m^{-2}) for 0-20 cm soil (Table 2.2). The pool size of DON was not affected by N fertilization but was significantly larger for balsam poplar compared to white spruce (Table 2.2a; $F_{1,8}$=7.1; p= 0.03), averaging 3733 ± 309 mg N·m^{-2} for balsam poplar and 2571 ± 254 mg N·m^{-2} for white spruce when control and fertilized plots were considered together.

Fertilization significantly increased average extractable pools of NO_3^--N in 20-30 cm mineral soil cores for both stand types when averaged across all time periods (Table 2.2b). In September 2000 (Figure 2.3), the last time mineral soil cores from the 20-30 cm depth interval were collected, NO_3^--N concentrations were significantly elevated in fertilized white spruce ($F_{1,4}$=15.2; p=0.02) and balsam poplar ($F_{1,4}$=8.9; p=0.04), compared to those collected during all other time periods. In contrast, NH_4^+-N concentrations in 20-30 cm cores were not significantly different for control and fertilized plots within either stand type (Table 2.2b) and did not increase over time (data not shown). As in 0-20 cm cores, the majority of soluble N for the 20-30 cm cores consisted of DON (Table 2.2b). DON at this depth did not differ significantly by stand or treatment type.

Net N Transformations

Across stand types, fertilized plots had significantly higher annual rates of net N mineralization (0-20 cm soil depth, Table 2.3) when calculated for the entire two-year study. Relative to control plots, annual net production of DIN was 62% higher in the

fertilized plots of balsam poplar ($F_{1,4}$=12.5; p= 0.02) and 77% higher in white spruce (marginally significant, $F_{1,4}$=4.3; p=0.10). Annual net N mineralization was also higher for control plots of balsam poplar than white spruce, although not significantly ($F_{1,\,5.76}$ = 3.6; p= 0.11).

When considering just the 2000-2001 period, fertilization increased annual net mineralization in white spruce but not balsam poplar stands (Table 2.3). Control plots of balsam poplar had significantly higher annual net mineralization than white spruce control plots during this period ($F_{1,\,7.81}$ = 6.4; p = 0.04).

For the two-year study period, fertilization increased annual net nitrification rates (mg NO_3^--N m^{-2} yr^{-1}), but only in balsam poplar (Table 2.3), where net nitrification was higher than in control plots during eight of the nine incubation periods (Table 2.4). Although fertilization did not increase average net nitrification in white spruce for the two-year period, this was largely due to unusually high net immobilization of NO_3^--N during July-August 2001 (Table 2.4) which offset the majority of incubation periods in which nitrification was either marginally higher or not statistically different in fertilized plots. For the 2000-2001 period fertilization significantly increased annual net nitrification across stand types (Table 2.3), but this was due principally to a fertilization response in balsam poplar (marginally significant, $F_{1,4}$=4.7; p=0.10) as white spruce stands did not have significantly higher nitrification during this period ($F_{1,4}$=3.9; p=0.12).

Fertilization tended to increase ammonification rates in white spruce, but lowered them in balsam poplar (Table 2.3, Table 2.4). This created a significant stand×treatment

interaction for the one-year (2000-2001) incubation period and a marginally significant interaction effect for the two-year incubation period.

There was a large amount of variation in net N transformations during the two overwinter incubation periods (Table 2.4). In the winter of 1999-2000, net N ammonification tended to be positive and accounted for a large portion of the yearly net N mineralization rate, but in 2000-2001 net N ammonification tended to be negative except in white spruce fertilized plots where it was positive. Net nitrification rates were also highly variable but were positive for all stand×treatment combinations during both overwinter periods.

Net rates of DON production were strongly positive for balsam poplar (697 ± 257 mg DON·m^{-2}·yr^{-1}) but strongly negative for white spruce (-680 ± 278 mg DON·m^{-2}·yr^{-1}) when computed for the two-year time period (Table 2.3; $F_{1,4}$=10.01; p=0.03). Fertilization did not significantly affect net DON production rates over this period for either stand type, although rates were somewhat lower in fertilized stands of balsam poplar relative to controls. A similar trend existed during the 2000-2001 period when there was a marginally significant effect from stand type on net DON production and a marginally significant effect due to fertilization. During this period white spruce control plots had significantly higher net DON production than fertilized plots (Table 2.3; $F_{1,4}$=9.8; p=0.03).

There were no significant stand or treatment effects on net N mineralization rates (mg DIN·m^{-2}·day^{-1}) for the 20-30 cm mineral soil cores (data not shown). Net N mineralization rates were very low during the four periods measured, and average net

mineralization for this soil depth was not significantly different from zero for any stand×treatment combination.

Microbial Biomass C&N

Fertilization did not have a significant affect on either microbial biomass C or N within either stand type (Figure 2.4); however, slightly larger pools of microbial N and slightly smaller pools of microbial C resulted in significantly lower microbial C:N in fertilized stands relative to control stands ($F_{1,4}$= 10.2; p=0.03) (9.4 ± 0.4 for control plots vs. 7.9 ± 0.4 for fertilized plots). There was no difference in microbial C:N between stand types ($F_{1,4}$= 0.4; p=0.56). When averaged across control and fertilized plots, balsam poplar stands had 88% higher microbial biomass C (Figure 2.4; $F_{1,4}$=36.7; p = 0.004), and 93% higher microbial biomass N, than white spruce ($F_{1,4}$=203.5; p < 0.0001).

Soil and Fine-Root C & N and Soil pH

Fertilization did not have a significant influence on either fine-root C:N ($F_{1,4}$=2.5; p=0.19) or %N ($F_{1,4}$=2.2; p=0.23) (Figure 2.4b). Fine-root C:N was significantly lower ($F_{1,4}$=18.2; p=0.01; Figure 2.4b), and fine-root %N significantly higher ($F_{1,4}$=29.2; p=0.006), in balsam poplar stands compared to white spruce. The 0-20 cm soil C:N (Table 2.2) was also significantly lower in balsam poplar compared to white spruce ($F_{1,4}$=53.96; p=0.002) but was unaffected by fertilization ($F_{1,4}$=0.17; p=0.7). Soil pH (Table 2.2) was generally lower in the fertilized plots of both stand types, but this difference was not significant ($F_{1,4}$ = 3.4; p=0.13). Across control and fertilized plots,

stand type had a marginally significant effect on 0-20 cm soil pH ($F_{1,4} = 6.7$; p=0.06) which was higher in balsam poplar than white spruce (Table 2.2).

Discussion

N Transformations and Pool Sizes From Intact Cores

Results from this study do not support our hypothesis that balsam poplar would be minimally impacted by N fertilization. Fertilizer additions brought about significant alterations to soil N transformations in balsam poplar by decreasing net ammonification and substantially increasing both annual net nitrification as well as pools of DIN (0-20 cm depth) and NO_3^--N (20-30 cm depth). Given our assumption that soil microbes (Clein & Schimel 1995; Schimel et al. 1996; Schimel et al. 1998) and perhaps plants in balsam poplar stands were N limited, and would quickly immobilize fertilizer additions, we were surprised by the speed and magnitude of response in this stand type. By 1999, just one year after initial fertilizer additions, the pool size of extractable NO_3^--N in balsam poplar was already appreciably higher in fertilized balsam poplar plots than in control plots for the 0-20 cm (Figure 2.2a) and 20-30 cm cores (Figure 2.3). Because negligible root biomass was observed at the 20-30 cm soil depth, we believe that an increase in NO_3^--N following fertilization is indicative of N leaching. Thus, it would appear that large-scale net immobilization of added N may not have occurred as we had anticipated or that the added N simply overwhelmed plant and microbial uptake.

Soil microbes in the balsam poplar stands of this study may not have been as N limited as we had originally thought or may have had very different soil characteristics

than those from the Clein and Schimel (1995) study. For example, their soil contained only forest floor organic material that likely had a much higher proportion of phenolic-rich leaf litter and could very well have been N limited. In contrast, we purposefully excluded the prior season of leaf litter from our 0-20 cm intact cores, but our soil did include several buried organic horizons. We suspect that our soil consisted of older, more highly decomposed organic material which had reduced heterotrophic N demand and, perhaps, also contained a higher density of N-fixing nodules.

An overall annual net nitrification rate that was dramatically higher in fertilized balsam poplar plots (Table 2.3) is consistent with previous suggestions that nitrification in poplar is primarily controlled by soil N availability rather than allelopathic inhibition from the large amount of secondary metabolites produced by this species (Clein & Schimel 1995; Schimel et al. 1996; Uliassi et al. 2000). We believe that fertilizer additions produced an immediate increase in overall N availability such that soil nitrifiers could more successfully compete with heterotrophic microbes (and perhaps plants) for NH_4^+-N. This reasoning may seem counterintuitive given that control plots of balsam poplar already had a higher NH_4^+-N supply than white spruce; however, it is important to note that net nitrification was also substantially higher in control plots of balsam poplar compared to white spruce (Table 2.3). Thus, nitrification may already have been stimulated in balsam poplar prior to N additions. The much larger pool size of NH_4^+-N in balsam poplar control plots may be a function of the microbial biomass (larger in balsam poplar, Figure 2.4a) involved in N transformations combined with the temporal gap between NH_4^+-N production and immobilization -- a "snapshot" of a flux. Thus, NH_4^+-N

pool size may be less representative of N demand than it is of NH_4^+-N flow between pools. Long-term measurements of *in situ* gross N mineralization and nitrification and the microbial pool size would be needed to resolve this issue.

Arguably, fertilization produced an even greater change to soil N cycling and DIN pool sizes in white spruce. Annual rates of net N mineralization, driven by elevated ammonification (Table 2.3, Table 2.4), consistently increased with fertilization in white spruce plots but were only occasionally higher following fertilization in balsam poplar. This would indicate that N additions overwhelmed the ability of soil heterotrophs in white spruce to immobilize excess N. There was also a significantly larger absolute increase in the average pool size of DIN for 0-20 cm soil and NO_3^--N for 20-30 cm soil (though not significant) in white spruce compared to balsam poplar. Although, it is unclear if such stand-level differences were due to dissimilar plant or microbial N demand or were the result of contrasting patterns of N losses such as leaching or denitrification. The large spikes in NO_3^--N for 0-20 cm during July 2001 (Figure 2.2b) and 20-30 cm soil during September 2000 (Figure 2.3) in fertilized white spruce – the last time these depths were sampled – may indicate that nitrate leaching was increasing dramatically. In contrast, *in situ* denitrification has previously been shown to be negligible in these stands (Klingensmith & Van Cleve 1993a) and suggests that this process was not responsible for controlling DIN pool sizes following fertilization. However, that study only measured denitrification from July - September during a single growing season. We believe that denitrification may be an important source of

ecosystem N losses during the spring (mid-May to mid-June) when soil is at or near saturation following snowmelt.

Our results, which show that white spruce soils in interior Alaska responded quickly to fertilizer additions, with NO_3^--N leaching and increases in net N mineralization, are similar to other studies of northern coniferous forests exposed to long term N additions (Emmett et al. 1998b; Gundersen 1998). However, our study sites did not exhibit the consistent increases in nitrification or a drop in soil pH often associated with "N saturation" in coniferous stands. This may be due to the relatively recent nature of N additions in this study (< 4 years) and because the alkaline soils of the floodplain served to buffer the effects of N additions.

The measurement of net DON production during soil incubation experiments is not one that has been used widely in the literature (but see Neff & Hooper 2002) and may not be particularly meaningful to specific aspects of plant or microbial N demand. However, we presented measurements of DON production here in order to stimulate discussion of what is, increasingly, regarded as an important component of plant and microbial N uptake in the boreal forest (Nasholm et al. 1998; McFarland et al. 2002) and to highlight the higher DON production in balsam poplar relative to white spruce (Table 2.3). We speculate that input to the DON pool is tied to the heterotrophic breakdown of soil detritus as well as the release of organic leachates from decomposing leaves and roots. Thus, the measurement of net DON production may be an index of inputs into the soluble pool and indicate a larger or more active pool of detritus in balsam poplar compared to white spruce.

Plant N Demand

The lack of significant change in fine-root %N or C:N following several years of N additions (Figure 2.4b) mirrors results by Pregitzer et al. (2002) who measured fine-roots from these same plots shortly after the initiation of fertilization in 1998 and 1999. Since roots from our study were collected in August of 2001, after two additional years of N additions, our results might indicate that the C:N of these roots is relatively fixed or indicate that plant N demand was not as high as we had originally anticipated. It is also probable that some component of these stands (e.g., alder) is limited by phosphorus rather than N (Uliassi et al. 2000; Uliassi & Ruess 2002).

Perhaps a more meaningful indication of plant N limitations will come not from changes to tissue N concentrations but rather from ongoing studies of belowground processes examining the response of fine root production and turnover to increases in soil N availability. Minirhizotron-based estimates from control plots show that fine root production was 67% higher in balsam poplar than white spruce (Table 2.1) and likely play a large, albeit unknown, role in determining microbial N and C demand in these stands. A large portion of fine roots in this system die and decompose within a year of being produced (Ruess et al. *in review*). Fine roots have low C:N ratios, and may account for a substantial source of actively cycled soil N; however, there is uncertainty regarding how much fine root N may be retranslocated prior to root death or senescence (Gordon & Jackson 2000), and the amount of plant N retranslocation may vary widely depending upon plant and soil nutrient status (Salifu & Timmer 2001). The answer to this problem

has large implications for microbial C and N demand: If much of the N in fine roots is retranslocated prior to senescence then soil microbes should have a large N demand as they decompose a labile source of C; however, if much of the N remains in senesced roots then soil microbes should cycle a substantial portion of this back into soil during decomposition.

Ecosystem Nitrate Utilization

Could there be a common factor responsible for the immediate increase in extractable NO_3^--N pools observed following fertilization in both stand types for 20-30 cm soil? A plant and/or microbial preference for NH_4^+-N over NO_3^--N (or inability to utilize NO_3^--N) could be the answer. Ammonium, which dominates the salt-extractable DIN pool in these stands (NH_4^+-N:NO_3^--N = 18.2 in white spruce and 30.3 in balsam poplar; Table 2.2), is known to inhibit nitrate reductase activity in both plants (Larcher 1995) and microbes (Atlas & Bartha 1993; Myrold 1999). It has also been suggested that plants and microbes exposed to increased N deposition have a reduced ability to absorb NO_3^--N (Kjonaas et al. 1998), and Tietema (1998) reported no immobilization of NO_3^--N by soil microbes in sites with substantial N deposition in northwestern Europe. White spruce in particular is known to have a very limited capacity to utilize NO_3^--N (Kronzucker *et al.* 1995a; Kronzucker *et al.* 1995b;1997), while field and laboratory studies have shown that Norway spruce (*Picea abies*) and beech (*Fagus sylvatica*) have a reduced or complete inability to take up NO_3^--N when exposed to N fertilizer or when grown in soil with a high NH_4:NO_3 ratio (Gessler et al. 1998; Rennenberg et al. 1998).

Several other studies (Chapin et al. 1986; Hangs et al. 2003; Yarie 1993) also report low or negligible uptake of NO_3^--N, but substantial uptake of NH_4^+-N by balsam poplar, aspen (*Populus tremuloides*), green alder (*Alnus crispa*), paper birch (*Betula papyrifera*) and jack pine (*Pinus banksiana* Lamb.).

Thus, many coniferous and deciduous trees in the boreal forest generally may have a limited ability to utilize NO_3^--N, especially in the presence of larger amounts of DIN or NH_4^+, which may inhibit the uptake of NO_3^--N and/or suppress nitrate reductase. While the leaching of NO_3^--N to the 20-30 cm soil depth in this study could, in part, be attributed to the high mobility of NO_3^--N in the soil profile relative to NH_4^+-N, this may not fully explain why NO_3^--N was able to move through multiple buried organic horizons within a year after initial fertilizer additions.

Microbial C and N

Contrary to our prediction, N fertilization did not increase microbial biomass in balsam poplar stands; rather, biomass remained unchanged in both stand types after more than three years of N fertilization while microbial C:N was significantly lower across both stand types. Although we are skeptical that microbial C:N was actually reduced in response to fertilization, rather than changed by slight differences between control and fertilized plots, Tietema (1998) also observed lower microbial C:N ratios following N additions and found that microbial biomass C remained unchanged across a wide gradient of N deposition in coniferous forests across Europe. Aber et al. (1995, 1998) (hardwoods and conifers) and Gundersen (1998) (conifers) also concluded that microbial biomass did

not change following large-scale N addition, while Fisk & Fahey (2001) and Corre et al. (2003) (hardwoods) showed a significant decrease in chloroform-extractable biomass in N fertilized stands. Thus, the response of soil microbial biomass to long-term N additions would appear to be variable across forest ecosystems.

The much larger pool of microbial biomass in balsam poplar compared to white spruce (Figure 2.4a), but lack of change following fertilization, suggests that factors associated with plant composition and inputs (fine-root and litter quality) have a much stronger influence on microbial biomass in this ecosystem than short-term N inputs and availability. In this regard we reiterate our view that the production and turnover of labile fine roots plays a major role in belowground carbon and nutrient cycling and, almost certainly, microbial biomass in the boreal forest where the ratio of belowground production to aboveground litter fall inputs is high relative to temperate forests (Ruess et al. 1996, Ruess et al. 2003). We predict that, with longer term N additions in these stands, the degree to which plant belowground production and turnover may be altered with increased N availability will ultimately determine whether or not there are significant changes in microbial biomass.

Conclusions

The results from this study help to elucidate controls on successional patterns of ecosystem N cycling as well as the types of responses that could be expected as pristine high-latitude forests experience increased deposition of human-derived reactive N. Though current N deposition in Alaska's interior boreal forest is exceptionally low, N

deposition is already elevated beyond pre-industrial levels in parts of the boreal forests in Canada and Russia (Holland et al. 1999), and global N deposition is predicted to increase substantially during the next several decades (Galloway et al. 1994; Galloway et al. 1995). The 100 kg $N \cdot ha^{-1} \cdot yr^{-1}$ applied during this study is in excess of any conceivable near-term increase in human-induced N deposition to interior Alaska; however, primary productivity in these stands is considerably higher than other plant communities (e.g., black spruce) in this region. Thus, the responses of soil N cycling observed here (e.g., the leaching of NO_3^- and the alteration of N mineralization and nitrification) could occur with substantially smaller inputs of N in other pristine boreal communities which have much lower N demand. We believe that both deciduous and coniferous high-latitude forests, in which state factors such as light and temperature play a critical role in limiting nutrient cycling and primary production during a brief growing season, are particularly vulnerable to the effects of N deposition compared to temperate systems with higher overall ecosystem N demand.

Acknowledgements

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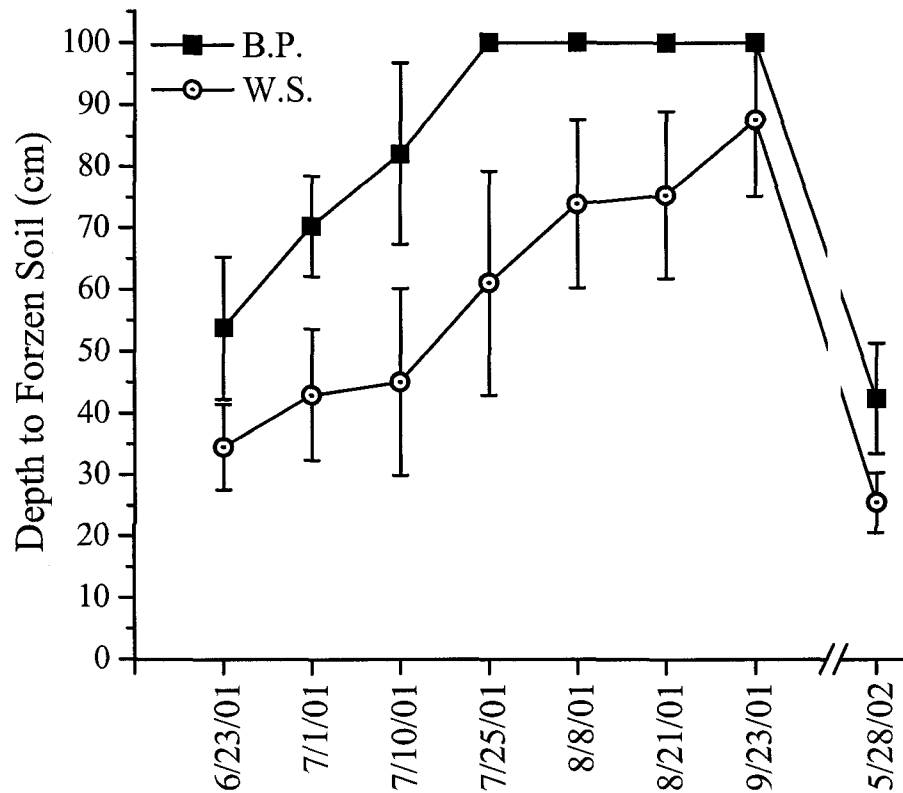
Figure 2.1. Growing season depth-of-thaw for stands of balsam poplar (BP) and white spruce (WS). Measurements were taken between June 2001 and May 2002 using a 1 m frost probe. Values are mean ± 1 S.E. and n=3 plots per stand type. In balsam poplar stands, the exact depth of thaw after July 10 can only be characterized as > 1 m.
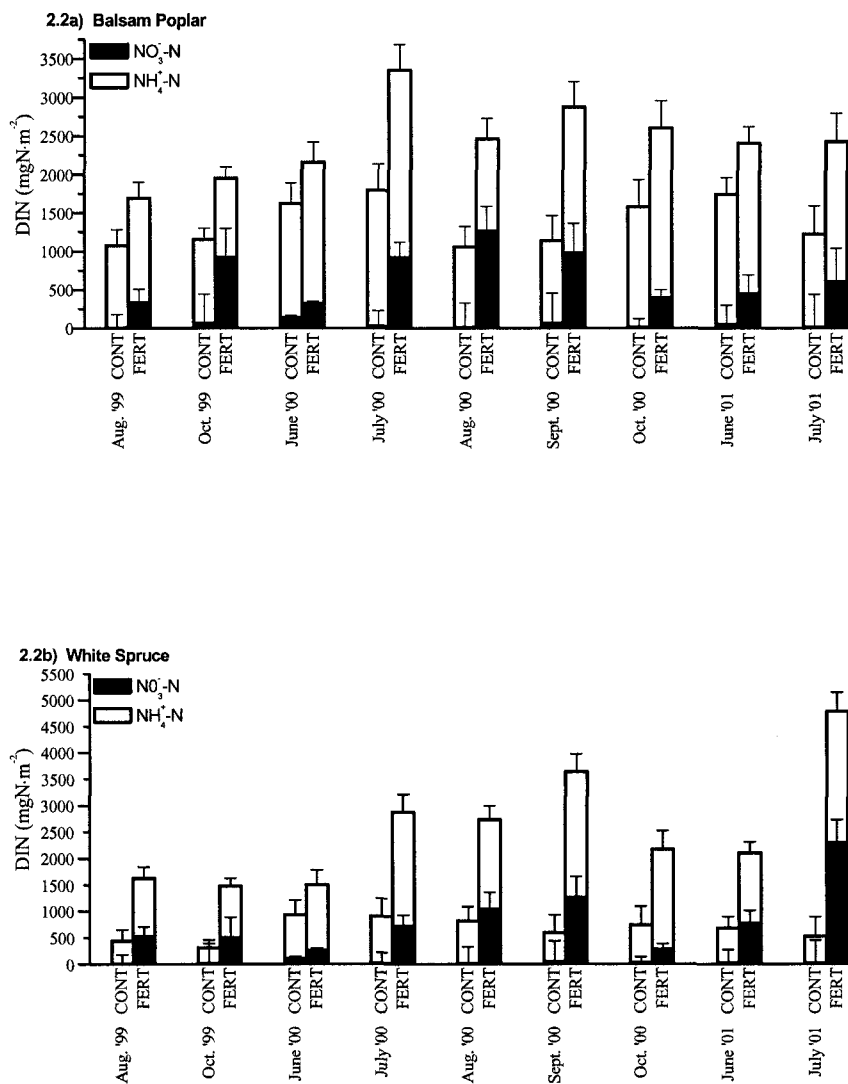
Figure 2.2. Pool size (mgN·m^2) of 0.5M K$_2$SO$_4$-extractable inorganic N for 0-20 cm soil depth. Soil cores were collected at each of the 9 incubation periods for control (CONT) and fertilized (FERT) plots of a) balsam poplar and b) white spruce (WS). Ammonium-N begins at the top of the NO$_3$-N bar, thus bar height indicates total the sum of NO$_3$-N + NH$_4^+$-N or DIN. Values are means (+1 S.E.) and n=3 for each stand×treatment combination.
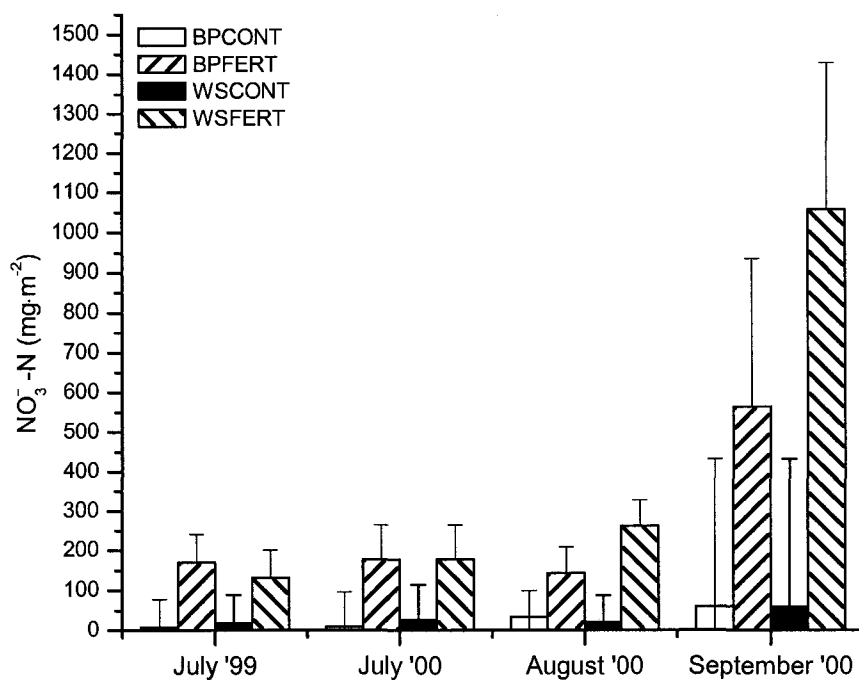
Figure 2.3. Pool size (mg·N m^{-2}) of 0.5M K$_2$SO$_4$-extractable NO$_3^-$-N from 20-30cm soil depth. Soil cores were collected from control (CONT) and fertilized (FERT) plots of balsam poplar (BP) and white spruce (WS) at four times during the two year study. Values are means + 1 S.E. and n=3 replicate plots for each stand×treatment combination. Graph is from untransformed data but data were square-root transformed before ANOVA.
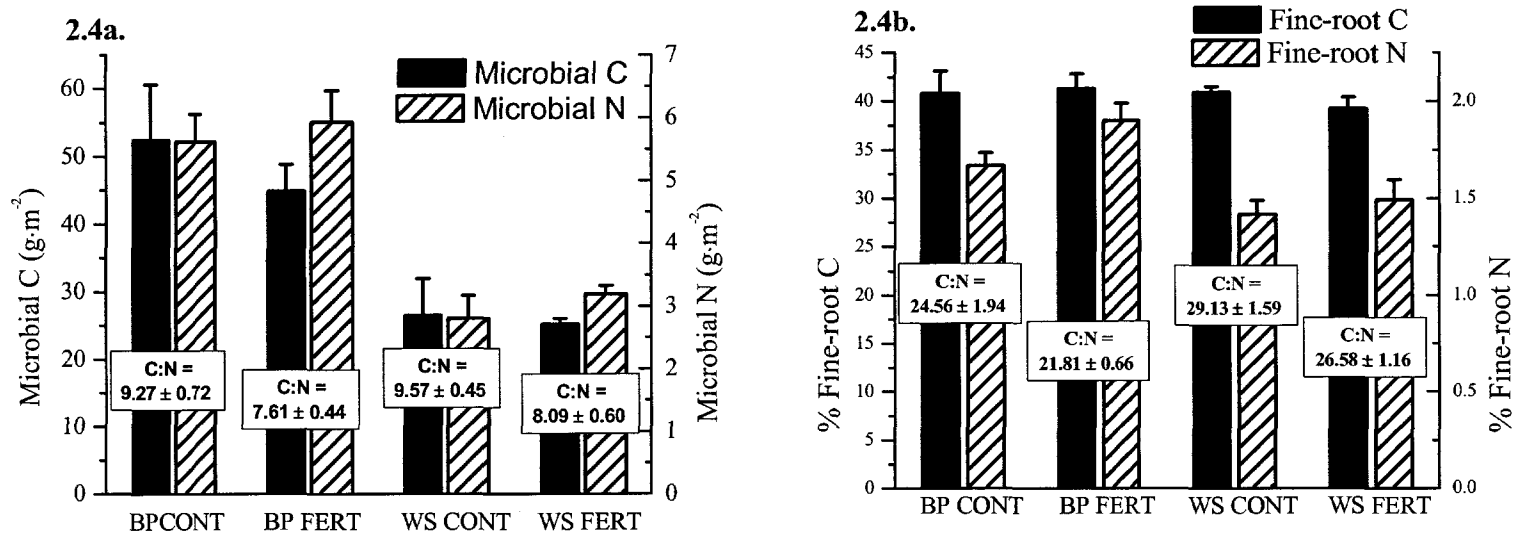
Figure 2.4. Microbial biomass C, N (g m^{-2}) and C:N. (2.4a); and Fine-root (<0.5 mm) %:C, % N and C:N (2.4b) for 0-20 cm depth soil. Values were determined from soil cores collected in control (CONT) and fertilized (FERT) plots of balsam poplar (BP) and white spruce (WS). Balsam poplar stands had significantly higher microbial biomass C, N, fine-root % N and significantly lower fine-root C:N than white spruce, but only microbial C:N was significantly affected (reduced) by N fertilization. Values are means + 1 S.E. and n=3 replicate plots for each stand×treatment combination.

Table 2.1. Select stand structure and biomass production data. Values are for balsam poplar and white spruce stands on the Tanana River floodplain.

Stand Type	Species	[1]Stem Density (stems ha^{-1})	[1]Basal Area (m^2 ha^{-1})	[1]Aboveground Tree Biomass (kg ha^{-1})	[2]Aboveground Production (kg ha^{-1} yr^{-1})	[1,3]F Litterfall (kg ha^{-1} yr^{-1})	[4]Fine Root Production (kg ha^{-1} yr^{-1})
Balsam poplar	*Populus balsamifera*	763 ± 93	36.7 ± 2.9	170411 ± 14079	5236 ± 355	2585 ± 202	3036 ± 428
	Alnus tenuifolia	485 ± 77	1.8 ± 0.3				
White spruce	*Picea glauca*	518 ± 36	38.9 ± 5.5	203089 ± 26837	4541 ± 833	1020 ± 311	1814 ± 605
	Alnus crispa/tenuifolia	148 ± 158	0.6 ± 0.7				

Biomass, production and litterfall values are expressed as kg oven-dry (65°C) mass. N=6 for each stand type.

[1]Ruess, unpublished data
[2]Includes trees, shrubs and bryophytes (Ruess et al. *in review*).
[3]Litterfall includes leaves and needles, fine wood (< 10 cm) and reproductive litter.
[4]Stand-level estimates of fine root production derived from minirhizotrons (Ruess et al. *in review*).

Table 2.2. Soil C and N content and pH. Values are for a) 0-20 cm soil and b) 20-30 cm soil cores. Cores were collected in control (CONT) and N fertilized (FERT) plots of balsam poplar and white spruce. Values are means ± 1 S.E. taken from a repeated-measures ANOVA of nine sampling events spanning the two-yr study period (August '99-July '01). There are n=3 replicate plots for each stand×treatment combination.

2.2a.) 0-20 cm soil

Stand	Treatment	pH	Soil C $(gC \cdot m^{-2})$	Soil N $(gN \cdot m^{-2})$	C:N	NH$_4$-N $(mgN \cdot m^{-2})$	NO$_3$-N $(mgN \cdot m^{-2})$	DON $(mgN \cdot m^{-2})$
Poplar	CONT	7.62	5174	298	17.36	1333	44	3910
	(± S.E.)	(0.22)	(367)	(25)	(0.37)	(58)	(5)	(319)
						**	**	
	FERT	7.06	5220	289	18.06	1749	687	3556
	(± S.E.)	(0.34)	(330)	(23)	(0.43)	(66)	(262)	(587)
		P=0.06		*	**			*
Spruce	CONT	6.43	4569	193	23.67	626	34	2499
	(± S.E.)	(0.49)	(784)	(41)	(1.31)	(166)	(2)	(502)
						***	**	
	FERT	6.18	3997	174	22.97	1690	853	2642
	(± S.E.)	(0.12)	(412)	(18)	(0.09)	(291)	(220)	(256)

Table 2.2 Continued. Soil C and N content and pH for 20-30 cm soil.

2.2b.) 20-30 cm soil

Stand	Treatment	pH	Soil C $(gC \cdot m^{-2})$	Soil N $(gN \cdot m^{-2})$	C:N	NH_4-N $(mgN \cdot m^{-2})$	NO_3-N $(mgN \cdot m^{-2})$	DON $(mgN \cdot m^{-2})$
Poplar	CONT	7.71	959	61	15.72	225	28	869
	(± S.E.)	(0.28)	(178)	(11.5)	(0.75)	(44)	(6)	(125)
							*	
	FERT	7.70	1881	94	20.01	157	264	945
	(± S.E.)	(0.22)	(749)	(29)	(2.95)	(13)	(64)	(201)
Spruce	CONT	7.14	1181	67	17.63	302	31	836
	(± S.E.)	(0.34)	(311)	(20)	(0.56)	(89)	(9)	(240)
							*	
	FERT	6.51	1220	72	16.94	220	407	736
	(± S.E.)	(0.42)	(647)	(29)	(2.15)	(51)	(232)	(370)

Asterisks between stands indicate significance of stand-level effects; those between treatments indicate significant contrasts, where: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2.3. Annual net N mineralization, nitrification, ammonification and DON production (mg $N \cdot m^{-2} \cdot yr^{-1}$) for 0-20 cm depth soil. Values were based on the entire two-years of the buried-bag study and on the complete one-year period from June 2000-June 2001 ($\pm$ 1 S.E; n=3 experimental plots for each stand×treatment combination). Estimates were calculated by summing plot-level production of individual N species during either the one-year period from June 2000 to June 2001 or the two-year period from August 1999 to July 2001. P-values between control (CONT) and N-fertilized (FERT) means are for contrasts, p-values at bottom of table are from the split-plot ANOVA with 1 and 4 degrees of freedom. Significant, $p < 0.05$, and marginally significant, $p < 0.10$, p-values are in bold.

Stand	Treat.	Net Min. (two-year)	Net Min. (2000-2001)	Net Nit. (two-year)	Net Nit. (2000-2001)	Net Ammon. (two-year)	Net Ammon. (2000-2001)	Net DON Prod. (two-year)	Net DON Prod. (2000-2001)
p-values for ANOVA	Stand	**0.06**	0.21	**0.03**	**0.07**	0.75	0.49	**0.03**	**0.09**
	Fert.	**0.02**	**0.07**	**0.05**	**0.04**	0.63	0.83	0.16	**0.06**
	Stand×Fert	0.36	**0.10**	**0.07**	0.90	**0.09**	**0.02**	0.33	0.15
B. Poplar	CONT	2972 (± 733)	3210 (± 1220)	849 (± 570)	999 (± 670)	2123 (± 179)	2212 (± 551)	1104 (± 371)	-377 (± 582)
	Contrast p-values	**0.02**	0.84	**0.02**	**0.10**	0.34	**0.05**	N/A	0.58
	FERT	4824 (± 791)	3222 (± 587)	3266 (± 488)	2548 (± 742)	1558 (± 480)	675 (± 239)	289 (± 162)	-651 (± 437)
W. Spruce	CONT	1365 (± 373)	933 (± 280)	138 (± 113)	24 (± 107)	1227 (± 344)	908 (± 229)	-586 (± 406)	-1288 (± 712)
	Contrast p-values	**0.10**	**0.03**	0.9	0.12	0.13	**0.04**	N/A	**0.04**
	FERT	2453 (± 385)	3455 (± 532)	262 (± 249)	828 (± 234)	2191 (± 512)	2627 (± 528)	-774 (± 462)	-2693 (± 225)

Table 2.4. Total DIN produced during each incubation period. The amount of DIN produced (Prod.) (in mg $N \cdot m^{-2}$) is divided into a.) net N mineralization or total production, b.) nitrification, and c.) ammonification (0-20 cm soil depth) for control (CONT) and fertilized (FERT) plots of balsam poplar (BP) and white spruce (WS). The parameters (mg $N \cdot m^{-2}$) are expressed as cumulative values for each incubation period. Daily rates can be calculated by dividing production estimates by the number of days in the incubation period. Values are means $\pm$ 1 S.E. from n=3 plots for each stand $\times$ treatment combination.

Net DIN Production/ Mineralization		Aug - Sept.'99	Oct.'99- June'00	June - July'00	July - Aug.'00	Aug. - Sept.'00	Sept. - Oct.'00	Oct.'00- June'01	June - July'01	July - Aug.'01
	Length of Incubation (days)	38-39	235-236	28-29	29	25-26	39-40	232-233	33	29
BPCONT	Prod.	753.6	1054.7	1345.9	-42.9	1113.8	999.4	-206.3	13.0	595.1
	($\pm$ S.E).	(154.2)	(204.0)	(259.4)	(342.8)	(467.7)	(71.8)	(227.1)	(127.2)	(129.0)
BPFERT	Prod.	1643.1	2507.0	1031.3	-769.8	2320.1	882.3	-241.6	598.6	1160.8
	($\pm$ S.E.)	(180.4)	(774.7)	(362.4)	(309.3)	(220.8)	(263.7)	(280.1)	(605.5)	(111.2)
WSCONT	Prod.	395.2	676.6	81.5	-90.9	390.6	631.5	-80.2	-7.8	588.0
	($\pm$ S.E.)	(113.5)	(147.5)	(22.3)	(56.4)	(256.3)	(205.5)	(161.8)	(113.8)	(136.1)
WSFERT	Prod.	1130.4	857.1	495.0	204.4	2074.3	90.3	590.9	146.4	-944.2
	($\pm$ S.E.)	(417.1)	(308.3)	(74.2)	(434.8)	(764.5)	(731.3)	(283.1)	(983.0)	(1058.3)
Net NO_3^--N Production/ Nitrification										
BPCONT	Prod.	399.2	29.1	364.9	192.8	157.0	197.1	86.6	59.8	120.2
	($\pm$ S.E.)	(244.6)	(59.3)	(238.6)	(118.8)	(107.1)	(138.7)	(70.3)	(68.8)	(61.6)
BPFERT	Prod.	888.3	895.4	550.1	-93.9	1006.9	835.4	249.3	851.8	998.9
	($\pm$ S.E.)	(111.6)	(651.6)	(270.1)	(236.1)	(290.6)	(180.5)	(193.4)	(373.9)	(450.2)
WSCONT	Prod.	130.9	19.0	-73.5	36.5	25.4	4.5	31.2	21.3	66.1
	($\pm$ S.E.)	(8.8)	(25.9)	(18.2)	(19.3)	(17.8)	(21.5)	(47.4)	(34.5)	(43.5)
WSFERT	Prod.	105.7	37.4	36.5	123.2	534.8	-214.7	348.4	276.1	-751.0
	($\pm$ S.E.)	(168.5)	(149.9)	(80.7)	(180.3)	(383.6)	(433.8)	(161.4)	(770.8)	(514.1)

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Table 2.4 Continued. Net NH₄ Production/Ammonification.

Net NH_4^+-N Production/ Ammonification										
BPCONT	Prod.	354.4	1025.7	981.0	-235.7	956.8	802.3	-292.9	-46.8	474.9
	(± S.E.)	(93.3)	(262.8)	(158.0)	(232.9)	(379.1)	(68.0)	(161.3)	(62.0)	(99.7)
BPFERT	Prod.	754.8	1611.6	481.2	-675.9	1313.2	46.9	-490.9	-253.3	161.9
	(± S.E.)	(182.0)	(248.0)	(351.5)	(78.4)	(144.8)	(201.3)	(105.0)	(385.2)	(351.7)
WSCONT	Prod.	264.3	657.6	155.0	-127.5	365.3	627.0	-111.4	-29.1	522.0
	(± S.E.)	(110.0)	(146.5)	(17.3)	(71.7)	(239.6)	(193.9)	(143.7)	(140.2)	(107.3)
WSFERT	Prod.	1024.7	819.7	458.5	81.2	1539.6	305.0	242.5	-129.7	-193.1
	(± S.E.)	(264.1)	(164.8)	(107.9)	(292.3)	(587.7)	(403.8)	(337.7)	(261.8)	(551.9)

Chapter 3

Soil Solution Nitrogen and Dissolved Ions During Mid and Late Succession of an

Undisturbed Boreal Forest Ecosystem.[1]

Abstract

We investigated the prediction that the transition from mid- to late succession forests in

interior Alaska would bring about an increase in the proportion of dissolved inorganic

nitrogen (DIN) below the rooting zone, relative to dissolved organic nitrogen (DON).

This prediction was based on the hypothesis that decreased nitrogen (N) demand in late

succession would facilitate leaching losses of DIN, while the production and export of

DON pool would remain relatively stable. The study was conducted in mature stands of

balsam poplar (mid succession) and white spruce (late succession) located along the

Tanana River in interior Alaska. Soil solution samples were collected over two growing

seasons at two different depths within the soil profile (12 cm – rooting zone and 40 cm –

mineral soil below rooting zone) using tension lysimeters. In contrast to our prediction,

soil solution DIN and DON concentrations were very similar across balsam poplar and

white spruce stands. Most N in solution at the 12 cm and 40 cm depths consisted of

DON (~92 % of total dissolved N (TDN) at 12 cm and ~79 % of TDN at 40 cm). Across

stands, nitrate dominated the DIN pool (84-98%) at all depths, with ammonium generally

[1]Brenner, R.E., Jones, J.B. Jr., and Boone, R.D. and Ruess, R.W. (in prep). Soil Solution Nitrogen and
Dissolved Ions During Mid and Late Succession of an Undisturbed Boreal Forest Ecosystem.

< 0.03 mg NH_4^+-N $\cdot L^{-1}$. Nitrate concentration in deeper mineral soil (40 cm) was significantly higher than in the surface horizon (12 cm) (0.17 ± 0.12 mg NO_3^--N L^{-1} and 0.46 ± 0.12 mg NO_3^--N L^{-1}, respectively). Significant positive correlations between soil moisture potentials at all depths and the rate of discharge from the Tanana River (Adj.-R^2= 0.92 for white spruce and 0.56 for balsam poplar; post-snowmelt), combined with results from principal component and cluster analyses, suggest that dissolved ions in the active layers of both stand types are derived from Tanana River water that moves into the surface horizons via hyporheic (capillary) flow during the growing season. Therefore, river water is probably also contributing to the influx of biologically important nutrients into the surface soils of late-succession stands that contain frozen soil throughout the growing season. Leaching and hyporheic flow are likely both important processes influencing soluble N concentrations in these stands; however, the relative influence of these processes across seasons and throughout succession remains to be determined.

Introduction

Due to the critical role of nitrogen (N) in regulating net ecosystem production in many regions, N inputs and losses from forest ecosystems have been the subject of much attention in biogeochemical research (Sollins et al. 1980; Tietema et al. 1997; Seely et al. 1998; Vitousek & Field 2001). During the 1970s a hypothesis of nitrogen (N) retention in forest ecosystems was developed (Vitousek & Reiners 1975; Gorham et al. 1979) proposing that N will generally be retained in aggrading, mid-succession, forests but

increasingly lost from late-succession (old growth) forests as a decrease in net ecosystem productivity induces a reduction in ecosystem N demand. A refinement of this hypothesis added that substantial losses of dissolved organic nitrogen (DON) may occur regardless of biological N demand (Hedin et al. 1995, Vitousek et al. 1998), and several studies across a wide range of forest types have shown that DON often makes up the dominant form of N lost from forest ecosystems (Sollins et al. 1980; Currie et al. 1996; MacLean et al. 1999; Perakis & Hedin 2002). This is probably because a substantial fraction of DON and associated DOC is comprised of recalcitrant, biologically-unavailable forms (Qualls & Haines 1991; Stepanauskas et al. 2000; Yano et al. 2000), and DON losses from ecosystems occur without extensive plant or microbial utilization. Thus, while biotic N demand likely plays a considerable role in the retention of N forms that are readily available to plants and soil microbes (e.g., NO_3^-, NH_4^+ and amino acids), all successional stages may have N leaching losses dominated by DON, provided that anthropogenic inputs of reactive N are low.

Floodplain stands of balsam poplar and white spruce in Alaska's interior encompass a dramatic primary successional transition (Viereck et al. 1993a) in a region with very low deposition of reactive N (< 0.3 kg $N \cdot ha^{-1} \cdot yr^{-1}$ wet + dry deposition) (http://www.epa.gov/castnet/sites/den417.html). During the course of approximately 150 years, deciduous, mid-succession stands of balsam poplar (*Populus balsamifera*) completely succumb to dominance by coniferous, late-succession stands of white spruce (*Picea glauca*), which shade out balsam poplar. Net primary productivity (Viereck et al. 1983), the abundance of N-fixing alder (*Alnus tenuifolia and A. crispa*) (Viereck et al.

1993a), as well as net soil N transformations (Klingensmith & Van Cleve 1993b, Van Cleve et al. 1993c) all decline during this transition. Furthermore, the N demand of vegetation shifts from high demand in balsam poplar stands to lower demand in white spruce stands (Van Cleve et al. 1983). Integral to the changes in composition, productivity and nutrient cycling are the development of a nearly continuous moss cover, a decline in soil temperatures and the associated occurrence of frozen soil throughout an increasing portion of the growing season in white spruce stands (Van Cleve et al. 1991).

In addition to decreased plant N demand, floristic succession on the floodplain may also bring about an alteration of overall ecosystem N retention. Soil N accumulates rapidly during early succession and throughout much of the balsam poplar stage, but shows no net gain as white spruce become dominant (Van Cleve & Viereck 1981; Van Cleve et al. 1993a). Reduced rates of N accumulation during late succession forests are almost certainly linked to a decrease in the abundance of N-fixing alder; however, the plateau in N accumulation may also indicate an acceleration of N losses during this successional stage. Studies of N fixation by thinleaf alder (*Alnus tenuifolia*) (Uliassi & Ruess 2002) suggest that much of the N fixed does not end up in the soil and thus may be lost from the system during the balsam poplar stage. This is a period in which N is still accumulating in the soil and soil heterotrophs are thought to be N limited due to a rich supply of labile phenolics in poplar litter (Clein & Schimel 1995; Schimel et al. 1998; Schimel et al. 1996). Thus, N losses on the floodplain, perhaps from an unavailable DON fraction, may occur throughout succession, even during periods of high soil and plant N demand. While N fixation inputs into white spruce stands are currently

unknown, soil microbes in this stand type are thought to be C limited (Flanagan & Van Cleve 1983) and thus may have an even more reduced capacity to immobilize biologically-available forms of N than during mid-succession. Low to undetectable rates of denitrification across a wide range of successional sequences on the Tanana River floodplain in interior Alaska (Klingensmith & Van Cleve 1993a) also indicate that N leaching is the primary route of N loss from this system.

The objective of this study was to examine the relative abundance of dissolved inorganic nitrogen (DIN) vs. DON in the soil solution of mid and late stages of a primary successional sequence. Our intention was not to determine an input/output budget of N in these stands; rather, our study assumes that the composition of soil solution N collected below the rooting zone (e.g. NO_3^-, NH_4^+ and DON) will define the dominant form of N leached from these stands. Assuming that water fluxes and DON leaching losses are fairly constant across this relatively brief ($\sim$100-150 year) successional transition, stands which have lower biotic N retention and higher leaching losses of DIN should also have a relatively lower ratio of DON:DIN below the rooting zone compared to stands in with high N demand and low levels of leaching losses. Thus, the ratio of soil solution DON:DIN should be indicative of the relative ecosystem N retention of biologically-available N. Accordingly, we predicted that the soil solution of late-succession white spruce stands would have a lower ratio of DON:DIN than is found in mid-succession balsam poplar soils. In addition, we examined the relationship between major ions in the soil solution and river water to evaluate the importance of subsurface flow (hyporheic flow) from the river on the chemistry of soil water in the surface horizons.

Methods

Study Site

This study took place at the Bonanza Creek Long Term Ecological Research Site (BNZ-LTER), approximately 30 km south of Fairbanks, Alaska (64°45' N, 148°18' W). Replicate research plots (15 m × 15 m) were located within stands of balsam poplar and white spruce on islands spread throughout the active floodplain (n=3 plots per stand type). All plots were located on high terraces (>3 m) formed by the frequent deposition of fluvial material. Precipitation in this region is low, averaging 269 mm, and is exceeded by potential evapotranspiration of approximately 466 mm. Mean annual air temperature is -3.7 °C with extremely cold winters (to -50 °C) and warm summers (to 35 °C) (Viereck et al. 1993b). All sites contained frozen soil throughout a large portion of the growing season; however, balsam poplar sites generally became ice-free by August while some white spruce sites contained frozen soil until early October (Table 3.1). Soils in these sites are classified as Typic Cryofluvents (Viereck et al. 1983; Van Cleve et al. 1993b) and generally consist of silt with occasional pockets of sand. Due to past flooding, all sites contained multiple buried organic layers; however, the number and depth of these varied among sites. Mineral soils are alkaline due to the high concentration of $CaCO_3$ originating from the primary (glacial) weathering of carbonate rock in the Alaska Range (Marion et al. 1993a; Marion et al. 1993b).

Balsam poplar sites (LTER sites BP1, BP2 and BP3) consisted of mature, uneven stands with some individuals exceeding 100 years of age and a dense understory of thin-

leaf alder (*Alnus tenuifolia* Nutt.), rose (*Rosa acicularis*) and infrequent white spruce. White spruce sites (LTER sites 4A, 4B and 4C) consisted of both mature and senescing stands 200+ years in age with an understory of alder (*A. crispa* and/or *A. tenuifolia*), rose and feather mosses (*Hylocomium splendens* and *Pleurozium schreberi*). Alder was a much smaller component of the understory in white spruce sites than in balsam poplar and was nearly absent at one site (4C). A complete description of plant and soil characteristics for the floodplain can be found in Viereck et al. (1993) and on the Bonanza Creek LTER website (*http://www.lter.uaf.edu/BCEF_index.htm*).

Soil Water and River Sampling

Tension lysimeters (Prenart Equipment ApS, Frederiksberg, Denmark) were installed during late June 2000 in both stand types. In each plot, lysimeters were installed at the 12-cm (n=5) and 40-cm depths (n=4). In the case of white spruce the soil depth was measured from the base of the live moss layer whereas in balsam poplar it was calculated from underneath the previous year's litter. Lysimeters were installed at approximately a 30° angle in order to minimize disturbance to the soil surrounding the lysimeter head. In addition, we attempted to limit damage to the moss ground cover in white spruce by installing board walks, from which lysimeter and tensiometer installation and sampling took place. During lysimeter installation a 1-m long hole was bored into the soil using a metal rod. The lysimeter and associated tubing were inserted to the end of the hole which was first backfilled with a slurry of silica flour and then with a slurry of mineral silt from the shore of the Tanana River. An additional 1-m of tubing ran

underneath the litter layer from the opening of the borehole to a shallow pit where a glass collection bottle was placed. Lysimeters were left undisturbed for one month after installation, and the first two sampling periods in 2000 were excluded from the analysis.

Tensiometers (Soil Measurement Systems, Tucson, Arizona) were installed concurrent with lysimeters in an array of 14, 32 and 50-cm depths (n=3 arrays per plot). Tensiometer tubes were filled with water the day prior to lysimeter sampling and soil water potentials were measured with a pressure transducer the day of lysimeter sampling. The transducers are sensitive to ± 0.1 kPa (1 mbar). The difference in soil water potential at the 14 and 32 cm or 32 and 50 cm depths were used to determine the direction of vertical flux of water within the soil profile. For example, a more negative water potential at 32 cm than 50 cm indicates that water is moving up through the profile.

Approximately 24 hours prior to sample collection, lysimeter tubes were connected with air-tight fittings to 2-L Prenart bottles. Bottles and tubing were then evacuated to -40 kPa using a hand pump (Soil Measurement Systems, Santa Barbara, California). The following day, water in lysimeter bottles was poured into acid-washed Nalgene[©] bottles that were placed on ice packs in a cooler for transport to the laboratory. Samples were filtered with pre-leached glass fiber filters (1 μm pore diameter), and pH and conductivity were measured. Samples then were frozen until further analysis. Water samples were also collected from the adjacent Tanana River during most sampling periods. River water was collected from the bow of a boat which was held into the oncoming current in the middle of the main channel. Lysimeters were sampled 20 times from August 2000 until October 2001, approximately weekly during frost-free periods

(late May – early October), although sample collection was briefly terminated during periods when soil was dry.

Nitrate and ammonium concentrations were measured on an API 300 segmented-flow autoanalyzer (Astoria-Pacific Inc., Clackamas Oregon) using standard colorimetric protocols (Bundy & Meisinger 1994). Dissolved organic N (DON-N) was determined by subtracting DIN (nitrate + ammonium) from total persulfate-digestible N (Cabrera & Beare 1993). Anion (chloride and sulfate) and cation (calcium, magnesium, sodium and potassium) concentrations were analyzed on a Dionex DX-320 ion chromatograph (Dionex Corp., Sunnyvale California). As a check on our autoanalyzer we also re-measured ammonium and nitrate in most samples via ion chromatography during the analysis of anions and cations. Total organic carbon (TOC) on a random subset of 157 samples spanning the entire experiment was determined using a Shimadzu TOC-5000 (Shimadzu Corp, Kyoto Japan).

Statistical Analysis

The concentrations of elements collected within each plot at a given depth were averaged such that each plot represented a single replicate (n=3 plots per stand type). Data were analyzed with a split-plot ANOVA design using PROC MIXED in SAS (SAS 1999). Stand type was used as the between-subject (whole plot) factor, and depth was the within-subject (split-plot) factor. Season (spring, summer or autumn) was also included in the model as a repeated measures factor. Significant effects were further analyzed using paired contrasts. A Kenward and Roger correction (Littell et al. 2002) was

implemented because exact F-tests were generally not possible. For all analyses the homogeneity of variance assumption was tested using Levene's test. Data were square-root transformed when the assumptions of homogeneity of variance was violated (Zar 1999), and an additional analysis was performed. Principal components analysis (PCA) and cluster analysis (Single Linkage, Euclidian distance) were used to investigate the relationship between dissolved ions from the Tanana River with those from each stand×depth combination of the soil solution. The first two factor scores from the PCA were further analyzed in a MANOVA and, when there were significant effects, paired contrasts were used to determine significant differences between groups. Data presented in Tables and Figures are means ± 1 standard error (S.E.) from untransformed data. Significance for all tests was established at the $P \leq 0.05$ level with values between 0.05 and 0.10 considered "marginally" significant.

Results

The largest portion (79-92%) of total dissolved nitrogen (TDN) in the soil solution at all stands and depths was comprised of DON (Figures 3.1 and 3.2, Table 3.2). In addition, DON concentrations across stand types were significantly higher at the 12 cm, than 40 cm, depth ($F_{1,8.64}=9.84$; p=0.01; 3.05 ± 0.27 for 12 cm vs. 1.85 ± 0.27 for 40 cm). This difference between depths was significant for white spruce ($F_{1,9.68}=7.69$; p=0.02) but only marginally significant for balsam poplar ($F_{1,10.1}=3.24$; p=0.10). The ratio of DON:DIN was not significantly different between stand types but was significantly higher at the 12 cm than 40 cm depth (Table 3.2). Averaged across stand

types, DON concentrations at the 12 cm depth were significantly higher during the summer period compared to the fall ($F_{1,108}=3.91$; p=0.05) (Figure 3.2). The summer was also the period when soil moisture content was lowest (see below), suggesting that DON concentration was influenced by dilution from snowmelt during the spring and precipitation in autumn.

Nitrate accounted for the largest fraction (84-98%) of the soil solution DIN pool with ammonium concentrations low throughout the study, averaging <0.03 mg NH_4^+-N·L^{-1} (Figure 3.1). In contrast to trends observed for DON, soil solution nitrate concentration was significantly higher ($F_{1,3.73}= 7.69$; p = 0.05) at the 40 cm depth (0.46 ± 0.12 mg NO_3^- -N L^{-1}) compared to the 12 cm depth (0.17 ± 0.12 mg NO_3^--N L^{-1}) when averaged across stands (Figure 3.1 and 3.2). However, within stands the difference between depths was only marginally significant for white spruce ($F_{1,3.89}$ =5.45; p=0.08) and was not significant for balsam poplar ($F_{1,4.06}$=2.45; p=0.19). Nitrate concentration did not change significantly across seasons ($F_{1,95.7}$ = 1.05; p = 0.31), although, the highest mean nitrate concentration observed in the study was from the 40 cm depth of white spruce during the fall (mean nitrate = 0.61 mg NO_3^--N·L^{-1}; Figure 3.2).

Averaged across all stands, soil solution DOC was significantly higher at 12 cm than 40 cm (Table 3.2: $F_{1,4}= 11.92$; p= 0.03). But, as with other solutes, this difference was only significant for white spruce ($F_{1,4}= 9.30$; p= 0.04) and not for balsam poplar ($F_{1,4}$ = 3.357; p= 0.14). DON and DOC concentrations were positively correlated across all sampling periods and sites (adj.-R^2 = 0.82, p <0.001; data not shown). As such the ratio of DOC:DON was fairly consistent across all depths and stand types, ranging from 34.7

in the 12 cm depth of balsam poplar to 40.5 in the Tanana River (Table 3.2) although there were not any significant difference.

Of the measured cations and anions only potassium and chloride concentrations exhibited consistent depth-wise patterns, with highest concentrations at the 12 cm depth for both stand types ($p < 0.01$; Table 3.3). Although there were some exceptions, cation concentrations in soil solution and river samples generally followed the pattern of Ca > Mg > K > Na > Li, and for anions SO_4 > Cl. A cluster analysis of standardized ion concentrations (Figure 3.3) shows that ions in the Tanana river were most closely associated with those from the 40 cm depth (Euclidian distance of 1.97 for white spruce and 2.40 balsam poplar) and more distantly associated with 12 cm depth (Euclidian distance of 2.93 for white spruce and 2.94 for balsam poplar). Factor scores generated from a principal components analysis of ion concentrations generally show ions from the Tanana River and 40 cm soil solution to be grouped much more tightly on the factor plane than those from the 12 cm soil solution (Figure 3.4). The first two PCA-generated factors explained >83% of the total variance. Factor 1 was highly correlated with SO_4, Ca, Na, and Mg and Factor 2 was highly correlated with Cl and K. A MANOVA of the factor scores yielded a highly significant site effect ($p < 0.0001$; data not shown). For Factor 1, pair contrasts indicate significant differences ($p < 0.02$) between ions in the Tanana River and those in the soil solution at all depths except white spruce 40 cm (Table 3.4). For Factor 2, paired contrasts yielded significant contrasts between ions in the Tanana River and the soil solution at the 12 cm depth of balsam poplar (Table 3.4). Electrical conductivity did not differ significantly between stand types or depths, but, like

all the elements measured, electrical conductivity was lower in the Tanana River samples compared to the soil solution (Table 3.2). The pH of soil solution and river samples was consistently above 8 throughout the course of the study. The pH of the soil solution did not vary significantly by stand type, soil depth, or season (p > 0.10) and ranged from 8.2 to 8.5.

Soil water potentials were highest, and often positive indicating saturated soil conditions, during the spring at all depths (Figure 3.5). The high values were the result of snowmelt, which saturated soil and, in some cases, pooled water on top of frozen soil during the spring flush. Standing water was most evident at the 50 cm depth of both stands, where water potentials remained positive until late June or early July. Water potentials at all depths dropped sharply in late June and early July as soil surfaces dried out. Water potential increased in late July as the discharge of the Tanana River rose and rains began, and decreased in the fall as the river level fell. There was a significant correlation between discharge of the Tanana River and soil water potential, but only for the period after July 10 (Figure 3.6) when surface horizons had begun to dry following the spring flush. Based on differences in water potentials between depths, we determined that for white spruce plots the downward infiltration of water roughly tracked precipitation events (Figure 3.7). In contrast, only during the spring flush was downward infiltration of water observed in balsam poplar.

Discussion

Contrary to our prediction, soil water DIN concentrations did not differ significantly between mid-succession balsam poplar and late-succession white spruce stands. Instead, both stand types had comparable concentrations of DIN, most of which (84-98 %) consisted of NO_3-N (Figure 3.1). The similar ratio of DON:DIN at the 40 cm depth of balsam poplar and white spruce (4.0 in balsam poplar and 3.8 in white spruce) (Table 3.2) also runs counter to our prediction that, due to greater plant and microbial demand for DIN in balsam poplar, this ratio would be higher in balsam poplar than in white spruce. While N demand in balsam poplar stands may indeed exceed that in white spruce as the result of a more labile pool of litter C (Schimel et al. 1996; Schimel et al. 1998) and higher rates of above- and belowground plant primary production (Ruess et al. *in press*), we do not believe there is definitive evidence that plants or microbes in this stand type are N limited. Mature poplar stands on the floodplain do have lower rates of N fixation inputs than the pure alder stands of early succession; however, N inputs from alder are still substantial during this stage ($\sim$38 kg $N \cdot ha^{-1} \cdot yr^{-1}$ Uliassi et al. 2000; Uliassi & Ruess 2002; Anderson et al. 2004). We suspect that N inputs may roughly equal or exceed the high biological N demand created from a labile litter pool and high rates of primary productivity. There is also a strong possibility that phosphorus, rather than N, is a more limiting nutrient to plants in these floodplain forests (Chapin et al. 1983; Uliassi & Ruess 2002), which grow on a very young alkaline soil with negligible amounts of mineral weathering. The uniform pool size of soil solution DIN below the rooting zone and uniform ratio of DON:DIN observed throughout the dramatic transition from mid to

late succession could indicate that successional stage does not play a critical role in regulating soil N retention in these forests. Many floodplain successional stages may have losses of DON and nitrate during some portion of the growing season, and, even though N fixation may decline later in succession, these inputs could continue to exceed N demand.

On the other hand, there is some evidence that soil microbes in balsam poplar stands do immobilize a higher proportion of nitrate produced from nitrification than those in white spruce soil. In our previous work we investigated monthly rates of *in situ* net nitrification over the course of two and a half years within these research plots (Brenner et al. *In Press*). Within balsam poplar stands the soil had higher mean rates of net nitrification than white spruce during all nine of the incubation period of the study and had yearly mean rates of net nitrification that were several times larger than in white spruce stands (849 mg $NO_3^- - N$ m^{-2} yr^{-1} in balsam poplar vs. 138 mg $NO_3^- - N$ m^{-2} yr^{-1} in white spruce). That higher concentrations of nitrate were not observed below the rooting zone of balsam poplar suggests that soil microbes may play a larger role in immobilizing nitrate in this stand type. However, an examination of gross rates of nitrification, combined with accurate estimates of nitrate retention would be needed to evaluate this possibility.

Because DON comprised the largest portion of soluble N in the soil solution at the 40 cm depth it likely constitutes the dominant form of soluble N loss from both of the successional stages in this study. While the bioavailability of DON at this depth is unknown, DON from streams of boreal forest ecosystems has been shown to have a high

proportion (81-72% at baseflow) of biologically-unavailable constituents (Stepanauskas et al. 2000). The same is likely true in these stands where there was a relatively high ratio of DOC:DON in the soil solution at all depths (34 - 41; Table 3.2). It is unknown if the soil solution from these stands serves as a major source of labile C for soil heterotrophs; however, the high ratio of DOC:DON exceeds by several fold the C:N ratio of substances such as amino acids which are known to be a direct sources for plant N uptake in the boreal forest (Nasholm et al. 1998; Persson & Nasholm 2001; McFarland et al. 2002). Thus, our study lends support to the idea that a sizeable fraction of the DON in forest ecosystems is not readily useable for plants and indicates that DON losses in this system are not necessarily controlled by biological N demand (Vitousek et al. 1998).

Interestingly, while DON significantly decreased with depth, nitrate concentration increased significantly with depth across stands (Figure 3.1). Given that nitrate made up the majority of the DIN pool and is highly mobile in soil (Vitousek et al. 1982; Miller & Gardiner 1998), nitrate leaching losses might be proportionally much higher than would be indicated by its relative abundance in the soil solution N pool. The increase in nitrate with depth is consistent with previous reports of soil solution chemistry for the floodplain in which the surface (20 cm) nitrate concentration was lower than at 50 cm (Yarie et al. 1993). The higher concentration of nitrate relative to ammonium in soil solution and Tanana River water throughout this study, particularly in the deeper mineral soil (Figure 3.1 and 2), is not consistent with predictions that DIN losses from pristine (low N deposition) forest ecosystems should have a very low ratio of nitrate:ammonium (Perakis & Hedin 2002). Although nitrate in soil solution at 40 cm cannot necessarily be

considered "removed" from the ecosystem, this depth is below the zone where ~ 90 % of fine root production occurs (Ruess et al. *in press*) and thus may not be readily available to most of the plants and soil microbes. Overall, our study reaffirms that "pristine" ecosystems with low rates of anthropogenic N deposition generally have soil solution and stream N pools consisting primarily of DON (MacLean et al. 1999, Sollins et al. 1980, Hedin et al. 1995; Stepanauskas et al. 2000).

Annual leaching losses of soluble N from floodplain soils of interior Alaska are difficult to estimate due to the unpredictable nature of water movement through seasonally frozen soil (Kane & Chacho 1990), as well as uncertainty in estimating water movement and soluble N concentrations in the mineral soil during the early spring (late April-May) and early winter (October to December). During late fall and early winter the collection of soil solution with lysimeters is difficult due to the freezing of lysimeter tubing. Moreover, our access to floodplain research plots is nearly impossible during the process of river freeze-up in October when travel by boat is not possible. However, three main factors suggest that N leaching losses likely occur during the early winter in this ecosystem: 1) Much of the upper 1 m of mineral soil remains unfrozen for two or more months after surface horizons freeze, making infiltrate to groundwater possible; 2) Depending upon the stand type, plant N demand is probably negligible (white spruce) or non-existent (balsam poplar) during early winter, and N immobilization by soil microbes is likely much diminished relative to the summer period due to low soil temperature; and 3) reduced evapotranspiration following leaf-fall (balsam poplar) and the onset of near

freezing conditions should increase the possibility of downward infiltration of water following precipitation events.

Differences between the infiltration of growing-season precipitation to deeper soil in balsam poplar and white spruce stands (Figure 3.7) might create dissimilar rates of N leaching losses in these stand types. Our study indicates that, for the sampling periods after snowmelt, downward infiltration to the 50 cm depth was never evident following rain events in balsam poplar. In contrast, infiltration of rain to the 50 cm depth was often observed in white spruce stands. This apparent difference between stand types might partially be explained by greater retention of water in the surface organic matter and moss in white spruce which would increase the time it takes water potentials to equilibrate with the mineral soil and would allow these events to be detected by manual tensiometer measurements, such as those used in this study. In other words, the moss carpet in white spruce stands may act as a sponge that holds onto rain water which would then move into the mineral soil in the days following a rain event. We believe that substantially higher stand-level transpirational water loss by plants (e.g., poplar, alder, rose) in balsam poplar is preventing the downward infiltration of rain water to the 50 cm depth. Therefore, despite a similar composition of N in the soil solution in both stand types, a larger amount of infiltration into the deeper mineral soil in white spruce following rain should result in greater N leaching during the growing season in this stand type. However, N losses due to downward infiltration are also complicated by the greater prevalence of frozen soil in white spruce (Table 3.1), which could both prevent water from infiltrating

to groundwater and prevent groundwater from moving into surface horizons during the first half of the growing season.

Soil water potential at all depths was quite high (wet soil) after snowmelt in the early spring, and then became progressively more negative (drier soil) until mid-July (Figure 3.5). Overall, we found that soil water potential during the growing season on the floodplain was significantly correlated with the height of the Tanana River (Figure 3.6). Part of this relationship could be due to the onset of rain during mid-July (Figure 3.7). However, despite the continuation of rain events, soil water potentials at all depths actually decreased during mid August, as did the discharge from the Tanana River. This reaffirms our belief that over the course of the growing season river height is generally more important in maintaining the soil moisture of the unsaturated zone than are rain events. The rise of the Tanana River during the growing season elevates groundwater closer to surface horizons (Viereck et al. 1993b) through the process of hyporheic flow -- the mixture of river water with groundwater and subsequent capillary rise into surface horizons. This process likely helps explains how soil water status can remain favorable to plants (generally > -20 kPa) in an ecosystem which has annual losses from evapotranspiration (466 mm) that are much higher than annual inputs from precipitation (269 mm).

In addition to the significant correlation between river discharge and soil moisture potential, several lines of evidence from our soil solution ion data also suggest that hyporheic flow could be a major factor controlling the movement of dissolved substances from riverwater and groundwater into the unsaturated zone of the soil profile – especially

at the 40 cm depth. A tree diagram generated from a cluster analysis of major dissolved ions (Figure 3.3) shows that river water ions have a strong linkage (shortest Euclidian distances) with the soil solution at the 40 cm depths of both stand types. The ratios of major ions (excluding sulfate) in the 40 cm soil solution to those of the same ion in the Tanana River (e.g., [Ca] in soil solution: [Ca] river water) were fairly constant in both stand types (2.3 – 5.4 in balsam poplar and 2.0 – 3.4 in white spruce), suggesting that the soil water originated from a common source (Table 3.3). The ratio of nitrate and DON at the 40 cm depths to those of river water also ranged from 4-5 and thus may also originate from river or groundwater. Similarly, Yarie et al. (1993) found significant positive correlations between many individual ions in Tanana River water and those collected from tension lysimeters in these stand types. Lastly, a principal components analysis of major ions anecdotally suggests that ions in the Tanana River (particularly K and Cl) may be closely associated with those in the 40 cm soil solution (Figure 3.4). Contrasts of PCA factor scores did indicate a significant difference between river water ions and those of the 40 cm soil solution in balsam poplar for the first PCA factor (p = 0.0008; Table 3.4). However, there were not any significant differences between factor scores from river water and the 40 cm depth of white spruce (p = 0.11 and p = 0.68 for Factor 1 and 2 in paired contrasts, respectively; Table 3.4). Also, the overall distribution of the two factors which explained > 83% of the total variance in the ion data seems to indicate that dissolved ions from the 40 cm soil solution clusters closely to those from river water (Figure 3.4). In contrast, the factor scores of ions from the surface soil solution are spread over a much wider range across the factor plane and overlap greatly with the

clusters from 40 cm and the Tanana River. This is probably due to evaporation at the soil surface which concentrates ions during dry periods and dilutes them during snow melts and rain events.

Thus, when compared to the explanation that precipitation flushes dissolved ions out of the system via downward flow, hyporheic flow seems to better explain the concentration of dissolved ions in surface soils. Hyporheic flow almost certainly plays some role in influencing the movement of nitrate and DON during parts of the growing season and further complicates our ability to understand N losses from this system. It is also possible that the higher concentrations of NO_3^- observed at 40cm in both stand types was the result of nitrification at the stream-soil interface with subsequent capillary rise to toward the surface. The hyporheic zone can be a significant source of NO_3^- when oxygen-rich river water with low bio-available DOC create conditions favorable for nitrification (Jones & Holmes 1996). The drop in NO_3^- near the surface could be the result of increased microbial immobilization fueled by labile C from root exudates and decomposing litter and fine roots.

Nevertheless, it also seems plausible that DON observed at 40 cm originates near the soil surface as this is where the vast majority of N inputs from symbiotic N fixation and decomposition (root and litter) occur. It is our view that both hyporheic flow and the downward leaching of the soil solution are important processes in the biogeochemistry of the floodplain and do not necessarily represents mutually exclusive mechanisms for the movement of N and other dissolved solutes in this ecosystem. For example, hyporheic flow might be the dominant process during late July as frozen soil dissipates (Table 3.1),

soil and air temperatures are near their maximum and, most importantly, glacial melt increases river discharge and elevates groundwater towards the soil surface (Viereck et al. 1993b; Figure 3.6). In contrast, runoff and the downward leaching of solutes might be more prevalent following snowmelt and during the late fall ("shoulder" periods) when river level is relatively low, precipitation inputs are relatively high and surface soils are wettest.

Conclusions

Our study indicates that DON is the most prevalent form of N in the soil solution below the main rooting zone and may be the dominant form of N lost from this ecosystem via leaching. However, our results also suggest that the capillary rise of Tanana River water within forests (hyporheic flow) may replenish dissolved ions in the unsaturated zone and plays at least some role in the seasonal movement of riverine N into the soil active layer. Soil solution N pools and the ratio of DON:DIN did not differ across stands, and thus did not support our prediction that late successional stands have a larger proportion of DIN below the rooting. Perhaps more importantly, our study brings to light several unresolved questions regarding the potential pathways of N losses in this and other high latitude forest ecosystems: 1) How do differences in stand-level water requirements influence the movement of soil water to mineral horizons, and what role do these differences play in altering N leaching losses?; 2) What are the rates of N leaching following the onset of freezing air temperatures during the late fall/early winter period when surface horizons remain unfrozen?; 3) To what degree does the presence of frozen

soil during the growing season influence N leaching losses and runoff during this period?; and 4) To what extent does hyporheic (capillary) flow contribute to stabilized and biologically-available N in these forests and how do these patterns change throughout succession? The answer to these questions will further our understanding of the factors regulating primary productivity and carbon sequestration in this and other sub-arctic ecosystems that are strongly influenced by seasonally frozen soil.

Acknowledgments

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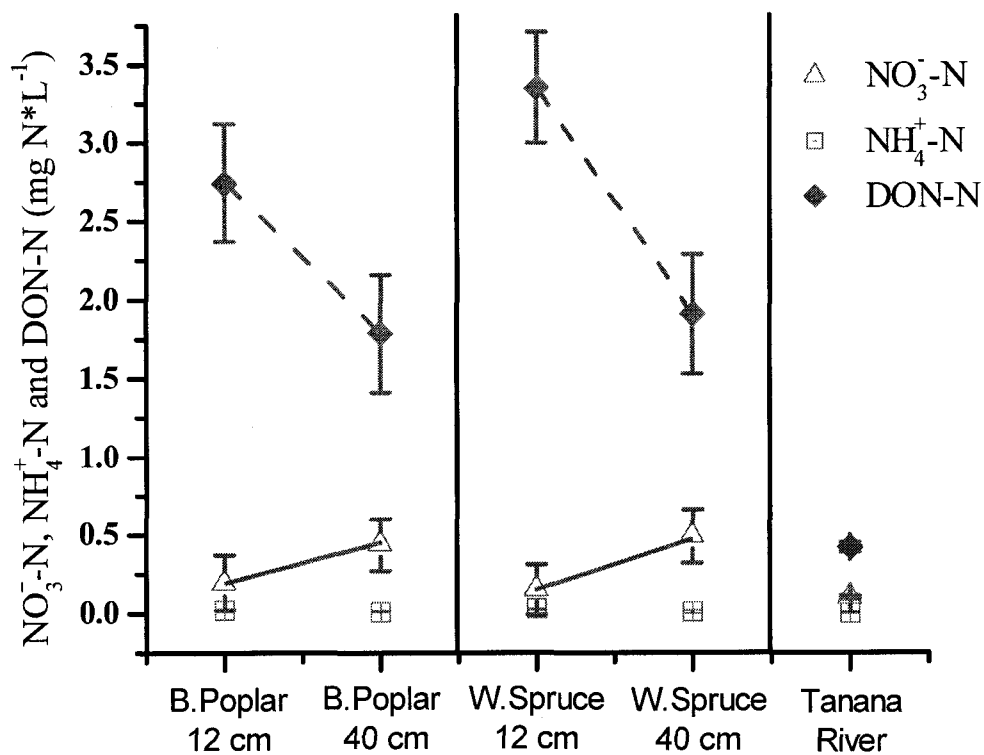
Figure 3.1. Soil solution NO_3^--N, NH_4-N and DON-N concentrations. The soil solution was taken from balsam poplar and white spruce stands and the Tanana River. Values are means obtained from a repeated-measures ANOVA. Samples were collected 20 times from August 2000 to October 2001 (n=3 for each stand×depth combination during each collection period).
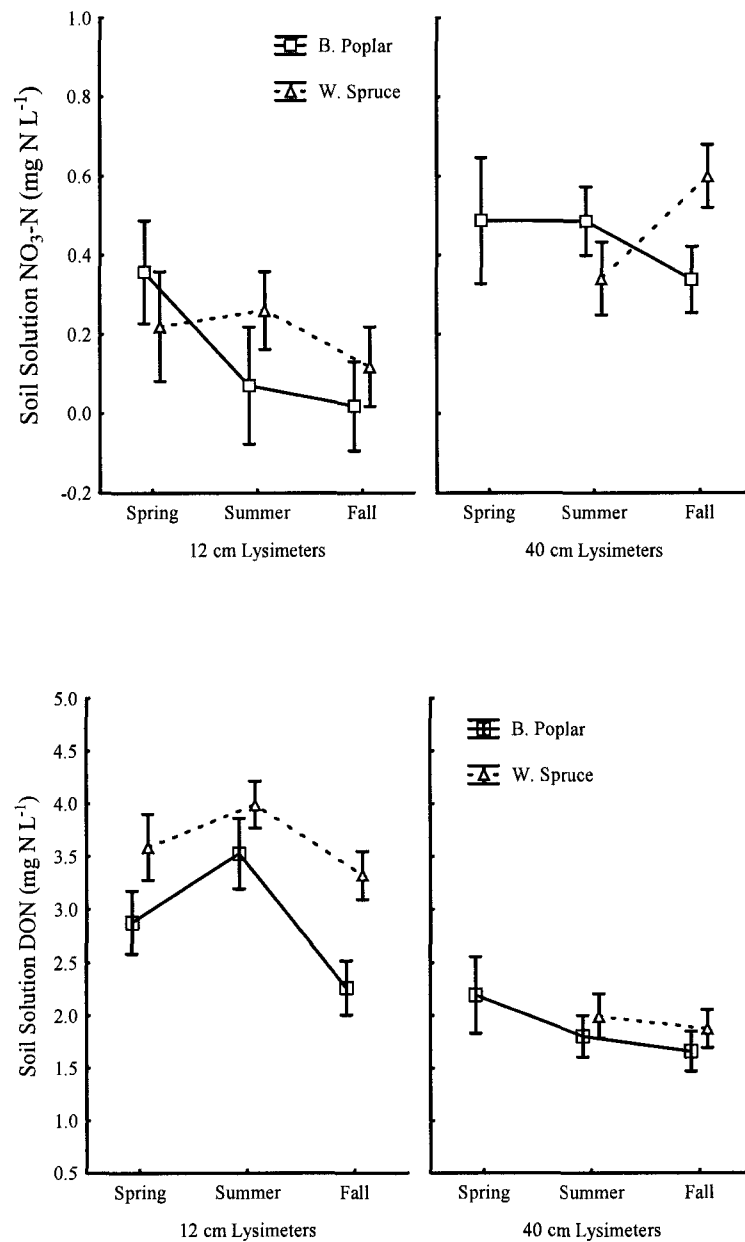
Figure 3.2. Mean seasonal soil solution concentrations of a.) NO_3^--N and b.) DON averaged for 2000-2001. Values are mean ± 1 S.E. obtained in a repeated measures ANOVA with n=3 replicate plots per stand*depth combination. There were no significant seasonal trends for nitrate (p>0.05); however, across stands DON was significantly lower at the 12 cm depth during the fall compared to the summer period.
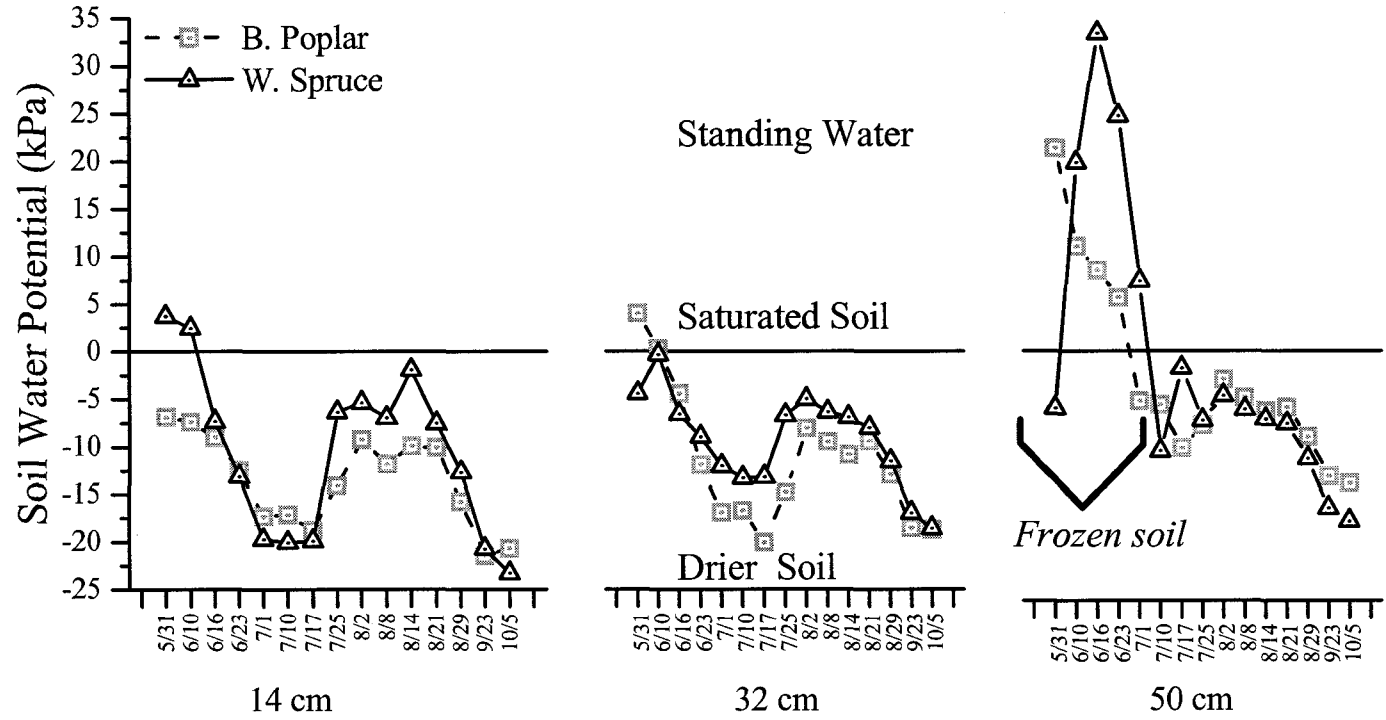
Figure 3.3. Mean soil water potentials (kPa) for stands of balsam poplar and white spruce during 2001. N = 3 replicate sites per stand.
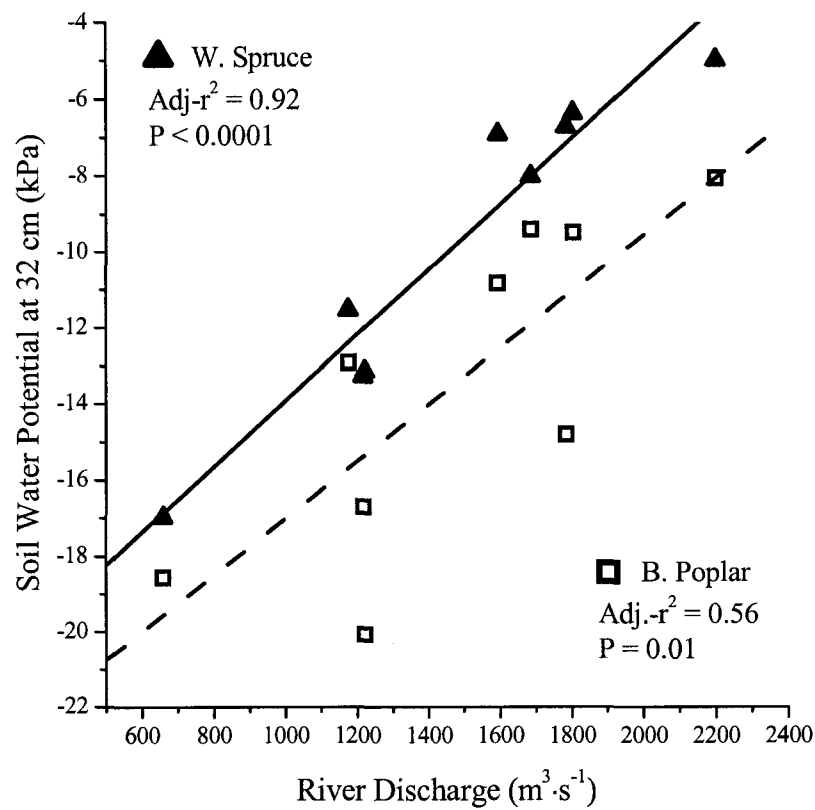
Figure 3.4. Mean soil water potential (kPa) vs. discharge of the Tanana River ($m^3 \cdot s^{-1}$). Soil water potential was measured at the 32 cm depth of soil in stands of balsam poplar and white spruce from July 10 to September 23 during 2001. Soil water potential values are the mean taken from n=3 plots per stand type. The dashed regression line is for balsam poplar and the solid line for white spruce.
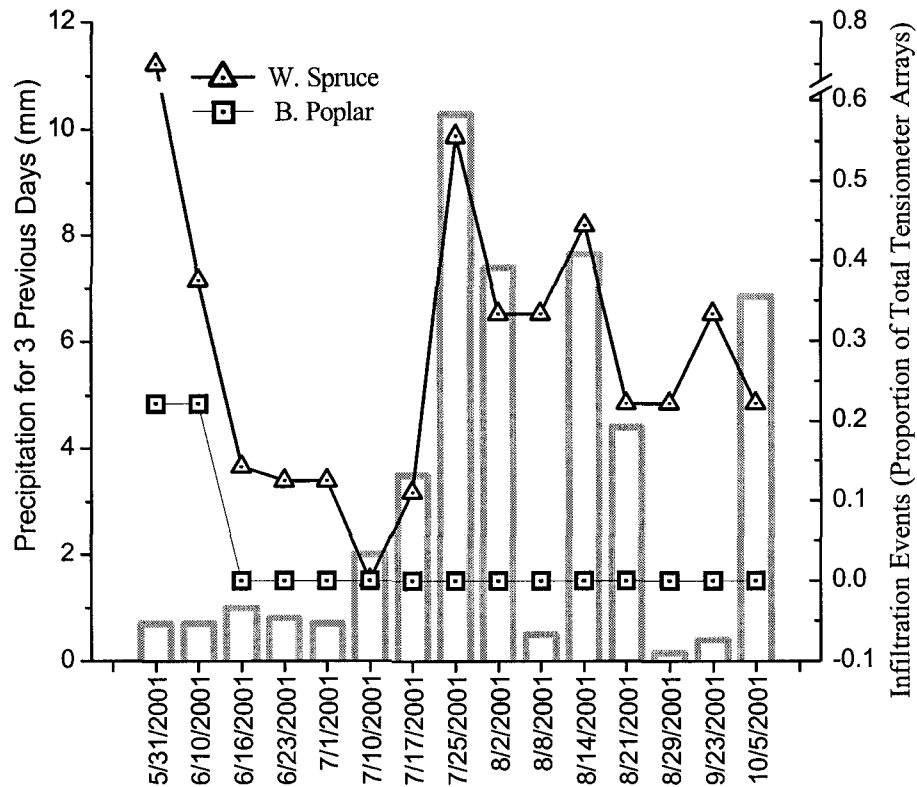
Figure 3.5. Downward infiltration and precipitation. This figure shows the proportion of tensiometer arrays within each stand type in which soil water is infiltrating downward (line + symbol) and cumulative precipitation for three days prior to sampling (bars) on each sampling day during 2001.

Single Linkage -- Euclidian Distance

Linkage Distance
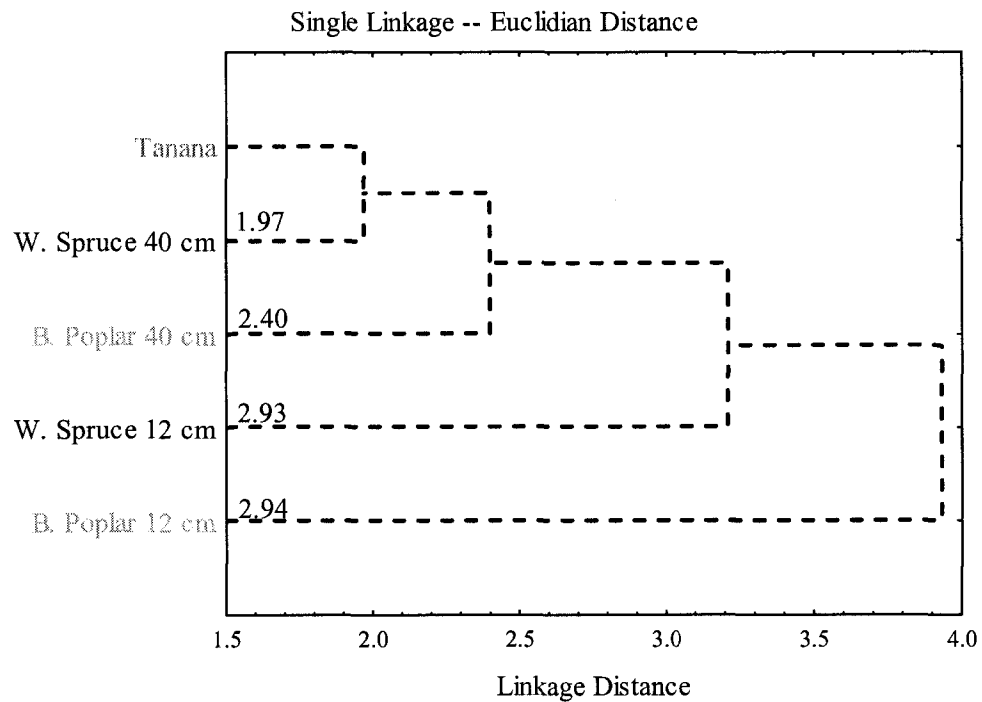
Figure 3.6. Cluster analysis associations of anions and cations. This dendrogram shows the associations between stand*depth levels and the Tanana River obtained from a cluster analysis of anion and cation concentrations. Values next to each soil solution category are the Euclidian distances from this group to the Tanana River.

Projection of the cases on the factor-plane (1 x 2)
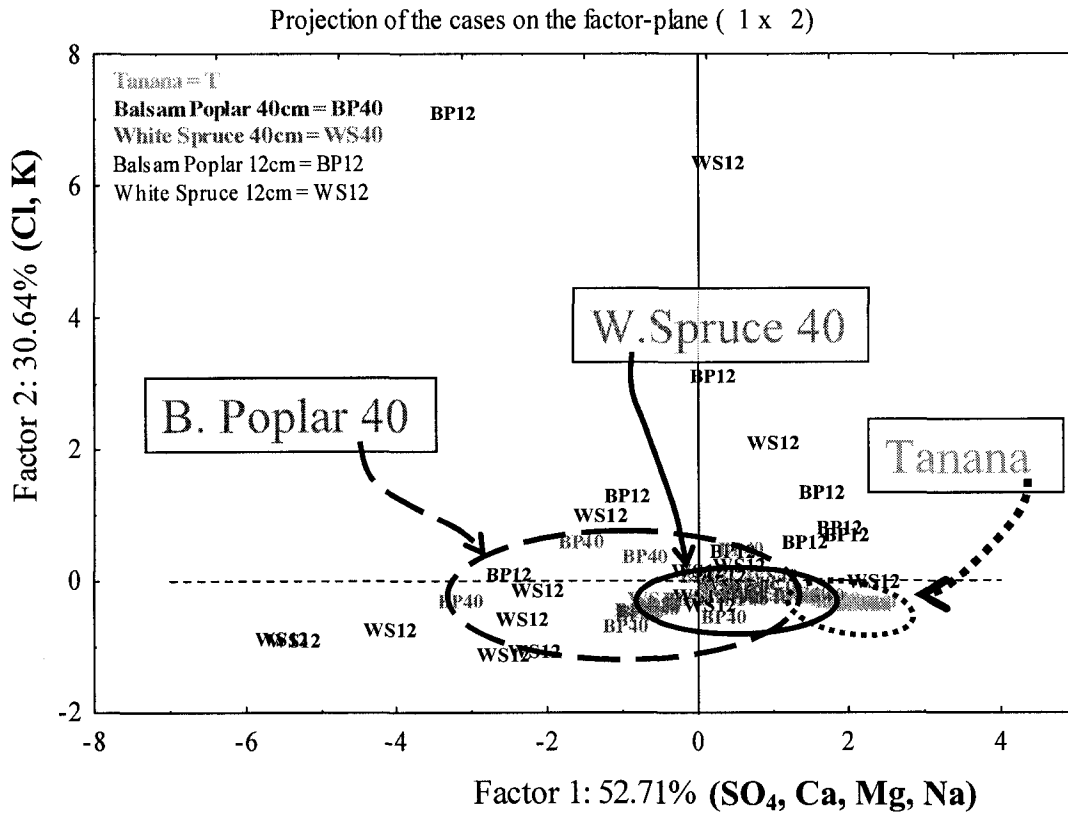
Figure 3.7. The grouping of the two principal factors from a principal component analysis of major ions. Ions are from the Tanana River and the soil solution. Factor 1 explained 52.71% of the total variance and was highly correlated with SO4, Ca, Mg and Na; Factor 2 explained 30.64% of the total variance and was highly correlated with Cl and K.

Table 3.1. Depth of active layer (depth to frozen soil) (cm) in stands of balsam poplar and white spruce. Values are the range of mean values from n=3 replicate sites.

Date	B. Poplar	W. Spruce
May 28	30 - 59	16 - 33
June 23	36 - 75	25 - 48
July 10	53 – N.F.	30 - 75
August 21	N.F.	53 – N.F.
Sept. 23	N.F.	63 – N.F.

* N.F. = No frozen soil detected in upper 1 m.

Table 3.2. Select characteristics for Tanana River water and the soil solution. The soil solution is from stands of balsam poplar and white spruce. Values are means ($\pm$ 1 S.E.) and all concentrations are in $mg \cdot L^{-1}$.

	BP 12cm	BP 40 cm	WS 12cm	WS 40cm	Tanana River
pH	8.50[a] (0.07)	8.37[a] (0.11)	8.20[a] (0.10)	8.30[a] (0.09)	8.41[a] (0.12)
Electrical Conductivity ($\mu S \cdot cm^{-1}$)	283.54[ab] (86.81)	539.24[b] (204.05)	394.75[ab] (232.13)	335.17[ab] (92.42)	164.46[a] (10.65)
DON-N: DIN-N[†]	27.6[a] (16.9)	5.7[b] (2.5)	24.0[a] (9.5)	6.6[b] (4.2)	4.3[b] (0.5)
DOC-C	95.18[ab] (6.57)	68.18[b] (2.25)	127.70[a] (25.61)	76.75[b] (11.19)	16.83[c] (1.19)
DOC-C: DON-N	34.7[a]	38.2[a]	38.1[a]	40.1[a]	40.5[a]

Superscript with the same letters are not significantly different ($p < 0.05$).

[†]Means were transformed prior to analysis.

Table 3.3. The concentration (mg·L^{-1}) of major ions from the Tanana River and the soil solution. The soil solution was from stands of balsam poplar and white spruce. All values are means (± 1 S.E.).

Cations	BP 12cm	BP 40 cm	WS 12cm	WS 40cm	Tanana River
Ca	31.28 (7.73)	42.89 (7.61)	31.47 (10.42)	37.83 (6.17)	18.57 (1.63)
Mg	14.24 (6.47)	35.87 (13.75)	22.55 (13.53)	22.87 (7.81)	6.67 (1.51)
K	20.35 (4.08)	8.36 (0.37)	17.59 (2.77)	6.05 (1.81)	2.64 (0.26)
Na	5.74 (3.38)	13.50 (7.24)	12.31 (7.98)	7.93 (3.41)	3.34 (0.39)
Li	0.01 (0.01)	0.001 (0.002)	0.02 (0.01)	0.003 (0.001)	0.001 (0.0003)
Anions					
SO$_4$	35.99 (19.07)	127.58 (92.61)	134.98 (113.93)	49.86 (27.59)	35.80 (4.06)
Cl	21.03 (3.22)	5.49 (1.09)	11.81 (5.05)	2.80 (0.71)	1.18 (0.12)

Table 3.4. P-values from planned contrasts between ions in Tanana River water and the soil solution. Contrasts follow a MANOVA of the first two factor scores from a principal component analysis. The factor scores were square-root transformed prior to the MANOVA and were highly correlated (>76% correlation) with the ions listed beneath them.

	Wilks Multivariate test	Factor 1 (SO_4, Ca, Mg, Na)	Factor 2 (Cl, K)
BP 12 cm	< 0.0001	0.02	.0006
BP 40 cm	0.002	0.0008	0.86
WS 12 cm	< 0.0001	< 0.0001	0.64
WS 40 cm	0.21	0.11	0.68

Chapter 4

The Temperature Dependence of Soil Nitrogen and Carbon Mineralization in Mid and Late Succession Forests on the Boreal Floodplain.

Abstract

We examined the temperature sensitivity of gross and net N mineralization and hetrotrophic respiration for soils from mid- and late- successional boreal forest in Alaska. Soil from surface and buried organic horizons from balsam poplar (mid succession) and white spruce (late succession) stands was incubated at four temperatures (5, 10, 15 and 20°C) at 50% water holding capacity. Gross N mineralization was measured after three weeks while net N mineralization and CO_2 respiration were measured periodically for 182 and 320 days, respectively. Across stands, gross rates of N mineralization (per g soil N) were more than an order of magnitude higher than net rates. Gross N mineralization was higher for white spruce soil than for balsam poplar across all incubation temperatures, except at 5°C where the difference was not significant (p>0.05). There was also a higher rate of increase (significantly steeper slope and higher Q10 values) in gross N mineralization with temperature in white spruce than balsam poplar. Soil respiration

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[1]Brenner, R.E., Mack, M.C., Boone, R.D. and Vogel, J.V. (in prep). The Temperature Dependence of Soil Nitrogen and Carbon Mineralization in Mid and Late Succession Forests on the Boreal Floodplain.

rates (per g soil C), the temperature sensitivity of soil respiration determined from Q_{10} and activation energy (E_a), and the cumulative amount of C respired during the entire incubation were also generally higher in white spruce compared to balsam poplar soil.

Soil temperature appeared to have a larger role in limiting the turnover of C and N during late succession than *a priori* indicators of organic matter quality such as C:N and lignin:N ratios. Due to the unique nature of the floodplain environment, caution should be used in extrapolating our results to a larger scale or other systems. Nevertheless, compared to the soil of mid-succession stands, increases in soil temperature brought on by the warming of high-latitude forests could result in a proportionately larger increase in the mineralization of N and C from the cold-dominated soils of late-succession stand types (i.e., white spruce and black spruce).

Introduction

In high-latitude ecosystems low soil temperature inhibits the microbially-mediated breakdown of organic matter and consequent mineralization of nitrogen (N) (Klingensmith & Van Cleve 1993; Schmidt et al. 1999; Jonasson et al. 2001) and carbon (C) (Goulden et al. 1998; Hobbie et al. 2000; Neff & Hooper 2002). Therefore, the ongoing and future warming of northern high-latitude regions (Houghton et al. 1996; Serreze et al. 2000; Overland et al. 2004) has large implications for soil C and N cycling in a variety of boreal forest community types where plant primary productivity is generally thought to be N limited (Nasholm et al. 1998; Persson & Nasholm 2001) and

where soil organic matter often tends to accumulate due to the wide-spread occurrence of cold soils or permafrost conditions. However, the magnitude by which soil C and N cycling will be altered by future climatic warming will also depend in part upon biologically-driven modifications to the soil environment from plant contributions to above- and belowground litter production.

In the boreal forest, soil temperature and the composition and decomposability of soil organic matter can be dramatically altered as a consequence of plant successional events (Flanagan & Van Cleve 1983; Fox & Van Cleve 1983; Van Cleve et al. 1996). On the floodplain ecosystems of Alaska's interior a major modification to the soil chemical and physical environment occurs when mid succession stands of balsam poplar (*Populus balsamifera*) succumb to dominance by white spruce (*Picea glauca*) (Viereck et al. 1983; Van Cleve et al. 1991) during the advent of late succession. As balsam poplar is shaded out by white spruce there is a decrease in the amount of leaf litterfall, which enables moss to establish in the understory (Viereck et al. 1993a). Progressively thicker layers of moss act as insulation and inhibit soil warming during the summer months such that surface horizons remain frozen throughout an ever-increasing portion of the growing season. Eventually the soil becomes permanently frozen year-round (permafrost) with only a shallow active layer unfrozen during the growing season. Plant litter produced during late succession contains higher ratios of lignin:N and C:N than in mid succession and is generally thought to be more recalcitrant to microbial breakdown (Van Cleve & Viereck 1981; Flanagan & Van Cleve 1983). Thus, the successional transition from a deciduous to coniferous-dominated landscape in the boreal forest is a fundamental turning point in

which plant species composition mediates declines in soil temperature, organic matter decomposition and rates of nutrient cycling.

The accumulation of organic matter in boreal floodplain forests has an additional element of complexity in that flooding events periodically deposit mineral substrate on top of the forest floor. The soil profile becomes stratified with a surface forest floor/litter layer and multiple buried organic and mineral horizons of varying thickness and composition. Flooding events eventually create terraces 3-4 meters above the average height of the river such that by the later part of mid-succession (mature balsam poplar) the inundation of surface soil is relatively infrequent. However, buried organic horizons remain within the soil profile, and constitute an important portion of the overall soil organic matter. Buried organic horizons likely persist because they are insulated from the higher surface temperatures that would facilitate decomposition and, in later succession stands, this portion of the soil profile remains frozen throughout a considerable portion of the growing season. Buried organic horizons are also known to be areas that are actively colonized by fine roots (Ruess *pers comm.*) and thus may actually accumulate or be replenished by labile forms of C and N over time.

In this study we used organic soil throughout the top 20 cm (surface and buried organic horizons) to investigate the influence of soil temperature on C and N mineralization during mid (balsam poplar) and late (white spruce) succession in forest stands on a boreal floodplain. Our goal was to simultaneously examine the influence of soil temperature on these two aspects of organic matter breakdown. In particular, we wanted to test whether field studies showing generally higher rates of net N

mineralization in the soil of mid versus late succession communities of this ecosystem (Figure 4.1; Klingensmith & Van Cleve 1993; Van Cleve et al. 1993; Brenner et al. *In Press*) could be attributed to a decrease in soil temperature during late succession or might be related to some other factor such as more recalcitrant organic matter. For example, if the recalcitrant nature of organic matter inhibits C and N mineralization rates in white spruce soil, then, compared to balsam poplar soil, gross N mineralization and CO_2 respiration should be less responsive to increases in temperature. However, if soil temperature is the main factor controlling organic matter breakdown in white spruce soil, then overall rates of soil C and N cycling in white spruce should be more sensitive to increases in temperature relative to balsam poplar. To accomplish these goals we measured gross and net N cycling during an initial (~1month) period across a range of temperature treatments (5, 10, 15 & 20°C). In order to compare the longer-term pool size of potentially mineralizable soil C in these two successional sequences, we periodically measured soil C mineralization for 316 days. In an effort to better understand the influence of temperature on the nature of C being mineralized (e.g., labile or recalcitrant) we also tracked the $^{13}CO_2$ isotopic signature of respiration at various times throughout the course of the study.

Methods

Study Sites

Soils for this experiment were collected from mature stands of balsam poplar and white spruce, located within and adjacent to the Bonanza Creek Long Term Ecological

Research Site (BNZ-LTER), approximately 30 km south of Fairbanks, Alaska USA
(64°45' N, 148°18' W). Balsam poplar sites (LTER sites BP1, BP2 and BP3) contained
trees 80-100 years old with a substantial understory of rose (*Rosa acicularis*) and N-
fixing thinleaf alder (*Alnus tenuifolia*). White spruce sites (LTER sites FP4A, FP4B and
FP4C) generally consisted of trees 200+ years old and an understory of alder (*A. crispa*
and *A. tenuifolia*) and rose; however, alder was not present at the FP4C site. White
spruce soils were covered by a carpet of moss approximately 10-15 cm thick
(*Hylocomium splendens* and *Pleurozium schreberi*). All research sites were on islands
within the active portion of the Tanana River floodplain and all soil profiles contain
multiple buried organic horizons as a result of past flooding events. The buried organic
horizon closest to the soil surface was likely the result of a massive flood in 1967.
Frozen soil in balsam poplar sites was gone by the end of July but persisted throughout
the entire growing season in white spruce sites (Brenner et al. *In Press*). Soil temperature
was generally highest around the second week in August in both stand types with
maximum values ranging from 10-14°C at 5 cm to ~5°C at 20 cm (LTER unpublished –
See Reference section). Select soil characteristics for these stands can be found in Table
1 and a complete overview of the climate, soil and vegetation of these stands can be
found in Viereck *et al.* (1993a; 1993b).

Experimental Design

Intact soil cores, 0-20 cm in length, were randomly collected from 15 × 15 m
plots during September of 2001 using a 5.8 cm diameter hand corer and immediately

frozen. The soil cores began in the surface litter layer and included dead moss and decomposed leaves; however, live moss and the previous 1-2 years of senesced leaves were pushed aside prior to coring. After approximately one month, cores were thawed at 4°C and organic layers, which included multiple buried organic horizons and surface organic layers, were removed and placed in a common container. Soils were then sieved to 5.6 mm to remove course roots and woody litter. All the soil from each stand type was then homogenized and adjusted to 50% water holding capacity (WHC) with the addition of de-ionized water. Approximately 120 g of soil from each stand type was placed in 20, ~473 cm^3 canning jars to be used for the determination of gross and net N cycling rates. An additional 20 g of soil from each stand type was placed in 20 canning jars (same size) to monitor C respiration over time. Twenty N mineralization and C mineralization canning jars from each stand type were randomly assigned to four temperature treatments (5, 10, 15 or 20°C with 5 replicate soil samples for each stand×temperature combination) and placed in the dark in their respective incubation chamber to begin day 1 of the experiment. All processing was completed within 48 hours of thawing.

Net and Gross N mineralization Rates

Gross N mineralization and consumption was measured between days 21 to 23 of the incubation using ^{15}N pool dilution (Davidson et al. 1991; Hart et al. 1994b). The pool dilution procedure was conducted when microbial processes in the soil had stabilized following thawing and sieving. Stability was characterized as a leveling off in the rate of soil respiration (see below) observed immediately after sieving and initial incubation.

The pool dilution procedure consisted of injecting a solution containing a label of 99% $^{15}NH_4$-N into each of the "N Mineralization" canning jars using a needle and syringe and then mixing the soil and solution with a sterile spoon. The labeled solution added did not exceed 10% of the overall NH_4 pool. Fifteen minutes following injection a sample of soil was mixed with 0.5 M K_2SO_4, shaken for 2 hours, and vacuum filtered through glass-fiber filters (1 μm nominal pore size). All N Mineralization jars were then covered and incubated at their respective temperatures for 48 hours, at which time another soil sample was removed and extracted using the same procedure. To examine microbial immobilization of the ^{15}N label (Davidson et al. 1991), a soil sample was also removed at this time and fumigated with $CHCl_3$ for 24 hours in a vacuum chamber before extraction with 0.5 M K_2SO_4. Subsamples of fumigated and non-fumigated extracts were digested in serum vials using a modified potassium persulfate digestion protocol (Cabrera & Beare 1993) in which dissolved N is oxidized to NO_3. During this procedure several different concentrations of phenylalanine were used as an internal digestion standard.

The concentration of NO_3 and NH_4 in the soil extracts was determined using standard colorimetric techniques on an API 300 segmented flow autoanalyzer (Astoria-Pacific Inc., Clackamas Oregon, USA). The NO_3 in the post-digestion product was determined in the same manner and microbial N was calculated as the difference of total soluble N in fumigated and non-fumigated extracts expressed on a per gram dry soil and per g soil N basis. The amount of ^{15}N within pools of NH_4, DON, and microbial biomass (post-digestion product as NO_3) was determined by first using a diffusion procedure to trap soluble N onto acidified filter paper following the protocol of (Khan et al. 1998).

The atom percent ^{15}N enrichment (APE = [(mole fraction of ^{15}N)/(mole fraction of $^{14+15}N$) x 100]) of the N trapped on the acidified disks was then measured on an isotope-ratio mass spectrometer at the University of Illinois (Mulvaney et al. 1990). Gross NH_4 production and consumption were calculated using the equations of Kirkham & Bartholomew (1954) and microbial immobilization of the $^{15}NH_4$ label was calculated using the non-linear method of Davidson et al. (1991).

Net N cycling was determined periodically throughout the experiment by removing soil samples and measuring the concentration of NH_4 and NO_3 in the N Mineralization jars. This was done on three occasions prior the gross N mineralization procedure (days 0, 8 and 21), during gross mineralization (day 23), and at day 182.

Carbon Mineralization and $\delta^{13}C$ of Heterotrophic Respiration

Soil CO_2 respiration was monitored throughout the course of the experiment by capping the C mineralization canning jars and measuring the change in headspace CO_2 concentration over the course of 2-5 days (Robertson et al. 1999). Gas sampling occurred by first venting and capping the jars, then injecting and extracting an initial ~10 cc sample (C_0) through a rubber septa using a needle and syringe. The initial injection was made so that the samples did not remain under vacuum during the incubation period. After 2-4 days, a final 10 cc sample (C_X) was extracted. The concentration of CO_2 in C_0 and C_X samples was measured with a LICOR 6200 infra red gas analyzer (LICOR, Lincoln, Nebraska, USA) that had been modified with a sample-injection port. The concentration of headspace CO_2 was kept below 4% while jars were capped and the soil

was exposed to moist air in the incubators between headspace samplings. Heterotrophic respiration was calculated as the amount of C evolved during the incubation, expressed on a per g soil or per g C basis. The C mineralization experiment was ended by placing all jars into the 20°C incubator where they remained from days 316 through 320 in order to compare the relative of amount of microbially available C remaining within the two stand types.

The amount of C respired per g soil C during the incubation was determined by fitting second-order exponential decay functions to the respiration rates of individual incubation jars during the entire 316 day incubation (modified from Robertson et al. 1999):

$$C_T = (k_1 \times C_1 \times e^{(-k_1 \times t)} + (k_2 \times C_2 \times e^{(-k_2 \times t)}) + y_0 \tag{1}$$

where C_T is the total amount of C available to soil microbes at a particular point in time, C_1 is the pool size of the most labile C (μg C·g soil^{-1} or μg C·g soil C^{-1}), k_1 is the rate constant for the labile pool (day^{-1}), C_2 is the pool size of the intermediate pool, k_2 is the rate constant for the intermediate pool , e is the base of the natural logarithm, t is the amount of time from the start of the incubation (days) and y_0 is the asymptote of the curve near the steady state. This function was then integrated across time to yield the cumulative amount of C respired. Curve fitting and integration were done with the Origin 7.5 software package (OriginLab Corp, Northampton, MA, USA). Both respiration rates and cumulative amounts of C respired were expressed per g soil C and were corrected for C lost throughout the experiment.

The temperature sensitivity of C respiration and gross N mineralized between each sequential 5°C incubation intervals was investigated by a Q_{10} coefficient (Kirschbaum 1995):

$$Q_{10} = (\frac{k_2}{k_1})^{[\frac{10}{(T2-T1)}]}$$

(2)

where Q_{10} is the coefficient for the exponential relationship between C or N mineralization and temperature normalized to a 10°C temperature interval; T1 and T2 are a cooler and warmer incubation temperature separated by 5°C, respectively, and k_1 and k_2 are the rates of C or N mineralization or cumulative amounts of C respired for the cooler and warmer incubation temperatures, respectively.

The temperature sensitivity of C mineralization was also examined through the calculation of activation energy (E_a). Activation energy is conventionally defined as the amount of energy needed to move a substrate from the ground state to the transition state. In the context of this work a higher E_a at a given temperature does not necessarily translate to a pool of available C that is more recalcitrant to heterotrophic utilization but rather that that the process of C mineralization is more temperature sensitive. Activation energy was determined from C mineralization rates at day 24 of the incubation and for the cumulative amount of C respired during the incubation. This was done by solving for E_a in a modified Arrhenius-type equation (Lloyd & Taylor 1994):

$$R = R_x e^{(\frac{E_a}{T_x K})(1-\frac{T_x}{T})}$$

(3)

where R is the cumulative amount of C respired at temperature T (in Kelvin), R_x is the cumulative amount of C respired at a reference temperature (T_x), E_a is the energy of activation (Joules·mole^{-1}) and K is the universal gas constant (8.314 Joules mole^{-1}K^{-1}).

At three times during the incubation (days 31, 84, 232 of the incubation) the δ^{13}C of CO_2 from C_0 and C_X headspace subsamples was measured on a Europa PDZ 20-20 isotope-ratio mass spectrometer (SerCon Ltd, Cheshire, U.K.). A mixing model was then used to calculate the source signature of δ^{13} CO_2 from heterotrophic respiration.

The concentration of microbial C and DOC in the 0.5 M K_2SO_4 extracts obtained at the end of the gross N mineralization procedure were determined using the persulfate digestions procedure mentioned previously. The post-digestion solution was quite acidic (pH 2-3); thus, the dissolved carbon oxidized during the digestion ended up as CO_2 in the headspace of the serum vials. The headspace air was sampled by inserting a needle through the septa of the serum vial and withdrawing a 10-12 cc sample into a syringe. The air was analyzed for CO_2 using the method described previously for respired CO_2. Along with digestion blanks, five concentrations of phenylalanine were used as an internal digestion standard. The amount of CO_2 evolved from the phenylalanine standards was used to develop a regression equation for calculation of the amount of dissolved C in the samples. Soluble C in the digestion solution was also calculated separately with the use of a pressure-volume equation:

$$n = \frac{PV}{RT} \tag{4}$$

where n is the number of moles of CO_2-C in the headspace of the serum vial following digestion, P is the pressure (atmospheres) inside the serum vial headspace (ambient

pressure + post-digestion pressure inside serum vial), V is the headspace volume (cm^3) or

total serum vial volume minus the volume of the digestion solution, R is the gas constant

of 82.05 (ml·atm/mole·K) and T is the ambient temperature in degrees Kelvin.

Measurements were made after the serum vials were cooled to ambient laboratory

temperature following the digestion.

The pressure-volume (actual C in solution) and internal standard-based

determinations of the C in the digested standard were compared and the efficiency with

which the phenylalanine standards were digested to CO_2 was calculated as:

$$\%E_d = \frac{C_{ph}}{C_{total}} \times 100 \tag{5}$$

where %E$_d$ is the digestion efficiency expressed as the percentage of total C in solution

digested to CO_2, C$_{ph}$ is the amount of C digested to CO_2 as calculated from a pressure-

volume equation of phynylalanine stds (blank corrected) and C$_{total}$ is the amount of

phenylalanine-C in the original digestion solution.

Statistical Analysis

Experimental results were analyzed using a factorial ANOVA or repeated

measures ANOVA in the General Linear Model (GLM) or General Regression Model

(GRM) modules of the Statistica software package (Statsoft 2004, Tulsa, OK, USA).

Tukey's Honestly Significant Difference (HSD) multiple comparison test was used to

determine significant differences within and between soil types for measurements during

a single sampling period (e.g., gross NH$_4$ mineralization); however, the HSD test was

determined to be overly conservative when comparing values for repeated measures analysis across multiple time periods, and in these situations (e.g., soil respiration over time), Fisher's Least Significant Difference (LSD) method was used. Linear and polynomial regression curves were used to examine the relationship between incubation temperature and C and N cycling processes within a given time period. Statistical significance was determined at $\alpha = 0.05$ with values between 0.05 and 0.10 considered "marginally" significant.

Results

Gross and Net Nitrogen Transformations

For white spruce there was a highly significant linear relationship (Adj.-R^2 = 0.93, p < 0.0001) between temperature and gross NH_4^+ mineralization (μg NH_4^+-N g soil N^{-1} day^{-1}), and gross NH_4^+ mineralization for this soil type increased significantly (p < 0.001) with each successive 5°C increases in incubation temperature (Figure 4.2). Gross mineralization rates per g soil N in white spruce soil were also larger than in balsam poplar for the entire range of incubation temperatures. For balsam poplar soil there was a significant linear relationship between gross NH_4^+ mineralization and temperature from 5-15°C (Adj-R^2 =0.70, p < 0.0001). However, gross NH_4^+ mineralization decreased dramatically in balsam poplar soils at 20°C, where the rate was similar to that found at 5°C (Figure 4.2a). Excluding the 20°C incubation temperature, there was a significantly steeper rate of increase in gross NH_4^+ mineralization with temperature in white spruce compared to balsam poplar (p = 0.0003 for stand × temperature interaction), such that

stand-level differences in gross NH_4^+ mineralization between 5-15°C became increasingly larger with increasing temperature (Figure 4.2a). In addition, Q10 values for gross NH_4^+ mineralization were significantly higher for white spruce soil than balsam poplar between at all temperature intervals (Figure 4.3).

Gross NH_4^+ consumption, which includes microbial immobilization and adsorption, followed the same pattern as gross NH_4^+ mineralization (Figure 4.2b) and absolute rates of NH_4^+ consumption closely matched gross mineralization in both stand types. Gross consumption also was highly correlated with temperature in white spruce (Adj.-R^2 = 0.97, p = 0.008), where NH_4^+ consumption increased steadily with each 5°C increase in incubation temperature, and in balsam poplar from 5-15°C (Adj.-R^2 = 0.99, p = 0.02). Patterns of gross consumption and mineralization differed in that rates of consumption did not decrease as dramatically at 20°C in balsam poplar as did gross mineralization.

Trends in the gross microbial immobilization of NH_4^+ were less pronounced than for either gross mineralization or consumption (Figure 4.2c). In balsam poplar soil, gross immobilization of NH_4^+ increased linearly with temperature from 5-20°C (Adj.-R^2 = 0.94, p < 0.0001), and there was not a decrease in gross immobilization at 20°C as was observed for gross mineralization and consumption. In white spruce there was a progressive decline in microbial immobilization of NH_4^+ with temperature between 5-15°C (Adj.-R^2 = 0.67, p < 0.0001) but an increase between 15-20°C (regression not shown).

The net N mineralization rate (per g soil N) measured during the first 21 days of the study, was significantly higher in white spruce soil than balsam poplar at all

temperatures (Figure 4.4a; $p < 0.001$) except at 15°C where the difference was not significant ($p = 0.50$). Across stand types there were widely contrasting patterns of net ammonification and nitrification that contributed to the overall net rate. For white spruce soil there was a net production of NH_4^+-N and a strong positive correlation between temperature and net NH_4^+-N production when fit with an exponential growth equation (Figure 4.4b; $R^2 = 0.98$). Net nitrification in white spruce was near zero at all incubation temperatures (Figure 4.4c). In contrast, for balsam poplar soil there was a net production of NO_3^--N and net nitrification made up the only positive contribution to overall net N mineralization. This is because there was always a net consumption of ammonium in balsam poplar and net ammonification did not respond to increased temperature. There was a strong exponential relationship between net nitrification and incubation temperature (R^2 of exponential fit = 0.96).

The concentration of NO_3^--N rose steadily in all balsam poplar soils throughout the course of the experiment and also increased with incubation temperature (Figure 4.5a). In white spruce, the pool size of NO_3^--N remained near zero until day 182 when concentrations increased dramatically and were greater than or equal to those found in balsam poplar stands. In balsam poplar soil the pool size of NH_4^+-N decreased steadily from days 0 to 182 (Figure 4.5b), and did not appear to be correlated to temperature, while in white spruce NH_4^+-N increased from day 0 to 21 and also generally increased with incubation temperature. However, the concentration of NH_4^+-N in white spruce soil changed only slightly between days 21 and 182.

The ratio of gross-to-net DIN mineralization was around 20 in white spruce across all incubation temperatures (Figure 4.6a). However, in balsam poplar this ratio was significantly higher than white spruce soil at 5°C (102.2 ± 8.4) and 10°C (87.2 ± 26.3), while at 15°C and 20°C it was not significantly different between stand types.

Carbon Mineralization

Heterotrophic respiration per gram soil C increased with incubation temperature in both stand types throughout the course of the incubation (Figure 4.7) and respiration rates were significantly higher ($p < 0.05$) in white spruce compared to balsam poplar soils during many of the sampling periods. During the period in which gross N processes were measured (week 3), soil respiration per g soil C was significantly higher ($p < 0.01$) in white spruce compared to balsam poplar at every incubation temperature (Figure 4.8). The cumulative amount of CO_2-C respired during the entire 316 day incubation was also significantly higher for white spruce compared to balsam poplar for all incubation temperatures (Figure 4.9). In addition, the difference between rates in white spruce and balsam poplar increased with incubation temperature, especially at 15 and 20°C where there was substantially more C respired per g initial soil C.

Incubating all soils at 20°C during days 316 to 320 reversed the previous trend in respiration such that an inverse relationship developed between the previous incubation temperature and respiration (Figure 4.10). During this period respiration per g soil C was very similar between stand types. The only significant difference was at 10°C where respiration was slightly higher for balsam poplar than white spruce soil ($p = 0.01$).

Q_{10} values calculated from the entire amount of C respired during the incubation were not significantly different between stand types for the 5-to-10°C or 10-to-15°C intervals but were dramatically higher for white spruce than balsam poplar at the 15-to-20°C interval (Figure 4.11a; $p < 0.0001$). Q_{10} decreased steadily with incubation temperature in balsam poplar but remained flat until the 15-to-20°C interval in white spruce. The energy of activation (E_a) decreased with incubation temperature in balsam poplar but increased with incubation temperature in white spruce. The energy of activation was significantly higher ($p < 0.0001$) for white spruce than balsam poplar at 10, 15 and 20°C. Compared to balsam poplar stands, Q_{10} values calculated at day 24 of the incubation were significantly higher for white spruce soil at the 15-to-20°C (1.90 ± 0.04 for balsam poplar vs. 2.19 ± 0.05 for white spruce; $p < 0.01$) interval and marginally higher for white spruce at the 5-to-10°C (2.04 ± 0.03 for balsam poplar vs. 2.20 ± 0.06 for white spruce; $p = 0.06$) and 10-to-15°C (2.20 ± 0.08 for balsam poplar vs. 2.34 ± 0.05 for white spruce; $p = 0.08$) intervals (Figure 4.11b). The energy of activation was significantly ($p < 0.05$) higher for white spruce soil at 10, 15 and 20°C.

As was the case for the ratio of gross-to-net N mineralization, the ratio of the CO_2-C respiration rate to net N mineralization (per g soil) during the pool dilution study was significantly higher at 5°C ($p = 0.006$) and 10°C ($p = 0.01$) in balsam poplar than in white spruce soil (Figure 4.6b). However, the ratio was similar between stand types at the 15 and 20°C incubation temperatures. In contrast, the ratio of CO_2 respiration to gross NH_4-N mineralization was significantly higher ($p \leq 0.0001$) for white spruce than

balsam poplar soil at all incubation temperatures except for 20°C (Figure 4.6c). The ratios in both soil types generally increased with temperature.

Microbial Biomass C and N

Microbial biomass C per g soil C measured at day 23 of the incubation was not significantly different between stand types (p > 0.23; table 2). There was a significant inverse correlation between temperature and microbial biomass C in balsam poplar (R = - 0.62, p = 0.004) and white spruce (R = -0.64, p = 0.003). However, this correlation only resulted in a slight decrease in biomass C with temperature. Microbial biomass N per g soil N was larger in white spruce than balsam poplar at all incubation temperatures (p < 0.0002). There was no relationship between incubation temperature and microbial biomass N.

DOC and DON

Dissolved organic carbon (DOC) concentrations (per g soil C), measured during the analysis of gross N cycling rates (day 23), were higher in balsam poplar soil than in white spruce across all incubation temperatures (table 2). Per g soil N, dissolved organic nitrogen (DON) concentrations were similar between stand types, except at the 20°C incubation temperature where the concentration was slightly higher for balsam poplar soil (table 2).

δ¹³C of Respiration

The $\delta^{13}C$ signature of heterotrophic respiration became increasingly more enriched over time with mean values generally between -30‰ and -27‰ early in the incubation and increasing to between -26‰ and -24.5‰ range by day 232 (Figure 4.12). Within a given sampling date there was a trend toward more depleted values with increasing temperature. The trend of heterotrophic respiration being progressively more enriched over time but more depleted with increasing temperature was similar for both stand types.

Discussion

Gross and Net N Mineralization

Despite patterns observed *in situ* showing that net soil N mineralization slows during the transition from mid-succession balsam poplar stands to late-succession white spruce stands (Klingensmith & Van Cleve 1993; Van Cleve et al. 1993; Brenner et al. *In Press*), laboratory estimates of gross (Figure 4.2a) and net (Figure 4.4) N mineralization rates (expressed per g soil N) in this study were greater in white spruce soil than in balsam poplar soil across all incubation temperatures. Moreover, the faster rate of increase in gross N mineralization (steeper slope) with increasing temperature and higher Q_{10} values (Figure 4.3) in white spruce soil compared to balsam poplar suggests that N mineralization in white spruce soil is more sensitive to increases in temperature. These data also suggest that organic matter "quality" with respect to N cycling does not necessarily decrease during this successional transition and that decreased soil

temperature could be the primary driver of the lower rates of *in situ* net N mineralization within the soil of late succession forests.

Because of the relatively high rates of NH₄ consumption (Figure 4.2b) and immobilization (Figure 4.2c), gross N mineralization rates were generally about 20 times those of net mineralization at day 23 of the incubation when gross N mineralization was measured (Figure 4.6a). Using the average daily rate of N mineralization between days 0 and 21, the ratio of gross-to-net N mineralization was very consistent across all incubation temperatures in white spruce; however, this ratio was considerably higher (ratio of ~100) at the 5°C and 10°C incubation temperatures in balsam poplar. Initially, this would seem to indicate that incubation temperature had a much stronger influence on microbial N demand in balsam poplar soil compared to white spruce (higher N consumption relative to production). However, we suspect that the decrease in this ratio with temperature may also be indicative of the limitations associated with laboratory incubations. By the time of the pool dilution procedure (days 21-23), an appreciable amount of inorganic N had built up inside of those soils that had been incubated at higher temperatures (Figure 4.5). Thus, the influence of temperature on rates of N cycling was confounded by the higher absolute amounts of inorganic N within the soils at the start of the pool dilution procedure. We believe that the steady build up of inorganic N may have served to alleviate microbial N demand in balsam poplar at the higher temperatures while the pool of active C was decreasing rapidly. Thus, soil microbes did not have an adequate supply of C to immobilize inorganic N at the higher incubation temperatures, resulting in higher net rates of N mineralization and a lower ratio of gross-to-net

mineralization. Nevertheless, when comparing the two stand types the substantially lower amount of net N mineralization and higher ratio of gross-to-net N mineralization in balsam poplar for the 5°C and 10°C incubation temperatures indicates that this soil type probably had a higher initial N demand. This would be in line with previous studies suggesting that microbes in balsam poplar soil are relatively N limited (Clein & Schimel 1995; Schimel *et al.* 1998) compared to other boreal forest communities which have been hypothesized to be limited by available energy (labile C) (Flanagan & Van Cleve 1983).

Similar to patterns observed *in situ* (Van Cleve et al. 1993; Brenner et al. *In Press*), a large portion of the calculated rate of net N mineralization (Figure 4.4) consisted of nitrification in balsam poplar soil but was dominated by ammonification in white spruce. We did not measure gross rates of nitrification in this study; therefore, we are not able to resolve the mechanisms behind this phenomenon and this topic warrants attention in future research. Possible explanations for the disparity in N forms produced during net N mineralization include higher rates of gross nitrification in balsam poplar soil, greater consumption of NO_3^- by microbes in white spruce soil, or the inhibition of soil nitrifiers in white spruce soil. Of these, it seems that consumption of NO_3^- in white spruce during the first several weeks of the incubation is the most likely possibility. Coniferous stands can have negligible net rates of nitrification but substantial amounts of gross NO_3^- production when measured by the $^{15}NO_3^-$ pool dilution technique (Stark & Hart 1997). This occurs because microbial immobilization of NO_3^- can equal or exceed that of gross NO_3^- production. In the present study, the concentration of NO_3^- increased

dramatically in the white spruce soil when it was last measured on day 182 (Figure 4.5a) and this would indicate that nitrifying bacteria were not chemically inhibited within this soil type. At some point prior to day 182 the pool of labile C had probably decreased to the extent that soil microbes could no longer immobilize NO_3^-, allowing the concentration to increase rapidly (Hart et al. 1994a).

There were two results pertaining to the influence of temperature on N mineralization that were puzzling to us. First, both gross N mineralization and consumption in balsam poplar soil decreased substantially at the 20°C incubation temperature, although we observed a linear increase between 5-15°C. Second, there was an exponential increase in net N mineralization (Figure 4.4) and C respiration (Figure 4.8) with temperature but only a linear increase in gross NH_4^+ production and consumption with temperature. Do these disparities accurately reflect what occurs *in situ* or could they be an artifact of examining these processes in a laboratory incubation experiment? Both results could be explained by a violation of one of the assumptions of the pool dilution method – that the $^{15}NH_4^+$ label is not re-mineralized back into the NH_4^+ pool once it has been taken up by soil microbes (Hart et al. 1994b). Such a re-mineralization would make it appear that the gross production of NH_4^+ was lower than it actually was. This is because gross rate calculations are based upon the dilution of the $^{15}NH_4^+$ label by the production of predominantly $^{14}NH_4^+$ during the depolymerization of organic molecules. If re-mineralization did occur during the 48 hours between the injection of the ^{15}N label and sample extraction it would likely have increased with temperature along with the turnover of microbial biomass. Across stands, a temperature-

dependent increase in re-mineralization could have caused the disappearance of the exponential trend from the actual gross rate of production while a particularly high rate of re-mineralization at 20°C in balsam poplar soil could have produced the appearance of a decrease in gross mineralization between 15-20°C.

If the "no re-mineralization" assumption is increasing violated at higher temperatures it severely confounds the ability to study the temperature dependence of gross N processes. This is because incubation length would need to be longer at low temperatures (e.g., -5 to 5°C) in order to have measurable amounts of production, but would need to be shorter at higher temperatures (> 20°C) to account for re-mineralization. Another possibility is that the observed lower gross rate at 20°C in balsam poplar soil reflects the disappearance of labile organic matter prior to the time that the pool dilution study had begun. This could occur if balsam poplar soil was at or near this temperature *in situ* which would allow a broad range of organic constituents (both labile and refractory) to be accessible to enzymatic breakdown. However, if this were the case then there also should have been an associated decrease in respiration at this temperature, and there was not. Whatever the reason behind the linear trends in gross NH_4^+ production with increasing temperature or the drop at 20°C in balsam poplar, it is clear from this study that incubating soil above temperatures that normally occur *in situ* will not necessarily result in a maximization of gross N transformation rates.

C Mineralization

Results from this study suggest that, across a wide range of temperatures, soils from late succession stands of white spruce contained a higher proportion of labile C than soils from mid succession stands of balsam poplar. Over the course of the incubation, soils from white spruce stands respired a significantly larger amount of C (per g soil C) at all incubation temperatures compared to that from balsam poplar stands (Figure 4.9). The difference in respired C in white spruce vs. balsam poplar also increased with incubation temperature. Moreover, activation energy for C respiration in white spruce soil was significantly higher than in balsam poplar from 10-20°C (Figure 4.11), indicating greater temperature sensitivity. Activation energy throughout the entire experiment (data not shown) was also almost always higher in white spruce. The Q_{10} of respiration was also generally higher in white spruce than balsam poplar. Overall, these results indicate that, compared to soil from balsam poplar stands, soil from these white spruce stands had: 1) a proportionately larger pool of available C for microbial breakdown across a wide range of temperatures; 2) respiratory losses of C that were generally more sensitive to increases in temperature; and, 3) respiratory temperature sensitivity that increased with incubation temperature.

It was somewhat surprising that the soil from white spruce stands had a larger fraction of microbially-available C than the soil from balsam poplar stands. Microbes in balsam poplar soils are thought to have a rich supply of C available to them due to the large annual inputs of poplar leaves containing labile, low molecular weight phenolics (Clein & Schimel 1995, Schimel et al. 1998). In contrast, it has been reported that

evergreen litter generally decomposes more slowly than litter from deciduous species (Hobbie et al. 2000; but, see Ruess *et al. in press-b*). In addition, the decomposability or "quality" of soil organic matter is thought to decrease sharply in late succession evergreen stands of the boreal forest as soil is formed from recalcitrant litter with a high lignin content and high ratio of C:N (Flanagan & Van Cleve 1983; Vance & Chapin 2001). However, previous studies suggesting that late-succession coniferous litter decomposes more slowly than mid-succession deciduous litter may not translate entirely to this study in which a combination of surface and buried organic horizons were incubated. The coarsely sieved soil of this study probably contained a large amount of labile fine roots that decompose rapidly (Ruess et al. *in press-a;* Ruess et al. *in press-b*) and such a pool might also have been quite sensitive to increases in temperature. The organic soil layers at the bottom of our white spruce cores also contained plant material from previous successional stages (e.g., alder and poplar) that may have been of relatively high quality. Such material would be at or near freezing for much of the year in white spruce stands in which frozen soil was detected throughout the growing season. Therefore, results showing that white spruce soil had larger amounts of C respired per g soil C at all temperatures, and respiration that was more temperature sensitive than balsam poplar soil, may reflect belowground turnover much more than surface litter which, we speculate, may contain a higher portion of recalcitrant material.

Our results are also in agreement with other studies suggesting that initial measures of low soil "quality" such as high ratios of C:N and lignin:N (table 1) and low pH may not always be good indicators of the relative rates of soil C and N mineralization

(Giardina et al. 2001; Hobbie et al. 2002). This is probably because the relative rates of decomposition in this and other high-latitude ecosystems are controlled to a greater degree by conditions in the field (e.g., low temperature or high moisture) rather than by soil chemistry (Weintraub & Schimel 2003). Therefore, it should not be assumed that soils from older coniferous successional seres in the boreal forest always have more recalcitrant organic matter than that of younger deciduous stands.

Relationships Between C and N Mineralization

The mineralization of C and N generally appeared to be linked in this study with significant linear correlations in both stand types between C respiration and gross and net N mineralization ($p < 0.0001$). However, for both stand types, with increasing temperature the amount of C respired tended to increase relative to the amount of gross N produced (Figure 4.6c). The proximate cause for this disparity is the exponential increase in C respiration with increasing temperature but linear increase of gross N mineralization with increasing temperature. Again, this might be due to a violation of the assumptions involved in the ^{15}N pool dilution technique (increasing re-mineralization of immobilized ^{15}N at higher temperature). It is our belief that the proportion of C-to-N mineralized is actually consistent across temperatures and that the increase of this ratio at higher temperatures represents the degree by which re-mineralization of the ^{15}N label reduced gross rates in both stand types.

The ratio of C-to-net N mineralization (Figure 4.6b) provides further evidence that there was a reduction in microbial N demand with increasing temperature in the

balsam poplar soil. As was the case for the ratio of gross:net N mineralization (Figure 4.6a), the ratio of C-to-net N mineralization measured during the third week of the study was very constant across all incubation temperatures in white spruce (125-150) and at the 15 and 20°C incubation temperatures in balsam poplar (~75). However, this ratio was substantially higher at the 5°C and 10°C incubation temperatures (300-325) in balsam poplar. Between stands, we believe that microbial demand for N was highest in balsam poplar soil but this demand was rapidly satiated at the higher incubation temperatures (15°C and 20°C) due to the increase in the pool size of DIN (Figure 4.5) and the simultaneous loss of labile C by week three of the incubation (Figure 4.7).

$\delta^{13}C$ of Heterotrophic Respiration

Time and temperature induced opposing trends on the isotopic composition of heterotrophic respiration (Figure 4.12). The approximately 2.5 to 3‰ enrichment of the $\delta^{13}CO_2$ signature between day 31 and 232 is comparable to trends observed in other studies (Andrews et al. 1999; Kohzu et al. 1999) and represents a source pool of available C that becomes increasingly enriched with time. The same situation occurs with depth in soil, perhaps because a given cohort of C -- a pool of C that enters the soil as litter at a point in time -- becomes increasingly composed of the enriched, reprocessed, constituents of soil microbes due to the respiratory loss of lighter isotopes (Ehleringer et al. 2000). This may involve the preferential digestion of enriched labile compounds over more depleted and recalcitrant compounds that, in situ, would be lost as DOC (Tu & Dawson In Press). However, in the short term (days to months) this mechanism would appear to

be an unsatisfactory explanation for the results of our study in which a 15°C increase in temperature resulted in a 1-2‰ depletion of respired CO_2 and, presumably, also the pool of C available to soil heterotrophs. We had expected higher incubation temperatures to be a good surrogate for time, in that a faster turnover of available C pools would cause the respiratory signature to become enriched more quickly than at the lower incubation temperatures. Instead, our results suggest that at higher incubation temperatures isotopically depleted substrates such as lignin and lipids (Gleixner et al. 1993; Fernandez et al. 2003) represent a larger portion of the C pool that is available to soil microbes. A shift towards the utilization of these more recalcitrant substrates at higher temperatures could be the result of a temperature-induced change in the microbial community (Andrews et al. 2000) or fractionation during substrate utilization (Henn & Chapela 2000; Henn et al. 2002). Another possibility is that the labile and enriched substrates are exhausted rapidly at higher temperatures, leaving only the more recalcitrant and depleted substrates by day 31. While we cannot rule out fractionation during C uptake, the utilization of more depleted and recalcitrant substrates at higher temperatures to us seems the most parsimonious explanation given the higher activation energy required for their enzymatic depolymerization. Over longer time periods our data suggest that the overall enrichment of available C pools will continue at all incubation temperatures and, we suspect, the influence of temperature on $\delta^{13}CO_2$ will be lessened as labile and intermediate pools of plant-derived C are used up.

Conclusions

Results from this study suggest that the depolymerization of organic C and N is generally linked in both stand types, with increasing temperature resulting in increases in C respiration as well as gross and net N mineralization. However, significantly higher Q_{10} values for C and N mineralization, higher activation energy, and a greater cumulative amount of C respired at all incubation temperatures in white spruce than in balsam poplar soils indicate that the soil of this late-succession stand type has a proportionately larger pool of microbially-available organic matter that is relatively more sensitive to microbial utilization with increasing temperature. Given scenarios predicting a warming of high-latitude ecosystems during the coming decades, boreal floodplain ecosystems that have deep stores of C (buried organic horizons) protected from microbial degradation by low soil temperatures could be landscapes that are particularly susceptible to C loss. This should be of particular concern given the substantial decrease in the areal extent of permafrost in Alaska's floodplain forests during recent decades (Jorgenson et al. 2001).

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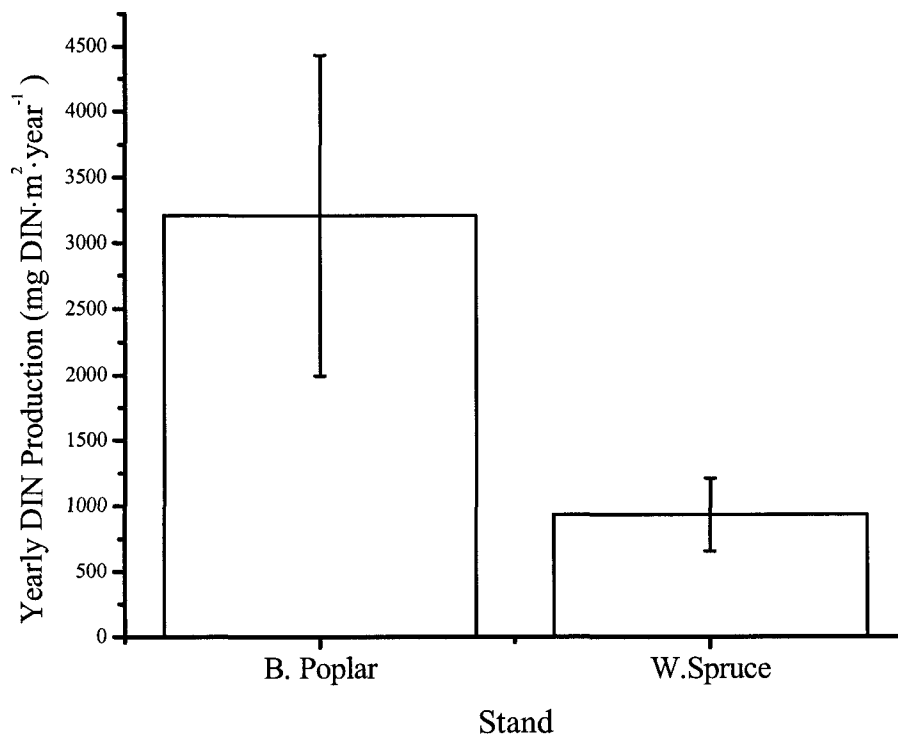
Figure 4.1. Average yearly net N mineralization rate from intact soil cores (0-20cm). The soil cores were incubated in stands of white spruce and balsam poplar during 2000-2001. Each replicate core was incubated for approximately one month during the snow free period and over the course of the winter from October to May. The soil included decomposed surface litter material as well as buried organic and mineral horizons. Values are means ± 1 S.E. from n=3 replicate plots per stand type.

4.2a)
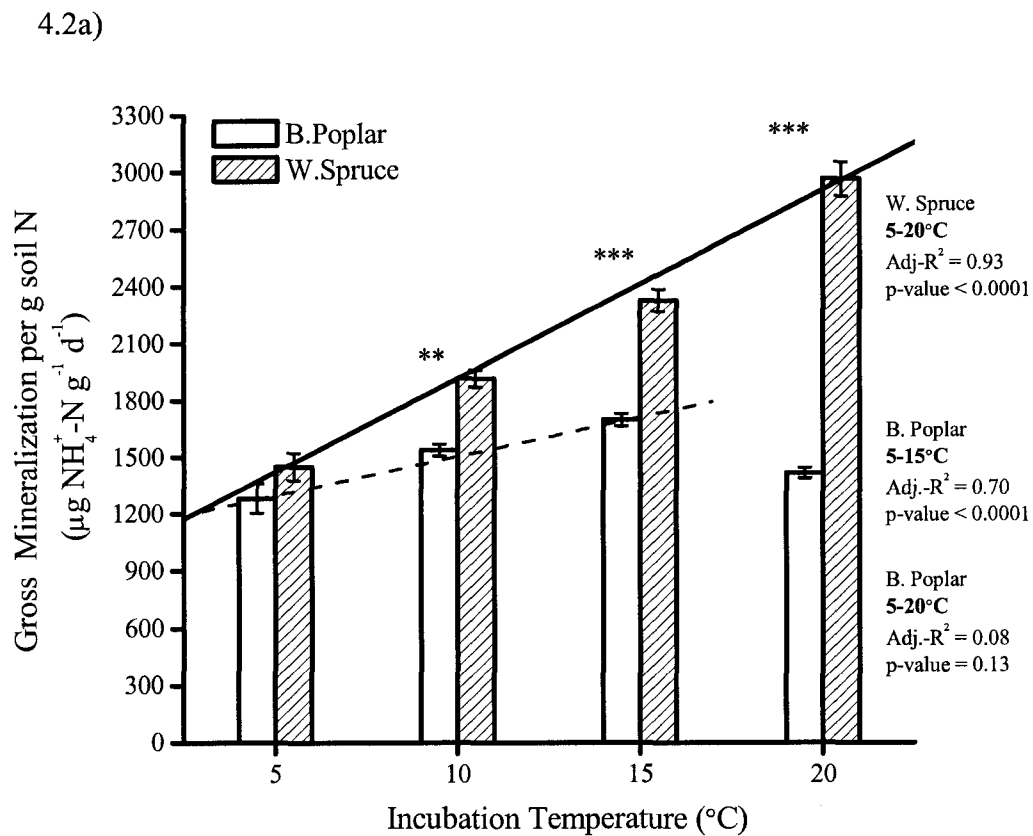
Figure 4.2a. Gross NH_4^+-N mineralization.

Figure 4.2. Bar graphs of a) gross mineralization, b) gross consumption and c) gross microbial immobilization. Rates were determined from organic soils of balsam poplar and white spruce incubated at 5, 10, 15 and 20°C and all values are expressed as μg NH_4^+-N·g soil N^{-1}·day^{-1}. For Figure 4.2a, the solid line is the linear regression of gross mineralization and temperature in white spruce from 5-20°C and the dashed line is the regression in balsam poplar from 5-15°C. Values in Figures are means (±1 S.E.) from n=5 replicate samples and asterisks indicate the following significant differences between stand types * p < 0.05, ** p < 0.01, *** p < 0.001.

4.2b)
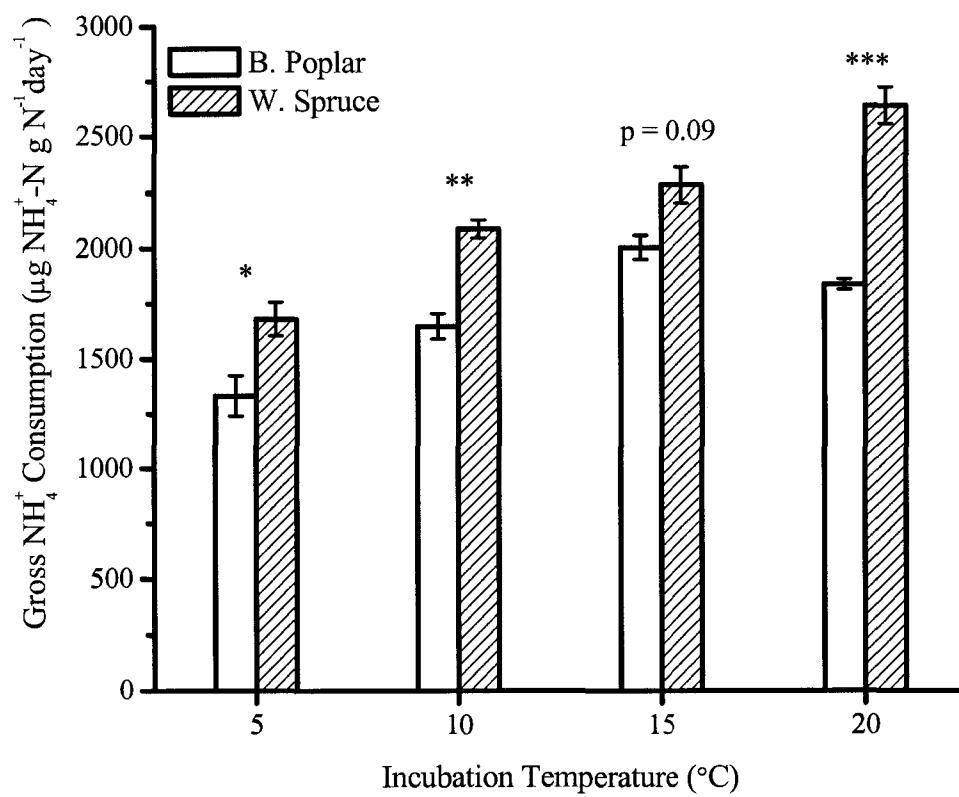
Figure 4.2b. Gross NH_4^+-N Consumption.

4.2c)
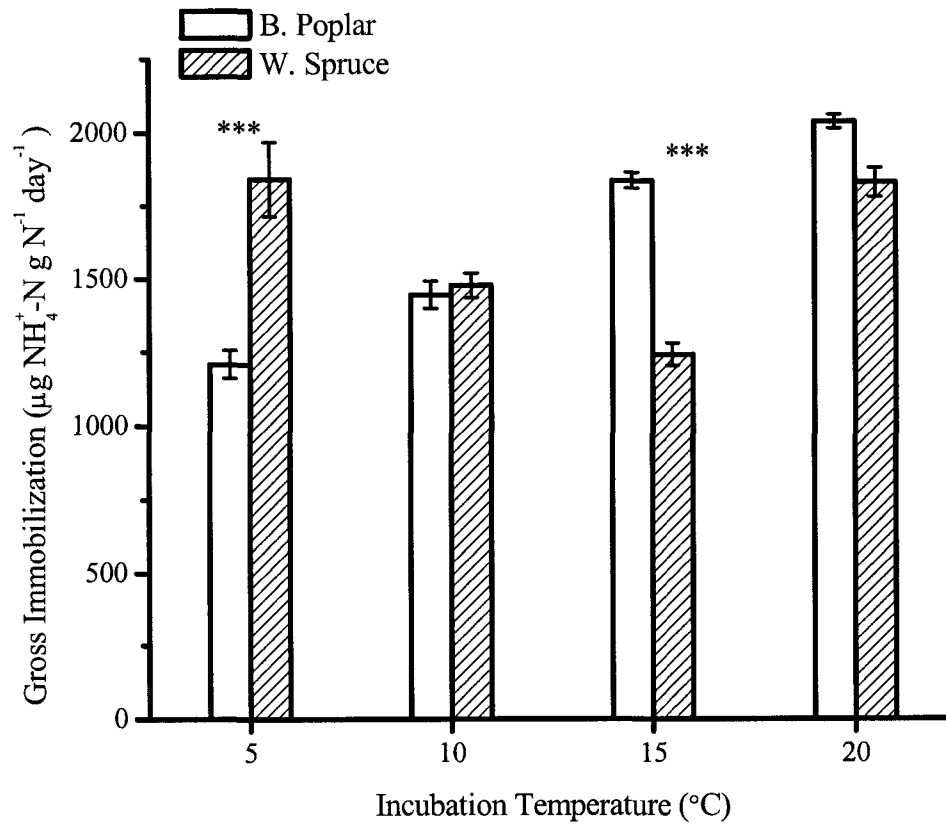

Figure 4.2c. Gross microbial immobilization of NH_4^+-N.
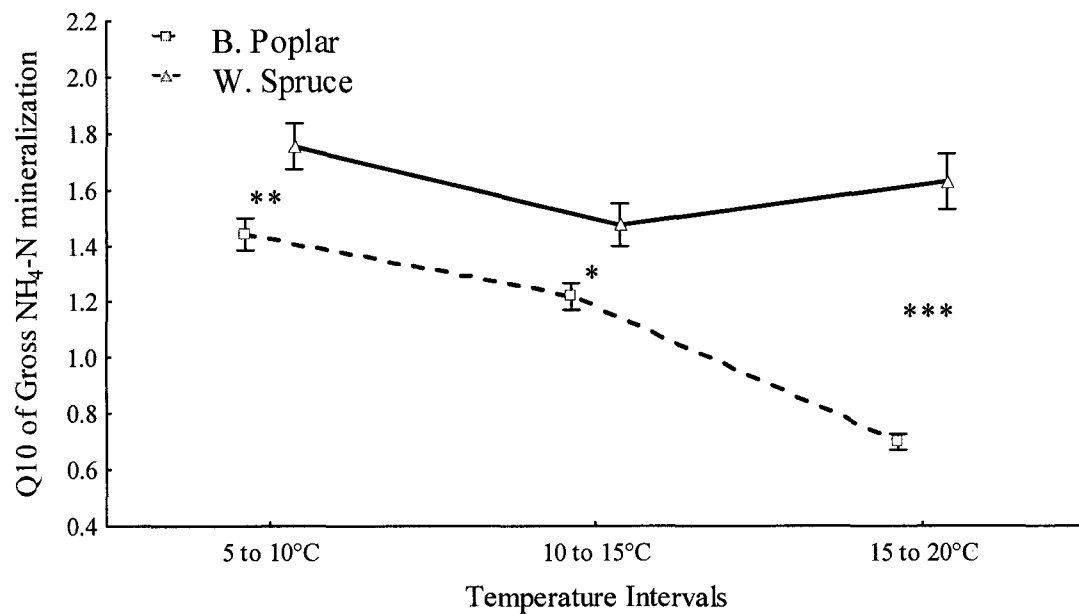
Figure 4.3. Q_{10} values determined from gross NH_4^+-N mineralization rate and incubation temperature. The Q_{10} describes the increase in gross NH_4^+-N mineralization normalized to a 10°C increase in incubation temperature. Each point is the mean (±1 S.E.) Q_{10} from n=5 replicate samples (incubation jars) per stand×temperature combination. Asterisks indicate the following significant differences between stand types * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4.4a)
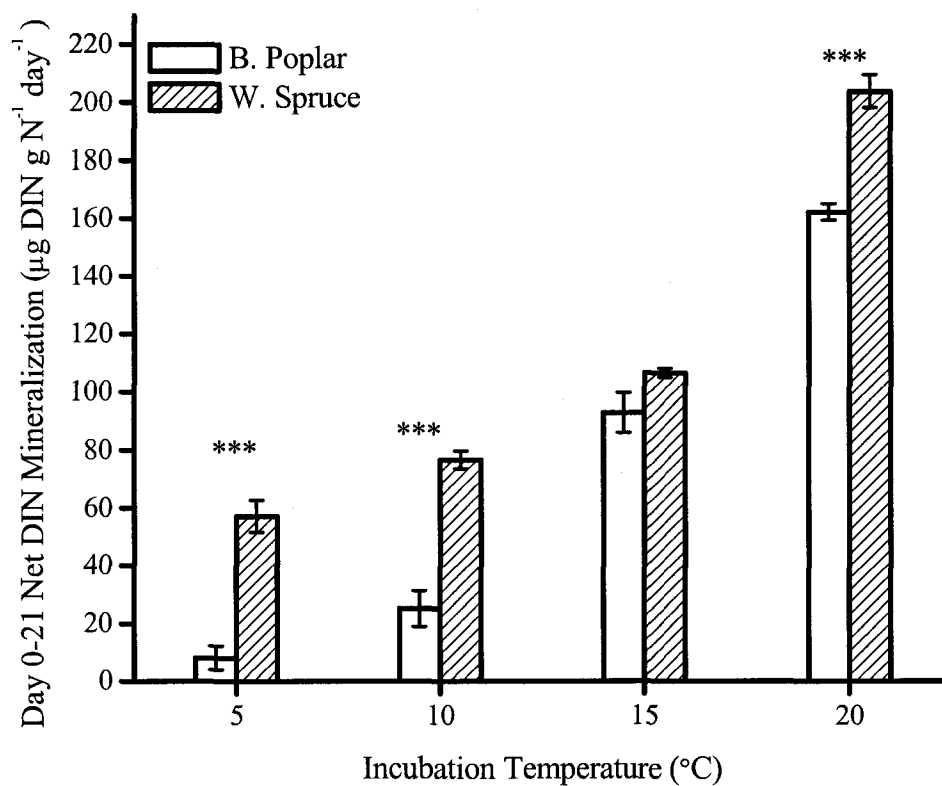
Figure 4.4a. Net N mineralization at day 21 of the incubation.

Figure 4.4. Mean (±1 S.E.) rates of **a)** net N mineralization, **b)** ammonification and **c)** nitrification (μg N·g soil N^{-1}·day^{-1}) in organic soils of balsam poplar and white spruce taken from the Tanana River floodplain. Rates were determined at day 21 of the incubation during the addition of the ^{15}N labeled solution.
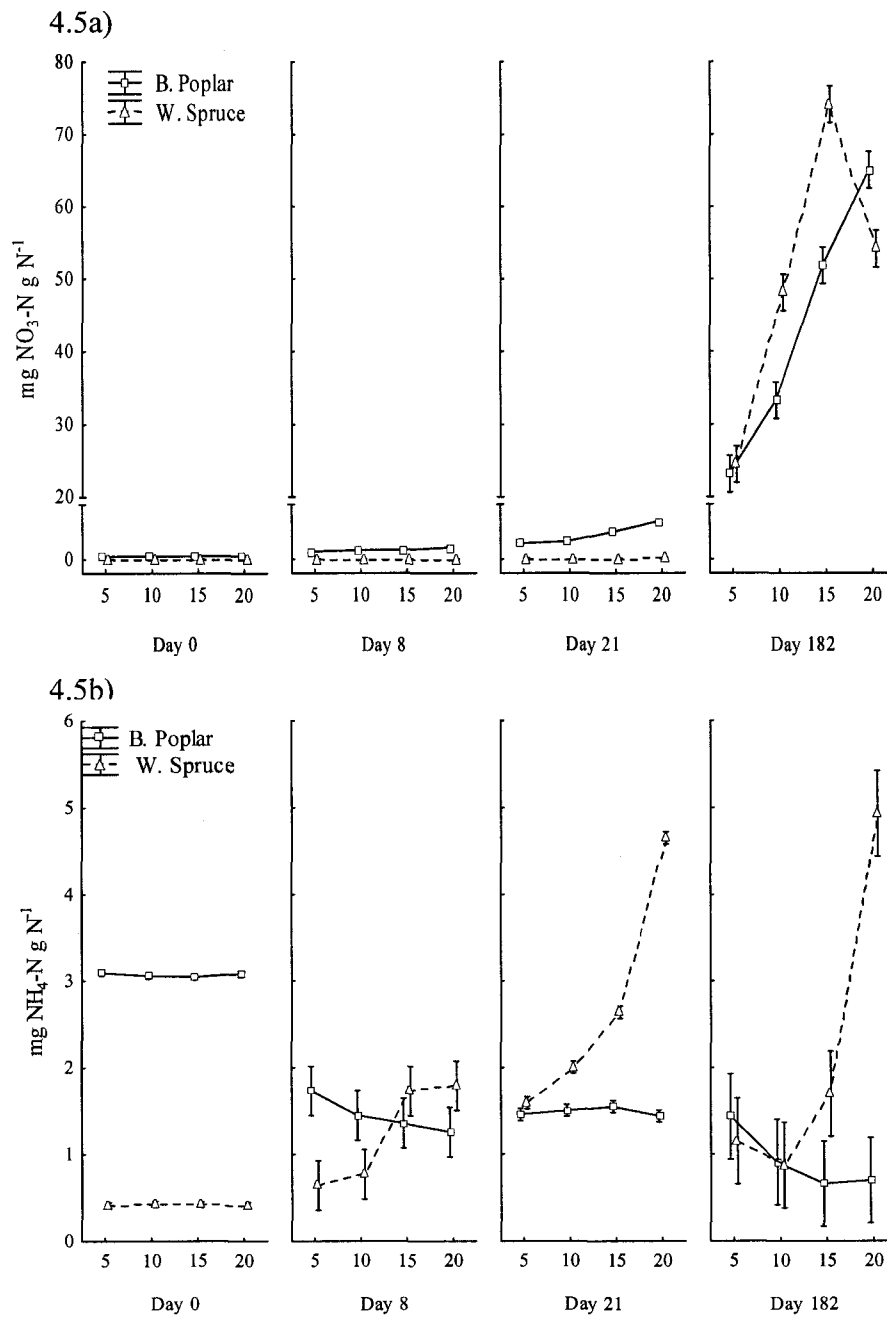
4.4b)
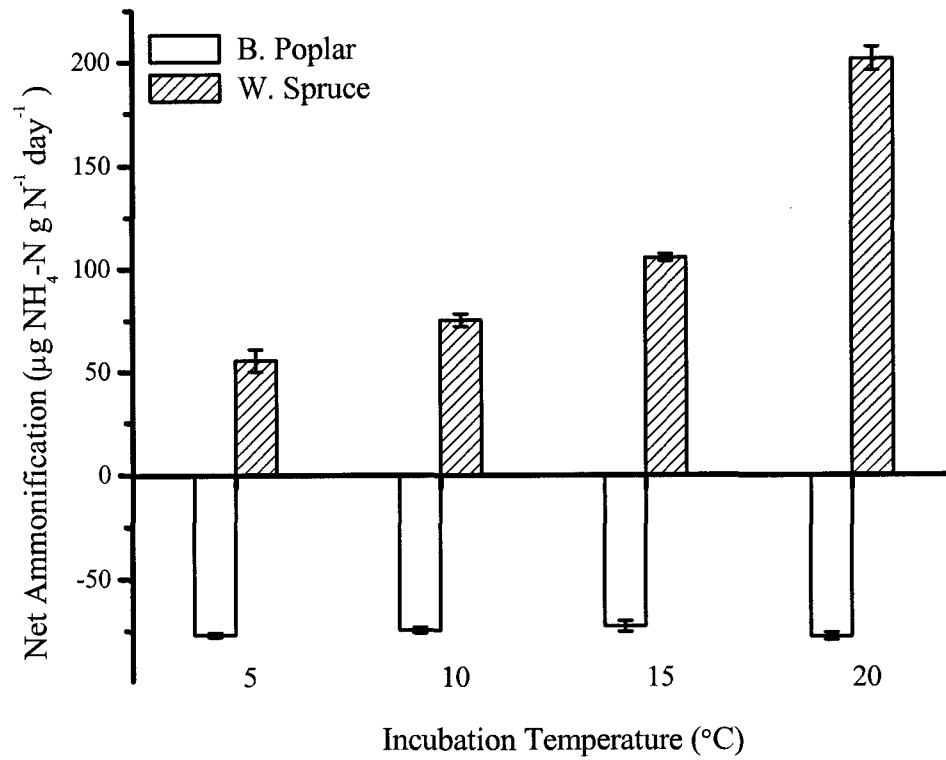
**Figure 4.4b.** Net ammonification at day 21 of the incubation.

4.4c)
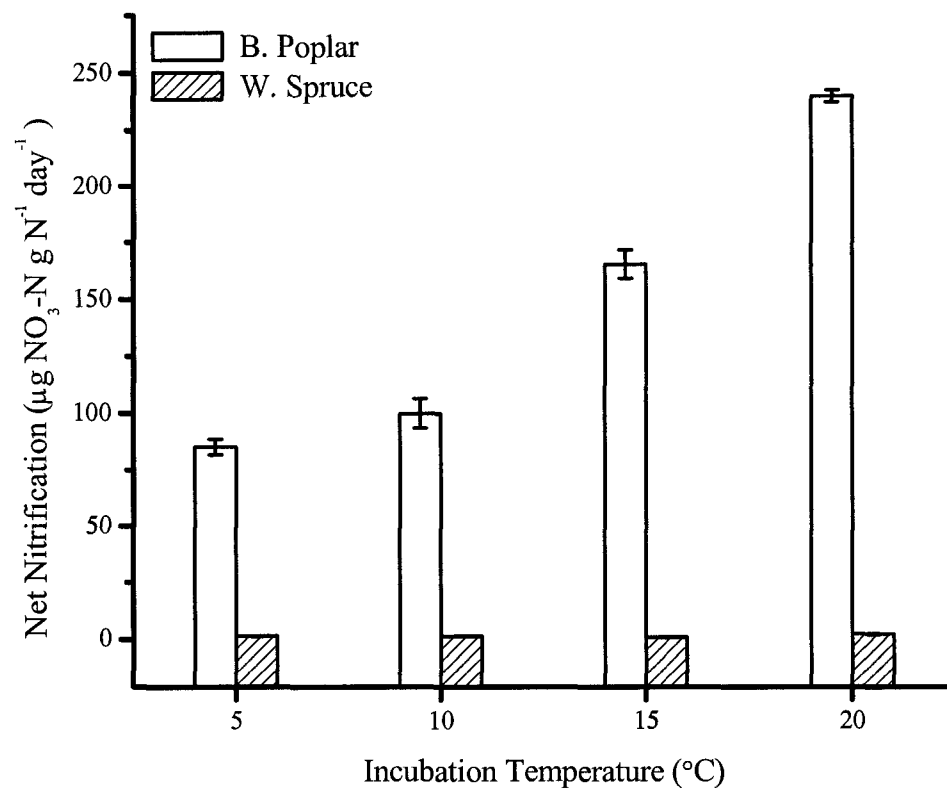
Figure 4.4c. Net Nitrification at day 21 of the incubation.

Figure 4.5. The mean concentration of **a)** NO_3^--N and **b)** NH_4^+-N over time. The concentrations were determined at days 0, 8, 21 and 182 of the incubation by extracting soil with a solution of 0.5M K_2SO_4. Values are means (± 1 S.E.) determined from a repeated measures ANOVA.

4.6a)
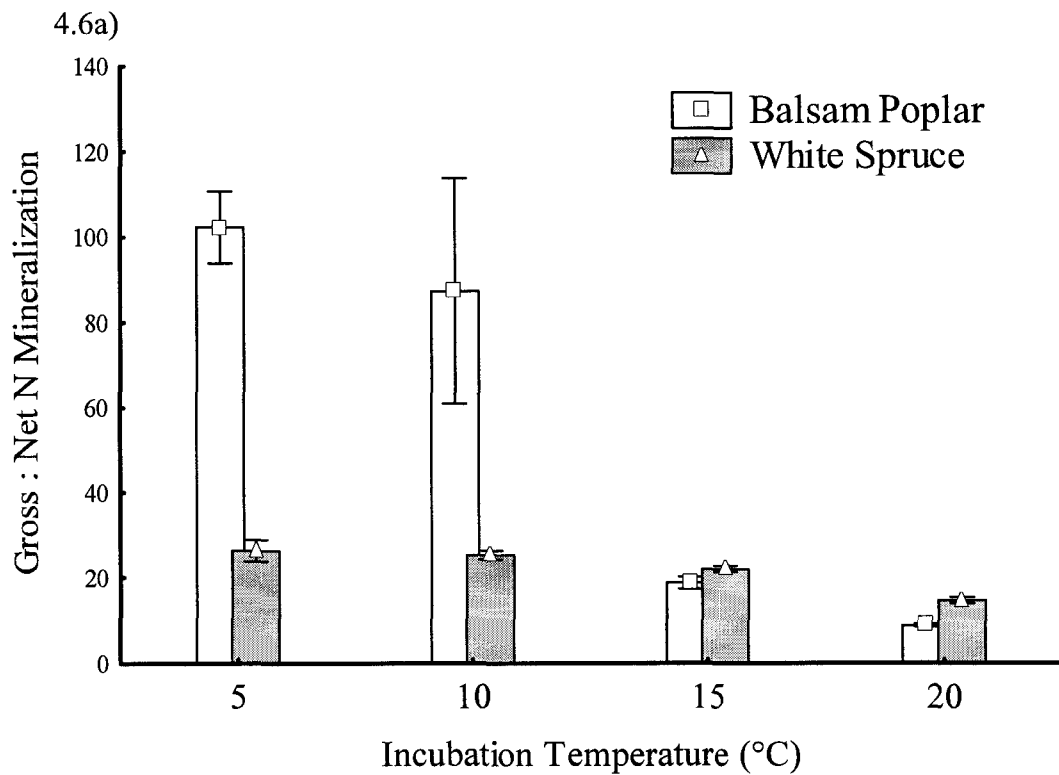
Figure 4.6a. The ratio of gross:net N mineralization at day 21 of the incubation.

Figure 4.6. Ratio of a) gross-to-net DIN mineralization, b) CO_2-C respired-to-net N mineralization and c) CO_2-C respired-to-gross N mineralization at day 21 of the incubation. Values are means (± 1S.E.) and n=5 for each stand×temperature combination from untransformed data. Data were square-root transformed for statistical tests.
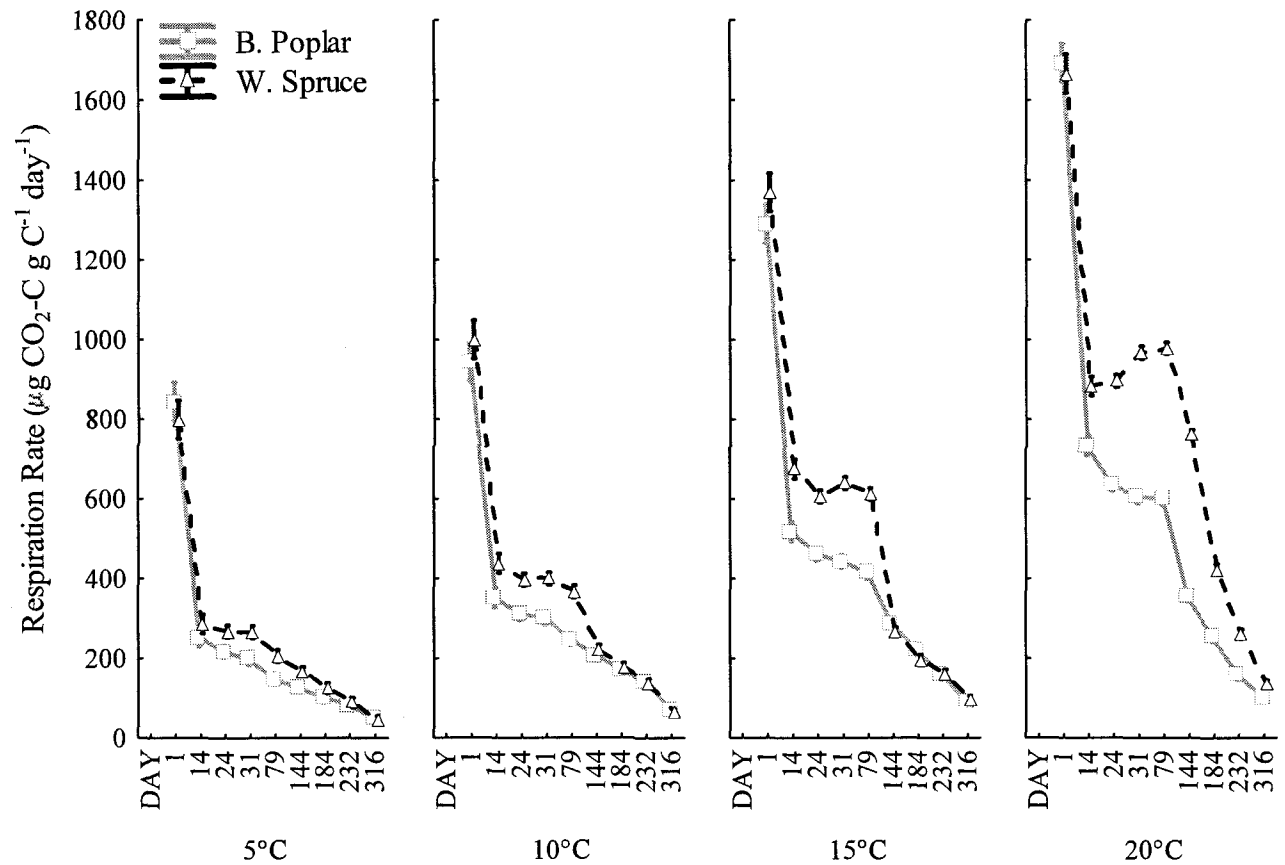
4.6b)
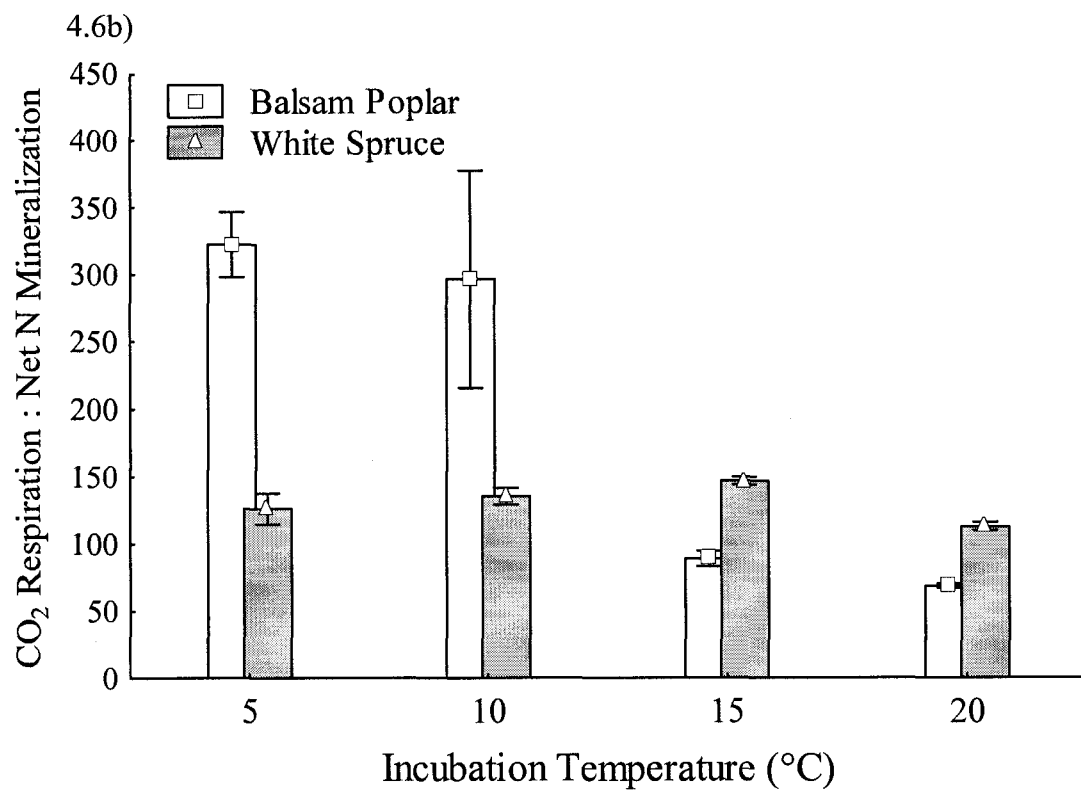
Figure 4.6b. The ratio of C respired to net N mineralized at day 21 of the incubation.
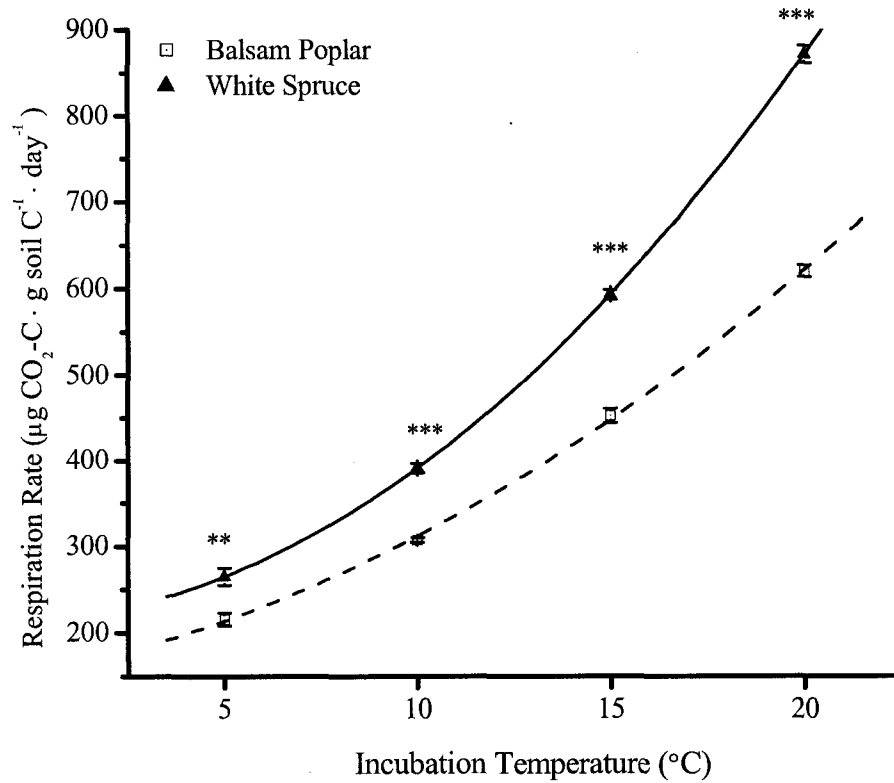
4.6c)
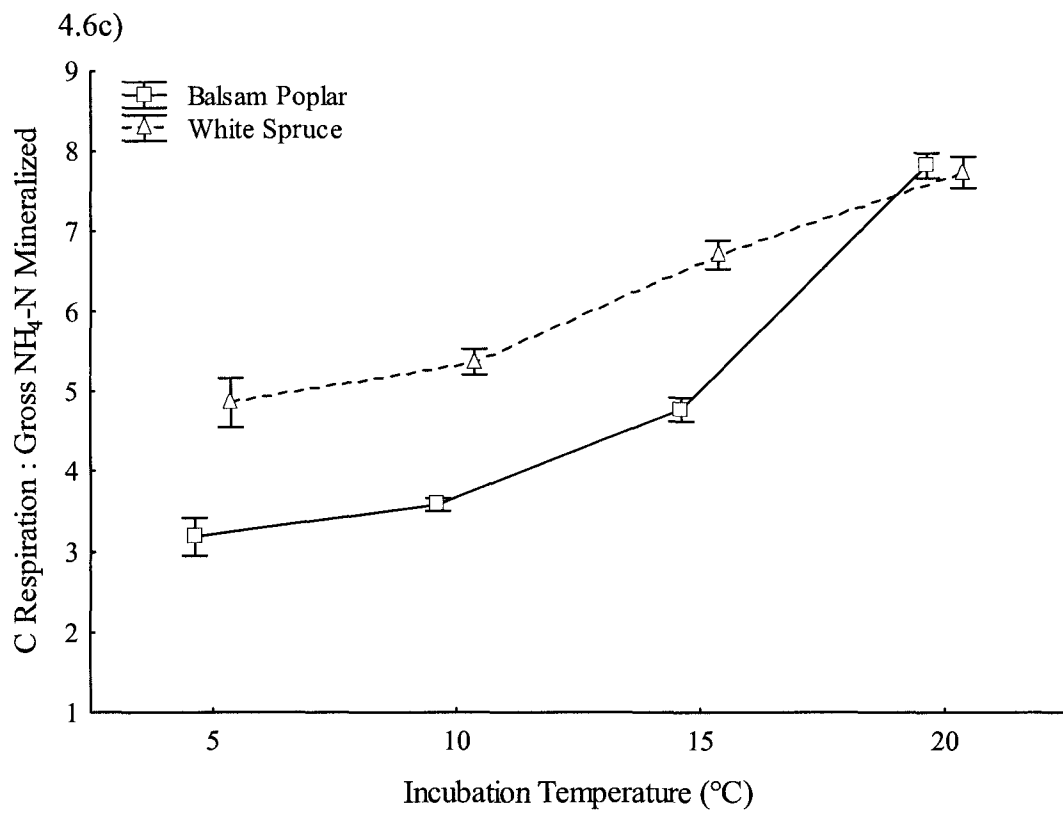
Figure 4.6c. The ratio of C respired to gross NH_4^+-N mineralized at day 21 of the incubation.

Figure 4.7. Soil respiration rate per g C for select days during the 316 day incubation. Rates are least-squares means ±1 S.E. calculated from a repeated measures ANOVA.

Figure 4.8. Soil Respiration per g soil C at day 24 of the incubation. Here, logarithmic growth curves have been fit to the rates in each stand type. Asterisks indicate the following significant differences between stand types * p < 0.05, ** p < 0.01, *** p < 0.001.
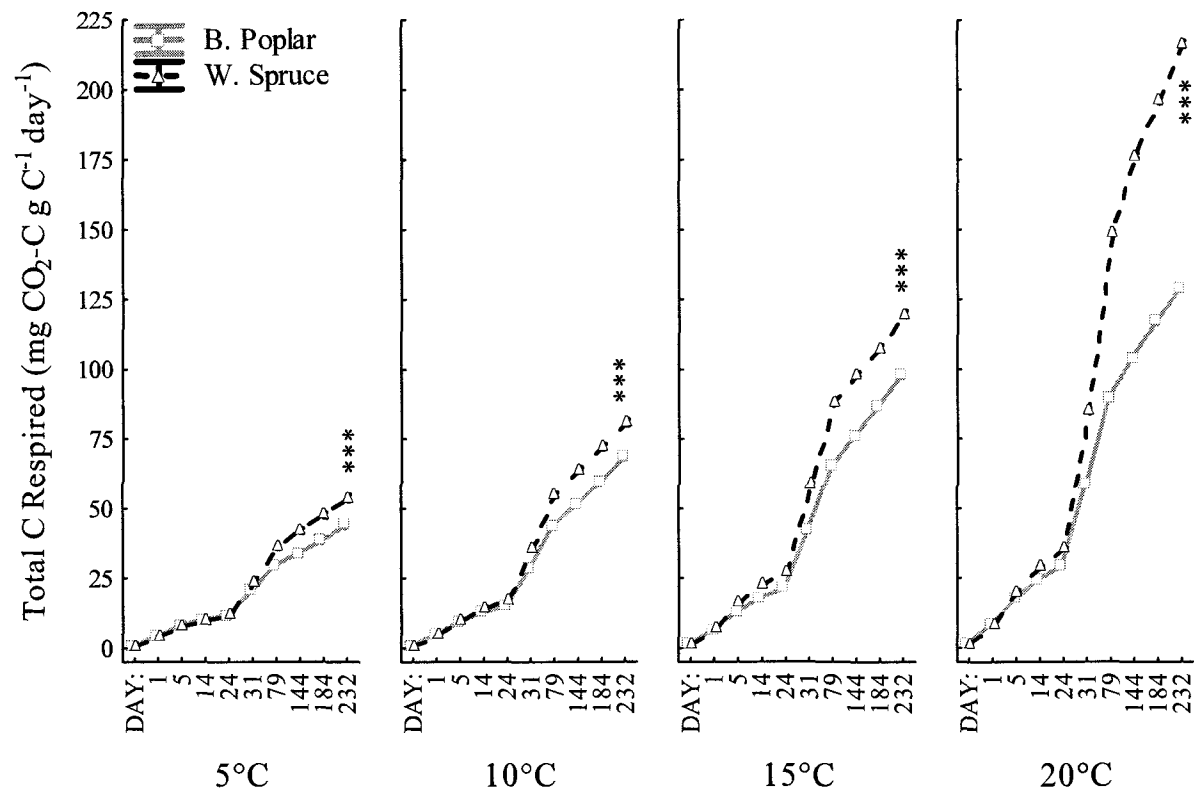
Figure 4.9. Cumulative amount of C respired during the course of the incubation. Values are means 1 ± S.E. (Least square means) (mg CO_2-C g soil C^{-1}) generated from a repeated measures ANOVA on select dates over time. Asterisks at day 316 indicates significant differences (*** = $p<0.001$) in the cumulative amount of C respired in white spruce and balsam poplar.
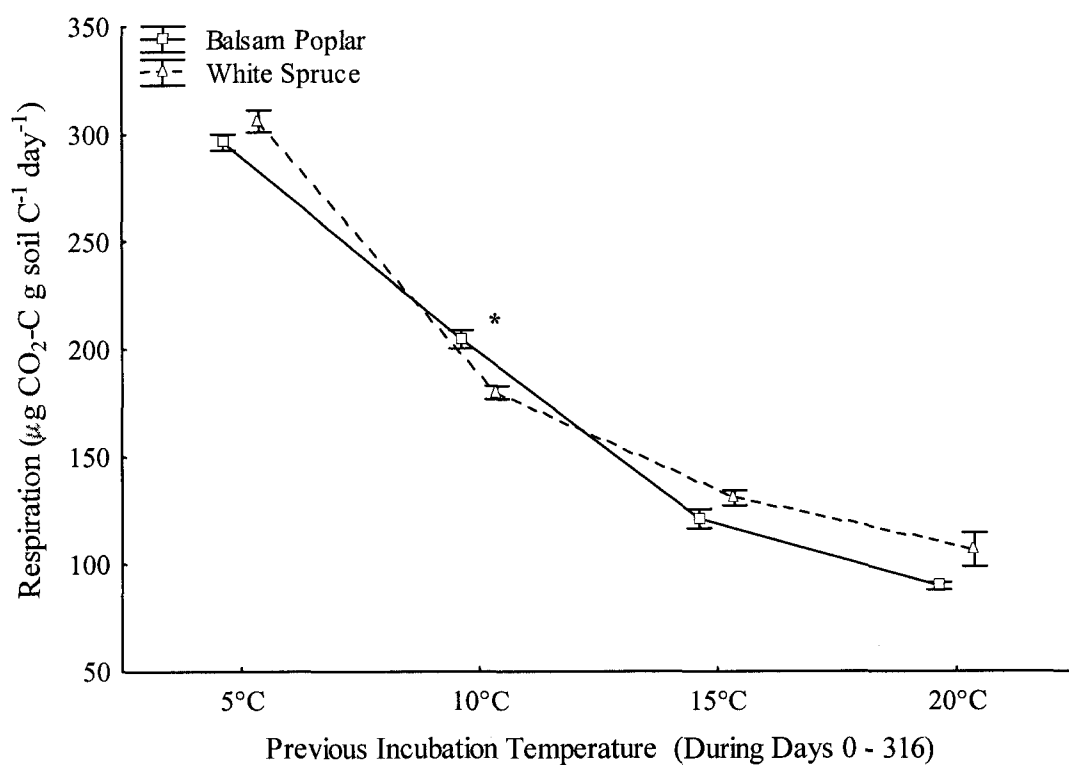
Figure 4.10. Respiration per g soil C at the end of the incubation (days 316 – 320). Values are means ±95% confidence intervals from n=5 replicate jars per stand×treatment combination.
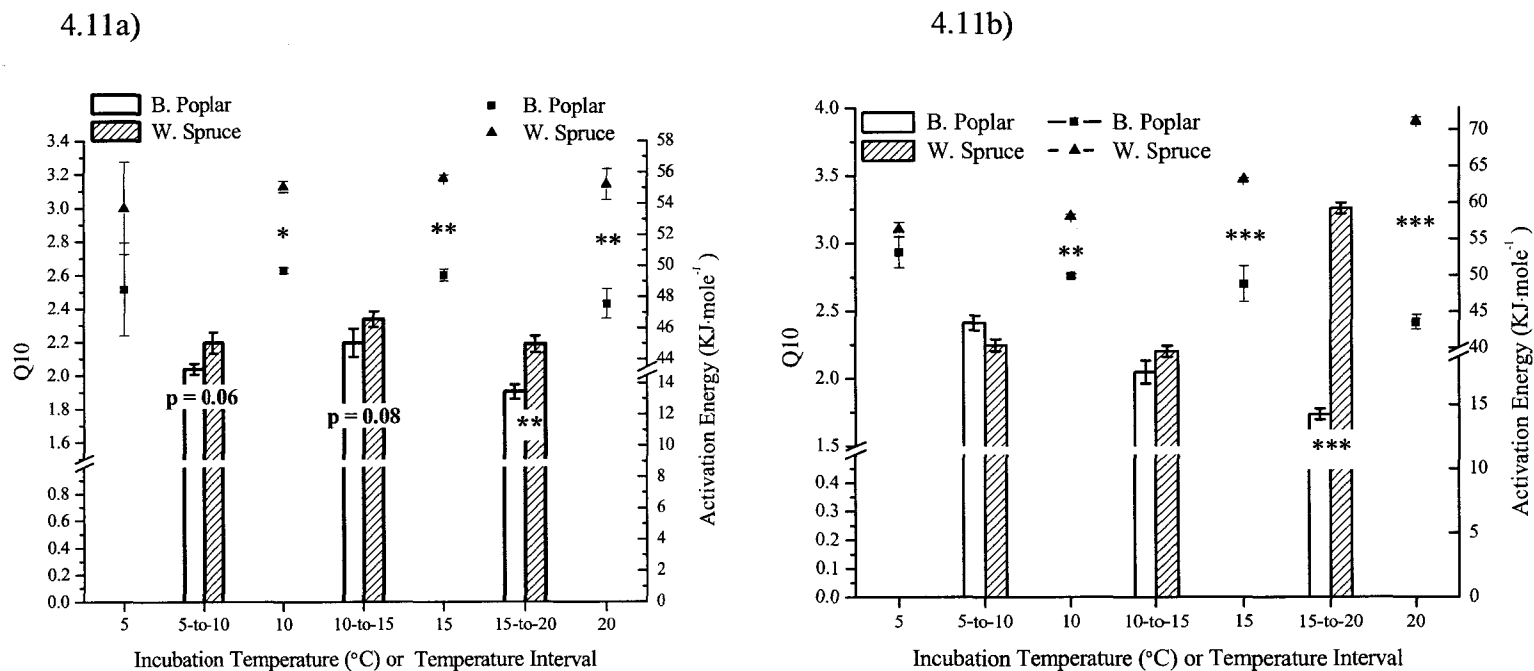
Figure 4.11. Average energy of activation (E_a) (scatterplot) and Q_{10} values (bar graph) for microbial respiration. Figure 4.11a is from day 24 of the incubation and 4.11b is the cumulative values for the entire 316 day incubation. Values are means ± 1 S.E. from n=5 replicate samples per stand×temperature combination. Asterisks indicate significant differences between stand types at a given temperature (for E_a) or temperature interval (Q_{10}). *$p < 0.05$, **$p<0.01$ and other p-values indicate marginally significant results ($0.05 \leq p \leq 0.10$).
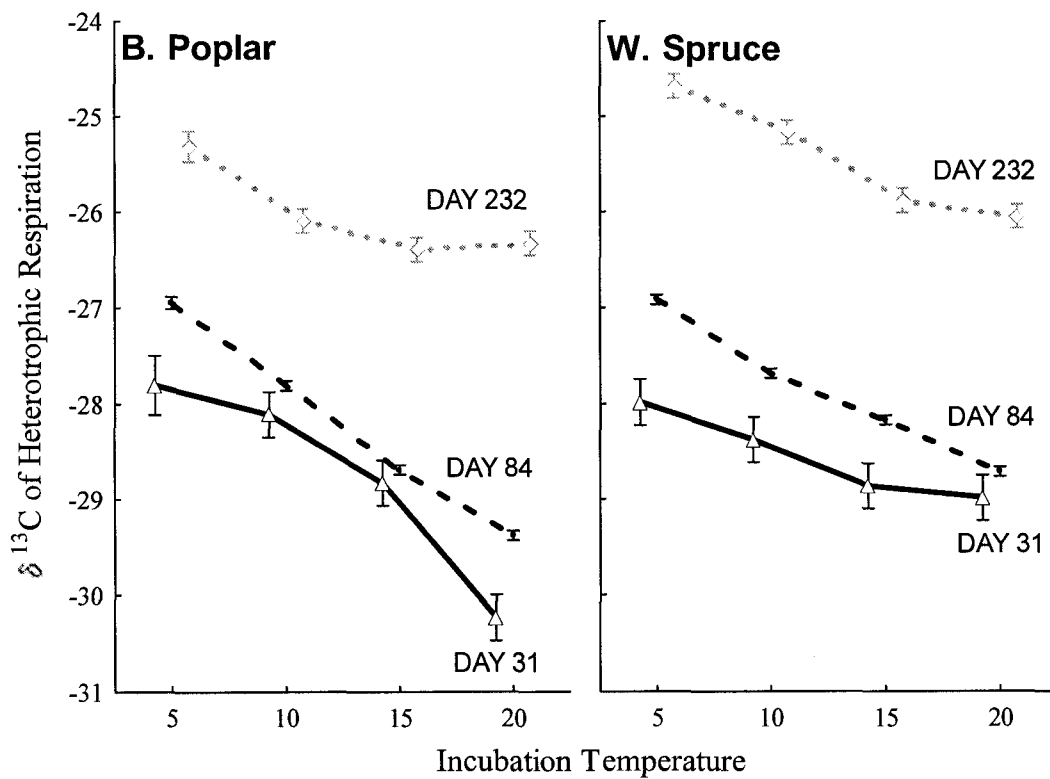
Figure 4.12. $\delta^{13}CO_2$ of microbial respiration at days 31, 84 and 232 of the incubation. Values are means (±1 S.E.) from n = 5 replicate incubation jars per stand×temperature combination obtained from a repeated measures ANOVA.

Table 4.1. Select soil characteristics of balsam poplar and white spruce soil. The characteristics were determined prior to the beginning of the incubation and are expressed on an oven-dry (105°C) basis. Values are means (±1 S.E.) calculated from n=4 replicate samples per stand type.

	% C	% N	C:N	Lignin:N[†]	[NH₄-N] ugN g⁻¹	[NO₃-N] ugN g⁻¹	δ ¹³C	δ ¹⁵N
B. Poplar	20.18 (0.03)	1.13 (0.006)	17.86	20.0 (0.6)	34.67 (0.59)	5.39 (0.17)	-28.02 (0.02)	-1.79 (0.12)
W. spruce	18.59 (0.08)	0.70 (0.004)	26.56	27.0 (0.6)	2.97 (0.11)	0.28 (0.003)*	-27.01 (0.09)	-0.87 (0.002)

[†] Lignin-to-N ratio taken from Van Cleve et al. 1993.

* The small standard error for NO_3-N is due to many white spruce samples having nitrate concentrations that were near the detection limits of 0.02 ppm NO_3-N.

Table 4.2. Microbial biomass C and N, DOC and DON at day 23 of the incubation. Biomass values were not corrected for extraction efficiency. Values are means ±1 S.E. from n=5 replicate soil samples per stand × temperature combination.

Incubation Temperature (°C)	Microbial C (μg C g soil C^{-1})		Microbial N (μg N g soil N^{-1})		DOC (μg C·gsoil C^{-1})		DON (μg N·gsoil N^{-1})	
	BP	WS	BP	WS	BP	WS	BP	WS
5	5,808 (162.6)	5,549 (91.9)	14,055 (71.1)	16,941*** (91.3)	4,631*** (47.8)	3,688 (43.3)	3,613 (45.0)	3,574 (86.0)
10	5,661 (91.2)	5,556 (156.2)	14,764 (181.7)	17,722*** (202.9)	4,457*** (22.2)	3,286 (138.8)	3,467 (18.8)	3,252 (62.5)
15	5,515 (56.1)	5,224 (69.9)	14,710 (204.3)	19,239*** (420.6)	3,989*** (32.6)	3,391 (36.1)	3,132 (22.0)	3,312 (53.9)
20	5,313 (124.4)	4,827 (245.7)	14,973 (167.2)	18,822*** (227.2)	3,875*** (43.6)	3,184 (139.6)	3,192 (35.5)	3,771*** (93.2)

Asterisks (***) indicate a significant difference of p< 0.001 between stands for a particular incubation temperature.

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Chapter 5

Concluding Remarks on the Influence of Succession on Soil N Transformations

Studies and their Conclusions

In this research, *in situ* nitrogen (N) additions, chemical analysis, and laboratory soil incubations were used to investigate several aspects of soil N and C transformations, N demand and N retention in forest stands which encompass a dramatic successional transition in the boreal forest. Nitrogen is believed to limit plant productivity in many boreal communities; thus, a better understanding of soil N transformations is critical in elucidating the controls over ecosystem processes and should improve forecasting of the impact that climate change may have on the net balance of C in the boreal forest. The main assumption when this research began was that higher levels of productivity in mid-succession stands of balsam poplar combined with a labile source of C in balsam poplar litter would result in an overall higher demand for N by plants and microbes in this stand type compared to late-succession stands of white spruce. It was also assumed that the overall quality of soil organic matter decreased during the transition from mid- to late succession. The following is a summary of results and conclusions from the studies conducted to investigate potential changes in soil N transformations in mid and late succession stands.

In the first study, N fertilizer additions (100 kgN ha^{-1} yr^{-1}) were used to compare the relative demand for N by soil microbes in stands of balsam poplar and white spruce. Nitrogen additions induced an immediate increase in net N mineralization relative to

control plots in both white spruce and balsam poplar. This increase was likely due to the inability of soil heterotrophs to immobilize a significant portion of the added N. In other words, the gross production of mineral N continued as soil microbes broke down organic matter, but the amount of available labile C in these soils was not sufficient for microbes to utilize the excess in fertilized plots. Microbial biomass also did not change as a result of N additions, and excess nitrate leached below the main rooting zone in N fertilized plots shortly after N additions were applied. Combined, these results suggest that, soil microbes in balsam poplar stands, relative to soil microbes in white spruce stands, did not have a larger N demand as we had predicted. However, there were large differences in the type of N transformations that were stimulated as a result of N additions. Nitrogen fertilization additions resulted in the stimulation of net nitrification in balsam poplar soil but increased net ammonification only in white spruce soil. Thus, either nitrification was inhibited in white spruce soil or any nitrate produced was quickly immobilized. The examination of gross nitrification rates with a $^{15}NO_3$ pool dilution technique is needed to resolve this question. In addition, because the amount of N applied to these plots quickly overwhelmed the ability of soil microbes to immobilize it, incremental levels of N additions may need to be applied in order to fully determine the relative demand for N by soil microbes in mid and late succession stands.

The assumption of higher biological N demand in balsam poplar than in white spruce was further investigated by examining the various organic and inorganic constituents of N found in soil water. For this study tension lysimeters were installed within and below the main rooting zone in both stand types. It was predicted that the

ratio of soil solution dissolved organic N (DON) to dissolved inorganic N (DIN) below the main rooting zone would be much higher in balsam poplar stands compared to white spruce stands. This prediction was based on the assumption that plants and microbes were N limited in balsam poplar stands and that DIN would be exhausted in the soil solution as it moved down through the soil profile.

Soil water was collected approximately every week for two growing seasons (2000-2001), and we determined that the ratio of DON:DIN was nearly identical in the two stand types and decreased significantly with depth in both stands. This occurred as a result of higher concentrations of nitrate, but lower concentration of DON, in the deeper mineral soil. Tensiometer measurements and a multivariate analysis of dissolved ions both indicated that the soil solution often moves up through the soil profile from ground/river water during the growing season (hyporheic flow) in these stands. Thus, the rise and fall of the Tanana River also likely contributes to plant and microbial N requirements throughout at least the beginning of late succession. This result may also help to explain the higher concentration of nitrate in the deeper soil, as nitrate moving up through the soil profile may not be utilized until it gets to the surface horizon where there is a sufficient supply of labile C to fuel microbial immobilization and plants roots also gain access to it.

In the third study, the influence of temperature on the mineralization of soil C and N was investigated. Soil temperature generally declines in late succession due to the development of an insulating moss layer, and it has also often been stated that late succession brings about a decline in the quality or decomposability of soil organic matter

and litter. Results from past studies and this dissertation have shown a general decline in *in situ* net N mineralization rates in late succession stands of white spruce compared to mid succession stands of balsam poplar. However, since both temperature and organic matter quality can control the mineralization of N in soil, it is not clear whether lower temperatures or a decrease in organic matter quality is more responsible for this decline. In this study soil from mid and late succession stands were incubated at 5, 10, 15 and 20 °C and C mineralization and gross and net N mineralization were measured.

Across the range of incubation temperatures, rates of C mineralization (per g soil C) and net and gross N mineralization (per g soil N) were almost always significantly higher in white spruce soil than in balsam poplar soil. Additionally, compared to balsam poplar soil, the mineralization of soil C and N in white spruce was also generally more sensitive to temperature increases (higher Q_{10} and activation energy). Thus, it would appear that the quality of organic matter was actually higher in these late succession stands, the opposite of what has typically been suggested. These results suggest that a warmer climate will bring about a larger respiratory loss of soil C from late succession stands.

Based on the results from these studies we argue that there are more similarities than differences with respect to soil N transformations in mid and late succession. Although rates of *in situ* N mineralization were often higher in mid succession, the biological demand for N was not shown to be appreciably higher in mid succession as had been predicted. In addition, the results do not support the conventional thought that, in late succession, there is a decline in the quality of the organic material in soil. Thus,

we believe that other factors such as temperature, moisture, N-fixation inputs or perhaps even riverine influences are more responsible for the high rates of productivity in mid succession stands.

Future Research

Several decades of research on the boreal floodplain of Interior Alaska have greatly improveed our knowledge of the biological, physical and chemical properties that contribute to the functioning of this ecosystem (Van Cleve & Viereck 1981; Flanagan & Van Cleve 1983; Van Cleve et al. 1983; Van Cleve et al. 1993b; Viereck et al. 1993; Clein & Schimel 1995; Ruess et al. 1996; Uliassi & Ruess 2002). However, as is often the case, this dissertation and the studies that have come before it also bring to light many additional questions. The following categories highlight areas of nutrient cycling on the floodplain that deserve further attention.

(1) <u>Organic N Uptake</u>. What proportion of plant N uptake consists of organic vs. inorganic N (McFarland et al. 2002)? Which plant species are able to utilize organic N and what processes control the uptake of the various N forms?

(2) <u>Denitrification</u>. How much gaseous N is lost from the floodplain through the process of denitrification (Klingensmith & Van Cleve 1993)? Does the rate of denitrification change seasonally and throughout succession as the availability of water and labile C is altered?

(3) <u>Nitrification</u>. What mechanisms are responsible for the substantial decline in net rates of nitrification between mid and late succession (Van Cleve et al. 1993a;

Brenner *et al. In Press*)? Does gross nitrification decline in the soil of late succession stands or does immobilization of NO₃ increase?

(4) <u>Nitrate Reduction by Plants and Microbes</u>. Do high relative concentrations of extractable NH₄ repress nitrate reductase in plants and microbes and contribute to leaching losses of nitrate? Does the persistently high ratio of ammonium:nitrate in soil extracts from mid and late stands affect the ability of plants and soil microbes to take up and utilize (reduce) nitrate (Kronzucker *et al.* 1995a; Kronzucker *et al.* 1995b;1997)?

(5) <u>Phosphorus vs. N Limitation</u>. Does the availability of phosphorus, rather than N, limit plant growth during some portion of the floodplain successional sequence such as when alder or balsam poplar are the dominant vegetation types (Uliassi & Ruess 2002)?

(6) <u>Hydrological Inputs and Losses of N</u>. What is the role of hydrology in controlling the net balance of N inputs on the Tanana River floodplain (Yarie *et al.* 1993 Chapter 3, this dissertation)? How do N inputs from the Tanana River (from deposition and hyporheic flow) compare to N losses via leaching and overland flow? What is the role of successional stage, permafrost, and terrace height in influencing this balance?

(7) <u>Nitrogen Fixation</u>. How much of the N fixed by the *Alnus-Frankia* symbiosis (Van Cleve 1971; Uliassi *et al.* 2000; Uliassi & Ruess 2002) actually becomes available to other plants and how much of it quickly enters a slow or recalcitrant organic pool (Kaye *et al.* 2003)?

Addressing these questions will further improve our understanding of how N availability influences plant growth in the most productive stand types of Alaska's boreal

forest. This, in turn, will provide valuable insights into the potential consequences of a changing climate to net carbon storage, plant-microbe competition for resources, and plant community composition.

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