ECOPHYSIOLOGICAL RESPONSES OF FOUR BOREAL TREE SPECIES TO SOIL NITROGEN SUPPLY

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ABSTRACT

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The ecophysiological responses of four boreal tree species, trembling aspen (*Populus tremuloides* Michx.), black spruce (*Picea mariana* (Mill.) B.S.P.), white spruce (*Picea glauca* (Moench) Voss), and jack pine (*Pinus banksiana* Lamb.) were examined at six different levels (from 25 to 775 ppm) of soil nitrogen (N). At the 50th and 100th day of treatment, two-year-old conifer seedlings and six-month-old trembling aspen seedlings were measured for gas exchange, foliar N concentration, growth and biomass allocation traits.

The gas exchange and resource use efficiency responses varied with species. The additional N input had no significant effect on photosynthesis (A, $\mu mol/m^2/s$) and positive effect on transpiration (E, $mmol/m^2/s$), and beyond 175 ppm N treatment it produced negative effect on whole seedling photosynthetic capacity (A_t, $\mu mol/s/seedling$) in trembling aspen. A, E and A_t in conifers responded negatively to N treatment, except for a brief positive response from 25 to 125 ppm N in jack pine. ΦPSII revealed relevant relationship with A but only for jack pine and white spruce. We found explicit positive response of foliar N concentration and negative response of PNUE to increasing soil N availability. However, the trends of PWUE between species varied across six N treatments and possibly due to luxury consumption. The PWUE was positively correlated with soil N supply in only white spruce and was negatively correlated in other species. The sufficient soil N availability for optimum gas exchange and nutritional status were at 75 ppm N for black spruce and at 125 for the other three species.

Overall, the growth response of aspen to N was more pronounced than that of conifers due to its fast growing nature. Significant growth response of aspen occurred between the 75 to 175 ppm N treatments. Substantial growth reduction occurred when aspen seedlings were induced to excessively high soil N concentrations (375 and 775 ppm), specifically due to the vulnerability of these seedlings to pest damages. This is also related to the significant increase in foliar N concentration at these N treatments. Greatest growth was achieved at 25 ppm N addition rate in black spruce and jack pine, and most notables at 125 ppm N in white spruce. However, these N levels are not conclusive when optimum soil N concentration(s) for gas exchange parameters, particularly for A_t, in these seedlings are taken into consideration. Nonetheless, at highest soil N supply (375 and 775 ppm), the growth of conifer seedlings was not as adversely affected as that of aspen seedlings. Our results also proved strong significance of N availability on biomass allocation between root and shoot components and height growth in all species. Root production was significantly

suppressed as soil N availability increased in the conifers. The opposite was found in aspen. In conifers, the proportions of total seedling biomass allocated to roots (or the root-to-shoot ratio, R/S), at all N treatments, were highest in white spruce, followed by black spruce then jack pine. The R/S ratio in aspen was comparable with, but followed an opposite pattern to, white spruce. The stem and foliage productions in all studied species showed negligible positive response to increasing soil N treatments. However, with the exception of aspen, the order of the species allocating highest to lowest portions of its total biomass to the foliage followed the exact opposite pattern as that of root allocation percentage. Aspen showed lesser biomass allocation to the foliage than conifers. However, it allocated highest percentages of total seedling biomass to the stem, at all N treatments, followed in sequential order by black spruce, white spruce and jack pine.

When all ecophysiological parameters are considered, our results indicated that low soil N availability, such as that at 25 ppm, does not adversely affect the boreal seedling growth and survival as much as it does at excessively high soil N availability (e.g., 375 and 775 ppm). Within the sufficient N regimes (i.e., 75 - 175 ppm), the most suitable soil N concentration for black spruce is at 75 ppm and at 125 ppm for other species.

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INTRODUCTION

Nitrogen (N) has been recognized as an essential element for plant growth and development (*e.g.*, Field and Mooney 1986, Marschner 1995, Miller and Donahue 1995). It is required in the highest quantity among all essential elements (*cf.* phosphorus, potassium, sulphur) in order for plants to grow healthily (Brady 1990, Marschner 1995). However, available N is notably the most deficient element in the Boreal forest (Krause *et al.* 1977), a land mass covering 11% of the earth's terrestrial surface (14.7 million km^2) (Bonan and Shugart 1989). The Boreal forest is the most economically important forest of Canada, which accounts for 74% of total forested area of the country (Bonan 1990).

The availability of soil N in the Boreal forests is dependant on a number of factors. For example, the high variation of total and available N in the soil, the typically limited amount of available N in the Boreal forests' soil, and the recent concern of increasing atmospheric N pollutants as well as the rate of N cycling due to the elevation global temperature, all of which can contribute to crucial structural and functional changes in the Boreal forest ecosystems (Bonan and Shugart 1989, Bonan 1990, MacDonald *et al.* 1992, Pare *et al.* 1993). The total soil N in the Boreal forest varied from 1,330 kg/ha in Quebec (Weetman and Agar 1983) to 4,541 kg/ha in Ontario (Timmer *et al.* 1983). As well, the input of available N from N mineralization, fixation and precipitation varied from 9.2 kg/ha/yr in upland coniferous (spruce) forests to 52.5 kg/ha/yr in broad-leaved (birch-aspen) forests (Ruess *et al.* 1996).

Despite the above differences, generally over 97% of the total soil N is in forms that are not readily available to plants (Donahue *et al.* 1995). In a sub-Boreal spruce forest, the reported available soil nitrate-N and ammonium-N (NO_3-N and NH_4-N) combined and total mineralizable (i.e., not readily available) soil N were 226 ppm (17.3%) and 1,081 ppm (82.7%), respectively (Driscoll et al. 1999). The causes of the much lower available soil N compared to the total amount of soil N are wet substrate, low soil temperature and low frequencies of natural fire, which are all characteristics of the Boreal forests (Bonan and Shugart 1989) that restricts the rates of organic matter decomposition and nutrient mineralization (Van Cleve and Jarie 1986). Furthermore, because of the present intensive forest management practices within the Boreal forests, the deleterious effects of soil N are likely due to greater biomass removals, shorter rotations, etc. than in the past (Timmer *et al.* 1983, Kimmins 1997).

On the contrary, the amount of available soil N could be enhanced due to the increasing N input from atmospheric N pollutants and the result of global climate change; *e.g.,* elevating soil temperature (Hom and Oechel 1983, Nihlgard 1985, Pastor and Post 1988, MacDonald *et al.* 1992, Zak *et al.* 1993). The forest decline in some parts of Europe was attributed to high N input from the atmospheric pollution (Nihlgard 1985). Various locations along the Great Lakes had shown increasing annual N deposition, as nitric acid rain, from 10 to 40 kg/ha (MacDonald *et al.* 1992). The increasing soil temperature was also found to speed up the rate of N cycling due to more rapid rates of organic matter decomposition and mineralization (MacDonald *et al.* 1992).

The above conditions may create either favourable or toxic environments for plant growth and survival, as well as the competitiveness of the species, in the Boreal forests.

However, our understanding of the response of different Boreal species to changes in soil N, in which the increasing amount of available N is anticipated from the overall picture, is still limited. A thorough study of forest tree ecophysiological and growth responses to a wide range of soil N is important for interpreting the possible ecological transformation, particularly the structural dynamics and nutrient fluxes, of various Boreal forest ecosystems. Numerous studies done in the past have compared very few species, and rarely did they provide good comparisons between deciduous and coniferous species. For example, effects of different N applications have been investigated for trembling aspen (*Populus tremuloides;* Coleman *et al.* 1998), three deciduous species (i.e., trembling aspen, sugar maple; *Acer saccharum*, and white birch; *Betula paperifera*) (Kinney and Lindroth 1997), Douglas fir (*Pinus radiata*; Van Hove *et al.* 1992), black spruce and white spruce (*Picea mariana* and *P. glauca*; Patterson *et al.* 1997), jack pine (*Pinus banksiana*; Cantin *et al.* 1997), black spruce and jack pine (Colombo and Smith 1987), and American elm (*Ulmus americana*; Walters and Reich 1989).

Nitrogen significantly affects ecophysiological traits as well as the growth and survival of plants. Since N is the prime constituent of amino acids, growth regulators, and chlorophyll (Chapin III 1980) that drive the processes of photosynthesis (A) and transpiration (E), these gas exchange parameters rely heavily on N in order to function properly. E tends to have a positive relationship with A (Hunt *et al.* 1985b). Both A and E generally increase with increasing N input, depending on the species and stage of development (Tan and Hogan 1995, Kubiske *et al.* 1997). Increasing N application enhanced height and diameter increments (Van den Driessche 1989, Catin *et al.* 1997), total leaf area (TLA, Sabate and Gracia 1994), foliar nitrogen concentration (N_f, Coleman *et al.* 1998), and photosynthetic

water-use-efficiency (PWUE, Green and Mitchell 1992, Liu and Dickman 1996). On the other hand, increased N restricted photosynthetic nitrogen-use-efficiency (PNUE, Birk and Vitousek 1986, Kubiske *et al.* 1997) and biomass allocation to the roots (R/S ratio, Fetene *et al.* 1993, Ibrahim *et al.* 1997). There are substantial differences between species in N requirement and allocation. For example, trembling aspen not only required more N for optimum growth but also allocated higher N content to foliage than did the conifers (Dang *et al.* 1997).

The objective of this study was to investigate ecophysiological responses to a range of soil N conditions (25 to 775 ppm) in four important Boreal tree species; specifically, trembling aspen (*Populus tremuloides* Michx.), black spruce (*Picea mariana* (Mill.) B.S.P.), white spruce (*Picea glauca* (Moench) Voss), and jack pine (*Pinus banksiana* Lamb.). Two main hypotheses were established: (1) increasing N soil availability (up to a toxic level) would increase A, E, PWUE, TLA and N_f, whereas PNUE and R/S ratio would decline in all four species and (2) aspen seedlings would be most responsive to increasing soil N followed by jack pine, white and black spruces. The N regime at 25 ppm is the lowest possible level when the optimum concentrations for other essential nutrients are to be maintained. Except for the available soil N concentration reported for a black spruce forest (226 ppm) by Driscoll *et al.* (1999), others usually reported soil N in terms of mass over area (*e.g.*, kg/ha), which is difficult for us to relate to these values in terms of concentrations. We used 775 ppm N, three times the concentration reported by Driscoll *et al.* (1999), as the highest treatment.

LITERATURE REVIEW

NITROGEN AVAILABILITY

The accessibility of soil nitrogen (N) to plants is a function of the amount of total and available N in the soil, the variation in total amount soil N and the input of atmospheric N (Aber *et al.* 1989, Pare *et al.* 1993, Lovett 1994, Donahue *et al.* 1995). Each of these factors can cause crucial functional and structural changes in the Boreal forest ecosystems. A study on post-fire (140 years) N content in the forest floor indicated 82.7% of the N in the soil profile was currently unavailable (Driscoll *et al.* 1999).

The availability of N is also influenced by the stage of forest succession and species composition. Forest succession (the latter stages) in the Boreal forest can lead to a decline in nutrient availability, especially N, because a large proportion of the ecosystem total is accumulated in the soil organic matter (Bormann and Sidle 1990, Pastor *et al.* 1988). Bonan (1990) and Bormann and Sidle (1990) showed that total N concentration and the rate of N mineralization in the soil decreased over time in some Boreal forest regions. As for the effect of species composition on N availability, Pare *et al.* (1993) found higher available N under white birch dominated stands than in stands dominated by trembling aspen, white spruce and eastern red cedar (*Thuja occidentalis*). Also, areas with high coniferous compositions tended to restrict the N cycling due to the slow rate of organic matter decomposition, thus restricting the N availability (Pare *et al.* 1993).

The amount of soil N varies significantly between locations in Boreal forest (see introduction for different amount of soil N reported by Timmer *et al.* (1983), Weetman and

Agar (1983) and Ruess *et al.* (1996). Furthermore, since rates of organic matter decomposition and mineralization tend to respond positively to increases in soil temperature, the rate of soil N cycling within an ecosystem can be enhanced by increasing soil temperature (MacDonald *et al.* 1992, Pastor and Post 1988, Zak *et al.* 1993). Recently, it has been postulated that soil temperature is likely to increase as a result of global climate change. Increasing soil temperature has been found to create higher soil moisture content, soil solutes and needle N contents, which in turn was coupled with higher rates of net photosynthesis and dark respiration (Hom and Oechel 1983).

Over the past decade, higher amounts of atmospheric N, attributed to industrial pollution, have been reported. Aber *et al.* (1989) and Lovett (1994) found that increased atmospheric N deposition resulted in an increase of soil N availability in some temperate forests. In the Netherlands, the emission of volatilized ammonia (NH_3) from animal manure increased N input into forests 10 to 20 times higher than the normal situation (Heij and Schneider 1991). In normal, unpolluted conditions, the annual average rate of atmospheric N deposition is approximately 5 kg/ha/yr (MacDonald *et al.* 1992). However, the annual N deposition (as nitric acid rain) ranged from 10 to 40 kg/ha/yr in some polluted locations along the Great Lakes (MacDonald *et al.* 1992).

NITROGEN-PHOTOSYNTHESIS RELATIONSHIP

Nitrogen is important to plant growth because of its involvement in photosynthesis or carbon assimilation (A), an essential mechanism providing energy and structural substrates for plant growth and reproduction (Field and Mooney 1986). Nitrogen is a key constituent of amino acids, plant growth regulators, and chlorophyll (Chapin III 1980, Marschner 1995).

Black (1968) indicated that N is also found in hormones, the "organic substances that exert important regulatory effects on metabolism when present in only minute quantities", and is a component of the respiratory-energy carrier, adenosine triphosphate (ATP).

Larger plants are the end product of the production of greater amount of protein and enzymes, such as RuBP and PEP carboxylase (Moorby and Besford 1983). Because of the involvement of these substances in the CO_2 fixation of A, leaves grow larger and a larger surface area is subsequently available for photosynthesis (Russell 1973). N is a key component of RuBP and PEP carboxylase and other enzymes, hence N is essential for cell growth and tissue renewal (Russell 1973) and maintenance of the photosynthetic apparatus (Chapin and Kedrowski 1983, Small 1972). As a result, N supply has found to be directly proportional to the amount of leaf area available for A (Russell 1973, Sabate and Gracia 1994). This in turns enhances carbon assimilation capacity, as found in the foliage of *Picea mariana* (Hom and Oechel 1983), *Pinus banksiana* (Tan and Hogan 1995), *P. radiata* D. Don. (Squire 1983), *P. sylvestris* (Kellomaki and Wang 1997), *P. taeda* (Green and Mitchell 1992), *Populus tremuloides* (Kubiske *et al.* 1997), *Pseudotsuga menziesii* (Mitchell and Hinckley 1993), *Ulmus americana* (Walters and Reich 1989), *Chenopodium album* (C_3) and *Amaranthus retroflexus* (C_4) (Sage and Pearcy 1987b), and Amazonian tree species (Reich *et al.* 1994).

The increase in N supply can sometimes produce contradicting results. Sheriff *et al.* (1986) found that N supply alone increased foliar N and P but did not increase A rate in *P. radiata*, unless growth is carried out in full sunlight. They have also shown that foliar N concentration is negatively related to diffusive conductance, quantum yield and maximum A, differing from the results reported by Hunt *et al.* (1985a, 1985c). Ibrahim *et al.* (1997)

concluded that N supply had no effect on A per unit leaf mass in *Populus balsamifera x P. trichocarpa*.

There is also evidence that great variations between and within species exist in response to N. The mass-based A is generally lower in evergreen species than in deciduous species. However, evergreen species possess higher potential for photosynthate production in their lifetime because of the higher N reabsorption rate (*i.e.*, higher internal N recycling) as well as greater leaf longevity (Small 1972). Within species, variations in photosynthetic capacity due to the location in canopy and/or leaf age have been found, regardless of N availability. For example, higher photosynthetic rates were found in young, growing leaves (6 to 10 μmol $CO_2/m^2/s$) as compared to that of mature leaves (2 to 8 μmol $CO_2/m^2/s$) (Fetene *et al.* 1993). Field *et al.* (1983) also indicated that photosynthetic capacity decreased with increasing leaf age, however, leaf N content also tends to decline in older foliage. So, it may be that the different amount of N contained in different aged leaves is responsible for the variation in A. Moreover, A on reproductive branches (*i.e.*, fruit-, seed- and flower- bearing branches) was found to be lower than on non-reproductive branches due to reduction in leaf area and N content per unit leaf area, apparently as a cost of reproduction (Karlson 1994).

High rates of A as well as plant biomass production at high levels of N supply can be further enhanced by elevating leaf internal CO_2 concentration (C_i). Mitchell and Hinckley (1993) found that C_i decreased as N supply increased from 10 to 125 mg N/L (or ppm N), which may have resulted in a CO_2 limitation to A, but as N supply increased beyond 125 mg N/L, C_i steadily increased to enhance A. Brown (1991) and Kellomaki and Wang's (1997) experiments on trembling aspen and Scots pine, respectively, showed that a decrease in foliar

N concentration was related to long-term CO_2-enrichment that resulted in a decrease in plant relative growth rate (Brown 1991, Kellomaki and Wang 1997).

CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence emission has been proven a useful tool to determine photosynthetic activity *in vivo* (Conroy *et al.* 1986, Hawkins and Lister 1985, Krause and Weis 1991, Papageordiou 1975, Toivoinen and Vidaver 1984, Vidaver *et al.* 1989). Fluorescence assessment has been used to provide information about the physiological status of white spruce (Vidaver *et al.* 1989). The usage of this method is described in detail in Lambers *et al.* (1998) and Krause and Weis (1991). Generally, the chlorophyll fluorescence emission is an indicator of Photosystem II (PSII) activity, quantifying the quantum yield (Φ_p), in isolated photosynthetic membranes (Lambers *et al.* 1998). It is normally referred to as Φ_{II} since it originates mainly from PSII (Lambers *et al.* 1998). As well, "in intact chloroplasts or whole leaves (needles) in the presence of CO_2, they can be indicators of complete photosynthesis" (Lichtenthaler and Grumbach 1975, Sivak and Walker 1983, Walker *et al.* 1983). For the purpose of this thesis, the quantum efficiency of photosystem II is referred to as ΦPSII.

Values for ΦPSII vary based on the degree of illumination to which the samples are exposed. Under dark-incubated conditions, the values for ΦPSII are around 0.8 (relative units) in healthy leaves (Lambers *et al.* 1998). Under illuminated conditions ΦPSII has values equal to or lower than that of dark-incubated samples, and the difference increases with increasing irradiance (Lambers *et al.* 1998). When irradiance increases from lower than 250 $\mu mol/m^2/s$ to higher than 1250 $\mu mol/m^2/s$ in the C_3 plant *Flaveria pringlei*, photosynthesis

increases from below 10 μmol/m^2/s to approximately 30 μmol/m^2/s, but Φ_{II} decreases from below 0.8 to approximately 0.45 (Krall and Edwards 1992).

NITROGEN-TRANSPIRATION RELATIONSHIP

Transpiration (E) is the rate of water loss from leaves, through the stomates, and is a necessary consequence of photosynthesis in terrestrial plants (Farquhar *et al.* 1980). E tends to increase as A increases, hence E possesses an indirect relationship with N supply or leaf N concentration. Hunt *et al.* (1985b) found that increasing nitrate-N supply increased E as well as A. They also found that if plants were subjected to low N supply over a period of time, E continued to increase while A stopped at 10 μmolN/m^2/s. On the other hand, under the induction of high N supply E increases at the same rate as it is under low N supply, but A continued to increase to 50 μmolN/m^2/s (Hunt *et al.* 1985b). In short, high N supply enhanced A more than it enhanced E.

NITROGEN-STOMATAL CONDUCTANCE RELATIONSHIP

Soil and atmospheric moisture conditions almost always affect the relationship between nitrogen and stomatal conductance (g_s). g_s responded similarly to A when the plant was subjected to changes in soil N and water status (Hunt *et al.* 1985b, Walters and Reich 1989). Under the same soil or atmospheric moisture conditions, along with high leaf N, A and g_s declined at a much faster rate than that of leaf with low leaf N (Hunt *et al.* 1985b, Walters and Reich 1989). Also, the response of g_s to water-stress was positively correlated to leaf N concentrations (Hunt *et al.* 1985b, Liu and Dickman 1996).

NITROGEN-IRRADIANCE RELATIONSHIP

The distribution of N nutrient between and within leaves is dependent on irradiance in order to optimize (Hilbert 1990) and maximize (Field *et al.* 1983) photosynthetic carbon gain by the plant. The review by Natr (1992) showed the beneficial effects of irradiance in aiding N to function effectively throughout the photosynthesis process. Inadequate N supplies limit the potential for acclimation of photosynthesis to irradiance (PAR) level during growth (Osmond 1983); when there is sufficient supply of N, whole plant production is proportional to the amount of radiation absorbed by the plant (Natr 1992). Mitchell and Hinckley (1993) found that elevating the light intensity could further enhance the rate of carbon assimilation at high N supply. At lower levels of irradiance, the partitioning of N into chlorophyll and thylakoids was enhanced, regardless of N treatments (Osmond 1983, Evans 1989), but the electron transport capacity per unit chlorophyll decreases, hence decreasing photosynthesis capacity (Evans 1989).

NITROGEN-WATER INTERACTION AND WATER-USE-EFFICIENCY

Walters and Reich (1989) found that adequately watered seedlings were more responsive to N supply than water-stress seedlings and that at high N levels, A and g_s were very sensitive to water-stressed. Natr (1992) concluded: "the interaction between N and water stress plays an important role not only in leaf expansion and stomatal opening, but also in modifying net photosynthetic rate" Also, limited N supply in plants can intensify drought or other stress situations (Walters and Reich 1989), leading to faster rates of leaf senescence, protein and chlorophyll degradation, and reductions in net photosynthesis (Ogren 1988).

Furthermore, unless both N and water is supplied in abundant amounts, the growth and development of individual plant parts (*e.g.*, roots) can not persist to produce healthy plants. Squire *et al.* (1987) discovered that well watered and well fertilized *P. radiata* seedlings had an increase in the concentration of fine roots, whereas high N supply with limited water appear to inhibit root growth, hence reducing seedling growth and survival. DeVisser *et al.* (1994) indicated that N nourishment did not enhance growth any further even at optimal by water supply and nutrients. In fact, excess fertigation (*i.e.*, excess N fertilization and irrigation combined) to reduced the fine root mass and overall root growth (DeVisser *et al* 1994).

Increasing available N can enhance instantaneous photosynthetic water use efficiency (PWUE, the net CO_2 fixation per unit of H_2O transpired or A/E ratio) provided that the microenvironment (*e.g.*, CO_2, water and light intensity) is adequate (Sheriff *et al.* 1986, Squire *et al.* 1987, Reich *et al.* 1989, Liu and Dickman 1996). High foliar N content, as opposed to low foliar N, can produce greater effects on A than E, resulting in increased PWUE (Green and Mitchell 1992). In contrast, Mitchell and Hinckley (1993) suggested that there was no significant difference in PWUE between different levels of N supplies unless ambient CO_2 and light intensity were enhanced. Sinclair *et al.* (1984) also indicated that "PWUE depends on the relative concentration differences in water and CO_2 between leaf and air"

NITROGEN-USE-EFFICIENCY

Photosynthetic nitrogen use efficiency (PNUE) is defined as the "net CO_2 exchange per unit of leaf N" (Reich *et al.* 1989) and is an important parameter for evaluating plant

productivity. Ingestad and Kahr (1985) found that PNUE was positively related to plant relative growth rate, and that the order from highest to lowest PNUE among some tree species was as follows: broadleaved species > lodgepole pine > Scots pine = Norway spruce. Black spruce and white spruce possessed similar PNUE under low N conditions (Patterson *et al.* 1996). The high PNUE of a certain species could either reflect its acclimation to habitats which have relatively higher constant N mineralization (Vitousek 1982), or the adaptation to infertile habitats with periodic N flushes (Chapin III 1980).

Above a certain minimum N supply (*i.e.*, to initiate and ensure full functions for the photosynthetic process) a negative relationship is typically observed between the N availability and PNUE, because low PNUE can result from luxury consumption under excess N supply (Chapin III 1980). Birk and Vitousek (1986) observed that foliar nitrogen concentration or N supply decreased. It has been reported that PNUE increased in loblolly pine (*Pinus taeda*). Similar situation was noted for perennial herb (*Solidago altissima*) (Hirose and Werger 1987), evergreen dwarf shrub (*Rhododendron lapponicum*) (Karlson 1994), trembling aspen (Kubiske 1997), and American elm (Reich *et al.* 1989).

It is also important to understand the relationship between the PNUE of plant and certain physiological factors (*e.g.*, mean residence time of N in plant, instantaneous A) and environmental factors (*e.g.*, irradiance) (Berendse and Aerts 1987, Evans 1988). Berendse and Aerts (1987) explained that "there is an evolutionary trade-off between properties that lead to high N productivity and those that lead to a long mean residence time of N in the plant" These authors suggested that in measuring PNUE, one has to consider two components: the mean residence time of N in plant and the instantaneous A per unit of N in the plant. This concept helps one to fully understand how a plant can utilize N and be able to

adapt to low nutrient regimes. Hence, the model he proposed for PNUE is the ratio of A to mean residence time (Ln). Furthermore, Terashima and Evans (1988) found that PNUE is independent of N level but dependent on irradiance, because PNUE is highest in leaves that grow under maximum (100%) irradiance.

TRADE-OFF BETWEEN PHOTOSYNTHETIC NITROGEN AND WATER-USE EFFICIENCY

Normally, PWUE is inversely related to PNUE because leaves that produce greater photosynthates per unit of leaf N tend to produce the lowest photosynthates per unit of water transpired (Field *et al.* 1983). This negative relationship and rank reversal between PWUE and PNUE were reported for five evergreen species (Field *et al.* 1983). Patterson *et al.* (1997) also found a PNUE-PWUE trade-off in black spruce and white spruce and indicated that "each species maximizes the use efficiency of the most limiting resource, while minimizing the concominant reduction in the use efficiency of the other resource" However, when plants are subjected to limited supplies of both water and nitrogen, they tend "to utilize each resource with suboptimal efficiency" (Patterson *et al.* 1997).

NITROGEN ALLOCATION AND CONCENTRATION

The priority for N allocation varies between different parts of the same plant. N allocation is generally greater in: (1) the upper and outer layers of the canopy compared to the lower and inner layer of the canopy, (2) leaves than in stems and roots, and (3) younger foliage than in older foliage (Hom and Oechel 1983). These authors also indicated that N concentration in the current year's growth of black spruce was highest, maintaining 90% of

the maximum value(s) in age-classes 1 to 8 years old and staying at a constant level of 70% in the older age-classes. Even the young leaves of annual species, such as of *Solidago altissima*, also contained higher N content per unit area than older ones, and higher N concentration occurred at the upper and outer layers of the canopy (Hirose and Werger 1987). The same was true for Douglas fir (Sheriff *et al.* 1986). In *Quercus ilex*, N allocation to leaves was higher than to stems under increasing N supply (Sabate and Gracia 1994). In white spruce and Douglas-fir, van den Driessche (1989) indicated that the percentages of N allocation to shoots and roots increase with N supply, but the proportion of N allocated to shoots is much greater than that to the roots.

The pattern of N allocation varies with species and N supply. Dang *et al.* (1997) found that aspen is likely to be more sensitive to N stress than coniferous species because a greater proportion of leaf N is allocated to the photosynthetic apparatus in aspen than in conifers. For a given N availability, N content per unit leaf area is directly proportional to the dry mass per unit leaf area, and this relationship changes with N availability (Walters and Reich 1989).

The level of N concentration in foliage (Nf) also varies with species and literature. Foliar N levels are more commonly reported in terms of a mass-based Nf (Nf_m, % or g/kg) rather than an area-based Nf (Nf_a, g/m^2). The reported optimum and critical ranges of Nf_m for species such as trembling aspen, jack pine, black and white spruces vary slightly in the literature. For aspen species, published optimal Nf_m were 34.1 gN/kg in 100-day-old trembling aspen (Brown 1991), 40 gN/kg in 4-month-old trembling aspen (Coleman and Smith 1998) and 35 gN/kg in 90-day-old Balsam Spire poplar [*Populus balsamifera* var. *Michauxii* (Henry) x *Populus trichocappa* var. *Hastata* (Dode) Farwell.] (Ibrahim *et al.*

1997). For conifers, optimal Nf_m, for 26-week-old seedlings of culture grown jack pine, black and white spruces were 29.2, 29.6 and 21.2 gN/kg, respectively (Swan 1970, Swan 1971). Swan (1970, 1971) also indicated that the sufficient Nf_m range for good to very good growth of jack pine, black and white spruces seedlings was from 15 – 25 gN/kg (actual reported values were in percentage), however foliar age was not specified. As well, the recommended optimal Nf_m for coniferous container seedlings were from 13 – 35 gN/kg (Landis *et al.* 1985a) or more specifically from 16 – 20 gN/kg (Swan 1970, Swan 1971, Meyer *et al.* 1997).

NITROGEN-GROWTH AND BIOMASS ALLOCATION

The increase in N supply does not only result in an increase of leaf N concentration, but also in larger and more numerous leaves (*i.e.,* greater photosynthetic or light harvesting surface), greater total growth and the allocation of carbon to shoots rather than roots (Walters and Reich 1989). Leaf N content was also realized as an important determinant of plant productivity (Coleman and Smith 1998, Ibrahim *et al.* 1997, Swan 1972) as well as the efficiency of photosynthetic apparatus (Chapin III 1980, Karlson 1994, Kubiske 1997, Reich *et al.* 1989). According to Kozlowski *et al.* (1991), the most important effect of N on plants is the production of photosynthetic surface or leaf area, which will result in higher production of photosynthate for growth and reproduction. As the amount of photosynthate increases, the photosynthetically active duration of foliage will also increase (Brady 1990, Foth 1984). In addition, growth response of trees due to increasing N supply is in the order of leaves, roots and then stems (Etter 1970).

The increase in nitrate-N supply has been found to increase various growth and physiological parameters (Hunt *et al.* 1985c). For example, it enhanced the relative growth rate of the whole plant (RGR; mass-based), relative leaf growth rate (RLGR; area-based), unit leaf rate (ULR; a change in mass per unit area), total N_f and chlorophyll concentrations (Hunt *et al.* 1985a), and total leaf area (TLA) (Hunt *et al.* 1985c). In Douglas fir, N application increased stem growth rate, as a result of the combined effect of increased photosynthesis efficiency (*i.e.*, PNUE), foliage production and decreased summer water stress in trees (Fife and Nambiar 1997) but did not extend growth duration during the growing season. This study also showed positive relationship between Nf and leaf area index (LAI). Ibrahim *et al.* (1997) found that low N supply resulted in a 50% reduction in growth of Balsam Spire poplar as a result of decreased TLA, total number of leaves per tree, mean leaf area per leaf, and specific leaf area (SLA; unit leaf area per unit leaf dry-weight).

The carbon allocation within the plant is strongly controlled by nutrient availability (Robinson 1986, Hilbert 1990, Mooney and Winner 1991). Higher productivity is often achieved at high N nutrition since rapid growth is associated with "a relatively large investment of N in photosynthesizing tissue" (Lambers *et al.* 1998). N supply affects the biomass distribution through a "shift of the relative sink strengths of roots and shoots" (Fetene *et al.* 1993). Decreasing shoot sink-strength occurs at low N supply, and this results in an increase of root to shoot biomass ratios (Birk and Vitousek 1986, Colombo and Smith 1987, Burke *et al.* 1992, Fetene *et al.* 1993, Gezelius and Nashom 1993, Kinney and Lindroth 1997, Van Cleve and Oliver 1997). Brown (1991), Ibrahim *et al.* (1997) and Coleman *et al.* (1998) found that the relative proportion of roots increases and that of foliage decreases as soil N availability declines in *Populus* species.

The variations in R/S ratio and/or the proportion of dry-mass allocated to roots reported are dependent on the species and its growing stage. Highest seedling dry-mass was achieved with R/S ratios of 0.42 and 0.22 in six month-old jack pine and black spruce seedlings, respectively (Colombo and Smith 1987). Ledig and Perry (1965) suggested that as trees become larger the shoot-to-root (S/R) ratio decreased (*i.e.*, R/S ratio increased). However, the increasing biomass allocated to roots due to plant aging could be used in root respiration, rather than root biomass accumulation, as there are high demands for maintenance processes such as respiration and protein turnover (Lambers *et al.* 1998). Consequently, the R/S ratios tended to decrease with further increasing age since total root biomass increases at a lower rate than that of the aboveground biomass, and this "decreases the respiratory burden of roots" (Lambers *et al.* 1998). So, is there a limit and what would that limit be, to which the increasing R/S ratio ceases as plants age?

VARIATIONS IN NITROGEN REQUIREMENT AMONG TREE SPECIES

Plants require N in highest quantity compared to other nutrients for optimal growth; however, the N requirement can vary depending on the species, organ and developmental stage (Marschner 1995). Because of adaptation to nutrient-poor sites, coniferous-evergreen species are believed to require lower N nutrition than broadleaved-deciduous species (Small 1972). Spruce species in turn often require higher amount of nutrients for growth than pine species (Ingestad 1979). N application resulted in greater growth responses in jack pine than in black spruce (Hoy 1973, Morrison and Foster 1995). Many researchers recommended 50 to 100 ppm N applications for spruce species and 20 to 80 ppm N applications for pine species (Swan 1970, Swan 1971, Swan 1972, Ingestad 1979, Landis *et al.* 1994).

Different nutrient elements also interact with each other, for example, N application alone shows a lower response (0.86 m^3/ha/yr) than the application of N and P together (1.24 m^3/ha/yr) (Morrison *et al.* 1995). These authors also found that high rates of N application alone can suppress black spruce growth. Sheriff *et al.* (1986) indicated that the stem diameter and volume of *P. radiata* can be elevated up to 130% by applying N and P nutrients together, compared to the effects of applying N or P separately.

SYMPTOMS AND EFFECTS OF NITROGEN DEFICIENCY AND TOXICITY

Nitrogen deficiency conditions can significantly affect various plant physiological processes and morphology. N deficiency not only results in decreases of net photosynthetic rates but also dark respiration and photorespiration rates (Moorby and Besford 1983, Hak and Natr 1987). Stomatal and mesophyll resistance to CO_2 transfer increased significantly (Natr 1992). Tesarova and Natr (1986) noted that both final leaf area and dry weight can be reduced as much as 50% in N deficient plants. Gillespie and Chaney (1989) and Gezelius and Nasholm (1993) confirmed this. Furthermore, low N supply in plants can create a whole range of problems, such as decreases in: root to shoot ratio; sulfur nutrition due to lower net uptake of S compared to other macronutrients; protein concentration (less N available to be incorporated into protein); and the proportion of K and Mn in plant (Gezelius and Nasholm 1993). Low N supply was also related to the increases in free amino acid concentration in shoot, needles and stems, as well as the allocation of macronutrients to roots (Gezelius and Nasholm 1993).

Nitrogen deficiency conditions can affect net photosynthesic rate by modifying leaf anatomy (Natr 1992). Such conditions are known to reduce the ratio of mesophyll to whole

leaf volume (Rovenska and Natr 1981), but are also known to increase stomatal frequency per unit leaf area and per mesophyll volume (Pazourek and Natr 1981). Evans (1989) showed that N deficiency can cause (1) a decrease in the volume and quantity of cells and chloroplasts (including the distribution of chloroplasts within the cells), (2) an inhibition of RuBP carboxylase activity, and (3) a reduction in the amount of soluble protein per unit leaf area and per cell.

Visible symptoms such as chlorosis and stunted needles are commonly found on jack pine trees when N supply becomes deficient (Landis *et al.* 1992b, Meyer *et al.* 1997). In severe cases, the jack pine needles become short, stiff and necrotic at the end of the growing season (Meyer *et al.* 1997). Generally, older leaves at the bottom of the canopy turns from light green to yellow at the tips, and eventually the entire leaves turn yellow even though the tissues are still alive and turgid (Foth 1984).

High N concentrations in the plant can also change plant morphology by means of increasing leaf length, width and area, but decreasing leaf thickness (Marschner 1995). Although N enhances root growth, the rate of increase in shoot growth is much higher than that of root growth, leading to an increase of S/R ratio both in terms of dry weight and length (Marschner 1995). Shoot and leaf growth increase because high N supply increases cell division and production at the early stage and cell expansion at the latter stage of growth (Moorby and Besford 1983). Similarly, high N supply also increases the number of cells in younger roots and cell size in older roots (Moorby and Besford 1983).

If N supply exceeds the requirement by the plant, numerous problems may result. A large supply of N nutrient can encourage the production of soft, succulent tissue that is susceptible to mechanical injury as well as to diseases and insects (Brady 1990, Foth 1984).

Soft, large leaves are likely to become droopy and interfere with light interception (Marschner 1995). Excessive supply of N can lead to excess vegetative growth, causing plant lodging or falling over, with slightest wind and competition from other vegetation (*e.g.*, weeds) (Brady 1990). When the growth period is prolonged (due to high N supply), it is believed to be beneficial only for plant crops in regions having long growing periods; in cooler regions, the excessive N supply can delay the development of cold-hardiness and predispose plants to frost damages (Marschner 1995). Considering the increases in S/R ratio at high N supply, the ability of the root system to uptake nutrients and water is reduced at the late growing stage, particularly in dry areas (Marschner 1995). The high nutrient supply tends to stimulate the higher production of smaller roots but suppresses the growth in root length towards the late growing stage (Marschner 1995). If N nutrition is not supplied at an appropriate level (*i.e.*, either too low or too high) 4 to 8 days after seed germination, the total number of primary and secondary lateral roots decreased (Moorby and Besford 1983).

MATERIALS AND METHODS

PLANT MATERIALS

At the start of the experiment, two-month-old trembling aspen (*Populus tremuloides* Michx.) and one-year-old black spruce (*Picea mariana* [Mill.] B.S.P.), white spruce (*Picea glauca* (Moench) Voss), and jack pine (*Pinus banksiana* Lamb.) seedlings were used. Seedlings for the experiment were selected for uniformity in size and morphology. Aspen seedlings were grown from seeds at the Lakehead University's greenhouse. The coniferous seedlings were obtained from A&R Greenhouse Ltd. (Dorion, ON).

GROWING CONDITIONS

The growing medium used was a peat-vermiculite mixture (50/50, v/v). The seedlings were planted in 5 x 5 x 7 cubic-inch pots. The photoperiod was controlled; at 16 hours and natural light was supplemented by high pressure sodium lamps on cloudy days, early mornings and late evenings. According to Landis *et al.* (1992a), the optimum ranges of day and night temperatures for boreal seedling growth are 21 to 27°C and 15 to 24°C, respectively. Day and night temperatures at the greenhouses were set at approximately 25°C and 18°C, respectively. A RH of 65% is the optimum level recommended by Landis *et al.* (1992a). Relative humidity (RH) during the experiment was 50% to 80%. Soil acidity and salinity were in the range of 5.5 to 6.0 pH and 1.2 to 2.5 mS/cm, respectively, and were within the ranges recommended by Landis *et al.* (1992b). Aspen seedlings were irrigated

using fertilizer solutions every 3 days, and conifers were irrigated every 6 days due to their lower water demand.

Essential mineral nutrients other than N were provided at optimum concentrations, using water-soluble fertilizers. The rates recommended by Landis *et al.* (1992b) for Boreal seedlings were used, *i.e.*, 60, 150, 80, 40, 60, 4.00, 0.80, 0.50, 0.32, 0.15, 0.02, and 4.00 ppm for P, K, Ca, Mg, S, Fe, Mn, B, Zn, Cu, Mo, and Cl, respectively. Microfine SuperPhosphate (0-20-0), Muriate of potash (0-0-62), magnesium sulphate ($Mg(SO_4) \bullet 7H_2O$), and calcium nitrate ($Ca(NO_3)_2$, containing 18% Ca and 15.5% N) were used to supply the macronutrients. MicroMax® micronutrient was used for micronutrients. The irrigation water and growing medium were tested for nutrient contents and consequently subtracted from the total rate of fertilizer application. The chemical formulation is attached in Appendix A.

EXPERIMENTAL DESIGN AND NITROGEN TREATMENT

This experiment was a Split-Split Plot design (Mead 1988, Hicks 1993, Brown 1995) utilizing a 2 x 2 x 6 x 4 factorial treatment structure with 12 experimental units (i.e., seedlings). The factors were:

- Two blocks in each of the two greenhouses,
- Six N treatments in each block: 25, 75, 125, 175, 375, and 775 ppm N (as NH_4NO_3 and $Ca(NO_3)$),
- Four species: trembling aspen, black spruce, white spruce, and jack pine, and
- Twelve seedlings per species per N treatment per block, from which 3 were selected at each of the 2 measurements.

The linear model is presented below (Equation 1). A completed expected means square (EMS) table and the tests of null hypotheses for the experiment are in Appendix B.

$$Y_{ijklm} = \mu + G_i + B_{(i)j} + \delta_{(ij)}' + N_k + GN_{ik} + BN_{(i)jk} + \delta_{(ijk)}'' + S_l + GS_{il} + BS_{(i)jl} + NS_{kl} + GNS_{ikl} + BNS_{(i)jkl} + \varepsilon_{(ijkl)m} \quad \text{Equation 1}$$

where G = Greenhouse (i = 1, 2), B = Block (j = 1, 2), N = Nitrogen (k = 1, 2, ..., 6), and S = Species (l = 1, 2, 3, 4).

DATA COLLECTION AND ANALYSIS

The root collar diameter and height of all seedlings were measured at the beginning of the experiment. These parameters were measured again on the 50th day (Measurement 1) and 100th day (Measurement 2) of the experiment. Three seedlings were selected randomly from each block and N treatment on the 50th and 100th day to measure foliar gas exchange (*i.e.*, photosynthesis, A, and transpiration, E) using a PP-system CIRAS-1 gas exchange system and Parkinson leaf chambers with automatic environmental control (PP-System, Haverhill, MA, USA). The quantum efficiency of Photosystem II (ΦPSII) was measured using a FMS2 fluorometer (Hansatech, Norfolk, England) simultaneously with the gas exchange measurement. The measurements were taken on the second or third fully expanded leaf from the top of the seedlings in aspen and on foliage at the upper part of the crown in conifers (excluding the tips). This was to minimize the effect of variations in gas exchange of foliage at the top *vs.* bottom or young *vs.* old foliage.

Following gas exchange measurements, the current foliage of conifer seedlings and the total foliage of aspen seedlings were harvested to determine total projected leaf area (TLA_{new}, cm^2) using a WinNeedle image analysis system (Regent, Quebec). Seedling components were oven-dried at 70°C over 48 hours for dry mass determination as well as

foliar nitrogen concentration analysis at the Environmental Laboratory, Lakehead University. Foliar N was analyzed using the colorimetric Skalar Methods (Skalar Analytical B.V. 1993). Sulfuric acid, potassium sulfate and mercuric sulfate were the catalysts used in the digestion process. The detailed procedure can be found in Skalar Analytical (1993). Total seedling photosynthetic capacity by current year foliage (A_t, A x total area of current year foliage) was calculated. The photosynthetic water-use-efficiency (PWUE, the net CO_2 fixation per unit of leaf H_2O transpired, *i.e.,* A/E,), and photosynthetic nitrogen-use-efficiency (PNUE, the net CO_2 fixation per unit of leaf N concentration, *i.e.,* A/Nf) were computed.

Although seedlings were selected for uniformity, there were still minor differences between individuals. To account for the possible effects of differences in the initial size, Analyses of Covariance (ANCOVA) were performed using the initial diameter as a covariate for diameter increment and the initial height as a covariate for height increment and seedling biomass variables. Gas exchange, foliar nitrogen concentration and photosynthetic resource-use-efficiency (PWUE and PNUE) variables were analyzed using a three-way Analysis of Variance (ANOVA). The Statistical Analysis System (SAS, SAS Institute Inc., Cary, NC, USA) software was used for all the analyses.

In the presentating the results, a graphical approach was used to investigate trends, rather than using tests for differences between the means. As well, the gas exchagne results for Measurement 1 was not presented in the thesis because there were some problems with the data.

RESULTS

GAS EXCHANGE AND FOLIAR NITROGEN

Measurement 1 (Foliar Nitrogen Only)

Mass-based foliar nitrogen concentration (Nf_m) showed a significant response to the interaction of nitrogen and species (N*S) ($p < 0.01$, Table 1). The pattern of response (Nf_m) of jack pine varied little with N treatments, whereas Nf_m stayed relatively constant from 25 to 175 ppm N and was generally positively related to the amount of N applied in the other three species (Figure 1A). The response of the area-based foliar nitrogen concentration (Nf_a) was significantly different only between species ($p < 0.01$, Table 1). This is attributed to the substantially higher Nf_a in jack pine compared to the other three species. Full ANOVA tables for these variables are found in Appendix C.

Overall, trembling aspen showed greater Nf_m but lower Nf_a at all N treatments than the coniferous species (Figures 1A and 1B). Among the conifers, black spruce had greater Nf_m than did white spruce at all N levels (Figure 1A). Nf_m in jack pine at lower N treatments (25 to 175 ppm N) was comparable to that of black spruce but was lower than that of black spruce at higher N treatments (375 and 775 ppm) (Figure 1A). At the highest N level, Nf_m remained relatively constant in jack pine but increased greatly in the spruces. However, in the area-based estimation jack pine showed highest Nf_a at most N treatments followed by black spruce, white spruce and trembling aspen (Figure 1B).

Table 1. Partial ANOVA table for foliar nitrogen concentration based on mass (Nf_m, g N/kg) and area (Nf_a, g N/m^2) for Measurement 1.

Source	DF	MS	Pr > F	MS	Pr > F
		Nf_m		Nf_a	
N [a]	5	527.10	**0.0001** [d]	20.27	0.3779
G*N [a]	5	39.74	0.2675	16.58	0.4767
S [b]	3	948.69	**0.0020**	393.77	**0.0001**
G*S [b]	3	22.34	0.7357	17.32	0.1396
B*S(G) [c]	6	51.31	**0.0295**	6.44	0.7989
N*S [c]	15	86.77	**0.0002**	20.46	0.1307
G*N*S [c]	15	58.27	**0.0038**	11.26	0.5862

[a] Test of hypothesis using B(G)*N as an error term.

[b] Test of hypothesis using B(G)*S as an error term.

Test of hypothesis using B(G)*N*S as an error term.

[d] Values in bold are significant at 95% C.I.

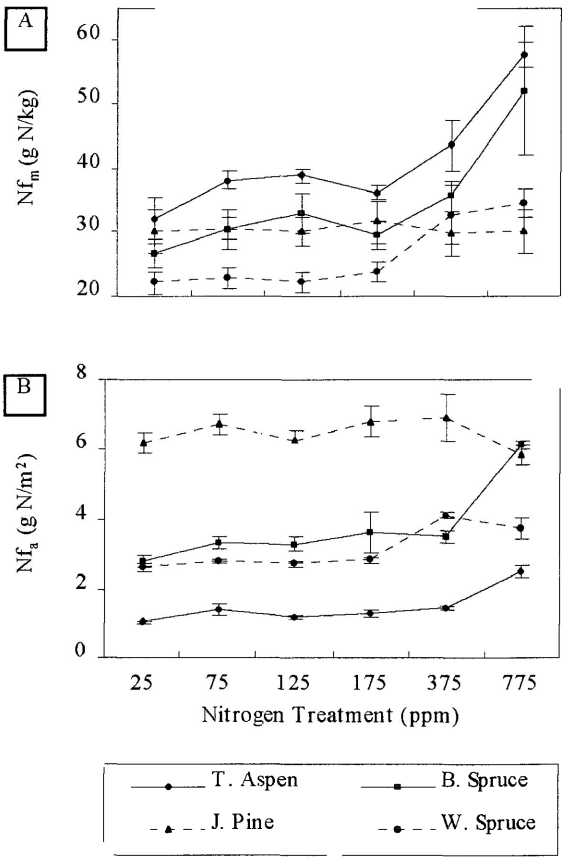

Figure 1. Foliar nitrogen concentration (mean ± S.E.M.) for Measurement 1. (Refer to Table 1 for definitions of abbreviations)

Measurement 2

Nitrogen-species interaction effects were significant in whole seedling photosynthetic capacity (A_t), transpiration (E), Nf_m, Nf_a, and in photosynthetic water-use-efficiency (PWUE) ($p < 0.05$, Table 2). However, other variables (*i.e.*, net photosynthetic rate, A; quantum efficiency of photosystem II, ΦPSII; and photosynthetic nitrogen-use-efficiency, PNUE) showed significant responses to N treatment and those were significantly different between species ($p < 0.05$, Table 2). PNUE, A_t and E also exhibited a significant response to the interaction of block, species and greenhouse (B*S*G; Table 2). Full ANOVA tables for these variables can be found in Appendix D.

Table 2. Partial ANOVA table for gas exchange and foliar nitrogen variables for Measurement 2.

Source	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
		A [a]		**A_t**		**E**		**ΦPSII**	
N [b]	5	155.75	**0.0003** [c]	461.85	**0.0137**	4.16	**0.0074**	0.0213	**0.0301**
G*N	5	11.68	0.4313	33.24	0.8584	0.81	0.3785	0.0084	0.2546
S	3	304.40	0.1014	4956.02	**0.0001**	5.84	0.3479	3.3499	**0.0001**
G*S	3	123.57	0.3509	367.47	**0.0362**	4.94	0.4089	0.0267	0.3616
B*S(G)	6	93.29	**0.0003**	66.08	0.4198	4.37	**0.0001**	0.0208	0.0555
N*S	15	23.99	0.1365	219.73	**0.0019**	1.38	**0.0378**	0.0105	0.3342
G*N*S	15	13.65	0.5682	24.57	0.9725	0.45	0.7680	0.0087	0.4899
		Nf_m		**Nf_a**		**PNUE**		**PWUE**	
N	5	809.63	**0.0001**	31.67	**0.0001**	44.99	**0.0001**	1.47	0.5709
G*N	5	23.03	0.5740	1.00	0.3604	2.12	**0.0470**	0.95	0.7571
S	3	1427.24	**0.0001**	133.00	**0.0001**	41.27	0.0743	170.89	**0.0001**
G*S	3	18.17	0.4868	0.58	0.5537	16.77	0.2904	3.45	**0.0137**
B*S(G)	6	19.80	0.1307	0.76	0.4447	10.64	**0.0006**	0.40	0.9291
N*S	15	73.08	**0.0001**	2.40	**0.0037**	2.45	0.2692	2.92	**0.0315**
G*N*S	15	11.30	0.4515	2.12	0.0470	2.78	0.1852	1.44	0.4009

[a] Net photosynthesis (A, μmol $CO_2/m^2/s$), total seedling photosynthesis capacity (A_t, μmol/CO_2/h/seedling), transpiration (E, mmol $H_2O/m^2/s$), photosystem II activity (ΦPSII, relative value), mass-based foliar nitrogen concentration (Nf_m, g N/kg), area-based foliar nitrogen concentration (Nf_a, g N/m^2), photosynthetic nitrogen use efficiency (PNUE, μmol $CO_2/m^2/s$/g N), and photosynthetic water use efficiency (PWUE, μmol CO_2/mmol H_2O).

[b] See Table 1 for the error terms used in testing for each of the above treatment combination.

[c] Values in bold are significant at 95% C.I.

Generally, A_t exhibited the greatest response to intermediate N regimes (*i.e.,* from 75 to 175 ppm N), which varied between species and resulted in species-nitrogen interaction effect (Figure 2C). Aspen achieved remarkably higher A_t at all N treatment than the conifers, of which jack pine showed higher A_t than the spruces. A_t in white spruce and aspen were relatively stable from 25 ppm N to 125 and to 175 ppm N, respectively, and then decreased with further increases in N (Figure 2C). A_t in black spruce and jack pine increased from 25 ppm N to 75 and to 125 ppm N, respectively, and then decreased significantly with further increasing in N (Figure 2C).

Distinct patterns of response of E occurred between species as well as across nitrogen treatments, resulting in the strong difference due to species-nitrogen effect ($p < 0.05$, Table 2, Figures 2B and 2E). E increased with increasing N in aspen, while it decreased with increasing N in black spruce and white spruce. In jack pine, E increased from 25 ppm N to 125 ppm N and then decreased with increasing N. Among the conifers, jack pine maintained greater E than the spruces at 125 ppm N treatment and beyond.

Different patterns of PWUE response occurred between species, resulting in a significant species-nitrogen interaction ($p < 0.05$, Table 2). The PWUE in aspen, was substantially lower than in conifers (Figure 2E). Although PWUE did not vary substantially between N treatments, PWUE in aspen and black spruce decreased slightly with increasing N, but the opposite was true for white spruce. In jack pine, PWUE varied little between N treatments. Moreover, at lower N treatments (25 and 75 ppm N), PWUE values in conifers were similar. As N increased beyond 75 ppm N, white spruce had slightly greater PWUE than did black spruce and jack pine. Beyond the 175 ppm N the PWUE in white spruce is further enhanced, while it decreased in black spruce and jack pine.

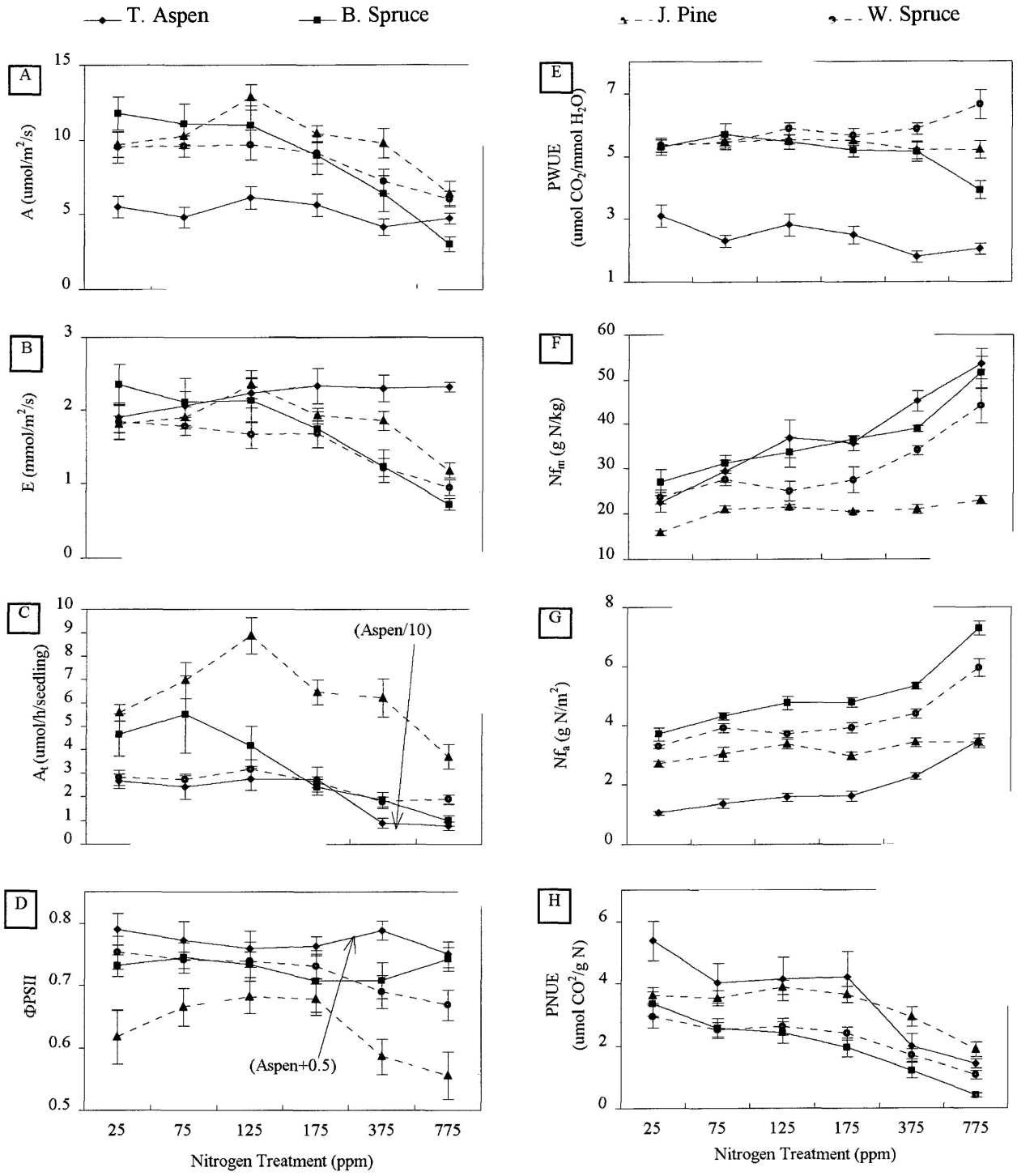

Figure 2. Gas exchange (mean ± S.E.M.) for Measurement 2.
(Refer to Table 2 for definition of abbreviations)

Nf_m and Nf_a showed significant responses to species-nitrogen interaction effect and were significantly different between species ($p < 0.01$, Table 2). As in Measurement 1, Nf_m and Nf_a responded positively to increasing N application in aspen, black and white spruces, and Nf_m and Nf_a jack pine showed the least response to increasing N addition (Figures 1 and 2). Nf_m in black spruce and trembling aspen were comparable and higher than those in white spruce and jack pine, which had the lowest Nf_m. Nf_a was highest in black spruce followed sequentially by white spruce, jack pine, and trembling aspen.

The net photosynthetic rate (A) displayed a significant response to increasing N treatment, without clear distinctions between the species (Table 2). In all conifers, there was a general decline of A beyond the 125 ppm N treatment. From 25 to 125 ppm N, A in black and white spruce stayed relatively stable, whereas it responded positively to N addition in jack pine (Figure 2A). In aspen, A did not vary significantly between N treatments.

The response of ΦPSII to both species and N treatment were significant ($p < 0.05$, Table 2). ΦPSII in white spruce was negatively related to increasing N, while in jack pine it increased from 25 to 175 ppm N, and dropping with further addition of N (Figure 2C). In aspen and black spruce, ΦPSII varied little and showed no obvious increasing nor decreasing trend with the addition of N. Among the conifers, ΦPSII values in black and white spruce were comparable and higher than jack pine's (Figure 2D).

The response of PNUE to the block-species interaction and to the main effect of N treatments was significant ($p < 0.05$, Table 2). Overall, PNUE responded negatively to increasing N in all four species (Figure 2H). In all species, there appeared to be a plateau between 75 and 175 ppm N. Aspen achieved the highest PNUE from 25 to 175 ppm N, but beyond which point it was lower than jack pine. Among the conifers, jack pine achieved the

greatest PNUE at all N treatments. The PNUE in white spruce was slightly lower than that in black spruce at 25 and 75 ppm N, but it exceeded that in black spruce beyond 125 ppm N.

GROWTH AND BIOMASS ALLOCATION

Measurement 1

A. Growth

Table 3 presents the ANOVA and ANCOVA results for those variables exhibiting significant responses (i.e., height and diameter increment, seedling dry-weight and total leaf area; TLA). Note the initial height and diameter was used as covariates for the ANCOVA for all growth variables after they were analyzed using the ANOVA, and the results for those variables that were significantly different due to the covariate effect are shown here. Only height growth responded significantly to a change in N, however, all four displayed significant responses to the greenhouse species interaction ($p < 0.05$). Only TLA exhibited a significant response to nitrogen-species interaction. The full ANOVA and ANCOVA tables for these variables can be found in Appendix E.

Total seedling dry weight, height and growths were significantly different due to species-greenhouse interaction (data not shown). The cause of these differences was primarily due to the different response of aspen, producing much more growth than the conifers (Figure 3). Also, greenhouse 2 tended to produce larger seedlings than greenhouse 1, because the overall day temperature in greenhouse 2 appeared to be slightly higher and the relative humidity appeared to be relatively lower than that in greenhouse 1. However, the response patterns to increasing N supply of each species were generally converged. Aspen showed remarkably higher height growth at 75, 125 and 175 ppm N treatments than at other

N levels (*i.e.*, at 25, 375 and 775 ppm N) and also compared to the growth achieved by the conifers at these N treatments. Among the conifers, black spruce achieved higher height increments than did the others, followed by white spruce at all N treatments. However, the diameter growth and total seedling weight in white spruce were greater than black spruce and jack pine.

Table 3. Partial ANOVA and ANCOVA table for growth variables for Measurement 1.

Source	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
		Height growth [a]		**Diameter growth** [a]		**Seedling Weight** [b]		**Total Leaf Area** [b]	
N [c]	5	153.6	**0.0482** [d]	0.508	0.6409	15.8	0.2901	43.9	0.1352
G*N	5	57.7	0.3500	0.319	0.8151	6.5	0.7031	14.1	0.6314
S	3	5612.1	**0.0001**	75.344	**0.0001**	2438.3	**0.0001**	6907.2	**0.0001**
G*S	3	1259.1	**0.0001**	3.643	**0.0246**	246.6	**0.0001**	117.8	**0.0001**
B*S(G)	6	13.8	0.9784	0.548	0.5305	1.7	0.9916	3.4	0.9836
N*S	15	135.5	0.0790	1.009	0.1341	16.0	0.3327	42.4	**0.0415**
G*N*S	15	49.5	0.7960	0.359	0.8764	8.5	0.8284	15.4	0.7070

[a] Variables analyzed using the ANCOVA.

[b] Variables analyzed using the ANOVA.

[c] See Table 1 for the error terms used in testing for each of the above treatment combination.

[d] Values in bold are significant at 95% C.I.

Species-nitrogen interaction significantly affected total leaf area (TLA) ($p < 0.01$, Table 3). Species differences were apparent with aspen, not unexpectedly, achieving the highest TLA at all N treatments (Figure 3C). TLA in aspen showed a bell-shaped response (being highest at 175 ppm N); however, there were no particular patterns in the conifers response to N treatments. The TLA in black spruce and jack pine were comparable while exchanging positions at virtually all N levels, but white spruce consistently exhibited the lowest values.

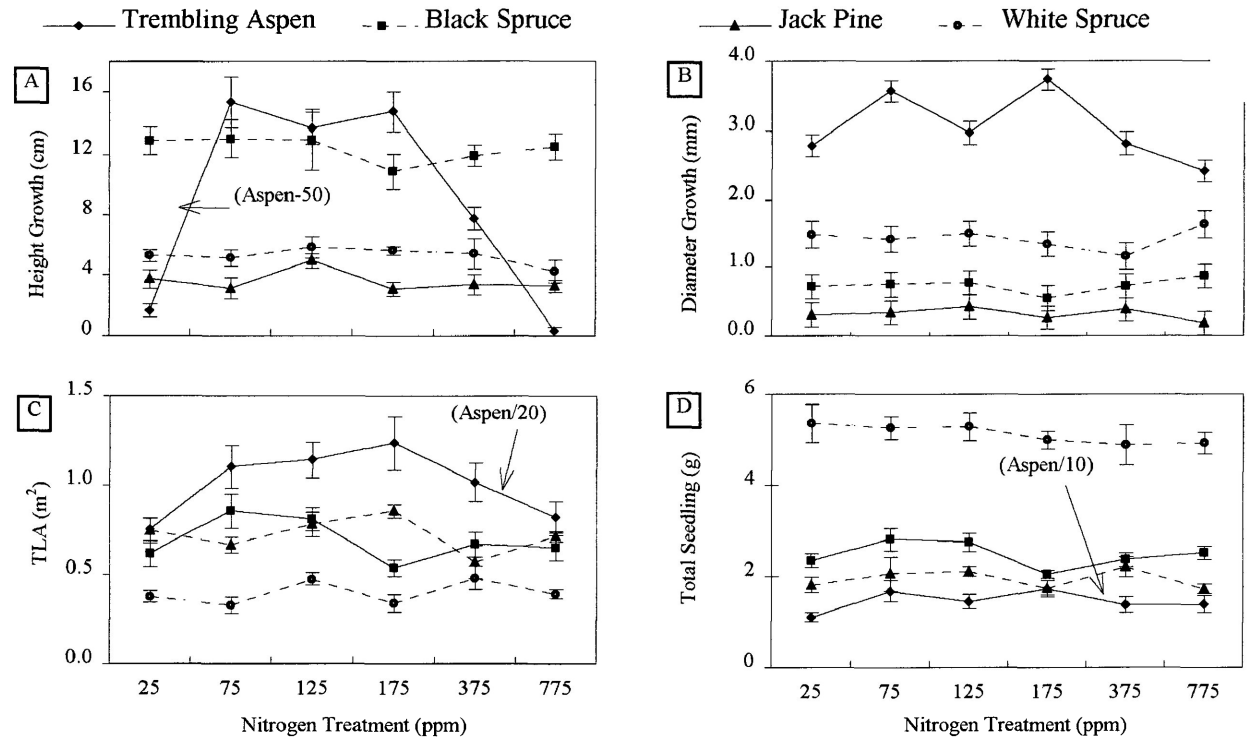

Figure 3. Growth and dry-weight (mean ± S.E.M.) for Measurement 1.

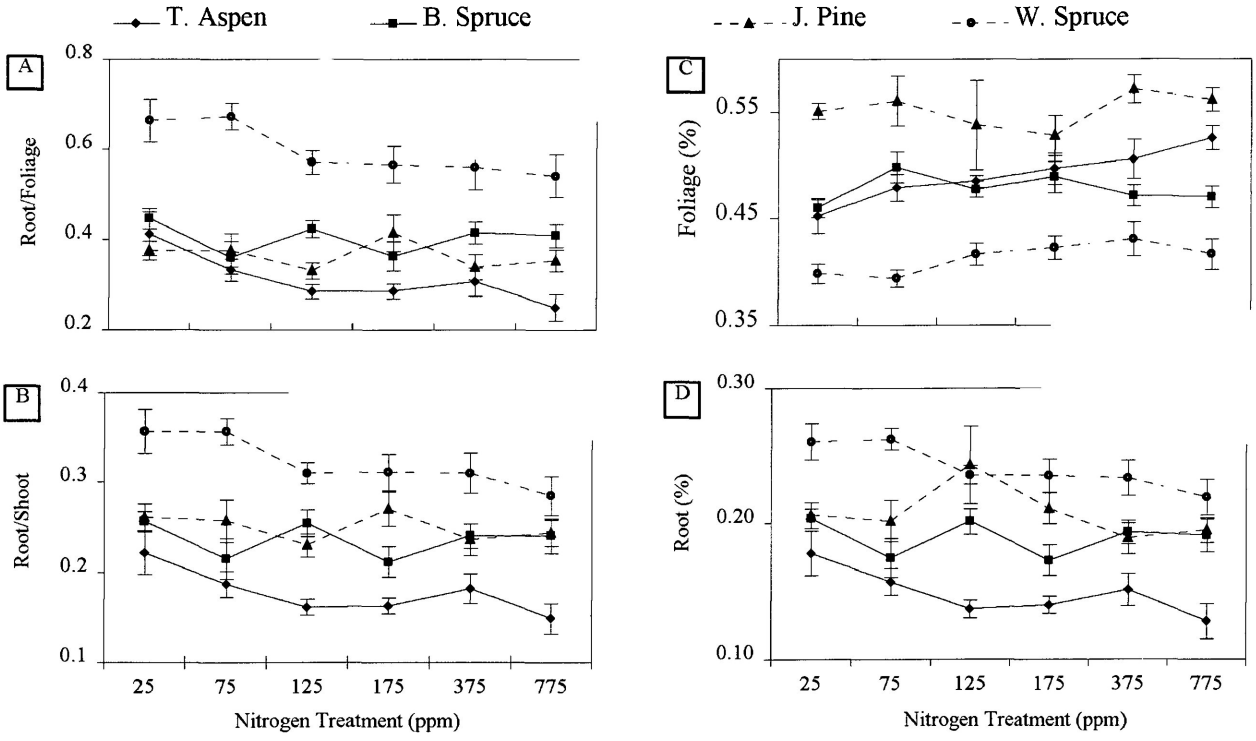

Figure 4. Biomass ratios and allocation (mean ± S.E.M.) for Measurement 1.

B. Biomass Ratios and Allocation

The species-nitrogen interaction effect was significant for both percent allocation to the foliage and to the root (Foliage% and Root%, respectively) ($p < 0.05$, Table 4), whereas root-to-foliage and root-to-shoot ratios (R/F and R/S, respectively) showed siginificant responses to species-greenhouse interaction ($p < 0.05$, Table 4). Interestingly, all four variables exhibited a significant species response which is reflected in the graphs (Figures 4A to D). For three of the variables, R/F, R/S and Root%, aspen and white spruce consistently exhibited the lowest and highest values, respectively. With respect to Foliage%, there were clear distinctions between the species, particularly white spruce and jack pine, with some cross over between aspen and black spruce. Higher N generally significantly decreased the R/F ratio (Figure 4A). Appendix G contains full ANOVA tables for biomass allocation variables. Generally, the trend for biomass allocation to foliage was opposite of that for roots (Figures 4C and 4D). The pattern was more obvious in white spruce and trembling aspen than in other species (Figure 4). The R/S ratio showed a similar trend to that of R/F ratio with increasing N supply, but N effect was not significant ($p = 0.06$, Table 3, Figure 4C).

Table 4. Partial ANOVA table for biomass allocation variables for Meaurement 1.

Source	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
		Root / Foliage [a]		**Root / Shoot**		**Foliage%**		**Root%**	
N [b]	5	0.0469	**0.0447** [c]	0.0119	0.0672	0.00562	0.0911	0.00528	0.3084
G*N	5	0.0332	0.1065	0.0118	0.0684	0.00162	0.6001	0.00397	0.4449
S	3	1.1085	**0.0001**	0.2535	**0.0001**	0.23428	**0.0001**	0.10667	**0.0011**
G*S	3	0.0937	**0.0028**	0.0332	**0.0036**	0.02417	**0.0323**	0.00614	0.3522
B*S(G)	6	0.0058	0.8816	0.0023	0.7352	0.00412	0.3630	0.00465	0.2989
N*S	15	0.0184	0.3094	0.0045	0.3430	0.00334	0.5482	0.00280	0.7025
G*N*S	15	0.0146	0.5054	0.0052	0.2307	0.00429	0.3305	0.00481	0.2521

[a] Root/Foliage = root-to-foliage ratio (g/g), Root/Shoot = root-to-shoot ratio (g/g), Foliage% = % allocation to foliage, Root% = % allocation to root.

[b] See Table 1 for the error terms used in testing for each of the above treatment combination.

[c] Values in bold are significant at 95% C.I.

Measurement 2

A. Growth

With the exception of root dry-weight, which responded significantly to species and greenhouse-species treatments, respectively, all growth variables responded significantly to species-nitrogen interaction ($p < 0.01$, Table 5). These relationships were displayed by the distinct response patterns as shown in Figure 5. The full ANOVA and ANCOVA tables for these variables can be found in Appendix F.

Table 5. Partial ANOVA and ANCOVA table for growth variables for Measurement 2.

Source	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
		Height Growth		**Diameter Growth**		**Total Leaf Area**		**Foliage Weight**	
N [a]	5	586.56	**0.0024** [b]	2.057	0.1371	58.69	**0.0034**	11.11	0.1196
G*N	5	127.45	0.1970	0.845	0.5182	3.92	0.7628	4.39	0.5064
S	3	10447.65	**0.0001**	92.771	**0.0001**	1547.92	**0.0001**	130.06	**0.0001**
G*S	3	545.16	**0.0162**	0.300	0.7084	3.47	**0.0527**	7.00	0.0848
B*S(G)	6	68.31	0.6323	0.625	0.7787	0.75	0.9932	1.94	0.4004
N*S	15	505.61	**0.0001**	2.354	0.0507	53.02	**0.0001**	8.19	**0.0002**
G*N*S	15	51.12	0.8938	0.638	0.8931	3.82	0.8432	2.87	0.1361
		Stem Weight		**Shoot Weight**		**Root Weight**		**Seedling Weight**	
N	5	14.10	**0.0296**	45.05	0.0537	6.25	0.1722	83.31	0.0640
G*N	5	4.43	0.3532	12.04	0.5365	1.54	0.7828	21.53	0.5846
S	3	1082.70	**0.0001**	1863.97	**0.0001**	305.41	**0.0001**	3663.00	**0.0001**
G*S	3	42.02	**0.0007**	75.85	**0.0075**	17.60	**0.0018**	163.42	**0.0044**
B*S(G)	6	1.56	0.6735	6.90	0.2388	0.93	0.8776	12.03	0.3874
N*S	15	11.88	**0.0001**	34.07	**0.0001**	2.19	0.5449	51.41	**0.0002**
G*N*S	15	3.52	0.1624	8.52	0.0924	1.48	0.8292	16.58	0.1635

[a] See Table 1 for the error terms used in testing for each of the above treatment combination.

[b] Values in bold are significant at 95% C.I.

The height growth in aspen was very responsive to increasing N treatment, while the conifers, particularly white spruce and jack pine were not responsive to N treatments. Similarly, aspen achieved very high TLA, foliage, stem, shoot and total seedling dry-weights compared to the conifers. However, among the conifers, jack pine achieved the greatest

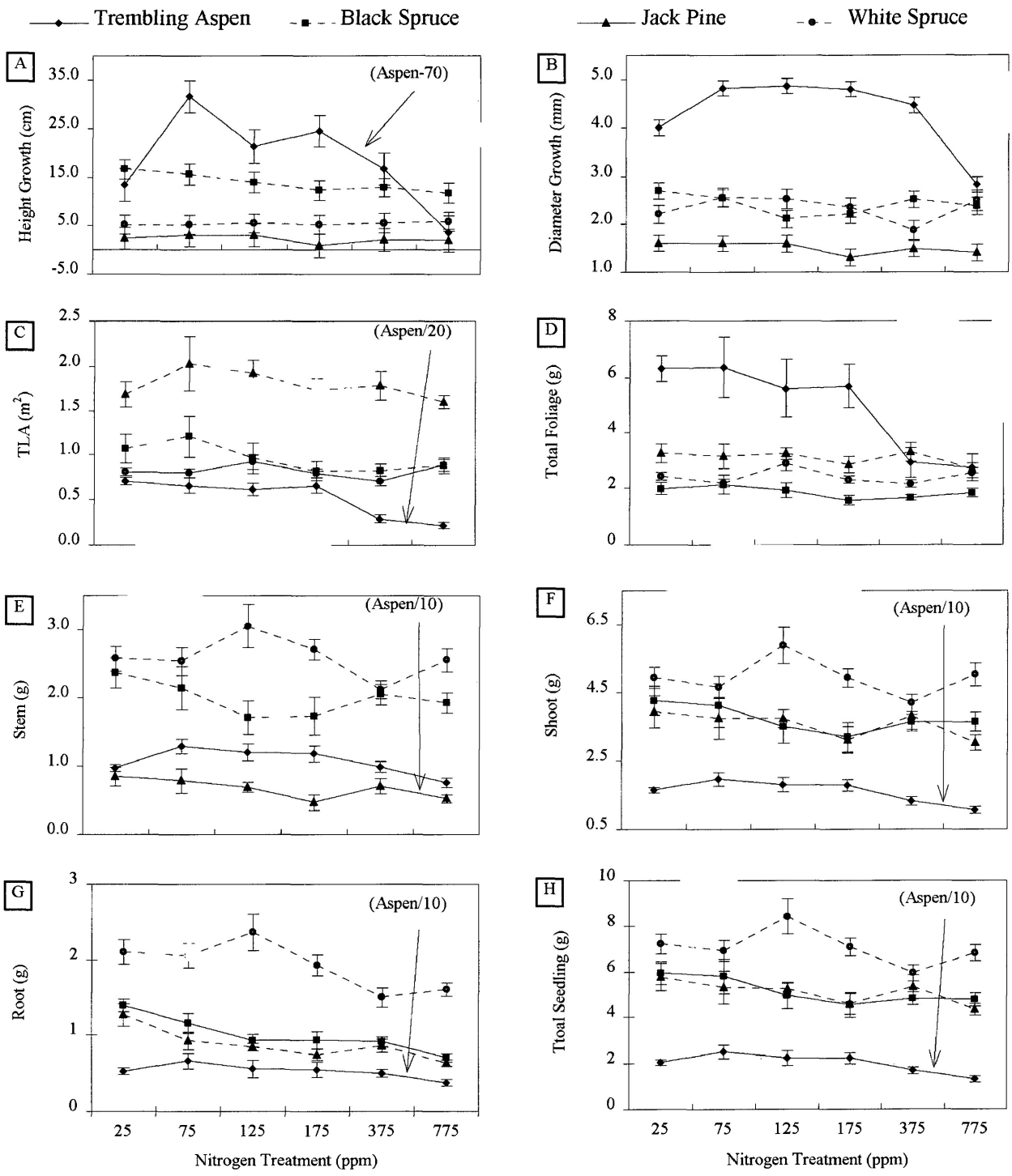

Figure 5. Growth and dry-weight (mean ± S.E.M.) for Measurement 2.

TLA and foliage dry-weight compared to the spruces at all N treatments (Figures 5C and 5D). Furthermore, on the area-based estimation black spruce achieved greater TLA than did white spruce, but the reverse was true based on the mass estimation. As for stem, shoot and total seedling dry-weights, white spruce realized higher biomass than black spruce and jack pine at all N treatments (Figures 5E, 5F and 5H). Furthermore, although the stem dry-weight in jack pine was lower than that in black spruce across the N treatments, it was comparable to black spruce when total shoot and seedling dry-weights were considered.

Height growth, TLA and stem dry- weight also responded significantly to nitrogen treatments ($p < 0.05$). The highest height increments in aspen occurred at intermediate N treatments, particularly at 75 ppm N (Figure 5A). Hence, similar results were found in total stem biomass (Figure 5E). Other than TLA in aspen, which showed highest result at 25 ppm N (Figure 5C), all growth variables were highest at 75 ppm N and significantly low beyond 175 ppm N (Figures 5D to 5H). Height increment in black spruce responded negatively, while white spruce and jack pine's height increments varied little, to increasing N (Figure 5A). An overall decreasing response of TLA to increase in N beyond 75 ppm N was found in black spruce and jack pine, while white spruce remained relatively similar at all N treatments (Figure 5C). As well, total stem weights in black spruce and jack pine were both highest at 25 ppm N, but highest stem weight for white spruce occurred at 125 ppm N (Figure 5E). This was also true for total foliage, shoot, root and total seedling dry-weights (Figures 5D to 5H).

As in Measurement 1, all growth variables showed significant differences between species ($p < 0.01$, Table 5). All growth parameters of aspen were substantially higher than those of coniferous species at all N levels (Figures 5A – 5H). In conifers, white spruce produced greater overall seedling growth than did jack pine and black spruce (Figure 5H),

since it was able to maintain both highest stem and root growth despite its lower foliage production (Figures 5D to 5G). The overall seedling weight of black spruce was comparable to that of jack pine (Figure 5J) but due to different reasons. While black spruce maintained higher root and stem biomass (Figures 5E and 5G), jack pine sustained higher foliage production (Figures 5C and 5D).

B. Biomass Ratios and Allocation

Species-nitrogen interaction significantly affected root-to-stem (R/St), R/F and R/S ratios, Foliage% and Root% (Table 6). The strong distinction in the responses of aspen compared to the conifers primarily contributed to the species-nitrogen interaction effect (Figures 6A to 6C). Highest ratios for black spruce and jack pine occurred at 25 ppm N. White spruce also showed highest R/St at 25 ppm N, but highest R/F and R/S were found at 75 ppm N. Lowest ratios in all conifers were at 775 ppm N. In aspen, highest R/F and R/S occurred at 375 and 775 ppm N. R/F and R/S ratios in aspen were relatively stable from 25 to 175 ppm N. R/St ratios in aspen were relatively stable across all N treatments. Appendix H contains full ANOVA tables for biomass allocation variables.

All biomass ratios and allocation variables differed significantly between species ($p < 0.01$) (Table 4). Trembling aspen had the lowest Foliage% but the highest Root% and Stem% at all N treatments, resulting in the highest R/F compared to the conifers (Figure 6). Among the conifers, black spruce allocated highest proportional resources to Stem (Figure 6E), resulting in least R/St (Figure 6B). White spruce showed highest Root% (Figure 6F) but lowest Foliage% (Figure 6D), therefore it showed greatest R/F and R/S (Figures 6A and 6C). Jack pine allocated highest resources to foliage (Figure 6D) resulting in lowest R/F (Figure 6A), whereas the reverse was true for percentage of stem allocation.

Table 6. Partial ANOVA table for biomass allocation variables for Measurement 2.

Source	DF	MS	Pr > F	MS	Pr > F	MS	Pr > F
		Root / Foliage [a]		**Root / Stem**		**Root / Shoot**	
N [b]	5	0.6517	0.3624 [c]	0.2034	**0.0034**	0.0282	**0.0425**
G*N	5	0.2004	0.8518	0.0134	0.7690	0.0035	0.8057
S	3	16.4008	**0.0005**	1.5676	**0.0001**	0.5064	**0.0001**
G*S	3	0.2714	0.6822	0.0489	0.1542	0.0190	**0.0501**
B*S(G)	6	0.5190	0.5363	0.0194	0.4804	0.0040	0.7952
N*S	15	1.329	**0.0323**	0.0404	**0.0566**	0.0212	**0.0097**
G*N*S	15	0.1610	0.9955	0.0078	0.9749	0.0019	0.9975
		Foliage%		**Stem%**		**Root%**	
N	5	0.0057	0.1644	0.00936	**0.0445**	0.0116	**0.0127**
G*N	5	0.0055	0.1737	0.00433	0.2445	0.0009	0.8283
S	3	1.7136	**0.0001**	1.00797	**0.0001**	0.1695	**0.0001**
G*S	3	0.0029	0.3038	0.00085	0.5798	0.0060	**0.0317**
B*S(G)	6	0.0012	0.8912	0.00120	0.8972	0.0010	0.8248
N*S	15	0.0134	**0.0142**	0.00401	0.3149	0.0061	**0.0068**
G*N*S	15	0.0040	0.6922	0.00270	0.6516	0.0006	0.9904

[a] Root/Foliage = root-to-foliage ratio (g/g), Root/Stem = root-to-stem ratio (g/g), Root/Shoot = root-to-shoot ratio (g/g), Foliage% = % allocation to foliage, Root% = % allocation to root.

[b] See Table 1 for the error terms used in testing for each of the above treatment combination.

[c] Values in bold are significant at 95% C.I.

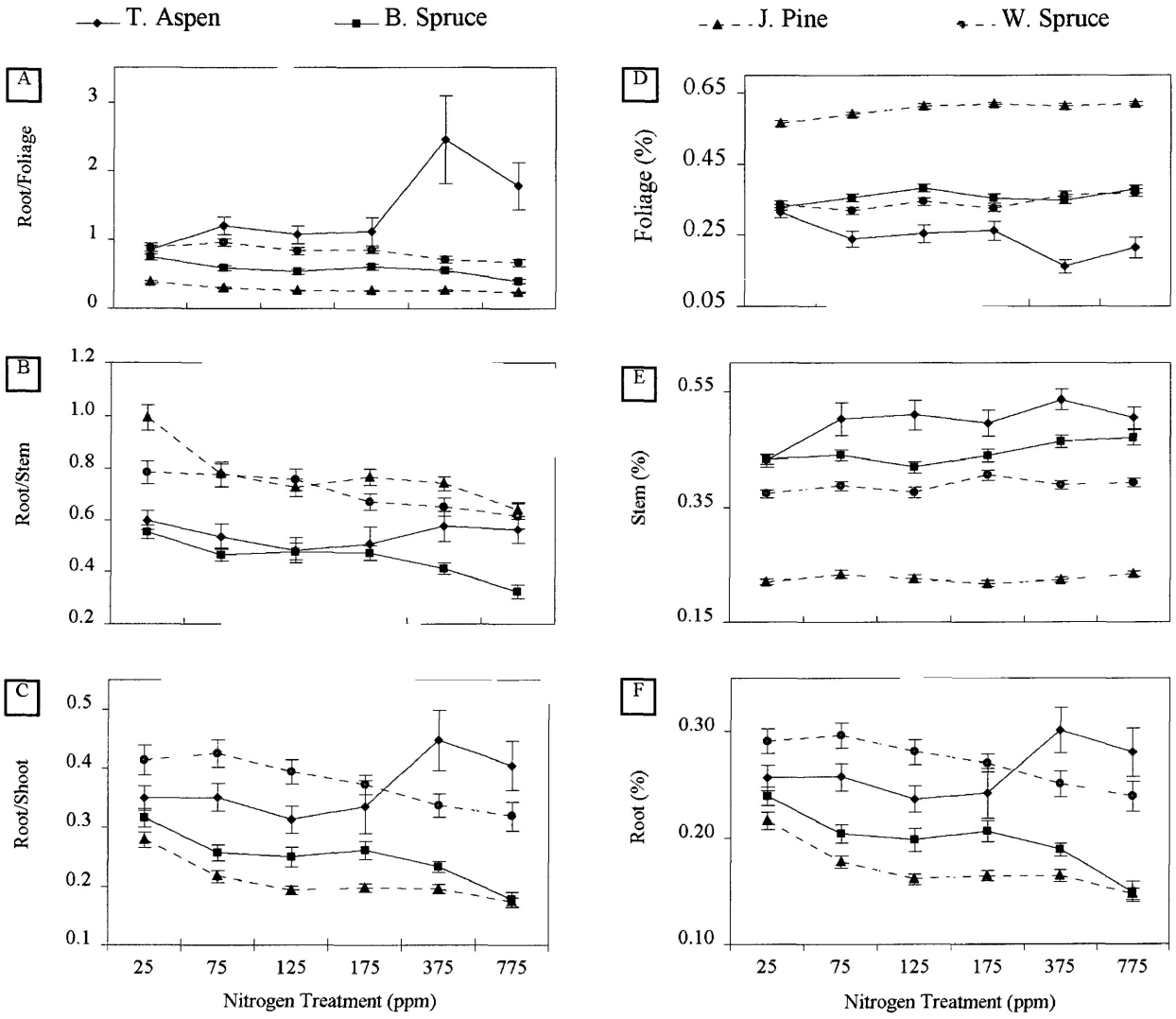

Figure 6. Biomass ratios and allocation (mean ± S.E.M.) for Measurement 2.

DISCUSSION

Foliar Nitrogen

Foliar N concentration (Nf) results in this study support the theory that leaf nitrogen (N) content is an important determinant of plant productivity (Swan 1972, Ibrahim *et al.* 1997, Coleman and Smith 1998) as well as the efficiency of photosynthetic apparatus (Chapin III 1980, Reich *et al.* 1989, Karlson 1994, Kubiske 1997). Optimum ranges of Nf for trembling aspen, jack pine, black and white spruces vary slightly in the literature. In spite of these variations, the mass-based foliar nitrogen concentration (Nf_m) values of this study were comparable to the literature. For example, from the lowest (25 ppm) to the highest (775 ppm) N treatments, the Nf_m of trembling aspen seedlings ranged from 32 – 58 gN/kg in Measurement 1 and 23 – 54 gN/kg in Measurement 2. The optimum Nf_m values reported for the same species varied from 34.1 – 40.0 gN/kg (Brown 1991, Coleman and Smith 1998). For spruce (*Picea* spp.) and pine (*Pinus* spp.) seedlings, the optimum Nf_m range was 13 – 35 gN/kg (Swan 1970, Swan 1971, Landis *et al.* 1985, Meyer *et al.*1997). The Nf_m in our conifer seedlings ranged from 22 – 35 and 16 – 52 gN/kg in Measurement 1 and in Measurement 2, respectively.

Generally, increases in N supply enhanced foliar N concentration in all four species, particularly for the area-based foliar N concentration (Nf_a). Black spruce concentrated greater N content in foliage than did white spruce and jack pine; jack pine contained the lowest foliar N at all N treatments on both mass and area basis. Trembling aspen achieved

lower Nf_a but higher Nf_m than did the conifers at all N treatments, because it produced greater total leaf area but lower total foliage weight than did the conifers.

Application of 375 and 775 ppm N appeared to have created the greatest increases in Nf, particularly the mass-based Nf, for aspen, black and white spruces seedlings and created luxurious to toxic N conditions for these seedlings (see discussion on Gas Exchange and Growth Response). In jack pine, Nf_m values were relatively stable across the N treatments, particularly in Measurement 1 (Figures 1 and 2). Toxic N conditions were evidenced by the substantial physiological and growth reductions meanwhile foliar N concentration continued to increase (Van den Driessche 1991). This was also evident in our study, especially in treatments beyond 175 ppm N (Figures 1 and 2). Conditions of luxury consumption of N can be detected when plant N concentration continues to increase whereas growth is stable (Van den Driessche 1991), and the luxury consumption range is intermediate between optimum and toxic N supply. In addition, an optimum Nf can be determined at the point where maximum photosynthesis (A) is achieved (Brix 1981; see discussion on Gas Exchange). Therefore, our results suggest that optimal Nf_m were 36.7, 21.6, 27.0, and 25.0 gN/kg and optimal Nf_a were 1.56, 3.35, 3.69, and 3.69 g/m^2, respectively for trembling aspen, jack pine, black spruce and white spruce (Figure 2). These Nf corresponded to N treatments at 25 ppm for black spruce and at 125 ppm for the other three species. However, the optimum N level for black spruce (25 ppm) seems a little doubtful since it is much lower than the reported optimum soil N condition (*i.e.*, 50 - 100 ppm N) for the spruces (Swan 1970, Swan 1971, Swan 1972, Ingestad 1979, Landis *et al.* 1994). Recalling that Nf in jack pine stayed relatively stable beyond the 125 ppm N treatment (Figure 2), this could imply that jack pine could not increase the N uptake when soil N was higher than 125 ppm and higher soil N was

probably toxic to the root system as suggested by the negative response of photosynthesis to higher N supply (Figure 2A).

Although the optimum Nf_m for each of the species in this study were within the optimal ranges reported by others, the Nf_m ranges covering from the lowest (25 ppm) to highest (775 ppm) N treatments for all the species were slightly higher than the values in the literature. This difference may be related to source and rate of nitrogen used in the experiment. NO_3-N generally enhances N uptake and accumulation by plants in less acidic environment, while N supplies that contain equal parts of NH_4-N and NO_3-N often yield greater growth compared to that of NO_3-N or NH_4-N alone (Marschner 1995, Nygren *et al.* 2000). In this study, N treatments contained slightly higher NO_3-N than NH_4-N because calcium nitrate ($CaNO_3$) was used for the calcium source, on top of the different rates of NH_4NO_3 source containing 17% NH_4 and 17% NO_3. Unfortunately the source of N was not clearly stated by others, who had reported the optimum Nf_m range (13 – 35 gN/kg) for conifers (Swan 1970, Swan 1971, Landis *et al.* 1985, Meyer *et al.*1997). In addition, our study utilized significantly higher N addition rates (especially the 375 and 775 ppm N), larger pots (5x5x7 in) as well as more frequent fertilization. These might also be factors contributing to higher Nf in seedlings of this study as compared to others'

Gas Exchange

In this study, the response of CO_2 assimilation or photosynthetic rate (A, $\mu mol/m^2/s$) varied with species as well as with N treatments. While A did not respond significantly to increasing N supply in trembling aspen, it responded negatively in the spruces and had a parabolic response in jack pine. In the literature, the extent to which A depends upon leaf N

also varied significantly between studies. For example, Cantin *et al.* (1997) and Coleman *et al.* (1998) concluded that increasing N input enhanced both A and foliar nitrogen concentration, while Ibrahim *et al.* (1997) found that N supply had no effect on A in poplar/aspen species (*Populus* spp.). Results for aspen in this study were consistent with the results by Boot *et al.* (1990), Wullschleger and Oosterhuis (1990), Bowman and Conant (1994), Ibrahim *et al.* (1997), and Kubiske *et al.* (1997). The insensitivity of trembling aspen could be explained by the ambient carbon dioxide (CO_2) utilized in our measurements. Kubiske *et al.* (1997) found that at elevated CO_2 the maximum leaf photosynthesis (A_{max}, $nmol.g^{-1}.s^{-1}$) were significantly higher in high N treatments than in low N treatments; however A_{max} were not significantly different between N treatments if ambient CO_2 was supplied. Moreover, the range of A in aspen in this study (4.3 – 6.1 $\mu mol/m^2/s$) were within the reported limits for a wide range of poplar clones (3 – 11 $\mu mol/m^2/s$; Ceulemans and Impens 1983).

Pest problems could have been a factor responsible for the insensitiveness of A in aspen seedlings to N treatment. Over the course of the experiment, many aspen seedlings were attacked by aphids, mites, thrips, and powdery mildew. These pests were generally controlled after two fumigation treatments. However, insect attacks were generally more severe in seedlings under high N treatments (*i.e.*, 375 and 775 ppm N), and heavy damages to the terminal leaders as well as some defoliation occurred. Upon Measurement 2 the seedlings at 375 and 775 ppm N showed fewer live leaves as measured by total foliage dry-mass and total leaf area than did lower N treatments (Figures 5A, 5C and 5D).

The vulnerability to insect attacks may be related to the biochemical content or nutritional status of the seedlings. Excess N supply can enhance the production of amino

acids that attract sucking insects such as mites, aphids and plant-hoppers (Dreyer and Campbell 1987, Salim and Saxena 1991, Marschner 1995). In this study, the foliar N concentration was significantly higher in seedlings under the 375 and 775 ppm N treatments and those seedlings suffered the most severe insect attack (Figure 2F). This suggests that aspen seedlings may not thrive at sites with high N supply. However, further research is necessary to confirm this conclusion.

In conifers, Brix (1981) demonstrated that the relationship between foliar N and CO_2 assimilation % of maximum in Douglas fir was parabolic. That is, CO_2 assimilation increased from a deficient foliar N concentration (10 gN/kg) to an optimum foliar N concentration (17 gN/kg) and then decreasing towards a supraoptimal or toxic foliar N concentration (23 gN/kg). Foliar N concentrations (16 – 52 gN/kg) for the conifers in this study were generally higher than the optimal suggested concentrations in the literature (13 – 35 gN/kg) (Swan 1970, Swan 1971, Landis *et al.* 1985, Meyer *et al.*1997). As well, A was inversely related to N supply, except for jack pine where A was enhanced as N increased from 25 to 125 ppm N (Figure 2). It is then logical to conclude that a N supply of 25, 75 and 125 ppm were sufficient for maximum photosynthtic rate for black spruce, white spruce and jack pine, respectively (Figure 2A).

Chlorophyll Fluorescence

The measurement of chlorophyll fluorescence has been proven to be a useful tool to determine the photosynthetic activity *in vivo* (Papageordiou 1975, Toivoinen and Vidaver 1984, Hawkins and Lister 1985, Conroy *et al.* 1986, Vidaver *et al.* 1989, Krause and Weis 1991). For example, it was used to provide information on the physiological status of white

spruce (Vidaver *et al.* 1989). Chlorophyll fluorescence can be used to estimate the photochemical quantum yield of Photosystem II (ΦPSII) (Lambers *et al.* 1998). In other instances, it was used as an indicator of photosynthesis (A) in intact chloroplasts or whole leaves (Lichtenthaler and Grumbach 1975, Sivak and Walker 1983, Walker *et al.* 1983, Lamontagne *et al.* 2000). This study showed that ΦPSII followed a similar trend as A in response to N treatment in white spruce and jack pine but not in trembling aspen and black spruce (Figures 2A and 2D). In black spruce, A decreased sharply with increasing N supply, while ΦPSII stayed relatively stable throughout N treatments. The similar responses of A and ΦPSII in white spruce and jack pine may suggest that the photochemical and biochemical reactions of A and ΦPSII were affected to a similar extent by N treatment. On the other hand, the lack of response of aspen and black spruce indicate that the biochemical processes were preferentially affected by N treatment in these species.

The values of ΦPSII in conifer seedlings are within the reasonable range (0.51 – 0.70) suggested by Lambers *et al.* (1998), who stated that illuminated samples had ΦPSII values equal to or less than 0.80. In fact, Lamontagne *et al.* (2000) reported ΦPSII values from 0.70 – 0.75 and 0.55 – 0.65 for black spruce samples that were taken at low and high light intensities, respectively. The values of ΦPSII for trembling aspen (0.20 – 0.24) were, however, consistently lower than those reported in the literature (*i.e.*, 0.45 – 0.80; Krall and Edwards 1992, Lambers *et al.* 1998). Therefore, whether the ΦPSII values measured for aspen in this study are within a tolerable range for this species is not clear, because no other studies have reported ΦPSII values for trembling aspen.

Photosynthetic Water Use Efficiency

Photosynthetic water-use-efficiency (PWUE) is a function of the rate of CO_2 assimilation (A) and the rate of transpiration (E), PWUE = A/E, and is dependent upon the plant genotype and physiological condition, including nutritional status (Van den Drissche 1991). The PWUE of fast-growing genotypes is generally higher than that of slow-growing genotypes (Blake *et al.* 1984). Some studies found that higher available soil N enhanced PWUE (Sheriff *et al.* 1986, Squire *et al.* 1987, Reich *et al.* 1989, Liu and Dickman 1996, Cantin *et al.* 1997), because N supply is generally positively related to foliar N concentration, which in turn often results in greater increase in A than in E (Green and Mitchell 1992). The PWUE from the present study is consistent with these findings only in white spruce, because as N addition increased, A decreased at a relatively slower rate than E, leading to a slight increase in PWUE (Figures 2A, 2B and 2E). Opposite relationships were observed in aspen and black spruce. Jack pine showed almost no changes in PWUE as N treatment increased, since the response of A and E resembled each other. Given that the soil water content was relatively stable during the experiment, the results suggest that aspen and black spruce may not be able to use water more efficiently in an increasing soil N environment. However, a similar microenvironment may be advantageous for white spruce and indifferent for jack pine, in terms of PWUE.

Photosynthetic Nitrogen Use Efficiency

Photosynthetic nitrogen-use-efficiency (PNUE) is a function of the rates of CO_2 assimilation (A) and foliar N concentration (Nf) (PNUE = A/Nf, Van den Drissche 1991). This study showed that increasing N supply significantly decreased the PNUE in all four

species (Figure 2H). This negative relationship is probably related to the disproportional increase of A with increasing N supply and toxic consumption, *i.e.*, A decreased while Nf increased substantially (Figures 2A, 2G and 2F).

Under low soil N supply, plants that are able to maintain high PNUE are believed to have a higher capacity to persist on low N sites (Brown 1978) and thus will also achieve greater production than those with lower PNUE on the same habitat (Schlesinger *et al.* 1989, Lambers *et al.* 1998). Generally, slow-growing species, such as some evergreen species, achieve their PNUE with a low N productivity and a high mean residence time, whereas fast-growing species, such as deciduous trees, have a considerably higher N productivity but a low mean residence time (Lambers *et al.* 1998). The results of this study support this theory. Although PNUE overlapped somewhat between species at different N treatments, it is obvious that deciduous species (trembling aspen) achieved higher overall PNUE than the conifers except at 375 and 775 ppm N, where it achieved lower PNUE than jack pine (Figure 2H). Among the conifers, jack pine had the highest PNUE at all N treatments, followed in sequential order by white spruce and black spruce. This is consistent with the fact that jack pine generally grows faster than the spruces regardless of the N availability. Black spruce was able to use N more efficiently than white spruce at low N regimes (25 and 75 ppm N), but the opposite was true in the richer N environment. This condition was reflected in A and whole seedling photosynthetic capacity (At) (Figures 2A and 2C), in which A and At in black spruce were less than that in white spruce at high N treatments. This result was in agreement with Patterson *et al.*'s (1997) conclusion that white spruce would grow faster than black spruce with increasing nutrient availability.

Growth Response

In our study, major differences in individual growth components occurred between (1) time of measurement, (2) species and (3) N treatments. In Measurement 1, aspen responded to N supply in terms of height increment and total leaf area. In Measurement 2, the growth of aspen seedlings were responsive to N addition rates up to 375 ppm in all growth components, *i.e.*, height and diameter increments, total foliage, stem and total seedling dry-mass. Seedlings at this stage of development were probably better adjusted to the experimental conditions upon Measurement 2, and therefore all tested variables showed significant responses to soil N supply. However, such trends were not as apparent in conifer seedlings

Generally, the growth components in conifer seedlings showed a lack of response to N treatments in both measurements. However, Colombo and Smith (1987) found that height and diameter growth in black spruce and jack pine seedlings correlated positively, up to a certain toxic N level, with N fertilization rate. The difference in the sensitivity to N between deciduous and coniferous species may be related to the nature of their growth capacity. Aspen has a greater potential to grow faster than the conifers and thus was able to take advantage of the increased N supply.

The results from this study suggested that aspen grew best at 75 ppm N addition rate, since highest total foliage dry-mass, height increment, stem, root and total seedling dry-mass production were achieved at this treatment upon Measurement 2. This is in spite of the fact that maximum A was achieved at 125 ppm N, and total leaf area and diameter growth at 75 ppm N were slightly less than that at 25 and 125 ppm N, respectively (Figures 5B and 5C). At the toxic N treatments (375 and 775 ppm N), growth constituents in aspen were reduced

significantly. However, this reduction was primarily due to insect damage to the foliage at high N regimes (refer to the discussion on Gas Exchange for further speculation on pest damage). The question remains: could the seedling at 375 and 775 ppm N treatments function as well as or better than that at 175 ppm N if pests were absent ?

Among the conifers, white spruce produced the greatest overall seedling dry-mass. Black spruce and jack pine were comparable in total seedling dry-mass, but they possessed different strategies in carbohydrate accumulation (see discussion on Biomass Allocation). Jack pine and black spruce seedlings grew best under the 25 and 75 ppm N regimes, and white spruce seedlings grew best under the 125 ppm N regime. More specifically, root and stem productions were greatest at 25 ppm N, whereas greatest foliage production was achieved at 75 ppm N in jack pine and black spruce. The 125 ppm N treatment enhanced all growth components in white spruce.

Biomass Allocation

Increases in N supply decreased biomass allocation to roots (*i.e.*, lower R/S ratio) in all coniferous seedlings, however the pattern of response varied with species (Figures 4 and 6). This finding was consistent with the literature (Colombo and Smith 1987, Brown 1991, Burke *et al.* 1992, Fetene *et al.* 1993, Ibrahim *et al.* 1997, Kinney and Lindroth 1997, Van Cleve and Oliver 1997, Coleman *et al.* 1998). R/S ratios in jack pine (0.17 - 0.28) were much lower for two-year-old seedlings than the values for six month-old seedlings (0.45 - 1.00) reported by Colombo and Smith (1987), while R/S ratios in black and white spruces were within the range reported by others (*e.g.*, Van den Driessche 1977, Colombo and Smith 1987 and Patterson *et al.* 1997). The differences in seedling age may be the contributing factor for

the differing R/S ratio between this study and that of Colombo and Smith (1987). R/S ratios tend to decrease as seedling age increases, since as plants age the total root biomass increases at a lower rate than the shoot biomass (Lambers *et al.* 1998).

The decrease in R/S ratio with increasing N in this study was primarily attributed to the increase in stem rather than in foliage production. This relationship reflected by the decrease in root-to-stem ratios but stable root-to-foliage ratios as N treatment increased (Figure 6). Regardless of N addition rates, white spruce allocated a higher amount of seedling biomass to the belowground biomass than did black spruce, followed by jack pine (Figure 6F). Jack pine invested most of its carbohydrate production in its foliage while black spruce invested its carbohydrate production in its root and stem (Figure 5). Despite the different strategies in their biomass allocation, as N supply decreased all conifer seedlings increased the dry-mass allocation to roots (Figure 6F) at the expense of foliage production (Figure 6D). The above discussion indicates that conifer seedlings may not thrive in excessively high soil N conditions, because the expansion of the root mass is inhibited. However, white spruce seedlings would be the least vulnerable and jack pine seedlings would be most vulnerable in such high soil N environment. Nevertheless, the above relationships did not appear to apply to trembling aspen in varying soil N supply.

The proportion of total biomass allocated to shoot and root production in trembling aspen followed an opposite pattern to that in conifers. R/S ratios (Figure 6C) and proportions of root dry-mass (Figure 6F) in aspen were consistent with Brown (1991) and Kinney and Lindroth's (1997) results. Of the three deciduous species that they examined, Kinney and Lindroth (1997) found that R/S ratio in trembling aspen responded positively to N treatment, whereas R/S ratio of sugar maple (*Acer saccharum* Marsh.) responded negatively, and red

oak (*Quercus rubra* L.) was insensitive to N treatments. As well, Brown (1991) concluded that the increase of dry matter partitioning to roots in trembling aspen was most pronounced in high-N seedlings. In Balsam Spire poplar, however, R/S ratio decreased as N addition increased (Ibrahim *et al.* 1997).

The results on aspen in this study are not conclusive because of the insect damage. But our results suggest that at very high N supply (375 and 775 ppm N), trembling aspen seedlings allocated a greater percentage of total dry-mass to the roots at the expense of foliage production. The lower allocation to foliage in aspen than in conifers, however, may simply reflect the effect of insect damage to foliage rather than the acclimation strategy of the species.

CONCLUSION

In summary, this study shows that conifer seedlings functioned best within the 25 – 125 ppm nitrogen (N) range based on gas exchange and growth parameters. Black spruce grew particularly well from 25 to 75 ppm N treatments, whereas jack pine and white spruce grew best from 75 to 125 ppm N treatments. Generally, N supply at 125 ppm or greater created luxury consumption (125 to 375 ppm) to toxic (375 to 775 ppm) conditions for the conifers. Black spruce was the most vulnerable to high N environment by showing the greatest reduction in growth, photosynthesis, transpiration and water and nitrogen-use-efficiency. Jack pine showed better capability over white spruce to persist under low N availability as well as under supraoptimal (175 ppm N) and toxic (375 and 775 ppm) N environment. Trembling aspen yielded higher biomass production within the 75 – 175 ppm soil N regime than at 25 and beyond 375 ppm N treatments. And, although the photosynthesis of aspen was insensitive to increasing N availability, the whole seedling photosynthetic capacity was relatively higher from 25 to 175 ppm N than that at 375 and 775 ppm N. At excessively high N supply, particularly at 775 ppm, aspen seedlings could barely survive, probably not because of toxic N conditions but rather because of the high vulnerability to disease and insect damages. Further research is required to confirm this explanation. Pest problems in the greenhouse, however, are very difficult to prevent, particularly in the summer season.

The results of this research raised some concerns with respect to the forest management and silvicultural practice within the Boreal forests. They are particularly useful

in dealing with the potential growth and production of Boreal tree species as they responded to variable or increasing soil and/or atmospheric nitrogen sources. The results of individual species can provide background information indicating the growth capacity of the four boreal tree species and perhaps their competitiveness when grown together under a particular N availability.

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APPENDIX A

CHEMICAL FORMULATION

Table A1. Essential nutrient formulation (without nitrogen from NH_4NO_3 source)

	Macronutrient Concentration (ppm)					
	Total-N	P	K	Ca	Mg	S
Target level	25	40	100	50	30	40
Water test (minus)	0	0	0.5	15	2.9	2
Growing medium test (minus)	0	0	6.2	3.8	4.3	0
To add	**25**	**40**	**93**	**31**	**23**	**38**

Sources of Fertilizer	**Amount Required per Treatment (mg/L)**
Microfine SuperPhosphate (20% P)	**458.30**
Muriate of Potash (62% K_2O)	**180.70**
Calcium nitrate (19% Ca, 15.5% N)	**172.22**
Epsom Salt (9.8% Mg, 12.9% S)	**234.88**
MicroMax Micronutrient mix + 12% S	**50.00**

Table A2. Nitrogen treatment formulation

Sources of Nitrogen:	Ammonium Nitrate (NH_4NO_3)	(34-0-0)	(17% NH_4, 17% NO_3)
	Calcium nitrate ($CaNO_3$)	(15.5-0-0)	(19% Ca, 15.5% N)

Target levels (NH_4NO_3 + $CaNO_3$) (ppm)	Total Nitrogen in $CaNO_3$ (ppm)	Total Nitrogen in NH_4NO_3 (ppm)	**Amount Required per Treatment (from NH_4NO_3) (mg/L)**
25	25	0	**0.00**
75	25	50	**147.06**
125	25	100	**294.12**
175	25	150	**441.08**
375	25	350	**1029.41**
775	25	750	**2205.88**

APPENDIX B

Table B1. The expected mean squares table associated with Equation 1

Source	2 R i	2 R j	6 F k	4 F l	3 R n	Expected Mean Square	Degrees of Freedom
G_i	1	2	6	4	3	$\sigma^2 + 72\sigma_{\delta'}^2 + 72\sigma_B^2 + 144\phi(B)$	1
$B_{(i)j}$	1	1	6	4	3	$\sigma^2 + 72\sigma_{\delta'}^2 + 72\sigma_B^2$	2
$\delta_{(ij)}'$	1	1	6	4	3	$\sigma^2 + 72\sigma_{\delta'}^2$	0
N_k	2	2	0	4	3	$\sigma^2 + 12\sigma_{\delta''}^2 + 12\sigma_{BN}^2 + 24\sigma_{GN}^2 + 48\phi(N)$	5
GN_{ik}	1	2	0	4	3	$\sigma^2 + 12\sigma_{\delta''}^2 + 12\sigma_{BN}^2 + 24\sigma_{GN}^2$	5
$BN_{(i)jk}$	1	1	0	4	3	$\sigma^2 + 12\sigma_{\delta''}^2 + 12\sigma_{BN}^2$	10
$\delta_{(ijk)}''$	1	1	1	4	3	$\sigma^2 + 12\sigma_{\delta''}^2$	0
S_l	2	2	6	0	3	$\sigma_\varepsilon^2 + 18\sigma_{BS}^2 + 36\sigma_{GS}^2 + 72\phi(S)$	3
GS_{il}	1	2	6	0	3	$\sigma_\varepsilon^2 + 18\sigma_{BS}^2 + 36\sigma_{GS}^2$	3
$BS_{(i)jl}$	1	1	6	0	3	$\sigma_\varepsilon^2 + 18\sigma_{BS}^2$	6
NS_{kl}	2	2	0	0	3	$\sigma_\varepsilon^2 + 3\sigma_{BNS}^2 + 6\sigma_{GNS}^2 + 12\phi(NS)$	15
GNS_{ikl}	1	2	0	0	3	$\sigma_\varepsilon^2 + 3\sigma_{BNS}^2 + 6\sigma_{GNS}^2$	15
$BNS_{(i)jkl}$	1	1	0	0	3	$\sigma_\varepsilon^2 + 3\sigma_{BNS}^2$	30
$\varepsilon_{(ijkl)m}$	1	1	1	1	1	σ_ε^2	192
Total							287

Table B2. Tests of null hypotheses associated with Equation 1.

Hypothesis	Test Statistics	F-Distribution
$\phi(G) = 0$	MS (G) / MS (δ')	No test
$\sigma_B^2 = 0$	MS (B) / MS (δ')	No test
$\sigma_{\delta'}^2 = 0$	MS (δ') / MS (N)	No test
$\phi(N) = 0$	**MS (N) / MS (BN)**	**F (5, 10)**
$\sigma_{GN}^2 = 0$	MS (GN) / MS (BN)*	F (5, 10)
$\sigma_{BN}^2 = 0$	MS (BN) / MS (δ")	No test
$\sigma_{\delta''}^2 = 0$	MS (δ") / MS (S)	No test
$\phi(S) = 0$	**MS (S) / MS (BS)**	**F (3, 6)**
$\sigma_{GS}^2 = 0$	MS (GS) / MS (BS)*	F (3, 6)
$\sigma_{BS}^2 = 0$	MS (BS) / MS (BNS)*	F (6, 30)
$\phi(NS) = 0$	**MS (NS) / MS (BNS)**	**F (15, 30)**
$\sigma_{GNS}^2 = 0$	MS (GNS) / MS (BNS)*	F (15, 30)
$\sigma_{BNS}^2 = 0$	MS (BNS) / MS (ε)	No test

* The tests for GN, GS, BS, and GNS are conservative.

APPENDIX C

ANOVA TABLES FOLIAR NITROGEN VARIABLES

Measurement 1

Dependent Variable: Nf_MASS_1

Source	DF	Sum of Squares	Mean Square	F Value	
Model	95	9946.93032	104.70453		
Error	0	.			
Corrected Total	95	9946.93032			

R-Square	C.V.	Root MSE	N_MASS Mean
1.000000	0	0	33.1159

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	794.70796	794.70796		
B(G)	2	102.32783	51.16391		
N	5	2635.51861	527.10372		
G*N	5	198.70103	39.74021		
B*N(G)	10	261.38646	26.13865		
S	3	2846.07809	948.69270		
G*S	3	67.01148	22.33716		
B*S(G)	6	307.85401	51.30900		
N*S	15	1301.48524	86.76568		
G*N*S	15	874.05625	58.27042		
B*N*S(G)	30	557.80335	18.59344	.	
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	2635.51861	527.10372	20.17	0.0001
G*N	5	198.701034	39.740207	1.52	0.2675
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	2846.07809	948.69270	18.49	0.0020
G*S	3	67.0114781	22.3371594	0.44	0.7357
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	307.854015	51.309002	2.76	0.0295
N*S	15	1301.48524	86.76568	4.67	0.0002
G*N*S	15	874.056253	58.270417	3.13	0.0038

Dependent Variable: Nf_AREA_1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	2485.18470	26.15984	2.18	0.0001
Error	192	2305.05600	12.00550		
Corrected Total	287	4790.24070			

R-Square	C.V.	Root MSE	N_MASS Mean
0.518802	87.37401	3.46490	3.96559

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	1.40421	1.40421	0.12	0.7327
B(G)	2	0.33008	0.16504	0.01	0.9863
N	5	101.36964	20.27393	1.69	0.1391
G*N	5	82.91411	16.58282	1.38	0.2330
B*N(G)	10	169.73354	16.97335	1.41	0.1765
S	3	1181.29793	393.76598	32.80	0.0001
G*S	3	51.96185	17.32062	1.44	0.2317
B*S(G)	6	38.62745	6.43791	0.54	0.7803
N*S	15	306.87353	20.45824	1.70	0.0528
G*N*S	15	168.93479	11.26232	0.94	0.5230
B*N*S(G)	30	381.73758	12.72459	1.06	0.3905
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	101.369639	20.273928	1.19	0.3779
G*N	5	82.9141059	16.5828212	0.98	0.4767
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	1181.29793	393.76598	61.16	0.0001
G*S	3	51.9618510	17.3206170	2.69	0.1396
B*S(G)	6	38.6274521	6.4379087	0.51	0.7989
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
N*S	15	306.873526	20.458235	1.61	0.1307
G*N*S	15	168.934793	11.262320	0.89	0.5862

APPENDIX D

ANOVA TABLES FOR GAS EXCHANGE AND FOLIAR NITROGEN VARIABLES

Measurement 2

Dependent Variable: A_2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	3863.36481	40.66700	11.37	0.0001
Error	192	686.91513	3.57768		
Corrected Total	287	4550.27995			

R-Square	C.V.	Root MSE	A Mean
0.849039	23.42114	1.89148	8.07594

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	16.728292	16.728292	4.68	0.0318
B(G)	2	39.097551	19.548775	5.46	0.0049
N	5	778.761241	155.752248	43.53	0.0001
G*N	5	58.435560	11.687112	3.27	0.0074
B*N(G)	10	109.152378	10.915238	3.05	0.0013
S	3	913.206023	304.402008	85.08	0.0001
G*S	3	370.720740	123.573580	34.54	0.0001
B*S(G)	6	559.771724	93.295287	26.08	0.0001
N*S	15	359.880325	23.992022	6.71	0.0001
G*N*S	15	204.706150	13.647077	3.81	0.0001
B*N*S(G)	30	452.904830	15.096828	4.22	0.0001
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	778.761241	155.752248	14.27	0.0003
G*N	5	58.4355601	11.6871120	1.07	0.4313
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	913.206023	304.402008	3.26	0.1014
G*S	3	370.720740	123.573580	1.32	0.3509
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	559.771724	93.295287	6.18	0.0003
N*S	15	359.880325	23.992022	1.59	0.1365
G*N*S	15	204.706150	13.647077	0.90	0.5682

Dependent Variable: Atotal_2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	26177.4800	275.5524	6.94	0.0001
Error	192	7617.9709	39.6769		
Corrected Total	287	33795.4509			

R-Square	C.V.	Root MSE	ATOTAL Mean
0.774586	77.96450	6.29896	8.07927

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	794.3102	794.3102	20.02	0.0001
B(G)	2	69.1326	34.5663	0.87	0.4201
N	5	2309.2308	461.8462	11.64	0.0001
G*N	5	166.2354	33.2471	0.84	0.5242
B*N(G)	10	900.0878	90.0088	2.27	0.0157
S	3	14868.0714	4956.0238	124.91	0.0001
G*S	3	1102.4005	367.4668	9.26	0.0001
B*S(G)	6	396.4471	66.0745	1.67	0.1315
N*S	15	3295.9921	219.7328	5.54	0.0001
G*N*S	15	368.5661	24.5711	0.62	0.8571
B*N*S(G)	30	1907.0059	63.5669	1.60	0.0316
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	2309.23076	461.84615	5.13	0.0137
G*N	5	166.235420	33.247084	0.37	0.8584
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	14868.0714	4956.0238	75.01	0.0001
G*S	3	1102.40051	367.46684	5.56	0.0362
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	396.447055	66.074509	1.04	0.4198
N*S	15	3295.99215	219.73281	3.46	0.0019
G*N*S	15	368.566145	24.571076	0.39	0.9725

Dependent Variable: E_2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	141.524528	1.489732	7.76	0.0001
Error	192	36.879267	0.192080		
Corrected Total	287	178.403794			

R-Square	C.V.	Root MSE	E Mean
0.793282	24.14519	0.43827	1.81514

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.3055014	0.3055014	1.59	0.2088
B(G)	2	4.2817681	2.1408840	11.15	0.0001
N	5	20.8193278	4.1638656	21.68	0.0001
G*N	5	4.0441319	0.8088264	4.21	0.0012
B*N(G)	10	6.7802153	0.6780215	3.53	0.0003
S	3	17.5117861	5.8372620	30.39	0.0001
G*S	3	14.8220569	4.9406856	25.72	0.0001
B*S(G)	6	26.2173597	4.3695600	22.75	0.0001
N*S	15	20.6436472	1.3762431	7.16	0.0001
G*N*S	15	6.7405097	0.4493673	2.34	0.0043
B*N*S(G)	30	19.3582236	0.6452741	3.36	0.0001
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	20.8193278	4.1638656	6.14	0.0074
G*N	5	4.04413194	0.80882639	1.19	0.3785
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	17.5117861	5.8372620	1.34	0.3479
G*S	3	14.8220569	4.9406856	1.13	0.4089
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	26.2173597	4.3695600	6.77	0.0001
N*S	15	20.6436472	1.3762431	2.13	0.0378
G*N*S	15	6.74050972	0.44936731	0.70	0.7680

Dependent Variable: OPSII_2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	11.0679747	0.1165050	13.79	0.0001
Error	192	1.6215333	0.0084455		
Corrected Total	287	12.6895080			

R-Square	C.V.	Root MSE	OPSII Mean
0.872215	17.10971	0.09190	0.53712

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.0437587	0.0437587	5.18	0.0239
B(G)	2	0.0156424	0.0078212	0.93	0.3979
N	5	0.1065809	0.0213162	2.52	0.0307
G*N	5	0.0419642	0.0083928	0.99	0.4228
B*N(G)	10	0.0535368	0.0053537	0.63	0.7837
S	3	10.0496344	3.3498781	396.65	0.0001
G*S	3	0.0800844	0.0266948	3.16	0.0258
B*S(G)	6	0.1245604	0.0207601	2.46	0.0259
N*S	15	0.1567677	0.0104512	1.24	0.2467
G*N*S	15	0.1308344	0.0087223	1.03	0.4233
B*N*S(G)	30	0.2646104	0.0088203	1.04	0.4112
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	0.10658090	0.02131618	3.98	0.0301
G*N	5	0.04196424	0.00839285	1.57	0.2546
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	10.0496344	3.3498781	161.36	0.0001
G*S	3	0.08008437	0.02669479	1.29	0.3616
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	0.12456042	0.02076007	2.35	0.0555
N*S	15	0.15676771	0.01045118	1.18	0.3342
G*N*S	15	0.13083437	0.00872229	0.99	0.4899

Dependent Variable: Nf_MASS_2

Source	DF	Sum of Squares	Mean Square	F Value	
Model	95	10874.6314	114.4698		
Error	0	.			
Corrected Total	95	10874.6314			

R-Square	C.V.	Root MSE	N_MASS Mean
1.000000	0	0	31.1175

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	281.32954	281.32954		
B(G)	2	93.57262	46.78631		
N	5	4048.16811	809.63362		
G*N	5	115.12753	23.02551		
B*N(G)	10	287.68295	28.76830		
S	3	4281.72719	1427.24240		
G*S	3	54.50514	18.16838		
B*S(G)	6	118.79438	19.79906		
N*S	15	1096.18305	73.07887		
G*N*S	15	169.45365	11.29691		
B*N*S(G)	30	328.08725	10.93624	.	
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	4048.16811	809.63362	28.14	0.0001
G*N	5	115.127525	23.025505	0.80	0.5740
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	4281.72719	1427.24240	72.09	0.0001
G*S	3	54.5051375	18.1683792	0.92	0.4868
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	118.794379	19.799063	1.81	0.1307
N*S	15	1096.18305	73.07887	6.68	0.0001
G*N*S	15	169.453650	11.296910	1.03	0.4515

Dependent Variable: Nf_AREA_2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	653.622699	6.880239	42.90	0.0001
Error	192	30.792533	0.160378		
Corrected Total	287	684.415232			

R-Square	C.V.	Root MSE	N_MASS Mean
0.955009	11.26261	0.40047	3.55576

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.657422	0.657422	4.10	0.0443
B(G)	2	7.549524	3.774762	23.54	0.0001
N	5	158.324628	31.664926	197.44	0.0001
G*N	5	4.972815	0.994563	6.20	0.0001
B*N(G)	10	8.028626	0.802863	5.01	0.0001
S	3	398.994613	132.998204	829.28	0.0001
G*S	3	1.749025	0.583008	3.64	0.0139
B*S(G)	6	4.571260	0.761877	4.75	0.0002
N*S	15	36.020008	2.401334	14.97	0.0001
G*N*S	15	9.852621	0.656841	4.10	0.0001
B*N*S(G)	30	22.902157	0.763405	4.76	0.0001
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	158.324628	31.664926	39.44	0.0001
G*N	5	4.97281528	0.99456306	1.24	0.3604
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	398.994613	132.998204	174.57	0.0001
G*S	3	1.74902500	0.58300833	0.77	0.5537
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	4.57125972	0.76187662	1.00	0.4447
N*S	15	36.0200083	2.4013339	3.15	0.0037
G*N*S	15	9.85262083	0.65684139	0.86	0.6100

Dependent Variable: PNUE_2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	620.714005	6.533832	5.70	0.0001
Error	192	219.985067	1.145756		
Corrected Total	287	840.699072			

R-Square	C.V.	Root MSE	PNUE Mean
0.738331	39.11773	1.07040	2.73635

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	1.184517	1.184517	1.03	0.3105
B(G)	2	4.170862	2.085431	1.82	0.1648
N	5	224.951116	44.990223	39.27	0.0001
G*N	5	10.605693	2.121139	1.85	0.1047
B*N(G)	10	6.236259	0.623626	0.54	0.8570
S	3	123.800490	41.266830	36.02	0.0001
G*S	3	50.297529	16.765843	14.63	0.0001
B*S(G)	6	63.832891	10.638815	9.29	0.0001
N*S	15	36.808375	2.453892	2.14	0.0097
G*N*S	15	41.637036	2.775802	2.42	0.0030
B*N*S(G)	30	57.189238	1.906308	1.66	0.0222
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	224.951116	44.990223	72.14	0.0001
G*N	5	10.6056934	2.1211387	3.40	0.0470
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	123.800490	41.266830	3.88	0.0743
G*S	3	50.2975288	16.7658429	1.58	0.2904
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	63.8328910	10.6388152	5.58	0.0006
N*S	15	36.8083747	2.4538916	1.29	0.2692
G*N*S	15	41.6370358	2.7758024	1.46	0.1852

Dependent Variable: PWUE_2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	670.723322	7.060245	13.51	0.0001
Error	192	100.327400	0.522539		
Corrected Total	287	771.050722			

R-Square	C.V.	Root MSE	PWUE Mean
0.869882	15.43113	0.72287	4.68448

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	1.156467	1.156467	2.21	0.1385
B(G)	2	8.811253	4.405627	8.43	0.0003
N	5	7.349120	1.469824	2.81	0.0178
G*N	5	4.733689	0.946738	1.81	0.1123
B*N(G)	10	18.246001	1.824600	3.49	0.0003
S	3	512.681459	170.893820	327.05	0.0001
G*S	3	10.361182	3.453727	6.61	0.0003
B*S(G)	6	2.418388	0.403065	0.77	0.5933
N*S	15	43.725301	2.915020	5.58	0.0001
G*N*S	15	21.662620	1.444175	2.76	0.0007
B*N*S(G)	30	39.577841	1.319261	2.52	0.0001
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	7.34911979	1.46982396	0.81	0.5709
G*N	5	4.73368924	0.94673785	0.52	0.7571
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	512.681459	170.893820	423.99	0.0001
G*S	3	10.3611816	3.4537272	8.57	0.0137
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	2.41838819	0.40306470	0.31	0.9291
N*S	15	43.7253010	2.9150201	2.21	0.0315
G*N*S	15	21.6626205	1.4441747	1.09	0.4009

APPENDIX E

ANOVA AND ANCOVA TABLES FOR GROWTH VARIABLES

Measurement 1

Dependent Variable: HEIGHT_1 (cm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	96	174028.006	1812.792	49.86	0.0001
Error	191	6944.392	36.358		
Corrected Total	287	180972.399			

R-Square	C.V.	Root MSE	HT1 Mean
0.961627	26.80144	6.02977	22.4979

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	2730.0888	2730.0888	75.09	0.0001
B(G)	2	15.2068	7.6034	0.21	0.8115
N	5	768.2195	153.6439	4.23	0.0011
G*N	5	288.4446	57.6889	1.59	0.1656
B*N(G)	10	455.6374	45.5637	1.25	0.2598
S	3	16836.3228	5612.1076	154.36	0.0001
G*S	3	3777.4035	1259.1345	34.63	0.0001
B*S(G)	6	83.0648	13.8441	0.38	0.8907
N*S	15	2033.0860	135.5391	3.73	0.0001
G*N*S	15	742.5167	49.5011	1.36	0.1699
B*N*S(G)	30	2231.3440	74.3781	2.05	0.0021
HT0	1	136.9875	136.9875	3.77	0.0537
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	768.219548	153.643910	3.37	0.0482
G*N	5	288.444553	57.688911	1.27	0.3500
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	16836.3228	5612.1076	405.38	0.0001
G*S	3	3777.40355	1259.13452	90.95	0.0001
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	83.0648308	13.8441385	0.19	0.9784
N*S	15	2033.08599	135.53907	1.82	0.0790
G*N*S	15	742.516694	49.501113	0.67	0.7960

Dependent Variable: DIAMETER_1 (mm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	96	330.214361	3.439733	11.91	0.0001
Error	191	55.164947	0.288822		
Corrected Total	287	385.379308			

R-Square	C.V.	Root MSE	DIA1 Mean
0.856855	39.11383	0.53742	1.37399

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	29.878644	29.878644	103.45	0.0001
B(G)	2	1.338583	0.669292	2.32	0.1013
N	5	2.540103	0.508021	1.76	0.1232
G*N	5	1.593165	0.318633	1.10	0.3601
B*N(G)	10	7.337215	0.733721	2.54	0.0067
S	3	226.031290	75.343763	260.87	0.0001
G*S	3	10.930079	3.643360	12.61	0.0001
B*S(G)	6	3.286889	0.547815	1.90	0.0833
N*S	15	15.140985	1.009399	3.49	0.0001
G*N*S	15	5.382259	0.358817	1.24	0.2433
B*N*S(G)	30	18.963771	0.632126	2.19	0.0008
DIA0	1	5.691253	5.691253	19.71	0.0001
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	2.54010278	0.50802056	0.69	0.6409
G*N	5	1.59316525	0.31863305	0.43	0.8151
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	226.031290	75.343763	137.54	0.0001
G*S	3	10.9300788	3.6433596	6.65	0.0246
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	3.28688893	0.54781482	0.87	0.5305
N*S	15	15.1409846	1.0093990	1.60	0.1341
G*N*S	15	5.38225911	0.35881727	0.57	0.8764

Dependent Variable: TOTAL SEEDLING WEIGHT_1 (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	9525.09138	100.26412	28.49	0.0001
Error	192	675.64967	3.51901		
Corrected Total	287	10200.74104			

R-Square	C.V.	Root MSE	TOTWT Mean
0.933765	31.27227	1.87590	5.99861

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	459.39857	459.39857	130.55	0.0001
B(G)	2	8.17596	4.08798	1.16	0.3151
N	5	78.73615	15.74723	4.47	0.0007
G*N	5	32.62216	6.52443	1.85	0.1042
B*N(G)	10	109.11137	10.91114	3.10	0.0011
S	3	7315.03387	2438.34462	692.91	0.0001
G*S	3	739.88015	246.62672	70.08	0.0001
B*S(G)	6	10.46188	1.74365	0.50	0.8113
N*S	15	240.09454	16.00630	4.55	0.0001
G*N*S	15	127.06451	8.47097	2.41	0.0032
B*N*S(G)	30	404.51222	13.48374	3.83	0.0001
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	78.7361486	15.7472297	1.44	0.2901
G*N	5	32.6221611	6.5244322	0.60	0.7031
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	7315.03387	2438.34462	1398.42	0.0001
G*S	3	739.880146	246.626715	141.44	0.0001
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	10.4618764	1.7436461	0.13	0.9916
N*S	15	240.094543	16.006303	1.19	0.3327
G*N*S	15	127.064508	8.470967	0.63	0.8284

Dependent Variable: TLA_1 (cm^2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	23465.3249	247.0034	26.41	0.0001
Error	192	1795.9057	9.3537		
Corrected Total	287	25261.2306			

R-Square	C.V.	Root MSE	LNEWAREA Mean
0.928907	55.62162	3.05838	5.49854

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	218.9627	218.9627	23.41	0.0001
B(G)	2	7.5682	3.7841	0.40	0.6678
N	5	219.3467	43.8693	4.69	0.0005
G*N	5	70.5242	14.1048	1.51	0.1891
B*N(G)	10	199.4357	19.9436	2.13	0.0238
S	3	20721.5686	6907.1895	738.45	0.0001
G*S	3	533.2722	177.7574	19.00	0.0001
B*S(G)	6	20.3367	3.3894	0.36	0.9020
N*S	15	635.8249	42.3883	4.53	0.0001
G*N*S	15	231.0532	15.4035	1.65	0.0649
B*N*S(G)	30	607.4320	20.2477	2.16	0.0010
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	219.346675	43.869335	2.20	0.1352
G*N	5	70.5242236	14.1048447	0.71	0.6314
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	20721.5686	6907.1895	2037.85	0.0001
G*S	3	533.272186	177.757395	52.44	0.0001
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	20.3366542	3.3894424	0.17	0.9836
N*S	15	635.824883	42.388326	2.09	0.0415
G*N*S	15	231.053168	15.403545	0.76	0.7070

APPENDIX F

ANOVA AND ANCOVA TABLES FOR GROWTH VARIABLES

Measurement 2

Dependent Variable: HEIGHT_2 (cm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	96	258255.944	2690.166	63.66	0.0001
Error	191	8071.893	42.261		
Corrected Total	287	266327.837			

R-Square	C.V.	Root MSE	HT2 Mean
0.969692	24.02968	6.50086	27.0535

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	1912.3964	1912.3964	45.25	0.0001
B(G)	2	176.6333	88.3167	2.09	0.1265
N	5	2932.7785	586.5557	13.88	0.0001
G*N	5	637.2394	127.4479	3.02	0.0121
B*N(G)	10	701.2541	70.1254	1.66	0.0929
S	3	31342.9562	10447.6521	247.22	0.0001
G*S	3	1635.4816	545.1605	12.90	0.0001
B*S(G)	6	409.8867	68.3144	1.62	0.1445
N*S	15	7583.0615	505.5374	11.96	0.0001
G*N*S	15	766.8495	51.1233	1.21	0.2671
B*N*S(G)	30	2823.9120	94.1304	2.23	0.0006
HT0	1	607.0472	607.0472	14.36	0.0002
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	2932.77847	586.55569	8.36	0.0024
G*N	5	637.239396	127.447879	1.82	0.1970
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	31342.9562	10447.6521	152.93	0.0001
G*S	3	1635.48161	545.16054	7.98	0.0162
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	409.886689	68.314448	0.73	0.6323
N*S	15	7583.06152	505.53743	5.37	0.0001
G*N*S	15	766.849545	51.123303	0.54	0.8938

Dependent Variable: DIAMETER_2 (mm)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	96	452.849915	4.717187	16.02	0.0001
Error	191	56.238385	0.294442		
Corrected Total	287	509.088300			

R-Square	C.V.	Root MSE	DIA2 Mean
0.889531	20.63240	0.54262	2.62997

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	45.151870	45.151870	153.35	0.0001
B(G)	2	2.362946	1.181473	4.01	0.0196
N	5	10.283219	2.056644	6.98	0.0001
G*N	5	4.227075	0.845415	2.87	0.0159
B*N(G)	10	9.412579	0.941258	3.20	0.0008
S	3	278.311759	92.770586	315.07	0.0001
G*S	3	0.898632	0.299544	1.02	0.3861
B*S(G)	6	3.748929	0.624822	2.12	0.0526
N*S	15	35.309471	2.353965	7.99	0.0001
G*N*S	15	9.565781	0.637719	2.17	0.0088
B*N*S(G)	30	35.159111	1.171970	3.98	0.0001
DIA0	1	6.901482	6.901482	23.44	0.0001
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	10.2832189	2.0566438	2.18	0.1371
G*N	5	4.22707463	0.84541493	0.90	0.5182
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	278.311759	92.770586	148.48	0.0001
G*S	3	0.89863226	0.29954409	0.48	0.7084
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	3.74892933	0.62482155	0.53	0.7787
N*S	15	35.3094714	2.3539648	2.01	0.0507
G*N*S	15	9.56578126	0.63771875	0.54	0.8931

Dependent Variable: TLA_2 (cm^2)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	6112.67384	64.34394	13.40	0.0001
Error	192	921.77273	4.80090		
Corrected Total	287	7034.44658			

R-Square	C.V.	Root MSE	LNEWAREA Mean
0.868963	62.60770	2.19110	3.49972

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	23.72457	23.72457	4.94	0.0274
B(G)	2	0.12580	0.06290	0.01	0.9870
N	5	293.42238	58.68448	12.22	0.0001
G*N	5	19.59731	3.91946	0.82	0.5393
B*N(G)	10	76.77961	7.67796	1.60	0.1092
S	3	4643.77305	1547.92435	322.42	0.0001
G*S	3	10.41222	3.47074	0.72	0.5394
B*S(G)	6	4.49432	0.74905	0.16	0.9877
N*S	15	795.22867	53.01524	11.04	0.0001
G*N*S	15	57.31733	3.82116	0.80	0.6813
B*N*S(G)	30	187.79860	6.25995	1.30	0.1466
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	293.422378	58.684476	7.64	0.0034
G*N	5	19.5973069	3.9194614	0.51	0.7628
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	4643.77305	1547.92435	2066.51	0.0001
G*S	3	10.4122153	3.4707384	4.63	0.0527
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	4.49432083	0.74905347	0.12	0.9932
N*S	15	795.228667	53.015244	8.47	0.0001
G*N*S	15	57.3173264	3.8211551	0.61	0.8432

Dependent Variable: FOLIAGE WEIGHT_2 (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	851.078588	8.958722	5.31	0.0001
Error	192	323.887600	1.686915		
Corrected Total	287	1174.966187			

R-Square	C.V.	Root MSE	LTOTWT Mean
0.724343	42.37274	1.29881	3.06521

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	81.642901	81.642901	48.40	0.0001
B(G)	2	1.270514	0.635257	0.38	0.6867
N	5	55.560317	11.112063	6.59	0.0001
G*N	5	21.951219	4.390244	2.60	0.0265
B*N(G)	10	47.723786	4.772379	2.83	0.0027
S	3	390.167401	130.055800	77.10	0.0001
G*S	3	20.991779	6.997260	4.15	0.0071
B*S(G)	6	11.633681	1.938947	1.15	0.3354
N*S	15	122.818136	8.187876	4.85	0.0001
G*N*S	15	43.109033	2.873936	1.70	0.0528
B*N*S(G)	30	54.209819	1.806994	1.07	0.3758
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	55.5603167	11.1120633	2.33	0.1196
G*N	5	21.9512194	4.3902439	0.92	0.5064
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	390.167401	130.055800	67.08	0.0001
G*S	3	20.9917792	6.9972597	3.61	0.0848
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	11.6336806	1.9389468	1.07	0.4004
N*S	15	122.818136	8.187876	4.53	0.0002
G*N*S	15	43.1090333	2.8739356	1.59	0.1361

Dependent Variable: STEM WEIGHT_2 (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	3969.21360	41.78120	24.32	0.0001
Error	192	329.86567	1.71805		
Corrected Total	287	4299.07927			

R-Square	C.V.	Root MSE	STEMWT Mean
0.923271	33.03992	1.31074	3.96715

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	154.05976	154.05976	89.67	0.0001
B(G)	2	2.99309	1.49655	0.87	0.4201
N	5	70.49451	14.09890	8.21	0.0001
G*N	5	22.14088	4.42818	2.58	0.0278
B*N(G)	10	35.20738	3.52074	2.05	0.0305
S	3	3248.08956	1082.69652	630.19	0.0001
G*S	3	126.07238	42.02413	24.46	0.0001
B*S(G)	6	9.36433	1.56072	0.91	0.4899
N*S	15	178.25858	11.88391	6.92	0.0001
G*N*S	15	52.78780	3.51919	2.05	0.0141
B*N*S(G)	30	69.74534	2.32484	1.35	0.1163
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	70.4945111	14.0989022	4.00	0.0296
G*N	5	22.1408819	4.4281764	1.26	0.3532
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	3248.08956	1082.69652	693.72	0.0001
G*S	3	126.072378	42.024126	26.93	0.0007
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	9.36432639	1.56072106	0.67	0.6735
N*S	15	178.258578	11.883905	5.11	0.0001
G*N*S	15	52.7878014	3.5191868	1.51	0.1624

Dependent Variable: SHOOT WEIGHT_2 (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	7537.31762	79.34019	14.48	0.0001
Error	192	1051.72813	5.47775		
Corrected Total	287	8589.04575			

R-Square	C.V.	Root MSE	SHWT Mean
0.877550	33.28654	2.34046	7.03125

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	459.85336	459.85336	83.95	0.0001
B(G)	2	7.56294	3.78147	0.69	0.5026
N	5	225.26738	45.05348	8.22	0.0001
G*N	5	60.18782	12.03756	2.20	0.0562
B*N(G)	10	139.13015	13.91301	2.54	0.0067
S	3	5591.91199	1863.97066	340.28	0.0001
G*S	3	227.54326	75.84775	13.85	0.0001
B*S(G)	6	41.41521	6.90254	1.26	0.2777
N*S	15	511.07197	34.07146	6.22	0.0001
G*N*S	15	127.80014	8.52001	1.56	0.0896
B*N*S(G)	30	145.57340	4.85245	0.89	0.6410
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	225.267379	45.053476	3.24	0.0537
G*N	5	60.1878236	12.0375647	0.87	0.5365
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	5591.91199	1863.97066	270.04	0.0001
G*S	3	227.543264	75.847755	10.99	0.0075
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	41.4152111	6.9025352	1.42	0.2388
N*S	15	511.071968	34.071465	7.02	0.0001
G*N*S	15	127.800140	8.520009	1.76	0.0924

Dependent Variable: ROOT WEIGHT_2 (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	1235.72173	13.00760	8.16	0.0001
Error	192	305.98673	1.59368		
Corrected Total	287	1541.70846			

R-Square	C.V.	Root MSE	ROOTWT Mean
0.801527	55.56861	1.26241	2.27181

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	62.347222	62.347222	39.12	0.0001
B(G)	2	2.291211	1.145606	0.72	0.4886
N	5	31.269390	6.253878	3.92	0.0021
G*N	5	7.711774	1.542355	0.97	0.4387
B*N(G)	10	32.036297	3.203630	2.01	0.0342
S	3	916.234325	305.411442	191.64	0.0001
G*S	3	52.787981	17.595994	11.04	0.0001
B*S(G)	6	5.547967	0.924661	0.58	0.7459
N*S	15	32.774196	2.184946	1.37	0.1649
G*N*S	15	22.138040	1.475869	0.93	0.5362
B*N*S(G)	30	70.583325	2.352778	1.48	0.0626
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	31.2693903	6.2538781	1.95	0.1722
G*N	5	7.71177361	1.54235472	0.48	0.7828
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	916.234325	305.411442	330.30	0.0001
G*S	3	52.7879806	17.5959935	19.03	0.0018
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	5.54796667	0.92466111	0.39	0.8776
N*S	15	32.7741958	2.1849464	0.93	0.5449
G*N*S	15	22.1380403	1.4758694	0.63	0.8292

Dependent Variable: TOTAL SEEDLING WEIGHT_2 (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	14577.3958	153.4463	13.87	0.0001
Error	192	2124.7590	11.0665		
Corrected Total	287	16702.1548			

R-Square	C.V.	Root MSE	TOTWT Mean
0.872785	35.75898	3.32663	9.30292

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	860.4335	860.4335	77.75	0.0001
B(G)	2	17.0849	8.5425	0.77	0.4636
N	5	416.5336	83.3067	7.53	0.0001
G*N	5	107.6669	21.5334	1.95	0.0886
B*N(G)	10	275.1611	27.5161	2.49	0.0080
S	3	10988.9845	3662.9948	331.00	0.0001
G*S	3	490.2495	163.4165	14.77	0.0001
B*S(G)	6	72.1732	12.0289	1.09	0.3716
N*S	15	771.2005	51.4134	4.65	0.0001
G*N*S	15	248.6943	16.5796	1.50	0.1089
B*N*S(G)	30	329.2138	10.9738	0.99	0.4850
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	416.533563	83.306713	3.03	0.0640
G*N	5	107.666882	21.533376	0.78	0.5846
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	10988.9845	3662.9948	304.52	0.0001
G*S	3	490.249508	163.416503	13.59	0.0044
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	72.1732111	12.0288685	1.10	0.3874
N*S	15	771.200457	51.413364	4.69	0.0002
G*N*S	15	248.694304	16.579620	1.51	0.1635

APPENDIX G

ANOVA TABLES FOR BIOMASS ALLOCATION VARIABLES

Measurement 1

Dependent Variable: ROOT/FOLIAGE_1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	5.17666667	0.05449123	5.28	0.0001
Error	192	1.98233333	0.01032465		
Corrected Total	287	7.15900000			

R-Square	C.V.	Root MSE	RS_TOTLF Mean
0.723099	24.28931	0.10161	0.41833

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.03042222	0.03042222	2.95	0.0877
B(G)	2	0.02520556	0.01260278	1.22	0.2973
N	5	0.23425417	0.04685083	4.54	0.0006
G*N	5	0.16579028	0.03315806	3.21	0.0083
B*N(G)	10	0.13516111	0.01351611	1.31	0.2279
S	3	3.32533889	1.10844630	107.36	0.0001
G*S	3	0.28098333	0.09366111	9.07	0.0001
B*S(G)	6	0.03486111	0.00581019	0.56	0.7596
N*S	15	0.27524028	0.01834935	1.78	0.0403
G*N*S	15	0.21887083	0.01459139	1.41	0.1441
B*N*S(G)	30	0.45053889	0.01501796	1.45	0.0701
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	0.23425417	0.04685083	3.47	0.0447
G*N	5	0.16579028	0.03315806	2.45	0.1065
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	3.32533889	1.10844630	190.78	0.0001
G*S	3	0.28098333	0.09366111	16.12	0.0028
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	0.03486111	0.00581019	0.39	0.8816
N*S	15	0.27524028	0.01834935	1.22	0.3094
G*N*S	15	0.21887083	0.01459139	0.97	0.5054

Dependent Variable: ROOT/SHOOT_1

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	1.29888750	0.01367250	4.63	0.0001
Error	192	0.56740000	0.00295521		
Corrected Total	287	1.86628750			

R-Square	C.V.	Root MSE	RS_TOT Mean
0.695974	22.09456	0.05436	0.24604

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.00001250	0.00001250	0.00	0.9482
B(G)	2	0.00566944	0.00283472	0.96	0.3850
N	5	0.05936667	0.01187333	4.02	0.0017
G*N	5	0.05894167	0.01178833	3.99	0.0018
B*N(G)	10	0.03996389	0.00399639	1.35	0.2054
S	3	0.76059306	0.25353102	85.79	0.0001
G*S	3	0.09952639	0.03317546	11.23	0.0001
B*S(G)	6	0.01361944	0.00226991	0.77	0.5959
N*S	15	0.06759444	0.00450630	1.52	0.0995
G*N*S	15	0.07828611	0.00521907	1.77	0.0420
B*N*S(G)	30	0.11531389	0.00384380	1.30	0.1488
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	0.05936667	0.01187333	2.97	0.0672
G*N	5	0.05894167	0.01178833	2.95	0.0684
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	0.76059306	0.25353102	111.69	0.0001
G*S	3	0.09952639	0.03317546	14.62	0.0036
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	0.01361944	0.00226991	0.59	0.7352
N*S	15	0.06759444	0.00450630	1.17	0.3430
G*N*S	15	0.07828611	0.00521907	1.36	0.2307

Dependent Variable: FOLIAGE_1 (%)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	1.12476908	0.01183967	5.44	0.0001
Error	192	0.41794733	0.00217681		
Corrected Total	287	1.54271641			

R-Square	C.V.	Root MSE	FOLIAGE Mean
0.729083	9.654342	0.04666	0.48327

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.03425653	0.03425653	15.74	0.0001
B(G)	2	0.01018276	0.00509138	2.34	0.0992
N	5	0.02810685	0.00562137	2.58	0.0275
G*N	5	0.00811007	0.00162201	0.75	0.5906
B*N(G)	10	0.02142328	0.00214233	0.98	0.4586
S	3	0.70282834	0.23427611	107.62	0.0001
G*S	3	0.07249618	0.02416539	11.10	0.0001
B*S(G)	6	0.02470399	0.00411733	1.89	0.0841
N*S	15	0.05005397	0.00333693	1.53	0.0967
G*N*S	15	0.06440130	0.00429342	1.97	0.0190
B*N*S(G)	30	0.10820580	0.00360686	1.66	0.0231
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	0.02810685	0.00562137	2.62	0.0911
G*N	5	0.00811007	0.00162201	0.76	0.6001
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	0.70282834	0.23427611	56.90	0.0001
G*S	3	0.07249618	0.02416539	5.87	0.0323
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	0.02470399	0.00411733	1.14	0.3630
N*S	15	0.05005397	0.00333693	0.93	0.5482
G*N*S	15	0.06440130	0.00429342	1.19	0.3305

Dependent Variable: ROOT_1 (%)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	0.68716244	0.00723329	2.68	0.0001
Error	192	0.51896333	0.00270293		
Corrected Total	287	1.20612578			

R-Square	C.V.	Root MSE	ROOT Mean
0.569727	26.45415	0.05199	0.19653

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.00162450	0.00162450	0.60	0.4391
B(G)	2	0.01131947	0.00565974	2.09	0.1260
N	5	0.02637894	0.00527579	1.95	0.0876
G*N	5	0.01983375	0.00396675	1.47	0.2022
B*N(G)	10	0.03808228	0.00380823	1.41	0.1787
S	3	0.31999758	0.10666586	39.46	0.0001
G*S	3	0.01841403	0.00613801	2.27	0.0817
B*S(G)	6	0.02790097	0.00465016	1.72	0.1181
N*S	15	0.04191858	0.00279457	1.03	0.4222
G*N*S	15	0.07215839	0.00481056	1.78	0.0399
B*N*S(G)	30	0.10953394	0.00365113	1.35	0.1176
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	0.02637894	0.00527579	1.39	0.3084
G*N	5	0.01983375	0.00396675	1.04	0.4449
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	0.31999758	0.10666586	22.94	0.0011
G*S	3	0.01841403	0.00613801	1.32	0.3522
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	0.02790097	0.00465016	1.27	0.2989
N*S	15	0.04191858	0.00279457	0.77	0.7025
G*N*S	15	0.07215839	0.00481056	1.32	0.2521

APPENDIX H

ANOVA TABLES FOR BIOMASS ALLOCATION VARIABLES

Measurement 2

Dependent Variable: ROOT/FOLIAGE_2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	104.425132	1.099212	4.12	0.0001
Error	192	51.280067	0.267084		
Corrected Total	287	155.705199			

R-Square	C.V.	Root MSE	RS_TOTLF Mean
0.670659	67.22013	0.51680	0.76882

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.4140500	0.4140500	1.55	0.2146
B(G)	2	0.8388014	0.4194007	1.57	0.2106
N	5	3.2584778	0.6516956	2.44	0.0359
G*N	5	1.0021958	0.2004392	0.75	0.5867
B*N(G)	10	5.2831236	0.5283124	1.98	0.0376
S	3	49.2025125	16.4008375	61.41	0.0001
G*S	3	0.8142472	0.2714157	1.02	0.3866
B*S(G)	6	3.1141097	0.5190183	1.94	0.0758
N*S	15	19.9429750	1.3295317	4.98	0.0001
G*N*S	15	2.4144403	0.1609627	0.60	0.8707
B*N*S(G)	30	18.1401986	0.6046733	2.26	0.0005
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	3.25847778	0.65169556	1.23	0.3624
G*N	5	1.00219583	0.20043917	0.38	0.8518
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	49.2025125	16.4008375	31.60	0.0005
G*S	3	0.81424722	0.27141574	0.52	0.6822
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	3.11410972	0.51901829	0.86	0.5363
N*S	15	19.9429750	1.3295317	2.20	0.0323
G*N*S	15	2.41444028	0.16096269	0.27	0.9955

Dependent Variable: ROOT/STEM_2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	7.71575000	0.08121842	4.04	0.0001
Error	192	3.85780000	0.02009271		
Corrected Total	287	11.57355000			

R-Square	C.V.	Root MSE	RS_STEM Mean
0.666671	22.90889	0.14175	0.61875

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.00568889	0.00568889	0.28	0.5953
B(G)	2	0.05445556	0.02722778	1.36	0.2604
N	5	1.01683750	0.20336750	10.12	0.0001
G*N	5	0.06699861	0.01339972	0.67	0.6490
B*N(G)	10	0.26721944	0.02672194	1.33	0.2168
S	3	4.70292222	1.56764074	78.02	0.0001
G*S	3	0.14658333	0.04886111	2.43	0.0664
B*S(G)	6	0.11615556	0.01935926	0.96	0.4512
N*S	15	0.60525694	0.04035046	2.01	0.0165
G*N*S	15	0.11676250	0.00778417	0.39	0.9811
B*N*S(G)	30	0.61686944	0.02056231	1.02	0.4400
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	1.01683750	0.20336750	7.61	0.0034
G*N	5	0.06699861	0.01339972	0.50	0.7690
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	4.70292222	1.56764074	80.98	0.0001
G*S	3	0.14658333	0.04886111	2.52	0.1542
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	0.11615556	0.01935926	0.94	0.4804
N*S	15	0.60525694	0.04035046	1.96	0.0566
G*N*S	15	0.11676250	0.00778417	0.38	0.9749

Dependent Variable: ROOT/SHOOT_2

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	2.43972778	0.02568135	4.15	0.0001
Error	192	1.18926667	0.00619410		
Corrected Total	287	3.62899444			

R-Square	C.V.	Root MSE	RS_TOT Mean
0.672288	26.22206	0.07870	0.30014

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.01253472	0.01253472	2.02	0.1565
B(G)	2	0.00861806	0.00430903	0.70	0.5000
N	5	0.14109861	0.02821972	4.56	0.0006
G*N	5	0.01732778	0.00346556	0.56	0.7310
B*N(G)	10	0.07996528	0.00799653	1.29	0.2379
S	3	1.51930833	0.50643611	81.76	0.0001
G*S	3	0.05689583	0.01896528	3.06	0.0293
B*S(G)	6	0.02394861	0.00399144	0.64	0.6946
N*S	15	0.31775417	0.02118361	3.42	0.0001
G*N*S	15	0.02794167	0.00186278	0.30	0.9950
B*N*S(G)	30	0.23433472	0.00781116	1.26	0.1777
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	0.14109861	0.02821972	3.53	0.0425
G*N	5	0.01732778	0.00346556	0.43	0.8157
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	1.51930833	0.50643611	126.88	0.0001
G*S	3	0.05689583	0.01896528	4.75	0.0501
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	0.02394861	0.00399144	0.51	0.7952
N*S	15	0.31775417	0.02118361	2.71	0.0097
G*N*S	15	0.02794167	0.00186278	0.24	0.9975

Dependent Variable: FOLIAGE_2 (%)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	5.67309316	0.05971677	34.45	0.0001
Error	192	0.33280067	0.00173334		
Corrected Total	287	6.00589383			

R-Square	C.V.	Root MSE	FOLIAGE Mean
0.944588	10.79915	0.04163	0.38552

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.00591328	0.00591328	3.41	0.0663
B(G)	2	0.00077753	0.00038877	0.22	0.7993
N	5	0.02858368	0.00571674	3.30	0.0070
G*N	5	0.02779582	0.00555916	3.21	0.0084
B*N(G)	10	0.02860492	0.00286049	1.65	0.0952
S	3	5.14095343	1.71365114	988.64	0.0001
G*S	3	0.00887648	0.00295883	1.71	0.1670
B*S(G)	6	0.01172027	0.00195338	1.13	0.3481
N*S	15	0.20096230	0.01339749	7.73	0.0001
G*N*S	15	0.06119233	0.00407949	2.35	0.0040
B*N*S(G)	30	0.15771310	0.00525710	3.03	0.0001
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	0.02858368	0.00571674	2.00	0.1644
G*N	5	0.02779582	0.00555916	1.94	0.1737
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	5.14095343	1.71365114	877.28	0.0001
G*S	3	0.00887648	0.00295883	1.51	0.3038
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	0.01172027	0.00195338	0.37	0.8912
N*S	15	0.20096230	0.01339749	2.55	0.0142
G*N*S	15	0.06119233	0.00407949	0.78	0.6922

Dependent Variable: STEM_2 (%)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	3.33064916	0.03505946	20.03	0.0001
Error	192	0.33600800	0.00175004		
Corrected Total	287	3.66665716			

R-Square	C.V.	Root MSE	STEM Mean
0.908361	10.75632	0.04183	0.38892

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.00089959	0.00089959	0.51	0.4743
B(G)	2	0.00072681	0.00036341	0.21	0.8127
N	5	0.04681631	0.00936326	5.35	0.0001
G*N	5	0.02166931	0.00433386	2.48	0.0335
B*N(G)	10	0.02697156	0.00269716	1.54	0.1274
S	3	3.02389587	1.00796529	575.97	0.0001
G*S	3	0.00255295	0.00085098	0.49	0.6922
B*S(G)	6	0.00717766	0.00119628	0.68	0.6631
N*S	15	0.06018857	0.00401257	2.29	0.0052
G*N*S	15	0.04055440	0.00270363	1.54	0.0929
B*N*S(G)	30	0.09919613	0.00330654	1.89	0.0057
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	0.04681631	0.00936326	3.47	0.0445
G*N	5	0.02166931	0.00433386	1.61	0.2445
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	3.02389587	1.00796529	842.59	0.0001
G*S	3	0.00255295	0.00085098	0.71	0.5798
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	0.00717766	0.00119628	0.36	0.8972
N*S	15	0.06018857	0.00401257	1.21	0.3149
G*N*S	15	0.04055440	0.00270363	0.82	0.6516

Dependent Variable: ROOT_2 (%)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	95	0.78985487	0.00831426	4.75	0.0001
Error	192	0.33597400	0.00174986		
Corrected Total	287	1.12582887			

R-Square	C.V.	Root MSE	ROOT Mean
0.701576	18.54536	0.04183	0.22556

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	1	0.00216701	0.00216701	1.24	0.2672
B(G)	2	0.00277478	0.00138739	0.79	0.4540
N	5	0.05846046	0.01169209	6.68	0.0001
G*N	5	0.00462432	0.00092486	0.53	0.7545
B*N(G)	10	0.02230147	0.00223015	1.27	0.2473
S	3	0.50850890	0.16950297	96.87	0.0001
G*S	3	0.01797357	0.00599119	3.42	0.0183
B*S(G)	6	0.00606783	0.00101131	0.58	0.7477
N*S	15	0.09250635	0.00616709	3.52	0.0001
G*N*S	15	0.00995893	0.00066393	0.38	0.9830
B*N*S(G)	30	0.06451125	0.00215037	1.23	0.2043
Tests of Hypotheses using the Type III MS for B*N(G) as an error term					
N	5	0.05846046	0.01169209	5.24	0.0127
G*N	5	0.00462432	0.00092486	0.41	0.8283
Tests of Hypotheses using the Type III MS for B*S(G) as an error term					
S	3	0.50850890	0.16950297	167.61	0.0001
G*S	3	0.01797357	0.00599119	5.92	0.0317
Tests of Hypotheses using the Type III MS for B*N*S(G) as an error term					
B*S(G)	6	0.00606783	0.00101131	0.47	0.8248
N*S	15	0.09250635	0.00616709	2.87	0.0068
G*N*S	15	0.00995893	0.00066393	0.31	0.9904