Current uptake of ^{15}N –labeled ammonium and nitrate in flooded and non-flooded black spruce and tamarack seedlings

M. Anisul ISLAM[1] and S. Ellen MACDONALD*

Department of Renewable Resources, University of Alberta, Edmonton, Alberta, T6G 2H1. Canada.

[1] Present address: Department of Forestry and Natural Resources, Purdue University, 715 West State Street, West Lafayette, IN 47907 USA.

*Corresponding Author:
Phone: (780) 492-3070
Fax: (780) 492-4323
E-mail: ellen.macdonald@ualberta.ca

Running Head: Uptake of 15N in black spruce and tamarack

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Text pages 22, Tables 2, Figures 3

Abstract

- We investigated the effects of flooding for three weeks on physiological responses and uptake of NH_4^+ and NO_3^- by black spruce (*Picea mariana* (Mill.) BSP.) and tamarack (*Larix laricina* (Du Roi) K. Koch) seedlings fertilized with labeled $(^{15}NH_4)_2SO_4$ or $K^{15}NO_3$ in a growth chamber experiment.
- Flooding reduced photosynthesis (*A*), transpiration (*E*), water use efficiency (WUE), and current uptake of NH_4^+ and NO_3^- in both species.
- Under flooding, there were no significant differences between the two species in uptake of either NH_4^+ or NO_3^- at the whole-plant level but black spruce had higher translocation of NH_4^+ to the shoots than did tamarack.
- Under non-flooded conditions, black spruce seedlings exhibited higher uptake of both NH_4^+ and NO_3^- than did tamarack and demonstrated preferential uptake of NH_4^+ (19.67 mg g^{-1}dw) over NO_3^-(12.31 mg g^{-1}dw after three weeks). In contrast, non-flooded tamarack seedlings had equal uptake of NH_4^+ (4.96 mg g^{-1}dw) and NO_3^-(4.97 mg g^{-1}dw).
- We hypothesize that the ability of tamarack to equally exploit both $^{15}NH_4^+$ and $^{15}NO_3^-$ would confer an advantage over black spruce, when faced with limitations in the availability of different forms of soil nitrogen.

Keywords: ammonium, flooding, gas exchange, nitrate, photosynthesis.

Introduction

Nitrogen (N) is a limiting factor for trees growing in boreal peatlands (Bonan and Shugart, 1989; Mugasha et al., 1993). This has been attributed to low soil temperature and anaerobic soil conditions, which inhibit root activity and nutrient uptake while also resulting in slow rates of decomposition, N mineralization and nitrification, and activity of soil fungi and fauna (Campbell, 1980; Van Cleve and Alexander, 1981; Mugasha et al., 1993). Trees which grow in the boreal peatlands of western Canada, such as the evergreen black spruce (*Picea mariana* (Mill.) BSP.) and deciduous tamarack (*Larix laricina* (Du Roi) K. Koch), experience significant fluctuations in depth of water table during the growing season (Dang et al., 1991). Flooding results in depletion of soil oxygen; thus oxygen availability for tree roots decreases when water tables rise close to the peat surface (Kozlowski, 1984; Mannerkoski, 1985). Furthermore, the limited oxygen available is taken up quickly by plant roots, microorganisms, and soil reductants (Ponnamperuma, 1972). Anaerobic soil conditions result in a lowering of soil redox potential (Eh), leading to progressively greater demand for oxygen within the soil and creating an additional stress on the plant roots. With decreasing redox potential (Eh) during anaerobiosis, soil nitrate availability decreases to zero while ammonium availability increases (Armstrong et al., 1994).

Plants take up inorganic N in the form of ammonium and nitrate. There is a greater energy requirement for assimilation of nitrate than for ammonium because once nitrate ions enter a plant cell, they are reduced to ammonium ions and this process requires energy (Pate, 1983; Raven et al., 1992). Organic acid is required to counter OH^- generated in nitrate assimilation and this process requires as much as 15% of a plant’s energy production (Chapin et al., 1987). Ammonium assimilation is less costly as root respiration provides the energy and reductant required for glutamine and glutamate synthesis (Chapin et al., 1987; Oaks and Hirel, 1985). Tamarack has the ability to transport oxygen to its roots under anaerobic conditions and is thus able to sustain limited root respiration under flooding (Conlin and Lieffers, 1993). Black spruce lacks that ability and relies solely on fermentative glycolysis in low temperature anoxic conditions. Net assimilation and foliar N of tamarack have been shown to be positively correlated with soil ammonium availability, which is higher in wetter, lower, and colder microtopographic positions in boreal peatlands (Astridge, 1996). In black spruce, photosynthesis and foliar N were correlated more with nitrate availability (Astridge, 1996). If tamarack can sustain root respiration under anaerobic conditions, like those found in peatlands, we expect it would be better able to assimilate ammonium than black spruce. This could help explain its ability to thrive in peatlands as a deciduous conifer.

The mechanisms of NH_4^+ and NO_3^- uptake of conifers have been investigated (Bassirirad et al., 1997; Hangs et al., 2003; Kronzucker et al., 1995 a, b, 1996; Marschner et al., 1991; Malagoli et al., 2000;) and several species demonstrated a clear preference for ammonium over nitrate. There have been no studies of ammonium and nitrate uptake in black spruce or tamarack. The objective of this study was to examine current uptake of ammonium and nitrate in these two boreal conifers under flooded and non-flooded conditions in order to provide insight into their respective nutrient acquisition strategies given nitrogen limitations and variation in the availability of different forms of nitrogen in peatlands. We used labeled $(^{15}NH_4)_2SO_4$ and $K^{15}NO_3$ since this approach allowed us to trace and quantify the current uptake of N that entered into the plant under study (Nômmik, 1990).

Material and methods

Plant material and growing conditions

One-year-old nursery grown containerized black spruce (*Picea mariana* (Mill.) B. S. P.) and tamarack (*Larix laricina* (Du Roi) K. Koch) seedlings were obtained from Bonnyville Forest Nursery (6-15A) in a dormant condition and were placed at 4^0C to acclimate for a week. Seedlings of both species were of similar size (heights: 20.1 - 24.4 cm). Seedlings were then transplanted to 3.78 liter pots containing a planting medium of (1:3 v/v): Pro-Mix BX (Canadian Sphagnum peat moss 75% by volume, perlite, vermiculite, pH adjusted Dolomitic and Calcitic Limestone) and sand (Premier Horticulture Inc., Riviere-du-Loup, Que. Canada) and placed in a growth chamber with 21^oC/18^oC day/night temperature, 65-70% relative humidity and 16-h photoperiod with photosynthetically active radiation (PAR) of 400 μmol m^{-2} s^{-1} provided by fluorescent lamps. Seedlings were watered to near container capacity every other day. After about three weeks, when all the buds had flushed and the seedlings were in an actively growing stage, we commenced treatment application. Although these seedlings received conventional nursery fertilization during their first growing season, they did not receive any further fertilization during winter hardening, storage or during the three week period prior to the start of the experiment. Initial shoot N concentrations ranged from 1.1 to 1.4% in black spruce and 0.8 – 1.4% in tamarack seedlings while N concentration in roots ranged from 1.0 to 1.2% in black spruce and 0.8 - 0.9% in tamarack seedlings. N concentrations observed here are within the range of N found for these species in other studies (Miller and Hawkins, 2003; Wanyancha and Morgenstern, 1985).

Treatment application and experimental design

Black spruce and tamarack seedlings received a single application of 150 mg ^{15}N per pot [simulating operational silvicultural prescription of 200 kg N ha^{-1} (Amponsah et al. 2004)] as either labeled $(^{15}NH_4)_2SO_4$ or $K^{15}NO_3$ (5% enriched, Sigma-Aldrich, Canada) dissolved in water. The single application rate (150 mg ^{15}N seedling^{-1}) adapted in this study is adequate to study current uptake and retranslocation processes and has been successfully implemented in other studies (Mead and Preston 1994; Preston and Mead 1994; Amponsah et al. 2004). Additionally, Nǒmmik and Larson, (1989) found no significant differences in ^{15}N recovery between split doses *versus* a single application. Chelated micronutrients (EDTA 42% and DTPA 13%) were applied at the rate of 0.03 g L^{-1} to prevent any deficiency (Salifu and Timmer, 2003). A total of 168 seedlings were used in this experiment. Half of the seedlings from both black spruce and tamarack were randomly placed in individual plastic tubs where flooding was imposed by submerging the seedlings to root-collar level. Non-flooded seedlings were carefully watered to ensure that ^{15}N fertilizer was not lost due to excess watering. Plastic saucers were placed under each pot and any leached solution after irrigation was re-applied to the pots. Variables other than treatment were standardized (e.g., seedling size, pot size, soil texture, irrigation, fertilization, etc). Seedlings were randomly allocated to treatments and randomly selected for physiological measurements; individual seedlings were regarded as replicates. The experiment was a 2 × 2 × 2 × 3 factorial design, testing form of N supply (NH_4^+ and NO_3^-), species (black spruce and tamarack), flooding (flooding and non-flooding), treatment duration (1 week, 2 week, and 3 week), and their interactions. Gas exchange (photosynthesis, transpiration, and water use efficiency; for details see below) was measured 1, 2, and 3 weeks after treatment imposition. On each measurement day seven seedlings were randomly selected from each species × treatment combination. After measurement of gas exchange the seedlings were destructively harvested for N analysis (see below). We did not attempt to evaluate seedling growth in the current study because previous studies (Islam and Macdonald, 2004; Islam et al., 2003) showed that 3-4 weeks of flooding exerts significant influence on the physiological functioning of both black spruce and tamarack but does not cause any differential growth.

Measurements

^{15}N analysis:

At the end of each week after completing gas exchange measurements, seedlings were harvested and partitioned into shoots and roots. Roots were washed free of planting medium. Both roots and shoots were oven dried for 72 h at 68°C, ground with a Wiley mill to pass a 20 mesh sieve and then pulverized in a vibrating-ball mill (Retsch, Type MM2, Brinkmann Instruments Co., Toronto, Ontario, Canada) in preparation for mass spectrometer analysis. Ethanol was used to clean the mill between samples after vacuum cleaning (Binkley et al., 1985). All plant samples were then run for total N and ^{15}N analysis in an elemental analyzer (NA 1500, Carla Erba Elemental Analyzer, Milan, Italy), which was connected to a continuous flow Stable Isotope Ratio Mass Spectrometer

(VG 10; Middlewich, Cheshire, U.K). The mass spectrometer was comprised of an automatic Dumas system (Carlo Erba) for total N and a flow-through system for the N gas generated for isotope ratio analysis using a triple collector system. The N isotopic ratio was calculated for samples of roots and shoots using the delta (δ) notation as:

$$\delta^{15}\text{N} = [(\text{atom\% } ^{15}N)_x \, / \, (\text{atom \% } ^{15}N)_{Std} \; -1] * 1000$$

where, (atom%^{15}N)x and (atom%^{15}N)std are the respective N isotope ratios of the sample and the standard (0.3666, International Atomic Energy Agency) (Hauck et al., 1994). Plant uptake of ^{15}N derived from labeled fertilizer (NDLF) was then calculated following the equation of Salifu and Timmer (2003):

$$NDLF = TN \left[\frac{A - B}{C - B} \right]$$

where, TN is total plant N content (mg), A is the atom% ^{15}N in fertilized plant tissues, B is atom% ^{15}N in natural standard (0.366 or control), and C is atom% ^{15}N in applied fertilizer. Total plant N content for a seedling [TN (mg)] was estimated as N concentration multiplied by the seedling tissue dry weight. This plant uptake of ^{15}N value (NDLF) was subsequently divided by the plant sample dry weight to obtain the concentration of ^{15}N present in the tissue (roots, shoots or whole seedling). This aided comparison of the two species, given their different sizes / sinks strengths, and is appropriate for comparison of differences in uptake of the two N forms by a given species since there were no effects of flooding or N form on seedling growth during the three week duration of treatment.

Gas exchange

Physiological responses of the seedlings to the treatments was assessed by measurement of photosynthesis (*A*), transpiration (*E*) and water use efficiency (WUE; net assimilation rate divided by transpiration rate) of seven flooded and non-flooded black spruce and tamarack seedlings each week using an infrared gas analyzer equipped with a automatic conifer cuvette (LCA-3, Analytical Development, Hoddesdon, U.K.). Photosynthesis measurements were performed at 21 ± 1 °C. The uppermost shoots of a randomly selected seedling from each species and treatment combination were placed in the cuvette for gas exchange measurements. Gas exchange and all other physiological measurements were taken 1, 2, and 3 weeks after flooding imposition. Relative humidity of air into the cuvette was maintained at approximately 18 %, which is sufficient to prevent stomatal closure due to vapor pressure deficit. Light levels of 1050 μmol m^{-2} s^{-1} were applied during the gas exchange measurements. Needles were carefully detached from the stem after gas exchange measurements and their surface area measured by computer scanning (Sigma Scan 3.0, Jandel Scientific, San Rafael, CA, USA). Photosynthesis and transpiration were calculated as described by Caemmerer and Farquhar (1981) and were expressed on a per unit needle area basis.

Data analysis:

Data were analyzed using analysis of variance (using SAS version 9.1; SAS Institute Inc., Cary, NC) to determine the main and interactive effects of flooding, form of N, time, and species. The model was as follows:

$$Y_{ijklm} = \mu + N_i + S_j + NS_{ij} + F_k + NF_{ik} + SF_{jk} + N_iS_jF_k + T_l + TN_{il} + TS_{jl} + N_iS_jT_l + TF_{kl} + N_iF_kT_l + S_jF_kT_l + NSFT_{ijkl} + \varepsilon_{(ijkl)m}$$

Where, Y_{ijkl} = Physiological parameter (e.g., photosynthesis), μ = overall mean, N = form of N applied (i = 1, 2). S = species (j = 1, 2); F = flooding treatment (k = 1, 2); T = time (l = 1, 2, 3); ε = error term; m = 7 replication).

We used least-squared means to conduct post-hoc comparisons; because several of the higher-level interactions were significant, we focused post-hoc tests on: 1) comparisons between the two species for a given flooding treatment, N form, and a given day; 2) comparisons of flooded versus non-flooded seedlings of a given species for a given N form on a given day (gas exchange measurements only); and 3) comparisons between the two forms of N, for a given species, with a given flooding treatment, on a given day. We used an $\alpha = 0.01$ in these comparisons to reduce the overall probability of Type I error.

Results

Physiological responses

In general, flooding reduced photosynthesis (*A*), transpiration (E) and water-use efficiency (WUE) but there was variation over time in the effects of the flooding treatments and fertilizer form on the two species (Table 1, Figure 1). One week of flooding reduced *A* of NH_4^+ -fertilized tamarack and reduced *A* and *E* of black spruce for both fertilizer treatments (Figure 1). Two weeks of flooding resulted in lower *A* and WUE for tamarack and lower WUE for black under both fertilizer treatments but lower *A* only in NO_3^- -fertilized black spruce. By three weeks, flooded tamarack had lower *A* under both fertilizer treatments but lower *E* only with NO_3^- and lower WUE only with NH_4^+. The only physiological response of black spruce to three weeks of flooding was reduced *E* in NO_3^- -fertilized seedlings (Figure 1). Overall, tamarack exhibited higher rates of *A*, *E*, and WUE than black spruce but the differences between species tended to be greater under non-flooded conditions (Figure 1). Form of fertilizer only rarely influenced gas exchange (Table 1, Figure 1). Flooded tamarack had higher *A* and *E* with NH_4^+ than with NO_3^- at the three-week measurement; non-flooded black spruce had higher WUE with NO_3^- than with NH_4^+ at the two-week measurement (Figure 1). Flooded seedlings of both species did not exhibit any morphological adaptations (such as stem hypertrophy or production of adventitious roots) during this experiment (personal observation).

^{15}N uptake and distribution in plant tissues

In general, current uptake of fertilizer N by the seedlings (as indicated by ^{15}N concentrations in tissues) was significantly lower in flooded *versus* non-flooded seedlings, irrespective of species or N form (Figure 2A and 2B). Under flooding, there were no significant differences in uptake of $^{15}NH_4^+$ versus $^{15}NO_3^-$ for either species (Figure 2A and 2B). Flooded black spruce did, however, have higher uptake of both $^{15}NH_4^+$ and $^{15}NO_3^-$ to the shoots than did tamarack (Figure 2A and 2B).

In non-flooded conditions, black spruce which received $^{15}NH_4^+$ had higher concentrations of ^{15}N in the roots (weeks 1 and 3), shoots and whole plant (weeks 2 and 3) as compared to seedling that received $^{15}NO_3^-$ In contrast, tamarack showed no difference in ^{15}N concentration in tissues between seedlings receiving $^{15}NH_4^+$ versus $^{15}NO_3^-$ at any time (Figure 2A and 2B). In non-flooded conditions, ^{15}N concentrations in black spruce seedlings increased throughout the experiment for both the $^{15}NH_4^+$ and $^{15}NO_3^-$ treatments while for tamarack only $^{15}NO_3^-$ resulted in slight increases in ^{15}N concentrations of the roots and whole plant over time (Figure 2A and 2B). In non-flooded conditions, ^{15}N concentrations in the shoot, roots, and the whole plant were significantly higher for black spruce than for tamarack throughout the entire experiment for both the $^{15}NH_4^+$ and $^{15}NO_3^-$ treatments (Table 1, Fig. 2).

The greater uptake of N by black spruce than by tamarack was further reflected by the higher total N concentration in black spruce *versus* tamarack (Table 2), irrespective of flooding or N source. Total N concentration in roots of $^{15}NH_4^+$ and $^{15}NO_3^-$ - fertilized black spruce was significantly higher than in tamarack for both flooding treatments throughout the experiment, except for week three (Table 2). For non-flooded seedlings black spruce had higher shoot N concentration than tamarack for both $^{15}NH_4^+$ - fertilization throughout the entire experiment and for $^{15}NO_3^-$ - fertilization in weeks one and three (Table 2). Under flooding, there were no significant differences between the two species in terms of shoot N concentration for either N form.

Recovery of applied ^{15}N in whole plant tissues [(amount of ^{15}N found in plant tissue/total amount of applied ^{15}N per plant) *100] ranged from 0.22% to 1.73% in flooded seedlings and from 19.48% to 64.36% in non-flooded seedlings (Figure 3A and

3B). Percentage recovery of the applied $^{15}NH_4^+$ and $^{15}NO_3^-$ was higher in non-flooded black spruce than tamarack, by the end of the three week period (Figure 3A and 3B). Recovery of $^{15}NH_4^+$ and $^{15}NO_3^-$ in roots was somewhat greater in tamarack than black spruce, while recovery of $^{15}NH_4^+$ in shoots was greater in black spruce than tamarack, but these differences were not significant. There were also no differences in recovery of $^{15}NH_4^+$ versus $^{15}NO_3^-$ under flooding, for either species. Non-flooded black spruce, however, showed significantly greater recovery of $^{15}NH_4^+$ than $^{15}NO_3^-$. Tamarack showed significantly greater recovery of $^{15}NH_4^+$ than $^{15}NO_3^-$ one week into the experiment but this difference had disappeared by the subsequent weeks (Figure 3A and 3B).

Discussion

Our results suggest that, under non-flooded conditions, black spruce has a strong preference for ammonium while tamarack shows no such preference. We were not able to detect differences between the two species in their ability to take up ammonium *vs* nitrate when flooded.

The general trend of lower photosynthesis (*A*), transpiration (*E*) and water-use efficiency (WUE) in flooded (*vs* non-flooded) seedlings of black spruce and tamarack is consistent with our earlier studies (Islam et al., 2003; Islam and Macdonald, 2004). Still, there were no significant morphological signs of stress and seedlings of both species continued to photosynthesize under flooding although nutrient uptake was much less. Tamarack's ability to maintain relatively higher photosynthesis under flooding (*vs* non-flooded) as compared to black spruce is likely due to it's ethylene tolerance (Islam et al., 2003). In contrast to our expectations based on field observations (Astridge, 1996), there was no effect of form of N on gas exchange of either species.

Flooding inhibited the uptake of both $^{15}NH_4^+$ and $^{15}NO_3^-$ in both species. Inhibition of N uptake and transport due to root dysfunction or death could occur during flooding because of highly reduced soil conditions (DeLaune et al., 1998, 1999). Blockages in the vascular and aerenchyma systems may result from phytotoxin damage in highly reduced soils (Armstrong et al., 1996a, b, c). Uptake of both $^{15}NH_4^+$ and $^{15}NO_3^-$ was significantly higher in both the roots and shoots of non-flooded (*vs* flooded) seedlings of both species. Despite this, and the higher total N concentration of its foliage, *A* of black spruce per unit leaf area was significantly lower than tamarack, irrespective of flooding treatment. As has been reported previously under optimal growing conditions *A* of non-flooded tamarack was significantly higher than for black spruce (Islam et al., 2003; Islam and Macdonald, 2004). Since tamarack has a much lower leaf weight per unit area than black spruce, its rate of *A* per unit area or per unit N is significantly higher than in black spruce (Macdonald and Lieffers, 1990). Its leaf structure, therefore, is an important contributor to its highly efficient utilization of N (Tyrrell and Boerner, 1987).

Despite lower *A* non-flooded black spruce had significantly higher uptake of $^{15}NH_4^+$ and $^{15}NO_3^-$ than did non-flooded tamarack. This could be attributed to black spruce’s larger leaf biomass per unit surface area than tamarack (Mugasha and Pluth, 1994). Mugasha and Pluth (1994) suggested that longer retention of needles and higher dry mass per needle in black spruce results in a larger above-ground sink than in tamarack. The higher uptake of ^{15}N by non-flooded black spruce, and its relatively higher tissue N concentration than non-flooded tamarack, did not significantly affect its physiological functioning since its *A* remained the same for the entire experiment. This could be attributed to the lower photosynthetic N use efficiency (PNUE) of the evergreens (DeLucia and Schlesinger, 1995) which is associated with high specific leaf mass (SLM). The leaves of evergreens invest proportionally more N in nonphotosynthetic functions such as defensive compounds, and the leaves may also have relatively high cell wall resistance to gas diffusion (DeLucia and Schlesinger, 1995). In contrast, the lower uptake of ^{15}N in tamarack likely reflects a lower demand since it has a smaller N sink and is more efficient in nutrient retranslocation (Tyrrell and Boerner, 1987). Consequently, tamarack has a lighter-weight, annually replaced canopy, in which it invests less than black spruce.

The recovery of applied ^{15}N in non-flooded black spruce (64%) and tamarack (41%) was higher than has been previously reported (Knowles and Lefebvre, 1972; Salifu and Timmer, 2003). Knowles and Lefebvre (1972) reported about 8-12% recovery of applied ^{15}N (urea) in black spruce seedlings over one growing season. Salifu and Timmer (2003) achieved 12-19% recovery of $^{15}NH_4{}^{15}NO_3$ in black spruce over 60 to 120 days while Amponsah et al. (2004) reported 4% - 43% recovery of the applied ^{15}N in lodgepole pine seedlings. Like us, Salifu et al. (2008) achieved high recovery (68-69%) of ^{15}N applied to northern red oak (*Quercus rubra* L.) seedlings. This variation in recovery of applied ^{15}N among studies is likely due to differences in experimental conditions (*in situ vs ex situ*, pot sizes), plant nutrient status at the time of application, seedling age, stages of growth, or volatization.

While non-flooded black spruce clearly demonstrated superior ability to uptake $^{15}NH_4^+$ *vs* $^{15}NO_3^-$, non-flooded tamarack seedlings had equal uptake of both nitrogen forms. Preferential uptake of $^{15}NH_4^+$ (*vs* $^{15}NO_3^-$) has also been observed in several other evergreen conifers including European larch (*Larix decidua* Mill.; Malagoli et al., 2000), white spruce (Kronzucher et al., 1995a; Kronzucher et al., 1997), loblolly and ponderosa pine (Bassirirad et al., 1997) and Norway spruce (Buchmann et al. 1995), and this has generally been attributed to the greater energy requirement for nitrate assimilation. We find the ability of tamarack to equally uptake both NH_4^+ and NO_3^- is unique.

We expected tamarack to have superior ability to take up ammonium when flooded, since it can transport oxygen to its roots and maintain higher root respiration than black spruce under flooding (Conlin and Lieffers, 1993, Islam and Macdonald, 2004). Instead, we observed that non-flooded tamarack could equally acquire both ammonium and nitrate while black spruce preferred ammonium. Since ammonium is the available form of N in wet, hypoxic peatland sites, the inability to perform the root respiration required for uptake of ammonium might be a disadvantage for black spruce.

In peatlands, there is considerable variability in water level, daily, seasonally, inter-annually, and spatially between microsites and this has been related to photosynthesis, growth, and foliar nutrient concentrations of black spruce and tamarack (Lieffers and Macdonald, 1990; Dang et al., 1991; Astridge, 1996; Macdonald and Yin, 1999). Such variation very likely influences soil anaerobiosis, and thus the form of N availability. With the ability to take up both forms of N, tamarack is well suited to cope with variation in availability of different forms of N. Its ability to sustain root respiration during flooding (Islam and Macdonald, 2004) should give it a further advantage over black spruce in terms of nutrient acquisition.

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Table 1. Results of Analysis of Variance (*P* values) testing for effects of N form (NH_4^+ or NO_3^-), species (black spruce or tamarack), flooding (flooded or non-flooded), treatment duration (1 week, 2 week, or 3 week), and their interactions on photosynthesis (*A*); transpiration *(E)*; water use efficiency (WUE); current uptake of ^{15}N (as indicated by ^{15}N concentration) to roots, shoots, and whole seedlings; and recovery (%) of applied ^{15}N in roots, shoots and the whole seedling. (See also Figures 1 - 3).

Source	Response variable								
	A	*E*	WUE	Root ^{15}N conc	Shoot ^{15}N conc	Whole plant ^{15}N conc	% ^{15}N recovery in root	% ^{15}N recovery in shoot	% ^{15}N recovery in total plant
Fertilizer (fert)	0.0760	0.0628	0.4316	0.0001	0.0001	0.0001	0.0001	0.0003	0.0001
Species (spp)	0.0001	0.0012	0.0001	0.0001	0.0001	0.0001	0.3775	0.0001	0.0006
Flooding (trt)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Time	0.0001	0.0001	0.0001	0.0001	0.0015	0.0001	0.0001	0.0001	0.0001
fert*spp	0.8929	0.7076	0.8663	0.0008	0.0074	0.0004	0.4610	0.2252	0.2313
fert*trt	0.0908	0.7244	0.0009	0.0006	0.0002	0.0001	0.0004	0.0006	0.0001
fert*time	0.1585	0.0090	0.1611	0.0530	0.0253	0.4863	0.1556	0.1397	0.8371
Spp*trt	0.0001	0.2982	0.0049	0.0001	0.0001	0.0001	0.0610	0.0001	0.0001
trt*time	0.0001	0.1507	0.0044	0.0001	0.0025	0.0001	0.0001	0.0001	0.0001
spp*time	0.1778	0.0421	0.0001	0.0001	0.0316	0.0001	0.0007	0.0054	0.0001
fert*spp*trt	0.5167	0.3329	0.7697	0.0003	0.0129	0.0003	0.1366	0.3806	0.1317
fert*spp*time	0.4049	0.3216	0.3376	0.0102	0.2359	0.0712	0.1068	0.0241	0.0120
fert*trt*time	0.0227	0.0005	0.1540	0.0565	0.0286	0.4977	0.1978	0.1960	0.9162
spp*trt*time	0.0001	0.0001	0.2332	0.0001	0.0450	0.0001	0.0005	0.0094	0.0001
fert*spp*trt*time	0.0510	0.0091	0.1121	0.0118	0.2601	0.0849	0.0850	0.0441	0.0149

Table 2. Total nitrogen concentration (mg g^{-1} dry weight) in plant tissues of flooded and non-flooded black spruce and tamarack seedlings sampled 1, 2 and 3 weeks after fertilization with ^{15}N –labeled ammonium or nitrate. Values are means (S.E) of five seedlings; values with same letter within a treatment combination indicate that there was no significant difference between the two species at *P* <0.05.

Root	Treatment	Species	1 week	2 week	3 week
NH_4^+ fertilized	Flooded	Black spruce	10.4 ± (0.5)a	9.6 ± (0.3)a	10.6 ± (0.3)a
		Tamarack	9.1 ± (0.5)b	8.1 ± (0.3)b	8.1 ± (0.2)b
	Non-flooded	Black spruce	12.2 ± (0.4)a	12.7 ± (0.2)a	14.0 ± (0.4)a
		Tamarack	9.4 ± (0.2)b	9.4 ± (0.2)b	8.8 ± (0.4)b
NO_3^- fertilized	Flooded	Black spruce	9.9 ± (0.5)a	9.7 ± (0.3)a	9.5 ± (0.5)a
		Tamarack	8.6 ± (0.2)b	7.7 ± (0.3)b	9.5 ± (0.3)a
	Non-flooded	Black spruce	11.7 ± (0.4)a	11.9 ± (0.4)a	12.2 ± (0.4)a
		Tamarack	9.1 ± (0.6)b	10.3 ± (0.4)b	9.7 ± (0.3)b
Shoot					
NH_4^+ fertilized	Flooded	Black spruce	8.5 ± (0.2)a	7.7 ± (0.1)a	7.9 ± (0.1)a
		Tamarack	7.8 ± (0.1)a	7.1 ± (0.1)a	8.5 ± (0.1)a
	Non-flooded	Black spruce	14.1 ± (0.4)a	13.4 ± (0.6)a	12.7 ± (0.3)a
		Tamarack	9.6 ± (0.3)b	10.4 ± (0.5)b	9.9 ± (0.2)b
NO_3^- fertilized	Flooded	Black spruce	8.5 ± (0.3)a	8.2 ± (0.4)a	7.8 ± (0.3)a
		Tamarack	8.9 ± (0.3)a	8.8 ± (0.2)a	8.6 ± (0.2)a
	Non-flooded	Black spruce	12.1 ± (0.2)a	11.4 ± (0.4)a	13.3 ± (0.3)a
		Tamarack	11.0 ± (1.1)b	11.3 ± (0.2)a	10.2 ± (0.2)b

Figure 1. Photosynthesis (*A*), transpiration (*E*) and water use efficiency (WUE) of flooded and non-flooded black spruce and tamarack seedlings 1, 2 and 3 weeks after commencement of the treatments (flooding and fertilization with ^{15}N –labeled ammonium or nitrate). Values are means of seven seedlings. Asterisks indicate a significant difference between the species for a given time period under non-flooded (asterisks above) or flooded (asterisks below) conditions. For effects of flooding and N form see text.

Figure 2. Current uptake of applied fertilizer as indicated by concentration of ^{15}N (mg g^{-1} dry weight) in plant tissues of non-flooded (left column) and flooded (right column) black spruce and tamarack seedlings fertilized with $^{15}NH_4^+$ (Fig. 2A) or $^{15}NO_3^-$ (Fig. 2B) and sampled 1, 2 and 3 weeks after the start of the treatments. BS = black spruce and TAM = tamarack seedlings. Note different y-axis scales for flooded versus non-flooded seedlings. Values are means (S.E) of five seedlings. Asterisks under a bar (“BS vs Tam:”) indicate a significant difference between the two species for that flooding treatment, nitrogen form, and measurement time. Asterisks above the bars (“$^{15}NH_4^+$ versus $^{15}NO_3^-$:”) indicate a significant difference between seedlings fertilized with the two different forms of nitrogen for that species, measurement time and flooding treatment.

Figure 3. Percent (%) recovery of applied ^{15}N in plant tissues of non-flooded (left column) and flooded (right column) black spruce and tamarack seedlings fertilized with $^{15}NH_4^+$ (Fig. 3A) or $^{15}NO_3^-$ (Fig. 3B) and sampled 1, 2 and 3 weeks after the start of the treatments. Note different y-axis scales for flooded versus non-flooded seedlings. BS =

black spruce and TAM = tamarack seedlings. Values are means (S.E) of five seedlings**.** Asterisks under a bar (“BS vs Tam:”) indicate a significant difference between the two species for that flooding treatment, nitrogen form, and measurement time. Asterisks above the bars (“$^{15}NH_4^+$ versus $^{15}NO_3^-$:”) indicate a significant difference between seedlings fertilized with the two different forms of nitrogen for that species, measurement time and flooding treatment.

Figure 1

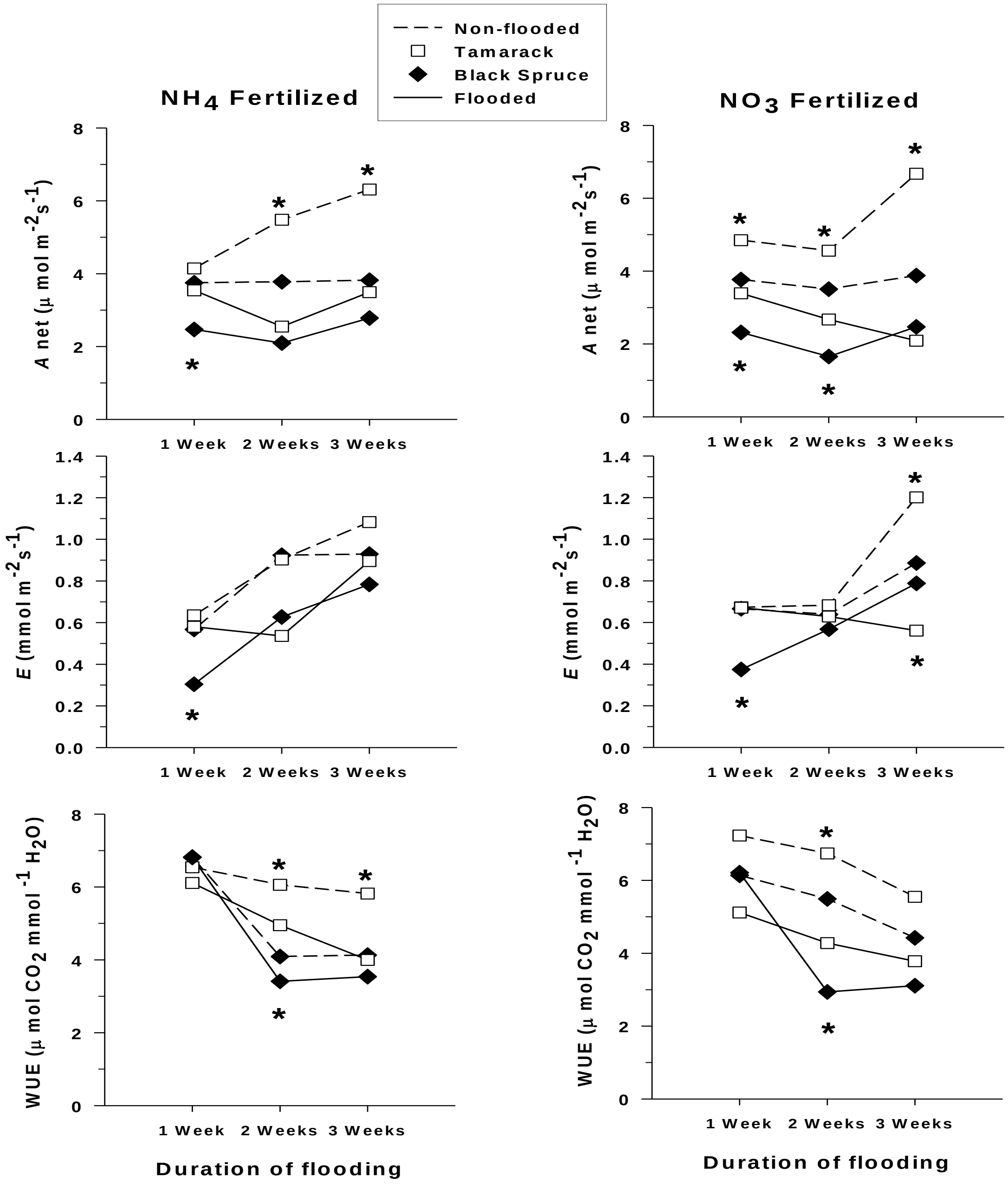

Non-flooded
Tamarack
Black Spruce
Flooded
NH4 Fertilized
NO3 Fertilized
A net (μ mol m-2s-1)
E (mmol m-2s-1)
WUE (μ mol CO2 mmol-1 H2O)
0
2
4
6
8
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1 Week
2 Weeks
3 Weeks
*
Duration of flooding
Duration of flooding

Figure 2A

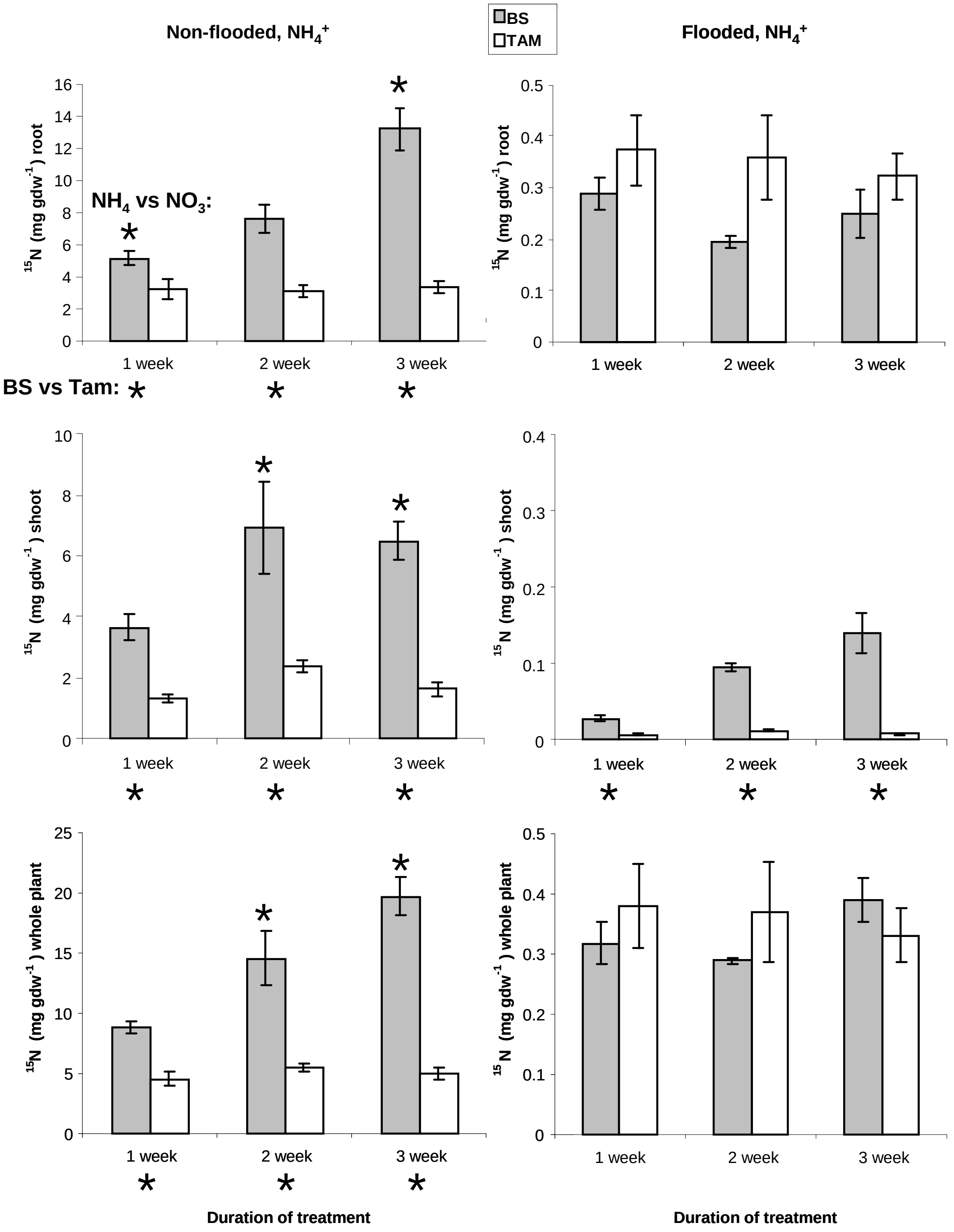

Non-flooded, NH_4^+
▨BS
☐TAM
Flooded, NH_4^+
^{15}N (mg gdw^{-1}) root
16
14
12
10
8
6
4
2
0
NH_4 vs NO_3:
*
*
1 week
2 week
3 week
BS vs Tam: * * *
0.5
0.4
0.3
0.2
0.1
0
^{15}N (mg gdw^{-1}) shoot
10
8
6
4
2
0
*
*
* * *
0.4
0.3
0.2
0.1
0
* * *
^{15}N (mg gdw^{-1}) whole plant
25
20
15
10
5
0
*
*
* * *
0.5
0.4
0.3
0.2
0.1
0
Duration of treatment
Duration of treatment

Figure 2B

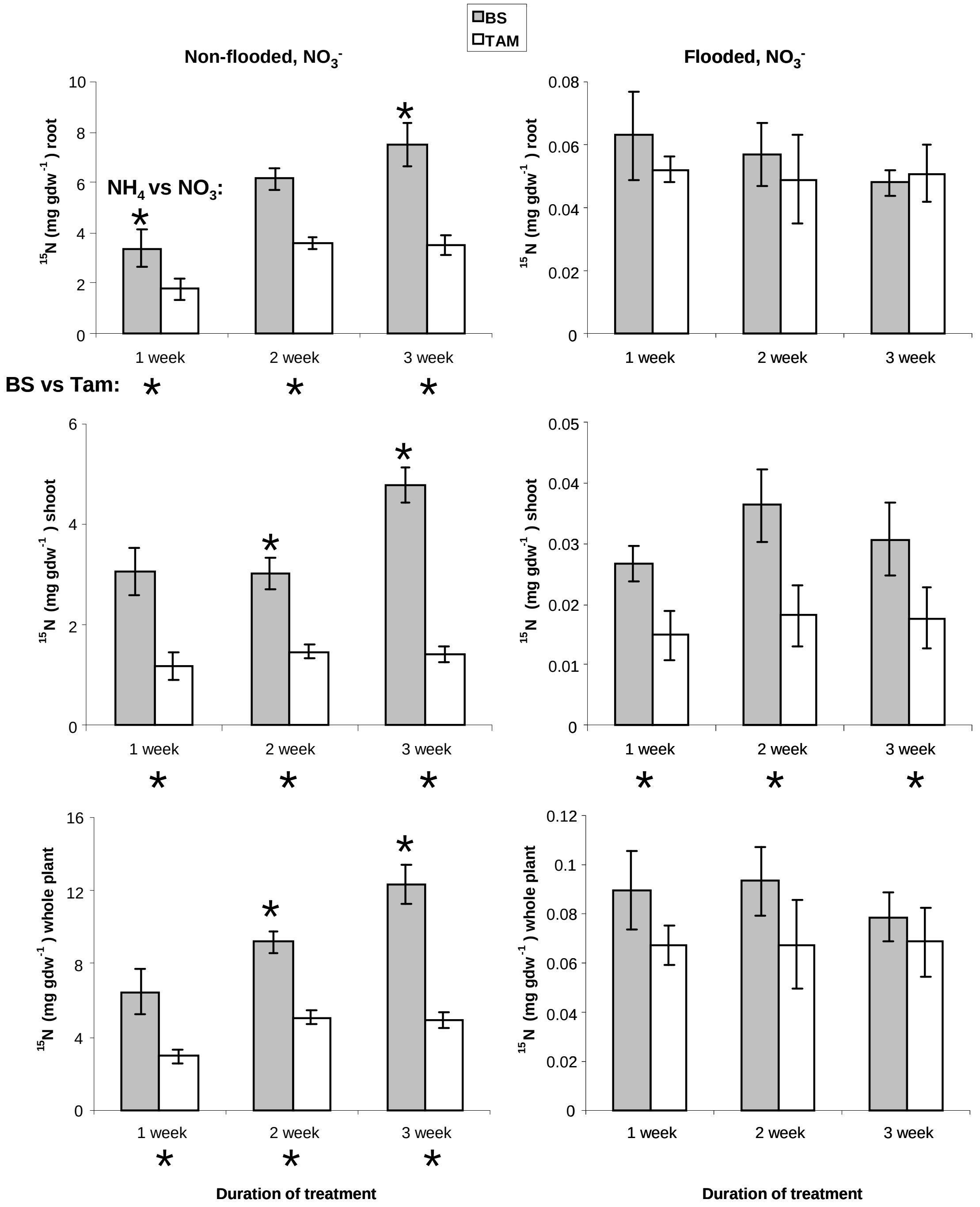
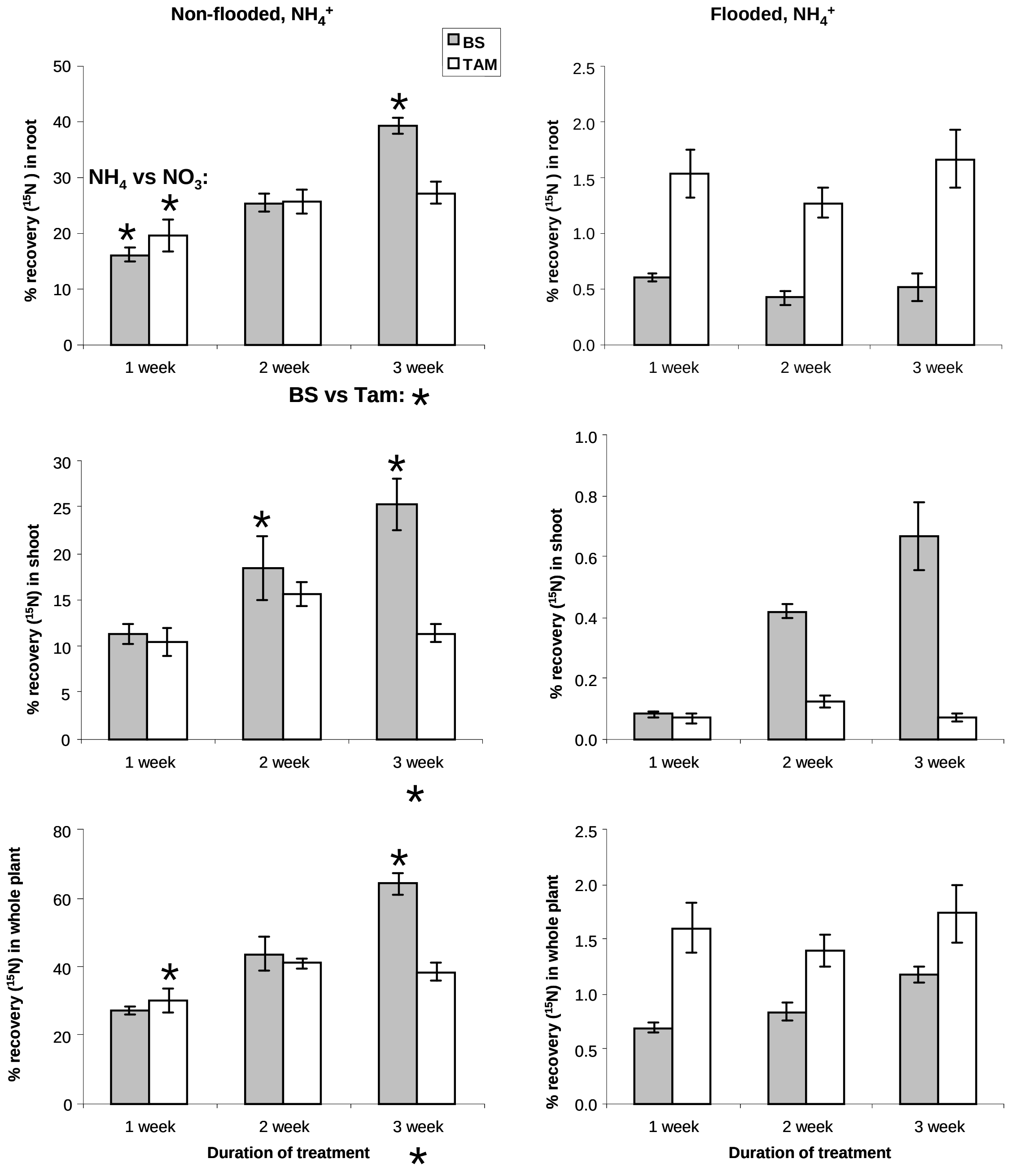
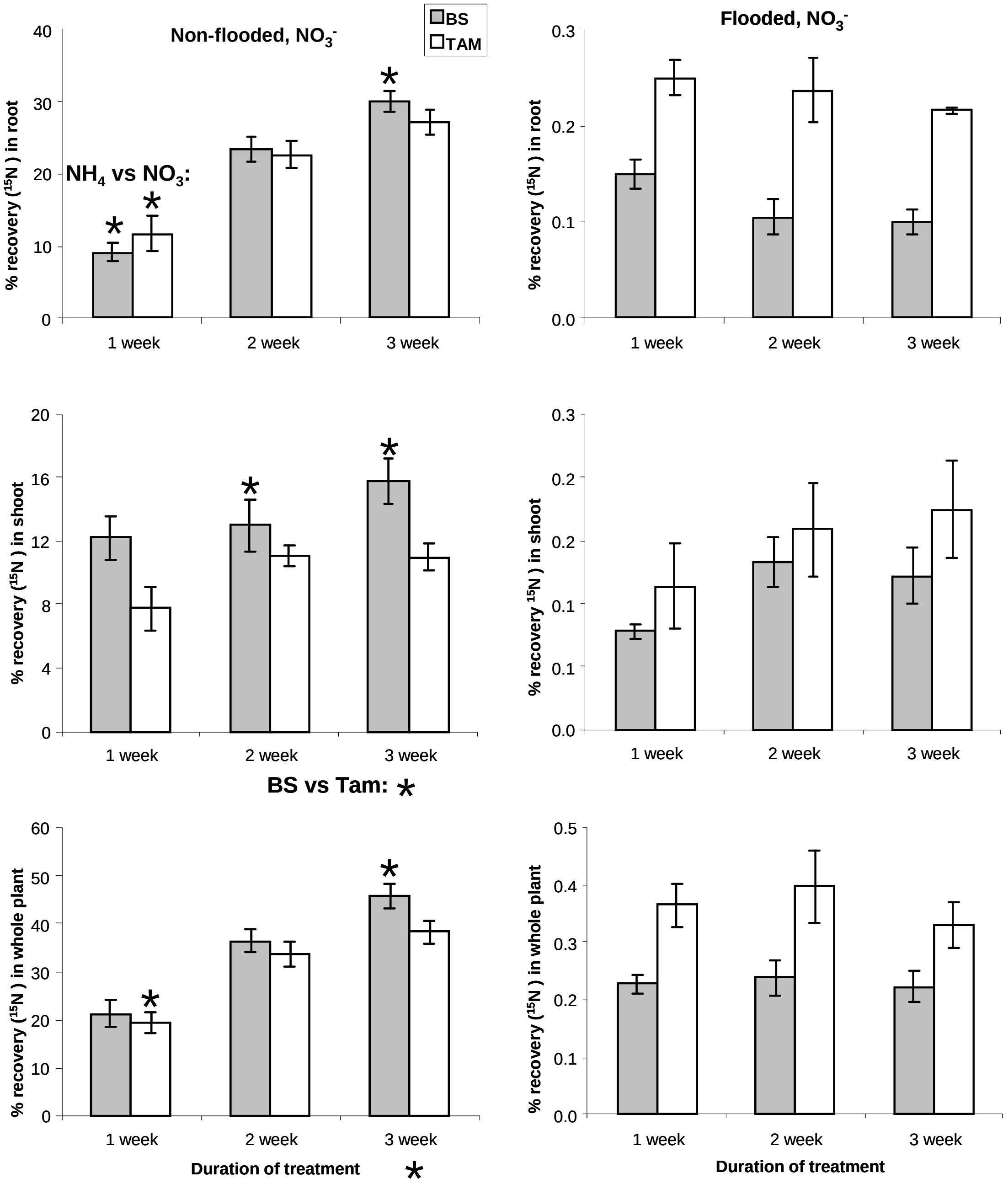

BS
TAM
Non-flooded, NO_3^-
Flooded, NO_3^-
^{15}N (mg gdw^{-1}) root
10
8
6
4
2
0
NH_4 vs NO_3:
*
*
1 week
2 week
3 week
BS vs Tam:
*
*
*
0.08
0.06
0.04
0.02
0
^{15}N (mg gdw^{-1}) shoot
6
4
2
0
*
*
*
*
0.05
0.04
0.03
0.02
0.01
0
*
*
*
^{15}N (mg gdw^{-1}) whole plant
16
12
8
4
0
*
*
*
*
*
0.12
0.1
0.08
0.06
0.04
0.02
0
Duration of treatment
Duration of treatment

Figure 3A



Non-flooded, NH4+
Flooded, NH4+
BS
TAM
% recovery (15N) in root
% recovery (15N) in shoot
% recovery (15N) in whole plant
NH4 vs NO3:
BS vs Tam: *
*
1 week
2 week
3 week
Duration of treatment
0
10
20
30
40
50
5
15
25
60
80
0.0
0.5
1.0
1.5
2.0
2.5
0.2
0.4
0.6
0.8

Figure 3B


Non-flooded, NO$_3^-$
BS
TAM
Flooded, NO$_3^-$
% recovery (^{15}N) in root
0
10
20
30
40
NH$_4$ vs NO$_3$:
*
*
*
1 week
2 week
3 week
0.0
0.1
0.2
0.3
% recovery (^{15}N) in shoot
0
4
8
12
16
20
*
*
% recovery ^{15}N) in shoot
BS vs Tam: *
% recovery (^{15}N) in whole plant
0
10
20
30
40
50
60
*
*
0.0
0.1
0.2
0.3
0.4
0.5
Duration of treatment
*