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Genotypic variation in nitrogen isotope discrimination in *Populus balsamifera* L. clones grown with either nitrate or ammonium

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ABSTRACT

Intraspecific variability in nitrogen use has not been comprehensively assessed in a natural poplar species. Here, a nitrogen isotope mass balance approach was used to assess variability in nitrogen uptake, assimilation and allocation traits in 25 genotypes from five climatically dispersed provenances of *Populus balsamifera* L. grown hydroponically with either nitrate or ammonium. Balsam poplar was able to grow well with either ammonium or nitrate as the sole nitrogen source. Variation within provenances exceeded significant provenance level variation. Interestingly, genotypes with rapid growth on nitrate achieved similar growth with ammonium. In most cases, the root:shoot ratio was greater in plants grown with ammonium. However, there were genotypes where root:shoot ratio was lower for some genotypes grown with ammonium compared to nitrate. Tissue nitrogen concentration was greater in the leaves and stems but not the roots for plants grown with ammonium compared to nitrate. There was extensive genotypic variation in organ-level nitrogen isotope composition. Root nitrogen isotope discrimination was greater under nitrate than ammonium, but leaf nitrogen isotope discrimination was not significantly different between plants on different sources. This can indicate variation in partitioning of nitrogen assimilation, efflux/influx (E/I) and root or leaf assimilation rates. The proportion of nitrogen assimilated in roots was lower under nitrate than ammonium. E/I was lower for nitrate than ammonium. With the exception of E/I, genotype-level variations in nitrogen-use traits for nitrate were correlated with the same traits when grown with ammonium. Using the nitrogen isotope mass balance model, a high degree of genotypic variation in nitrogen use traits was identified at both the provenance and, more extensively, the genotypic level.

1. Introduction

Nitrogen is a limiting nutrient in most natural ecosystems. However, nitrogen availability and predominant inorganic forms can vary across ecosystems and are dependent on soil and environmental factors (Pastor and Post, 1986). These differences in nitrogen availability may affect widely distributed tree species differently, indicating regional adaptation to the dominant form of nitrogen in the soil. Poplar species are widely distributed trees that have been identified as model systems for woody plant biology (Bradshaw et al., 2000; Jansson and Douglas, 2007). As such, poplars may be useful to identify intraspecific variation in nitrogen source preference. Balsam poplar (*Populus balsamifera* L.) has a geographic range extending through much of the boreal forest region across North America; extending from the Atlantic coast of Canada and New England to Alaska and above 65°N (Peterson and Peterson, 1992). Therefore, extensive variation in the dominant nitrogen form in the soil should exist across this geographic range. Balsam poplar has already been shown to contain clinal variation in phenology and adaptive photosynthetic traits (Soolanayakanahally et al., 2009). However, nitrogen-use traits have not been assessed in this expansive species. Preference for nitrogen can be identified by simply assaying biomass accumulation when grown with different nitrogen sources (DesRochers et al., 2007). Although biomass accumulation and nitrogen accumulation is a good indicator of overall nitrogen source preference, it does not provide detailed information on the underlying mechanisms contributing to differences in nitrogen source preference.

With the exception of organic nitrogen, uptake of nitrogen in plants is through uptake and assimilation of inorganic nitrogen by

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roots. Nitrogen uptake changes in response to variation in demand versus supply. Plants must exhibit some degree of plasticity to match uptake with demand for efficient growth and development. There are multiple genes controlling uptake and assimilation and each gene can have multiple isozymes (Glass et al., 2002). The role of such large gene families is not entirely clear. This, combined with a complex regulatory network for nitrogen use, makes it difficult to observe distinct changes in nitrogen-use as a consequence of single gene disruption. The complexity of the genetic and regulatory network of the nitrogen uptake and assimilation pathway has been emphasized by an extensive body of molecular work (Britto and Kronzucker, 2002; Glass et al., 2002; Lea and Azevedo, 2006; Miller and Cramer, 2005). Although there is a good functional understanding of nitrogen uptake and assimilation at the molecular level, the complexity of the system limits the integration of research on whole-plant uptake and assimilation into molecular physiology. As knowledge of these regulatory pathways and interactions becomes more complete, there will be a demand to integrate this valuable molecular knowledge into the whole-plant context (Hirel et al., 2001; Glass et al., 2002).

Careful assessment of nitrogen isotope discrimination may provide information on nitrogen fluxes and assimilation patterns that cannot be provided using biomass and nitrogen concentration measurements alone. A nitrogen isotope mass balance model proposed initially by Comstock (2001) and Evans (2001) and expanded on by Kalcsits et al. (2014) uses measurements of organ level nitrogen isotope fractionation under steady-state nitrogen conditions in combination with *a priori* knowledge of source $\delta^{15}N$ to identify variations in nitrogen fluxes and assimilation between plant parts and the substrate. In addition to organ-level biomass, nitrogen concentrations and unprocessed nitrogen isotope composition, traits related to nitrogen uptake and assimilation can also be calculated. Assimilation partitioning is a function of the difference in isotope composition between the two primary sites of assimilation (roots and leaves) and the proportion of overall plant nitrogen in the leaves. Partitioning of assimilation, efflux/influx (E/I) and translocation of inorganic nitrogen to the shoot can be calculated to reflect the ease of movement and supply, relative to demand, under steady-state nitrogen conditions. E/I can be obtained as a function of whole plant nitrogen isotope composition, partitioning of assimilation and the discrimination factor of either nitrate reductase or glutamine synthetase for nitrate or ammonium supplied trees, respectively.

For woody plants grown under steady-state nitrogen conditions, species level variation in nitrogen isotope composition has only been described in white spruce (*Picea glauca* (Moench) Voss) (Pritchard and Guy, 2005). In the field, intraspecific variation in nitrogen isotope composition has been reported in European beech (*Fagus sylvatica* L.) (Peuke et al., 2006) and Norway spruce (*Picea abies* (L.) Karst) (Gebauer and Schulze, 1991). However, plant nitrogen isotope composition under field conditions is typically affected by chemical, spatial and temporal heterogeneity in soil nitrogen. Interpretation of variation in nitrogen isotope composition at the whole plant and organ level requires careful control over the nitrogen substrate and measurement of organ level isotope composition (Kalcsits et al., 2014). In herbaceous plants under steady-state conditions, intraspecific variation in nitrogen isotope composition has been reported in barley (*Hordeum vulgare* L.) (Handley et al., 1997; Robinson et al., 2000; Kolb and Evans, 2003), wheat (*Triticale aestivum* L.) (Yousfi et al., 2009, 2012, 2013), and rice (*Oryza sativa* L.) (Yoneyama et al., 2001). This variation has been attributed to possible differences in nitrogen uptake or assimilation patterns that are thought to be a function of environment and genotype. Nitrogen isotope discrimination by plants can be used to interpret results obtained from such experiments (Comstock, 2001; Evans, 2001; Kalcsits et al., 2014), providing integrated information on nitrogen use at the whole plant and organ level that is difficult to measure using traditional assays.

There is increasing interest in using *Populus* species or their hybrids as feedstock for the growing biofuel industry (Yemshanov and McKenney, 2008; Sannigrahi et al., 2010). Rapid growth, ease of propagation and the ability to grow on marginal soils make poplar suitable for sustainable production of biofuel feedstock. However, as efforts to improve yields increase, the nitrogen demand of new high-yielding cultivars will likely increase. Therefore, identifying intraspecific variability in nitrogen fluxes and assimilation is a critical initial step in improving on N-use efficiency. Cost, time and complexity are all limitations to current assays available for measuring many of these traits. Although intraspecific variation in nitrogen use has been reported for a number of cereal species (Hirel et al., 2007) and *Arabidopsis thaliana* (Masclaux-Daubresse et al., 2010), the lack of an integrated approach to evaluate nitrogen-use traits limits interpretation of this genotypic variation (Hirel et al., 2007). Nitrogen isotope discrimination at natural abundance has the potential to be used as an integrated measure of nitrogen use in plants (Robinson, 2001; Evans, 2001; Kalcsits et al., 2014). Here, 25 genotypes were used from five climatically dispersed provenances of balsam poplar that extend from the prairie transition in the dry range of the species to the boreal forest-tundra transition zone to determine whether nitrogen source preference varies along a climatic gradient, and whether intraspecific variation exists for nitrogen-use traits when grown with nitrate or ammonium.

2. Materials and methods

2.1. Plant material and experimental design

Dormant branches taken from the previous-year growth of 25 genotypes of balsam poplar ranging from 51°N to 56°N were obtained from the Agriculture Canada Balsam Poplar (AgCanBaP) collection (Soolanayakanahally et al., 2009) at the AAFC-AESB Agroforestry Development Centre at Indian Head, Saskatchewan, Canada and stored at 4 °C for approximately three months to fulfill chilling requirements. The five provenances reflected a climatic gradient that extends from a prairie ecosystem northwards into the boreal forest of the Canadian Shield. The five provenances were: Outlook (OUT), Saskatchewan (51.1°N, 106.2°W), Saskatoon (SKN) (52.2°N, 106.4°W), Saskatchewan, Turtleford (TUR), Saskatchewan (53.2°N, 108.3°W), Cold Lake (CLK), Alberta (54.2°N, 110.1°W) and Gillam (GIL), Manitoba (56.4°N, 94.7°W). Two-node cuttings, approximately 6–8 cm long were arranged in a randomized complete block design with three blocks of two nitrogen treatments supplied as either 500 μM nitrate or 500 μM ammonium. Plants were grown for 45 days in a hydroponics solution until harvest. Complementary samples of each genotype were collected as reference samples (N = 3) and analyzed for starting nitrogen isotope composition and concentration.

2.2. Hydroponics system

The hydroponics system was comprised of six 1000L containers lined with 45 mil rubber pond liner (Firestone, USA) constructed in a greenhouse under ambient light conditions supplemented by sodium halide lighting (600 $\mu mol\,m^{-2}\,s^{-1}$) and 18/6 h day/night photoperiod. Temperatures in the greenhouse were maintained between 20 and 24 °C. Solution temperatures averaged approximately 20 °C. Each container had a floating raft that had a capacity for up to 32 plants. Unused plugs in the raft and the rest of the container were covered with black plastic to prevent algal growth from light infiltration into the hydroponics solution. The hydroponics solution was a modified 1/10th

strength Johnson's solution (Johnson et al., 1957) supplemented with either 250 μM $Ca(NO_3)_2$ or 250 μM $(NH_4)_2SO_4$. Final nutrient composition, excluding the nitrogen salts, was: 200 μM KH_2PO_4, 200 μM K_2S0_4, 100 μM $MgSO_4$, 100 μM $CaSO_4$, and micronutrients: 5 μM Cl, 2.5 μM B, 0.2 μM Mn, 0.2 μM Zn, 0.1 μM Mo, 0.05 μM Cu, and 50 μM Fe^{2+}. A centrifugal pump, with a pumping capacity of approximately 20 L per minute, provided circulation and aeration of the solution for each container. The solution was monitored periodically to ensure stable oxygen levels, pH and temperature. Powdered calcium carbonate ($CaCO_3$) was added to buffer pH in the range of 6–7.5. Media NH_4^+ and NO_3^- concentrations were assayed using the phenol-hypochlorite (Solorzano, 1969) and perchloric acid (Cawse, 1967) methods, respectively. The solution was completely replaced every 14 days to ensure that there was no substantial decrease (no more than 10%) in concentration of nitrate or ammonium over time that could result in major changes to the $\delta^{15}N$ of the hydroponics solution.

2.3. Sampling and natural abundance isotope analysis

After 45 days of growth, plants were separated into leaves, stems, roots and the original cutting. Samples were flash frozen in liquid nitrogen and stored at −80 °C until samples could be freeze-dried at −50 °C for two days. Once dried, roots, leaves, stems, cuttings and the reference cuttings were weighed. Samples were ground to a fine powder using a mortar and pestle and then ball milled (Fritsch Laborgeratebau, Terochem Scientific). Sub-samples of 3 ± 0.1 mg were weighed into tin capsules (Elemental Microanalysis Ltd., 8 × 5 mm, D1008) and analyzed for $\delta^{15}N$ and nitrogen concentration on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) (University of California Stable Isotope Facility, Davis, CA). Isotopic composition is expressed as $\delta^{15}N$ (‰):

$$\boldsymbol{\delta}^{15}\boldsymbol{N}(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \quad (1)$$

where R_{sample} is the $^{15}N/^{14}N$ isotope ratio of the sample and $R_{standard}$ is the isotope ratio of a known standard (air). Tissue nitrogen isotope composition was corrected for contaminating nitrogen from cuttings using the protocol described in Kalcsits and Guy (2013). Isotope discrimination is usually denoted by the equation (Farquhar et al., 1989):

$$\boldsymbol{\Delta}^{15}\boldsymbol{N}_{\boldsymbol{sample}} = \left[\frac{\left(\delta^{15}\boldsymbol{N}_{\boldsymbol{source}} - \boldsymbol{\delta}^{15}\boldsymbol{N}_{\boldsymbol{sample}}\right)}{\left(1 + \frac{\delta^{15}\boldsymbol{N}_{\boldsymbol{sample}}}{1000}\right)}\right] \quad (2)$$

The ammonium and nitrate salts used for the growth media had a $\delta^{15}N$ of −0.96 and +58.5‰, respectively. Leaf, root, stem and cutting $\delta^{15}N$ were converted to discrimination values prior to model calculations.

2.4. Mass balance calculations

Pre-existing nitrogen remobilized to growing tissues from vegetative cuttings was accounted for using the approach described in Kalcsits and Guy (2013). Then, the isotope mass balance model described in detail in Kalcsits et al. (2014) was used to calculate the proportion of nitrogen assimilated in the roots (P_{root}) and the efflux/influx ratio (E/I) of nitrogen movement between the substrate and the roots from measurements of nitrogen isotope composition, nitrogen concentration and biomass of roots and leaves. P_{root} can be estimated from the following:

$$\boldsymbol{P}_{root} = 1 - \left(\left(\frac{\mathbf{N}_{leafpool}}{\boldsymbol{N}_{total}}\right) \times \frac{Ti}{Tt}\right), \quad (3)$$

where $N_{leafpool}/N_{total}$ (Eq. (4)) is the proportion of total plant nitrogen found in the leaf pool and T_i/T_t (Eq. (6)) is the proportion of nitrogen translocated from the root to the leaves that is in inorganic form.

$$\frac{Ti}{Tt} = \frac{(\Delta^{15}\boldsymbol{N}_{leaf} - \boldsymbol{\Delta}^{15}\boldsymbol{N}_{root})}{\boldsymbol{\Delta}_{enz}}, \quad (4)$$

where Δ_{enz} is equal to the discrimination factor of nitrate reductase (15.4‰) for nitrate-grown cuttings (Ledgard et al., 1985) and glutamine synthetase (16.8‰) for ammonium-grown cuttings (Yoneyama et al., 1993).

$$\boldsymbol{f}_{\boldsymbol{stem\ leaf}} = \frac{\left(\Delta^{15}\boldsymbol{N}_{\boldsymbol{stem}} - \boldsymbol{\Delta}^{15}\boldsymbol{N}_{\boldsymbol{root}}\right)}{\left(\Delta^{15}\boldsymbol{N}_{\boldsymbol{leaf}} - \boldsymbol{\Delta}^{15}\boldsymbol{N}_{\boldsymbol{root}}\right)}, \quad (5)$$

where $\Delta\delta^{15}N_i$ is equal to the isotope discrimination of the root, stem or leaf.

$$\frac{N_{leaf\ pool}}{N_{total}} = \frac{Biomass_{leaf} \times [N]_{leaf} + f_{stem-leaf} \times Biomass_{stem} \times [N]_{stem}}{Biomass_{plant} \times [N]_{plant}} \quad (6)$$

where $[N]_i$ is the bulk tissue nitrogen concentration, $f_{stem\ leaf}$ is equal to the fraction of stem nitrogen that is from the leaves (Eq. (5)), and $Biomass_i$ is equal to the biomass of each plant fraction. The efflux/influx ratio for each plant can then be estimated from the following:

$$\frac{E}{I} = \frac{\boldsymbol{\Delta}^{15}\boldsymbol{N}_{plant}}{\boldsymbol{\Delta}_{enz} \times \boldsymbol{P}_{root}},$$

where $\Delta\delta^{15}N_{plant}$ can be calculated using the mass balance equation (Eq. (8)) divided by the discrimination factor of the enzyme multiplied by the proportion of nitrogen assimilation occurring in the roots.

$$\boldsymbol{\Delta}^{15}\boldsymbol{N}_{plant} = \frac{\boldsymbol{N}_{leafpool}}{\boldsymbol{N}_{total}} \times \boldsymbol{\Delta}^{15}\boldsymbol{N}_{leaf} + \left(1 - \frac{\mathbf{N}_{leafpool}}{\boldsymbol{N}_{total}}\right) \times \boldsymbol{\Delta}^{15}\boldsymbol{N}_{root} \quad (8)$$

2.5. Statistical analysis

Biomass, root:shoot ratio, $\delta^{15}N$, nitrogen concentration, total nitrogen of root, stem and leaf tissues, and model calculations were compared using a nested ANOVA with nitrogen source, genotype and provenance as fixed effects with genotype nested within provenance. The statistical model is as follows:

$$Y_{ij} = \mu + \alpha_i + \tau_j + \tau(\gamma)_{jk} + \beta_{ij} + \varepsilon_{ijk} \quad (9)$$

where, μ is the overall mean response, α_i is the effect due to the genotype, τ_j is the effect due to the nitrogen source, $\tau(\gamma)_{jk}$ is the effect due to genotype nested within provenance and β_{ij} is the effect due to any interaction between the genotype and nitrogen source. Analysis of variance procedure was carried out using Graphpad Prism 6 (La Jolla, CA, USA) to obtain estimates of the means, followed by Tukey's multiple comparison tests to separate means. Where necessary, data were log transformed to meet assumptions of homogeneity of variance and normality. Differences between treatments described as significant are those where $P<0.05$. For correlations, significance is indicated using ·, *, **, and *** to denote significance when $P<0.10$, $P<0.05$, $P<0.01$ and $P<0.001$.

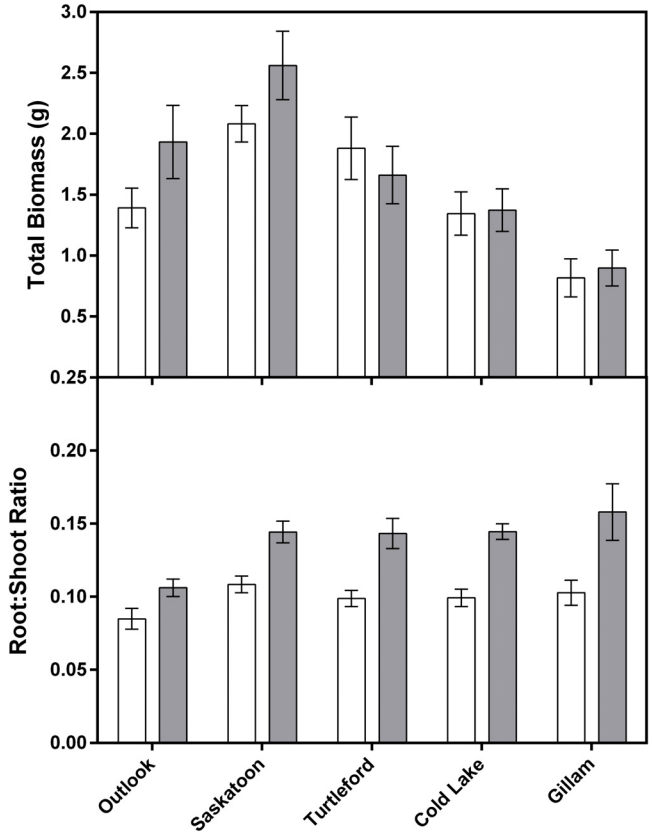

Fig. 1. Total plant biomass ±SE (N = 9–15) (top) and root:shoot ratio ±SE (bottom) for five provenances of *Populus balsamifera* L. grown with either 500 μM nitrate (white bars) or ammonium (grey bars).

3. Results

3.1. Root:shoot ratio and leaf nitrogen concentration were affected by nitrogen source but biomass was not

Nitrogen source had no significant effect on total plant biomass (Fig. 1). However, biomass for the two most southerly genotypes was notably greater when grown with nitrate than ammonium ($P < 0.10$) (Fig. 1). Generally, genotypes that were larger when grown with nitrate were also larger when grown with ammonium ($r = 0.543$; $P = 0.0059$) (Table 2). The root:shoot ratio was higher in all provenances when grown with ammonium than with nitrate (Fig. 1). This was largely caused by significant increases in leaf and stem biomass but not an increase in root biomass under nitrate. Similar to biomass, root:shoot ratios among genotypes grown with ammonium were correlated with root:shoot ratios of those same genotypes when grown with nitrate ($r = 0.581$; $P = 0.0023$).

Nitrogen concentrations were significantly higher in leaves and stems but not roots for plants grown with ammonium compared to nitrate (Table 1). Mean nitrogen concentration was 2.51, 0.42 and 2.03 μmol mg dw^{-1} under nitrate and 2.55, 0.76, and 2.18 μmol mg dw^{-1} under ammonium for roots, stems and leaves, respectively. Nitrogen concentrations in roots and stems were less variable than leaves (Table 1). The range in nitrogen concentration among genotypes was greater for roots in nitrate-grown plants, stems for ammonium grown plants and equal for leaves. There were no clinal geographic trends in leaf nitrogen concentration. Taking into account biomass and differences in nitrogen concentrations, the fraction of total plant nitrogen found in the leaves ($N_{leafpool}/N_{total}$) was not higher for nitrate-grown plants compared to ammonium-grown plants. Since net root uptake will be partly proportional to the inverse of the root:shoot ratio (Kalcsits et al., 2014), net root uptake was lower under ammonium than nitrate. Although there were genotypic differences in net root uptake of nitrogen, there were no differences between provenances (Table 1).

3.2. Nitrogen source affected organ-level Δ^{15} but not whole plant $\Delta^{15}N$

All genotypes were depleted in ^{15}N relative to their respective hydroponic solutions at the individual organ and whole plant level, showing discrimination against ^{15}N on both nitrogen sources (Fig. 2). Average $\Delta^{15}N$ was 10.1, 7.37 and 4.42‰ with nitrate and 8.75, 6.56 and 4.83‰ under ammonium for roots, stems and leaves, respectively. Whole plant $\Delta^{15}N$ was weighted more towards leaf $\Delta^{15}N$ since leaf nitrogen accounted for approximately 80% of total plant nitrogen. There were no clinal trends in nitrogen isotope composition among provenances (Fig. 3). Root and stem $\Delta^{15}N$ were greater in plants grown with nitrate than plants grown with ammonium, and differences between provenances were consistent across sources (Fig. 3). There were no significant correlations between nitrate and ammonium-grown plants for leaf and stem $\Delta^{15}N$ but there was a notable, but non-significant correlation for root $\Delta^{15}N$ between plants grown with nitrate and ammonium (Table 2) ($P < 0.10$).

3.3. Proportioning of nitrogen assimilation to the leaves was higher for NO_3^- than NH_4^+, but genotypic ranking was maintained on either source

The proportion of nitrogen translocated from the roots to the leaves that is in inorganic form (*Ti*/*Tt*) was greater for plants grown with nitrate than ammonium (Table 2) indicating more nitrogen assimilation in the leaves. Between 14 and 45% of leaf nitrogen

Table 1
Mean (±SE) (N = 9–15) provenance tissue nitrogen concentration (root [N], stem [N] and leaf [N]) (μmol mg dw^{-1}), the proportion of plant nitrogen allocated to the leaf ($N_{leafpool}/N_{total}$), and net root uptake (μmol mg dw^{-1} h^{-1}) for five climatically dispersed provenances of *Populus balsamifera* L. grown with either 500 μM nitrate or ammonium.

	Provenance				
	Outlook	Saskatoon	Turtleford	Cold Lake	Gillam
Nitrate					
Root [N]	2.54 ± 0.11	2.45 ± 0.06	2.54 ± 0.08	2.48 ± 0.10	2.52 ± 0.08
Stem [N]	0.42 ± 0.02	0.43 ± 0.01	0.39 ± 0.02	0.40 ± 0.02	0.46 ± 0.03
Leaf [N]	2.13 ± 0.08	2.03 ± 0.06	2.06 ± 0.06	1.96 ± 0.08	2.06 ± 0.11
$N_{leafpool}/N_{total}$	0.81 ± 0.01	0.77 ± 0.01	0.79 ± 0.01	0.78 ± 0.01	0.82 ± 0.01
Net Root Uptake	0.56 ± 0.04	0.43 ± 0.02	0.46 ± 0.02	0.43 ± 0.04	0.46 ± 0.03
Ammonium					
Root [N]	2.67 ± 0.07	2.51 ± 0.05	2.49 ± 0.06	2.57 ± 0.12	2.53 ± 0.05
Stem [N]	0.87 ± 0.09	0.80 ± 0.07	0.65 ± 0.05	0.71 ± 0.07	0.81 ± 0.10
Leaf [N]	2.28 ± 0.08	2.17 ± 0.07	2.15 ± 0.07	2.13 ± 0.11	2.24 ± 0.13
$N_{leafpool}/N_{total}$	0.83 ± 0.01	0.78 ± 0.01	0.77 ± 0.01	0.77 ± 0.01	0.76 ± 0.02
Net Root Uptake	0.49 ± 0.02	0.37 ± 0.02	0.37 ± 0.02	0.38 ± 0.02	0.37 ± 0.03

Table 2
Mean and genotype minimum and maximum total biomass (g), root:shoot ratio, tissue nitrogen concentration (root [N], stem [N] and leaf [N]) (μmol mg dw^{-1}), the proportion of plant nitrogen allocated to the leaf pool ($N_{leafpool}/N_{total}$), net root uptake (μmol mg dw^{-1} h^{-1}), tissue $\Delta^{15}N$, the proportion of leaf organic nitrogen assimilated in the leaf (*Ti*/*Tt*), the proportion of root assimilated nitrogen exported to the leaves as organic nitrogen (*To*/*Tr*), the proportion of plant nitrogen assimilation in the roots (P_{root}), efflux/influx (*E*/*I*), and tissue assimilation (μmol mg dw^{-1}).

		Nitrate			Ammonium				
Growth	Measurement	Mean ± SE	Genotype Minimum	Genotype Maximum	Mean ± SE	Genotype Minimum	Genotype Maximum	Source Effect[a]	Source Relationship[b]
	Total Biomass	1.86 ± 0.14	0.51	4.46	1.64 ± 0.10	0.33	3.48	0.077	0.543**
	Root:shoot ratio	0.10 ± 0.00	0.06	0.15	0.14 ± 0.00	0.09	0.20	<0.0001	0.581**
Nitrogen Partitioning	Root [N]	2.50 ± 0.04	2.08	2.93	2.55 ± 0.03	2.28	2.96	0.3082	0.205
	Stem [N]	0.42 ± 0.01	0.33	0.52	0.76 ± 0.03	0.47	1.25	<0.0001	0.329•
	Leaf [N]	2.04 ± 0.03	1.58	2.42	2.18 ± 0.04	1.63	2.65	0.0005	0.831***
	$N_{leafpool}/N_{total}$	0.79 ± 0.01	0.72	0.84	0.78 ± 0.01	0.72	0.85	0.0613	0.157
	Net Root Uptake	0.46 ± 0.01	0.29	0.64	0.39 ± 0.01	0.29	0.55	<0.0001	0.616***
Nitrogen Isotopic Discrmination	Root $\Delta^{15}N$	10.1 ± 0.25	6.88	13.44	8.75 ± 0.19	5.64	11.80	<0.0001	0.342·
	Stem $\Delta^{15}N$	7.37 ± 0.25	4.21	11.54	6.56 ± 0.20	3.27	9.35	0.0101	0.162
	Leaf $\Delta^{15}N$	4.42 ± 0.23	2.41	6.79	4.83 ± 0.19	1.62	6.84	0.1562	0.076
	Plant $\Delta^{15}N$	5.59 ± 0.21	3.32	7.94	5.70 ± 0.18	2.52	8.11	0.66947	0.180
Model Calculations	*Ti*/*Tt*	0.26 ± 0.01	0.14	0.45	0.23 ± 0.01	0.14	0.41	0.163	0.501*
	To/*Tr*	0.64 ± 0.01	0.58	0.73	0.63 ± 0.01	0.41	0.69	0.1285	0.462*
	P_{root}	0.80 ± 0.01	0.64	0.88	0.82 ± 0.01	0.71	0.89	0.018	0.591*
	E/*I*	0.31 ± 0.01	0.17	0.44	0.42 ± 0.01	0.20	0.58	<0.0001	0.280
	Leaf Assimilation	0.63 ± 0.03	0.44	1.01	0.59 ± 0.02	0.37	0.95	0.8561	0.573*
	Root Assimilation	18.36 ± 0.60	11.16	26.84	16.12 ± 0.43	11.62	21.97	<0.0001	0.496*

[a] P-value reported from a nested three-way ANOVA.
[b] Source relationship is the correlation for a genotypic mean for trait for NO_3^- versus NH_4^+. Pearson correlation coefficient (r-value) reported from linear regression analysis, significance denoted as *** P < 0.001, ** P < 0.01, * P < 0.05, · P < 0.10.

was assimilated in leaves under nitrate compared to 14–41% under ammonium (Table 2). There was no clinal trend between provenances. From the product of *Ti*/*Tt* and $N_{leafpool}/N_{total}$, an estimate for the partitioning of nitrogen (P_{root}) assimilation was calculated. Since $N_{leafpool}/N_{total}$ was not different between sources, P_{root} was largely a reflection of *Ti*/*Tt* where P_{root} was greater for plants grown with nitrate compared to ammonium (Table 2). Relative to nitrate, P_{root} under ammonium was less variable and was not different between provenances (Fig. 4). P_{root} under nitrate ranged from 0.64 to 0.88, indicating that for some genotypes, approximately one-third of assimilation occurred in the leaves. Genotypic differences in both *Ti*/*Tt* and P_{root} on the different sources were correlated with each other (Table 2) ($P < 0.05$), indicating that ranks were maintained and clonal variation in these traits is independent of source.

Although root assimilation accounted for between 60 and 80% of total plant nitrogen assimilation, root biomass was much smaller than leaf biomass. Therefore, assimilation rates per unit dry mass were approximately 15 times higher in roots than in leaves. Root assimilation was similar among provenances and was not significantly different between nitrogen sources (Fig. 6) (P < 0.05). Root assimilation rates were greater for nitrate than ammonium but leaf assimilation rates were not significantly different between plants grown on nitrate or ammonium (Table 2; Fig. 6).

3.4. Efflux/influx was lower for nitrate than ammonium and genotypic differences were not similar between sources

Efflux/Influx (*E*/*I*) was lower under nitrate than ammonium (Fig. 5). For ammonium, *E*/*I* ranged from 0.24 to 0.58 and for nitrate, it ranged from 0.19 to 0.48. However, there was a significant source by genotype interaction where some genotypes had higher *E*/*I* under nitrate than ammonium. In contrast, at the population level, provenances with high *E*/*I* under nitrate also had high *E*/*I* under ammonium (Table 2). There were no clinal patterns in *E*/*I* among provenances.

4. Discussion

The objective of this study was to determine whether there was intraspecific variation in nitrogen isotope composition and consequently, nitrogen fluxes, assimilation and allocation for balsam poplar grown with either steady-state nitrate or ammonium nutrition. The understanding of nitrogen uptake and assimilation in woody plants is incomplete. A first step towards understanding intraspecific variation in nitrogen-use traits is the assessment of time-integrated genotypic and environmental variation in nitrogen uptake, assimilation and allocation under steady-state conditions. Here, we report on the inherent variation in these traits using an isotope discrimination approach. However, the tightly controlled conditions needed for these analyses, as presently implemented, do not reflect the physiochemical and biotic variability that is normal to a natural environment. Further testing of the relationship between measured genotypic variation under controlled, common environment conditions to field-level growth and nitrogen-use is necessary.

Strong genotypic variation was uncovered in growth, nitrogen isotope discrimination and nitrogen-use traits calculated using an isotope mass balance model presented in Kalcsits et al. (2014). Intraspecific variation in nitrogen-use traits has not been described in many species. Masclaux-Daubresse et al. (2010) described intraspecific variation in nitrogen-use traits in *Arabidopsis thaliana* L. and reported high variability among populations when grown with nitrate. Singh and Arora (2001) reported significant genotypic variability in nitrogen uptake and allocation in *Triticum aestivum* L. However, there has not been any research to identify intraspecific variation for more than a few genotypes of poplar. Since poplar is a potential agricultural crop to supply growing bioenergy demand, the ability to grow on marginal soils that are often low in nitrogen availability, and to use applied nitrogen efficiently, is an important consideration. The high degree of natural variation observed here in balsam poplar suggests that selection for improved nitrogen use efficiency is possible.

4.1. Balsam poplar grew well on both sources of nitrogen

Here, nitrogen source did not affect plant biomass. In contrast, DesRochers et al. (2007) reported greater growth with ammonium than nitrate for pot-grown poplar genotypes, but this response was genotype-dependent. Healthy growth on both sources is consistent with balsam poplar being a generalist species (Peterson and

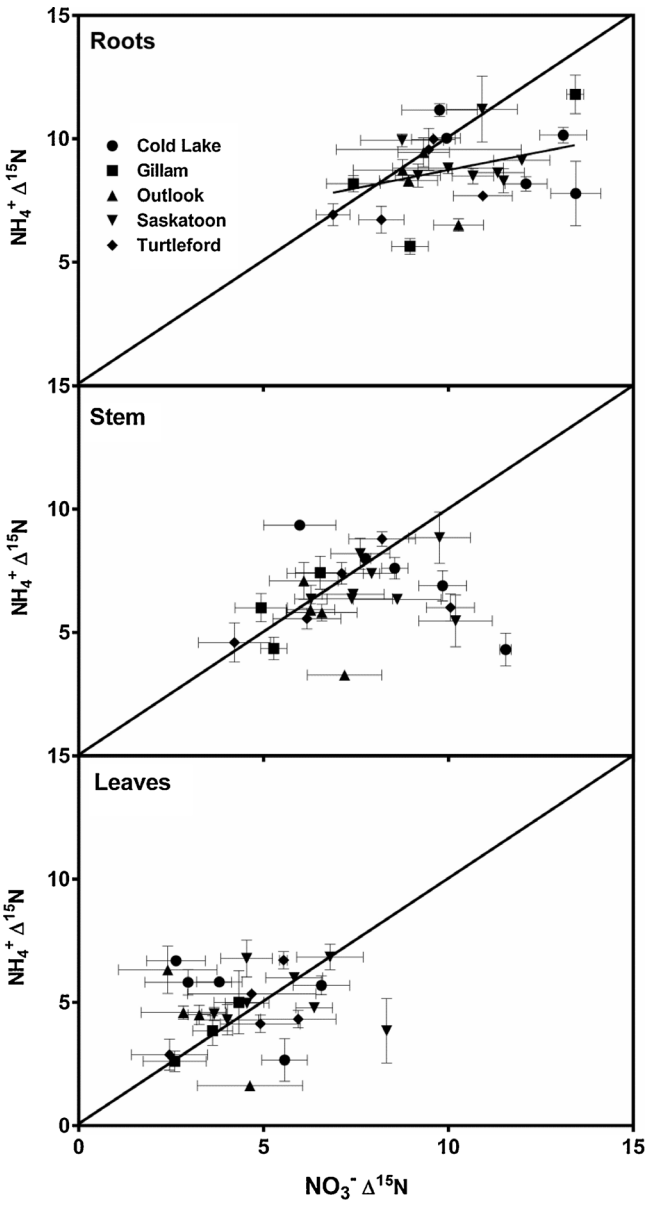

Fig. 2. Root, stem and leaf $\Delta^{15}N$ (N = 3) ±SE for 25 genotypes of *Populus balsamifera* L. from five provenances grown with either 500 μM nitrate (x axis) or ammonium (y axis). The solid lines represent the 1:1 line between the x-axis and the y-axis.

Peterson, 1992) that has the ability to acquire multiple forms of nitrogen. Similarly, Rennenberg et al. (2010) and Luo et al. (2013) suggested that other similar poplar species are capable of taking up both nitrate and ammonium. Plant biomass observed here in balsam poplar grown with ammonium corresponded well with previous measurements of hydroponically grown *Populus trichocarpa* for a similar period of time with ammonium (Buschhaus, 2007). Between species, Falkengren-Grerup (1995) reported large differences in biomass between nitrate and ammonium-grown plants where in many cases, greater growth occurred with nitrate nutrition, particularly in species that are found in less acidic soils. Furthermore, Cox and Reisenauer (1973) reported that *Triticum aestivum* was smaller when grown with ammonium compared to nitrate. In contrast, Pritchard and Guy (2005) reported higher growth with ammonium than nitrate for white spruce, a species known to prefer ammonium. Ariz et al. (2011) reported increased nitrogen isotope discrimination when grown with ammonium and a decreased nitrate:ammonium biomass ratio for species known

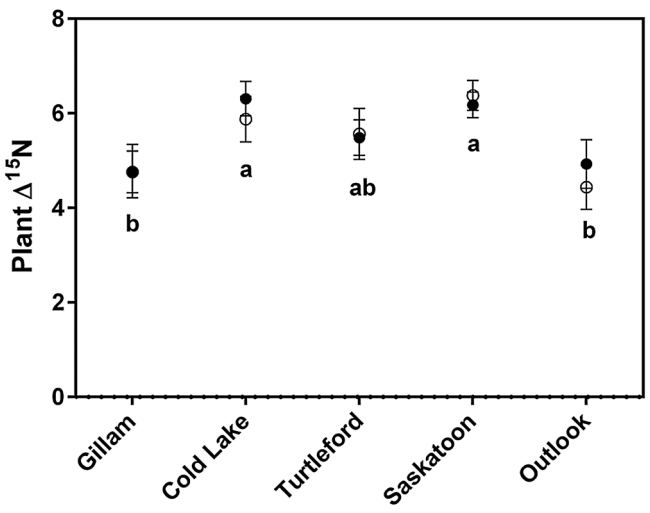

Fig. 3. Whole plant $\Delta^{15}N \pm SE$ (N = 9–15) of five provenances of *Populus balsamifera* L. grown with either 500 μM nitrate (open circles) or ammonium (closed circles).

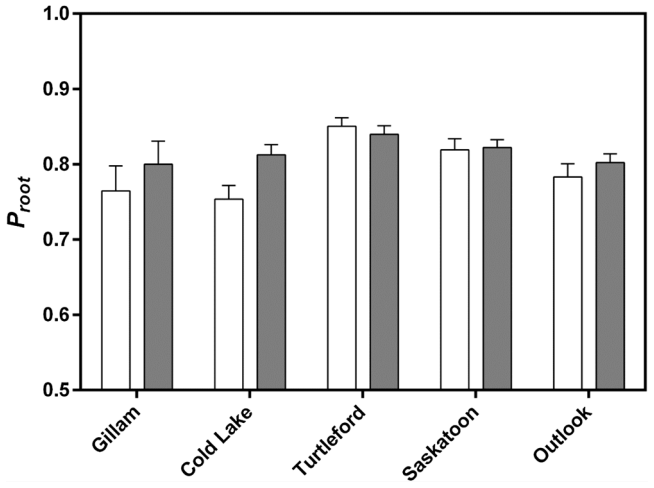

Fig. 4. The proportion of nitrogen assimilated in the root (P_{root}) ±SE (N = 9–15) of five provenances of *Populus balsamifera* L. grown with either 500 μM nitrate (white bars) or ammonium (grey bars).

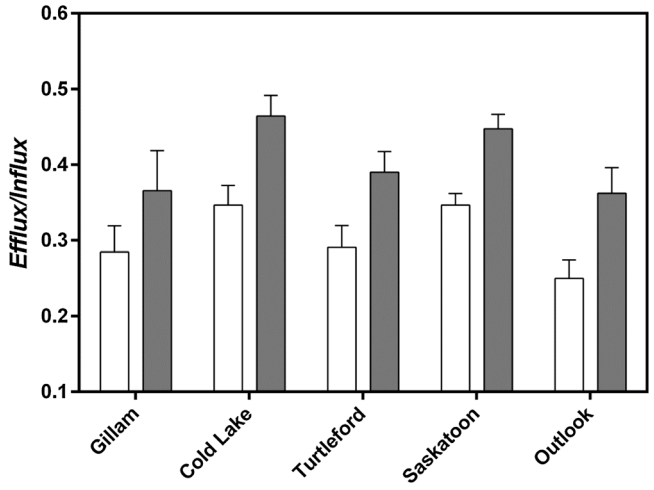

Fig. 5. Efflux/influx (E/I) ±SE (NN = 9–15) of five provenances of *Populus balsamifera* L. grown with either 500 μM nitrate (white bars) or ammonium (grey bars).

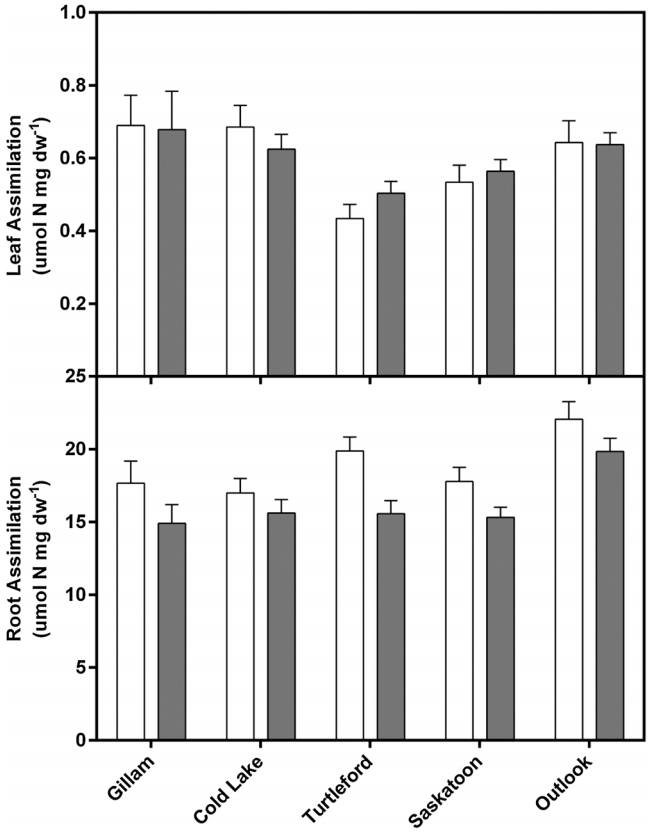

Fig. 6. Root and leaf assimilation rates (μmol N mg dw^{-1}) (lower) ±SE of five climatically dispersed provenances of *Populus balsamifera* L. grown with either 500 μM nitrate (clear) or ammonium (grey).

to be sensitive to ammonium. Here, we observed provenance level differences in nitrogen isotope composition and calculated nitrogen use traits. However, genotypic variation within provenances often exceeded variation between provenances (Table 1). Previously, within and between-provenance variation was reported for phenological and photosynthetic traits in balsam poplar and followed a clinal trend indicating selection for adaptive traits (Farmer, 1993; Soolanayakanahally et al., 2009). Balsam poplar has both seeds and pollen adapted for long-distance seed dispersal that likely increases gene flow and genetic diversity within a provenance (Keller et al., 2010). The limited number of genotypes and provenances used in this experiment prevents any firm conclusions regarding local adaptation to different nitrogen sources. There was no evidence of such adaptation despite the geoclimatic range covered. However, there appears to be a high degree of intraspecific variation and differential genotypic responses producing a range in growth and root:shoot ratios under nitrate and ammonium that may be indicative of variability in source preference.

4.2. Time-averaged nitrogen use traits are independent of growth and nitrogen source

Nitrogen isotope discrimination and root-to-shoot differences in isotope discrimination were similar between nitrogen sources. Here, we demonstrate genotypic differences in nitrogen use traits that extend across more than a single inorganic nitrogen source. Partitioning of assimilation between roots and leaves, leaf and root assimilation rates, and translocation of inorganic and organic nitrogen to the shoot all show a significant relationship between genotypic means under ammonium and nitrate. Interestingly, these variables are not correlated with growth (data not shown). Therefore, there must be some underlying mechanism that explains the observed genotypic consistency in nitrogen-use traits that is independent of nitrogen source. Variation in nitrogen uptake efficiency has been attributed to variation in root architecture (Hirel et al., 2007; Garnett et al., 2009), a trait that may contribute to nitrogen isotope composition under steady-state conditions. Although detailed analysis was not done, in the present study, root thickness and branching frequency appeared to vary between genotypes (data not shown). Genetic differences in root thickness, density and/or surface area may produce similar responses between the two nitrogen sources. Another possible explanation would be genotypic variation in nitrogen demand. Assimilatory demand is thought to impact rates of efflux relative to influx (Pritchard and Guy, 2005), and therefore, would also affect net nitrogen isotope discrimination, regardless of source.

5. Conclusion

Poplar has the capacity to grow well on both nitrate and ammonium when provided at an ecologically relevant nitrogen supply. However, there is considerable intraspecific variation in growth performance and in isotope discrimination at both whole plant and organ levels. Some of this variation is the same across inorganic nitrogen sources, suggesting that nitrogen demand rather than source-specific supply likely contributes to uptake, assimilation and allocation of nitrogen within balsam poplar. The exception to this was cycling of nitrogen across the root membrane where isotope mass balance modelling showed that genotypes responded differently to each source, indicating intraspecific variation in source preference. Identifying intraspecific variation in complex nitrogen-use traits will produce a better understanding of nitrogen fluxes and assimilation and better identify approaches to improve nitrogen-use traits in woody plants.

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