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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