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Water relations of pine seedlings in contrasting overstory environments

Matthew D. Powers [a,*], Kurt S. Pregitzer [b], Brian J. Palik [c], Christopher R. Webster [a]

[a] School of Forest Resources and Environmental Science, Michigan Technological University, 1400 Townsend Drive, Houghton, MI 49931, USA
[b] Department of Natural Resources and Environmental Science, University of Nevada, 1000 Valley Road, Reno, NV 89512, USA
[c] USDA Forest Service Northern Research Station, 1831 Hwy 169 East, Grand Rapids, MN 06514, USA

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ABSTRACT

Overstory conditions influence understory microclimate and resource availability, leading to gradients in evaporative demand and moisture availability that influence seedling water relations. Partial canopies may either reduce seedling moisture stress by ameliorating environmental conditions, or increase moisture stress by reducing soil moisture availability. This study used stable isotope ratios of oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) and mass-based foliar nitrogen concentrations to investigate changes in transpiration (E), stomatal conductance (g_s) and intrinsic water use efficiency (iWUE) of pine seedlings across an overstory gradient from open canopy gap environments to closed canopy forest. Foliar $\delta^{18}O$ increased sharply from basal areas of 0–10 $m^2\ ha^{-1}$ in *Pinus banksiana*, *Pinus resinosa*, and *Pinus strobus* seedlings, followed by a more gradual increase with further increases in basal area. Foliar $\delta^{13}C$ followed a similar, but less pronounced pattern in *P. banksiana* and *P. strobus* seedlings, and had no apparent relationship with overstory basal area in *P. resinosa* seedlings. The slope of the $\delta^{18}O$:$\delta^{13}C$ relationship was positive for every species. Foliar nitrogen concentrations were not correlated with overstory basal area. These results suggest seedling E declined as overstory basal area increased due to reductions in g_s, while iWUE increased slightly from open gaps to partial canopy environments. Open gap environments appear to provide sufficient moisture to sustain high leaf-level gas exchange rates in the species we studied, while relatively small increases in overstory basal area apparently promote rapid declines in g_s, leading to greatly reduced seedling water loss and small increases in iWUE.

1. Introduction

Interest in multi-cohort management in forest types traditionally managed using even-aged techniques has grown in recent years (Guldin, 1996; Lindenmayer and Franklin, 1997; O'Hara, 1998; Palik et al., 2002; Palik and Zasada, 2003; Zenner and Krueger, 2006), but we have a limited understanding of how many of the dominant tree species in these systems respond to the variable overstory environments associated with multi-cohort stand structures. Variation in resource availability and microclimate along structural gradients from canopy gaps and large clearings to closed forest conditions has been well documented (Chen et al., 1995; Palik et al., 1997; McGuire et al., 2001; Heithecker and Halpern, 2007). Variability among overstory conditions is correlated with seedling growth (Palik et al., 1997; McGuire et al., 2001), however, regeneration responses to different overstory environments may be complex and nonlinear in nature (Palik et al., 1997, 2003; Acker et al., 1998). This can make predictions of seedling growth and development in variable overstory environments difficult. Developing an understanding of the functional mechanisms driving regeneration responses among different overstory environments should help us make predictions about patterns of growth and development in those environments.

Light availability is the variable most closely correlated with seedling growth across different overstory environments (Palik et al., 1997; Drever and Lertzman, 2001; McGuire et al., 2001), and much of the research focused on understanding regeneration responses to different overstory environments has focused on relationships between light availability and seedling growth. Changes in soil moisture availability and evaporative demand, however, may also influence seedling development (Dang et al., 1997; Drever and Lertzman, 2001). For example, moisture stress can influence photosynthetic responses to light and other environmental variables (Maier and Tesky, 1992; Parker and Dey, 2008). This implies that understanding how different overstory conditions influence seedling water relations may be an important component of developing a broader understanding of regeneration dynamics in structurally complex forest stands with variable overstory conditions.

* Corresponding author. Tel.: +1 906 370 3288; fax: +1 906 487 2915.
E-mail addresses: mdpowers@mtu.edu (M.D. Powers), ksp@cabnr.unr.edu (K.S. Pregitzer), bpalik@fs.fed.us (B.J. Palik), cwebster@mtu.edu (C.R. Webster).

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Several interacting factors influence seedling water relations by causing both stomatal conductance (g_s) and transpiration (E) to increase as overstory abundance decreases. Soil moisture availability often increases following canopy gap formation or overstory density reductions (Dunlap and Helms, 1983; Aussenac and Granier, 1988; Breda et al., 1995). A number of studies suggest increases in soil moisture availability associated with decreased overstory densities have been shown to reduce stress in seedlings (Dunlap and Helms, 1983; Parker and Dey, 2008; Rodriguez-Calcerrada et al., 2008), which should lead to higher g_s in more open environments. Light availability in the understory also increases as overstory abundance decreases (Wetzel and Burgess, 2001; Palik et al., 2003; Boucher et al., 2007), and g_s generally increases with increasing irradiance (Kaufmann, 1976; Dalton and Messina, 1994; Shimazaki et al., 2007). Thus, changes in both soil moisture and light availability should lead to negative correlations between g_s and overstory abundance. This suggests E should also have a negative relationship with overstory abundance since large changes in g_s also influence E (Pallardy, 2008).

While declines in soil moisture availability associated with increasing overstory density should promote greater seedling moisture stress beneath denser canopies, changes in microclimate associated with different overstory environments also influence E and, therefore, plant moisture stress. Irradiance declines as canopy cover increases, which should decrease air temperatures and increase relative humidity (Dunlap and Helms, 1983; Chen et al., 1995; Aussenac, 2000; Heithecker and Halpern, 2007). As a result, leaf to air vapor pressure deficits (VPD) and potential evapotranspiration generally have an inverse relationship with overstory abundance. The increased VPD associated with open environments can result in higher E, which can increase seedling moisture stress in open environments compared to partial canopy environments (Dalton and Messina, 1994; Aussenac, 2000; Dumais and Prevost, 2008). Excessive moisture stress may lead to stomatal closure (Comstock and Ehleringer, 1984; Dang et al., 1997), which would limit the rate of carbon assimilation (A). In this situation, a partial canopy environment may be beneficial to seedlings because the shaded understory conditions would limit evaporative demand and E (Dalton and Messina, 1994; Aussenac, 2000).

Understanding the relative importance of stomatal and evaporative controls on seedling E in different overstory environments would provide useful insights about patterns of seedling moisture stress that could influence growth and development. Stable isotope ratios of oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) in seedling foliage may offer insights into the physiological and microclimatic factors driving water relations because they are thought to provide integrated signals of leaf-level processes that occurred during tissue formation (Dawson et al., 2002). While direct gas exchange measurements provide quantitative data about instantaneous rates of leaf-level physiological processes, analyses of stable isotopes in plant tissues may offer a qualitative estimate of relative rates of these same processes integrated over one or more growing seasons. Foliar oxygen isotope ratios ($\delta^{18}O$) are influenced by several factors including source water $\delta^{18}O$, evaporative and diffusional processes that occur during transpiration, and oxygen exchange between organic molecules and water. In well-mixed conditions, leaf water enrichment is proportional to $1 - e_a/e_i$, where e_a is water vapor pressure in the atmosphere and e_i is water vapor pressure inside the leaf, and enrichment is positively correlated with ε^k, the kinetic fractionation of water associated with diffusion across the stomata and leaf boundary layer (Craig and Gordon, 1965; Dongmann et al., 1974; Barbour, 2007; Farquhar et al., 2007).

Since E decreases and e_a/e_i increases as VPD decreases and relative humidity increases, leaf water enrichment should be positively correlated with E when differences in E are primarily driven by changes in evaporative demand. The kinetic fractionation of water, however, is negatively correlated with g_s so leaf water enrichment should be negatively correlated with E when differences are primarily driven by changes in g_s (Farquhar et al., 2007). Assuming the isotopic composition of source water is similar, these relationships suggest a positive correlation between foliar $\delta^{18}O$ and E when evaporative demand is the primary driver of changes in E, and a negative correlation between $\delta^{18}O$ and E when stomatal regulation is the primary driver of changes in E (Table 1). Since VPD and g_s generally decrease as overstory abundance increases, we might expect decreasing foliar $\delta^{18}O$ in seedlings growing along a gradient from open to partial canopy environments if VPD is the primary driver of changes in E, or increasing foliar $\delta^{18}O$ across the same gradient if g_s is the primary driver of changes in E.

Table 1
Expected changes in gas exchange and environmental variables influencing foliar oxygen isotope ratios of tree seedlings as overstory abundance increases and either (a) declines in soil moisture and stomatal conductance drive changes in transpiration or (b) declines in evaporative demand drive changes in transpiration.

Variable[a]	(a)	(b)
g_s	↓	≈
VPD	≈	↓
rH	≈	↑
E	↓	↓
e_a/e_i	≈	↑
ε^k	↑	≈
$\delta^{18}O$	↑	↓

[a] g_s: stomatal conductance; VPD: vapor pressure deficit; rH: relative humidity; E: transpiration; e_a: water vapor pressure in the atmosphere; e_i: water vapor pressure in the leaf; ε^k: kinetic fractionation of water molecules associated with diffusion; $\delta^{18}O$: oxygen isotope ratio.

The carbon isotope ratio ($\delta^{13}C$) of foliage is inversely proportional to the ratio of the leaf's intercellular CO_2 concentration (c_i) to the concentration of CO_2 in the atmosphere (Farquhar et al., 1982). This relationship exists because the carboxylating enzyme Rubisco discriminates against the heavier ^{13}C, and the rate of discrimination declines as c_i declines (Park and Epstein, 1961). The balance between A and g_s is the primary driver of c_i, so there is a positive correlation between foliar $\delta^{13}C$ and intrinsic water use efficiency (iWUE), the ratio of A/g_s (Farquhar et al., 1982, 1989). Since $\delta^{18}O$ may provide information about evaporative processes, and $\delta^{13}C$ provides information about the ratio of A to g_s, several authors have suggested $\delta^{18}O$ and $\delta^{13}C$ can be used together to understand the relative rates of leaf-level gas exchange processes (Saurer et al., 1997; Scheidegger et al., 2000; Barbour et al., 2002). While these studies focused primarily on understanding the sources of variability in $\delta^{13}C$, the dual isotope approach should also be applicable to studies focused on understanding sources of variability in $\delta^{18}O$, particularly across gradients in overstory abundance where the primary environmental factor associated with changes in photosynthesis (light) follows predictable patterns.

In this study, we evaluated relationships between overstory basal area, foliar $\delta^{18}O$, and foliar $\delta^{13}C$ in seedlings of three pine species to better understand trends in water relations across variable overstory environments. We used a dual isotope approach to examine trends in E, g_s, and iWUE. We used this information to partition the relative importance of stomatal and evaporative influences on transpiration in pine seedlings growing across a range of overstory basal areas. Specifically, we believed that foliar $\delta^{18}O$ would either (1) increase as overstory abundance increased as a result of declining g_s and E, or (2) decrease as overstory