Ecophysiological responses of black walnut (Juglans nigra) to plantation thinning along a vertical canopy gradient

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A B S T R A C T

Ecophysiology of black walnut (Juglans nigra L.) along a vertical canopy gradient was studied in a mixed species plantation for the first (2007) and second (2008) growing seasons after thinning to understand response mechanisms to increased resource availability. Parameters were affected by the thinning treatment in 2008 only. Thinned trees showed increased light-saturated maximum photosynthesis ($A_{max}$) from 2007 to 2008 compared to non-thinned trees. This response was likely due to increased light levels near thinned trees (vs. changes in water or nutrient status), because thinning did not affect midday leaf water potential ($Ψ_{md}$), average daily soil water content (SWC), or leaf nitrogen content per unit area ($N_a$). Plantation thinning did not increase relative diameter growth during the experimental period. This may be due to low thinning intensity and mortality prior to thinning that reduced competition from first-tier neighbors. Certain leaf traits such as leaf mass per unit area (LMA) and $N_a$ increased from the bottom to the upper canopy position, but did not influence thinning responses. Distribution patterns of photosynthetic parameters through the vertical canopy gradient were less defined than leaf structural traits such as LMA and $N_a$. Findings reflect black walnut’s large variability in response to thinning.

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1. Introduction

Black walnut (Juglans nigra L.) is generally associated with several species in the Central Hardwood Forest Region (CHFR) of the United States, including sugar maple (Acer saccharum Marsh.), yellow-poplar (Liriodendron tulipifera L.), hickories (Carya spp.), and many oak (Quercus spp.) species. Black walnut is site-sensitive and grows best on deep, fertile, well-drained, nearly neutral pH soils (Williams, 1990). Soil textures such as sandy loam, loam, or silt loam are generally appropriate. Although not a major component of forest stands in the CHFR, it is currently one of the most economically valuable species in North America and, therefore, commonly established in plantations. Black walnut is becoming more intensively managed to promote economic returns. There has been a large amount of research involved in black walnut tree improvement. Beineke (1989) summarizes 20 years of work with superior tree selection, grafting, and progeny testing. Hemery et al. (2005) and Michler et al. (2004) provide more recent examples of applied research in genetics, nursery culture, and site management. Morphological attributes, such as diameter growth, were also recently studied in walnut species (Bohanek and Groninger, 2003; Díaz and Fernández-López, 2005; Nicolescu, 1998).

Thinning is widely practiced in black walnut plantation management, a treatment typically conducted in single-cohort stands to improve the future growth of residual trees by altering stand density. Treatment response, however, is notoriously difficult to predict in this species. Black walnut tends to break bud later and senesce earlier than its associate species, thereby shortening its growing season. Crown architecture is open and foliage often develops leaf anthracnose, further reducing growing season length. Black walnut is also very sensitive to drought and soil conditions. To better understand the possible role that ecophysiology may play in the response of black walnut to thinning, it is important to examine the relative importance of environmental factors, such as light, water, and nutrients, to the thinning response. Ecophysiological traits of walnut species past the sapling stage are still poorly documented (Le Roux et al., 1999). Only one published report was found on thinning black walnut past the sapling stage (Gauthier and Jacobs, 2009) and two in other angiosperms: sessile oak (Quercus petraea (Matt.) Liebl.) stands in France (Bréda et al., 1995) and paper birch (Betula papyrifera Marsh.) stands in British Columbia, Canada (Wang et al., 1995). The small number of studies can be explained in part by the difficulty in collecting in situ gas exchange data on trees past the sapling stage. Additionally, many ecophysiological parameters can vary from the bottom to the top of the canopy as influenced by the light intensity regime (Reich et al., 1990; Ellsworth and Reich, 1993; Turnbull et al., 2002). Investigating these changes could provide further insight into black walnut treatment response.
The objectives of this experiment were to determine: (i) if plantation thinning influences ecophysiological parameters of black walnut in a mixed plantation setting; (ii) whether changes in light, water, or nutrient status after thinning play a more important role in mediating physiological and tree growth responses; and (iii) how these parameters vary along a vertical canopy gradient.

2. Materials and methods

2.1. Site description

A mixed hardwood plantation established in 1994 and located in Lafayette, IN, USA (40°23′09″N, 86°55′56″W) was selected for study (Table 1). Mean annual temperature, precipitation, and frost-free intervals are 10.1 °C, 937 mm, and 233 days, respectively (National Climatic Data Center, 2004). Elevation is 190 m on less than 2% slope. Billett loam is the dominant soil type on this former agricultural land, characterized by well-drained soils favorable for black walnut growth. The site index of walnut at this location is 21 m at age 50 (Schlesinger and Funk, 1977). The site was planted at 2.4 m × 2.4 m spacing. Black walnut, white oak (Quercus alba L.), and northern red oak (Quercus rubra L.) 1 + 0 bare-root seedlings, 30–45 cm in height at time of planting, were provided by the Indiana Department of Natural Resources, Vallonia state nursery. Planting pattern was not consistent, i.e., species did not alternate evenly among or between rows. A combination of mowing and herbicide was applied at time of planting as well as for the first three years after planting to reduce herbaceous competition. Herbicide was applied in narrow bands for expansion. The thinning treatment was meant to release one black walnut tree on all four sides and leave one black walnut tree non-thinned as a control, but high mortality of northern red oak (Table 1) prevented implementation of this design. Hence, thinning was performed according to the planting pattern (Fig. 1). Four trees surround each measurement tree based on cardinal directions (N, S, E, W); thus, forming a diamond pattern of first-tier competitors. A full description of first-tier competitors is presented in Table 2. Residual stocking near (2.4 m) thinned trees after treatment was zero. The thinning treatment was meant to release one black walnut tree on all four sides and leave one black walnut tree non-thinned as a control, but high mortality of northern red oak (Table 1) prevented implementation of this design. Hence, thinning was performed according to the planting pattern (Fig. 1). Four trees surround each measurement tree based on cardinal directions (N, S, E, W); thus, forming a diamond pattern of first-tier competitors. A full description of first-tier competitors is presented in Table 2. Residual stocking near (2.4 m) thinned trees after treatment was zero. The reduction was due in part to mortality (29%) and to thinning (71%). In comparison, residual stocking near control trees was 83%, with a 17% loss from mortality. Second-tier competitors, i.e., those located at NW, NE, SW, and SE corners, were not removed. Thinning was conducted prior to bud break in March 2007.

Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>dbh (cm)</th>
<th>Height (m)</th>
<th>Density (stems ha⁻¹)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. nigra</td>
<td>7.7 ± 0.3</td>
<td>6.7 ± 0.2</td>
<td>498 ± 16</td>
<td>89</td>
</tr>
<tr>
<td>Q. alba</td>
<td>13.6 ± 0.3</td>
<td>9.8 ± 0.2</td>
<td>498 ± 23</td>
<td>89</td>
</tr>
<tr>
<td>Q. rubra</td>
<td>8.4 ± 0.4</td>
<td>8.3 ± 0.2</td>
<td>327 ± 53</td>
<td>58</td>
</tr>
<tr>
<td>All trees</td>
<td>10.2 ± 0.3</td>
<td>8.3 ± 0.2</td>
<td>1324 ± 75</td>
<td>79</td>
</tr>
</tbody>
</table>

Fig. 1. Graphic representation of thinned (above) and control (below) replicates within each block (1–6). Species codes for first-tier competitors are as follows: black walnut (BW; Juglans nigra), northern red oak (RO; Quercus rubra), and white oak (WO; Quercus alba). Tree status is indicated by legend: each measurement tree is black walnut.
Table 2
Mean (±SE) diameter at breast height (dbh) and height of measurement trees and their first-tier competitors prior to plantation thinning in March 2007. Species include black walnut (Juglans nigra), white oak (Quercus alba), and northern red oak (Quercus rubra).

<table>
<thead>
<tr>
<th>Tree status</th>
<th>Species</th>
<th>Sample size (n)</th>
<th>dbh (cm)</th>
<th>Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alive</td>
<td>J. nigra</td>
<td>3</td>
<td>12.5 ± 1.7</td>
<td>8.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Q. rubra</td>
<td>5</td>
<td>8.1 ± 1.1</td>
<td>8.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Q. alba</td>
<td>12</td>
<td>7.9 ± 0.8</td>
<td>7.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>20</td>
<td>8.7 ± 0.7</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td>Cut</td>
<td>J. nigra</td>
<td>4</td>
<td>10.9 ± 1.7</td>
<td>8.5 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Q. rubra</td>
<td>3</td>
<td>8.9 ± 2.0</td>
<td>7.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Q. alba</td>
<td>10</td>
<td>8.4 ± 1.0</td>
<td>7.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>17</td>
<td>8.8 ± 0.7</td>
<td>7.6 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>J. nigra</td>
<td>6</td>
<td>13.0 ± 0.2</td>
<td>9.2 ± 0.2</td>
</tr>
<tr>
<td>Thinned</td>
<td>J. nigra</td>
<td>6</td>
<td>14.7 ± 0.6</td>
<td>9.6 ± 0.3</td>
</tr>
</tbody>
</table>

relative size of competitors within a defined search radius of the study tree (Vanclay, 2006). The higher the index, the greater the amount of competition from neighbors:

\[
C_I = \frac{\sum_{j=1}^{N} d_i^2}{\sum_{j=1}^{N} d_j^2} (1)
\]

where CI is the competition index for the study tree \(i\), \(d_i\) is the dbh of competitor \(j\), \(d_i\) is the dbh of study tree \(i\), \(D_i\) is the difference between \(i\) and \(j\), and \(N\) is the number of competitors of study tree \(i\) within a defined search radius (2.4 m in this study) (Radtke et al., 2003).

Study trees were remeasured in dbh at the end of the first and second growing seasons after treatment to measure growth (dbh at end of growing season – initial dbh) as well as relative growth ((growth/initial dbh) × 100). Cut trees were moved outside measurement plots and mowing was conducted prior to beginning gas-exchange measurements in each growing season to provide uniform understory conditions. Grass (Achnatherum spp.) and poison ivy (Toxicodendron radicans (L.) Kunz) were the most common species mowed.

2.3. Gas exchange

Light-response curves were developed for each level of thinning and vertical canopy gradient for the first (2007) and second (2008) growing seasons after thinning. For each block, control and thinned trees were sampled at all three canopy positions between 10:00 and 15:00 h on clear days. Only 1 block was measured per day and the sampling order alternated between treatments so that time of day was not confounded with treatment effects. A mobile lift (Altec Rover 37-GR, Altec Industries, Inc.; Birmingham, AL) was used to reach the leaves. Gas exchange was measured on one fully expanded leaf per tree on the northeast side of each canopy position with a portable photosynthesis system (LI-6400, Li-Cor, Inc.; Lincoln, NE). Light-response curves were developed using near-ambient [CO\(_2\)] of 380 μmol mol\(^{-1}\) and relative humidity (RH) between 50 and 75% in the cuvette to emulate external conditions (Lewis et al., 1999). Temperature inside the chamber was adjusted to outside temperature (\(T_{SW}\)) based on an external sensor attached to the chamber. Ambient light levels (photomultiplier flux density, PPDF) were also monitored using a cuvette-mounted sensor at the location of gas-exchange measurements. Ambient light was recorded as the mean of three measurements in full sun for each light-response curve, i.e., each thinning treatment and vertical canopy position. Net photosynthesis (\(A\)) was measured as a function of PPDF inside the cuvette according to the following steps: 1500, 2000, 1000, 500, 300, 200, 100, 50, and 0 μmol m\(^{-2}\) s\(^{-1}\). The 1500 μmol m\(^{-2}\) s\(^{-1}\) light level was measured prior to the 2000 μmol m\(^{-2}\) s\(^{-1}\) level because it is generally recommended to prepare the chamber for light-response curves in C3 plants at 1500 μmol m\(^{-2}\) s\(^{-1}\) (Li-Cor, Inc., 2003). Measurements were taken in June, except for block 6, which was measured in early July 2007. Photosynthetic parameters were derived from each light-response curve by fitting a linear regression line between the 0 and 200 μmol m\(^{-2}\) s\(^{-1}\) light range, with light compensation point (\(L_c\), μmol photons m\(^{-2}\) s\(^{-1}\)) determined when \(y = 0\), dark respiration rate (\(R_d\), μmol CO\(_2\) m\(^{-2}\) s\(^{-1}\)) determined when \(x = 0\), and apparent quantum efficiency (\(Q_e\), μmol CO\(_2\) μmol\(^{-1}\) photons) as the slope of the line. Light-saturated maximum photosynthesis (\(A_{max}\), μmol CO\(_2\) m\(^{-2}\) s\(^{-1}\)) was measured according to the PPFD level providing the highest A along each response curve.

2.4. Leaf water potential, leaf morphology, and soil parameters

Leaves sampled for gas exchange were also measured for midday leaf water potential (Ψ\(_{m}\)) in 2008 using a pressure chamber to determine water stress (PMS Instruments Co.; Corvallis, OR). After water potential was measured, leaves were placed between two moistened paper towels and inserted in a plastic bag, then deposited in a large cooler with ice. Leaves were brought back to the laboratory and stored at 5 °C. Leaf area, including petiole, was measured using a leaf area meter (LI-3100c, LI-Cor, Inc.), leaf mass per unit area, leaf water potential (Ψ\(_{w}\)), and soil water content sensors were inserted to a depth of 3 cm of the soil profile. Grass (Achnatherum spp.) and poison ivy (Toxicodendron radicans (L.) Kunz) were the most common species mowed.

2.5. Statistical analyses

A mixed linear models approach was used in statistical analyses to account for nested treatments and repeated measures on the same experimental units over time. Thinning, vertical gradient, and time were classified as fixed effects. Block (1–6) and tree (1–12) were classified as random effects, with tree as the subject for repeated measures. Based on experimental design, thinning × vertical gradient, thinning × time, and vertical gradient × time interactions were tested. Parameters were also tested for differences within one year. Treatment means and associated variance were reported by averaging experimental units depending upon statistical significance. For a thinning effect only, \(n = 18\) (6 study trees × 3 canopy positions). For a vertical gradient effect only, \(n = 12\) (6 study trees per treatment × 2 treatments). When values are reported by thinning treatment and vertical gradient, \(n = 6\). The vertical gradient effect was not tested on soil parameters. Least square means were used to distinguish levels of interaction effects, i.e., change over time and changes within one year. Non-normal distribution of errors and homogeneity of variance were assessed visually. Non-parametric tests (Kruskal–Wallis) were used for dbh data that could not be normalized using transformations. Differences were deemed significant when \(p < 0.05\). All statistical analyses were conducted using SAS (SAS Institute, Cary, NC). Photosynthetic parameters derived by regression were calculated in JMP (SAS Institute, Cary, NC).
3. Results

3.1. Thinning interaction effects

Thinning × time interactions from the first (2007) to the second (2008) growing season after thinning were found in $A_{\text{max}}$, $I_c$, PPFD, and $Q_e$ (Table 3 and Fig. 2). While $A_{\text{max}}$ and $Q_e$ increased over time regardless of thinning treatment, all parameters showed greater increases over time in thinned trees than in control trees (Fig. 2). Light levels increased over time near thinned trees ($p < 0.0001$), but remained similar near control trees (Fig. 2). No differences were found in 2007, but light was much higher near thinned trees ($1618 \pm 82 \mu \text{mol m}^{-2} \text{s}^{-1}$) than near control trees ($860 \pm 174 \mu \text{mol m}^{-2} \text{s}^{-1}$, $p < 0.0001$) in 2008 (Fig. 2). While not statistically different, ambient light levels presented for each year, thinning treatment, and vertical canopy position suggest a possible trend of increased light at the bottom canopy position near thinned trees in 2008 (Table 4). A decrease over time was detected in $I_c$ regardless of thinning treatment, but the decrease was greater in thinned trees than in control trees (Fig. 2). Within each year, $I_c$ was higher in thinned trees than in control trees in 2007 ($p = 0.01$); values were similar in 2008 (Fig. 2).

3.2. Vertical canopy gradient interaction effects

A vertical gradient × time interaction was found in $R_d$ only (Table 3 and Fig. 2). $R_d$ varied over time along the vertical gradient (Fig. 2). In 2007, $R_d$ was lower at the bottom of the canopy than at

Table 3
Mixed linear models and repeated measures analysis of ecophysiological parameters of thinned and non-thinned black walnut ($Juglans nigra$), along a vertical gradient, from the first (2007) to the second (2008) growing season after plantation thinning. Abbreviations are as follows: light-saturated maximum photosynthesis ($A_{\text{max}}$), light compensation point ($I_c$), leaf mass per unit area (LMA), leaf nitrogen content per unit area ($N_a$), midday leaf water potential ($\Psi_{\text{md}}$), ambient light (photon photosynthetic flux density, PPFD), apparent quantum efficiency ($Q_e$), dark respiration ($R_d$), and soil water content (SWC).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Year</th>
<th>Factor</th>
<th>df</th>
<th>$F$-value</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{\text{max}}$</td>
<td>Both</td>
<td>Thinning × time</td>
<td>1</td>
<td>10.52</td>
<td>0.0024</td>
</tr>
<tr>
<td>$I_c$</td>
<td>Both</td>
<td>Thinning × time</td>
<td>1</td>
<td>4.88</td>
<td>0.031</td>
</tr>
<tr>
<td>PPFD</td>
<td>Both</td>
<td>Thinning × time</td>
<td>1</td>
<td>13.76</td>
<td>0.0005</td>
</tr>
<tr>
<td>$Q_e$</td>
<td>Both</td>
<td>Thinning × time</td>
<td>1</td>
<td>7.53</td>
<td>0.0072</td>
</tr>
<tr>
<td>SWC</td>
<td>2007</td>
<td>Thinning</td>
<td>1</td>
<td>13.62</td>
<td>0.03</td>
</tr>
<tr>
<td>$R_d$</td>
<td>Both</td>
<td>Vertical gradient × time</td>
<td>2</td>
<td>5.30</td>
<td>0.008</td>
</tr>
<tr>
<td>LMA</td>
<td>2007</td>
<td>Vertical gradient</td>
<td>2</td>
<td>26.62</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LMA</td>
<td>2008</td>
<td>Vertical gradient</td>
<td>2</td>
<td>27.49</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$N_a$</td>
<td>2007</td>
<td>Vertical gradient</td>
<td>2</td>
<td>14.20</td>
<td>0.0001</td>
</tr>
<tr>
<td>$N_a$</td>
<td>2008</td>
<td>Vertical gradient</td>
<td>2</td>
<td>25.83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$\Psi_{\text{md}}$</td>
<td>2008</td>
<td>Vertical gradient</td>
<td>2</td>
<td>4.47</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Fig. 2. Mean ($n = 18 \pm \text{SE}$) ecophysiological parameters in thinned (●) and non-thinned (○) black walnut ($Juglans nigra$) for the first (2007) and second (2008) growing seasons after plantation thinning. From top left to bottom right: light-saturated maximum photosynthesis ($A_{\text{max}}$), light compensation point ($I_c$), apparent quantum efficiency ($Q_e$), and ambient light (photon photosynthetic flux density, PPFD). Statistical differences: *$p < 0.05$; those located between dates indicate a time × thinning interaction.
3.3. Main effects

Vertical gradient effects were found in LMA, Iac, and leaf \( \psi_{\text{mat}} \). Both LMA and \( I_a \) were lower at the bottom canopy position than the upper canopy position (\( p < 0.0001 \)) in 2007 and 2008 (Fig. 3). Leaf \( \psi_{\text{mat}} \) also varied along the canopy in 2008 (\( p = 0.02 \)); the bottom crown position averaged \( 1.53 \pm 0.08 \) MPa compared to the upper position with mean \( 1.96 \pm 0.09 \) MPa (\( p < 0.0001 \)).

A thinning effect was found in SWC only; SWC was higher near thinned trees in 2007 (\( p = 0.03 \)), but not in 2008 (Fig. 4). All other parameters, including \( T_{\text{air}} \) and \( T_{\text{soil}} \) (Fig. 4), showed no significant main effects.

3.4. Tree growth response

Morphological features of all trees located inside study blocks prior to thinning showed that black walnut was larger and taller than oak species (Table 1). The degree of competition (CI) prior to treatment was similar near thinned trees (0.74 ± 0.14) compared to control trees (0.92 ± 0.13, \( p = 0.16 \)). In 2007, thinned trees were slightly larger than control trees prior to beginning the experiment (14.7 ± 0.6 cm vs. 13.0 ± 0.2 cm, \( p = 0.01 \)) and for the first year after thinning (15.5 ± 0.5 cm vs. 13.8 ± 0.3 cm, \( p = 0.02 \)). Diameter growth was similar between treatments. In 2008, thinned trees were again larger than control trees, but the diameter increase was not significant in both absolute (1.5 ± 0.2 cm vs. 1.1 ± 0.2 cm, \( p = 0.08 \)) and relative terms (15.7 ± 1.3% vs. 14.8 ± 1.1%, \( p = 0.52 \)). A thinning treatment \( \times \) time interaction was found in CI (\( p = 0.001 \)). By the end of the second growing season, mean CI near control trees (0.92 ± 0.13) was identical to pre-thin levels (\( p = 0.97 \)) while CI near thinned trees decreased from 0.74 ± 0.14 to zero (\( p = 0.002 \)). Thus, CI near thinned trees was also lower than CI near control trees in 2008 (\( p < 0.0001 \)).

4. Discussion

Our first objective was to determine if plantation thinning influences ecophysiological parameters of black walnut in a mixed plantation over a two-year period. Compared to control trees, results suggest thinned trees showed no measurable improvement in ecophysiological status in 2007, and moderate improvement in 2008. During the second growing season, thinned trees exhibited higher increases in \( A_{\text{max}} \) and \( Q_{\text{e}} \) as well as lowered \( I_c \). These measurements do not represent a change after thinning in the traditional sense, because gas exchange was not measured prior to thinning, but the magnitude of change from 2007 to 2008 in thinned compared to control trees suggests a physiological response to thinning was detected. Hegyi’s competition index measured before and after thinning also suggests the thinning treatment had some impact on the immediate surroundings (2.4 m radius) of thinned trees. While the lack of response in 2007 may be due in part to water stress, absence of a substantial growth response in diameter after two years may be attributed to thinning intensity, lack of a consistent planting pattern, and high mortality of northern red oak prior to treatment. Black walnut is sensitive to

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th>Vertical canopy position</th>
<th>Ambient light level (( \mu \text{mol m}^{-2} \text{s}^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>Control</td>
<td>Bottom</td>
<td>549 ± 160</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
<td>1110 ± 189</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Upper</td>
<td>1094 ± 241</td>
</tr>
<tr>
<td></td>
<td>Thinned</td>
<td>Bottom</td>
<td>725 ± 235</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
<td>990 ± 149</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Upper</td>
<td>1096 ± 127</td>
</tr>
<tr>
<td>2008</td>
<td>Control</td>
<td>Bottom</td>
<td>108 ± 29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
<td>1027 ± 305</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Upper</td>
<td>1445 ± 169</td>
</tr>
<tr>
<td></td>
<td>Thinned</td>
<td>Bottom</td>
<td>1533 ± 149</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Middle</td>
<td>1653 ± 156</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Upper</td>
<td>1670 ± 141</td>
</tr>
</tbody>
</table>

Fig. 3. Mean (n = 12 ± SE) dark respiration (\( R_d \)), leaf mass per unit area (LMA), and leaf nitrogen content per unit area (\( I_a \)) in black walnut (\textit{Juglans nigra}) for the first (2007; ) and second (2008; ) growing seasons after plantation thinning as influenced by vertical position along the crown. Statistical differences (\( p < 0.05 \)); means not followed by the same letter within a year, and * above the x-axis between years.
water stress (Ni and Pallardy, 1991; Loewenstein and Pallardy, 1998), and for the combined months of May and June, precipitation was 276 mm in 2008 compared to 153 mm in 2007, with an historical average of 218 mm (National Weather Service, 2008). Additionally, SWC was higher near control trees in 2007; this suggests water stress may have played a role in the lack of response during the first growing season. Soil water may have been higher near control trees in 2007 because they were smaller than thinned trees, and thus may have been less impacted by any water deficits. First-tier competitors near control trees could have provided more shade on the ground, which contributed to less evaporation. In a thinning study of 10-year-old loblolly pine (Pinus taeda L.), Cregg et al. (1990) found that plant water relations were relatively unaffected by thinning treatments and differences in transpiration observed during the first growing season. Soil water may have been higher near control trees in 2007 because they were smaller than thinned trees, and thus may have been less impacted by any water deficits.

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Our second objective was to determine whether changes in light, water, or nutrient status after thinning has the greatest influence in mediating physiological and tree growth responses. Results suggest light was the only factor that varied according to treatment over time. The increase in $A_{\text{max}}$ is probably due to increased incident irradiance. Although no statistically significant interactions were found between thinning and vertical gradient, the biological significance of trends suggested by the data may help explain results. Light levels near thinned trees more than doubled at the bottom of the canopy from 2007 to 2008 (725–1533 μmol m$^{-2}$ s$^{-1}$), but decreased from 550 to 108 μmol m$^{-2}$ s$^{-1}$ under control trees during the same period. Data also suggests treatment differences in ambient light at middle and upper canopy positions were less pronounced. Thus, higher light levels near thinned trees at the bottom of the canopy may help explain the greater increase in $A_{\text{max}}$, while similar light levels at the middle and upper canopy positions may provide reason for the overall competitiveness and physiological performance of control trees. Lack of treatment differences in light levels in 2007, however, was unexpected and could be due in part to measurement error, e.g., partial shading of sensor by neighboring leaves during some measurements. Thus, we suggest that collecting estimates of ambient light while measuring light-response curves may not provide adequate characterization of the
light environment due to large variability when using this light measurement method (Fig. 2). Thinning of 19-year-old black walnut in a monocultural plantation showed increased light levels near thinned trees one year after thinning compared to non-thinned trees (Gauthier and Jacobs, 2009). Light levels reported were similar to those found in our study. Thinning also lowered water stress and increased leaf N content in thinned trees. Thinning of 9- to 13-year-old paper birch in Canada increased A of trees in thinned compared to non-thinned stands during the subsequent two growing seasons (Wang et al., 1995). Increased A was attributed to changes in light, foliar N concentration, and leaf area, but light was not directly measured. Thus, levels cannot be compared with results from our study. Increased radial growth of 43-year-old sessile oak two seasons following thinning in France was attributed in part to tree water relations as measured by higher predawn leaf water potential ($\psi_{pd}$) of thinned trees (Bréda et al., 1995). Many additional studies report improvements in $\psi_{pd}$ of gymnosperms after thinning (Succoff and Hong, 1974; Whitehead et al., 1984; Donner and Running, 1986; Aussenac and Granier, 1988; Pothier and Margolis, 1990; Skov et al., 2004). These results are consistent with ecophysiological responses found in this study in terms of light only because plantation thinning did not influence the water (SWC, leaf $\psi_{pd}$) or nutrient ($N_{a}$) status of thinned trees over the two-year period. Increased precipitation in 2008 could suggest that competition for water was not a limiting factor during the second growing season.

Some photosynthetic parameters did not respond to thinning as anticipated. Lowered I and increased Qc in thinned trees, from 2007 to 2008, are responses generally associated with increased shading, because trees typically acclimate to shade by initiating A at lower light levels and increasing quantum efficiency (Kozlowski and Pallardy, 1997; Lewis et al., 1999). Using one-year-old black walnut seedlings and a custom-made shade apparatus, Dean et al. (1982) demonstrated a 44% increase in Qc under the densest shading when compared to an unshaded control. Thinning increased light in 2008 and lowered CI near thinned trees; an increase in Qc and a lowering of Qe were more likely to occur. While increased light and lowered competition suggests thinning created a different environment near thinned trees compared to control trees, it appears thinning intensity may have not been sufficient to induce change in these photosynthetic parameters. Pre-thinning northern red oak mortality also lowered the effectiveness of the thinning treatment. Several studies report black walnut, and walnut species in general, adapt quickly to changes in environmental conditions such as light (Cochard et al., 2007) and are less likely to exhibit measurable physiological differences. Koike et al. (2001) found that the change in A between sun and shade leaves within the crown of Japanese walnut (Juglans ailanthifolia Carr.) was weaker than six other deciduous angiosperms. Dean et al. (1982) also reported that black walnut seedlings can maintain considerable photosynthesis under heavy shade. Frak et al. (2001) demonstrated that photosynthetic capacity of one-year-old hybrid walnut (Juglans nigra × regia) seedlings increased 25–45% within 20 days of transfer from low to high light conditions. The rapid acclimation of walnut may help explain some similarities among thinning treatments, such as increased $A_{max}$ in control trees from 2007 to 2008, although ambient light levels remained similar during that period.

Our last objective was to determine if ecophysiological parameters of black walnut vary along a vertical canopy gradient. Variations were found in $R_{d}$, LMA, $N_{a}$, and leaf $\psi_{pd}$. Position along the crown can have considerable influence on physiological parameters (Jurik et al., 1988; Bolstad et al., 1999). Increases in either LMA or $N_{a}$ along the canopy as indicated for both growing seasons, were reported previously in deciduous angiosperms (Reich et al., 1990; Ellsworth and Reich, 1993; Turnbull et al., 2002). These reflect changes in mesophyll thickness and N investments in the photosynthetic apparatus (Evans and Seemann, 1989) as influenced by the light intensity regime. Changes in $R_{d}$ along the canopy are also likely a reflection of the light environment (Lewis et al., 2000; Kenzo et al., 2006). Leaves at the bottom of the canopy are more conservative than leaves at the top of the canopy, and thus will tend to have lower respiration rates and water intake to maintain carbon balance under low light levels. This can also help explain increased leaf $\psi_{pd}$ reported from the bottom to the top of the canopy in 2008. Of interest in this study, however, is the finding of such differences considering that the length of the vertical gradient is only 5 m; previous studies reported gradients of 10 m or more. This suggests the open crown architecture of black walnut is highly variable in terms of physiological responses. Overall, distribution patterns of photosynthetic parameters such as $Q_{c}$, $A_{max}$ and leaf $\psi_{pd}$ along the canopy were less obvious than leaf structural traits such LMA and $N_{a}$, as was reported by Turnbull et al. (2002) for other deciduous angiosperms of the temperate region.

5. Conclusions

This experiment was established to investigate ecophysiological responses, along a vertical canopy gradient, of black walnut for the first (2007) and second (2008) growing seasons after plantation thinning. Overall, results indicate black walnut did not respond in 2007, possibly due to water stress. In 2008, thinned trees showed increased $A_{max}$ and $Q_{c}$ and lowered I compared to control trees. Increased $A_{max}$ was mainly due to increased light levels. Evidence of improved water or nutrient status was limited because no treatment differences were detected in SWC, leaf $\psi_{pd}$, and leaf $N_{a}$. Some photosynthetic parameters did not respond as anticipated: lowered I and increased Qc found in thinned trees would be expected from a decrease in light levels. Plantation thinning did not generate a substantial relative diameter growth increase. This may be due to low thinning intensity and mortality prior to thinning that reduced competition from first-tier neighbors. Many parameters varied along the vertical canopy gradient, but they did not influence thinning response. LMA, $N_{a}$, $R_{d}$, and leaf $\psi_{pd}$ increased from the bottom to the upper part of the canopy. Overall, patterns of photosynthetic parameters through the vertical canopy gradient were not as pronounced as leaf structural traits such LMA and $N_{a}$.

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