

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

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ABSTRACT

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.

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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 ($40^{\circ}23'09''N$, $86^{\circ}55'56''W$) was selected for study (Table 1). Mean annual temperature, precipitation, and frost-free intervals are $10.1^{\circ}C$, 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 $2.4\text{ m} \times 2.4\text{ m}$ spacing. Black walnut, white oak (*Quercus alba* L.), and northern red oak (*Quercus rubra* L.) 1+0 bareroot seedlings, 30–45 cm in height at time of planting, were provided by the Indiana Department of Natural Resources, Vallynia 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 near the seedlings. Current density is $1324 \pm 75 \text{ stems ha}^{-1}$ and basal area is $10.8 \pm 0.4 \text{ m}^2 \text{ ha}^{-1}$. No silvicultural treatments were applied prior to initiating this experiment.

2.2. Experimental design and thinning treatment

A randomized complete block design was established in this study, with thinning as the main plot and vertical gradient nested within the thinning treatment. The thinning treatment can be classified as a partial release. Six blocks were established in an attempt to account for potential differences in site variation and weather conditions between sampling dates. Each block was composed of six rows, with six trees in each row for a total of 36 trees, excluding mortality. In each block, two black walnut trees were selected based on similar form, height, and diameter at breast

Table 1

Mean ($\pm \text{SE}$) stand-level estimates of diameter at breast height (dbh), height, density, and survival of black walnut (*Juglans nigra*), white oak (*Quercus alba*), and northern red oak (*Quercus rubra*), prior to plantation thinning in March 2007.

Species	dbh (cm)	Height (m)	Density (stems ha^{-1})	Survival (%)
<i>J. nigra</i> (n=60)	13.6 ± 0.3	9.8 ± 0.2	498 ± 23	89
<i>Q. alba</i> (n=56)	7.7 ± 0.3	6.7 ± 0.2	498 ± 16	89
<i>Q. rubra</i> (n=38)	8.4 ± 0.4	8.3 ± 0.2	327 ± 53	58
All trees (n=154)	10.2 ± 0.3	8.3 ± 0.2	1324 ± 75	79

height (dbh). One tree served as an untreated control while the other was thinned. The number of trees sampled was based in part on preliminary gas-exchange measurements taken on black walnut trees for a similar study and adjusted to account for blocking and vertical gradient effects; full details on sample size estimates can be found in Gauthier and Jacobs (2009). To evaluate the effect of the vertical gradient, tree crowns were divided into thirds, lower ($5.6 \pm 0.3 \text{ m}$), middle ($7.6 \pm 0.3 \text{ m}$), and upper ($9.6 \pm 0.2 \text{ m}$), based on relative proportions specific to each tree. Randomization constraints required classification of the vertical gradient as nested within the thinning treatment. Based on average dbh, basal area, and stand density, crown competition factor (CCF) in this mixed species plantation was estimated at 150 (Schlesinger and Funk, 1977; USDA Forest Service, 1981). CCF is a unitless measure of competition that integrates tree size and stand density. Values greater than 100 reflect crowding; values less than 100 indicate room 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 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.

Hegyi's (1974) competition index (CI) was used to provide a quantitative estimate of competition from first-tier neighbors prior to thinning. It is computed (1) as the sum of the distance-adjusted

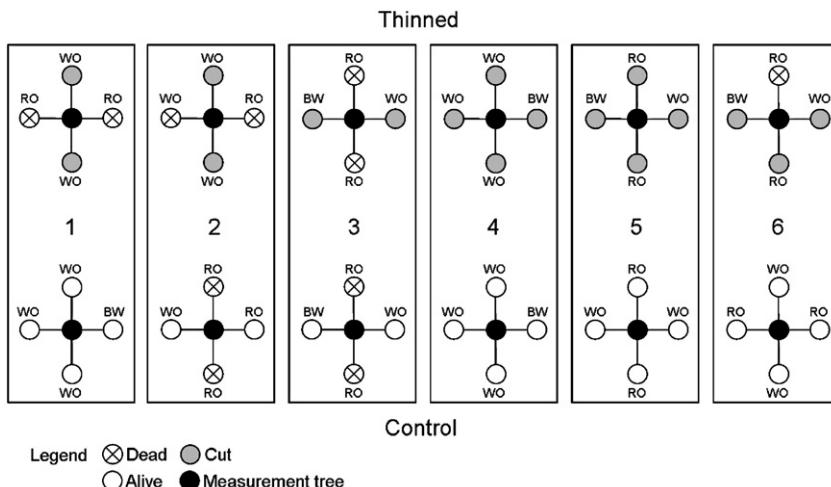


Fig. 1. Graphic representation of thinned (above) and control (below) replicates within each block (1–6). Species codes for first-tier competitors are as follows: black walnut (BW; *Juglans nigra*), northern red oak (RO; *Quercus rubra*), and white oak (WO; *Quercus alba*). Tree status is indicated by legend; each measurement tree is black walnut.

Table 2

Mean (\pm 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*).

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

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:

$$CI_i = \sum_{j=1}^{N_i} \frac{d_j}{d_i} \times \frac{1}{D_{ij}} \quad (1)$$

where CI_i is the competition index for the study tree i , d_j is the dbh of competitor j , d_i is the dbh of study tree i , D_{ij} the distance between i and j , and N_i 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) \times 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.) Kuntze) 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 [CO₂] 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_{air}) based on an external sensor attached to the chamber. Ambient light levels (photon photosynthetic flux density, PPF) 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 PPF 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 2000 μmol m⁻² s⁻¹ light range, with light compensation point (I_c , μmol photons m⁻² s⁻¹) determined when $y = 0$, dark respiration rate (R_d , μmol CO₂ m⁻² s⁻¹) determined when $x = 0$, and apparent quantum efficiency (Q_e , μmol CO₂ μmol⁻¹ photons) as the slope of the line. Light-saturated maximum photosynthesis (A_{max} , μmol CO₂ m⁻² s⁻¹) was measured according to the PPF 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_{md}) 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-3100, Li-Cor, Inc.), dried at 65 °C for at least 48 h, ground, and weighed for determination of leaf mass per unit area (LMA). Leaf N was measured using a LECO CNS-2000 Analyzer (LECO Corp.; St. Joseph, MI). Leaf N_a was computed as N concentration \times LMA (Wilson et al., 2000).

Soil temperature (T_{soil}) and water content (SWC) were measured in four of the 6 blocks by installing one data logger on the NE side of each measurement tree (Onset Computers, Bourne, MA). Each logger was equipped with one soil moisture sensor (ECH2O dielectric aquameter, Decagon Devices Inc.; Pullman, WA) and one 12-bit soil temperature sensor (Onset Computers, Bourne, MA) located ~2 m away from the bole of each measurement tree. Soil temperature sensors were inserted to a depth of 3 cm of the soil profile. Soil water content sensors were inserted to vertically monitor average water content in the top 20 cm of the soil profile. Measurements were taken every hour and averaged daily.

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 \times vertical gradient, thinning \times time, and vertical gradient \times 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 \times 3 canopy positions). For a vertical gradient effect only, $n = 12$ (6 study trees per treatment \times 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).

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_{\max}), light compensation point (I_c), leaf mass per unit area (LMA), leaf nitrogen content per unit area (N_a), midday leaf water potential (Ψ_{md}), ambient light (photon photosynthetic flux density, PPFD), apparent quantum efficiency (Q_e), dark respiration (R_d), and soil water content (SWC).

Variable	Year	Factor	df	F-value	p-value
A_{\max}	Both	Thinning × time	1	10.52	0.0024
I_c	Both	Thinning × time	1	4.88	0.031
PPFD	Both	Thinning × time	1	13.76	0.0005
Q_e	Both	Thinning × time	1	7.53	0.0072
SWC	2007	Thinning	1	13.62	0.03
R_d	Both	Vertical gradient × time	2	5.30	0.008
LMA	2007	Vertical gradient	2	26.62	<0.0001
LMA	2008	Vertical gradient	2	27.49	<0.0001
N_a	2007	Vertical gradient	2	14.20	0.0001
N_a	2008	Vertical gradient	2	25.83	<0.0001
Ψ_{md}	2008	Vertical gradient	2	4.47	0.02

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_{\max} , I_c , PPFD, and Q_e (Table 3 and Fig. 2). While A_{\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. 3). R_d varied over time along the vertical gradient (Fig. 3). In 2007, R_d was lower at the bottom of the canopy than at

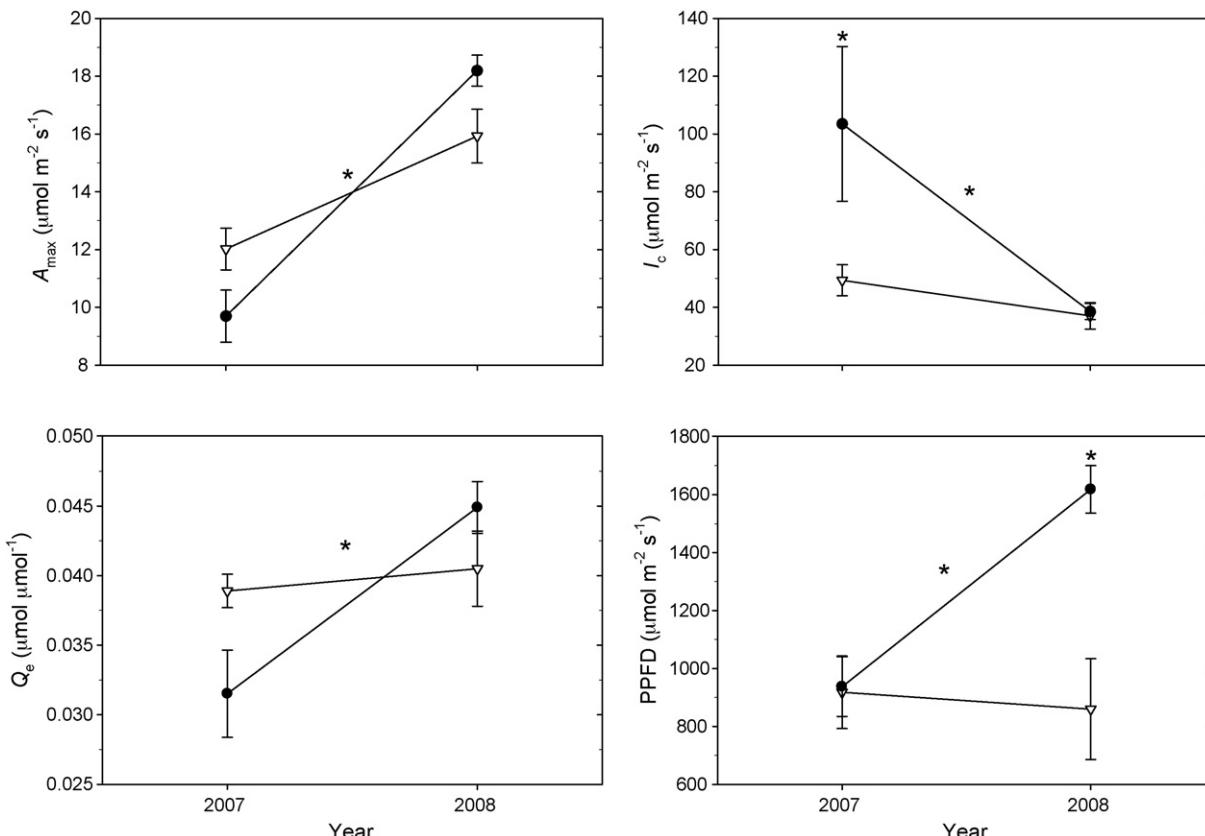


Fig. 2. Mean ($n = 18 \pm \text{SE}$) ecophysiological parameters in thinned (●) and non-thinned (▽) black walnut (*Juglans nigra*) for the first (2007) and second (2008) growing seasons after plantation thinning. From top left to bottom right: light-saturated maximum photosynthesis (A_{\max}), light 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.

Table 4

Mean ($n=6 \pm SE$) ambient light levels (photon photosynthetic flux density) near 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.

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

the top of the canopy ($p < 0.0001$, Fig. 3). In 2008, the bottom and middle canopy positions did not differ, but both were lower than the upper canopy position ($p < 0.0001$, 0.004, Fig. 3).

3.3. Main effects

Vertical gradient effects were found in LMA, N_a , and leaf Ψ_{md} . Both LMA and N_a were lower at the bottom canopy position than the upper canopy position ($p < 0.0001$) in 2007 and 2008 (Fig. 3). Leaf Ψ_{md} also varied along the canopy in 2008 ($p = 0.02$): the bottom crown position averaged -1.53 ± 0.08 MPa compared to the upper position with mean -1.96 ± 0.09 MPa ($p < 0.0001$).

A thinning effect was found in SWC only; SWC was higher near control trees compared to thinned trees in 2007 ($p = 0.03$), but not in 2008 (Fig. 4). All other parameters, including T_{air} and T_{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 ($15.7 \pm 1.3\%$ vs. $14.8 \pm 1.1\%$, $p = 0.52$). 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_{max} and Q_e as well as lowered I_c . These

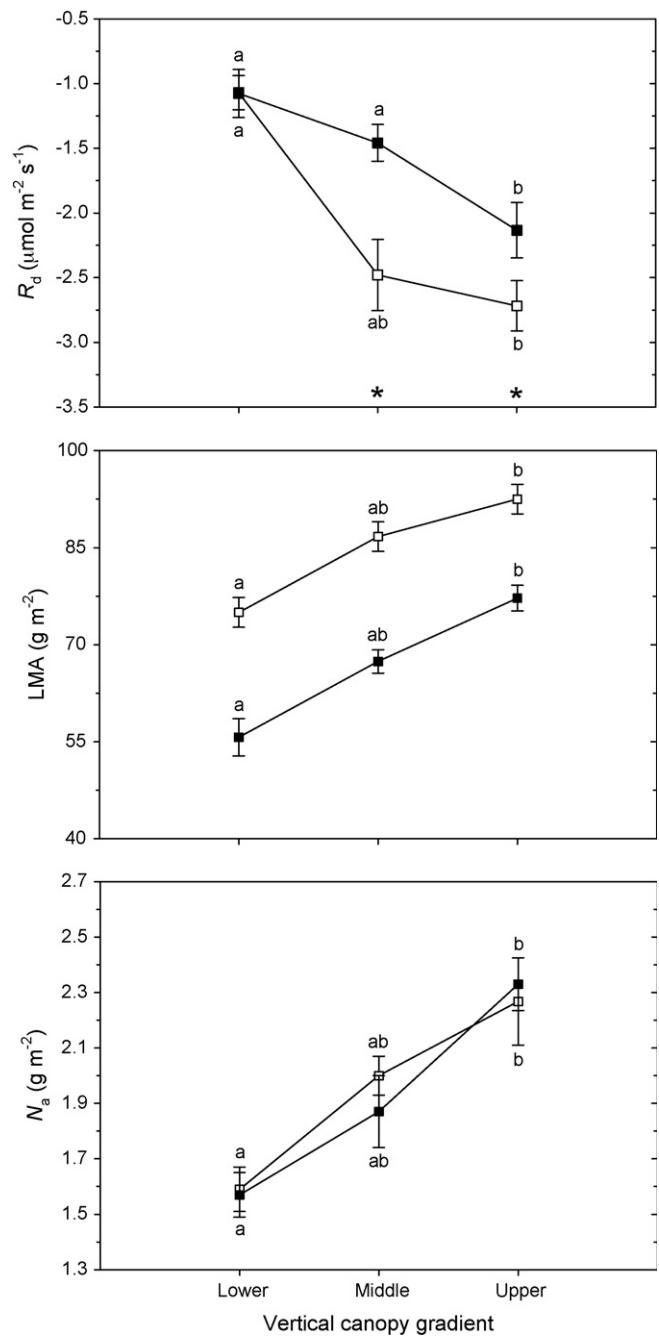


Fig. 3. Mean ($n = 12 \pm SE$) dark respiration (R_d), leaf mass per unit area (LMA), and leaf nitrogen content per unit area (N_a) in black walnut (*Juglans nigra*) for the first (2007; □) and second (2008; ■) growing seasons after plantation thinning as influenced by vertical position along the crown. Statistical differences ($p < 0.05$): means not followed by the same letter within a year, and * above the x-axis between years.

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

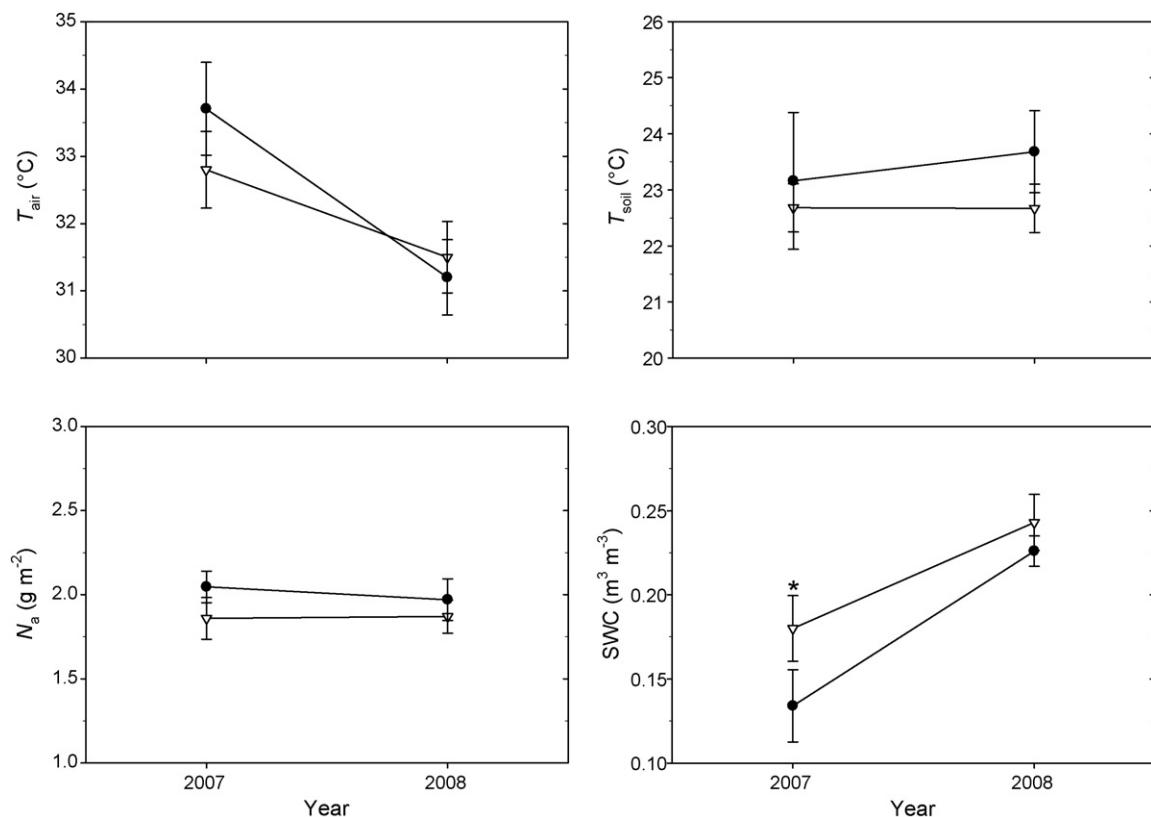


Fig. 4. Mean ecophysiological parameters in thinned (●) and non-thinned (▽) black walnut (*Juglans nigra*) for the first (2007) and second (2008) growing seasons after plantation thinning. From top left to bottom right: air temperature (T_{air}), soil temperature (T_{soil}), leaf nitrogen content per unit area (N_a) and average daily soil water content (SWC). Both T_{air} and N_a had six experimental units per treatment; T_{soil} and SWC had four. Statistical differences: * $p < 0.05$.

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 were more likely related to differences in light interception and crown exposure. Similar findings were reported by Gravatt et al. (1997), suggesting improvements in water relations may not always occur after thinning or may be ephemeral depending upon thinning intensity. Although pre-thinning estimates of crown competition suggest thinning was necessary, larger block size that would have allowed thinning on a radius wider than 2.4 m around study trees may have provided measurable increases in water status in 2007 or 2008. In black walnut, a partial release will generate less of a growth response than a complete release (Clark, 1967; Phares and Williams, 1971; Schlesinger and Funk, 1977; Bohanek and Groninger, 2003). It should be noted, however, that mortality of northern red oak prior to thinning reduced competition near measurement trees. Although mean CI values prior to thinning were not significantly different among treatments, lower CI near thinned trees compared to non-thinned trees in some of the blocks indicates that some of the thinned trees had already experienced a

slight release prior to thinning that may help explain the lack of response in 2007. No increase in CI near control trees two years after thinning also suggests non-thinned trees were competing well for resources compared to their first-tier neighbors. These factors limited the effectiveness of the thinning treatment despite a favorable decrease in CI near thinned trees compared to pre-thin competition levels. The short measurement period and inherent dbh measurement error could also account for part of the lack of differences.

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_{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 ($725\text{--}1533 \mu\text{mol m}^{-2} \text{s}^{-1}$), but decreased from 550 to 108 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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_{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 (Ψ_{pd}) of thinned trees (Bréda et al., 1995). Many additional studies report improvements in Ψ_{pd} of gymnosperms after thinning (Sucoff 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 Ψ_{md}) 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_c and increased Q_e 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 Q_e under the densest shading when compared to an unshaded control. Thinning increased light in 2008 and lowered CI near thinned trees; an increase in I_c and a lowering of Q_e 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 Ψ_{md} . 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 Ψ_{md} 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_e and A_{max} 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_e and lowered I_c 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 Ψ_{md} , and leaf N_a . Some photosynthetic parameters did not respond as anticipated: lowered I_c and increased Q_e 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 Ψ_{md} 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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