



Carbon and nitrogen accumulation within four black walnut alley cropping sites across Missouri and Arkansas, USA

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Abstract Agroforestry systems that integrate useful long-lived trees have been recognized for their potential in mitigating the accumulation of atmospheric fossil fuel-derived carbon (C). Black walnut (*Juglans nigra*) is frequently planted and cultivated in North America for its valuable lumber and edible nuts, and is highly amenable to the integration of understory crops or livestock in agroforestry systems. However, little is known about C content in black walnut trees, including the amounts of C assimilated into lignocellulosic tissues within different tree compartments. Therefore, allometric equations for above- and below-ground compartments of 10-year-old black walnut trees across diverse locations were developed. Ten grafted black walnut trees from each of four sites across the midwestern USA were destructively harvested for

above- and below-ground biomass, and dry biomass weight (DW_w), C (C_w) and nitrogen (N; N_w) stocks were quantified. Soils surrounding the harvested trees were sampled and analyzed for soil organic C (SOC) and total N (TN). Total DW_w ranged from 27 to 54 kg tree⁻¹, with woody tissues containing an average of 467 g kg⁻¹ C and 3.5 g kg⁻¹ N. Woody tissues differed in C_w and N_w across location, and above-ground sections contained more C and less N compared with most root tissues. The slopes of the allometric equations did not differ significantly among locations, while intercepts did, indicating that trees only differed in initial size across locations. SOC and TN did not vary in distance from the trees, likely because the trees were not yet old enough to have impacted the surrounding soils. Our results establish a

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foundation for quantifying C and N stocks in newly established black walnut alley cropping systems across diverse environments.

Keywords *Juglans nigra* · Allometry · Root–shoot ratio · Log–log models

Introduction

While numerous studies have estimated the carbon (C) accumulation potential for forests (e.g., Harmon 2001; Nepal et al. 2012; Woodbury et al. 2007), Nair et al. (2009) specifically proposed agroforestry as a strategy for impactful C assimilation; this is further supported by data from Cardinael et al. (2018a, b). Agroforestry systems that integrate woody species with other crops or livestock can provide a high marginal rate of return for producers (Benjamin et al. 2000), including the potential sale of C credits as such markets emerge (Udawatta and Jose 2012). Alley-cropping and silvopasture practices that incorporate long-lived woody plants into productive agricultural systems can sequester fossil fuel-derived C into stable and benign ligneous forms for decades (Verchot et al. 2007); mean C residence time depends on tree species and longevity, decomposition rate, and ultimate use of lumber and other wood products (Profft et al. 2009). Carbon assimilation that occurs in agroforestry farming systems is being recognized as a viable strategy for sequestration under the Clean Development Mechanism of the Kyoto Protocol to the United Nations Framework Convention on Climate Change (UNFCCC 2006), as well as by the Intergovernmental Panel on Climate Change (Cardinael et al. 2018a). For example, Udawatta and Jose (2012) estimated that agroforestry could offset current US CO₂ emission by 33%, with alley cropping accounting for 10% of the potential C sequestered by agroforestry practices. However, detailed studies are necessary to accurately quantify the amounts and rates of C assimilation in lignocellulosic biomass and in soils associated with various agroforestry species and practices to encourage adoption of such systems (Dollinger and Jose 2018; Udawatta and Jose 2012). Nitrous oxide, often released from agricultural systems as a result of excessive N fertilization or under-utilization of N fertilizers by crops, has also been

implicated in climate change (Ravishankara et al. 2009); therefore N assimilation into lignocellulosic tissues within agroforestry systems also merits study.

Black walnut (*Juglans nigra* L.) is indigenous to eastern North America and is well known for its high-value timber and edible nut production (Câmara and Schlegel 2016; Chenoweth 1995; Wolz and DeLucia 2019). The species is often cultivated and managed for lumber and veneer production, while intensive orchards of trees grafted to improved cultivars specifically for nut production are emerging (Reid et al. 2009). Black walnut trees are relatively fast growing under ideal conditions of soil type, soil fertility, and moisture, and are of interest in terms of C assimilation because they are frequently cultivated and because of their longevity. Most black walnut nut production orchards are established as widely spaced alley cropping systems, wherein other agricultural crops can be produced for several years until canopy closure (Garrett and Harper 1998). Black walnut alley cropping-based nut production systems can provide multiple income streams to producers while providing significant ecosystem services, including long-term C and N assimilation. However, little is known about C and N distribution and cycling in black walnut trees and surrounding soils, especially those grown in alley cropping practices. A better understanding of C stocks within black walnut production systems may also increase opportunities for the sale of C credits as that market expands. Data on the assimilation and distribution of N in such trees will contribute to more efficient N fertilizer use, less N runoff, and less evolution of nitrous oxide from agroforestry systems (e.g., Wolz et al. 2018).

Allometric equations are commonly used in estimating biomass production, and C and N accumulation by trees (e.g., Chojnacky et al. 2014; Dold et al. 2019; Jacobs et al. 2009; Jenkins et al. 2004; McPherson et al. 2016). However, the allometry of trees managed in agroforestry systems and within different environments is still poorly understood. Most allometric equations are derived from forest-grown trees that differ in their growth rate and canopy architecture compared with trees in alley cropping growth conditions, which can lead to substantial biomass over- and under-estimations (Zhou et al. 2014). Trees in agroforestry systems are less exposed to mutual shading compared with forest trees, and have a higher branch biomass and different tree

architecture with greater specific gravity (dependent on species) to support the larger tree canopy (Zhou et al. 2011; Schroth et al. 2015). In addition, trees have a high physiological and morphological plasticity to adapt to resource limitations, such as water, nutrients, and solar radiation, all of which change with site-specific climatic and soil conditions (Grams and Andersen 2007; Stovall et al. 2013), and which impact allometric equations (Lines et al. 2012). To the best of our knowledge these site-specific differences have not been addressed for black walnut allometry in alley cropping systems specifically to address C and N accumulation. Furthermore, previous studies of black walnut did not comprehensively estimate above- and below-ground C and N accumulation. Several studies investigated black walnut roots in agroforestry systems without considering below-ground C (Awaz et al. 2018; Germon et al. 2016; Zhang et al. 2015), or used general allometric equations from literature (Cardinael et al. 2017). Other studies focused only on black walnut above-ground compartments (Dupraz et al. 1999; Zellers et al. 2012). Ares and Brauer (2004) and Brauer et al. (2006) estimated nut production of black walnut varieties with allometric equations across different sites in the Midwestern US.

Site- and management-specific allometric equations for different tree species under various agroforestry practices need to be developed to enable better understanding of biomass production, C accumulation, and nutrient uptake in agroforestry systems. The objectives of this study were to quantify soil and tree biomass C and N in black walnut alley cropping systems across four latitudinally distinct environments

of similarly aged trees, and to establish site-specific allometric equations.

Materials and methods

Study locations and establishment

Ten grafted black walnut trees of similar age and origin from each of four different alley-cropping practices were destructively harvested, and the soil surrounding these trees extensively cored and sampled for this study. The four study sites are located in Missouri and Arkansas, USA (Table 1). Significant details on site location, design and establishment, alley vegetation establishment and management, soil chemistry, and weed and pest management were previously described (Brauer and Jones 2003; Burner et al. 2015; Coggeshall et al. 2003; Sauer et al. 2015; Thomas et al. 2008), with some relevant details summarized in Table 1. Specific details on site and tree fertilization at all four sites are provided in Thomas et al. (2008). The sites, arrayed north to south, were as follows:

New Franklin, MO (NF): The site was at the University of Missouri's Horticulture and Agroforestry Research Center near New Franklin in central Missouri. The soil was a Marshall silt loam (fine-silty, mixed, mesic Typic Hapludolls), a deep, well-drained, gently sloping, upland soil with moderate permeability and high water-holding capacity that formed on deep loess deposits (Grogger and Landtiser 1978). Site preparation began in summer 2000, then in February 2001, 180 trees were transplanted into nine rows

Table 1 Site descriptions (north to south) for the four black walnut alley cropping practices studied: New Franklin, MO (NF), Mount Vernon, MO (MV), Fayetteville, AR (FV), and Boonville, AR (BN)

Factor	NF	MV	FV	BN
Latitude N (°)	39.02	37.08	36.09	35.09
Longitude W (°)	92.76	93.87	94.19	93.97
Elevation (m)	197	378	427	139
USDA hardiness zone	6a	6a	6b	7b
Annual rainfall (mm)	975	1106	1094	1140
Tree spacing (m)	9.1 × 9.1	12.2 × 9.1	15 × 9.1	12.2 × 7.6
Soil type	Typic Hapludolls	Fluventic Hapludolls	Typic Fragiudults	Typic Hapludults
Site index for black walnut ^a	65	80	60–65	55–60

^aCarmean et al. (1989): site index is a relative prediction of tree growth based on a soil's suitability for a given tree species; the figures here are predicted black walnut tree height (in feet units) at 50 years. Data, respectively, from Grogger and Landtiser (1978), Hughes (1982), Harper et al. (1969) and Garner et al. (1980)

covering 1.5 ha. Weeds and vegetation within a 1 m radius of each tree were suppressed with two annual applications of glyphosate herbicide; alley vegetation consisted of mixed grasses and weeds that were mowed periodically (Coggeshall et al. 2003; Thomas et al. 2008).

Mt. Vernon, MO (MV): The site was located at the University of Missouri's Southwest Research Center near Mt. Vernon in southwest Missouri. The soil was an alluvial Huntington silt loam (fine-silty, mixed, mesic Fluventic Hapludolls), a deep, level, well-drained soil that infrequently floods (Hughes 1982). The site was prepared by killing existing vegetation with glyphosate herbicide in fall 2000, and trees were transplanted in February 2001. The orchard had 120 trees in nine rows covering 1.4 ha. Weeds and vegetation within a 1 m radius of each tree were suppressed with two applications of glyphosate herbicide annually; vegetation beyond this area consisted of mixed grasses dominated by tall fescue (*Festuca arundinacea* Schreb.) that was mowed periodically (Coggeshall et al. 2003; Thomas et al. 2008).

Fayetteville, AR (FV): The site was at the Arkansas Agricultural Research and Extension Center at Fayetteville in northwest Arkansas. The slope at the site was south-facing, ranging from 1 to 8%. Soils at the site formed from loamy deposits and cherty limestone residuum. Most of the site was Captina silt loam (fine-silty, mixed, mesic Typic Fragiudults), which has a fragipan at 40–60 cm depth (Harper et al. 1969). Glyphosate herbicide was applied in April and July 1999 to kill existing vegetation. In November 1999, 91 black walnut trees were transplanted into five rows covering 1.25 ha. Weeds and vegetation near trees were suppressed with a weed barrier fabric (1 m²) for the first 5 years, and thereafter with periodic applications of glyphosate herbicide within a 1-m radius. Alleys were initially planted to orchardgrass (*Dactylis glomerata* L.) that was harvested as hay through 2005, after which mixed grasses and weeds were mowed periodically (Sauer et al. 2015).

Booneville, AR (BN): The site was located at the USDA-ARS Dale Bumpers Small Farms Research Center, near Booneville, in west-central Arkansas. The soil at the higher elevation was an Enders silt loam (clayey, mixed, thermic Typic Hapludults) that transitions into a Leadvale silt loam (fine-silty, siliceous, thermic Typic Fragiudults) down a 5% slope. Both soils have low natural fertility, and are deep,

moderately well drained, with slow water permeability and medium water-holding capacity (Garner et al. 1980). The site was chisel-plowed in November 1999, after which trees were transplanted in December 1999. The orchard contained 72 trees in nine rows, and covered 0.7 ha. Approximately 40% of the trees suffered extensive damage due to a very heavy nut load in summer of 2002 and were replaced in January 2003 with similarly grafted trees. For this study, three trees from the 1999 planting, and seven from the 2003 planting were tested. Weeds and vegetation near trees were suppressed with a 1 m² weed barrier fabric. Alley vegetation consisted of mixed tall fescue and bermudagrass [*Cynodon dactylon* (L.) Pers.] that was mowed periodically (Brauer and Jones 2003; Burner et al. 2015).

Soil core sampling and analysis

Prior to tree excavation, soil cores were collected around each of the 40 study trees using a hydraulic soil probe (a utility vehicle-mounted #5-UV Model GSRPSUV at NF and MV; and a truck-mounted #15-SCS Model GSRPS at FV and BN; Giddings Machine Co., Fort Collins, CO). Soil cores (4.4 cm at NF and MV; 5.7 cm at FV and BN) were taken at opposing 1, 2, and 3 m radius positions measured from the center point of each tree trunk. Cores were taken in the direction of and within the tree rows at FV (east–west), NF, and MV (both north–south), whereas at BN, cores were taken in all four cardinal directions. Thus, six cores per tree were collected at NF, MV, and FV, and 12 cores at BN. Cores were collected to a depth of 100 cm at the four sites if possible. In some cases (especially at FV), impediments (rocks and fragipan) in the soil profile prevented collection of the deeper samples. Soil cores from each position were separated into vertical sections of 0–10, 10–20, 20–50, and 50–100 cm from the soil surface. A total of 891 soil samples were collected for analysis. Samples were initially stored at < 0 °C, then later dried in a ventilated greenhouse (warm ambient temperature). Dried samples were ground with a hammer mill (Model C-H, Viking Manufacturing Co., Manhattan, KS) to < 3 mm, then a $\approx$ 15 g subsample was further ground on a roller mill (Bailey Manufacturing Inc., Norwalk, IA) for 12 h to create a fine powder. A subsample of the powder was analyzed for soil organic carbon (SOC) and total nitrogen (TN) concentration

via the dry combustion method using a Flash 1112 or 2000 elemental analyzer (Thermo Finnigan, San Jose, CA).

Tree resource, harvest, and biomass data collection

The black walnut trees harvested from the four sites were all 2 years old from seed when transplanted, plus 8–11 years post-transplanting age (Table 2). All were grafted to superior nut-producing cultivars and established for agroforestry and nut production studies. Trees at NF and MV were produced identically and simultaneously in our own nursery for an initial multi-location rootstock study (Coggeshall et al. 2003; Thomas et al. 2008). They were all ‘Kwik-Krop’ scions grafted to potted 1-year-old rootstocks (either ‘Kwik-Krop’, ‘Sparrow’, or ‘Thomas’ seedlings) in early Spring 2000, then maintained in the nursery that summer. Trees for FV were identical except that they were produced and transplanted 1 year earlier (Sauer et al. 2015). Trees from BN included ‘Emma K’, ‘Kwik-Krop’, ‘Saubert’, and ‘Thomas’ scions grafted to ‘Kwik-Krop’ and ‘Thomas’ seedling rootstocks in various combinations (Brauer and Jones 2003; Burner et al. 2015). Trees at BN were produced in a similar manner as the other trees, but by Forrest Keeling Nursery (Elsberry, MO).

Forty black walnut trees (ten per site) were selected for analysis, and harvested in 2011 as follows: NF—week of April 4, MV—week of March 21, FV—week of June 21, BN—week of March 28. Above-ground tree biomass was measured through modified methods described by Jacobs et al. (2009). Tree trunk diameter was measured at 137 cm above soil level (diameter at breast height; DBH). Trees were then felled and height measured. Above-ground tissues were separated into trunk, large branches (≥ 12 cm diameter), and small branches (< 12 cm diameter), and fresh weight (FW) of all harvested materials determined. At time of harvest, leaves had emerged only at FV, but were not sampled. Cross-section samples of the trunks (approx. 5 cm thick) were collected at 30, 60, 120, and 180 cm above soil level, and representative samples of large and small branches were selected. Fresh weight was determined for all samples, which were then dried in a propane-fired dryer (≈ 50 °C) for several days until the weights stabilized, and then dry weight (DW) was measured. The fresh and dry weights of the samples

were then correlated to total tree biomass on a percent basis.

Below-ground tree biomass was determined at MV and BN by digging around the root system to a radius of 1.5 m and about 1.4 m deep with a backhoe, then lifting the main root system from the ground using a backhoe and chains, making every attempt to maintain the integrity of the root system. While this sampling protocol was not able to obtain 100% of the roots, this was a uniform method that secured the largest roots in order to accomplish the project objectives. Soil was removed from the roots with a pressure washer. Root systems were then separated into large roots (≥ 1.6 cm diameter), small roots (< 1.6 cm diameter), and root bole (the major solid, central, woody below-ground structure). Very fine feeder roots were not analyzed. A cross-section sample of the root bole (approx. 5 cm thick) was collected for each tree, as were sub-samples of large and small roots. Fresh and dry weights for samples and total below-ground biomass were determined as above.

Woody tissue C and N analysis

Woody tissue samples from small branches and roots were cut into small (< 1 cm) pieces with a pruning shear. For larger-diameter tissues, a hand-held electric drill with 8 mm-diameter drill bit was used to extract wood shavings in a representative manner from the sample. All samples were ground to 20 mesh ($< 841 \mu\text{m}$). Carbon and N concentrations of 298 woody samples were determined by dry combustion analysis of a 150 mg subsample using a Vario Max C/N analyzer (“Aspar 185” method; Elementar, Inc., Mt. Laurel, NJ). A subsample of each analyzed sample was dried for 24 h at 65 °C to determine moisture concentration, after which C and N values were corrected to a dry matter basis. Sample results were then correlated to total tree C and N content on a percent dry-weight basis.

Dry weight, C and N stock, and log–log models

The total above- and below-ground dry weight (DW_w), carbon (C_w), and nitrogen (N_w) stock was calculated as the sum of each above- and below-ground woody tree component n :

Table 2 Black walnut tree sample characteristics: average ($\pm$ SE) tree age and size, above- and below-ground DW_w, C_w and N_w, and tree compartment C and N from four alley cropping sites in Missouri and Arkansas, USA in 2011

Variable	Sub-variable	NF	MV	FV	BN
Tree age/size	Tree age (year) ^a	10.0	10.0	11.0	8.9
	Height (m)	5.4 $\pm$ 0.4	5.2 $\pm$ 0.2	4.5 $\pm$ 0.2	7.0 $\pm$ 0.4
	DBH (cm)	10.9 $\pm$ 1.1	9.5 $\pm$ 0.6	7.1 $\pm$ 0.5	15.0 $\pm$ 1.0
Above ground	DW _w (kg tree ⁻¹)	36.8 $\pm$ 8.3	17.0 $\pm$ 2.2	10.2 $\pm$ 1.2	36.0 $\pm$ 3.4
	r	0.61 $\pm$ 0.01	0.59 $\pm$ 0.01	0.58 $\pm$ 0.01	0.62 $\pm$ 0.02
	C _w (kg tree ⁻¹)	17.0 $\pm$ 3.8 a ^b	8.0 $\pm$ 0.1 b	4.8 $\pm$ 0.5 b	17.1 $\pm$ 1.6 a
	N _w (kg tree ⁻¹)	0.091 $\pm$ 0.022 a	0.054 $\pm$ 0.005 ab	0.023 $\pm$ 0.003 b	0.040 $\pm$ 0.003 b
Below ground	DW _w (kg tree ⁻¹)	–	9.6 $\pm$ 1.1	–	18.0 $\pm$ 2.2
	r	–	0.44 $\pm$ 0.01	–	0.48 $\pm$ 0.01
	C _w (kg tree ⁻¹)	–	4.4 $\pm$ 0.5 a	–	8.2 $\pm$ 1.1 a
	N _w (kg tree ⁻¹)	–	0.095 $\pm$ 0.011 b	–	0.120 $\pm$ 0.017 a
RS	(kg kg ⁻¹)	–	0.575 $\pm$ 0.02	–	0.509 $\pm$ 0.048
Trunk C _n and N _n					
at 30 cm	C (g kg ⁻¹)	461.0 $\pm$ 1.6	472.0 $\pm$ 0.7	469.4 $\pm$ 0.7	472.4 $\pm$ 1.1
	N (g kg ⁻¹)	1.9 $\pm$ 0.2	1.7 $\pm$ 0.2	1.2 $\pm$ 0	1.1 $\pm$ 0.1
at 60 cm	C (g kg ⁻¹)	459.2 $\pm$ 6.5	471.8 $\pm$ 1.4	469.2 $\pm$ 0.7	476.2 $\pm$ 1.1
	N (g kg ⁻¹)	1.8 $\pm$ 0.1	1.8 $\pm$ 0.2	1.4 $\pm$ 0.1	1.2 $\pm$ 0.1
at 120 cm	C (g kg ⁻¹)	465.8 $\pm$ 1.6	471.8 $\pm$ 1	468.0 $\pm$ 0.7	476.0 $\pm$ 1.0
	N (g kg ⁻¹)	1.7 $\pm$ 0.2	2.4 $\pm$ 0.2	1.6 $\pm$ 0.1	1.1 $\pm$ 0.1
at 180 cm	C (g kg ⁻¹)	466.4 $\pm$ 2.5	466.4 $\pm$ 2.7	466.5 $\pm$ 0.9	476.4 $\pm$ 0.9
	N (g kg ⁻¹)	1.6 $\pm$ 0.2	2.6 $\pm$ 0.1	1.8 $\pm$ 0.2	1.0 $\pm$ 0.1
Branch C _n and N _n ^c					
Large	C (g kg ⁻¹)	–	475.3 $\pm$ 0.9	472.1 $\pm$ 1.2	477.5 $\pm$ 0.5
	N (g kg ⁻¹)	–	2.4 $\pm$ 0.1	2.2 $\pm$ 0.1	1.4 $\pm$ 0.1
Small	C (g kg ⁻¹)	461.7 $\pm$ 1.2	472.7 $\pm$ 0.9	473.9 $\pm$ 1.3	–
	N (g kg ⁻¹)	4.3 $\pm$ 0.6	8.4 $\pm$ 0.3	5.4 $\pm$ 0.2	–
Root C _n and N _n ^d					
Large	C (g kg ⁻¹)	–	468.9 $\pm$ 1.1	–	460.9 $\pm$ 2.3
	N (g kg ⁻¹)	–	9.0 $\pm$ 0.8	–	3.9 $\pm$ 0.5
Small	C (g kg ⁻¹)	–	441.3 $\pm$ 3.8	–	442.5 $\pm$ 2.3
	N (g kg ⁻¹)	–	16.7 $\pm$ 1	–	9.6 $\pm$ 0.6
Root bole	C (g kg ⁻¹)	–	453.5 $\pm$ 8.7	–	–
	N (g kg ⁻¹)	–	2.7 $\pm$ 0.2	–	–

DW_w, dry weight; DBH, tree diameter (cm) at 137 cm above soil level; r, DW:FW ratio; RS, root-shoot ratio; C_n, carbon concentration; C_w, carbon stock; N_n, nitrogen concentration; N_w, nitrogen stock; NF, New Franklin MO; MV, Mt. Vernon MO; FV, Fayetteville AR; BN, Booneville AR

^aPost-transplanting age; all trees were 2 years old from seed when planted

^bMeans across rows with the same letters are not different according to the Tukey Test ($p < 0.05$)

^cLarge branches ≥ 12 cm diameter, small branches < 12 cm diameter

^dLarge roots ≥ 1.6 cm diameter, small roots < 1.6 cm diameter

$$DW_w = \sum_{i=1}^n \bar{r}_n * FW_n \quad (1)$$

$$C_w = \sum_{i=1}^n \bar{r}_n * FW_n * \bar{C}_n \quad (2)$$

$$N_w = \sum_{i=1}^n \bar{r}_n * FW_n * \bar{N}_n \quad (3)$$

where DW_w = total above- or below-ground dry weight (kg); $\bar{r}_n$ = average DW:FW ratio of sample DW divided by sample FW of tree component n (i.e., trunk, branches, roots) (kg kg^{-1}); FW_n = measured tree component fresh weight (kg); C_w = total above- or below-ground carbon stock (kg); C_n = average sample carbon concentration of tree component n (g kg^{-1}); N_w = total above- or below-ground nitrogen stock (kg); N_n = average sample nitrogen concentration of tree component n (g kg^{-1}).

The sample tree root–shoot ratio (RS) was calculated as the quotient of below- and above-ground DW_w . An allometric equation (log–log model) with DBH as independent variable, and DW_w , C_w , and N_w as dependent variables was developed:

$$\ln(y) = \ln(a) + b * \ln(x) \quad (4)$$

where y is the response variable (above- and below-ground DW_w , C_w , and N_w , all in kg), a is the intercept, x is the explanatory variable DBH (cm), and b is the slope.

Equation (4) was used to calculate total DW_w , C_w , and N_w for the FV site using DBH measurements for the ten excavated trees plus 38 additional similar-age-and-size black walnut trees at the site that were not excavated (Sauer et al., 2015). To minimize the bias introduced by logarithmic transformation, a correction factor proposed by Shen and Zhu (2008) was used as a multiplier, which yielded reliable estimates and predictions (Clifford et al. 2013). The correction factor was calculated with a , b , and the model estimate of σ , and ranged from 1.004 to 1.028.

C accumulation (dC_w), and N uptake rates (dN_w) for the FV site were calculated on the tree stand and average tree as C_w divided by years after planting (i.e., 11 years). The same calculation was used for N_w uptake and DW_w growth.

Statistical analyses

Differences among above- and below-ground tree tissue, and site for C_w and N_w were statistically analyzed with two-way analysis of variance (ANOVA) with site and tissue as factors ($p \leq 0.05$), using the *aov*- and *anova*-function in R (R Foundation for Statistical Computing, Vienna, Austria). Differences among means were statistically analyzed with Tukey Test, using the *TukeyHSD*-function in R. An analysis of covariance (ANCOVA) with site as covariate was used in Eq. (4) to determine if the slope and intercepts were significantly different among sites ($p \leq 0.05$). A step by step approach was used to find the most parsimonious model (i.e., from differences in slopes and/or intercepts to no differences among sites). The log–log models and ANCOVA were calculated in R using the *lm*-, *step*-, and *anova*-function. Differences in SOC and TN among sites, soil depth, and distance to the tree were statistically analyzed with three-way ANOVA and Tukey Test in R.

Results

Tree C_w and N_w across sites

Mean tree height and DBH varied among sites, ranging from 4.5–7.0 m and 7.1–15.0 cm, respectively, and increased in the order $FV < MV < NF < BN$ (Table 2). Similarly, the above- and below-ground DW_w varied among sites, with average above-ground DW_w ranging from 10.2 kg tree^{-1} at FV to 36.0 kg tree^{-1} at NF, and below-ground DW_w 9.6 kg tree^{-1} at MV and 18.0 kg tree^{-1} at BN. Total tree DW_w for these ≈ 10 -year-old trees ranged from 26.6 kg tree^{-1} at MV to 54.0 kg tree^{-1} at BN. The DW:FW ratio (r) was higher for above-ground tissues compared with below-ground tissues. The root–shoot ratio (RS) was 0.575 and 0.509 kg kg^{-1} at the MV and BN site, respectively. While the soil site index (Table 1; Carmean et al. 1989) was least favorable at BN, other factors such as higher annual rainfall, warmer weather, and a longer growing season at that more southerly site may have influenced tree growth there.

Differences in C_n and N_n concentrations in trees were noted among the four sites and among tissues (Table 2). Interestingly, while woody tissues at BN

Table 3 Log–log model coefficients for DW_w , C_w (both kg), and N_w (g), with mean intercept a , mean slope b , and coefficient of determination R^2

Above-ground	Variables	Parameters	NF	MV	FV	BN
	DW _w	a	-0.500*	-0.948***		
		b	1.663***			
		R ²	0.90			
	C _w	a	-1.286***	-1.711***		
		b	1.670***			
		R ²	0.90			
	N _w	a	0.655ns		0.168***	-0.375***
		b	1.505***			
		R ²	0.78			
Below-ground	Variables	Parameters	MV		BN	
	DW _w	a	-0.861ns			
		b	1.371***			
		R ²	0.75			
	C _w	a	-1.656**			
		b	1.372***			
		R ²	0.75			
	N _w	a	2.551***			
		b	0.836***			
		R ²	0.44			

Note that these models were developed with a DBH range of 4.5–18.3 cm, and that predicted values outside that range may not be correct

DW_w , dry weight; C_w , carbon stock; N_w , nitrogen stock; DBH, tree diameter (cm) at 137 cm above soil level; NF, New Franklin MO; MV, Mt. Vernon MO; FV, Fayetteville AR; BN, Booneville AR

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant

generally had higher C_n , they had the lowest N_n , with tissues at NF being the opposite (lowest C_n but among the highest N_n). One possible hypothesis for the lower level of N at BN is that those trees were already producing heavy nut crops; the nuts might have been aggressive sinks for mobile N in the trees. Above ground tissues (both trunks and large branches) tended to have higher C_n but lower N_n compared with below-ground tissues. The total above- and below-ground C_w ranged from 4.8–17.1 and 4.4–8.2 kg C tree⁻¹, respectively. The total above- and below-ground N_w ranged from 0.023–0.091 and 0.095–0.120 kg N tree⁻¹, respectively. Above-

ground C_w was significantly higher at NF and BN than at MV and FV, while N_w was higher at NF than at FV and BN. Mean C_w across all sites was significantly higher in above-ground tissues (11.7 kg tree⁻¹) compared with below-ground (6.3 kg tree⁻¹), while N_w was higher in below-ground tissues (0.108 kg tree⁻¹) compared with above-ground (0.052 kg tree⁻¹). Site $\times$ tree tissue interactions for C_w and N_w in this study were not statistically significant ($p < 0.05$).

The above-ground DW_w , C_w , and N_w log–log models were significantly different in intercepts among sites, but slopes were not different (Table 3; Figs. 1, 2). The above-ground log–log models

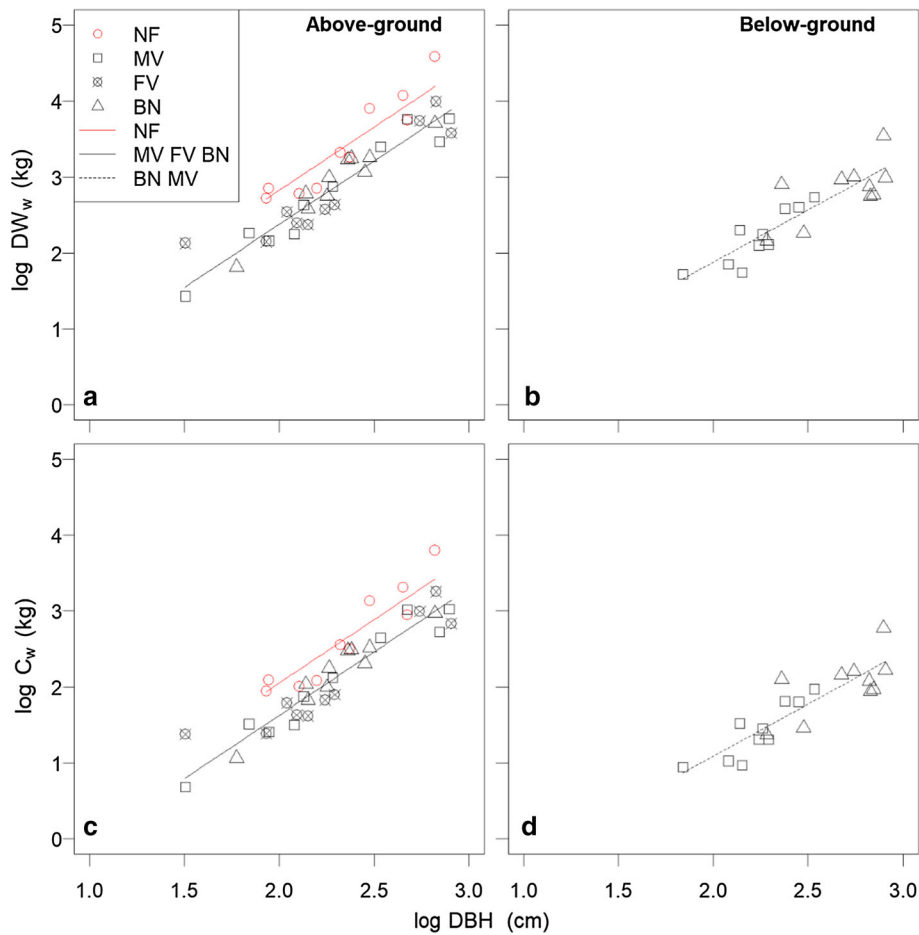


Fig. 1 Site-specific log–log models with above-ground (a, c) and below-ground (b, d) dry weight (DW_w) (a, b) and carbon (C_w) (c, d) as dependent, and DBH as independent variable,

respectively. All sites have the same slope, but intercepts differ in above-ground DW_w and C_w on the NF site. DBH = tree diameter (cm) at 137 cm above soil level

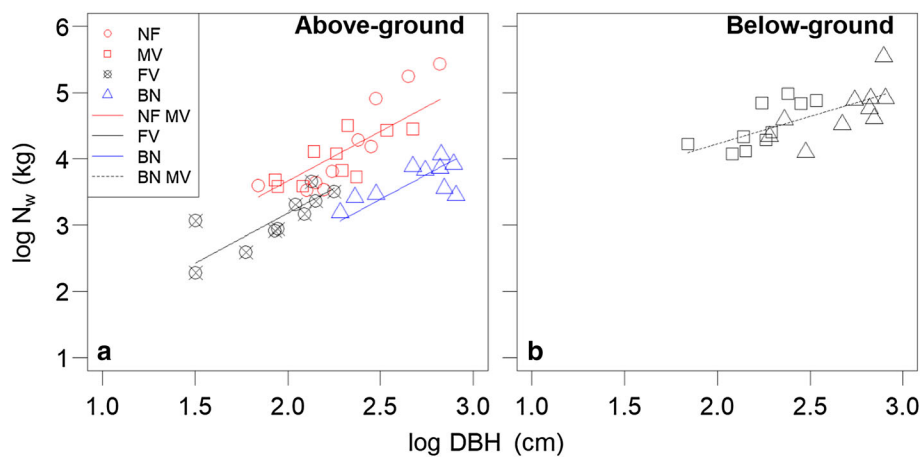


Fig. 2 Site-specific log–log models with above-ground (a) and below-ground (b) nitrogen (N_w) as dependent, and DBH as independent variable, respectively. All sites have the same

slope, but intercepts differ in above-ground N_w among sites. DBH = tree diameter (cm) at 137 cm above soil level

Table 4 Statistical analysis^a of soil organic carbon (SOC) and total N (TN) concentrations in soil core samples from four black walnut alley cropping sites in Missouri and Arkansas, USA, at three distances and four depths adjacent to the trees in Spring 2011

NF, New Franklin MO; MV, Mt. Vernon MO; FV, Fayetteville AR; BN, Booneville AR

^aMeans within sub-columns with the same letters are not different according to the Tukey Test ($p < 0.05$); *** $p < 0.001$; ns not significant

^bWithout BN and FV site, low sample size

Variable	Sub-variable	SOC (g kg ⁻¹)	TN (g kg ⁻¹)
Site	NF	8.1 ± 0.3d	1.14 ± 0.03b
	MV	9.2 ± 0.4c	1.17 ± 0.03b
	FV	12.6 ± 0.6a	1.21 ± 0.05ab
	BN	10.3 ± 0.5b	1.27 ± 0.04a
	<i>p</i>	***	***
Distance (m)	1	9.5 ± 0.4	1.17 ± 0.03
	2	9.6 ± 0.4	1.19 ± 0.03
	3	10 ± 0.4	1.21 ± 0.03
	<i>p</i>	ns	ns
	Depth (cm)		
	0–10	16.3 ± 0.4a	1.71 ± 0.03a
	10–20	9 ± 0.2b	1.14 ± 0.02b
	20–50	5.7 ± 0.1c	0.87 ± 0.02c
	50–100 ^b	4.1 ± 0.1c	0.77 ± 0.02c
	<i>p</i>	***	***
Site × distance	<i>p</i>	ns	ns
Site × depth	<i>p</i>	***	***
Distance × depth	<i>p</i>	ns	ns
Site × dist × depth	<i>p</i>	ns	ns

explained 78–90% of the variation. The site-specific DW_w and C_w intercepts were significantly higher at NF than at BN, MV, and FV. The N_w intercept was different and increased in the order $BN < FV < NF = MV$. The slope and intercept of the below-ground DW_w , C_w , and N_w log–log models at BN and MV were not different, and explained 44–75% of the variation.

The total black walnut stand and individual tree average DW_w , C_w , and N_w were calculated for the FV site (1.25 ha) using the site-specific log–log model equation (Eq. 4; Table 3). Total above-ground tree stand DW_w was 691 kg ha⁻¹ with an average of 18.0 kg tree⁻¹. Total C_w was 327 kg ha⁻¹ with an average C_w of 8.5 kg tree⁻¹. Carbon accumulation rate was 29.7 kg ha⁻¹ year⁻¹. Total N_w was 1.46 kg ha⁻¹ and on average 38.1 g tree⁻¹. The N_w uptake rate was 133.1 g ha⁻¹ year⁻¹.

SOC and soil TN

Significant differences in background SOC and soil TN were noted among the four sites (Table 4). The SOC and TN did not differ with increasing distances from the tree trunks collectively or across strata at the various soil depths, and there were no significant site × distance interactions. The highest SOC and TN were consistently found in the top 10 cm of soil, with

consistent and significant decreases in SOC and TN with increasing depth. We also found consistent site × depth interactions.

Discussion

Above- and below-ground biomass C and N

Despite similar tree age, differences in mean tree heights and DBH as well as in DW_w , C_w and N_w were detected among sites, likely due to varying site, climatic, and management factors. The fragipan soil at FV was the least suitable for black walnut trees and was manifested in poor tree growth overall. This may have limited the uptake of soil N through reduced root exploration of the soil profile. Zhang et al. (2017) noted increasing RS with increasing age for plantation-grown *Juglans regia*, but their RS at similar tree age (0.27–0.30) was lower compared with this study. Our above-ground C_n concentrations were within the range of temperate angiosperm tree species (Thomas and Martin 2012), higher than reported by Zhang et al. (2017) for *J. regia* (421.6–464.6 g kg⁻¹) and by Cardinael et al. (2017) for hybrid walnut (*J. regia* × *J. nigra*) (445.7 and 428.6 g kg⁻¹), yet lower than reported by Lamloom and Savidge (2003) for *J. nigra*

(491.7 g kg⁻¹). The below-ground C_n was within the range of *J. regia* reported by Zhang et al. (2017). N_n was higher than typical N ranges of wood tissue of 0.7–1.2 g kg⁻¹ (Chave et al. 2009), especially the below-ground tissue. The wide range of RS and C_n within *Juglans sp.* and the significant differences of C_w and N_w among sites and tissues indicate that more data are needed to accurately quantify the C and N accumulation in above- and below-ground tree compartments.

Log–log models

The log–log model intercept differed among sites, i.e., DW_w , C_w , and N_w at NF and MV were higher at a given DBH than at FV and BN. The above-ground intercepts increased along site latitudes, despite the growth-stunting conditions at the FV site (Tables 1, 3). This could indicate that above-ground intercepts were primarily influenced by environment. The slopes were not significantly different among sites, i.e., the DW_w , C_w , and N_w increased per unit of DBH at the same rate. Site-specific differences were not detected for below-ground models, i.e., amount and rate of root DW_w , C_w , and N_w in relation to DBH were equal among sites. To the best of our knowledge, there are no allometric equations previously published for *J. nigra* DW_w , C_w , or N_w . Previous studies focused on nut production or wood volume allometry (Ares and Brauer 2004; Brauer et al. 2006), of which the latter can be used for DW_w calculation. A generalized DW_w model with $a = -2.5095$ and $b = 2.5437$ was proposed for trees of the *Juglandaceae* family (Chojnacky et al. 2014), and previous open-grown *J. nigra* models focused on DBH growth over time or other allometrics (Cabanettes et al. 1998; McPherson et al. 2016). The study by Cardinael et al. (2017) was limited by using a generalized model for below-ground biomass estimations and non-destructive measurements of *J. nigra*. The black walnut log–log models herein can predict DW_w , C_w , and N_w for a wide DBH range (4.5–18.3 cm), with site-specific intercept adjustment (Table 3). We suggest selecting the coefficients with the most comparable conditions for use in other applications. Also, note that R^2 values of the N_w log–log models were low, and we suggest using mean N_n concentration and DW_w coefficients to calculate total N uptake.

SOC and soil TN concentrations across sites

The significant differences in SOC and TN among sites were likely due to inherent local environmental and pedological conditions and characteristics, as management factors among sites were relatively similar. The highest SOC and TN in the upper soil layer is a consistent pattern commonly observed in a variety of soils and settings (e.g., Burner et al. 2013; Jobbagy and Jackson 2000). The SOC and TN did not differ with distance from the trees which likely had not been established long enough (i.e., ≈ 10 years) to spatially affect SOC concentrations. While tree roots should have been present in most of this soil profile (0–100 cm deep and 1–3 m laterally from trunk; except at FV due to fragipan), we were not able to attribute any of these SOC and TN differences to the presence of tree roots. Furthermore, control of perennial grasses and weeds within the drip line might have impacted tree root growth and SOC accumulation due to lack of cover and associated roots in those areas (Brauer et al. 2004; Burner et al. 2015; Mulia and Dupraz 2006). Cardinael et al. (2017) found high variation in SOC topsoil stocks in a 6-year-old hybrid walnut (*J. regia* $\times$ *J. nigra*) agroforestry system, with both significant increase and no change in SOC compared to monocropping, while older agroforestry systems showed consistent significant increases. Cardinael et al. (2018b) underscore the complexity of SOC and TN dynamics in agroforestry systems, of which tree roots are only one of numerous interacting and competing factors, both biological and physical.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest associated with this research or publication.

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