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Responses of mature roadside trees to root severance treatments

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ABSTRACT

Conflicts between trees and infrastructure installation or repair occur frequently in the urban environment and may result in damage to or complete removal of tree roots. We monitored the responses of trees to linear root cutting and examined the effects of the presence of a road within tree root zones. Mature *Quercus virginiana* Mill trees (n = 31) were exposed to three root pruning treatments (plus a control) consisting of a trench on one side of the tree, offset by a distance as a multiple of trunk diameter (3x, 6x and 12x). Roots were severed manually, and their diameters were measured at the cut point to estimate a total cross-sectional area of severed roots. The total severed root cross-sectional area was subsequently expressed as a ratio of whole trunk cross-sectional areas (termed $Ar_{(BH)}$), as well as trunk conductive sapwood areas (termed $As_{(BH)}$) at breast height (1.4 m). The shortest horizontal distance between the tree bases and the edge of a nearby road was also recorded and included in statistical analyses as a continuous variable. Linear root cutting reduced shoot extension and leaf area by as much 146.13 mm ($p < 0.01$) and 2.92 cm^2 ($p < 0.001$) respectively, one growing season after root severance. Trees nearer the road had significantly reduced trunk diameter growth ($p < 0.05$), independent of root severance effects, i.e. 12x, 6x and 3x treatments. Pre-dawn leaf water potential was negatively affected for all treatments ($p < 0.05$) and the symptoms of water stress persisted in the 3x treatment for 440 days. $Ar_{(BH)}$ and $As_{(BH)}$ ratios were regressed against physiological (predawn leaf water potential (Ψ), stomatal conductance (g_s), leaf temperature (T) and two chlorophyll fluorescence variables (Fv/Fm and Fv/Fo)) and growth (trunk diameter, shoot extension and leaf area) responses. $Ar_{(BH)}$ was a significant predictor of Ψ, Fv/Fm, Fv/Fo, shoot extension and leaf area responses after one growing season, although model strength varied (R^2 = 0.75, 0.14, 0.16, 0.18, 0.24 respectively). To avoid sustained water stress symptoms, linear root cutting on *Q. virginiana* should not be undertaken closer to trees than six times DBH, equating to ≈ 25% root system loss.

1. Introduction

City streets are often modified to accommodate an increasing need for utility and network services, due to urban growth and development (Sánchez et al., 2013). Similarly, upgrades to pedestrian and vehicular transport networks are often carried out to meet modern engineering requirements and to accommodate population growth (Chi, 2012; Duranton and Turner, 2012; Chen et al., 2014). Whilst trenchless technologies exist for utility works, their use can be contingent on detailed plans for existing buried infrastructure (Metje et al., 2007), and so many of these types of activities involve trenching works and excavations in the public transport corridor (Rogers et al., 2012). These types of ground alterations can place nearby urban trees at risk of root damage, leading to partial destruction or removal of root systems (Jim, 2003; North et al., 2017) and contributing to increased urban tree mortality (Hauer et al., 1994). Linear excavations which sever roots at distances equating to six times the trunk diameter at breast height (DBH ≈ 1.4 m), may result in the removal of 24.5% of the root system of trees with a 35 cm DBH (Day et al., 2010). Trenching works that sever roots closer than three times DBH, can significantly reduce tree stability when compared to trees that have not had their roots removed (Smiley, 2008). Besides negatively affecting tree stability (Smiley et al., 2014), removing roots can lead to reduced growth (Pretzsch et al., 2016) and vitality (Watson, 1998) as well as altered physiological processes (Benson et al., 2019a).

During greenfield-type site developments, root pruning and root care practices often involve setting aside an area of ground around a tree (a tree protection zone) to define an area of protection, or isolation from construction work (Standards Australia, 2009; British Standards Institute, 2012; Fite and Smiley, 2016). In the urban environment, where trees are often planted in narrow berms alongside roads and footpaths (Jim, 1997), complete isolation from utility or infrastructure

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works may not be possible, and arboricultural specialists may need to rely on experience or other guidelines when making decisions about root pruning and root care. Occasionally, local government regulatory frameworks contain specifications on root pruning for infrastructure-related works around urban trees (eg. The City of Charlottesville, 2009; The City of Boroondara, 2010; The City of Rancho Cucamonga, N.D), although empirical evidence supporting these guidelines may be limited (Auckland Council, 2018).

Some guidance documents recommend that root pruning should be offset from tree trunks by no less 2 m (The City of Regina, 2000), five (The City of Bellevue, 2009) or three (Fite and Smiley, 2016) times the trunk diameter. The discrepancies between these guidelines may be due to a lack of research in specific root pruning practices (Costello et al., 2017). Other root pruning guidelines consist of fixed diameter thresholds, above which roots should not ordinarily be removed. The National Joint Utilities Group (2007) in the United Kingdom recommend that this threshold be set at 25 mm in diameter, as too does the British Standards Institute (2012). In some regions of New Zealand, the threshold set by local authorities may permit the removal of roots as large as 80 mm in diameter (Auckland Council, 2018). The disadvantage of using a fixed diameter threshold, is that cumulative root loss relative to tree size cannot be accounted for, as it can be when a trunk diameter-defined trenching offset is adopted.

To account for cumulative root loss using individual roots, recent work (Benson et al., 2019a, b) has focused on using tree allometry and the principles of the Pipe Model Theory (Shinozaki et al., 1964a, b) to quantify the impact of root severance treatments. The Pipe Model describes the relationship between the conductive sapwood area in the trunk, and other tissues such as leaves (Grabosky et al., 2007; Lubczynski et al., 2017) and root systems (Kaipiainen and Hari, 1985; Gould and Harrington, 2008). These initial root pruning studies revealed significant relationships between the ratio of severed root cross-sectional area to trunk or trunk sapwood cross-sectional areas and the physiological and morphological responses of two different species of *Acer* (Benson et al., 2019b) as well as *Quercus virginiana* (Benson et al., 2019a), when roots were severed in multiple linear trenches at 30 cm from the tree base, and in circumferential trenches made incrementally closer to the tree, respectively. This type of allometric relationship has also been employed when investigating the effects of stability loss following root cutting, revealing significant correlations between the force required to pull standing trees to 1 °, and the ratio of severed root cross-sectional area (CSA) to trunk CSA (Σ(severed root CSA) / trunk CSA at 1.37 m) (Smiley et al., 2014).

Recent root pruning research using *Quercus virginiana* has replicated the types of root severance which may be encountered on a development site, where root loss may occur on all sides (Benson et al., 2019a); for example, due to grade changes. The purpose of this investigation was to replicate the types of root severance which may occur on one side of the tree in a single trench; for example, during utility trenching. Specifically, our objectives were to:

- Investigate the responses of mature landscape trees in response to linear root cutting (trenching), at varying distances (as a function of trunk diameter).
- Investigate how these responses may be affected by the presence of a road.

In doing so, we also investigate the use of allometry and the relationships between total severed root cross-sectional area and trunk cross-sectional area, to quantify root pruning doses. The easiest way to account for cumulative root loss is to provide an encroachment threshold - at which root loss should not occur - as several guidance documents do (e.g. Standards Australia, 2009; Fite and Smiley, 2016). However, in some regions of the world, the guidelines provide individual root pruning thresholds, specifying a maximum root diameter (e.g. Auckland Council, 2018). The purpose of investigating the allometric method was to investigate whether this would be a suitable alternative to selective root removal, and to account for cumulative root loss and tree size.

2. Materials and methods

2.1. Study sites and trees

The study was conducted using 31 *Quercus virginiana* Mill trees (mean trunk diameter at 1.40 m = 34.20 cm (δ = 4.40 cm); mean height = 8.86 m (δ = 1.09 m)) planted from 170 L containers into a loamy sand (mean bulk density = 1.43 g/cm^3 (δ = 0.09 g/cm^3), n = 5) in 2005 at the University of Florida's Gulf Coast Research and Education Center, Balm, Florida, USA (27° 45' 41.76" N. 82° 13' 41.01" W). The trees formed part of the landscape at the research centre and were planted in a lawn area surrounding the grounds (mean spacing = 8.14 m (δ = 5.6 m)). The root zones of the trees had not been altered since planting. A low traffic-volume asphalt road surface ran adjacent to the lawn area that formed the trees' growing environment. The distance from the edge of the road to the tree bases ranged from 2.3 m to 9.1 m. A diagrammatic representation of the experimental site is depicted in Fig. 1. To account for the presence of the road, the shortest horizontal distance between the road edge and the centre of each tree at its base was measured (Road_D).

Mean annual rainfall at the site is 1216.62 mm and mean annual temperature is 21.07 °C (FAWN, N.D.). Temperature and rainfall were recorded using an on-site weather station with data loggers (Campbell Scientific, Logan, Utah, USA) (Fig. 2). In September of 2017, the site and surrounding area were affected by Hurricane Irma (Cangialosi et al., 2018). Though all trees remained standing following the hurricane, they were subjected to high precipitation and sustained winds exceeding 80 km/h.

2.2. Sapwood area determination

The allometric principals of the root cross-sectional area ratios used in previous studies (Benson et al., 2019a, b) relate to the Pipe Model Theory of tree form (Shinozaki et al., 1964a, b). The Pipe Model relates trunk sapwood cross-sectional area with other peripheral tree

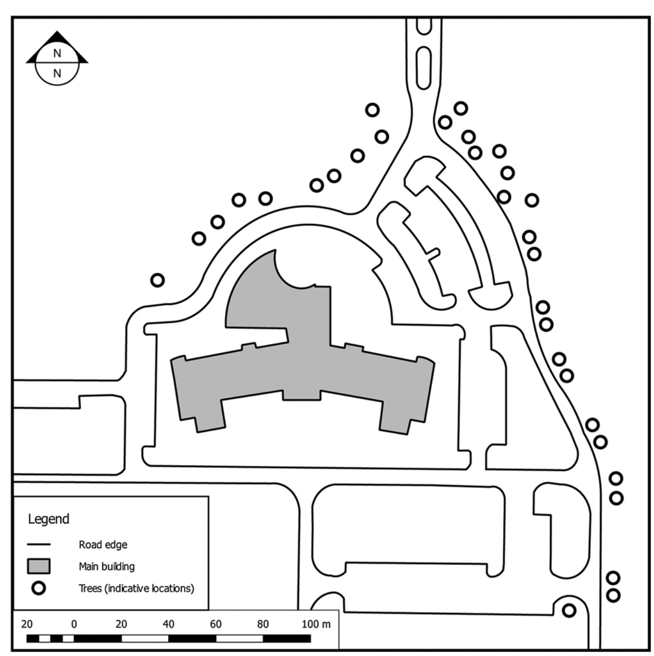


Fig. 1. Diagrammatic representation of the experimental site depicting approximate tree locations relative to the surrounding features.

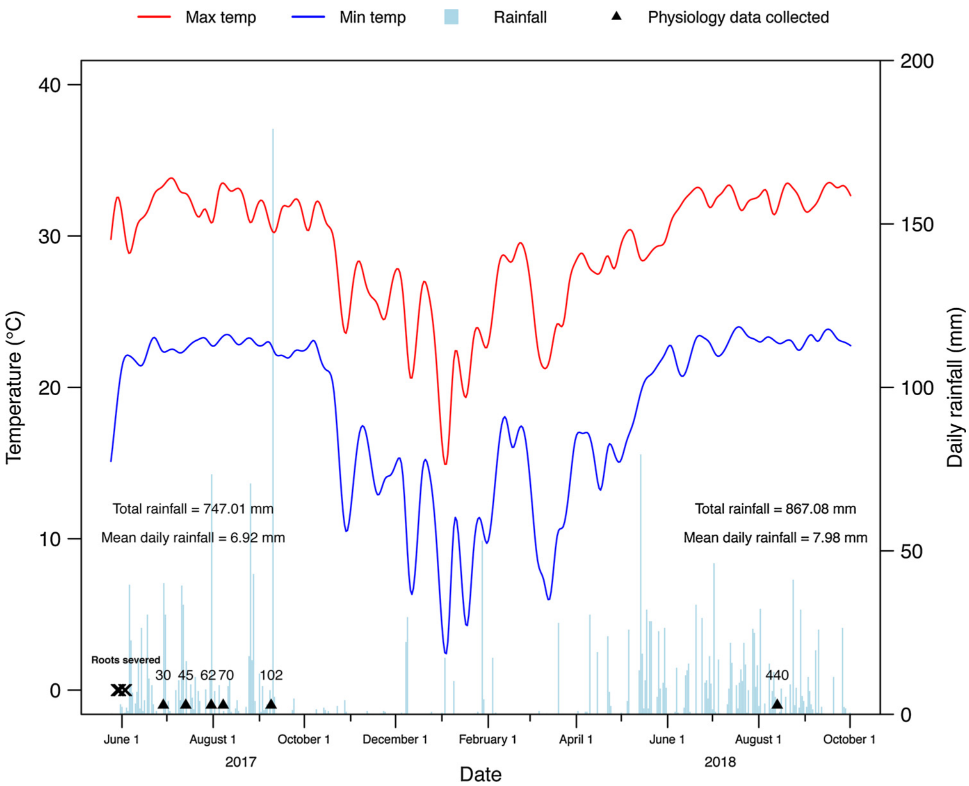


Fig. 2. Meteorological conditions throughout the experimental period. Triangles denote days on which physiological data were gathered with corresponding numbering denoting the number of days following root severance. Mean daily, and total precipitation in mm for the first 102 days of the study as well as the corresponding period in 2018 are shown.

structures. Accordingly, to align with this previous work, we estimated the sapwood area of our study trees. Destructive sampling of tree trunks was not possible, so a minimally-invasive technique using electrical resistance tomography (ERT) was used to estimate sapwood areas (Guyot et al., 2013; Wang et al., 2016; Benson et al., 2018) using a multichannel, multi-electrode electrical resistance tomograph (PiCUS TreeTronic, Argus Electronic GmbH, Rostock, Germany). The tomograph passes an electrical current through a series of small nails driven into the tree circumferentially, the geometry of which is accurately plotted using the accompanying wireless caliper unit (PiCUS Calliper, Argus Electronic GmbH, Rostock, Germany).

To determine the sapwood area, we followed the method set out by Benson et al. (2018) and applied the correction for *Q. virginiana*. The method estimates the sapwood – heartwood (SW/HW) interface of 16 equally oriented radii, allowing the sapwood area to be determined by summing up the 16 sapwood area sectors.

2.3. Root pruning treatments

Trees were randomly allocated to one of three treatment groups with eight replicates of each treatment plus seven controls. Root pruning treatments consisted of a single linear trench, offset from the tree base at a distance equivalent to 3 (3x), 6 (6x) or 12 (12x) times the trunk diameter at 1.4 m.

Between the 31st May and 6th June 2017, trenches ≈ 500 mm deep, ≈ 100 mm wide and ≈ 10.3 m long were excavated using pneumatic soil displacement (Air Spade, Guardair, Chicopee, MA, USA). The ≈ 10.3 m long trench ensured that the maximum chord length of the root zone of the largest tree in the 3x treatment was attained. That is, the 10.3 m trench completely bisected the root zone of all trees in each treatment. The chord lengths (trench length) required for each tree in their treatments were calculated according to Eq. (1).

$$Chord\ length = 2r \sin\frac{\varphi}{2} \tag{1}$$

Where: r = the root zone radius (Day et al., 2010) plus half of the trunk diameter at ground level and φ = the angle subtended at the tree base by two equally oriented radii (r) forming a sector.

Roots spanning the trenches were severed manually using a sharp saw or loppers during the same period, and fragments of each root were completely excised before filling in the trenches with the original soil material. After first removing all loose soil material, root cross-sectional area ratios for whole trunk area ($Ar_{(BH)}$) and trunk sapwood area ($As_{(BH)}$) at 1.4 m were determined for each tree following the methods prescribed by Benson et al (2019b), using Eqs. (2)–(5).

$$RCSA = \frac{\pi(d_1 \times d_2)}{4} \tag{2}$$

$$TCSA = \frac{C^2}{4\pi} \tag{3}$$

$$Ar_{(BH)} = \frac{\sum RCSA}{TCSA_{(BH)}} \tag{4}$$

$$As_{(BH)} = \frac{\sum RCSA}{TCSWA_{(BH)}} \tag{5}$$

Where; $Ar_{(BH)}$ = the area ratio of roots expressed as a proportion of the trunk cross sectional area at breast height (BH) (1.4 m), $As_{(BH)}$ = the sapwood area ratio of roots expressed as a proportion of the trunk sapwood area at breast height; C = trunk circumference in cm at breast height; d = individual root diameter at the point of severance in cm; RCSA = root cross sectional area of an individual root in cm^2; $TCSA_{(BH)}$ = trunk cross-sectional area at breast height in cm^2; $TCSWA_{(BH)}$ = trunk conductive sapwood area at breast height in cm^2.

The use of pneumatic soil displacement not only enabled us to carefully remove roots from the trees, it also enabled us to ensure that roots from other nearby treatment or control trees were not inadvertently removed, by tracing their growth and examining morphology where required.

2.4. Tree responses to root pruning - Physiology

Pre-dawn leaf water potential (Ψ), stomatal conductance (g_s), leaf temperature (T), chlorophyll fluorescence variables (Fv/Fm and Fv/Fo) and volumetric soil moisture (θ) were measured periodically (approximately every 10 to 14 days, providing the meteorological conditions were favourable) throughout the experimental period (June to September inclusive 2017), and again on August 14th, 2018 (a final measurement date 440 days after root severance). The months of May to October are often described as the 'rainy season' in Florida, with regular (often daily) precipitation events during the afternoon (usually after 14:00). Pre-dawn leaf water potential was measured in situ between 03:00 and 05:00 using a pressure chamber (PMS Instruments, Albany, Oregon, USA) from one fully expanded leaf at the second node proximal to the terminus of a new twig from each tree. Stomatal conductance and leaf temperature measurements were made using an SC-1 leaf porometer (Meter Environment, Washington, USA) calibrated to local conditions using the SC-1 Leaf Porometer Calibration Kit (part# 30425, Meter Environment, Washington, USA). Chlorophyll fluorescence variables were measured using an OS30P+ (Optisciences, Hudson, New Hampshire, USA) following a 45-minute dark adaption period.

Conductance and fluorescence data were gathered from three fully expanded sun leaves at the second node proximal to the terminus of an equivalent number of new twigs between 08:00 and 11:00, using the same leaves for both sets of measurements. Only leaves in full sun were sampled. Seasonal mean values for each physiology response for each tree were established using the arithmetic mean of all physiology data gathered during the 2017 growing season (June to September inclusive, 2017).

Using a handheld data-logger (Pro-Check, Meter Environment, Washington, USA) equipped with a soil moisture probe (GS3, Meter Environment, Washington, USA), volumetric soil moisture data were recorded during sunrise (06:00 – 07:00) at two locations (one to the north and one to the south) in the upper 200 mm of the soil approximately 5 m from the trunks of each tree near the outer edge of the 'drip line', where fine root activity was expected (Gilman, 1989; Day et al., 2010; Rahman et al., 2019). Individual soil moisture readings were recorded for each tree and used to derive a site mean water content for each measuring period as well as a seasonal mean value for each tree.

2.5. Tree responses to root pruning – Tree growth

Using a conventional measuring tape, trunk circumferences at 500 mm, 1 m and at 1.4 m (breast height) were recorded immediately prior to root severance treatments and again at the end of the 2017 growing season (28th September) and again on 10th August 2018 (436 days after roots were cut). Logistical constraints precluded gathering the growth data for year 2 during September. Shoot growth was measured using 18 new terminal shoots per tree and leaf area was measured using 10 sun leaves harvested from the third node proximal to the terminus of an equivalent number of new twigs from each tree using an LI-3100C leaf area meter (LI−COR Devices, Nebraska, USA). Shoots and leaves were harvested from the upper apical region of each tree, accessed using a mobile work platform.

2.6. Statistical analyses

2.6.1. Treatment effects

All data were analysed using R statistical software version 3.4.4 (R Core Team, 2018). ANCOVA analyses were undertaken for all physiological and morphological response variables using the root pruning treatment (control, 12x, 6x and 3x) as a discrete variable. Soil moisture was introduced as a continuous variable as well as the distance of each individual tree from the road edge (Road_D). To account for the distance of the road from each tree relative to its size, DBH was also introduced as a continuous variable along with the interaction term with the road (Road_D : DBH). We also tested for a spatial effect (East or West) of the experimental layout. Finding none, data were pooled for analysis.

For physiology responses, individual soil moisture data for each tree on each day that data were gathered were used. For growth responses, a seasonal mean for each tree was established and introduced into the analyses. Models were simplified by stepwise regression using the step() command, specifying "both" in the direction term. The models with the lowest Akaike's Information Criterion (AIC) value from the stepwise analyses, were examined using the Anova() command in the "car" package (Fox and Weisberg, 2011), and any remaining non-significant terms were sequentially removed (beginning with interaction terms). Final models were selected based on the statistical comparison tests between models using the anova() command, selecting the most statistically significant model. Statistically significant differences between treatment and control were identified by the significance of the treatment intercepts from the simplified ANCOVA models, using the summary() command.

2.6.2. Continuous variables

Additionally, ANCOVA analyses were undertaken for all physiological and morphological response data using root cross-sectional area ratios at 1.4 m ($Ar_{(BH)}$ and $As_{(BH)}$) as the explanatory variables. Soil moisture, the road and DBH were introduced into the analytical models in the same way as the treatment effects analyses. Stepwise regression, model simplification and selection were undertaken using the step(), Anova() and anova() commands, as was described for the treatment effects.

Unless otherwise indicated, statistical significance for categorical and continuous responses is reported at $p \leq 0.05$.

3. Results and discussion

3.1. Sapwood areas and root cross sectional area ratios

Trunk sapwood areas at 1.4 m for the study trees were between 203.68 cm^2 and 677.24 cm^2 (mean sapwood radius = 8.32 cm (δ = 2.44 cm); mean sapwood width % of DBH = 49% (δ = 14%)) and ranging from 71% to 84% of the total trunk cross-sectional area. Root cross-sectional area ratios expressed as proportions of trunk sapwood areas ($As_{(BH)}$) are thus distinct from the ratio of root to whole trunk cross-sectional area ($Ar_{(BH)}$). Root cross-sectional area ratios at breast height (BH; 1.40 m) ($Ar_{(BH)}$ and $As_{(BH)}$) increased as the distance of the treatment to the tree base decreased (12x to 3x) ($p < 0.0001$). Across the range of treatments, mean total severed root cross-sectional areas were between 0.05 and 0.19 times the trunk cross-sectional area at 1.4 m ($Ar_{(BH)}$), and 0.11 and 0.44 times the trunk conductive sapwood area at the same height ($As_{(BH)}$). Mean values for $Ar_{(BH)}$ and $As_{(BH)}$ ratios plus or minus one standard error (in parentheses) are presented in Table 1. The relationship between total severed root cross-sectional area and treatment is seen in Fig. 3.

Table 1
Mean $Ar_{(BH)}$ and $As_{(BH)}$ ratios for each root pruning treatment ± one standard error (in parentheses). Different letters in a single column denote a significant difference between treatments.

Treatment	$Ar_{(BH)}$	$As_{(BH)}$
12x	0.05 (0.02) a	0.11 (0.04) a
6x	0.08 (0.02) a	0.21 (0.04) a
3x	0.19 (0.02) b	0.44 (0.09) b

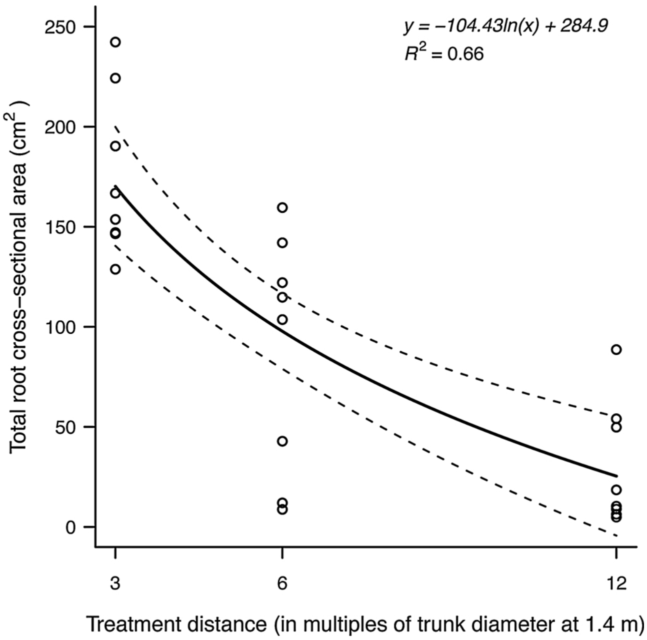


Fig. 3. Relationship between the distance of the trench from the tree base (treatment) and total severed root cross-sectional area. Dashed lines denote 95% confidence intervals.

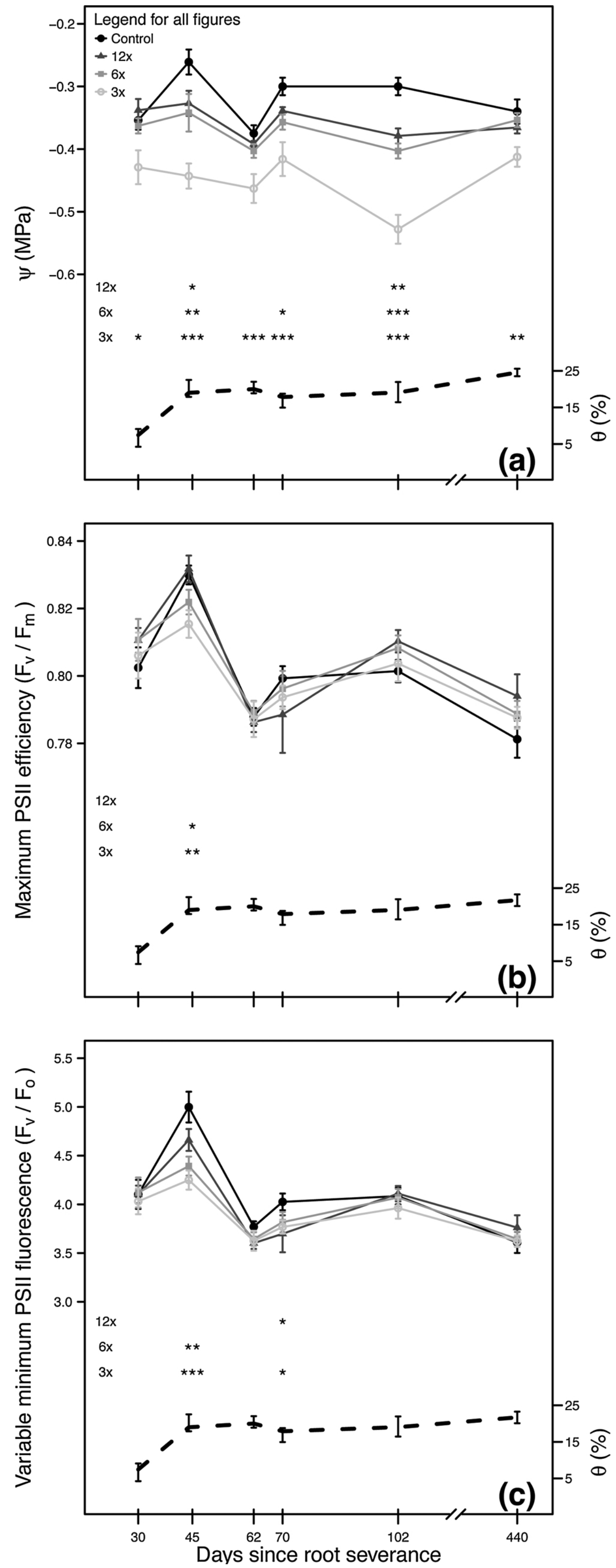
3.2. Tree physiological responses to root pruning treatments

Pre-dawn leaf water potential was negatively affected by the trenching treatment (Fig. 4a, Supplementary table 1). Significant differences between control trees and all treatments were observed on one or more of the measuring days. Significant treatment effects were sustained to day 440 only in the 3x treatment (a difference of −0.07 MPa from control trees), which showed statistical separation from the control trees on each of the other five days on which data were gathered.

The negative effects we observed in the Ψ response of the root pruned trees are consistent with the responses of other species involved in root pruning (Wang et al., 2014; Dong et al., 2016) and water stress (Epron et al., 1992; Kubiske et al., 1996; Zwack et al., 1998; Fini et al., 2013a) studies, including *Q. virginiana* (Cavender-Bares et al., 2007; Benson et al., 2019a). Whilst there was some variability in the significance between different treatments and the control, it is evident that severe root loss (3x treatment) can have persisting negative effects on leaf water status. Statistical separation between the 12x treatment and the control trees observed on day 102, may have been influenced by fine-root mortality following the hurricane (Herbert et al., 1999).

The photosynthetic process was also negatively affected, particularly on day 45 when the maximum photosystem II (PSII) quantum efficiency (Fv/Fm) was significantly different from the control for the 6x (a difference of -0.01) and 3x (a difference of -0.02) treatments (Fig. 4b, Supplementary table 2). The variable minimum fluorescence (Fv/Fo) values for the 6x (a difference of -0.47) and 3x (a difference of -0.70) treatments were also significantly different ($p < 0.01$) from the control on day 45 (Fig. 4c, Supplementary table 3). Negative effects on the photosynthetic mechanism were absent 102 days after treatment, possibly a result of new root growth in the backfilled trenches alleviating some of the initial dysfunction, in combination with the arrival of regular precipitation events and concomitant ground water recharge.

No effect of the root pruning treatment on the g_s response was observed (Supplementary table 4). This is consistent with the anisohydric behaviour of *Q. virginiana* (Beeson, 2014). This type of hydraulic strategy enables stomata to remain open during periods of reduced leaf turgor, to maintain photosynthetic rates (Sade et al., 2012; Martínez-Vilalta et al., 2014). The sclerophyllous nature of the leaves of live oak



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(Monk, 1987), goes some way towards explaining this behaviour, since it adds rigidity to the guard cells (Lo-Gullo and Salleo, 1988; Oertli et al., 1990; Salleo and Lo-Gullo, 1990).

Fig. 4. Treatment mean pre-dawn leaf water potential (Ψ) in MPa (a); mean maximum photosystem II photochemical efficiency (Fv/Fm) (b); and mean variable minimum fluorescence (Fv/Fo) (c) plotted against number of days since roots were severed. Asterixis in the inset matrix show significant differences between control and treatment (left hand side) on a particular day with the following significance codes; * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$. Dashed lines denote mean soil water volume (%) throughout the study. Error bars show ± one standard error.

3.2.1. Effects of the road on tree physiology

The road (Road_D) was absent from each of the Ψ ANCOVA models, although remained significant for stomatal conductance and leaf temperature responses on day 45 (and leaf temperature on day 70) with a negative slope term (i.e. trees nearer the road had higher g_s and T), when the maximum temperature recorded by the weather station was 34.03 °C (the hottest of all data gathering days).Whilst there may be other factors which could have influenced this behaviour, one possible, and likely reason is that it is due to increased radiant heat over the asphalt surface (Kjelgren and Montague, 1998; Montague and Kjelgren, 2004). Increasing leaf temperature resulted in elevated g_s ($p \leq 0.01$) on each of the data gathering days (data not shown). The photosynthetic responses we observed on the same day (which both retained Road_D as a significant variable in the ANCOVA models), may indicate temporary damage to the photosynthetic mechanism, borne from the initial effects of root loss-induced water stress in combination with increased transpiration and leaf temperature (Ashraf and Harris, 2013). The road was absent from the ANCOVA models for g_s and T responses on day 440, which may be due to the evaporative cooling effect of increased precipitation (Fig. 2) during the 2018 growing season (Lu, 2011; Li et al., 2014). The results suggest that *Q. virginiana* is susceptible to photosynthetic perturbations during periods of increased temperature in conjunction with a root loss-induced water stress.

3.3. Tree growth responses to root pruning treatments

The root pruning treatments negatively affected shoot extension and leaf area responses in *Q. virginiana* after one growing season, but not trunk diameter growth (Fig. 5, Supplementary table 11). Whilst shoot elongation responses in mature trees may exhibit some variability, the root pruning treatments resulted in a 97.29 mm reduction in the 12x treatment, a 146.13 mm reduction in the 6x treatment and a 132.24 mm reduction in the 3x treatment at the end of the first growing season relative to control trees. Leaf area responded in the same fashion, and we recorded reductions in leaf area of 2.17 cm^2 in the 12x treatment, 2.72 cm^2 in the 6x treatment and 2.92 cm^2 in the 3x treatment at the end of the first growing season relative to control trees. Root removal often negatively affects shoot elongation in the growing season following root loss, although the duration of the effect is variable (Young and Werner, 1982; Ferree, 1989; Autio and Greene, 1994; Khan et al., 1998; Watson, 1998; Fini et al., 2013b; Dong et al., 2016). Reducing leaf area in response to root loss-induced water stress, may serve to reduce overall tree transpiration (Struve and Joly, 1992; Liu and Stützel, 2004; Pallardy, 2008).

Treatment effects on shoot elongation and leaf area responses were absent after the second growing season, supporting the premise that *Q. virginiana* is tolerant to root removal (Matheny and Clark, 1998) and supporting previous results showing variability in the shoot elongation response. Furthermore, since the trenches were backfilled following root severance, it is likely that new root growth contributed towards restoring the root:shoot ratio in the treatment trees, enabling shoot elongation and leaf area responses to return to expected norms (i.e. no statistical separation from control).

3.3.1. Effects of the road on tree growth

The road remained significant in the trunk diameter growth models for the 2017 growing season with a positive slope term (i.e. the trunks of trees closer to the road grew less than those further away). Whilst roots directly below paved surfaces may experience favourable conditions (Wagar and Franklin, 1994; Nicoll and Armstrong, 1998), and tree growth may be affected by a range of factors (Cienciala et al., 2016), the response we observed may allude to the constraints placed upon trees by the built environment (McPherson, 2001; Grabosky and Gilman, 2004; Celestian and Martin, 2005; Day and Amateis, 2011; Chen et al., 2017; Sand et al., 2018). The road was absent from the 2018 ANCOVA models using the categorical treatment variables.

3.3.2. Tree responses using severed root cross sectional area ratios

The ability of the allometric relationship between trunk and root cross-sectional areas to predict tree responses to root pruning during the growing season immediately following root loss, varied between responses. Scatterplots and regression lines for the relationships between $Ar_{(BH)}$ and $As_{(BH)}$ and Ψ, Fv/Fm and Fv/Fo responses using 2017 seasonal mean data, as well as the data gathered on day 440 are shown in Supplementary Fig. 1. The full ANCOVA model statistics are presented in Supplementary table 6 to Supplementary table 10. $Ar_{(BH)}$ was able to account for 75% of the variability (calculated as R^2) in the pre-dawn leaf water potential response ($p < 0.001$) in the growing season following root removal using mean data, revealing explanatory power comparable to previous work using the same species when roots were severed in circumferential trenches (Benson et al., 2019a). $Ar_{(BH)}$ accounted for 14% of the Fv/Fm response ($p < 0.05$) and 16% of the Fv/Fo response ($p < 0.05$) in the first growing season following treatment using mean data. Model significance was retained ($p = 0.001$) for the Ψ response on day 440, although is absent for each of the fluorescence responses, possibly illustrating that hydrological response variables are better-suited for this type of investigative purpose, and that $Ar_{(BH)}$ is more useful in the short-term only. No significant relationships are reported between g_s or leaf temperature using the allometric variables.

Scatterplots and regression lines for the relationships between $Ar_{(BH)}$ and $As_{(BH)}$ and the above-ground growth responses are seen in Supplementary Fig. 2. Full ANCOVA model statistics are shown in Supplementary table 12. $Ar_{(BH)}$ and $As_{(BH)}$ remained significant in the ANCOVA models for all growth responses in 2017. In combination with soil moisture effects, $Ar_{(BH)}$ accounted for 21% of the variability (R^2) in trunk diameter growth, 18% of the shoot extension response in isolation and 24% of the variability in the leaf area response. Treatment effects on shoot elongation using $Ar_{(BH)}$ and $As_{(BH)}$ remained in 2018 in combination with effects of the road, perhaps indicating a sustained negative effect of built infrastructure on this growth response. Although we again acknowledge that shoot growth responses may behave with some variability.

4. Conclusions

Root pruning negatively affected the growth and physiology of *Q. virginiana* in Central Florida. Significant differences were observed between all root pruning treatments and the control for the pre-dawn leaf water potential response, highlighting that root pruning induces water stress in the affected trees. Growth responses were negatively affected by the root pruning treatments, with significant differences between treatment and control emerging at the 12x treatment for leaf area and shoot extension responses after one growing season. The results perhaps highlight the importance of roots peripherally located in the total root system. Although *Q. virginiana* was susceptible to the negative effects of root removal; 440 days after root severance, trees exposed to the 6x and 12x treatments were able to regain physiological function, and tree growth amongst all treatments had returned to expected norms, likely due to new root growth in the backfilled trenches of these lesser treatments.

The allometric variables appeared to be best suited for short-term tree responses, with statistical models losing significance and

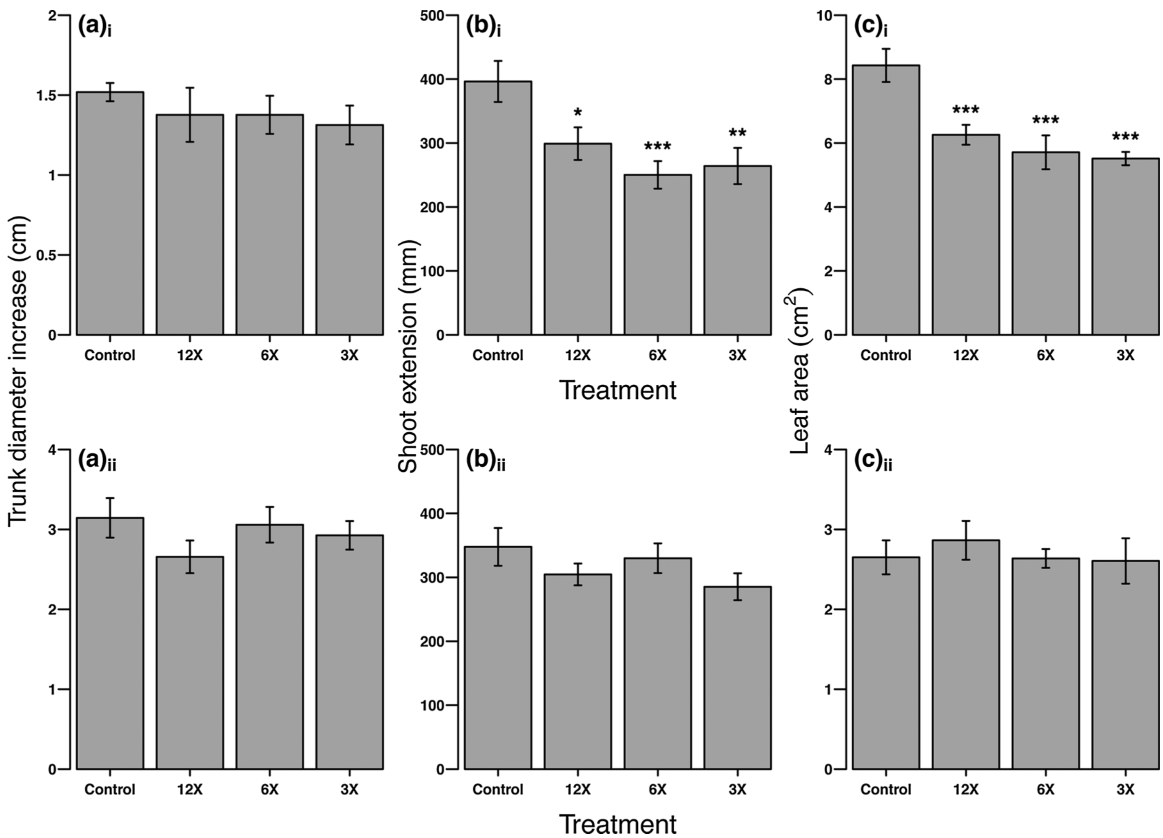

Fig. 5. Bar plots showing trunk diameter (a), shoot elongation (b) and leaf area (c) growth responses to the root pruning in 2017 (i) and 2018 (ii). Significant differences between treatment and control based on the ANCOVA analyses are denoted by asterixis above each bar with the following significance codes; * p ≤ 0.05 ** p ≤ 0.01 *** p ≤ 0.001. Error bars show ± one standard error (SE).

explanatory power (R^2) over time. The main purpose of using the allometric variables was to investigate a potential alternative to root pruning guidelines which use fixed root diameters, enabling practitioners to account for cumulative root loss during selective root pruning practises. In the context of this study, it is simpler to prescribe a trunk diameter-defined offset at which root loss should not occur. Since we observed physiological recovery of the 6x and 12x treatments, we conclude that linear root cutting should not be undertaken at distances closer than six times DBH in *Q. virginiana,* equating to ≈ 25% root system loss.

Trees which were closer to the road had reduced trunk diameter increases compared to those further away, as well as elevated stomatal conductance and leaf temperature on certain days. Whilst tree growth and physiology may be affected by numerous factors, these results suggest that the presence of built infrastructure in the root zones of *Quercus virginiana* in Central Florida, produces negative effects and may hinder tree growth and development over time.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ufug.2019.126448.

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