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ORIGINAL ARTICLE

Autumn flooding disrupts seasonal nitrogen storage and impacts spring growth in *Quercus texana* seedlings

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Abstract

Key message **Autumn flooding reduced nitrogen uptake and fine root mass. Leaf nitrogen resorption increased, but overall autumn nitrogen accumulation was negligible in flooded seedlings. Subsequently, spring survival and growth were decreased.**

Abstract Since nitrogen (N) is often limiting in terrestrial ecosystems, N is conserved in trees by resorption before leaf senescence. Bottomland forests are prone to flooding, which could reduce N uptake, and may decrease phloem transport, which is essential for N resorption. Therefore, we hypothesized that autumn flooding may diminish both N uptake and N resorption in trees, and this would reduce spring growth. Two-year-old *Quercus texana* seedlings either had no flood or had their complete root system flooded during dormancy induction, the period in autumn when trees prepare metabolically and physiologically for winter dormancy. We measured seedling growth and nutrient contents before flooding and after leaf fall and determined impacts of autumn flooding on growth during the subsequent spring. Autumn flooding resulted in a small increase in N resorption from leaves. In non-flooded seedlings, much more N accumulated in stems and roots than the amount resorbed from leaves, suggesting that there was substantial N uptake during the autumn dormancy induction period. However, flooding severely reduced accumulation of N in roots and stems during autumn, probably by directly reducing uptake and by increasing fine root mortality. Winter survival was reduced 50% by autumn flooding. Autumn-flooded seedlings that survived winter had greater new root growth in spring than non-flooded seedlings, but substantially decreased stem diameter growth. Our results indicate that *Q. texana* seedlings which are flooded during dormancy induction may be less competitive the following spring due to fine root mortality and reduced nutrient storage, which negatively impact spring growth.

Keywords Nitrogen uptake · *Quercus texana* · Autumn dormancy induction · Wetland forests · Nitrogen resorption

Introduction

Bottomland (wetland) hardwood forests cover millions of acres in the Mississippi Alluvial Valley, providing a major carbon sink for carbon sequestration, as well as a multitude of ecosystem functions and services such as foraging habitat for migratory birds (Twedt et al. 1998; Schoenholtz et al. 2001). Bottomland hardwood sites may be flooded annually during late autumn through early spring, both intentionally, in the case of green tree reservoirs (GTRs), and naturally via normal weather events (Gardiner 2001), exposing trees to both long-term and short-term flood stress (Parker 1950). Oak (*Quercus*) species, such as Nuttall oak (*Q. texana*), are ecologically and economically important in bottomlands (Twedt et al. 1998; Gardiner 2001; Gardiner et al. 2010). Oaks vary in their ability to tolerate flooding from moderately intolerant to moderately tolerant (Gardiner 2001). *Q.*

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texana is classified as moderately flood tolerant based on its response to short-term flooding during active growth (Parker 1950; Pezeshki and Anderson 1996). Although flooding during autumn is common in bottomland hardwood forests, there is little known about the impacts of flood during autumn dormancy induction, when specialized physiological and metabolic processes prepare trees for leaf senescence and dormancy (Crawford 2003).

The various physiological effects of flooding on trees during the growing season have been well studied and include decreased stomatal conductance, photosynthesis and transpiration, and ultimately reduced growth (Parent et al. 2008; Rasheed-Depardieu et al. 2015). Flooding stresses trees by causing hypoxia (low oxygen) in the root zone (summarized by Parent et al. 2008), since oxygen diffuses much more slowly through water than in air. Hypoxia severely limits aerobic respiration in the roots, which may lead to tissue death if flooding is persistent (Parent et al. 2008). Some trees have physiological and morphological adaptations to tolerate reduced oxygen availability during flood stress. For example, to meet immediate energy demands tree roots experiencing hypoxia may employ anaerobic respiration (Pezeshki and DeLaune 2012), which produces ATP, although at a much lower rate per molecule of glucose than aerobic respiration. Bottomland oaks may tolerate short-term flooding via physiological mechanisms, which are not fully understood (Pezeshki and Anderson 1996), and perhaps limited morphological responses such as the formation of hypertrophied lenticels and adventitious roots (Gardiner 2001), but they cannot tolerate long-term flooding during the growing season (Gardiner and Hodges 1996). During the autumn dormancy induction period, shoot growth typically ceases, although root growth may continue into the late autumn (Teskey and Hinckley 1981; Kuhns et al. 1985). Thus, flooding during autumn is not expected to alter shoot growth or biomass immediately, but could reduce root growth or result in lateral root death.

Flooding may also reduce nutrient uptake by roots (Smethurst et al. 2005). In slash pine, direct measurements of potassium uptake by roots indicated that uptake was reduced under hypoxia (Fisher and Stone 1990). In various oak species, flooding or root hypoxia during the growing season reduced uptake of nutrients, including nitrogen (N) (Pezeshki et al. 1999; Gardiner and Krauss 2001; Kreuzwieser et al. 2002). Of the mineral nutrients, N is often limiting in forest ecosystems (Cooke and Weih 2005), and so both N uptake and internal N conservation are essential in trees. N uptake may continue during late autumn, even as shoots enter dormancy (Millard and Grelet 2010). Thus, we hypothesized that flooding during autumn could reduce N uptake during the autumn dormancy induction period. Additionally, since N stored over winter is used for spring growth (Millard and Grelet 2010), it is possible that flooding during the autumn dormancy induction period could negatively impact initial spring growth.

N resorption during autumn senescence is a crucial mechanism for N conservation, which enables trees to enter winter dormancy with greater N reserves that can be remobilized and used for spring growth (van Cleve and Apel 1993; Cooke and Weih 2005). N resorption is the process in which leaf proteins are broken down into mobile forms of N, which are transported through the phloem into woody tissues during autumn to be stored over winter (Wildhagen et al. 2010; Babst and Coleman 2018). It has been suggested that flooding could impair phloem transport of carbohydrates to the roots of trees, based on the reduction of carbohydrate pools in waterlogged roots (Sloan et al. 2016). Since N resorption relies on phloem transport of amino-N from leaves to sink tissues, and the bulk flow of phloem sap is dependent on carbohydrate loading and unloading according to the pressure-flow hypothesis (Babst and Coleman 2018; Knoblauch et al. 2016), it is possible that autumn N resorption may be reduced in flooded trees. However, it is not clear whether flooding inhibits only phloem unloading locally in flooded tissues, or if it reduces phloem transport globally. Therefore, it is also possible that N resorption is maintained in flooded trees, but that N is moved preferentially to non-flooded tissues, such as branches that are above the waterline during soil flooding.

To test the hypotheses that (1) flooding during autumn could reduce N uptake during the autumn dormancy induction period, and (2) autumn N resorption may be reduced in flooded trees, we compared pre-senescence N levels in late summer before flooding with N levels immediately after leaf fall in stems, leaves, and roots of *Q. texana* seedlings that were either exposed or not exposed to flooding during autumn. We also measured growth and biomass, and compared tissue contents of other nutrients. Finally, to test whether flooding during autumn negatively impacts spring growth, a subset of the seedlings were maintained through the dormant period after the autumn flooding was ended, and the first flush of growth in spring was measured.

Materials and methods

Plant materials and growth

One-year-old bare root *Q. texana* seedlings were provided by the Arkansas Forestry Commission (Baucum Nursery, North Little Rock, AR, USA) in February 2017. Ninety seedlings, which were approximately 0.6 m tall and 10 mm diameter, were potted in 4:1 top soil:sand on February 22–24, 2017 in 9600 cm^3 tree pots (Stuewe & Sons Inc., Tangent, OR, USA), and were grown outside in southeast Arkansas (33.595254, −91.812545). Once potted, nine

Q. texana seedlings were placed into each of ten 0.42 m^3 tubs fitted with drain holes, which provided a means to impose and remove flood treatment without otherwise disturbing the seedlings. To mitigate slight leaf yellowing in mid-summer, we fertilized seedlings twice on July 21 and August 2, 2017 with 50 mL of 65 × diluted McCown's salts (0.035 g/L, which provided 0.06 mg $CaCl_2$, 0.36 mg $Ca(NO_3)_2.4H_2O$, 0.13 mg KH_2PO_4, 0.75 mg K_2SO_4, 0.14 mg $MgSO_4$, 0.30 mg NH_4NO_3, 0.19 µg $CuSO_4.5H_2O$, 27.92 µg FeNaEDTA, 4.72 µg H_3BO_3, 16.97 µg $MnSO_4.H_2O$, 0.19 µg $Na_2MoO_4.2H_2O$, 6.54 µg $ZnSO_4.7H_2O$ per seedling). Seedlings that were not sampled immediately after terminating flood treatment (30 per treatment) remained outside to overwinter, and were watered to field capacity every 2–3 days until the following spring.

Flooding treatment

The flooding treatment was initiated on September 20, 2017 to ensure that the treatment occurred during the autumn dormancy induction period. Although visible signs of senescence are not apparent until late November, the first biochemical processes that prepare *Q. texana* trees for senescence and dormancy begin by mid-September (Sample and Babst 2018). To simulate flooding, the drain holes were plugged and the tubs filled until water levels were slightly above the soil line of the seedlings. Flooding was isolated to the root zone to test the hypotheses that root flooding would reduce N resorption, and reduce N uptake. Tubs were placed in two rows, seedlings were randomly assigned to each tub, and treatments were systematically alternated such that every other tub was flooded during autumn (five tubs per treatment). The flood treatment was terminated after leaf abscission was complete and a subset of the seedlings was harvested on December 7, 2017. All remaining seedlings ($N = 30$ per flood treatment) were maintained with normal water (i.e., not flooded) during winter and the subsequent spring.

Anthocyanin measurements

Since anthocyanin accumulation can be a response to reduced carbohydrate export from leaves via the phloem (Arnold et al. 2004), anthocyanin levels on ten seedlings per treatment were measured on November 12, 2017 using a hand-held device (ACM-200_{plus}, Opti-Sciences, Hudson, NH, USA), which measures transmittance of light through the leaf around 525 nm, the range of wavelengths that free anthocyanins absorb, and at a reference wavelength 931 nm. The ACM-200_{plus} device provides an anthocyanin content index (ACI), which is the ratio of the transmittance at 931 nm to the transmittance at 525 nm. The ACI correlates well with extracted anthocyanin content in red leaves, but not green leaves (van den Berg and Perkins 2005). Leaves were all red at the time when we used the device.

Late summer and autumn sampling, and biomass

Ten seedlings each of pre-senescent, flooded post-senescent, and non-flooded post-senescent seedlings were harvested. One seedling was selected randomly from each of ten tubs for pre-senescent harvest, and two seedlings were selected randomly from each of five tubs for post-senescent harvest of flooded and non-flooded seedlings. To determine total N concentrations in seedling tissues before N resorption, ten pre-senescent seedlings were harvested before initiation of the flooding treatment on September 19, 2017. At this time of year, in the region where the study took place, the leaves are not visibly senescent, but the earliest stages of leaf senescence are initiated in *Q. texana*, e.g., protein degradation is underway (Sample and Babst 2018). For these pre-senescent seedlings, the entire seedling was harvested, keeping leaves, branches, main stem, taproot, and fine roots separate. Prior to leaf abscission, a clear plastic mesh net was placed over each branch of the seedlings to ensure no leaves were lost. For ten seedlings per treatment, we regularly collected the leaves as they abscised, and dried them. Leaves were kept in dry storage until all leaves had abscised, and all of the leaves for each seedling were pooled. Leaf abscission occurred from November 15, through December 1, 2017. Once leaf abscission was complete, we harvested branches, main stem, taproot and fine roots from each of the seedlings on December 4–7, 2017. All samples were oven dried for 5 days at 65 °C and were weighed on 2 consecutive days to ensure drying was complete and to obtain dry biomass for each tissue. At the time of each harvest, seedling height and basal diameter were recorded.

Spring sampling and biomass

Budbreak occurred from March 23 through April 9, 2018. Approximately 2 months later, on May 22–25, 2018, all seedlings that had broken bud were harvested (12 for autumn-flood treatment and 24 for non-flood treatment). Any seedlings that had not initiated new shoot growth by May 25 were assumed to be dead. At the time of harvest, we measured height, basal diameter, total length and diameter of new shoots and recorded the number of dead seedlings. Relative height and diameter growth were calculated as:

$$\frac{\text{Size}_{\text{harvest}} - \text{Size}_{\text{dormant}}}{\text{Size}_{\text{dormant}}} \quad (1)$$

Similar to the harvest in autumn, the entire seedling was harvested keeping leaves, new branches, main stem, taproot, fine roots, and new roots separate. All samples were oven

dried for 5 days at 65 °C and were weighed on 2 consecutive days to ensure drying was complete and to obtain dry biomass for each tissue.

Nutrient analysis

We examined the full suite of mineral macronutrients and most of the micronutrients in each of the tissues harvested in the autumn, after each sample was oven dried and ground using a Wiley mill with 20 mesh screen. Nutrient concentrations measured included N, phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and boron (B). N content was measured as N percentage of the sample weight using an Elementar Vario MAX CN analyzer (Elementar Vario, Ronkonkoma, NY, USA) following the Dumas method, which involves dry combustion, separation of the resulting gases on gas-selective trapping columns, and quantification of N_2 using a thermal conductivity detector (Nelson and Sommers 1996). We evaluated both tissue N concentration and tissue total N content (N concentration × tissue mass). Because we were particularly interested in the decrease of N in leaves, indicating resorption, and the increase of N in other tissues, indicating accumulation of N storage reserves, the change of total tissue N (ΔN) was calculated by subtracting the average for the pre-senescent control from the average of the flooded and non-flooded treatments. Standard errors were calculated to account for the variability in both pre-senescent and post-senescent measurements, by taking the square root of the sum of squared SEMs (Eq. 1).

$$\mathrm{SEM}_{\Delta N} = \sqrt{\mathrm{SEM}^2_{\mathrm{post-senescent}} + \mathrm{SEM}^2_{\mathrm{pre-senescent}}} \quad (2)$$

Concentrations of other nutrients were quantified at the Agricultural Diagnostic Laboratory of the University of Arkansas System Division of Agriculture by acid digestion, followed by inductively coupled plasma optical emission spectroscopy (ICP–OES) analysis (Campbell and Plank 1991), using a FHS16 ICP-OES (Spectro Arcos, Kleve, Germany). Similar to N, both concentrations and total nutrient contents were examined for each tissue.

Statistical analysis

Using a one-way analysis of variance in either SAS (version 9.4, Cary, NC, USA) or R software (R core team 2018), we tested for differences between treatments in height, basal diameter, relative height and diameter growth, total new shoot lengths, relative anthocyanin levels, biomass, and all nutrient concentrations. Sample sizes of autumn-flooded and non-flooded seedlings were different for spring growth and biomass measurements due to differences in survival over winter, but the ANOVA assumptions were not violated. The assumptions of normality and homogeneity in all one-way ANOVAs were visually checked using diagnostic plots, and were tested using a Chi-Square goodness-of-fit test and Levene's test respectively. When needed, data were log transformed, which corrected the violated assumptions. To test for pairwise differences between the pre-senescent, non-flood, and autumn-flooding treatment, least square means for each treatment were compared using a Tukey–Kramer multiple comparison post hoc analysis. For all tests, alpha was set a priori at 0.05.

Results

At the post-senescence harvest in the autumn, there was no significant increase in final heights or final basal diameters relative to the pre-senescence measurements in either flood treatment (Table 1). Similarly, there were no differences between post-senescence and pre-senescence biomass of most tissues (Table 1), indicating that no detectable growth occurred during the autumn dormancy induction period. The only significant difference in biomass was for fine roots,

Table 1 Size, biomass and anthocyanin measurements of *Quercus texana* seedlings harvested either pre-senescence (PS) in September or in December after autumn flood (F) or non-flood (NF) treatments were imposed during senescence

	Height (cm)	Diameter (mm)	Leaves (g dw)	Branches (g dw)	Main stem (g dw)	Taproot (g dw)	Fine roots (g dw)	Rel. anthocyanins levels (ACI)
PS	68.35 ± 2.20	10.69 ± 0.56	4.39 ± 0.36	2.85 ± 0.21	13.09 ± 2.50	26.64 ± 3.38	**9.00 ± 1.11a**	ND
NF	66.94 ± 2.12	11.17 ± 0.76	4.58 ± 0.22	2.73 ± 0.24	12.95 ± 0.99	23.37 ± 2.60	**9.36 ± 0.79a**	**9.93 ± 1.89a**
F	68.58 ± 5.79	12.59 ± 0.61	4.13 ± 0.24	3.19 ± 0.34	13.36 ± 1.60	26.62 ± 4.91	**5.66 ± 1.03b**	**30.17 ± 5.19b**

We measured height, stem basal diameter, and biomass as dry weight of leaves, branches, main stem, tap roots, and fine roots. Anthocyanin content index (ACI) is a ratio of transmittance of light through a leaf at wavelengths 931–525 nm, and so is unitless. ND indicates that no data were recorded. Bolded values show which measurements had significant differences, with different letters indicating which treatments were significantly different according to an ANOVA at $\alpha = 0.05$ or a Tukey's post hoc multiple comparisons test when needed. Values are mean ± SEM, and $n = 10$ for all treatments for all measurements

where autumn-flooded seedlings had a significantly lower fine root mass compared to non-flooded seedlings and pre-senescent seedlings (Table 1). The fact that non-flooded fine root mass that was measured post-senescence did not increase relative to pre-senescent fine root mass, but fine root mass of autumn-flooded seedlings was significantly lower than both indicates that seedlings in the flood treatment experienced fine root mortality.

There was a small decrease in leaf N concentrations during autumn senescence compared to the pre-senescent leaves harvested in September and the decrease was only statistically significant in autumn-flooded seedlings (Fig. 1a). On the contrary, branch N concentrations increased slightly during autumn dormancy induction in both non-flooded and autumn-flooded seedlings (Fig. 1b). N resorption is expected to result in decreased leaf N and increased branch N over the course of autumn dormancy induction. N concentrations measured after leaf fall were elevated significantly in the main stem, taproots, and fine roots of the non-flooded seedlings compared to the pre-senescent seedlings, which could be due to either N resorption from leaves or N uptake from soil. However, autumn-flooded seedlings did not experience a similar increase in N concentrations in the main stem or roots over the same time period (Fig. 1c–e).

Resorption of leaf N was very limited, as the decrease in leaf total N during senescence was not statistically significant (Fig. 2). In flooded seedlings, the total amount of N resorbed from leaves (i.e., decreased leaf total N during senescence) was roughly equal to the amount of N accumulated in the branches (i.e., increased branch total N during senescence) during autumn dormancy induction (Fig. 2). However, in non-flooded seedlings N resorption from leaves, which was near zero, could not account for the increase in N throughout all storage tissues combined, i.e., branches, stem, and roots (Fig. 2). Thus, the increased N in non-flooded seedlings must have been due to N uptake from the soil. While the total N in branches increased more in autumn-flooded than non-flooded seedlings, the opposite was true for the main stems, fine roots, and tap roots, where there was a much greater increase of N in non-flooded than flooded seedlings (Fig. 2). In fact, autumn-flooded fine roots had a net decrease of N (Fig. 2). Overall, the autumn-flooded seedlings had 40% less total whole-seedling N, which suggests that autumn flooding reduced N uptake from the soil.

Only K, P, and Cu concentrations decreased significantly in the leaves during autumn dormancy induction (Online Resource 1), possibly due to resorption. Leaf K decreased similarly in both autumn-flooded and non-flooded seedlings, but leaf P and Cu decreased only in autumn-flooded seedlings. Unlike N, flooding curbed the decrease in leaf K content (Fig. 3). Total K content increased in taproots over the course of autumn, although not significantly (Fig. 3; Online Resource 1), indicating a possible sink for resorbed leaf K.

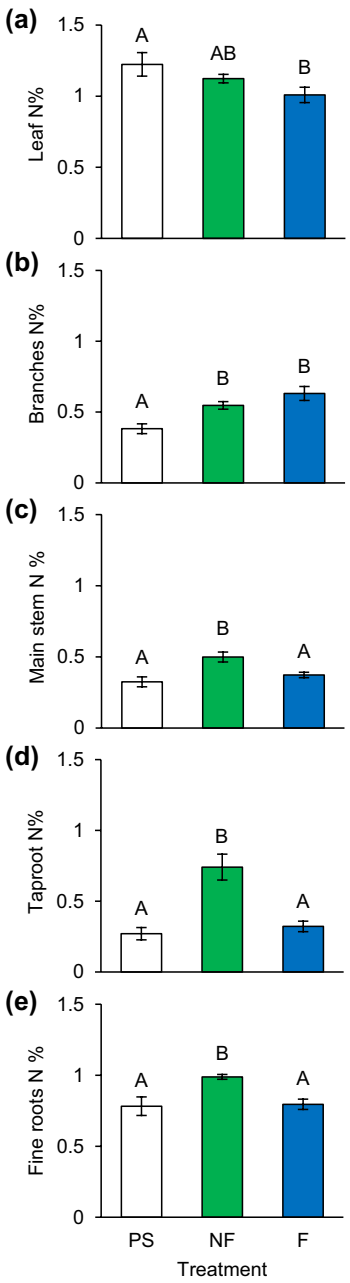

Fig. 1 Nitrogen concentration of leaves (**a**), branches (**b**), main stem (**c**), taproot (**d**), and fine roots (**e**) of *Quercus texana* seedlings for the pre-senescent (PS), and post-senescent non-flood (NF) and autumn-flood (F) treatments. ANOVA was significant only for fine roots. Different letters above bars represent significant differences according to a Tukey–Kramer multiple comparisons test, at $\alpha = 0.05$. Bars are means ± SEM, $n = 10$

In the absence of flood, P, Ca, S, Na, Zn, and Cu content generally increased in at least one tissue type during autumn (Fig. 3), indicating that uptake of these nutrients from soil occurred during the autumn dormancy induction period. Flooding during autumn severely reduced or eliminated the accumulation of Ca, S, Zn, Cu, and Mg. Autumn-flooded

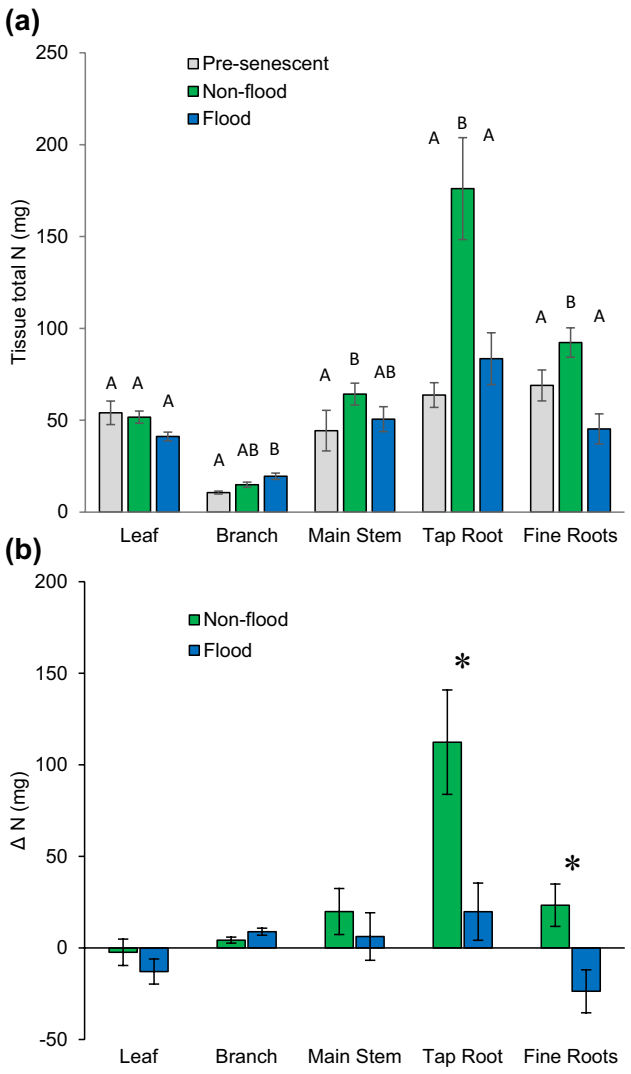

Fig. 2 Tissue total N content and change in N content for leaves, branches, main stem, taproot, and fine roots in pre-senescent seedlings, and autumn-flooded and non-flooded *Quercus texana* seedlings sampled immediately after leaf fall. **a** Bars for total N content indicate mean ± SEM. **b** Change in N content (ΔN) relative to the pre-senescent control seedlings is also shown, since changes may be indicative of processes such as N resorption and N uptake from soil. ΔN was determined by subtracting the average tissue N content prior to flooding from the average tissue N content after leaf fall. Error bars for ΔN indicate SEM, which was determined by taking the square root of the sum of squared SEMs for the pre-senescent total N and post-senescent total N ($n = 10$). Where ANOVA was significant for (**a**), different letters above bars indicate statistically significant differences according to a Tukey's post hoc multiple comparison procedure. Statistics were done on log-transformed data for leaves, main stem and tap roots, to meet the assumptions of ANOVA for normality. In panel (**b**), * asterisks indicate statistically significant differences in tissue total N between autumn-flood and non-flood treatments according to the Tukey's test for data in panel (**a**)

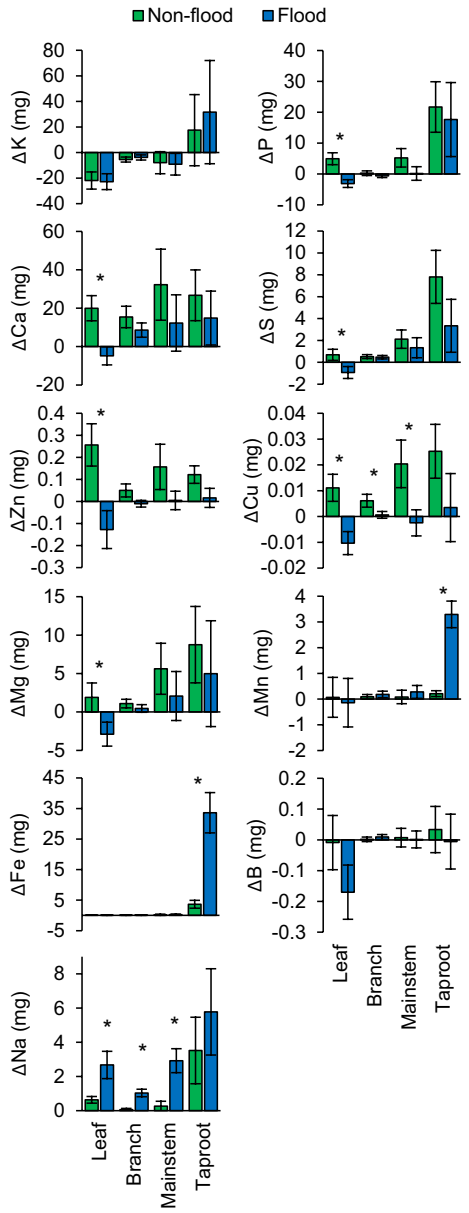

Fig. 3 Change in total contents of nutrients during the course of autumn for leaves, branches, main stems, and taproots in autumn-flooded and non-flooded *Quercus texana* seedlings. Change (Δ) was determined by subtracting the average tissue total nutrient content prior to flooding from the average tissue total nutrient content after leaf fall, and error from both pre-senescent and post-senescent measurements were propagated to the SEM of the Δnutrients as described above in Fig. 2. *Asterisks indicate statistically significant differences in tissue total nutrient content between autumn-flood and non-flood treatments according to a Tukey's post hoc multiple comparisons test between total nutrient contents of pre-senescent, autumn flood and non-flood treatments (Table S2). Bars are means ± SEM, $n = 10$ for all tissue/treatment combinations except $n = 9$ for branches and main stems in the autumn-flood and non-flood treatments

seedlings also accumulated Mn and Fe in roots during autumn, to about 400% and 450% higher concentrations, respectively, than non-flooded roots (Online Resource 1 and 2). Otherwise, only Na increased significantly in autumn-flooded seedlings (Online Resource 1 and 2). Thus, uptake of most, but not all, nutrients was impaired by autumn flooding.

During autumn leaf senescence, leaves of autumn-flooded seedlings appeared to be very red, which can indicate anthocyanin accumulation. Since anthocyanin accumulation can be a response to phloem impairment (Arnold et al. 2004), we compared anthocyanin levels of flooded seedlings and non-flooded seedlings with a nondestructive hand-held meter. Anthocyanins in autumn-flooded seedlings were threefold higher than non-flooded seedlings (Table 1).

By spring, 24 out of the remaining 30 seedlings (80%) in the non-flood treatment survived over winter, but only 12 out of 30 (40%) autumn-flooded seedlings survived over winter. Therefore, autumn-flooded seedlings had a 50% lower survival rate than non-flooded seedlings. Of the surviving seedlings, all broke buds between March 23 and April 9 (2.5 weeks), and the timing of spring budbreak was not significantly affected by previous exposure to autumn flooding. It is possible, but unlikely, that some of the seedlings that were presumed dead could have resprouted from the base of the stem later in the growing season. However, we monitored the seedlings until May 22 (total of 9.5 weeks after first bud break), and we have observed previously that resprouting after top dieback generally occurs within 9 weeks of initial bud break. All surviving seedlings were used for growth and biomass measurements. Among surviving seedlings, non-flooded seedlings had significantly higher relative basal diameter growth than autumn-flooded seedlings after the development of the first flush of leaves (Fig. 4b), but there was no difference between treatments for relative height growth (Fig. 4a, b). Similar to senescent seedlings in autumn, in spring there were no significant differences in leaf, branch, main stem, or taproot biomass (Table 2), and seedlings previously exposed to autumn flooding had significantly lower fine root biomass (Table 2). There was no difference in total seedling biomasses between the flooded and non-flooded seedlings. Autumn-flooded seedlings had significantly higher new root biomass (Table 2), and surprisingly there was increased total new shoot length for flooded seedlings (Fig. 4c), which appeared to be due to a bushy growth habit with many thin branches in the autumn-flooded seedlings.

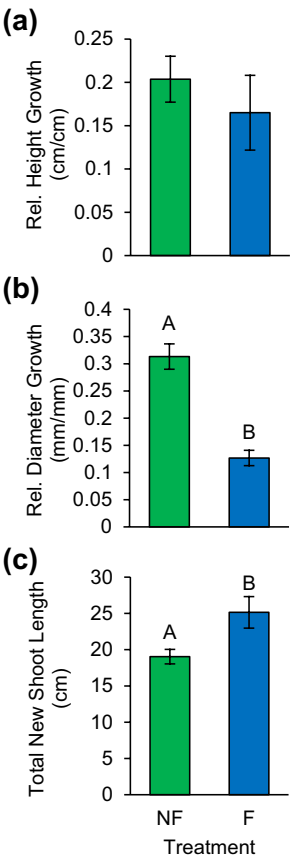

Fig. 4 Spring measurements of relative height growth (**a**), relative basal diameter growth (**b**), and total new shoot length (**c**) of *Quercus texana seedlings* that were flooded (F) or not flooded (NF) during the autumn dormancy induction period. Different letters show significant differences according to an ANOVA at $\alpha = 0.05$. Bars are means ± SEM, $n = 24$ (non-flood) and 12 (flood) due to differences in survival between flood treatments

Discussion

Autumn is an important time for nutrient uptake in *Q. texana* tree seedlings. All of the nutrients that we measured except boron accumulated in branches, stems, and/or roots during autumn senescence, in most cases without any evidence of resorption from leaves (i.e., Ca, S, Zn, Cu, Mg, Mn, Fe, B, Na). Previous studies of trees indicate that N resorption can vary from 0–90%, depending on developmental stage, and environmental conditions, and high internal N status may result in a reduced N resorption efficiency. Leaf N was about 1–1.2% in our study prior to initiation of leaf senescence, which is fairly low. However, the N concentration of winter storage tissues in non-flooded seedlings increased substantially during autumn (e.g., nearly threefold for tap roots). It is possible that the high N status, due to the large amount of N taken up from the soil in non-flooded seedlings, may have reduced the capacity to resorb N from leaves to already full sinks, or may have resulted in signaling to leaves that reduced N remobilization during leaf senescence. The total N content increased much more than could be accounted for by the decrease in total leaf N, suggesting that a substantial amount of N uptake may occur in the autumn. Several prior studies reported uptake of N from soil during the autumn dormancy induction period in trees (Millard and Thomson 1989; for review see Millard and Grelet 2010) but uptake during autumn may be even more important in the southern temperate region. N uptake by tree roots is dependent on soil temperatures (Dong et al. 2001), which remain warmer than air temperatures later into the season (Tsilingiridis and Papakostas 2014). In the southern United States, *Q. texana* may experience warm air temperatures until late in the autumn (Sample and Babst 2018). Thus, it is likely that N uptake by *Q. texana* roots continues during much of the autumn

Table 2 Spring tissue biomass of *Quercus texana* seedlings for the autumn-flood (F) and non-flood (NF) treatments

	Leaves (g dw)	Branches (g dw)	Main stem (g dw)	Taproot (g dw)	Fine roots (g dw)	New roots (g dw)
Non-flooded	7.93 ± 0.58	0.47 ± 0.05	10.83 ± 0.63	13.37 ± 0.72	**9.14 ± 0.62***	**0.13 ± 0.02***
Flooded	7.26 ± 0.92	0.37 ± 0.06	12.39 ± 1.24	14.84 ± 1.63	**6.89 ± 1.10***	**0.24 ± 0.05***

Leaf, branch, main stem, taproot, fine root, and new root biomasses are shown as dry weight (dw). Bolded values with asterisks to the left represent tissues that had significant differences according to an ANOVA at $\alpha = 0.05$. Values are means ± SEM; $n = 24$ non-flooded seedlings and 12 autumn-flooded seedlings, due to differences in survival between flood treatments

dormancy induction period as long as soil moisture is adequate and our data suggest that uptake during autumn could make a major contribution to *Q. texana* seedling nutrition.

Soil flooding apparently impeded uptake of N and most other nutrients during the autumn dormancy induction period. While non-flooded seedlings significantly increased the N concentrations in their main stems and roots during autumn senescence, autumn-flooded seedlings had stem and root N concentrations after leaf fall that were similar to pre-senescent control seedlings. Total seedling contents of P, Ca, Mg, S, Zn, Cu, and B were similarly reduced by autumn flooding, indicating that uptake of these nutrients during autumn dormancy induction tended to be impaired by root flooding. The reduced nutrient uptake in autumn-flooded seedlings may have been due to both physiological inhibition of nutrient uptake in living roots (Fisher and Stone 1990), and fine root mortality. Previous reports demonstrated that flooding during the growing season decreases uptake of N and other nutrients in trees (Pezeshki et al. 1999; Gardiner and Krauss 2001; Kreuzwieser et al. 2002), due to hypoxia (Fisher and Stone 1990). The exceptions are Fe, Mn, and Na, which tended to be hyperaccumulated under flood conditions in *Q. texana* seedlings, and in previous studies (Smethurst et al. 2005; Du et al. 2009; Wang et al. 2017). Overall, our observations indicate that uptake of N and most other nutrients by *Q. texana* is reduced by flooding in the autumn just as it is during the growing season, which could magnify the importance of conservation mechanisms, such as N resorption from leaves.

Only N, P, and K appeared to be resorbed from leaves, as indicated by a decrease in leaf total content, with a concomitant increase in total content in at least one of the storage tissues, although N and P resorption were only apparent in autumn-flooded seedlings, not non-flooded seedlings. Most of the resorbed N in autumn-flooded seedlings could be accounted for by N storage in branches, which is consistent with previous evidence of predominantly short-distance N transport, and N storage in young branches in oak (Bazot et al. 2013). Flooding may reduce phloem transport of carbohydrates to roots (Sloan et al. 2016), and the increased anthocyanin accumulation that we observed in the autumn-flood treatment can indicate restriction of carbohydrate export from leaves (Botha et al. 2000; Arnold et al. 2004). However, anthocyanin accumulation also may be a more general response to stress (Hoch et al. 2001; Morris and Wang 2007), and we found increased, not disrupted, short-distance N transport from senescing leaves to branches due to autumn flood. Thus, flooding of the root system may have disrupted phloem transport to the roots, but our results do not support the hypothesis that flooding disrupts phloem transport throughout the entire seedling. In fact, disruption of phloem transport to roots may have resulted in preferential transport of N to other sink tissues (i.e., stem, branches, and buds). The flood-induced increase in N resorption may have been a result of a stress signal from the roots such as 1-aminocyclopropane-1-carboxylic acid (ACC) the precursor to ethylene (Jackson 1997), or a response to low plant N status, which may lead to increased autumn N resorption efficiency (Millard and Thomson 1989). Future research should address the regulatory mechanism behind this flood-induced increase in autumn N resorption.

As expected, there was no growth of seedlings during the autumn dormancy induction period, and so no differences in growth between flood treatments during autumn. However, autumn flooding resulted in substantial fine root mortality in *Q. texana*, similar to previously reported effects of flooding during the growing season (Pezeshki et al. 1999; Anderson and Reza Pezeshki 2001). Thus, seedlings exposed to autumn flood experience a compound disadvantage; autumn-flooded seedlings enter winter dormancy with reduced total N reserves, and begin new growth in the subsequent spring with fewer fine roots and a diminished capacity for N uptake.

Indeed, autumn flooding resulted in increased seedling mortality over winter, and affected spring growth. Surviving seedlings had lower fine root mass than non-flooded seedlings after the first flush of growth was completed in the spring, but there was not a simple reduction in overall growth. Autumn-flooded seedlings had nearly double the new root biomass of non-flooded seedlings, indicating that resource allocation in the seedlings was oriented towards compensating for flood damage to the root system. Replacement of fine roots is important for recovering the ability to take up N. In some tree species, initial shoot growth in spring relies solely on N remobilization (Grassi et al. 2002; Guak et al. 2003; Millard et al. 2006), but other tree species initiate N uptake from the soil at the same time as N

remobilization (Millard et al. 2001; Frak et al. 2002). Since flooded *Q. texana* seedlings had both reduced N reserves and reduced fine root mass, it is logical that restoration of nutrient, and also water, uptake capacity are a high priority for autumn-flooded *Q. texana* seedlings, given the need to cope with potentially very dry conditions during summer, and the intense competition from other trees and herbaceous vegetation that is common in their native bottomlands.

The apparent compensatory growth of roots came at the expense of reduced stem diameter growth in autumn-flooded seedlings. Given that N stored over winter may make a substantial contribution to the N required for initial spring growth (Millard and Thomson 1989), this reduction in spring growth is not surprising. On the contrary, we found that autumn-flooded oak seedlings nearly matched the height growth of non-flooded seedlings, albeit with a shrubbier growth habit with more numerous, but shorter and thinner branches. This shrubbier growth habit is most likely a symptom of stress, rather than an adaptive response. Height growth is often a priority over diameter growth in the seedling stage, because competition for sunlight may be intense (King 1981). Favoring height growth over diameter growth may provide an adaptive advantage, but it may also entail risks. Diameter growth in young seedlings is mainly due to xylem formation. In other red oak species, like *Q. rubra* and *Q. phellos*, only the xylem produced in the current year contributes to water transport (Cochard and Tyree 1990; White 1993). Thus, while maintaining stem elongation and height growth may allow greater access to light, reduced diameter growth could reduce total stem xylem conductance, making seedlings more vulnerable to water stress if dry conditions prevail during the season following a major autumn flood event.

Conclusion

Our study indicates that N uptake from soil during autumn may make a major contribution to seedling N storage reserves just prior to overwintering, more so than autumn leaf N resorption on a whole-seedling level. N uptake was inhibited by autumn flooding, and this prevented N accumulation in the main stem and roots of seedlings flooded during the autumn dormancy induction period. As a consequence of reduced N content, N resorption from leaves increased in autumn-flooded *Q. texana* seedlings. Overall, if long-term flooding begins in autumn in bottomland hardwood systems before seedlings enter dormancy, it may result in less N storage accumulation prior to winter, lower survival over winter, reduced fine roots for nutrient and water uptake in spring, and reduced overall biomass of the surviving seedlings the following growing season. Thus, sustainable management of green tree reservoirs must account for the stress imposed on trees by flooding during autumn, when the processes that prepare trees for dormancy are active. Furthermore, the predicted increase in frequency or intensity of flooding in the future, such as that caused by hurricanes and tropical storms in late summer and autumn, could impact even forests that are moderately well adapted to transient flood, like the bottomland hardwood forests of the Southeastern United States.

Author contribution statement RDS performed the experiments and statistical analysis. BAB and RDS designed experiments, and wrote the manuscript together.

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