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Xylem vessel traits predict the leaf phenology of native and non-native understorey species of temperate deciduous forests

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Summary

1. Non-native understorey woody species have been shown to extend leaf display and inhabit vacant phenological niches in early spring and late autumn when growing with native counterparts in temperate deciduous forests across the world. Despite the potential competitive advantages, extended leaf duration also subjects non-native species to possible hydraulic risks associated with maintaining leaves during periods of increased frost probability. It remains unclear how non-native species are able to maintain xylem function within this context.

2. Leaf phenology in temperate deciduous trees has been shown to be a function of xylem anatomy, with earlier bud break associated with smaller xylem vessels due to the presumed resistance of smaller vessels to freezing-induced cavitation. We examined relationships between leaf phenology and xylem vessel traits across 82 native and non-native understorey deciduous woody species common to eastern U.S. deciduous forests. We hypothesized that non-native species possess xylem vessel traits associated with maximum hydraulic safety during frost-prone spring and autumn leaf display without compromising rapid growth rate.

3. Larger metaxylem vessels in non-native species were associated with both faster spring growth and delayed autumn leaf fall compared to native species. Non-native species also had smaller latewood vessel diameter, latewood vessel area percentage and a higher proportion of solitary vessels in the entire secondary xylem cross section compared to natives, potentially increasing their resistance to freezing- and/or drought-induced cavitation in autumn, thus allowing for delayed autumn leaf fall.

4. Native and non-native species exhibited similar dates of spring bud break and leaf emergence, consistent with similar xylem vessel size and vessel area percentage within metaxylem and earlywood. Within both groups, species with earlier bud and leaf emergence had a higher total percentage of vessel area within metaxylem and earlywood. This suggests understorey species need sufficient water to support their early spring growth at the risk of freezing-induced cavitation.

5. Our study suggests xylem vessel properties, along with cross-sectional spatial xylem vessel distribution, reflect the capacity of non-native plants to thrive in a new environment and deepen our understanding of the physiological mechanisms of successful invasions of non-native understorey woody plant species.

Key-words: delayed autumn leaf fall, extended leaf duration, metaxylem, solitary vessel percentage, spring leaf emergence, vessel area percentage, vessel diameter, xylem seasonal variation

Introduction

Non-native plant species that successfully establish and spread in native ecosystems often possess traits that confer high competitive ability compared to co-occurring native species. For example, leaf phenology – the timing of leaf emergence and senescence – has been implicated as a mechanism of invasion in temperate forests (Wolkovich, Cook & Davies 2014). Leaf phenology studies have indicated spring months before canopy closure is a critical

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carbon (C) gain period for native understorey deciduous woody species in eastern U.S. forests (Gill, Amthor & Bormann 1998; Augspurger, Cheeseman & Salk 2005). Conversely, extended autumn leaf display after canopy leaf fall seems to be rare in native understorey species (Augspurger, Cheeseman & Salk 2005; Fridley 2012), presumably due to lower autumn solar radiation levels (Fridley 2012), declining leaf area and photosynthetic capacity towards winter dormancy (Gill, Amthor & Bormann 1998; McEwan *et al.* 2009) and/or freezing temperature that causes winter-induced xylem cavitation (Wang, Ives & Lechowicz 1992). This produces vacant autumn phenological niches in native ecosystems; inhabiting these niches to exploit temporally available resources becomes significant for successful invasions (Wolkovich & Cleland 2010).

When growing with native species in temperate forests, some non-native invasive understorey woody species have been found to have advanced spring leaf emergence (Zotz, Franke & Woitke 2000; Xu, Griffin & Schuster 2007; McEwan *et al.* 2009), and most of those in eastern North America have been shown to exhibit a delay in autumn leaf fall compared to native species, independent of phylogeny (Fridley 2012). The temporal phenological disparity between native and non-native understorey species potentially enhances C gain in non-native species, which may benefit their long-term survival, affect their distribution and abundance and ultimately impact ecosystem processes including CO_2, water and energy movement (Polgar & Primack 2011). However, despite the potential competitive advantages, extended leaf duration also subjects non-native species to possible hydraulic risks associated with maintaining leaves during periods of increased frost probability in early spring and late autumn especially (Wolkovich & Cleland 2010). Although differences in foliar phenology between natives and non-natives have been implicated in numerous studies, it remains unclear how they are able to maintain xylem function within this context.

Plants that possess larger vessels are inherently more susceptible to freezing-induced cavitation (Hacke & Sperry 2001; Willson & Jackson 2006), with the trade-off that larger vessels confer highly efficient xylem lumen water transport (Ewers 1985; Tyree & Ewers 1991; Sperry, Hacke & Pittermann 2006). Air bubbles form during the freeze–thaw process, and larger vessels are less capable of redissolving embolisms compared to smaller vessels. This occurs for two reasons: (i) the time for bubble dissolution increases with increasing conduit diameter, and (ii) the dissolution only occurs at much less negative xylem pressure in larger vessels (Yang & Tyree 1992; Davis, Sperry & Hacke 1999; Pittermann & Sperry 2003), leading to blockages in the water-conducting pathway that disrupt the normal water transport capacity of a plant. Thus, possessing smaller xylem vessels is an adaptation for reducing the risk of freezing-induced cavitation (Davis, Sperry & Hacke 1999; Pittermann & Sperry 2006), which we hypothesize confers species with extended leaf duration, a competitive advantage in the context of potential early- and late-season C gain. In a classic synthesis, Lechowicz (1984) showed that earlier-leafing temperate trees tended to have smaller xylem vessels or a lower proportion of secondary xylem occupied by vessels compared to trees with later leaf emergence and *vice versa*. Similarly, in an examination of a broad spectrum of temperate woody plants in the Northern Hemisphere, Panchen *et al.* (2014) found that diffuse- and semi-ring-porous species leafed out 1–2 weeks earlier on average than ring-porous species, related to the smaller associated vessel diameter (VD). However, Smith *et al.* (2013) observed smaller average VD in non-native understorey woody species than their native counterparts, even though spring bud and leaf emergence was similar between natives and non-natives (Fridley 2012).

In temperate woody plants, xylem formation is composed of distinct developmental stages that may complicate attempts to link simple classes of xylem structure to leaf function. Primary growth includes the development of both protoxylem and metaxylem. Both developmental stages are located within each leaf trace and responsible for shoot elongation (Larson 1976). Morphologically, metaxylem vessels are larger and developed in strands compared to the much smaller and solitary protoxylem vessels. These morphological and structural traits theoretically endow metaxylem with more efficient water transport (Loepfe *et al.* 2007) and water storage capacity, which is critical to plant survival during prolonged water stress (Fisher, Tan & Toh 2002). As plants grow larger, secondary xylem vessel development (early- and latewood) is initiated from the vascular cambium, producing more vascular tissues to transport water and nutrients. In the spring, this process results in the production of earlywood, while latewood is developed later in the season.

According to the transition in vessel size and distribution between early- and latewood, there are three types of xylem porosity in general in hardwood species: ring-porous, semi-ring-porous and diffuse-porous. In ring- and semi-ring-porous species, earlywood vessels are usually larger than latewood vessels and can transport a large proportion of water when the plant water status is favourable. However, earlywood xylem vessels most likely become air-filled during winter drought in northern temperate areas, with only the smaller latewood vessels remaining conductive (Lo Gullo *et al.* 1995). Thus, we hypothesize that the timing of autumn leaf fall might be largely associated with the xylem anatomical properties of latewood. Higher resistance of smaller vessels to freezing-induced cavitation suggests that having smaller vessels in latewood might be a means to postpone autumn leaf senescence.

Overall, we expect those species of longest leaf display, including both earlier spring leaf emergence and later autumn leaf fall, to possess relatively small vessels in earlywood and even smaller vessels in latewood. Conversely, if some of these same species also exhibit fast spring growth during periods of high nutrient, light and water availability, larger metaxylem vessels may facilitate faster spring shoot extension via more efficient water transport. We hypothesize that woody forest invaders possess both traits in order to maximize both xylem function safety and early-season growth.

Here, we address relationships between spring and autumn leaf phenology and the stem xylem anatomy of 82 native and non-native understorey deciduous woody species, including 48 native and 34 non-native species belonging to 24 genera common to eastern U.S. temperate deciduous forests. We monitored leaf phenology during 2008–2010 in a common garden and examined xylem vessel characteristics in metaxylem, earlywood and latewood. We aimed to test three hypotheses: (i) non-native species in temperate deciduous forests are more likely to exhibit an optimal pattern of stem xylem structure than native species, including smaller vessels of early- and latewood; (ii) this xylem vessel pattern is correlated with extended leaf duration; and (iii) non-natives of fast growth potential have larger metaxylem vessels, allowing them to maximize both xylem function and growth potential.

Materials and methods

PLANT MATERIALS AND GROWING CONDITIONS

All plant material was obtained from a common garden at Syracuse University, Syracuse, NY, USA (43°03′N, 76°09′W), established in 2006–2007. Focal species included 48 native and 34 non-native understorey deciduous woody species, belonging to 24 genera, including 9 genera containing at least one native and one non-native member (Table S1, Supporting Information). Most species were grown in three replicate blocks, under 80% black shade cloth during the growing season to simulate deciduous forest conditions. Plants were established from wild individuals in central New York where possible and otherwise obtained from nursery stock of similar latitude. For details on garden establishment and maintenance, see Fridley (2012).

ANATOMICAL MEASUREMENTS

Five to six 1-year-old terminal branch stems were randomly sampled from individuals of each species for anatomical measurements in November 2010. A 1-cm-long segment was randomly cut from each original stem segment, immediately preserved in formalin–acetic acid–alcohol solution (FAA) and dehydrated in a graded series of ethanol–tertiary butanol (TBA) dilutions before infiltration with pure TBA. All the tissue samples were embedded into Paraplast Plus embedding medium (McCormick Scientific, Saint Louis, MO, USA). After polymerization, the transverse cross sections were cut using a HM355S rotary microtome (Microm International GmbH, Walldorf, Germany) into 20-μm-thick sections which were mounted onto slides. The slides were stained and examined using a light microscope (Axioskop 2 Plus; Carl Zeiss Microscopy, Jena, Germany) with a digital camera (Olympus Imaging Corp., Tokyo, Japan) for anatomical observations. See Smith *et al.* (2013) for more details of anatomical sectioning.

Xylem porosity type was identified for each species (Rowell 2012). In ring-porous species, the transition from earlywood to latewood is abrupt, with VD being much larger in earlywood than that in latewood. In diffuse-porous species, earlywood vessels are of approximately the same diameter as those in latewood. In semi-ring-porous species, vessel size and distribution patterns intermediate between ring- and diffuse-porous, with VD gradually decreasing in size from earlywood towards latewood. Each stem cross section was then examined for average xylem vessel size, maximum vessel size, vessel number, total vessel area and total xylem area. The anatomical measurements were conducted using IMAGEJ 1.44p software (http://rsbweb.nih.gov/ij/, National Institute of Health, Bethesda, MD, USA). Vessel size was then converted to VD assuming vessels were circular. Hydraulically weighted vessel diameter (d) was determined as follows:

$$d = 2(\Sigma r^5/\Sigma r^4),$$

where r is the radius of a conduit (Sperry *et al.* 1994). Vessel area percentage of the entire xylem cross section was determined as the proportion of the entire xylem cross section occupied by vessels. When calculating vessel area percentage of a specific zone, for example earlywood and latewood, the entire cross-sectional area was still used in the calculation. For example, vessel area percentage in earlywood and latewood was calculated as the proportion of the entire xylem cross section occupied by early- and latewood vessels, respectively. Vessel density was calculated as the number of vessels per mm^2 of xylem cross section. Solitary vessel percentage was estimated as the percentage of solitary vessels relative to the total number of vessels in the entire secondary xylem cross section. All of these parameters were measured in metaxylem, earlywood and latewood, respectively.

LEAF PHENOLOGY MONITORING

From 2008 to 2010, we monitored spring bud and leaf development in four stages, including ‘bud active’, ‘bud exposed’, ‘bud burst’ and true leaf emergence [for definitions of different stages of bud and leaf development, see Fridley (2012)], by photographs at 2- to 5-day intervals from early March to mid-May. We also randomly selected five healthy terminal branch stems to monitor leaf senescence and maximum rate of leaf production. For leaf senescence, total extant leaves on each stem were counted at 2-week intervals from July to December; 50% and 90% leaf fall were then estimated. Leaves were considered senesced when chlorosis was >50%, measured with a Chl meter (CCM-200; Opti-Sciences, Hudson, NH, USA). Leaf duration of each plant was determined from the date of true leaf emergence to the date of 90% leaf fall. Maximum rate of leaf production (i.e. maximum number of new leaves produced per day) for each species was estimated as the annual maximum number of new leaves produced by any stem over any 2-week period after initial leaf flush, standardized to leaves per day (Fridley & Craddock 2015). Total extant leaves on existing buds and new shoots on each branch were counted at each interval. A new leaf was counted once it had reflexed by 20°. Leaf phenology measurements were averaged over the 2008–2010 period for each species in analysis. Although there was a year effect, species did not vary in ranking in leaf phenology across years (Fridley 2012).

STATISTICAL ANALYSIS

The means of xylem anatomical traits between native and non-native species, or among three types of xylem porosity, were compared using Welch's test. *P*-values were adjusted using Benjamini–Hochberg correction to control a false discovery rate of <0·05 (Benjamini & Hochberg 1995; Waite & Campbell 2006). Average VD, hydraulically weighted VD and vessel area percentage within metaxylem and earlywood, and vessel area percentage in latewood were $\log_{10}$-transformed before conducting Welch's test to meet the assumption of normality. Wilcoxon tests examined mean differences in leaf phenological traits between native and non-native species, or among three types of xylem porosity, with *P*-values adjusted by Benjamini–Hochberg correction. A post hoc test was conducted to compare all types of xylem porosity in a pairwise manner using Steel–Dwass' test. Differences were considered significant if $P < 0{\cdot}05$. Pearson's correlation coefficients (r) were calculated for all pairwise combinations of leaf phenological traits and xylem anatomical traits, both between natives and non-natives and separately within native and non-native groups. When the correlation was significant, reduced major axis (RMA) regression (Niklas & Enquist 2001; Martínez-Cabrera *et al.* 2011) was performed to model the relationship between two variables. Cook's distance (D) was calculated to estimate the influence of potential outliers on regressions. A data point was considered as highly influential if $D \geq 1$. D-values of all the observations were <0·15 in this study. Statistical analyses mentioned above were performed in JMP PRO 11 (SAS Institute Inc., Cary, NC, USA). Because spring and autumn foliar phenology contrasts between this group of native and non-native species have been shown to be independent of phylogeny (Fridley 2012), we did not pursue phylogenetic correlations of xylem anatomy further in the present study.

Results

COMPARISONS OF XYLEM VESSEL TRAITS BETWEEN NATIVE AND NON-NATIVE SPECIES

Either within the unit of metaxylem and earlywood (Fig. 1) or within earlywood itself, we detected no differences in average VD, hydraulically weighted VD or vessel area percentage between native and non-native species. Non-native species average VD in the latewood, however, was 46% smaller compared to native species ($P < 0{\cdot}001$). Hydraulically weighted VD in latewood was also significantly smaller for non-native species over native species ($P < 0{\cdot}001$). Non-native species had on average a 98% smaller vessel area percentage in latewood compared to native species ($P < 0{\cdot}001$).

CORRELATIONS BETWEEN LEAF PHENOLOGY AND XYLEM VESSEL TRAITS

Non-native species had a higher maximum rate of leaf production compared to native species ($P = 0{\cdot}004$), and as predicted, their average metaxylem VD was 24% larger than that in native species ($P = 0{\cdot}046$). The positive correlation ($r = 0{\cdot}60$, $P < 0{\cdot}001$) between average metaxylem VD and maximum rate of leaf production indicates that species having larger metaxylem vessels have the capacity to produce more leaves per day over the growing season (Fig. 2).

As reported by Fridley (2012), there was no significant difference in bud and leaf emergence in the spring between

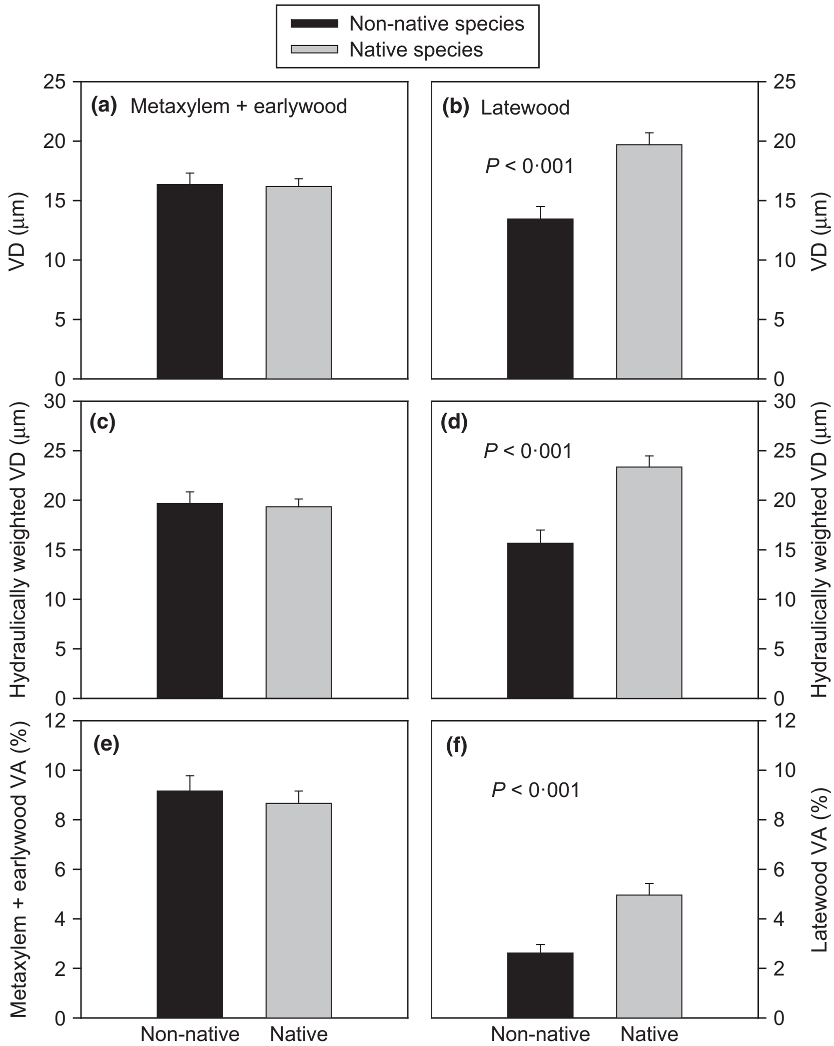

Fig. 1. Comparisons of average vessel diameter (VD; a and b), hydraulically weighted VD (c and d) and vessel area (VA) percentage (e and f) between native and non-native species in metaxylem and earlywood (a, c and e), and latewood (b, d and f). Black and grey bars represent non-native and native species, respectively. Level of significance of P-value is indicated where comparisons are significant according to Welch's test with Benjamini–Hochberg correction.

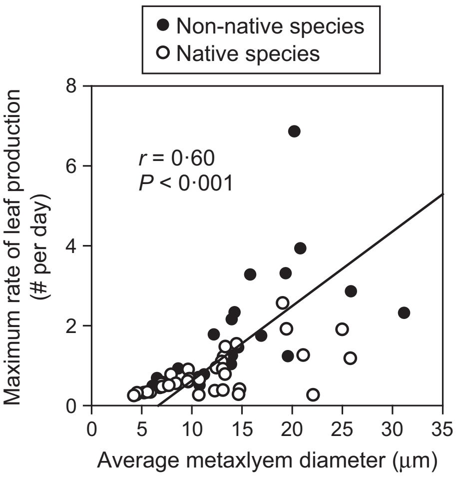

Fig. 2. Reduced major axis regression between average metaxylem vessel diameter and maximum rate of leaf production. Black and white circles represent non-native and native species, respectively. Correlation coefficient (r) and level of significance of P-value are indicated. The regression line is shown for the significant relationship.

native and non-native species. Across all native and non-native species, spring bud and leaf emergence, including bud active, bud exposed, bud burst and true leaf emergence, was negatively correlated with the sum of metaxylem and earlywood vessel area percentage (Fig. 3; Table S2), indicating that buds and leaves emerged earlier with increasing metaxylem and earlywood vessel area percentage.

Non-native species delayed autumn leaf fall compared to native species, as previously reported by Fridley (2012). Across all native and non-native species, the date of 50% and 90% autumn leaf fall was positively correlated with average metaxylem diameter (Fig 4a,b; Table S2), that is species with stems of larger metaxylem diameter tended to delay autumn leaf fall. Meanwhile, autumn leaf fall was negatively correlated with latewood vessel area percentage (Fig 4c,d). A higher proportion of solitary vessels in secondary xylem was also associated with delayed 50% autumn leaf fall (Fig. 4e), but not 90% leaf fall (Fig. 4f).

Leaf duration in non-native species was longer than that in native species by an average of 16 days ($P < 0.001$). Overall, the factors determining delayed autumn leaf fall were also correlated with longer leaf duration. Specifically, species possessing larger metaxylem diameter, or smaller latewood vessel area percentage, or higher proportion of solitary vessels in secondary xylem tended to have longer leaf duration (Fig. 5).

All leaf phenological traits were also compared among the three types of xylem porosity. Most of the traits were similar among three types of xylem porosity except 50% leaf fall ($P = 0.041$) and the maximum rate of leaf production ($P = 0.0084$, Table S5). Fifty-percent leaf fall was earlier in diffuse-porous species compared to semi-ring and ring-porous species. Maximum rate of leaf production was highest in ring-porous species and lowest in diffuse-porous species.

Discussion

We evaluated whether non-native woody species of forest understories would possess xylem vessel traits associated with maximum hydraulic safety during frost-prone spring

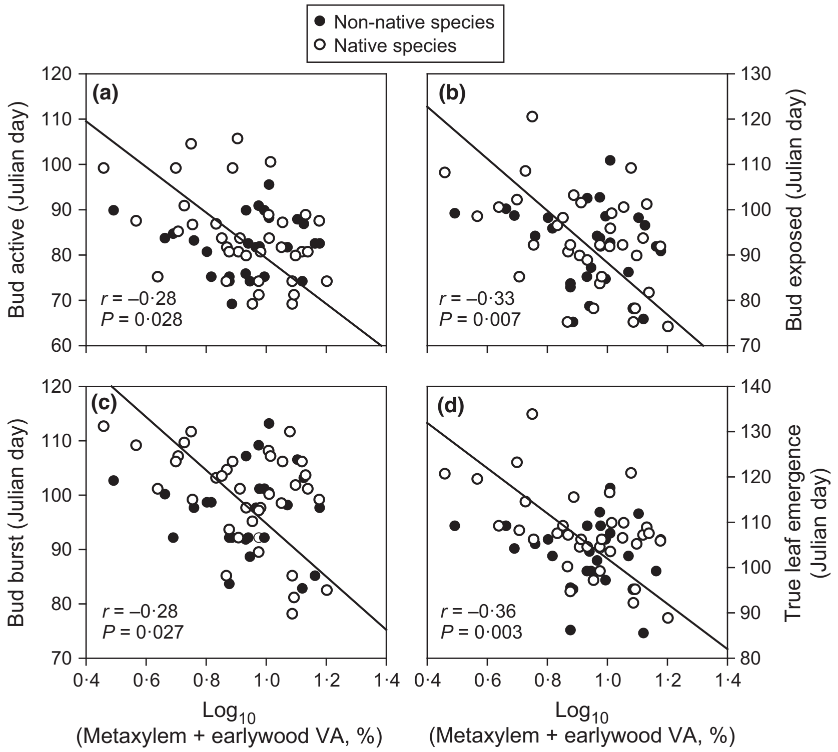

Fig. 3. Reduced major axis regression between bud active (a), bud exposed (b), bud burst (c) and true leaf emergence (d) with $\log_{10}$-transformed sum of metaxylem and earlywood vessel area (VA) percentage. Black and white circles represent non-native and native species, respectively. Correlation coefficient (r) and level of significance of P-value are indicated. The regression lines are shown for significant relationships.

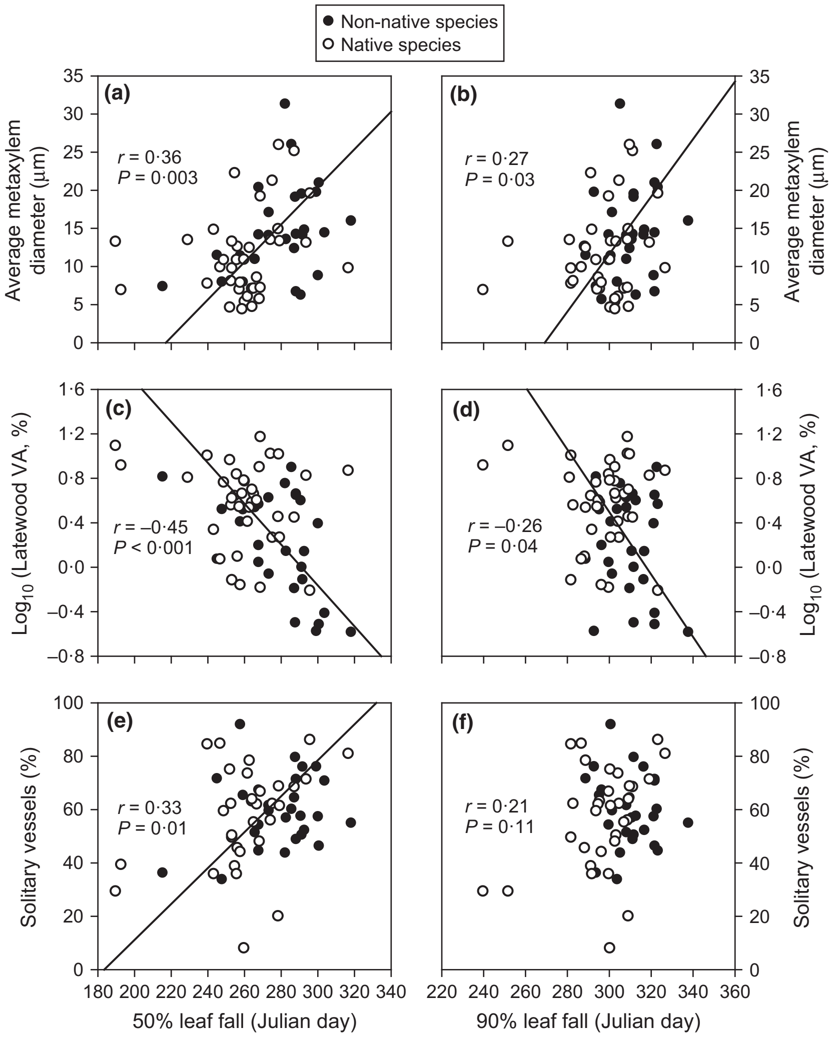

Fig. 4. Reduced major axis regression between 50% leaf fall (a, c and e) and 90% leaf fall (b, d and f) with average metaxylem diameter (a and b), $\log_{10}$-transformed latewood vessel area (VA) percentage (c and d) and proportion of solitary vessels in an entire secondary xylem cross section (e and f). Black and white circles represent non-native and native species, respectively. Correlation coefficient (*r*) and level of significance of *P*-value are indicated. The regression lines are shown for significant relationships.

and autumn leaf display without compromising fast spring growth, due to their implicated competitive advantages of extended leaf duration compared to co-occurring natives. Overall, our analysis of xylem vessel traits in 82 understorey natives and non-natives supported both hypotheses, consistent with the view that non-native invasive species are less susceptible to trade-offs between growth and stress tolerance, giving them a competitive advantage over co-occurring native species.

Larger metaxylem vessels in non-native species were associated with both faster spring growth and delayed autumn leaf fall compared to native species. Metaxylem vessels tended to be grouped together, especially in non-native species: they had a higher number of ≥5-vessel grouping compared to native species, as reported by Smith *et al.* (2013). Higher vessel grouping, usually with higher vessel density, can potentially result in a hydraulically functional advantage under stressed environments because it allows water to bypass cavitated vessels via alternative pathways created by intervessel pits connectivity within a vessel group (Loepfe *et al.* 2007; Robert *et al.* 2009). Moreover, wide metaxylem vessels have an important function of storing water in the large vessel lumens during cavitation (Holbrook 1995; Fisher, Tan & Toh 2002) and in parenchyma cells which are richer in primary xylem than secondary xylem (Carlquist 1962). During early spring, stored water and nutrients can be transported to the leaves facilitating maximum growth potential, and during late autumn, similarly, stored water can prolong non-native species' leaf display. We conclude that the wider and highly grouped metaxylem vessels ensured the continued growth in non-native species under water-stressed environments and allowed them to maintain leaves and capture a significant proportion of their annual C gain after canopy leaf fall (Fridley 2012).

In addition to wider metaxylem vessels, delayed autumn leaf senescence exhibited by non-native species was also associated with smaller latewood vessel area percentage. It is well-established that smaller xylem conduits are more resistant to freezing-induced cavitation (Hacke & Sauter 1996; Davis, Sperry & Hacke 1999; Cavender-Bares *et al.* 2005). Resistance to cavitation is an important factor in freezing or drought tolerance. Once most earlywood vessels become cavitated in late autumn (Lo Gullo *et al.* 1995;

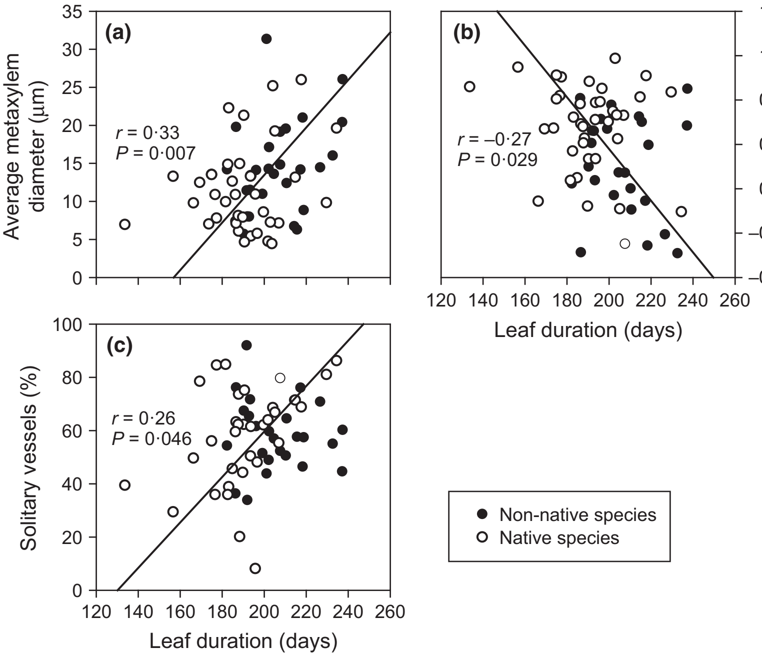

Fig. 5. Reduced major axis regression between leaf duration with average metaxylem diameter (a), $\log_{10}$-transformed latewood vessel area (VA) percentage (b) and proportion of solitary vessels in an entire secondary xylem cross section (c). Black and white circles represent non-native and native species, respectively. Correlation coefficient (*r*) and level of significance of *P*-value are indicated. The regression lines are shown for significant relationships.

Utsumi *et al.* 1999), smaller vessels in the latewood remain conductive, capable of transporting water for continued C gain. A smaller latewood vessel area percentage is mostly due to smaller average latewood VD, but is also influenced by the proportion of latewood in the entire xylem cross section (the smaller the proportion of latewood, the larger the proportion of metaxylem and earlywood). Under warm temperatures and abundant soil water, a larger proportion of metaxylem and earlywood contributes to more efficient hydraulic conductivity. Under freezing- or water-stressed conditions, since earlywood has a larger water storage capacity than latewood (Domec & Gartner 2002), a larger proportion of earlywood suggests a larger amount of stored water that can be used to help maintain leaves later in the season.

In addition to metaxylem diameter and latewood vessel area percentage, across all species we examined, autumn leaf fall was also positively correlated with the proportion of solitary vessels in the secondary xylem. As reported in Smith *et al.* (2013), non-native species had a higher proportion of solitary vessels in secondary xylem compared to natives. More solitary vessels (or less vessel groupings) in non-native species can decrease their vulnerability to cavitation by decreasing the probability of cavitation spread to adjacent vessels (Loepfe *et al.* 2007), resulting in greater resistance to freezing- and/or drought-induced cavitation during late autumn in non-natives. Although more solitary vessels can also decrease the potential maximum hydraulic efficiency, having wider and highly grouped metaxylem vessels may simultaneously balance the disadvantage from the aspect of safety (Robert *et al.* 2009).

Smith *et al.* (2013) observed smaller average VD with greater frequency in non-native understorey woody species when compared to native congeners. Since average VD was similar within the collective metaxylem and earlywood unit between native and non-native species, we suggest that smaller average VD in non-natives mainly resulted from smaller vessels within the latewood. Along with wider and highly grouped metaxylem vessels, smaller latewood vessel area percentage and a higher proportion of solitary vessels in secondary xylem, these specific anatomical features may facilitate non-native species' tolerance to frost and/or seasonal drought in late autumn, and contribute to a competitive advantage in which non-natives are able to maintain a longer growing season. This extended leaf display due to higher frost resistance in non-native species is in agreement with previous observations on an invasive deciduous shrub, *Lonicera maackii* (McEwan *et al.* 2009).

However, stem xylem vessel traits were not correlated with 90% leaf fall as well as with 50% leaf fall, suggesting that there are some unexplained environmental and physiological variations around these relationships, such as minimum freezing temperature, photoperiod and leaf cold acclimatization during frequent freeze–thaw events (Cavender-Bares *et al.* 2005). Also, most of the species in our study were diffuse- and semi-ring-porous; the few ring-porous species, such as *Rhamnus davurica*, exhibited both the smallest latewood vessel area percentage and the earliest 90% leaf fall, implying that smaller latewood vessels and/or vessel area percentage can help extend autumn leaf display provided that a minimum level of hydraulic capacity required for leaf activities is achieved.

As previously reported by Fridley (2012), native and non-native species had similar dates of bud and leaf emergence; here, we show this is supported by their similar xylem VD and vessel area percentage within the metaxylem and earlywood unit. Moreover, across all species, earlier bud and leaf emergence was associated with more, rather than less, total vessel area percentage of metaxylem and earlywood, suggesting that the spring leaf phenology of shrubs and lianas may be less constrained by xylem anatomy than that of trees (Lechowicz 1984). This implies that early spring growth may require sufficient water to potentially refill embolisms that occurred over the winter, rehydrate the stems and support early spring bud activities. Likewise, Sobrado (1993) found spring leaf emergence in tropical woody deciduous species was associated with a high wood water content in the spring. Some species develop new earlywood vessels immediately prior to bud break to guarantee sufficient water transport through functional vessels in the spring, such as *Fraxinus excelsior* (Sass-Klaassen, Sabajo & den Ouden 2011), *Prunus persica* (Améglio *et al.* 2002) and *Quercus petraea* (Michelot *et al.* 2012), implying that organogenesis and primary growth in buds is closely related to xylem cambium growth (Cochard *et al.* 2005).

Our spring leaf phenology results for understorey shrubs and lianas stand in contrast to those of Lechowicz (1984) and Wang, Ives & Lechowicz (1992), who showed that tree species with smaller vessels tend to leaf out earlier in spring. We suspect the difference can be attributed to their lack of separation of xylem vessel developmental stages within seasonal components of a xylem cross section. For example, in our data set, non-native species had smaller average VD within the entire xylem cross section compared to natives, but non-natives had similar dates of bud and leaf emergence as natives, most likely a result of similar VD and vessel area percentage within the collective metaxylem and earlywood unit. Secondly, our study included small statured woody species that may be more dependent on early leaf out to capitalize on light availability, such as higher photosynthetic photon flux density and higher ratio of red to far red light (Gill, Amthor & Bormann 1998). This again suggests that more water is required for early spring growth of understorey species before canopy closure, which can be achieved by possessing relatively larger vessels or vessel area percentage in metaxylem and earlywood despite the risk of freezing-induced cavitation. Thirdly, compared to shrubs, tree leaf phenology may be more constrained by some other plant functional traits that covary with leaf phenology and xylem vessel traits, such as plant height. Taller trees tend to leaf out later in spring and have shorter leaf duration (Seiwa 1999), because they have an advantage in light interception and higher temperatures during the middle to late growing season (Sun & Frelich 2011). Plant height has been found to be positively correlated with tree VD (Martínez-Cabrera *et al.* 2011), and larger vessels in deciduous trees are more likely to lose hydraulic conductivity over winter and constrain their timing of leaf emergence in spring (Wang, Ives & Lechowicz 1992). This implies an integration of vessel traits, plant height and leaf phenology in tree species. However, the correlations between plant height and vessel traits were weaker or not significant within shrubs (Martínez-Cabrera *et al.* 2011), suggesting that shrub leaf phenology may be independent of plant height, especially for the species in our study because they were growing without competition for light with neighbours (Fridley 2012).

In conclusion, we demonstrate that the extended leaf phenology of non-native understorey woody species in temperate forests is tightly linked to xylem vessel structure supporting an optimal physiology of hydraulic safety coupled with high growth potential early in the growing season. Specifically, having wider and highly grouped metaxylem vessels, smaller latewood vessel area percentage and higher proportion of solitary secondary vessels may facilitate leaf maintenance in non-native species during freezing- and/or water-stressed periods. This more optimal pattern of xylem structure attained by non-native species likely contributes to their success in the understorey of deciduous forests and their impact on native species and ecosystems (Polgar, Gallinat & Primack 2013).

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Data accessibility

Data deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad.76ph0 (Yin *et al.* 2015).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Table S1. List of 82 woody understorey species tested in this study.

Table S2. Pearson's correlation coefficient matrix for leaf phenological traits and xylem anatomical traits across 82 native and non-native species.

Table S3. Correlations between spring leaf phenology and total metaxylem and earlywood vessel area percentage within native and non-native species, respectively.

Table S4. Correlations between autumn leaf phenology, leaf duration and average metaxylem diameter, latewood vessel area percentage and solitary vessel percentage within native and non-native species, respectively.

Table S5. Comparisons of phenological traits and xylem vessel traits among three types of xylem porosity.