Application of herb-chronology: Annual fertilization and climate reveal annual ring signatures within the roots of U.S. tallgrass prairie plants

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ring signatures within the roots of U.S. tallgrass prairie plants

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Abstract

The relatively new field of herbaceous root chronology (“herb-chronology”) uses annual rings of secondary xylem in roots of perennial forbs to analyze belowground secondary growth as a function of annual growth environment. By using three tallgrass forb species from long-term experiments within Konza prairie of northeastern Kansas USA, we aim to find the effects of fertilization, growing season temperature, and precipitation on annual secondary growth. For two of the three species, we found annual rings were significantly larger between plots that were fertilized annually with phosphorus or nitrogen + phosphorus in contrast to unfertilized control plots. Rings also had significant variation with climatic variables. We found a consistent negative correlation with early season temperature for each species. However, early growing season precipitation proved to be far less consistent, with positive correlations only found in a few cases between species. Overall, we conclude that annual rings in these select tallgrass prairie species may not carry reliable climatic signatures; rather site-specific ecological factors, such as aboveground competition with neighbors, may be more important for annual ring patterns. In our discussion we propose a framework to help better disentangle the effects of site or climatic factors that may affect herbaceous annual ring variation.

Keywords: Annual rings, herb-chronology, grasslands, interannual climatic variability, fertilization, supplemental water

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Introduction

Annual growth rings in the secondary xylem of plants may be useful for linking past environmental conditions with annual growth. In the field of dendrochronology, annual ring widths from tree stems can be used in reconstructing long-term climatic histories from a diversity of time periods and plant communities worldwide (Conkey 1986; Matisons et al. 2012; Voelker et al. 2012). In addition to climatic reconstruction, annual rings are also useful for investigating stand history. For instance, a gradual decline in ring width helped resolve when exotic woody species first established and suppressed growth of natives in Ohio hardwood forests (Hartman and McCarthy 2007), or how severe drought limited growth of dominant canopy species in Mediterranean forests (Corcuera et al. 2004). Conversely, a gradual increase in annual growth could pinpoint the timing of community-wide disturbance events, which released growth and initiated succession of Appalachian old growth forests (Abrams et al. 1998; Hart et al. 2012).

Though dendrochronology is a valuable tool for vegetation science, it is only confined to studying the growth of species with perennial aboveground woody tissue. However, studying the growth of non-graminoid herbaceous species is just as important since herb species represent key components of grassland systems responsible for a major part of global net primary production (Hui and Jackson 2006). Being that annual net primary production in many grasslands is largely a function of annual climatic and site ecological factors (Knapp and Smith 2001), a technique similar to the study of rings in woody species is needed to investigate how yearly ecological and climatic conditions may impact plant growth and production in herbaceous species. Fortunately, certain techniques to monitor past growth may be applied to herbaceous species by using the annual rings of the secondary xylem within the taproot or rhizome of perennial forb species: a technique coined 'Herb-chronology' by Dietz and Ullmann (1998). 50 to 85% of all forb species sampled possess fairly to clearly demarcated growth rings (Dietz and Ullmann 1997). In the majority of cases these growth rings are found to be annual (von Arx and Dietz 2006). To date, the most common herb-chronology research is counting rings to assess population age structure (Dietz and Ullmann 1998; Dietz 2002; Pergl et al. 2006; Perkins et al. 2006).

In many respects herb-chronology has not reached its full potential. Dietz and Schweingruber (2002) stated that there is a lack of knowledge about annual growth rings in North American perennial forbs; since its beginning herb-chronology research has focused

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primarily on Central European grasslands. Furthermore, the true potential of using annual rings outside of population level studies, such as indicators of climatic or site-specific ecological factors, have only begun to be explored. Only a few studies in herb-chronology address these issues: For instance, Dietz and Ullmann (1998) unlocked past site history by discovering decreasing annual ring widths of herb species along a gradient of shade within an encroaching shrub. Dietz and von Arx (2005) found synchronous interannual variation in ring widths of perennial forbs across Europe and North America that were a function of El Niño Southern Oscillation (ENSO). In addition, Liu and Zhang (2010) found the same associations between ENSO and perennial forb root increments in an inner Mongolian grassland. Considerable amount of work has also been done linking alpine perennial forb annual ring increments to climatic conditions of the high altitudes, notably interannual variation in snowpack and/or drought (Dietz et al. 2004; Franklin 2013).

The tallgrass prairie of the central U.S. provides great potential for further exploring the possible site ecological and regional climatic signals perennial forbs may hold in their annual rings. Fire, mowing, and fertilization represent land management practices that affect annual production of these grasslands (Collins et al. 1998; Knapp and Seastedt 1998), which may influence annual ring width variation. In addition, these mesic systems have experienced great fluctuation in annual precipitation in the last few decades (Zhou et al. 2012), thus affecting productivity (Knapp and Smith 2001). However, little is known about the determinants of belowground annual production in prairie plants (Hui and Jackson 2006; Xu et al. 2012). Considering that about half of grassland net primary production is from belowground organic matter in the form of root biomass (Luo et al. 2009), any fluctuation in this belowground production could be important in sequestering carbon to long-term belowground sinks (Zhou et al. 2012), especially if annual belowground biomass production is tied to yearly aboveground production.

Using preexisting research plots with imposed treatments, we ask the following questions: 1. How do different levels of annual fertilization affect the annual ring width? 2. How does supplemental irrigation affect the annual ring width? 3. How do average monthly temperature and precipitation in different parts of the growing season relate to ring width? 4. How does the pairwise correlation of annual rings compare between site-specific ecological

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factors (intra-treatment correlation) and possibly the regional climate (inter-treatment correlation)?

Materials and methods

Study site and LTER experiment descriptions

Konza Prairie Biological Station (KPBS) is part of the National Science Foundation's Long Term Ecological Research (LTER) network and is jointly owned and operated by Kansas State University and The Nature Conservancy. Located on approximately 3,487 hectares of native tallgrass prairie in the Flint Hills region of northeastern Kansas (39.0833°N, 96.5833°W), Konza Prairie's LTER program consists of numerous long-term studies focusing on fire, grazing, and climatic studies and their impact on the structure, function, and composition of native tallgrass prairie vegetation. C4 grasses typical of undisturbed native tallgrass prairie dominate this area, consisting predominately of *Andropogon gerardii* (Big bluestem), *Schizachyrium scoparium* (Little bluestem), *Sorghastrum nutans* (Indiangrass), and *Panicum virgatum* (Switchgrass) (Freeman 1998). Forb species have lower biomass but contribute substantially to the diversity of the region (Turner and Knapp 1996). Mean annual air temperature at KPBS is 13 °C with the late summer months ranging from 20-33 °C and late winter months ranging between -9- 3 °C (Nippert and Knapp 2007). Average annual precipitation of the station is 835 mm, to which about three fourths of this coincides with the late spring and early summer of the growing season (Hayden 1998).

We utilized two ongoing experiments initiated in 1986. The first such experiment, titled "the belowground plot experiment" (Rice et al. 1998), monitors the interactive effects of annual mowing, burning, and fertilization on aboveground net primary production (ANPP), belowground net primary production (BNPP), plant species composition, plant N and P content, decomposition, soil chemistry, and soil biota composition. In total there are 64 12 m² plots set up in a split-plot design with main blocks arranged by fire treatment and further subdivided by mowing and fertilization treatments. Burning and mowing treatments are either plots that are burned and/or mowed once on an annual basis or plots that remain as unburned and/or unmowed control plots. It is important to note that mowing treatments were discontinued in 2003.

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Fertilization consisted of four treatments: an unfertilized control (C), nitrogen only (N), phosphorus only (P), or a nitrogen + phosphorus nutrient (N+P) addition. There are four replications of each of the 16 possible treatment combinations for a total of 64 plots. Annual burns are implemented every late June while fertilization is applied by hand broadcasting annually in early June using the following treatments: N = 10g N·m² as ammonium nitrate, P = 1g P·m² as superphosphate, and N+P = 10g N·m² + 1g P·m² (Rice et al. 1998).

The second experiment is a water supplementation study (Knapp et al. 1994), identified as the “irrigation transects experiment,” where the amount of water supplied is set to meet expected evapotranspirational demands per year. Watering events are contingent upon the timing of precipitation events, but usually are at the highest frequency during the late summer where precipitation is relatively low and temperatures the highest. Two 100-meter long irrigation lines span from the top of a rocky hillside down to lowlands below at a medium slope gradient. Twelve 1.5 meter tall high impact sprinkler heads are located at equal distance along the irrigation lines, with a spray rate of 8mm/hour to a radius of 15 meters from the head (Knapp et al. 1994). Each 100 meter irrigated span serves as a transect where water status of the most dominant grass species is measured on an annual basis, along with ANPP, within the three meter zone of maximum spray coverage of each individual sprayer (Knapp et al. 1994). Control transects that are also 100 meters long are located adjacent to each irrigation transect, but out of the spray zone of adjacent irrigation treatments.

Field and laboratory methods

In early September 2013, we visited the two experimental sites located at Konza Prairie and assessed which species provided frequent enough abundance amongst the different treatments. The overall focal species in our study were *Asclepias syriaca*, *Dalea purpurea*, and *Lespedeza capitata*. *A. syriaca* is a member of the milkweed family (Apocynaceae), with a root system that appears to be a rhizome, and an erect aboveground shoot that can reach about two meters in height. *D. purpurea* and *L. capitata* (both Fabaceae) are both taprooted perennials with multi-branched aboveground shoots that may reach at a maximum of 1.5 meters in length. KPBS represents the middle latitude in all three of the respective species ranges. *D. purpurea* and *L. capitata*, were found exclusively in plots that were burned annually while *A. syriaca* was too infrequent in unburned plots to have a sufficient sample size between burn treatments. Thus, for

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each species, we could not test between the burn treatments. Additionally, mowing treatments, which ceased in 2003, could also not be evaluated since the average number of rings was fewer than ten.

The analysis of the fertilization experiments consisted of comparisons between N + P, P, and C treatments for *A. syriaca* and comparisons between P and C treatments for *L. capitata* and *D. purpurea*. Replication amongst treatments was possible since species abundance was high enough between four, and in some cases five, replicates for each treatment for all three species. Up to five individuals per species were collected by sampling individuals closest to each of the four corners and center per plot, thus making the total sample size for each fertilization treatment between 20-25 individuals. The root collar (represented as the area directly beneath the root crown where previous shoots originated), was located and harvested after digging typically 1 -5 cm belowground, but in some cases as far as 10 cm below the surface. It should be noted however, that the 'root' collar for *A. syriaca* was probably anatomically stem material since in all cases it contained pith. For the irrigation transect, *L. capitata* was the only species abundant enough in treatment and control transects. Furthermore, we decided to only collect individuals from lower parts of the transect since *L. capitata* was nearly non-existent upslope. The same excavation methods were applied with sample size $n = 20$ per each transect (one irrigated and one control). We paid careful attention to collecting only individuals that were within the zone of even coverage from each sprayer along the irrigation transect.

Laboratory methods consisted of using a sledge microtome to cut approximately 20-30 μm thin sections of each root collar that were then stained with Toluidine Blue for 5 minutes. Toluidine Blue is a metachromatic dye for staining lignified structures. Thus, in a microsection stained lignified secondary walls of fiber cells in the latewood would be an indicator for discerning one annual ring to the next (see Figure 1). After the staining process was complete, specimens were photographed with a low power dissecting scope with a Leica digital camera. Magnification levels were noted for each image, along with a photographed 1mm scale for calibration. This subsequent analysis for measuring ring widths was performed in ImageJ (Abramoff et al. 2004) using the image stitching tool when necessary (Preibisch et al. 2009) and the line segment measuring tool. Ring widths were averaged across three radii that were each subjectively chosen to represent the variation in diameter along the span of each ring as accurately as possible.

Analytical Methods

Ring borders were identified by the proliferation of earlywood- like vessels, which are larger in area and higher in density, adjacent to the previous season's latewood, which for all species consisted of relatively few vessels. After ring widths were measured on each individual, we were able to construct the presumed age at time of sampling by counting total rings present, though we do note that this was not possible for all individuals since the more aged part of the root collar for about half of the individuals was too deteriorated to delineate ring boundaries. Since traditional age-detrending was not possible because there were few rings per individual, we decided to use age in 2013 as a covariate (for ones that had deteriorated older parts, we used the maximum amount of rings visible) when we applied ANCOVAs to test the variation in ring widths amongst the fertilization and irrigation treatments for each separate year. Due to the fact that most of individuals were between 3 – 6 years old at the time of sampling, as well as the sizable portion of individuals who had deterioration of the central root which blurred the older ring boundaries, we usually carried the analysis to either 2007 or 2008 since any year before these had considerably reduced sample sizes. Additionally, we used Tukey's post hoc comparisons for the three fertilization treatments with *Asclepias syriaca*. Obtaining historical climatic data from Konza's Climate Reference Network, we also took the average monthly temperature and precipitation between April and June and also July through September per year to test the climatic signal on ring width from either the early or late growing season. We used Pearson correlation to test between all ring widths per each species and experiment separately using these climatic variables. Finally, to detect the strength of intra- or inter-treatment correlations amongst individuals, we used macro functions in Microsoft Excel to calculate yearly pairwise correlations between series. From this analysis, the average correlation between series was calculated per treatment to determine intra-treatment correlations. To find the inter-treatment correlations using this technique we simply found the average correlation between series with all treatments included as a whole.

Results

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Growth rings were easily discernable for the three species in the study (Fig. 1). We assume that growth rings are annual for these three species since, upon excavation (late summer), the ring closest to cambium was always past the typical “earlywood” phase and somewhere in the “latewood” phase. Overall sample sizes decreased after transport from the field due to a handful of roots suffering from decomposition due to mold spore attack during transport. This highlights the importance of storing roots in a high enough concentration of alcohol during transport, at least in 40% ethanol is adequate (Schweingruber et al. 2011). As previously mentioned, a considerable amount of the roots were also decomposed in the older parts of the organ, rendering confident aging of these individuals too difficult to include in analyses. Though we cannot show the age distribution for our study we do highlight the sample depth per year pertaining to each experiment (Figure 2).

Overall, fertilization treatments had significant effects on annual ring width for the focal species of the belowground plots experiment, with the exception of *D. purpurea* (Data for *D. purpurea* are not shown). In *A. syriaca*, the years 2012, 2013, and average of all years combined had significant variation amongst treatments, with the general pattern of ring widths increasing from $C < P < N+P$. For 2012 the only significant difference was C being significantly less in average width than N+P (F-test = 3.59, $df = 2$, $P < 0.03$, Fig. 3a), while for 2013 both C and P were significantly less than N+P (F-test = 3.32, $df = 2$, $P < 0.04$, Fig. 3a). Though there were no significant differences between fertilization treatments from 2007 – 2011, when all years are combined, $C < NP$ was the overall significant difference (F-test = 5.39, $df = 2$, $P < 0.01$, Fig. 3a). For *L. capitata*, the years 2011-2013 and overall averages had significant differences between C and P treatments, with $C < P$ in each year (F-test = 2.81, $df = 1$, $P < 0.001$ (2013), F-test = 2.21, $df = 1$, $P < 0.02$ (2012), F-test = 1.72, $df = 1$, $P < 0.05$ (2011), and F-test = 2.12, $df = 1$, $P < 0.04$, (all years) respectfully, Fig. 3b). *D. purpurea* was the only species of the three to not have any fertilization treatment effect on annual rings. The irrigation transects yielded less clear patterns, with the control transects holding significantly larger rings than irrigated transects in 2008 and 2010 for *L. capitata* (F-test = 4.41, $df = 1$, $P < 0.04$, Fig. 4) only.

Climatic variables had significant correlations with annual ring increment of each of the three focal species in both experiments, however, the overall patterns were weak with rather small magnitudes of effect. For all 3 species a negative correlation with mean monthly temperature between April and June was significant in the belowground plots by using all years

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pooled together ($P < 0.05$): *A. syriaca* had a coefficient of -0.184 ($df = 288$, $P < 0.001$, Fig. 3a), *L. capitata* had a coefficient of -0.217 ($df = 183$, $P < 0.001$, Fig. 3b), and *D. purpurea* had a coefficient of -0.231 ($df = 184$, $P < 0.001$). *L. capitata* also had a negative association with monthly temperature between April and June in the irrigation transects ($r = -0.223$, $df = 172$, $P < 0.003$, Fig. 4). For monthly precipitation amount, average precipitation between April and June proved to be positively correlated with *D. purpurea* ring width in the belowground plots ($r = 0.206$, $df = 184$, $P < 0.004$) and also *L. capitata* in the irrigation transects ($r = 0.227$, $df = 172$, $P < 0.002$, Fig. 4).

Table 1 shows the intra- and inter-treatment correlations per species. We considered a strong mean pairwise correlation coefficient to be $> + 0.500$, which is the minimum accepted value typically used in dendrochronology for accurate cross-dating of ring series (Cook 1983). Overall, the only species to have consistently strong intra-treatment correlations for the fertilization treatments was *L. capitata*. The inter-treatment correlation was also relatively strong for *L. capitata* (0.5212, Table 1). For *A. syriaca* and *D. purpurea* within and amongst the fertilization treatments, the pairwise correlation was usually close to zero, thus showing weak series intercorrelation of annual rings towards the treatment. Lastly, *L. capitata* had overall weak series intercorrelation within and amongst the irrigation transects with pairwise correlations usually close to zero.

Discussion

Annual rings in our study were more consistently related to fertilization than to interannual climatic variation. We found a positive relation between fertilization and annual ring widths for two of our three species studied while climatic factors only amounted to weak relationships with ring variation. Specifically, we found a consistent negative correlation between early season temperature and ring width in conjunction with a more inconsistent positive relation with early season precipitation. The weak patterns with climate are further exemplified with overall weak inter-treatment correlations we found with each species. Though intra-treatment correlations were inconsistent in overall magnitude as well, the only ones that were strong were related to fertilization treatments for *L. capitata*.

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The general correspondence with fertilization compared to climate is discussed in the context of annual ring ontogeny in this discussion. We suggest that ring width variation may be a function of an internal carbon budget based on yearly competition for resources. The source of this yearly variation in competition is thought to be modulated by variation in interannual climate. Throughout this discussion, we suggest a framework for improving future herb-chronology studies aimed at matching annual ring signatures with past growth environment.

Physiological determinants of the relationship between belowground secondary growth and site conditions

Annual incremental growth of the root secondary xylem could be a function of conductance to sustain aboveground growth (von Arx et al. 2012; Olano et al. 2013) and enlargement to make storage room for photoassimilates (Bell and Ojeda 1999; Lacoite 2000). While specific changes in soil moisture and evaporative stress may impact the size and distribution of conductive tissues in the root annual ring (von Arx et al. 2012; Olano et al. 2013), the amount of metabolically expensive storage space (Chapin et al. 1990; Salisbury and Ross 1992) created in annual rings may be more tied with the overall carbon budget of the plant and competition between different carbon sinks (Wardlaw 1990). In fact, von Arx et al. (2006) found evidence that the timing of reproduction with a plant's life history could have an overall effect on ring width, likely due to the cost of reproduction on total carbon budget. Overall, the production and allocation of photoassimilates leading to belowground secondary growth could be strongly related to immediate growth conditions of the site, plant life history, and carbon budget.

The tallgrass prairie is seen as nitrogen limited, especially when annually burned (Ojima et al. 1994). Specifically with *A. syriaca*, we found that the Nitrogen + Phosphorus addition had on average the largest rings, perhaps from increased aboveground photosynthetic rates. On the other hand, individuals in control plots, in what can be seen as relatively N limited, had comparatively decreased ring sizes. Allocation to increased fine root biomass or any other organ may outcompete allocation towards belowground carbon sinks in this limiting condition (McCarthy and Enquist 2007). Furthermore, the strength of allocation competition between sinks may be inconsistent between individuals in this condition, which could explain the lack of pairwise correlation within lower level fertilization treatments for *A. syriaca*. In fact, *A. syriaca* is a rhizomatous species, which could help further explain the lack of pairwise correlations

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amongst individuals. Rhizomatous species should be analyzed with caution since the spatial spread of the plant as a whole may considerably differ between individuals. Thus, it may be hard to attribute ring variation to any treatment related factors if annual rings could be more sensitive to the conditions outside of the treatment area where a significant proportion of the plant may exist. Unfortunately, this may be a factor in our study, as it was part of the sampling design to choose plants close to the four corners of the treatment plots. If analyzing rhizomatous species, future studies should only sample individuals well within treatment boundaries.

L. capitata, one of the two nitrogen fixers in our study, had the greatest positive magnitude of effect from increased fertilization. Interestingly, *L. capitata* only occurred in annually burned control and phosphorus plots. Other closely related legume species are found to have increased frequency in burned areas (Towne and Knapp 1996), possibly due to a selective advantage these nitrogen fixers may have over non-nitrogen fixers in annual burned areas that become extremely nitrogen limited (Lauenroth and Dodd 1979; Ojima et al. 1994). The increased frequency of *L. capitata* in the phosphorus treatment may be because of the putative role of phosphorus in increasing rates of nitrogen fixation (Sprent and Raven 1985; Aerts and Chapin 2000; Reed et al. 2007), which is usually concomitant with increased rates of foliar biomass production in pastoral legume species (Ae et al. 1990; Smith 1992; Ayala et al. 2000). Increased ring size in phosphorus addition plots could be the result of the increased need for storage under higher rates of photosynthetic activity. The suggested physiological role that fertilization plays on increasing rates of nitrogen fixation could be driving the relative higher intra-treatment pairwise correlations for *L. capitata*. In this aspect, phosphorus addition may lead to a more consistent response in growth between individuals.

In contrast, considering *D. purpurea*'s lack of response to fertilization, biomass responses to nitrogen limitation and/or phosphorus availability may not be consistent between all legumes, as Towne and Knapp (1996) found in similar legume fertilization studies. In fact, increased nitrogen inputs may even negatively affect the legume-rhizobium mutualism and result in decreased foliar biomass in some legumes (Weese et al. 2015). The extent to which this negative reaction may exist may highly differ from plant to plant, possibly explaining the weakness of intra-treatment pairwise correlation we found with *D. purpurea*.

Given the overall positive impact fertilization had on ring sizes in our study, any future herb-chronology studies aiming to further connect ring width with aboveground productivity

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must consider a suite of additional factors to measure. Namely, closer observation between the correlative response between foliar biomass, reproductive biomass, photosynthetic rates, and secondary annual growth will help solidify the utility of using annual rings as markers of past site conditions and plant productivity. Furthermore, as seen with the inclusion of nitrogen fixers in our study, life history traits of individual species under study should be taken into consideration. Overall differences in physiology may play an important role in the patterns of annual ring variation related to site conditions.

Using series intercorrelations to determine the strength of common signals between different trees and the possible sensitivity to environmental factors is a common practice in dendrochronological research. Thus, for future herb-chronology studies we also strongly recommend incorporating pairwise correlation analysis, as we did in this study, to calculate series intercorrelations within different environmental conditions between sites within a study. One primary reason for doing so is that it will strengthen confidence that all series were properly cross-dated. An additional strength could be determining the relative ease of cross-dating for any one specific species, which could be particularly useful for herb-chronology where it is not yet known the extent to which root growth rings are indeed annual across species.

However, we do caution that pairwise correlation for climatic analysis should only be considered for longer lived individuals, where the majority of samples are at least 20 years old. Due to a likely low sample depth of earlier years, cross-dating using pairwise correlation likely becomes unreliable with anything less than 20 years old if the overall goal is to carry out proper correlative analysis with climate, including using such linked indices as “mean sensitivity” (Fritts 1976) and the “expressed population signal (EPS)” (Briffa and Jones 1990). Longer chronologies such as the work of Franklin (2013) and her 77 year-long chronology of a long-lived U.S. alpine herb, have done this effectively by using the afore mentioned indices in finding associations between ring width and climate. Nonetheless, for future studies with relatively short chronologies such as ours, intra- and inter- treatment pairwise correlation still has its advantages within and amongst sites that represent particular extremes in a persistent environmental condition. Strong intra-treatment pairwise correlations in this aspect could lead to considerable support of which environmental factors are most important in affecting annual ring sizes in short lived herbs. Correlations with interannual climate can still be done by using a rather short master

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chronology though we discuss potential pitfalls, as they were evident in our study, in the proceeding sections.

The suspected effect of competition on annual ring variation and the interactive effects with climate

In the tallgrass prairie, aboveground biomass of dominant grass types responds positively to annual precipitation. However, foliar grass biomass is negatively correlated with aboveground biomass of forb species (Briggs and Knapp 1995). In our study, we observed overall patchy distributions of large grass cover between experiments. The irrigated transects had visually more cover of dominant grasses than the nearby controls, as did unfertilized plots compared to ones that had fertilization. The weak relationship between precipitation and annual ring width could be because of the unequal presence of dominant grass species and their potential inhibitory effects on forb biomass production in wetter environments (Briggs and Knapp 1995). Reduced photosynthetic output from aboveground competition could lead to less allocation to belowground carbon sinks (Bloom et al. 1985; Chapin et al. 1990), and obscure the otherwise positive effect of precipitation on ring growth.

Overall hotter years bring about overall lower amounts of annual net primary productivity in North American grasslands (Knapp and Smith 2001; Knapp et al. 2002), especially in annually burned tallgrass prairie (Briggs and Knapp 1995). Though hotter temperatures could lead to a declined growth environment for forbs in general, competition with grasses could be amplified when high temperatures lead to increased evaporative stress. In an already stressful growth environment, forb species would have to simultaneously compete with dense superficial roots of neighboring grass for water (Kiaer et al. 2013). Thus, it shouldn't be surprising that we found a consistent negative relationship between temperature and ring size in our study. However, the overall patchy distribution of grasses may play a role in the weak pairwise correlations nonetheless.

Future studies may benefit from experimentally disentangling the apparent interaction between climate, competition, and their effects on belowground secondary growth in perennial forbs. In addition, since juvenile tree seedlings have a considerable difference in the factors they respond to in comparison with adult trees regarding their annual rings (*see Mendivelso et al. 2013 for example*), herb-chronology studies should also consider age as an additional factor

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when investigating rings in perennial forbs and how they respond to the environment. We attribute the lack of consistent pattern between ring width and the earlier years under analysis in our study to this possibility of juvenile sensitivity. Thus, experimentally manipulating competition levels in concert with the natural variation in interannual climate, as well as considering age as a factor, should help in determining the relative importance of site ecology and climate on annual ring variation. As before, we also suggest using pairwise correlations within and amongst treatments in this suggested experimental design. Strong pairwise correlations within an induced level of competition may give a reliable indication of importance of competition on ring growth. On the other hand, if there were a stronger inter-treatment correlation then this could warrant further investigation into correlative analysis with climate and its putative role on interannual ring variation.

Conclusions

Well-established fields such as dendrochronology have used annual growth rings to uncover the effects of past ecological and climatic events on the growth of woody plant populations in a variety of landscapes (Corcuera et al. 2004; Hartman and McCarthy 2007). Since herbaceous species constitute some of the biggest epicenters of belowground primary production (Luo et al. 2009), the field of herb-chronology warrants further investigation into whether or not annual rings in roots may be records of past site and climatic factors that affect plant production. In addition to a few other earlier herb-chronology studies, our work represents one of the first attempts to relate annual ring variation in herbaceous species with site growing conditions and/or interannual climate (Dietz et al. 2004; Dietz and von Arx 2005; Liu and Zhang 2010; Olano et al. 2011; Franklin 2013). Of these studies, ones with moderate length chronologies (at least ten years on average) found significant correlation between interannual ring variation and yearly climate (Dietz et al. 2004; Dietz and von Arx 2005; Liu and Zhang 2010). In our case however, our overall chronology can only be applied to the six year period between 2007 – 2013, where we found only weak associations with climate. Though this is a limitation in our study, we still advocate for future herb-chronology research in the U.S. tallgrass prairie region, being aware of the possibility of root rot, but also taking the initiative to investigate other perennial forbs which may possess longer chronologies in this diverse grassland environment (Briggs and Knapp 1995).

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Nonetheless, we found consistent relationships between fertilization and annual ring variation coupled with the weak association with yearly climate. Besides the fact that our overall chronology may have been too short to pick a stronger climatic signal, the patchy distribution of grasses in our study and our weak correlations with climatic factors could also point to the possible greater role of competition on annual ring growth increments. To further disentangle site ecology from climate, we suggest future studies utilize experimental frameworks manipulating certain site factors and using pairwise correlations to find whether site or climate (for chronologies of at least 20 years old) is the driving force behind annual ring variation. While we believe that weak pairwise correlations were attributable to the ecological factors previously mentioned, we highlight the possibility of false annual ring occurrence, which can lead to incorrect year assignments. Careful attention needs to be paid towards recognizing ring patterns in a selected target species (*see Schweingruber et al. 2011 for a good visual reference to many plant families*). If possible, verifying if the growth rings are indeed annual should always be the first step.

We also strongly recommend future studies incorporating physiological aspects on the individual plant level to better understand how annual rings in the roots of herbaceous species correspond to aboveground biomass and how this may differ between life history groups. In this aspect, age should also be considered as the juvenile stages of a plant may be more sensitive towards different environmental factors than older more mature individuals may be. Overall, if some of these improvements to herb-chronology research take place in future studies then the true potential of this emerging discipline in vegetation science may be realized.

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Tables and Figures

Table 1. Intra- and inter- treatment series correlations for each of the three species. Top part of the table shows the intra- and inter- treatment series correlations for the fertilization treatments while the bottom part shows the same for the irrigation transects. Values that are > 0.500 are bolded, signifying a strong intra- or inter- treatment series correlation. Ring widths were not transformed prior to correlation. Sample size represents the number of individuals included in each respective pairwise correlation.

	<u>Intra-treatment correlation</u>			<u>Inter-treatment correlation</u>
	<u>Control</u>	<u>P</u>	<u>NP</u>	
<i>Asclepias syriaca</i>	0.1999 (n=10)	0.0485 (n=20)	0.1808 (n=16)	0.1385 (n=46)
<i>Dalea purpurea</i>	0.2146 (n=23)	0.3505 (n=11)	X	0.2973 (n=34)
<i>Lespedeza capitata</i>	0.5865 (n=20)	0.5345 (n=16)	X	0.5212 (n=36)
	<u>Irrigation-intra correlation</u>			<u>Irrigation inter-treatment correlation</u>
	<u>Control</u>		<u>Irrigated</u>	
<i>Lespedeza capitata</i>	0.2217 (n=18)		0.0544 (n=18)	0.2061 (n=36)

Figure 1. Root collars of a) *Lespedeza capitata* and b) *Asclepias syriaca*. Approximately seven growth rings are visible in *L. capitata*, while ten are visible in *A. syriaca*. Specimens were stained with Toluidine Blue to help enhance ring boundaries. Proliferation of bands of earlywood-like vessels (larger in size and density compared to latewood vessels) were used to delineate ring borders. In the case of *A. syriaca*, shown here is the post-image processing in ImageJ to assign years from the outer to the most inner ring. Note the deterioration of the older rings in *L. capitata*, thus rendering unreliable age estimation in most individuals of this species as well as *D. purpurea*.

Figure 2. Yearly sample depth for A) *Dalea purpurea*, *Asclepias syriaca*, and *Lespedeza capitata* in the fertilization treatments and B) *Lespedeza capitata* in the irrigation transect experiment. All analyses were ceased around 2008 – 2007 due to the low sample depth for the years prior.

Figure 3. Average ring widths (95% CI) per year as a function of nutrition treatment for **A.** *Asclepias syriaca* and **B.** *Lespedeza capitata*. Letters denote significantly different treatments per year via post-hoc comparisons. The line graph projected above the bar graph shows the average monthly temperature between April and June per year to highlight the relationship with annual ring widths. This relationship was significant ($P < 0.05$) for both species, with the correlation coefficient = -0.184 for *A. syriaca* and -0.217 for *L. capitata*. Precipitation (not shown) was not significant in either case. Late season climatic variables were also not significant.

Figure 4. Average ring widths (95% CI) per year as a function of irrigation treatment for *L. capitata*. Letters denote significantly different treatments per year via post-hoc comparisons. The line graphs projected above the bar graph show the average monthly temperature and rainfall between April and June per year to highlight the relationship with annual ring widths. Climatic variables were significant ($P < 0.05$) with correlation coefficients = 0.227, and -0.223 for average monthly rainfall and temperature respectfully. Late season climatic variables were not significant.

Figure 1. Root collars of a) *Lespedeza capitata* and b) *Asclepias syriaca*. Approximately seven growth rings are visible in *L. capitata*, while ten are visible in *A. syriaca*. Specimens were stained with Toluidine Blue to help enhance ring boundaries. Proliferation of bands of earlywood-like vessels (larger in size and density compared to latewood vessels) were used to delineate ring borders. In the case of *A. syriaca*, shown here is the post-image processing in ImageJ to assign years from the outer to the most inner ring. Note the deterioration of the older rings in *L. capitata*, thus rendering unreliable age estimation in most individuals of this species as well as *D. purpurea*.

140x60mm (300 x 300 DPI)

Figure 2. Yearly sample depth for A) *Dalea purpurea*, *Asclepias syriaca*, and *Lespedeza capitata* in the fertilization treatments and B) *Lespedeza capitata* in the irrigation transect experiment. All analyses were ceased around 2008 – 2007 due to the low sample depth for the years prior.
214x313mm (300 x 300 DPI)

Figure 3. Average ring widths (95% CI) per year as a function of nutrition treatment for A. *Asclepias syriaca* and B. *Lespedeza capitata*. Letters denote significantly different treatments per year via post-hoc comparisons. The line graph projected above the bar graph shows the average monthly temperature between April and June per year to highlight the relationship with annual ring widths. This relationship was significant ($P < 0.05$) for both species, with the correlation coefficient = -0.184 for *A. syriaca* and -0.217 for *L. capitata*. Precipitation (not shown) was not significant in either case. Late season climatic variables were also not significant.

207x107mm (300 x 300 DPI)

Figure 4. Average ring widths (95% CI) per year as a function of irrigation treatment for *L. capitata*. Letters denote significantly different treatments per year via post-hoc comparisons. The line graphs projected above the bar graph show the average monthly temperature and rainfall between April and June per year to highlight the relationship with annual ring widths. Climatic variables were significant ($P < 0.05$) with correlation coefficients = 0.227, and -0.223 for average monthly rainfall and temperature respectively. Late season climatic variables were not significant.

279x340mm (300 x 300 DPI)