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Growth Rings of Roots in Perennial Forbs in Duolun Grassland, Inner Mongolia, China

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Abstract

Annual growth rings of roots in perennial forbs have been used in studies of climate change and the ecology of grasslands. However, little has been done in this aspect of research in China. In this study, we report the characteristics of growth rings in the main roots of 13 herb species sampled in Duolun of the Inner Mongolia grassland in northern China. The results show that around two thirds of the species possess clearly demarcated annual rings in the root xylem. Some species of the same genera show different patterns in anatomical structure of the root xylem. Standardized annual ring widths of three species, *Potentilla anserine* L., *Cymbaria dahurica* L. and *Lespedeza daurica* (Laxm.) Schindl., show a common linear trend, indicating a continued favorable growth condition in the sampling sites. Our results provide evidence that growth rings in roots of some perennial forbs in the Inner Mongolia grassland can serve as a new and useful indicator of past changes in the grassland environment.

Key words: China; growth ring; herb-chronology; perennial forbs.

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Annual growth rings in the main roots of perennial forbs have received increased attention in studies of climate change and disturbance ecology (Dietz and Ullmann 1998; Dietz and Arx 2005). The anatomical ring structure in the secondary xylem of permanent roots in some forbs has been found to be similar to the patterns in semi-ring porous woods, which have wider vessels at the beginning of growing season and narrower vessels in the late growing season (Dietz and Ullmann 1997; Dietz and Schweingruber 2002). The growth rings were verified to be true annual rings by comparing the number of rings of growing plants with their known age (Dietz and Ullmann 1997; Dietz and Fattorini 2002). These findings give rise to a possibility of developing herb chronology for understanding growth response of herbs to environmental disturbances and climatic fluctuations (Dietz and Ullmann 1998; Dietz and Schweingruber 2002; Dietz and Fattorini 2002; Dietz and Arx 2005; Arx et al. 2006), stand age structure and development (Dietz and Ullmann 1998), and life history changes of forbs species in different altitudes (Arx et al. 2006).

In China, there is only one report that the root of herb species *Glycyrrhiza uralensis* produces annual growth rings and the ring widths are affected by habitat conditions (Ye and Zhang 1998). To date, the characteristics of growth rings in many forbs species remain unknown. Grassland in Inner Mongolia is responding to global climate change and is one of the major vegetation types in northern China (Bai et al. 2004). The objectives of this paper are: 1) to examine the anatomical features of growth rings in main forbs species growing in the Duolun site of Inner Mongolia grassland; 2) to compare the patterns in growth rings of different species; and 3) to identify the sensitivity of growth rings in the main roots of the studied forbs species to environmental variation.

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Results

Anatomical structure

Of the 13 forbs species, growth rings in the roots could be distinguished clearly in eight species; root anatomical patterns could not be clearly interpreted as growth rings or varied considerably amongst individuals in four species; and one species lacked distinguishable growth rings in the roots (Table 1, Figure 1). Anatomical structures observed in the root cross-sections, that is, the size and orientation of cells and vessels in the earlywood and latewood, contributes to the delineation of growth rings (Figure 1A–H). Growth rings could be recognized because of the larger vessels or higher vessel density in the earlywood compared to those of the latewood.

Anatomical pattern differences

Different species of the same genera showed different anatomical patterns (Table 1, Figure 1). For example, *Potentilla anserine* L. showed clear-demarcated growth rings with narrow branching vessel rays, whereas *P. tanacetifolia* Willd. presented relatively distinct growth rings with wider branching vessel rays. In *P. bifurca* L. there were well-distributed vessels over the entire cross section, some of which had distinct growth rings while others did not. The species *P. multifida* L. did not show clear growth rings and had narrow vessel rays that were not branched, which is similar to *P. betonicaefolia* Poir. In addition, individual plants within the same species also had different anatomical ring patterns. For example, younger individuals of *P. betonicaefolia* exhibited more clear rings than older ones.

Annual trend of ring-width variation

Standardized ring-width series in three species, *P. anserine* L., *Cymbaria dahurica* L., and *Lespedeza daurica* (Laxm.) Schindl., exhibited a linear increasing trend with time but the slope of the trends was different from species to species (Figure 2). The ring widths of *P. anserine* increased significantly ($P = 0.026$, $r^2 = 0.350$) from 1991 to 2004, and *C. dahurica* showed a particularly significant increasing trend ($P = 0.001$, $r^2 = 0.514$) during the period 1988–2004. *L. daurica* also exhibited a similar trend, although it was less significant ($P = 0.163$, $r^2 = 0.204$).

Discussion

In this study, the proportion of the species having clear or relatively clear demarcated growth rings is 62% of the species sampled. This is slightly lower than in central Europe (approximately 66% and 80%, respectively; Dietz and Ullmann 1997; Schweingruber and Dietz 2001) and in North America (approximately 65%; Dietz and Schweingruber 2002). A relatively low number of species sampled (13 species) and the differences in grassland types may contribute to this slightly lower percentage. Further collection and analysis of more perennial forbs species would better illustrate the characteristics of growth rings in roots of forbs species in the Inner Mongolia grassland.

In our study, most species showed obvious differences in vessel diameter between earlywood and latewood (Figure 2). This observation is consistent with the pattern known from semi-ring porous woods with wider vessels in the earlywood that is formed at the beginning of the growing season and narrower vessels in the latewood that is formed in the latter part of the growing season (Fritts 1976). For identifying the annual rings in forbs' roots (herb-chronology), it is the best to

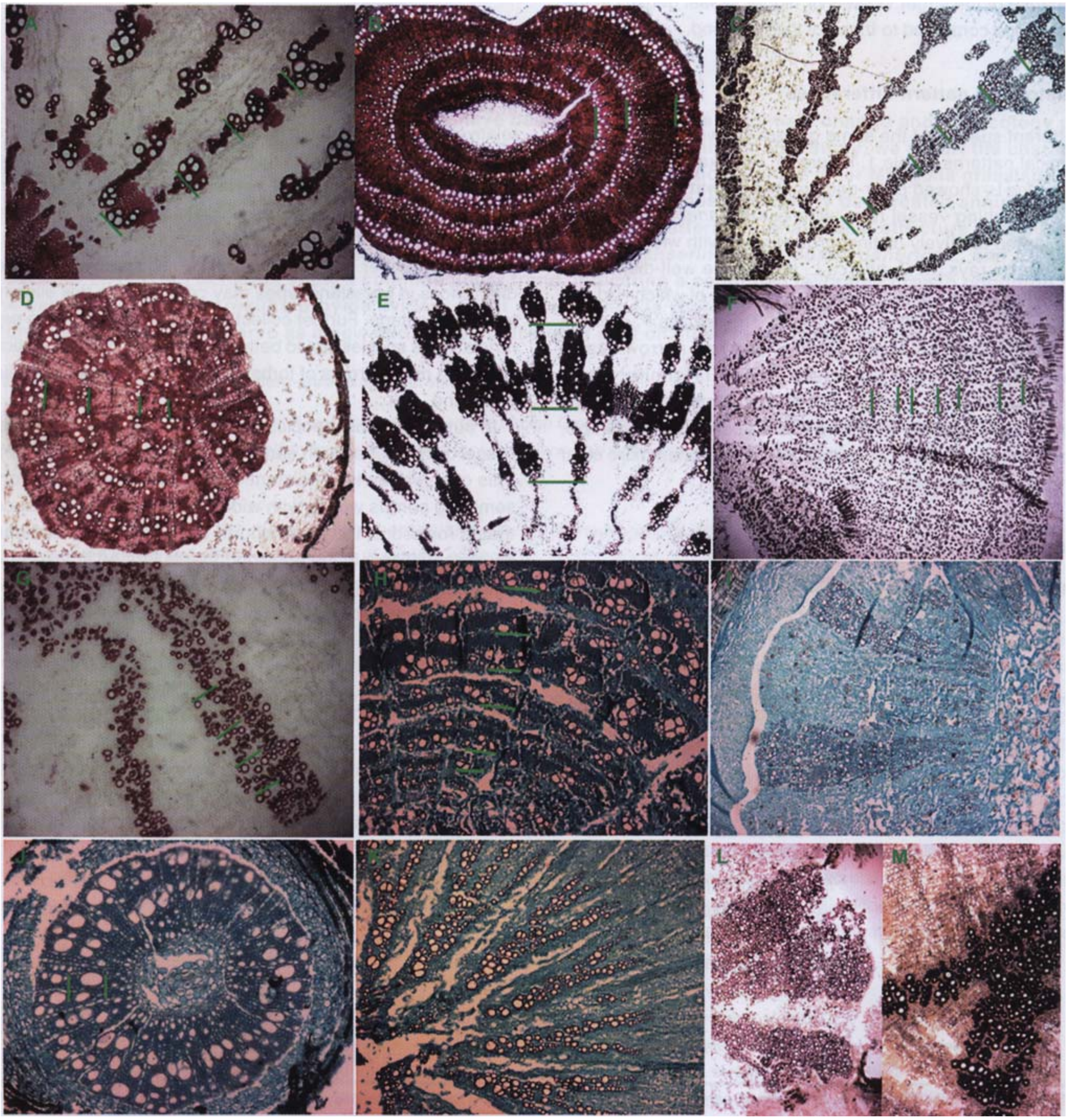
Table 1. Anatomical patterns in the secondary xylem of roots in 13 perennial forbs species sampled in Duolun County, Inner Mongolia, China.

Family	Species	Number of samples	Demarcation	Pattern
Rosaceae	*Potentilla betonicaefolia*	6	Varying	VL, VB
	P. anserine	15	Clear	VL, PI
	P. bifurca	12	Varying	VL
	P. tanacetifolia	6	Varying	VL, VB
	P. multifida	3	Varying	VL, VB
Leguminosae	*Melilotus dentatus*	4	Relatively	VL, VD
	Hyoscyamus niger	3	Clear	VL, VC
	Lespedeza daurica	31	Clear	VL, VD
Scrophularlaceae	*Cymbaria dahurica*	30	Clear	VL, VD
Caryophyllaceae	*Silene jenisseensis*	7	Relatively	VL, VB
	Dianthus chinensis	15	Relatively	VL, VD
Thymelaeaceae	*Stellera chamaejasme*	6	Clear	VL, VD
Umbelliferae	*Saposhinikovia divaricata*	4	Weak	[VL]

PI, concentric discontinuities in the lignified parenchyma; VB, zonal branching of vessel density; VC, broad vessel clumps spaced by linear vessels; VD, differential vessel density; VL, differential vessel lumina (Dietz and Ullmann 1997; Dietz and Schweingruber 2002). Anatomical pattern only weakly visible is given in brackets.

base observations on vessel size and the distribution patterns of vessels in the secondary xylem (Arx and Dietz 2005). Because many forbs show lignification only in the vessel cell walls, fluctuating light intensities often represent only irregularly occurring rings of lignified parenchyma cells (Werner 1978). The anatomical patterns in growth rings of perennial forbs vary from species to species, which is consistent with the observations of forbs rings in other regions (Dietz and Ullmann 1997; Dietz and Schweingruber 2002).

Roots are the dominant components for measuring net primary production (NPP), below-ground productivity and biomass (Matamala et al. 2003; Pucheta et al. 2004). Climate fluctuation and habitat conditions may influence plant growth, vessel characters (Stevenson and Mauseth 2004), and the



radial increment growth in roots of perennial forbs (Dietz and Ullmann 1998; Dietz and Schweingruber 2002; Dietz and Fattorini 2002; Dietz and Arx 2005; Arx et al. 2006). In our study, three species of different families (*P. anserine*, *L. daurica*, *C. dahurica*) showed similar trends in standardized ring-width series (Figure 2), indicating an increase in below-ground production and favorable growth condition for forbs in Duolun grassland. Sara et al. (2005) revealed that the central part of Inner Mongolia Autonomous Region might contain a noticeable increase in GPP that coincides with increasing precipitation shown by the satellite data during an 18-year period from 1982 to 1999. Cao et al. (2003) indicated that the terrestrial NPP increased with a rate of 0.32% year^{-1} in China based on remote sensing GLO-PEM. Our results provide further evidence for the sensitive and rapid response of radial increment growth in roots of perennial forbs species to a changing environment.

In summary, our results provide a new insight into environmental variation from anatomical characteristics of growth rings in the main roots of 13 perennial forbs in Duolun of the Inner Mongolia grassland. The identification of perennial forbs species that have clear growth rings may help researchers in their studies of herb chronology of particular species. Further study on growth ring characteristics from more species in the grassland will be useful for better understanding of climate and disturbance effects on grassland ecosystems.

Materials and Methods

Study area and field sampling

Thirteen species were collected from Duolun County (42°27' N, 116° 41' E, 1 380 m above sea level), a semi-arid area located in the central part of Inner Mongolia, China. According to the observed climate records from 1987 to 2004, mean annual total precipitation in Duolun is 387.2 mm, with the maximum monthly value occurring in July and August. Mean annual air temperature is 3.0 °C, with monthly mean temperature ranging from –16.2 °C in January to 19.4 °C in July.

From October 25 to 28, 2005, we collected root samples of perennial forbs species *Potentilla anserine* L. and *Lespedeza daurica* (Laxm.) Schindl. at Caimu Mountain site (42°30' N, 116° 45' E), and 11 species at Shisanlitan site (42°05' N, 116° 15' E) (Table 1). Main roots approximately 5–10 cm beneath the ground surface were cut off and 20 samples were collected from each species. The samples were put in airproof bags in the field and stored in a refrigerator in the laboratory before preparing cross-section slides.

Laboratory procedures

We prepared cross-sections (10–15 µm thickness) from the proximal end of the sampled roots by using a sledge microtome. These sections were stained with phloroglucinol and concentrated HCl (Dietz and Ullmann 1997; Dietz and Ullmann 1998). The vessels and other lignified parenchyma cells had reddish coloring and were clearly discernible after the treatment (Figure 1A–G). For the species of *Melilotus dentatus* Pers., *P. multifida*, *P. bifurca*, and *Saposhinikovia divaricata* Schischk., the cross-sections of roots were stained with safranin and fast green. Most cell types in the cross-sections exhibited green coloration, except for the vessels and lignified cells, which showed red coloring (Figure 1H–K). The species *L. daurica* and *Cymbaria dahurica* L. had very hard roots and it was difficult to make high-qualified cross-section slides.

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Figure 1. Patterns of growth rings in the secondary root xylem.

(A) *Stellera chamaejasme* L.
(B) *Cymbaria dahurica* L.
(C) *Potentilla anserine* L.
(D) *Lespedeza daurica* (Laxm.) Schindl.
(E) *Hyoscyamus niger* L.
(F) *Dianthus chinensis* L.
(G) *Silene jenisseensis* Willd.
(H) *Melilotus dentatus* Pers.
(I) *Potentilla multifida* L.
(J) *Potentilla bifurca* L.
(K) *Saposhinikovia divaricata* Schischk.
(L) *Potentilla tanacetifolia* Willd.
(M) *Potentilla betonicifolia* Poir.

The bar markers denote transition from latewood of the previous growing period to earlywood of the following one. See Table 1 for further information.

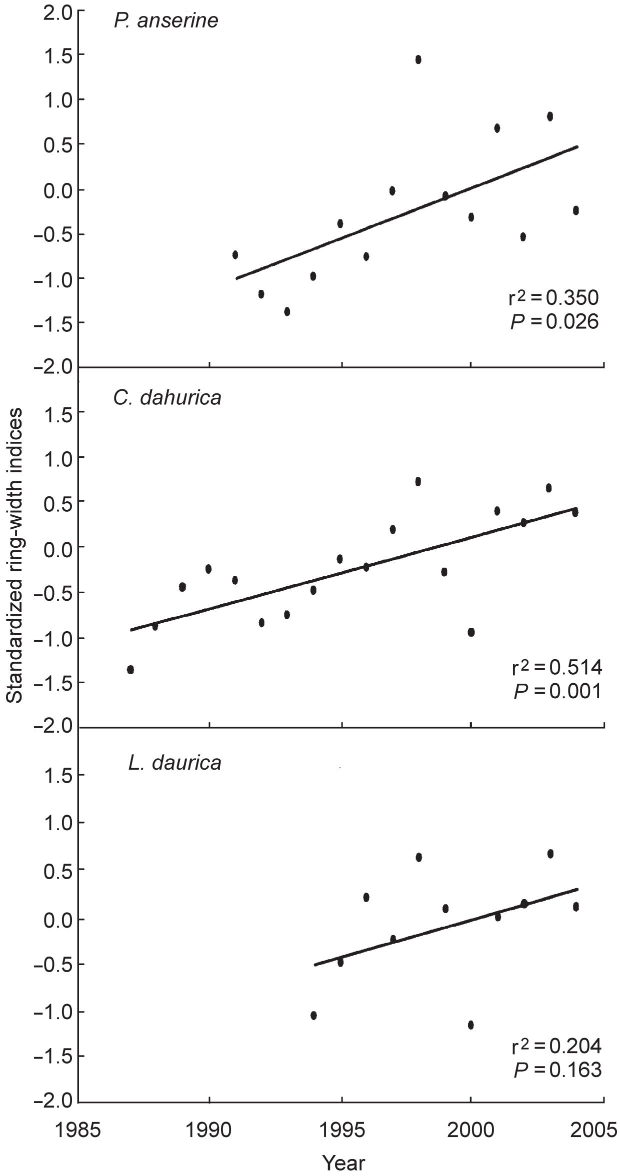

Figure 2. Standardized ring-widths series in roots of *Potentilla anserine* (top: W_p = –223.433 + 0.112 (year), P = 0.026, r^2 = 0.350), *Cymbaria dahurica* (medium: W_c = –156.351+0.078 (year), P < 0.001, r^2 = 0.514), *Lespedeza daurica* (bottom: W_l = –162.680 + 0.081 (year), P = 0.163, r^2 = 0.204).

Using a dissecting microscope with AxioVision 4.1 software (ZEISS Inc.), clear images of these sections were obtained. Growth rings in the main roots were identified by differentiating earlywood and latewood in the secondary xylem (Dietz and Ullmann 1997) (Figure 1). Ring widths at three different radii for each sample of the species *P. anserine, L. daurica*, and *C. dahurica* were measured using the AxioVision 4.1 software (ZEISS Inc.). The mean of the three radii measurements was calculated to represent the average growth condition in the sample.

Statistical analysis

The measured ring widths were standardized using the following method:

$$W = \frac{x - \bar{x}}{s}$$

where, W denotes the standardized ring widths, x is the measured ring widths, $\bar{x}$ and s represent the mean and standard deviation of the measured ring widths for each individual, respectively.

For three species, *P. anserine, L. daurica, C. dahurica*, the mean values of the ring widths for all individuals were calculated. We did not incorporate the ring in 2005 into analysis because it was not known if the growing season had completed by our sampling time in 2005. The first ring in the center was discarded in the analysis because some individuals appeared to have root decay in the center of the rings (Dietz and Ullmann 1997).

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References

Arx GV, Dietz H (2005). Automated image analysis of annual rings in the roots of perennial forbs. *Int. J. Plant Sci.* **166**, 723–732.

Arx GV, Edwards PJ, Dietz H (2006). Evidence for life history changes in high-altitude populations of three perennial forbs. *Ecology* **87**, 665–674.

Bai YF, Han XG, Wu JG, Chen ZZ, Li LH (2004). Ecosystem stability and compensatory effects in the Inner Mongolia grassland. *Nature* **431**, 181–184.

Cao M, Prince SD, Li K, Tao B, Small J, Shao X (2003). Response of terrestrial carbon uptake to interannual variability in China. *Global Change Biol.* **9**, 536–546.

Dietz H, Fattorini M (2002). Comparative analysis of growth rings in perennial forbs grown in an alpine restoration experiment. *Ann. Bot.* **90**, 663–668.

Dietz H, Schweingruber FH (2002). Annual rings in native and introduced forbs of lower Michigan, U.S.A. *Can. J. Bot.* **80**, 642–649.

Dietz H, Ullmann I (1997). Age-determination of dicotyledonous herbaceous perennials by means of annual rings: Exception or rule? *Ann. Bot.* **80**, 377–379.

Dietz H, Ullmann I (1998). Ecological application of 'herbchronology': comparative stand age structure analyses of the invasive plant *Bunias orientalis* L. *Ann. Bot.* **82**, 471–480.

Dietz H, Arx GV (2005). Climatic fluctuation causes large-scale synchronous variation in radial root increments of perennial forbs. *Ecology* **86**, 327–333.

Fritts HC (1976). *Tree Rings and Climate*. Academic Press, New York.

Matamala RM, Gonzàlez-Meler A, Jastrow JD, Norby RJ, Schlesinger WH (2003). Impacts of fine root turnover on forest NPP and soil C sequestration potential. *Science* **302**, 1385–1387.

Pucheta E, Bonamici I, Cabido M, Díaz S (2004). Below-ground biomass and productivity of a grazed site and a neighboring ungrazed exclosure in a grassland in central Argentina. *Aust. Ecol.* **29**, 201–208.

Sara B, Runnstrfm M, Seaquist JW (2005). Primary production of Inner Mongolia, China, between 1982 and 1999 estimated by a satellite data-driven light use efficiency model. *Global Planet Change* **45**, 313–332.

Schweingrugruber FH, Dietz H (2001). Annual rings in the xylem of dwarf shrubs and perennial dicotyledonous herbs. *Dendrochronologia* **19**, 115–126.

Stevenson JF, Mauseth JD (2004). Effects of environment on vessel characters in cactus wood. *Int. J. Plant Sci.* **165**, 347–357.

Werner PA (1978). On the determination of age in *Liatris aspera* using cross-sections of corms: Implications for past demographic studies. *Am. Nat.* **112**, 1113–1120.

Ye LQ, Zhang LM (1998). Preliminary study on the rhizome of *Glycyrrhiza uralensis*. *Chin. Wild Plant Resour.* **1**7, 30–31 (in Chinese).

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