Effect of climate on the growth of annual rings in the main roots of perennial forbs in an Inner Mongolian semi-arid grassland, China

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Abstract

Question: Is there a pattern in growth of annual rings in roots of perennial forbs in relation to climate and climate extremes in grassland ecosystems?

Location: Semi-arid grassland in Duolun (42°27′N, 116°41′E, 1380 m a.s.l.), central Inner Mongolia, China.

Methods: Main roots of three perennial species, *Potentilla anserina* L., *Cymbaria dahurica* L. and *Lespedeza daurica* Schindl., were sampled. Cross-sections (10–15-μm thick) were produced from the proximal end of sampled roots using a sledge microtome. Annual growth rings in the main roots were identified and measured by differentiating between earlywood and latewood in the secondary xylem. Relationships between annual growth rings and monthly mean temperature and total monthly precipitation were identified using correlation analysis. Differences in an annual ring width to the previous and following years were examined by calculating a distinctness score.

Results: The three perennial forbs showed clearly demarcated annual growth rings in all individuals and the same fluctuation patterns. Their ring widths were generally positively correlated with precipitation from April to October (except for August) and with temperature from February to June (except June for *L. daurica*), September to October, and the annual mean. Strong deviations of annual ring widths from their neighbour rings were observed in 1998 and 2000. The trend of absolute distinctness scores (D_m) increased significantly from 1988 to 2003, indicating an increase in the frequency of annual ring width variation.

Conclusions: Annual growth rings in the main roots of three perennial forb species can be used as an indicator of the influence of climate on below-ground grassland growth. The change in below-ground conditions and effects on the functioning of grassland should receive more attention in future studies.

Keywords: Annual growth rings; Belowground ecological process; Climate extremes; Inner Mongolian grassland; Perennial forbs.

Introduction

Below-ground ecological processes in grassland generate considerable interest because of their role in the global carbon cycle (Hungate et al. 1997; Jones & Donnelly 2004) and in the response of grassland ecosystems to global change (De Boeck et al. 2006; Antoninka et al. 2009; Shaw et al. 2009). Long-term changes in grassland soil organic matter and ecosystem carbon balance could be reflected in the response of root production in grasslands to elevated temperatures, altered precipitation, rising atmospheric CO_2 concentrations and nitrogen deposition (Norby & Jackson 2000). An important component of the global carbon cycle is net primary productivity (NPP). Below-ground net primary productivity (BNPP) comprises around 20-80% of total ecosystem productivity (Titlyanova et al. 1999; He et al. 2004). BNPP is strongly influenced by climate (Melillo et al. 1993; Gill & Jackson 2000; Gower et al. 2001; Schuur 2003; Hui & Jackson 2006), and climate warming leads to decreased below-ground productivity (Egli et al. 2004; De Boeck et al. 2007). Hui & Jackson (2006) found that the fraction of BNPP to total NPP decreased significantly with increasing mean annual temperature and precipitation across 12 grassland sites worldwide (ten sites located at northern high latitudes). In Inner Mongolian grassland, several studies have found that below-ground biomass decreased as mean annual temperature increased (Ni 2004; Fan et al. 2009), while biomass increased as mean annual precipitation increased (Fan et al. 2009). Understanding the relationship between biomass in grasslands and key climatic variables will be useful in predicting the influences of climate change on

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ecosystem productivity (Cao & Woodward 1998; Fan et al. 2009).

Predictions of grassland ecosystem responses to global change are challenging because of complex interactions among a myriad of above- and below-ground factors (Wolters et al. 2000). The responses of above-ground systems in grassland ecosystems to global change have already been studied extensively, and some important conclusions have been reached. Below-ground responses are still poorly understood because cryptic below-ground organs and organisms are difficult to observe. Therefore, additional data on influences of climate change on below-ground productivity and below-ground dynamics are required. Previous studies have demonstrated that annual rings in the main roots of perennial forbs are sensitive to climate fluctuations (Dietz & Fattorini 2002; Dietz & Schweingruber 2002; Dietz & Arx 2005). Dietz & Arx (2005) found that radial growth in main roots was affected by high temperatures during the El Niño year 1998, and that synchronous growth variations were seen across Switzerland and the United States.

Annual rings in forb roots have been examined mainly at high latitudes (Dietz & Ullmann 1997; Dietz & Fattorini 2002). The typical grassland at high latitudes in Inner Mongolia accounts for 10% of the total grassland of China (Chen & Wang 2000). The grassland in east Inner Mongolia is particularly sensitive to both natural environmental change and human disturbance (Yuan et al. 2007). In this paper, we report on the response of annual rings in main roots of perennial forbs to climate extremes in Inner Mongolian semi-arid grassland. The main objectives of our study were (1) to identify patterns of variation of annual ring width in main roots of three perennial forb species, *Cymbaria dahurica*, *Potentilla anserina* and *Lespedeza daurica*; and (2) to examine whether climate and its extremes affects the growth of annual rings in main roots.

Methods

Study area and field sampling

Samples were collected from the Duolun region (42°27′N, 116°41′E, 1380 m a.s.l.), a semi-arid grassland located in the centre of Inner Mongolia, China. According to the climate records from 1987 to 2004, mean annual total precipitation in Duolun is 387.2 mm, with the maximum monthly values occurring in July and August. Mean annual air temperature is 3.0°C, with monthly mean tempera-

tures ranging from − 16.2°C in January to 19.4°C in July (Fig. 1).

The three species studied, *C. dahurica* L., *P. anserina* L. and *L. daurica* Schindl., are all perennial herbaceous plants. *C. dahurica* grows mainly in north China, and the other two species are distributed widely in north, central and southwest China. They are all drought-tolerant species, and mainly grow in semi-arid and arid environments, such as grazed and abandoned grasslands. They all have an obvious main root. Plants were excavated and main roots were taken from approximately 5–10 cm beneath the ground surface. Thirty samples were collected from each species. These samples were placed in airtight bags in the field and stored in a refrigerator in the lab before preparation of cross-section slides.

Lab procedure

Cross-sections (10–15-μm thick) were produced from the proximal end of the sampled roots using a sledge microtome. These sections were stained with phloroglucinol and concentrated hydrochloric acid (Dietz & Ullmann 1997, 1998; Liu & Zhang 2007). After this treatment, the vessels and other lignified parenchyma cells were reddish in colour and were clearly discernible (Fig. 2).

Using a dissecting microscope, clear images of these sections were obtained. Growth rings in the main roots were identified by differentiating early-wood from latewood in the secondary xylem (Dietz & Ullmann 1997) (Fig. 2). Ring widths at three different radii for each sample were measured using the AxioVision 4.1 software (Zeiss Inc.). The mean value of the three radii measurements was calculated to represent average growth of the sample.
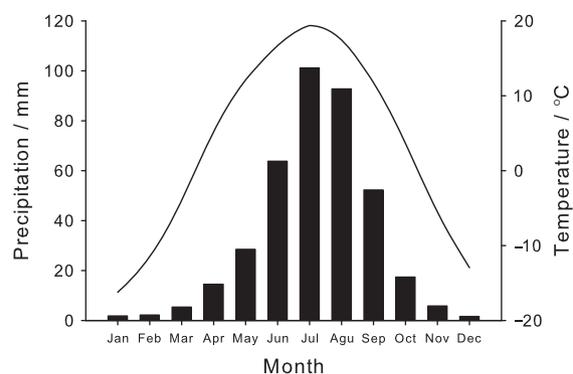

Fig. 1. Monthly mean temperature (line) and total monthly precipitation (bar) at Duolun County, China, from 1987 to 2004.

Annual ring width and statistical analysis

The measured annual ring widths were standardized using the following equation:

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

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

For each of the three species studied, the values of the standardized ring widths were averaged by year across individual samples. The border ring of 2005 and the centre ring were not incorporated into the analysis because it was not clear whether the growing season had finished at our sampling time in October 2005. The first ring in the centre was discarded from the analysis because some individuals appeared to have root decay in the centre of the roots.

A distinctness score (D) provided the sum of the differences in annual ring width to the previous and following years (Dietz & Arx 2005):

$$D_i = (W_{i-1} - W_i) - (W_i - W_{i+1})$$

where the indices $i-1$, i, $i+1$ denote the previous year, the year for which the D score is calculated and the following year, respectively. Marginal years could not be compared with the previous and following year, so their distinctness scores were not calculated. In addition, individuals that were younger than 4 years old were omitted because their rings were too scarce to calculate the D scores. The change in annual growth ring in 1 year relative to its two neighbouring years could be identified from the distinctness score value.

In order to determine whether particularly narrow or wide annual rings were significantly over-represented in specific years, a permutation test was employed. When several samples were compared, random patterns appeared because roots have the equal probability to develop particularly narrow or wide annual rings every year in the null model (Dietz & Arx 2005). Mean D scores (means of all individuals) were compared for each year, with a distribution of 9999 overall mean D scores obtained from the same data set (null distribution). Significant deviations were identified through comparing the observed scores with the scores of the null distribution (Manly 1999).

The mean value (D_m) of the absolute D_{abs} scores from the three species was calculated. Linear re-
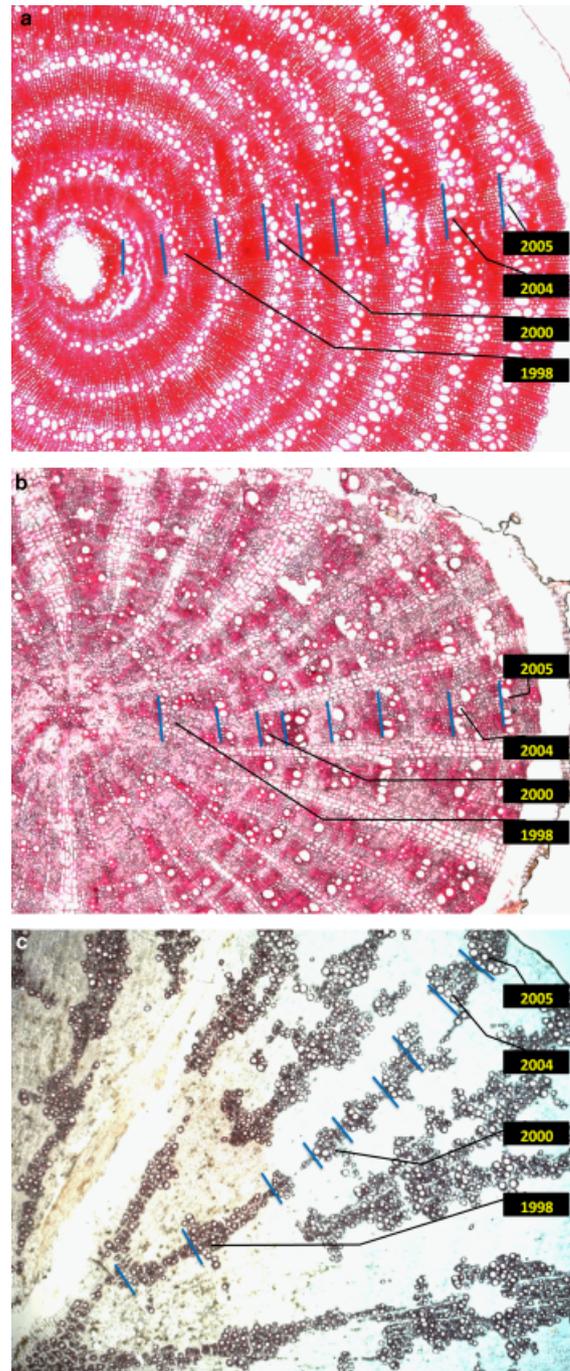
Fig. 2. Examples of annual rings in the secondary xylem of the main root of (a) *Cymbaria dahurica* (10 years old), (b) *Lespedeza daurica* (9 years old), and (c) *Potentilla anserina* (9 years old) as viewed through a microscope.

gression was used to identify annual trends in absolute distinctness scores.

Correlation coefficients were calculated between the mean series of the standardized annual
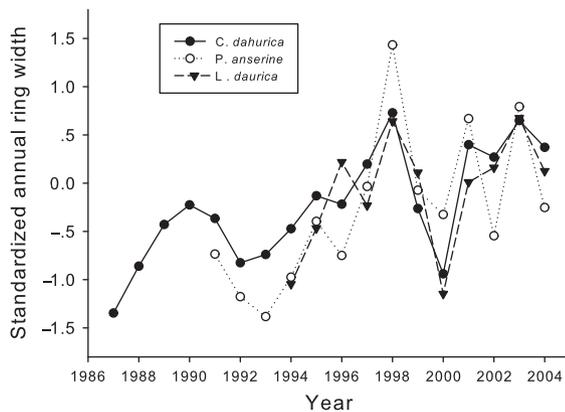
Fig. 3. Standardized annual ring widths of all samples for *C. dahurica* (●), *P. anserina* (○) and *L. daurica* (▼).

ring widths and climate data, monthly/annual mean temperature and monthly/annual precipitation.

Results

The three species, *C. dahurica*, *P. anserina* and *L. daurica*, showed clearly demarcated annual growth rings in all individuals (Fig. 2). These forbs are ring-porous species and the growth rings can be identified by the location of the large xylem vessels, which appear in earlywood. The oldest individuals for *C. dahurica*, *P. anserina* and *L. daurica* were 20, 15 and 13 years old, respectively. The three forbs species showed same fluctuation in patterns in annual ring widths during 1987–2004 (Fig. 3). All three species had the widest annual rings in 1998. The narrowest annual rings of different species appeared in different years. *C. dahurica*, *P. anserina* and *L. daurica* had the narrowest annual ring widths in 1987, 1993 and 2000, respectively.

The annual ring width in 2000 showed a significant deviation compared with neighbouring rings for each species ($D_{abs} = 1.90$, $P < 0.001$ for *C. dahurica*; $D_{abs} = 1.25$, $P = 0.0079$ for *P. anserina*; $D_{abs} = 2.31$, $P < 0.001$ for *L. daurica*). In 1998, a significant deviation in annual ring width appeared for *C. dahurica* ($D_{abs} = 1.29$, $P = 0.011$) and *P. anserina* ($D_{abs} = 2.68$, $P < 0.001$), but not for *L. daurica* ($D_{abs} = 0.60$, $P = 0.225$), although it was the widest ring, as was the case for the other species.

Mean values (D_m) of the absolute *D* scores in each year were calculated from the three species. The largest values of D_m appeared in 2000 and 1998. A significant increasing trend was observed ($r^2 = 0.66$, $P < 0.001$) in mean values (D_m) over the period from 1988 to 2003 (Fig. 4).
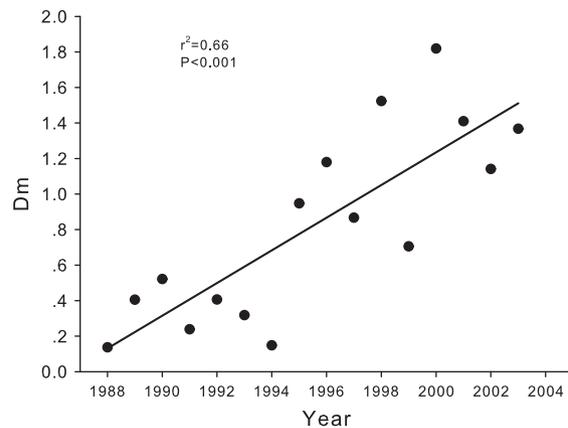

Fig. 4. Mean value of absolute *D* scores (D_m) for the three species (*C. dahurica*, *P. anserina* and *L. daurica*). The heavy line is the significant linear trend in D_m [$D_m = -183 + 0.092$(year); $r^2 = 0.66$; $P < 0.001$].

Results of the correlation analysis between standardized annual ring widths of the three forb species and the climate data are presented in Fig. 5. The ring widths of the three species were generally positively correlated with precipitation from April to October (except August), and with temperature from February to June (except June for *L. daurica*), September to October and the annual mean. For *C. dahurica*, the annual growth rings had a positive and significant correlation with precipitation in June and from September to October, with temperatures in February and March and the annual mean. For *P. anserina*, the annual growth rings had a positive correlation with precipitation in September and October, temperatures in September and October and the annual mean. For *L. daurica*, the annual growth rings had a significant and positive correlation with precipitation in September and November and temperature in September. Ring widths had significant negative correlations with precipitation in August for *C. dahurica* and *L. daurica* (also a negative correlation for *P. anserina* but not significant at the $P < 0.05$ level), and with temperature in July for *L. daurica* (also a negative correlation in June and August but not significant at the $P < 0.05$ level, Fig. 5).

Discussion

### *Relationship between root growth and climate*

Our data demonstrated that annual growth rings in the main roots of the three forbs were sensitive to climate fluctuations. The same pattern of
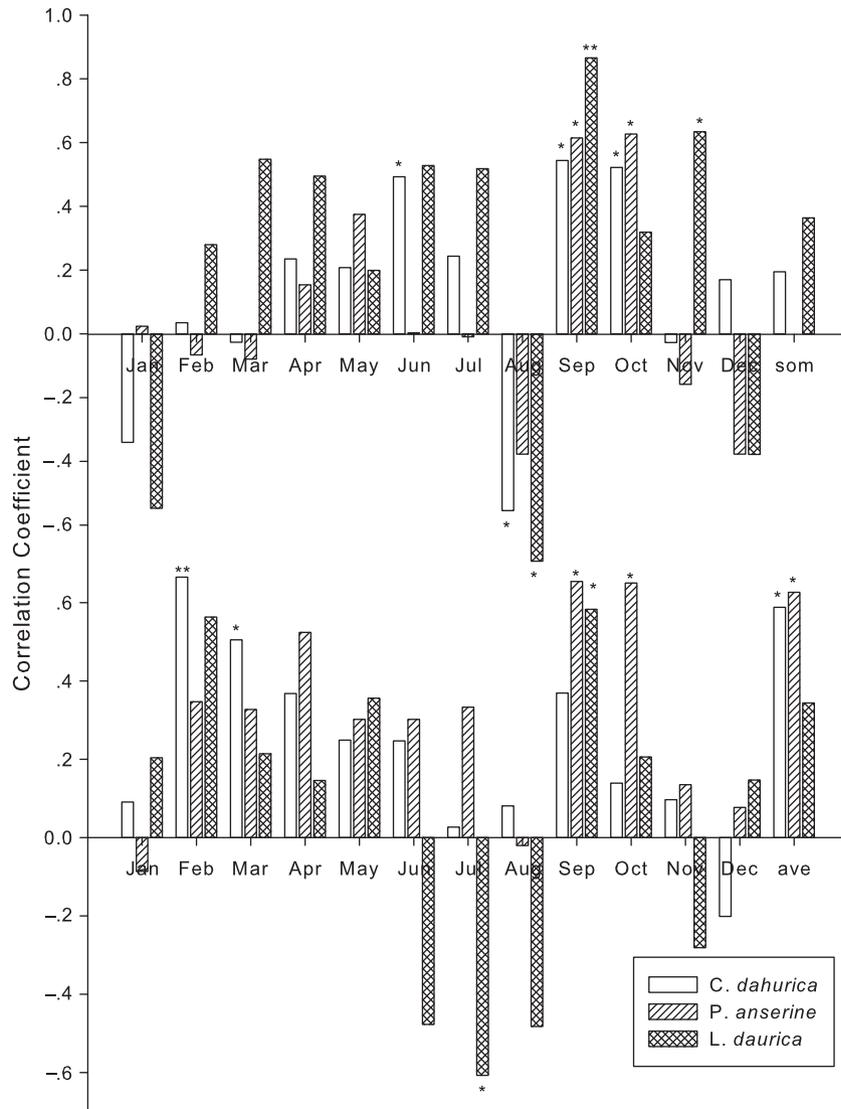
Fig. 5. Correlation coefficient between standardized monthly precipitation (top) and monthly mean temperature (bottom) with standardized annual ring widths of three species, *C. dahurica, P. anserina* and *L. daurica* ($^{*}P<0.05$, $^{**}P<0.01$). "som", annual precipitation; "ave", annual mean temperature.

annual ring widths in the secondary xylem of main roots was observed in all three perennial forbs, suggesting that a common factor, most likely climate, has a role in the growth of annual rings. According to the correlation analysis between annual ring widths and climate data, annual mean temperature and precipitation in the growing season are the major factors limiting the growth of annual rings.

Because mean annual air temperature is very low (3.0°C, Fig. 6) in Duolun region, from where samples were collected, temperature plays a major role in controlling the start and end of plant growth. High temperatures in spring melt the snow covering the grasslands and precondition the root growth. The growing season for below-ground organs is
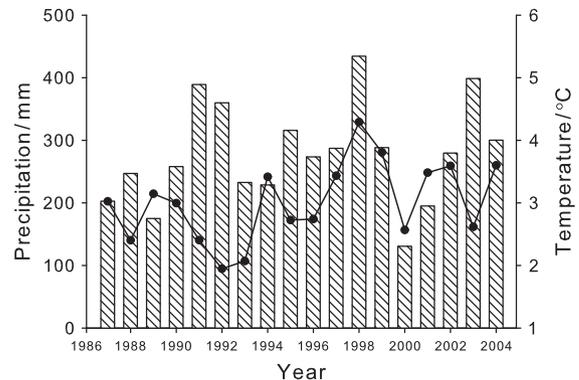
Fig. 6. Annual mean temperature (line) and total precipitation in April to July and September to October (bar) in each year from 1987 to 2004 in Duolun County, China.

likely to be different from the growing season for above-ground organs in grasslands (Zha et al. 2005). The active growth of above- and below-ground organs may occur at different times; in particular, the allocation of organic substances to roots might be delayed. Titlyanova et al. (1999) found that the below-ground organs of *Festuca pratensis* could be active up to September or October in grasslands. The climatic sensitivity differed among the studied species. For *L. daurica*, root growth was negatively correlated with temperature in June to August. High temperatures in the warm season (i.e. June to August) could enhance water deficit by increasing evapotranspiration and decreasing allocation of dry matter to roots (Carlen et al. 1999; Xu & Zhou 2005), thus inhibiting ring growth; while high temperatures in the cool season (i.e. early spring and late autumn) would result in wide annual rings because they enhance photosynthetic activity, advance the start date of the growing season and prolong the growth period (Jobbägye et al. 2002).

Precipitation during the growing season is important to the growth of plants (Yang et al. 1998), and water deficit is the most critical climate variable for grassland productivity (Zha et al. 2005). Precipitation affects photosynthesis, carbon assimilation and nutrient availability, for which water is essential (Yang et al. 1998), and is also a key factor at the end of the growing season (Jobbägye et al. 2002). It is reasonable that the annual growth rings in main roots of the forbs were positively correlated with precipitation in April to July and September to October in this semi-arid grassland area. The negative correlation between root growth and August precipitation might be related to nonlinear physiological processes in root respiration and resource allocation. Illeris et al. (2003) showed that the response of root respiration to precipitation was not fixed but depended on the amounts and frequencies of precipitation events. Water surplus in the rooting zone could displace air from the non-capillary pore space, thus inhibiting root respiration and growth (Polacek et al. 2006). Heavy precipitation could also reduce the growth of roots by decreasing allocation of dry matter to roots (Wang et al. 2003). Because the November temperature is already below zero and the plants had stopped growing, the positive correlation between root growth of *L. daurica* and November precipitation was difficult to explain from a physiological point of view. We consider that this correlation might be a statistical artifact that resulted from the short length of the climate data and ring sequences used in the correlation analysis.

Climate extremes affect growth of annual rings

According to meteorological data for the period from 1987 to 2004, monthly total precipitation and mean temperature in 1998 and 2000 showed significant deviations. The heaviest precipitation and highest temperatures in the study area occurred in 1998, with 490 mm annual precipitation and a temperature of 4.3°C each month on average. In 2000, precipitation and temperature had wide fluctuations, with monthly precipitation ranging from 12.9 mm in July to 231.3 mm in August, and temperature from -20.6°C in January to 22.4°C in July. These abnormal precipitation and temperature values were the result of extreme climatic events in 1998 and 2000. Easterling et al. (2000) described climate extremes such as extreme temperature events of freezing during the winters of 1983 and 1985 in Florida (Miller & Glantz 1988; Katz & Brown 1992), and extreme precipitation events (Ekström et al. 2005; Fowler et al. 2005) like droughts that have caused famine episodes in Africa (Glantz 1987; Katz & Brown 1992).

Due to the significant influence of abnormal precipitation and temperature in 1998 and 2000, there were wider annual ring widths in 1998 and narrower ring widths in 2000. The above-average precipitation from February to October (except August) in 1998 might be a decisive factor resulting in wider rings in the main roots of perennial forbs, whereas the water deficit from February to October (except August) in 2000 inhibited ring growth. August precipitation had a negative correlation with ring growth. The August precipitation in 1998 was 33 mm, obviously lower than the average value of 103.3 mm from 1987 to 2004. The August precipitation in 2000 was 231.3 mm, and more than half of the rainfall occurred in a single day.

The occurrence of wide rings in 1998 and narrow rings in 2000 might also be related to temperature. Temperature controls the start of the growing season (Jobbägye et al. 2002). The higher temperature in April 1998 (8.8°C compared to the average 5.2°C) may have preconditioned the start of the growing season, while lower temperatures in April 2000 (4.3°C) might have inhibited the start of the growing season. In addition, the above average July temperature in 2000 (22.4°C compared to the average 20.0°C) might have further inhibited the root growth in *L. daurica*.

In 1998 and 2000, the annual growth rings showed consistent variations compared to neighbouring rings in all three species, but the degree of variation in *L. daurica* in 1998 was not as significant

as in the other two species. This suggests that the annual growth rings in main roots of perennial forbs are sensitive to short-term climatic extremes, although the climatic sensitivity differs among species. *P. anserina* had the highest distinctness *D* value in 1998, while *C. dahurica* and *L. daurica* had the highest distinctness *D* values in 2000. Compared to the other two species, *L. daurica* was less sensitive to the 1998 climate event. Dietz & Arx (2005) found that radial increment growth in main roots of forbs had a considerable response to the 1998 El Niño event across Switzerland and the United States. The annual growth rings of *C. dahurica, P. anserina* and *L. daurica*, in the Duolun grassland may also reflect climate extremes in the 1998 El Niño year and also the 2000 La Niña year.

The mean value (D_m) of the three absolute *D* scores from the three species increased from 1988 to 2003, meaning an increase in intensity of annual ring width fluctuations during this period (Liang et al. 2009). We think this trend is not related to a plant aging effect but is related to climate variables, because the observed trend in root growth could be fairly well reconstructed from climate variables. The trend suggests that the below-ground condition for root growth is changing more frequently than in the past. This is important in view of results from other studies that extreme climate events might become more frequent in the future (Easterling et al. 2000; White et al. 2000; Stenseth et al. 2002; Sánchez et al. 2004; Goswami et al. 2006). Goswami et al. (2006) found significant rising trends in the frequency and magnitude of extreme rain events in a warming environment.

Conclusion

Recording the occurrence and predicting the influences of climate extremes on grassland ecosystems is difficult because great spatial variability means that real-time surveys rarely record such effects. For example, above-ground growth or abundance may not immediately and easily indicate short-term climate change. Our results demonstrate that radial growth in the main roots of perennial forbs contain climate signals. The same pattern of annual ring widths was observed in the secondary xylem of main roots in *C. dahurica, P. anserina* and *L. daurica* in the Duolun grassland of Inner Mongolia. The strong deviation in these annual growth rings in exceptional years, 1998 and 2000, indicate that the rings in main roots are sensitive to climatic extremes. Moreover, the magnitude of annual ring

width fluctuations showed an increasing trend from 1988 to 2003, suggesting the increased influence of climate on below-ground ecological processes. Linking the pattern in root growth ("herbchronology", Dietz & Ullmann 1998) obtained in this study to above-ground biomass productivity is worthy of further study.

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