Jessen, M.-T., **Roeder, A.**, **Roscher, C.** (2025):
Intensity and timing of land use influence annual increment in growth rings of *Galium mollugo* in temperate grasslands
Ann. Bot. **136** (7), 1485 - 1496

https://doi.org/10.1093/aob/mcaf158

Type of article: Original Article

Title: **Intensity and timing of land use influence annual increment in growth rings of *Galium mollugo* in temperate grasslands**

Maria-Theresa Jessen*[1,2], Anna Roeder [1,2], Christiane Roscher[1,2]

[1] Department of Physiological Diversity, Helmholtz Centre for Environmental Research – UFZ, Permoserstraße 15, D-04318 Leipzig, Germany

[2] German Centre for Integrative Biodiversity Research Halle-Jena-Leipzig (iDiv), Puschstraße 4, D-04103 Leipzig, Germany

Running title: Variation in annual growth rings of *Galium mollugo*

Contact information: maria-theresa.jessen@idiv.de

Abstract

Background and Aims: Current grassland management including fertilization, mowing and livestock grazing, substantially influences plant communities, however it is not fully understood how management can affect plant growth at the individual level. Most herbaceous dicotyledonous plant species form distinct annual rings in the xylem that reveal age but their annual width may also respond to changes in environmental conditions. Further is unclear at what scale, from the local plot level to the regional level, such secondary growth varies most.

Methods: Using cross sections of the oldest well-preserved detectable part of the rhizome of *Galium mollugo* agg., we determined the response of secondary growth to the intensity, timing, and type of grassland management for 1220 individuals across 60 populations in three different regions in Germany. In addition, we used environmental variables to examine the effects of climatic, soil, and diversity-related characteristics on ring width variation.

Key Results: The age of the studied *G. mollugo* individuals ranged between one and 17 years (average age six years). Secondary growth varied most among individuals within sites and only slightly among sites within regions and among regions, indicating a strong influence of local plant-plant interactions. Increasing overall management and land-use intensity decreased secondary growth while a late start of annual land use increased it. In addition, summer soil moisture was positively related while neighbour species richness was negatively related to secondary growth.

Conclusions: Our results indicate that annual secondary growth of a widespread herbaceous grassland species is sensitive to timing and intensity of land use and season water regimes. The results imply that trends of increasing land-use intensity along with prolonged summer heat waves will further hamper growth of *G. mollugo* which may translate to reduced performance and subsequently affect the overall plant community.

Keywords: climate, *Galium mollugo*, grassland, growth ring, land use intensity, management timing, secondary growth, xylem

Introduction

Grasslands are globally an important biome, which occupies more than one third of the terrestrial surface and provides many ecosystem functions and services (Bengtsson *et al.*, 2019). In Central Europe, most grassland originated as a result of human management. Traditional low-intense agricultural practices like grazing of domestic animals or haymaking have created plant communities, which belong to the most diverse ecosystems in Europe (Hejcman *et al.*, 2013). However, land-use change involving homogenization, abandonment and/or intensification has caused a notable decrease in grassland diversity, especially in the second half of the last century (Poschlod and WallisDeVries, 2002, Jandt *et al.*, 2022). Land-use intensification of grasslands includes higher fertilizer input, more frequent cuts in meadows or increased livestock densities in pastures. Changes in grassland management can affect plant demography and species coexistence due to altered resource competition (Hautier *et al.*, 2009, Socher *et al.*, 2013). Fertilization generally increases biomass production and vegetation density and intensifies competition for light (Hautier *et al.*, 2009, Socher *et al.*, 2012). Both, grazing and mowing can be seen as disturbances affecting vegetation structure, the occurrence and distribution of gaps and the availability of resources such as light and nutrients. Livestock grazing involves processes such as trampling, selective foraging and deposition of excreta by grazing animals and creates more heterogeneous swards, while mowing is a non-selective, more uniform process of biomass removal which can lead to homogenization of the vegetation (Tälle *et al.*, 2016).

Temperate grasslands are facing growing seasons characterized by much warmer temperatures as well as increased spring and reduced summer precipitation (Hari *et al.*, 2020, IPCC, 2021, Regionaler Klimaatlas Deutschland, 2022). While the severe impacts of prolonged droughts, increased temperatures and more extreme climate events on plant productivity in grasslands has been shown in various studies (e.g. Ciais *et al.*, 2005, Korell *et al.*, 2024), it is not well understood how climate change affect the growth dynamics of individual species under natural conditions.

One rather hidden aspect of plant growth is the formation of rings in the xylem of perennial herbaceous dicotyledonous grassland species. It is well known from dendroecology that xylem growth responds sensitively to variation in environmental conditions, i.e. poor growth conditions may be reflected by narrow annual growth rings, while better growth conditions are related to wider rings (Fritts, 1966). So far, only few studies attempted to explain differences in growth ring width in perennial dicotyledonous forb species among plant individuals within or between populations or fluctuations of ring widths among years (e.g. Dietz and Fattorini, 2002, Dietz and von Arx, 2005, von Arx *et al.*, 2006). In grassland species, this secondary growth, which can be derived from stem sections, has been found to respond to fertilization and clipping in an experimental context (Dee and Palmer, 2016, Dee and Palmer, 2019), but the effects of "real-world" anthropogenic land use on secondary growth are still unknown. Another important aspect in relation to land use is the timing of its application. Since growth ring formation starts with the main increment early in the growing season (Dietz and Ullmann, 1997, von Arx and Dietz, 2005), land-use activities in spring may have a greater impact on secondary growth than those implemented later in the year. However, the effects of timing in land use on ring width have not yet been studied.

Regarding the effects of climatic variables, it is already known that there can be a correlation between ring width and temperature in cold-limited grassland ecosystems (Liu and Zhang, 2010, Doležal *et al.*, 2020, Rai *et al.*, 2024). However, this relationship might be different in temperate ecosystems, where the formation of growth rings may be under stronger control of water availability, as the xylem formation can be water dependent (von Arx *et al.*, 2012, Olano *et al.*, 2013). The impact of climate on ring width also likely depends on other site- or even microsite-specific conditions (Dietz *et al.*, 2004, Klimešová *et al.*, 2013, Doležal *et al.*, 2020). In general, not much is known about variation in secondary growth in response to environmental conditions at different spatial resolutions. Some studies have found little variation across different geographic regions (Dietz and von Arx, 2005, Bär *et al.*, 2008) pointing to the importance of site-specific conditions determined by soil (e.g., soil texture, nutrients or acidity) or vegetation characteristics such as species richness. These factors influence resource availability and consequently plant-plant interactions such as competition which can alter annual ring growth (Dee and Palmer, 2016, Dee and Palmer, 2019). Even though some aspects influencing secondary growth are known, its potential has not been fully explored in ecological studies.

In the present study, we selected the widespread forb species *Galium mollugo* agg. to investigate the annual rings in the rhizome of 1220 individuals. The individuals originated from 60 populations from grassland areas in three different regions of Germany with different land-use types, intensities and times of application (*Biodiversity Exploratories*) (Fischer *et al.*, 2010). We also assessed effects of site-level climatic, edaphic and diversity-related variables on secondary growth variation. We asked 1) Does secondary growth of *G. mollugo* respond to different land-use types and varying land-use intensity and timing between years? 2) Does secondary growth of *G. mollugo* respond to between-year and site variation in spring and summer precipitation and/or temperature? 3) Do additional local site and/or microsite characteristics such as soil or diversity-related variables determine secondary growth in *G. mollugo*?

Methods

Study species

Galium mollugo agg. (Rubiaceae) is a widely distributed perennial grassland forb. It reproduces both sexually and vegetatively and is, because of its tall stature (30-100 cm; Jäger *et al.*, 2013), highly competitive with other meadow forb species (Mersereau and DiTommaso, 2003).

Originating from a bulbous cotylonedar node young individuals produce adventitious roots which form plants with a clumpy growth, while well-developed plants may also grow with longer belowground rhizomes (Mörchen, 1965). Compared to other forbs in temperate grasslands, individuals are relatively long-lived and growth rings are mostly clearly distinguishable (Roeder *et al.*, 2017), which makes the species particularly suitable for the analysis of secondary growth. Across Germany *G. mollugo* agg. has different subspecies (Bundesamt für Naturschutz, 2022). According to the results of a recent study about genetic diversity across multiple temperate grassland species (Durka *et al.*, 2025), *Galium album* Mill. (*G. mollugo* ssp. *album*) is the much more common species, but *G. mollugo* s. str. is also found almost everywhere in Germany. Both species show smooth transitions in their expression of morphological characteristics used for their identification (Krendl, 1967) . We did not further determine the subspecies and therefore refer to *G. mollugo* L. agg.

Field sites and experimental design

The study was conducted in the so-called *Biodiversity Exploratories (*Fischer *et al.*, 2010*)*. They are implemented in three different regions in Germany: *Schorfheide-Chorin* region (an UNESCO biosphere reserve embedded in a young glacial landscape in the lowlands of north-eastern Germany), the *Hainich-Dün* region (located in the National Park Hainich with surroundings in the hilly areas of Central Germany on calcareous bedrock), and *Schwäbische Alb* region (an UNESCO biosphere reserve in the low mountain area of south-western Germany on calcareous bedrock). The regions represent different landscape types and reflect a gradient with rising altitude, increasing precipitation and slightly decreasing annual mean temperatures from north-east to south-west Germany (Fig. 1A). Fifty grassland study plots within each region were established along local land-use gradients, where mean and range of land-use intensities were comparable among regions (Fischer *et al.*, 2010, Blüthgen *et al.*, 2012) and are typical for managed grasslands in Central Europe. They are managed by mowing or grazing, or a combination of both. Grazing involves different livestock types, mostly cattle or sheep, and less often horses (Vogt *et al.*, 2019). Land-use intensity was assessed yearly by gathering information from farmers to create a land-use intensity (LUI) index with the aim to reduce the complexity of three land-use components, i.e. mowing, grazing and fertilization, into a single dimension (Blüthgen *et al.*, 2012). The grazing part of the land-use index accounts for different livestock types by converting the grazing animals to livestock units (depending on type and age of the grazing species) and the duration of grazing (= number of livestock units per days and ha^{-1}). The mowing part of the land-use index quantifies the frequency of mowing (and mulching) as intensity (= number of cuts per year), which varies between no mowing and the maximum of four cuts per year, while one or two cuts per year are more typical (Vogt *et al.*, 2019). Fertilization includes the use of different fertilizer types such as mineral fertilizer, farmyard manure or slurry. In most cases manuring is done in early spring at the beginning of the growing season (between late February and mid-April) and can be expected to influence the current year`s growth. However, in some cases, manuring is done later in spring (until June), in autumn to early winter (between September and December) or several times per year. The fertilizer component of the land-use index also incorporates that nutrients are not immediately available from all manure types, but could also be later released for example from farmyard manure. In this case, the nitrogen input from manuring enters the fertilizer intensity of the next (two) growing season in the fertilization index (Vogt *et al.*, 2019). For each study site and year, the compound land-use intensity (LUI) was quantified as a continuous variable using the following equation,

$$LUI = \frac{G_p}{G_g} + \frac{M_p}{M_g} + \frac{F_p}{F_g}$$

where G_p is grazing intensity, M_p is mowing intensity and F_p is fertilization intensity on a study plot p for a given year. The three components are divided by the global mean (across all regions and years) of the respective component (G_g, M_g and F_g, respectively), and the square-root of the summed standardized components was extracted, according to Blüthgen *et al.* (2012). Information on mowing, grazing and fertilization was obtained from land owners (Vogt *et al.*, 2019), and calculations were done using the LUI calculation tool (Ostrowski *et al.*, 2020) implemented in BExIS (http://doi.org/10.17616/R32P9Q). Values of each component were used to quantify fertilization, mowing and grazing intensity, respectively (Supplementary Information Fig. S1A-D). We also calculated the intensity of management (MI) as square-rooted sum of standardized mowing and grazing (Supplementary Information Fig. S1E). In addition, we had data on the timing of land use per plot per year (i.e. the month, when first or the last mowing or grazing was done in a year) (Ayasse *et al.*, 2022), which we used in the statistical analysis as ordinal variable. Typical time for first mowing or grazing is May or June, but it also varies among sites and years (Supplementary Information Fig. S2).

Sampling, growth ring analyses and set-up of time series

Vegetation records from all grassland study plots of the *Biodiversity Exploratories* (Bolliger *et al.*, 2020) were screened to choose the study plots for sampling of *G. mollugo* with the aim to equally represent the regions of the *Biodiversity Exploratories* and to cover different grassland management types and land-use intensities within each region. Due to the lower occurrence of *G. mollugo* we sampled only ten plots in the region *Schorfheide-Chorin*, while in the *Hainich-Dün* region and *Schwäbische Alb* region, samples were collected on 26 and 25 grassland plots, respectively. On each grassland plot with a total size of 50 × 50 m, a strip of 50 × 2 m on the eastern side of the plot was available for sampling. Sampling was conducted between 2017 and 2019 in summer or early autumn (July to September) when the growth ring of the current year is formed and the vegetation is well developed to easily identify species. On each plot, 20 individuals were sampled along a transect with a minimum distance of one meter to each other taking the individual growing with the clumpy part closest to the sampling point. First, we identified all neighbouring species rooting within a minimum distance of 10 cm to the clumpy part of the focal individual to get an estimate of local species richness and composition. Afterwards, we carefully excavated the focal individual with a weeding trowel or small shovel and transported the plants in sealed plastic bags to the laboratory. There, we cleaned the samples from soil with tap water. A segment of the oldest stem portion (5-15 cm long; dependent on the growth of the individual) comprising the part with the cotyledon node if available (see Fig. 1B for a typical specimen) was excised from each plant and placed in plastic tubes with 70% ethanol. Later, the samples were cut using a microtome (Microtome type GSL1; Gärtner and Schweingruber, 2013). In cases, where the oldest part of the specimen could not be clearly assigned, especially when several rhizomes grew from the cotyledon node, cuts were made at different parts. Microtome sections were put on a slide into a drop of a glycerol-water mixture (1:1) and covered with a cover glass. Ring width was measured directly under the microscope (Axio-Scope A1 Microscope, Carl Zeiss Microscopy GmbH, Jena, Germany, equipped with a microscopic camera, Axiocam 105 colour; Carl Zeiss Microscopy GmbH, Jena, Germany) and analysed with the built-in measuring function of the microscope software (ZEN 2, blue edition, Carl Zeiss Microscopy GmbH, 2014). Images of the microsections were taken for documentation (Fig. 1C). If necessary, a polarized light filter was used to facilitate ring visibility and measure annual radial growth.

The age of the individuals was assessed by counting the number of annual rings. In total, 1219 individuals of *G. mollugo* were analyzed (500 individuals in *Schwäbische Alb* region, 519 individuals in *Hainich-Dün* region as measurements were not possible for one individual due to low ring distinctness, and 200 individuals in *Schorfheide-Chorin* region) (Roeder and Roscher, 2024). Due to low sample depth in the early years and the beginning of land-use and climate data availability starting in 2008, we decided to truncate our time series from 2008 to the respective year of sampling (i.e., 2017, 2018, or 2019) for the analyses of annual secondary growth.

Plot-level soil, climate and diversity-related data

Plot-level information on soil, climate and plant diversity was derived from the publicly available data of the *Biodiversity Exploratories*. Climate data were collected from weather stations located on each plot that record observations every ten minutes (Hänsel *et al.*, 2024). Here, we used air temperature measured 2 m above the ground, soil temperature measured 10 cm below the surface, soil moisture measured 10 cm below the surface, and precipitation based on RADOLAN (Weigl, 2017). For the statistical analyses, we calculated mean values for spring (March to May) and summer (June to August) for soil moisture, soil and air temperature, or the summed the values for precipitation across the respective months. Soil data were collected in repeated soil sampling campaigns. Here, we used C_{org}:N ratio (Schöning, 2023), soil pH (measured in $CaCl_2$) (Schöning, 2024) and clay and sand content (Schöning *et al.*, 2021) from soil cores of the upper 10 cm of the soil horizon. For species richness as diversity measure at plot level, we used species surveys conducted within a 4 x 4 m subplot of each plot yearly in spring (Bolliger *et al.*, 2020). For species richness in close proximity to the harvested *G. mollugo* individuals, we assessed species numbers recorded around the individual (see above) (Roscher and Roeder, 2024). For an overview of all variables see Table S1 in the Supplementary Information.

Statistical analysis

All statistical analyses were conducted with the statistical software R (version 4.2.2; R Core Team, 2022). The data were structured in a long format so that each ring width measurement was associated with the respective year of the ring.

To assess variation in plant age, mean ring width and to detect possible correlations of ring width series within regions, within plots and within individuals (i.e. first order autocorrelation), we used the function RwlInfo from the *detrendeR* package (Campelo, 2012). This function can calculate the age, the ring width, the correlation between series of a given data set based on a master series which is derived from all series in the data set and the first order autocorrelation (i.e., testing if there is a temporal dependency between ring width of two consecutive years) of each series. For the determination of age and mean ring width we used the entire data set with 1219 individuals, for the determination of inter series and first order autocorrelation we had to exclude 17 one-year-old individuals, because calculation of correlations was not possible. We double checked the output from the RwlInfo function by re-calculating all variables by hand. We tested for an effect of region on plant age, mean ring width, inter series and first order autocorrelation using mixed-effects models with plot as random effect to account for statistical dependencies of individuals collected on the same plot (i.e. the same population) using the *lme4* package (Bates *et al.*, 2015). We tested for differences between the regions using post-hoc Tukey's test from the *emmeans* package (Lenth, 2022).

To assess the effects of different land-use types and intensities, as well as the effects of land-use timing on secondary growth, we applied generalized additive mixed models (GAMM) using the packages *mgcv* (Wood, 2011*)* and *nlme* (Pinheiro and Bates, 2000). GAMMs allow to model both linear and nonlinear effects, which is useful when dealing with plant age because of nonlinear ontogenetic effects (Olano *et al.*, 2013). Fixed effects assuming a linear relationship were the overall land-use intensity, management intensity and the single components grazing, fertilization and mowing intensities of the year when the growth ring was formed as well as the timing (month) of the first management activity (i.e. grazing or mowing) in the respective year as ordinal variable. The components grazing, fertilization and mowing intensities were log-transformed after adding 1 (there were many zeros in the data), because of their non-normal distribution. We also lagged all variables by one year to test for potentially delayed effects on secondary growth (data not shown). The random effects in all models were sample (i.e. individual from which the ring widths were measured) nested in plot nested in region to account for the hierarchical design of the sampling. In addition, to account for temporal dependency, since the ring width and land-use data were time series data, we added a temporal autocorrelation structure of order 1 with the grouping factor sample nested in plot nested in region (Zuur *et al.*, 2009). To meet the assumptions of heteroscedasticity and normality of errors ring width was log-transformed. Prior to fitting the land-use variables we entered plant age at the ring level (i.e. the age of the individual, when the ring was formed) as a smoothed fixed effect modelled with a penalized cubic regression spline to account for a potential, nonlinear age-related effect on secondary growth and validated this by checking against the null model. We set k = 5 in the smoothing term to prevent the smoothing term from overfitting due to a skewed age distribution (i.e. limited number of individuals older than ten) (Wood, 2017). All models were fitted using maximum likelihood and were evaluated on a comparison of AIC and log likelihoods ratios. To provide the coefficients of determination showing the variance explained by fixed and random effects in the models we used the *MuMIn* package (Barton, 2024). We used the same approach to test for effects of climatic variables on secondary growth. Fixed effects tested against the model with only age as a smoothed fixed effect were air and soil temperature, soil moisture and precipitation separately for spring (March to May) and summer (June to August). We exclusively tested for an effect of climate variables in spring and summer since these represent conditions during the active growing season. Previous growth ring analyses have also found these seasons to be most influential (Olano *et al.*, 2013). To also test for effects of diversity-related and soil variables on secondary growth we ran further models with the same random effects using neighbour species richness (i.e. number of species in close proximity to the harvested individual) and plot species richness (from a 4 x 4 m area), as well as soil carbon to nitrogen ratio (Corg:N), clay and sand content and pH as explanatory variables. Neighbour species richness and plot species richness were log-transformed.

In a final step we started with the best performing (based on AIC) model of the previously mentioned models and added explanatory variables which had no correlation issue (Supplementary Information Fig. S3) in a stepwise manner always checking for significant improvement of the model, based on change in loglikelihood values, p-values and AICs, with the previous best model (forward model selection). The resulting final model had the same random effect and temporal autocorrelation structure as described above. Fixed effects in the final model were plant age modelled as a smoothed term, timing of the first land use, summer air and soil temperature and moisture, spring soil temperature and neighbour species richness (log-transformed) (Table 1). The final model was fitted using restricted maximum likelihood, as this is more conservative against overfitting than maximum likelihood in a GAMM context (Wood, 2017). Significance of the parametric and the smoothed terms in the final model was assessed with the summary function.

To understand the importance of environmental conditions of contrasting spatial resolution (i.e., hierarchical levels of the experiment; region, plot, individual plant) for secondary growth we estimated the variance explained by the random factors using the *rptR* package and *rpt* function with 1000 bootstrap iterations and 1000 permutations from the final model coded according to the model framework of *lme4* (Stoffel *et al*., 2017). This function calculates the repeatability *R*, which can explain variance in data, as variance among group means over the combined group and residual variance (Stoffel *et al.*, 2017).

Results

Characteristics of regional chronologies

On average, individuals in the *Schwäbische Alb* were 5.9 (± 0.1 s.e) years old, in the *Hainich-Dün* 6.4 (± 0.1) years old, and in the *Schorfheide-Chorin* 5.4 (± 0.2) years old (Fig. 2A, Supplementary Information Table S2). The oldest individual across the experimental regions was sampled in the *Hainich-Dün* region at 17 years of age. Individuals in the *Schwäbische Alb* region had a mean ring width of 199.87 (± 2.89) µm, in the *Hainich-Dün* region a mean ring width of 183.81 (± 3.20) µm and in the *Schorfheide-Chorin* region a mean ring width of 216.56 (± 6.96) µm (Fig. 2B, Supplementary Information Table S2). The mean correlation among the chronologies of individuals from the same plot and region was low: 0.29 (± 0.02) for *Schwäbische Alb* and *Hainich-Dün* and 0.32 (± 0.04) for *Schorfheide-Chorin* (Fig. 3A, Supplementary Information Table S2). In addition, the mean first order autocorrelation for individuals of each plot and region was low: -0.09 (± 0.01) for *Schwäbische Alb*, -0.06 (± 0.01) for *Hainich-Dün* and -0.10 (± 0.02) for *Schorfheide-Chorin* (Fig. 3B, Supplementary Information Table S2), expressing high interannual variation of radial growth independent from growth ring width of the previous year. Mean ring width from individuals in *Hainich-Dün* was significantly smaller than from individuals in *Schorfheide-Chorin* (Tukey HSD test: p = 0.011). None of the other characteristics measured differed significantly between the three regions.

Effects of land-use type, intensity, and timing on annual growth ring width

Plant age at the year of growth ring formation was nonlinearly associated with secondary growth (Table 2, Supplementary Information Fig. S4). After accounting for variation dependent on plant age, overall land-use intensity and management intensity were negatively associated with secondary growth, while of the single land-use types only grazing negatively affected secondary growth (Supplementary Information Table S3). Regarding the timing of land use, the timing of the first land use (mowing or grazing) in the year had a significant effect on secondary growth (Table 2). The later in the year the first land use occurred, the wider the annual growth rings became (Fig. 4A). Land use in the previous year did not affect secondary growth in the following year (analyses not shown).

Effects of plot-level climate, soil and diversity-related variables on annual growth ring width

Modelling of plot-level climate variables resulted in spring air temperature and summer precipitation as well as spring and summer soil moisture being positively associated with secondary growth, while spring precipitation and soil temperature and summer air and soil temperature were negatively associated with secondary growth (Supplementary Information

Table S3). Modelling of diversity-related variables revealed that neighbour species richness as well as plot-level species richness had a significant negative association with ring width (Supplementary Information Table S3). None of the soil variables included in the modelling process had a significant effect (Supplementary Information Table S3).

In the final model nonlinearly modelled plant age, timing of the first land use, spring and summer soil temperature, summer air temperature and soil moisture as well as neighbour level species richness significantly improved the model (Table 1). However, only nonlinearly modelled plant age, timing of the first land use and neighbour level species richness remained significant on a predictor level (Fig. 4, Table 2). Variation partitioning of the random effects in the full model showed that only little of the variance expressed as the repeatability R (see Methods) in secondary growth was explained by region ($R = 0.017$) and plot ($R = 0.06$) whereas the individual sample, i.e., the *G. mollugo* individuals from which secondary growth was measured, explained more variation ($R = 0.243$) (Supplementary Information Fig. S5).

Discussion

Variation in secondary growth of herbaceous plant species is increasingly used as a valuable “archive” to deduce plant responses to temporal or spatial changes in growth conditions, particularily in terms of climate (Liu and Zhang, 2010, Shi *et al.*, 2016, Dee and Stambaugh, 2019, Doležal *et al.*, 2020). Investigating time series of ring width in *G. mollugo* across managed grasslands in three different regions in Germany, we have found that overall land-use and management intensity negatively affected secondary growth and that secondary growth positively responded to first land-use application happening late in the year. The negative response of ring width to land use was mainly driven by grazing. Furthermore, neighbour and plot-level species richness had a negative relation to secondary growth. This highlights that variation in secondary growth can also be attributed to the intensity and timing of land use and to environmental conditions other than climate. Still, water availability, here precipitation in summer and soil moisture in spring and summer, did additionally increase secondary growth, whereas higher air and soil temperatures in summer decreased secondary growth. We also show that the variation in ring width is greatest within plots (i.e., among individuals of the same population), in contrasts to differences among regions or different study plots within regions.

Land-use intensity and timing of application related to ring width

We found that when the first management activity (i.e., grazing or mowing) was later in the year, this was associated with wider rings of the same year. Since the formation of growth rings is restricted to the active growing season a time bound effect of land management is plausible. Management that disturbs the shoot by removing photosynthetically active plant parts interrupts the transport of resources for plant growth and makes it necessary to invest primarily in the re-growth of the above-ground structures. This is important as ring width is strongly linked to the emergence of the shoot and was shown to be largest when the shoot has reached maximum size and assimilated carbon is allocated to root growth (Dee *et al.*, 2018). Consequently, management happening before the shoot is well developed will have a detrimental effect on secondary growth, while later management creates a longer period of disturbance free growth. We found no evidence of a carryover effect from late management to ring width of the next year (data not shown), underpinning the within year effect of land management on secondary growth. This shows that timing of grassland management is not only important to aboveground plants parts (Vermeire *et al.*, 2023), but also to the otherwise hidden aspect of secondary growth which can feed back to plant fitness and performance (Strock and Lynch, 2020).

Overall land-use and management intensity as well as grazing intensity were negatively associated with secondary growth. Furthermore, land-use intensity and management intensity, were equally important for ring width (Supplementary Information Table S3). The grasslands we examined in our study include pure pasture and meadows, but often both management types are combined. Therefore, our results suggest that both types of disturbance through land management (i.e., grazing and mowing), which is included in both variables, is driving the reduction of ring width. Again, this can be linked to the removal of the photosynthetically active aboveground plant parts hampering the resource supply needed for secondary growth and consequently leading to narrower rings. In addition, *Galium* has been observed to thrive well in unmanaged grasslands (Pavlu *et al.*, 2007) suggesting that it may benefit from its relative tall stature in undisturbed conditions. It remains to be tested if other perennial grassland forb species also exhibit intensity- and time-dependent management responses of secondary growth. Nonetheless our species-specific results suggest that low-intensity land use starting later in the growing season supports secondary growth.

Climate conditions related to ring width

We also found that summer precipitation and spring and summer soil moisture were positively associated with annual ring width, while summer air and spring and summer soil temperature were negatively associated with secondary growth. In agreement with our findings, various studies have already reported on positive effects of summer precipitation on ring width, as well as region and species-specific negative effects of temperature (e.g. Liu and Zhang, 2010, Shi *et al.*, 2016, Dee and Stambaugh, 2019, Doležal *et al.*, 2022). Studying four temperate grassland forb species, Doležal *et al.* (2022) have shown that the climate control of secondary growth can also vary among species. The shallow-rooting forbs (*Lychnis viscaria* Borkh., *Thymus pulegioides* L.) showed a positive response to higher summer precipitation resulting in wider rings width, while their secondary growth was negatively affected from high summer temperatures. From a physiological perspective sufficient water availability as indicated by levels of precipitation and soil moisture allow herbaceous plants to be more competitive in terms of growth because of increased hydraulic efficiency in the xylem (von Arx *et al.*, 2012, Dong *et al.*, 2022). The combination of little precipitation in concert with high temperatures reduces grassland productivity and vitality due to heat stress, increased evapotranspiration and water shortage leading to unfavorable growing conditions (De Boeck *et al.*, 2016, De Boeck *et al.*, 2008, Obermeier *et al.*, 2018, Kowalski *et al.*, 2024). Our study supports previous studies that such conditions translate to secondary growth and highlights another aspect of the critical nature of climate change with heat waves and drought events predicted for Central Europe (Hari *et al.*, 2020, IPCC, 2021, Regionaler Klimaatlas Deutschland, 2022). In addition, there is evidence that secondary growth and aboveground biomass production, as well as phenology timing, are linked (Doležal *et al.*, 2018, Dee *et al.*, 2018), which may suggest a possible cascading effect of climate on various aspects of plant growth and performance.

Importance of species richness at the local site scale

Considering vegetation characteristics at different spatial resolutions around the studied individuals we found a negative relationship between ring width and neighbour species richness and plot-level species richness. If the main mode of action of higher local richness is increased competition for resources, plants growing in species-rich surroundings might produce narrower growth rings, because they may invest more in aboveground than belowground structures to reduce light competition (Goldberg *et al.*, 2017). In the *Biodiversity Exploratories,* as in other agriculturally managed grasslands, species richness declines with increasing productivity at the community level (Socher *et al.*, 2012), which could point to intensified competition for belowground resources additionally hampering secondary growth. Furthermore, we cannot exclude that fast-growing large *Galium* individuals with wide rings simply occupied more space in their close vicinity and thus were associated with lower species richness at the local level.

Our finding that most differences in secondary growth of *G. mollugo* occur between individuals from the same plot, rather than between plots or regions, underscores the importance of microsite conditions on ring width in this species. Similar findings about the importance of local plant-plant interactions on ring width have been shown in forest trees responding to stand density (Ahmed *et al.*, 2024). For a deeper understanding of the influential drivers of ring width, it would be necessary to closely examine growth conditions both above and below ground in close proximity to plant individuals. This could include traits related to resource competition of neighbouring plants or nutrient or water availability in the root growth zone. In summary, our results imply that anthropogenic land use with its global trend of intensification and climate change with higher temperatures and reduced precipitation during the growing season can be critical aspects for secondary growth in herbaceous plants. It remains to be tested to what extent the effects on secondary growth caused by global change are transferred to the performance of individual plants and how different species respond to these drivers to assess to what extent entire plant communities might be affected.

Supplementary data

Supplementary data are available and consist of the following

Table S1: Overview of all modelled variables

Table S2: Descriptive statistics of the *G. mollugo* samples

Table S3: Log likelihood ratios and AICs of the generalized additive mixed models testing effects of land-use intensity and timing, climate, soil and diversity-related variables

Figure S1: Histograms showing the distribution of the intensity of the three different land use types, the combined land use intensity and the management intensity

Figure S2: Bargraph of the timing of the first land management activity

Figure S3: Correlation plot showing correlation values between all modelled variables

Figure S4: Bootstrap estimates variances of the different spatial resolutions

Literature cited

Ahmed S, Hilmers T, Uhl E, *et al.* 2024. Neighborhood competition modulates the link between crown structure and tree ring variability in monospecific and mixed forest stands. *Forest Ecology and Management,* 560. doi: 10.1016/j.foreco.2024.121839

Ayasse M, Fischer M, Weisser W, *et al.* 2022. *Land use in grasslands: raw data of yearly owner interviews.* Version 49. Biodiversity Exploratories Information System. Dataset ID= 26487.

Bär A, Pape R, Bräuning A, Löffler J. 2008. Growth-ring variations of dwarf shrubs reflect regional climate signals in alpine environments rather than topoclimatic differences. *Journal of Biogeography,* 35: 625-636. doi: 10.1111/j.1365-2699.2007.01804.x

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software,* 67: 1-48. doi: 10.18637/jss.v067.i01

Barton K. 2024. *MuMIn: Multi-Model Inference.* R package version 1.48.4. https://CRAN.R-project.org/package=MuMIn.

Bengtsson J, Bullock JM, Egoh B, *et al.* 2019. Grasslands—more important for ecosystem services than you might think. *Ecosphere,* 10: e02582. doi: 10.1002/ecs2.2582

Blüthgen N, Dormann CF, Prati D, *et al.* 2012. A quantitative index of land-use intensity in grasslands: Integrating mowing, grazing and fertilization. *Basic and Applied Ecology,* 13: 207-220. doi: 10.1016/j.baae.2012.04.001

Bolliger R, Prati D, Fischer M, Hölzel N. 2020. *Vegetation Records for Grassland EPs, 2008 - 2019.* Version 2. Biodiversity Exploratories Information System. Dataset ID= 26106. https://www.bexis.uni-jena.deddm/data/Showdata/26106?version=2

Bundesamt für Naturschutz. 2022. *FloraWeb.* https://www.floraweb.de/xsql/taxoquery.xsql?taxname=galium+mollugo&submit.x=0&submit.y=0&max-rows=10&skip-rows=0 (08.04.2022).

Campelo F. 2012. *detrendeR: Start the detrendeR Graphical User Interface (GUI).* R package version 1.0.4. https://CRAN.R-project.org/package=detrendeR.

Ciais P, Reichstein M, Viovy N, *et al.* 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature,* 437: 529-33. doi: 10.1038/nature03972

De Boeck HJ, Bassin S, Verlinden M, Zeiter M, Hiltbrunner E. 2016. Simulated heat waves affected alpine grassland only in combination with drought. *New Phytologist,* 209: 531-41. doi: 10.1111/nph.13601

De Boeck HJ, Lemmens CMHM, Zavalloni C, *et al.* 2008. Biomass production in experimental grasslands of different species richness during three years of climate warming. *Biogeosciences,* 5: 585–594. doi: 10.5194/bg-5-585-2008

Dee JR, Adams HD, Palmer MW. 2018. Belowground annual ring growth coordinates with aboveground phenology and timing of carbon storage in two tallgrass prairie forb species. *American Journal of Botany,* 105: 1975-1985. doi: 10.1002/ajb2.1198

Dee JR, Palmer MW. 2016. Application of herb chronology: Annual fertilization and climate reveal annual ring signatures within the roots of US tallgrass prairie plants. *Botany,* 94: 277-288. doi: 10.1139/cjb-2015-0217

Dee JR, Palmer MW. 2019. Utility of herbaceous annual rings as markers of plant response to disturbance: A case study using roots of a common milkweed species of the US tallgrass prairie. *Tree-Ring Research,* 75: 14-24. doi: 10.3959/1536-1098-75.1.14

Dee JR, Stambaugh MC. 2019. A new approach towards climate monitoring in Rocky Mountain alpine plant communities: A case study using herb-chronology and Penstemon whippleanus. *Arctic, Antarctic, and Alpine Research*, 51: 84-95. doi: 10.1080/15230430.2019.1585173

Dietz H, Fattorini M. 2002. Comparative analysis of growth rings in perennial forbs grown in an Alpine restoration experiment. *Annals of Botany,* 90: 663-8. doi: 10.1093/aob/mcf247

Dietz H, Ullmann I. 1997. Age-determination of dicotyledonous herbaceous perennials by means of annual rings: Exception or rule? *Annals of Botany,* 80: 377-379. doi: 10.1006/anbo.1997.0423

Dietz H, von Arx G. 2005. Climatic fluctuation causes large-scale synchronous variation in radial root increments of perennial forbs. *Ecology,* 86: 327-333. doi: 10.1890/04-0801

Dietz H, von Arx G, Dietz S. 2004. Growth increment patterns in the roots of two alpine forbs growing in the center and at the periphery of a snowbank. *Arctic, Antarctic, and Alpine Research,* 36: 591-597. doi: 10.1657/1523-0430(2004)036[0591:Gipitr]2.0.Co;2

Doležal J, Altman J, Jandová V, *et al.* 2022. Climate warming and extended droughts drive establishment and growth dynamics in temperate grassland plants. *Agricultural and Forest Meteorology,* 313. doi: 10.1016/j.agrformet.2021.108762

Doležal J, Dvorsky M, Börner A, Wild J, Schweingruber FH. 2018. *Anatomy, Age and Ecology of High Mountain Plants in Ladakh, the Western Himalaya* Cham: Springer.

Doležal J, Jandova V, Macek M, *et al.* 2020. Climate warming drives Himalayan alpine plant growth and recruitment dynamics. *Journal of Ecology,* 109: 179-190. doi: 10.1111/1365-2745.13459

Dong Y, Li Z, Keyimu M, *et al.* 2022. A comparative analysis of the hydraulic strategies of non-native and native perennial forbs in arid and semiarid areas of China. *Forests,* 13. doi: 10.3390/f13020193

Durka W, Michalski SG, Höfner J, *et al.* 2025. Assessment of genetic diversity among seed transfer zones for multiple grassland plant species across Germany. *Basic and Applied Ecology,* 84: 50-60. doi: 10.1016/j.baae.2024.11.004

Fischer M, Bossdorf O, Gockel S, *et al.* 2010. Implementing large-scale and long-term functional biodiversity research: The Biodiversity Exploratories. *Basic and Applied Ecology,* 11: 473-485. doi: 10.1016/j.baae.2010.07.009

Fritts HC. 1966. Growth-rings of trees: their correlation with climate. *Science,* 157: 973 - 979. doi: 10.1126/science.154.3752.973

Gärtner H, Schweingruber FH. 2013. *Microscopic Preparation Techniques for Plant Stem Analysis.* Remagen-Oberwinter: Verlag Dr. Kessel.

Goldberg DE, Martina JP, Elgersma KJ, Currie WS. 2017. Plant size and competitive dynamics along nutrient gradients. *The American Naturalist,* 190: 229-243. doi: 10.1086/692438

Hänsel F, Forteva S, Wöllauer S, Nauss T. 2024. *Open Climate Data of the Exploratories Project.* Version 6. Biodiversity Exploratories Information System. Dataset ID= 24766. https://www.bexis.uni-jena.de/ddm/data/Showdata/24766?version=6

Hari V, Rakovec O, Markonis Y, Hanel M, Kumar R. 2020. Increased future occurrences of the exceptional 2018-2019 Central European drought under global warming. *Scientific Reports,* 10: 12207. doi: 10.1038/s41598-020-68872-9

Hautier Y, Niklaus PA, Hector A. 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science,* 324: 636-8. doi: 10.1126/science.1169640

Hejcman M, Hejcmanová P, Pavlů V, Beneš J. 2013. Origin and history of grasslands in Central Europe - a review. *Grass and Forage Science,* 68: 345-363. doi: 10.1111/gfs.12066

IPCC. 2021. *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. doi:

Jäger EJ, Müller F, Ritz CM, Welk E, Wesche K. 2013. *Rothmaler Exkursionsflora von Deutschland Gefäßpflanzen: Atlasband*: Springer Spektrum Berlin, Heidelberg.

Jandt U, Bruelheide H, Jansen F, *et al.* 2022. More losses than gains during one century of plant biodiversity change in Germany. *Nature,* 611: 512-518. doi: 10.1038/s41586-022-05320-w

Klimešová J, Doležal J, Šťastná P. 2013. Growth of the alpine herb *Rumex alpinus* over two decades: effect of climate fluctuations and local conditions. *Plant Ecology,* 214: 1071-1084. doi: 10.1007/s11258-013-0232-8

Korell L, Andrzejak M, Berger S, *et al.* 2024. Land use modulates resistance of grasslands against future climate and inter-annual climate variability in a large field experiment. *Global Change Biology,* 30: e17418. doi: 10.1111/gcb.17418

Kowalski K, Senf C, Okujeni A, Hostert P. 2024. Large-scale remote sensing analysis reveals an increasing coupling of grassland vitality to atmospheric water demand. *Global Change Biology,* 30: e17315. doi: 10.1111/gcb.17315

Krendl F. 1967. Cytotaxonomie der Galium mollugo-Gruppe in Mitteleuropa (Zur Phylogenie der Gattung Galium, VIII.). *Österreichische Botanische Zeitschrift,* 114: 508-549.

Lenth RV. 2022. *emmeans: Estimated Marginal Means, aka Least-Squares Means.* R package, version 1.8.2. https://CRAN.R-project.org/package=emmeans.

Liu Y-B, Zhang Q-B. 2010. Effect of climate on the growth of annual rings in the main roots of perennial forbs in an Inner Mongolian semi-arid grassland, China. *Journal of Vegetation Science,* 21: 899-907. doi: 10.1111/j.1654-1103.2010.01199.x

Mersereau D, DiTommaso A. 2003. The biology of Canadian weeds. 121. *Galium mollugo* L. *Canadian Journal of Plant Science,* 83: 453–466. doi: 10.4141/P01-152

Mörchen G. 1965. Wuchsformen einheimischer Rubiaceen. *Hercynia,* 2: 352-370. doi: 10.25673/93633

Obermeier WA, Lehnert LW, Ivanov MA, Luterbacher J, Bendix J. 2018. Reduced summer aboveground productivity in temperate C3 grasslands under future climate regimes. *Earth's Future,* 6: 716-729. doi: 10.1029/2018ef000833

Olano JM, Almería I, Eugenio M, von Arx G, Tjoelker M. 2013. Under pressure: how a Mediterranean high-mountain forb coordinates growth and hydraulic xylem anatomy in response to temperature and water constraints. *Functional Ecology,* 27: 1295-1303. doi: 10.1111/1365-2435.12144

Ostrowski A, Lorenzen K, Petzold E, Schindler S. 2020. *Land use intensity index (LUI) calculation tool of the Biodiversity Exploratories project for grassland survey data from three different regions in Germany since 2006, BEXIS 2 module*. Version v2.0.0. doi: 10.5281/zenodo.3865579

Pavlu V, Hejcman M, Pavlu L, Gaisler J. 2007. Restoration of grazing management and its effect on vegetation in an upland grassland. *Applied Vegetation Science*, 10: 375-382. doi: 10.1111/j.1654-109X.2007.tb00436.x

Pinheiro JC, Bates D. 2000. *Mixed-Effects Models in S and S-PLUS.* NY: Springer New York.

Poschlod P, WallisDeVries MF. 2002. The historical and socioeconomic perspective of calcareous grasslands - lessons from the distant and recent past. *Biological Conservation*, 104: 361-376. doi: 10.1016/S0006-3207(01)00201-4

R Core Team. 2022. *R: A language and environment for statistical computing.* Version 4.2.2.

Rai S, Breme N, Jandova V, *et al.* 2024. Growth dynamics and climate sensitivities in alpine cushion plants: insights from *Silene acaulis* in the Swiss Alps. *Alpine Botany*. doi: 10.1007/s00035-024-00318-8

Regionaler Klimaatlas Deutschland. 2022. *Regionaler Klimaatlas Deutschland*. https://www.regionaler-klimaatlas.de/klimaatlas/2071-2100/jahr/durchschnittliche-temperatur/sachsen-anhalt/mittlereanderung.html (21.06.2022).

Roeder A, Roscher C. 2024. *Age and stem anatomical traits of Galium mollugo (collected between 2017-2019).* Version 11. Biodiversity Exploratories Information System. Dataset ID= 31543. doi: 10.71615/bexis.31543-11

Roeder A, Schweingruber FH, Fischer M, Roscher C. 2017. Growth ring analysis of multiple dicotyledonous herb species—A novel community-wide approach. *Basic and Applied Ecology,* 21: 23-33. doi: 10.1016/j.baae.2017.05.001

Roscher C, Roeder A. 2024. *Vegetation records (neighboring species) around focal individuals of four common forb species collected for analyses of age and stem anatomical traits in the grassland EP`s (collected between 2017-2019).* Version 6. Biodiversity Exploratories Information System. Dataset ID= 31362. https://www.bexis.uni-jena.de

Schöning I. 2023. *Soil carbon and nitrogen concentrations - soil sampling campaign 2017, all experimental plots (EPs), 0-10 cm.* Version 14. Biodiversity Exploratories Information System. Dataset ID= 23846. https://www.bexis.uni-jena.de/ddm/data/Showdata/23846?version=14

Schöning I. 2024. *Soil pH - soil sampling campaign 2011, all experimental plots (EPs), 0-10 cm.* Version 9. Biodiversity Exploratories Information System. Dataset ID= 14447. https://www.bexis.uni-jena.de/ddm/data/Showdata/14447?version=9

Schöning I, Solly E, Klötzing T, Trumbore S, Schrumpf M. 2021. *MinSoil 2011 - Soil Texture* Version 10. Biodiversity Exploratories Information System. Dataset ID= 14686. https://www.bexis.uni-jena.deddm/data/Showdata/14686?version=10

Shi S, Li Z, Wang H, *et al.* 2016. Roots of forbs sense climate fluctuations in the semi-arid Loess Plateau: Herb-chronology based analysis. *Scientific Reports,* 6: 28435. doi: 10.1038/srep28435

Socher SA, Prati D, Boch S, *et al.* 2013. Interacting effects of fertilization, mowing and grazing on plant species diversity of 1500 grasslands in Germany differ between regions. *Basic and Applied Ecology,* 14: 126-136. doi: 10.1016/j.baae.2012.12.003

Socher SA, Prati D, Boch S, *et al.* 2012. Direct and productivity-mediated indirect effects of fertilization, mowing and grazing on grassland species richness. *Journal of Ecology,* 100: 1391-1399. doi: 10.1111/j.1365-2745.2012.02020.x

Stoffel MA, Nakagawa S, Schielzeth H. 2017. rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods in Ecology and Evolution,* 8: 1639-1644. doi: 10.1111/2041-210x.12797

Strock CF, Lynch JP. 2020. Root secondary growth: an unexplored component of soil resource acquisition. *Annals of Botany,* 126: 205-218. doi: 10.1093/aob/mcaa068

Tälle M, Deák B, Poschlod P, Valkó O, Westerberg L, Milberg P. 2016. Grazing vs. mowing: A meta-analysis of biodiversity benefits for grassland management. *Agriculture, Ecosystems & Environment,* 222: 200-212. doi: 10.1016/j.agee.2016.02.008

Vermeire LT, Waterman RC, Reinhart KO, Rinella MJ. 2023. Grazing intensity and seasonality manipulate invasive annual grasses and native vegetation. *Rangeland Ecology & Management,* 90: 308-313. doi: 10.1016/j.rama.2023.04.001

Vogt J, Klaus VH, Both S, *et al.* 2019. Eleven years' data of grassland management in Germany. *Biodivers Data J,* 7: e36387. doi: 10.3897/BDJ.7.e36387

von Arx G, Archer SR, Hughes MK. 2012. Long-term functional plasticity in plant hydraulic architecture in response to supplemental moisture. *Annals of Botany,* 109: 1091-100. doi: 10.1093/aob/mcs030

von Arx G, Dietz H. 2005. Automated image analysis of annual rings in the roots of perennial forbs. *International Journal of Plant Sciences,* 166: 723-732. doi: 10.1086/431230

von Arx G, Edwards PJ, Dietz H. 2006. Evidence for life history changes in high-altitude populations of three perennial forbs. *Ecology,* 87: 665-74. doi: 10.1890/05-1041

Weigl E. 2017. *RADOLAN: Radar Online Adjustment Radar based quantitative precipitation estimation products*. doi:

Wood SN. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society (B)*, 73: 3-36. doi: 10.1111/j.1467-9868.2010.00749.x

Wood SN. 2017. *Generalized Additive Models - An Introduction with R.*2 ed. New York: CRC/Taylor&Francis.

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM. 2009. *Mixed effects models and extensions in ecology with R.* New York: Springer

Funding

The work was supported by the DFG Priority Program 1374 "Biodiversity Exploratories" (RO2397/9).

Data availability statement

The data is available in the Biodiversity Exploratories Information System (BEXIS; https://www.bexis.uni-jena.de/ddm/publicsearch/ and https://www.bexis.uni-jena.de/tcd/PublicClimateData/Index, id no. of the used data sets: 14686, 14447, 23846, 24766, 26106 (all public available), 26478 (not publicly available), 31362 and 31543, which both will be made public upon publication of the manuscript.

Author contributions

C.R. and A.R. planned the study and performed the field and laboratory work, A.R. analysed the microsections. M.T.J. analysed the data and wrote the manuscript with substantial support from C.R.

Acknowledgments

We thank Hans Schönewolf and Katharina Franke for support during field work, and Gabriele Rada (iDiv) for designing Figure 1. We thank the managers of the three Exploratories, Kirsten

Reichel-Jung, Iris Steitz, Sandra Weithmann, Florian Staub, Juliane Vogt and Miriam Teuscher and all former managers for their work in maintaining the plot and project infrastructure; Christiane Fischer and Jule Mangels for giving support through the central office, Michael Owonibi and Andreas Ostrowski for managing the central data base, and Markus Fischer, Eduard Linsenmair, Dominik Hessenmöller, Daniel Prati, Ingo Schöning, François Buscot, Ernst-Detlef Schulze, Wolfgang W. Weisser and the late Elisabeth Kalko for their role in setting up the Biodiversity Exploratories project. We thank the administration of the Hainich national park, the UNESCO Biosphere Reserve Swabian Alb and the UNESCO Biosphere Reserve Schorfheide-Chorin as well as all land owners for the excellent collaboration. Field work permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg.

Figure captions

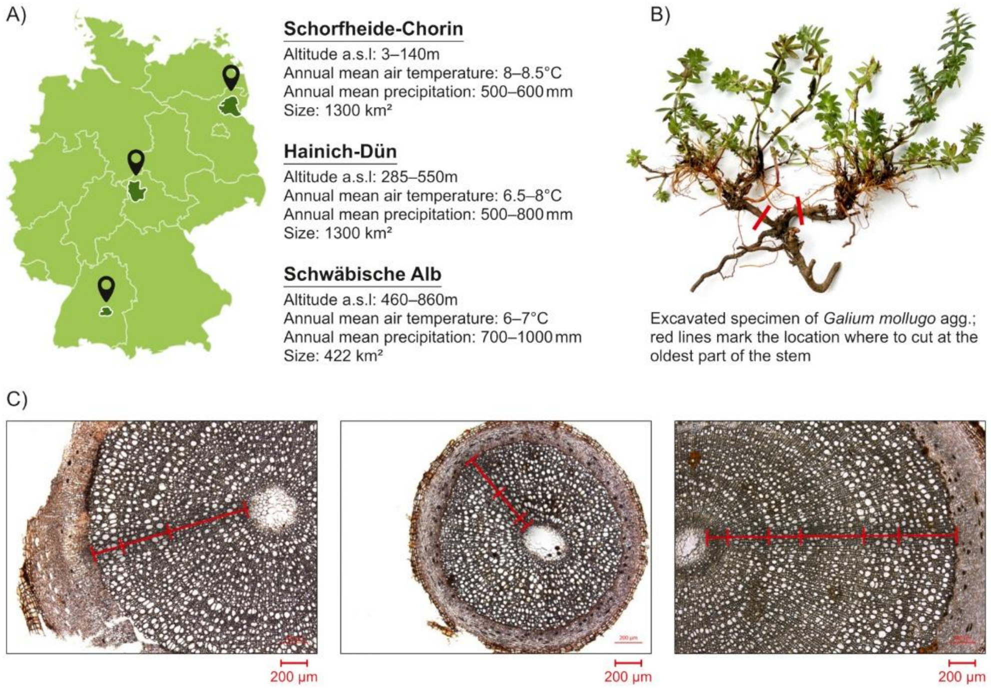

Figure 1: A) Location and characteristics of the three study regions, B) excavated *G. mollugo* individual, and C) exemplary microscopic images of stem cross sections of *G. mollugo* showing three different individuals with different rings width and age; demarcation of annual rings is marked with red horizontal lines along the examined radii. Source of the map shown in (A): picoStudio/stock.adobe.com.

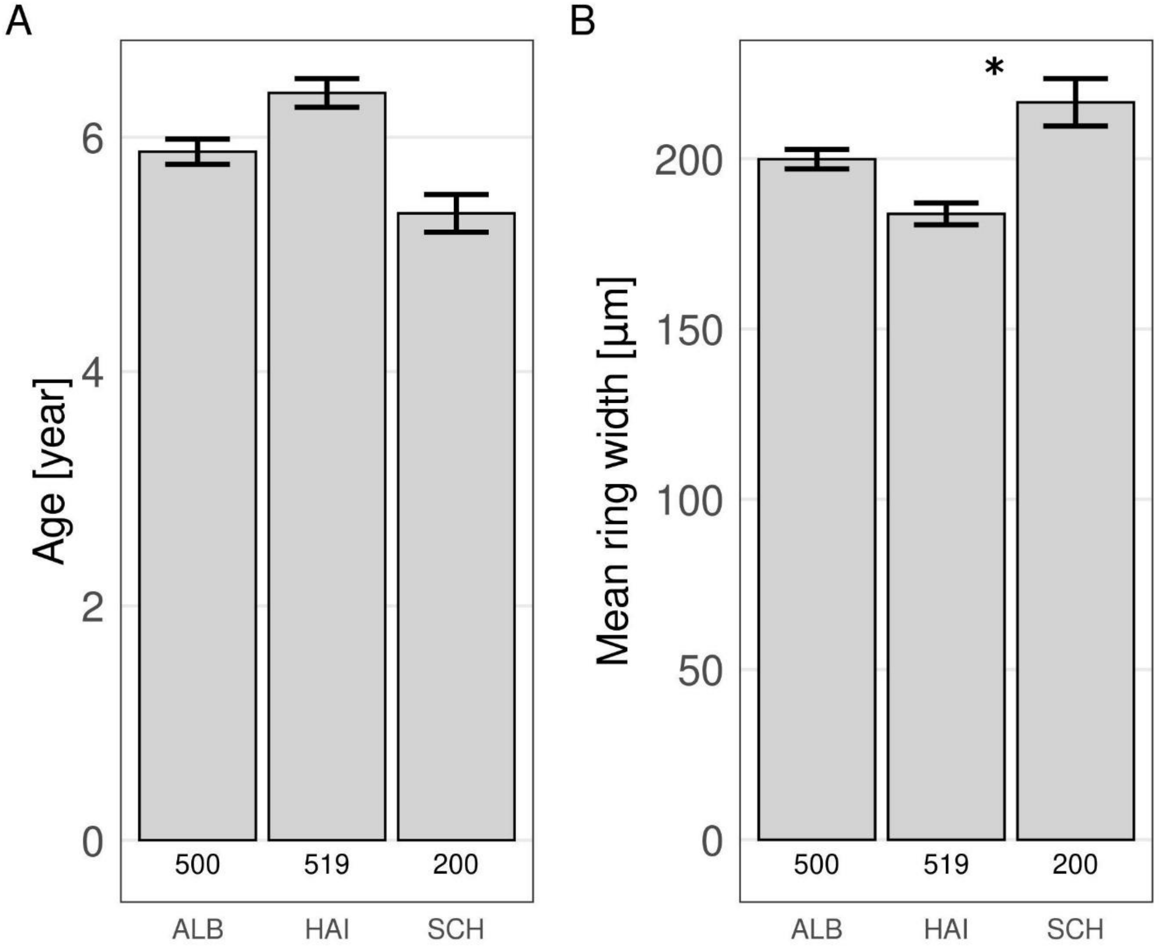

Figure 2. Descriptive statistics computed with RwlInfo separated by region (ALB = *Schwäbische Alb*, HAI = *Hainich-Dün*, SCH = *Schorfheide-Chorin*). A) Age calculated as number of growth rings counted. B) Mean growth ring width. All values are means (± 1 SE) across all samples per region. The numbers show the sample size per region. The asterisks shows significant differences ($p < 0.05$) between regions tested with Tukey's test. If no asterisk is shown there is no significant difference. One sampled individual in *Hainich-Dün* had no ring width measured and was therefore deleted from the data set.

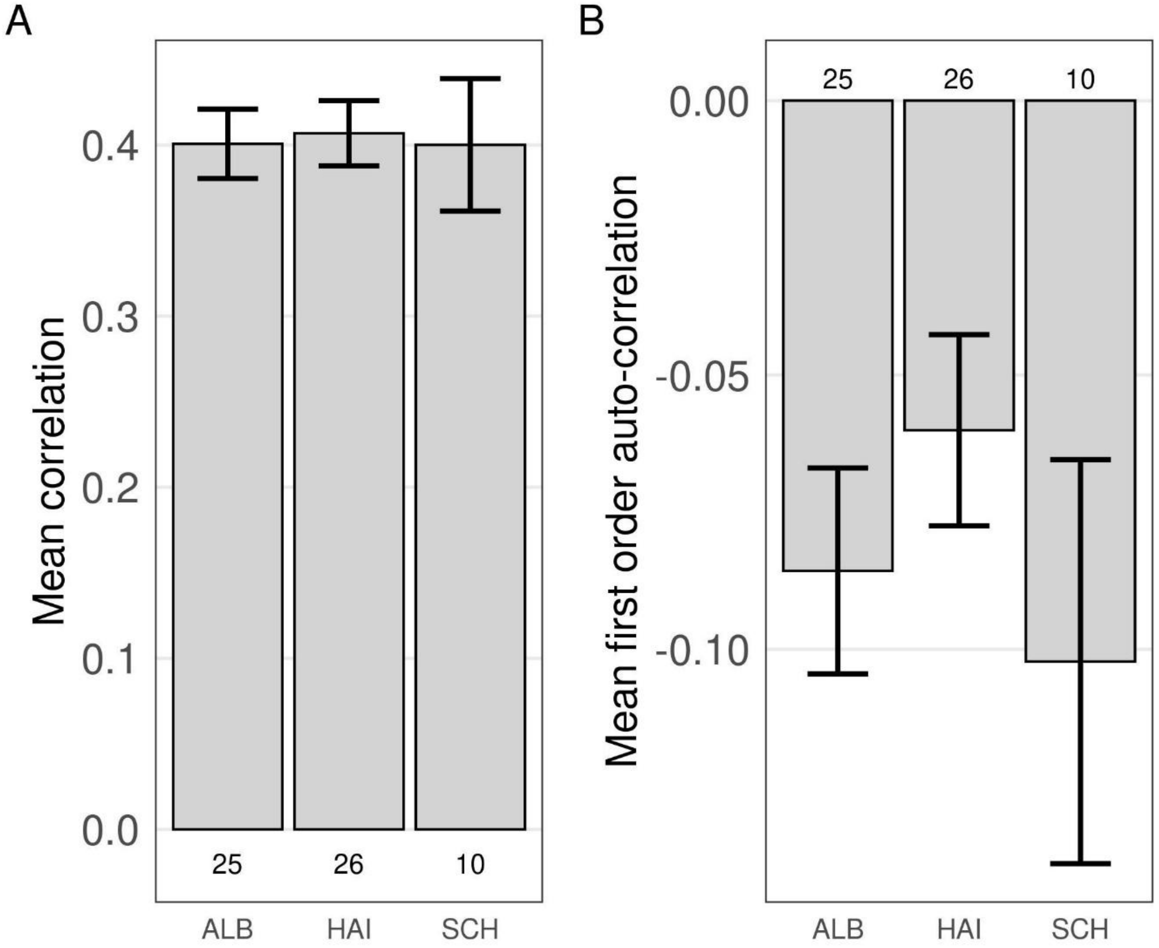

Figure 3. Descriptive statistics computed with RwlInfo separated by region (ALB = *Schwäbische Alb*, HAI = *Hainich-Dün*, SCH = *Schorfheide-Chorin*). A) Correlation of ring widths with the ring width of the master chronology (calculated from the mean ring width per year from all individuals per plot as the master chronology per plot) B) Mean first order autocorrelation calculated as the correlation between the current and the previous ring width. All values are means (± 1 SE) across all samples per plot and region. The numbers show the plot sample size per region. Note that the numbers differ from the numbers in Fig. 1 because of individual level and plot level calculations. There is no statistically significant difference between any of the measurements per region.

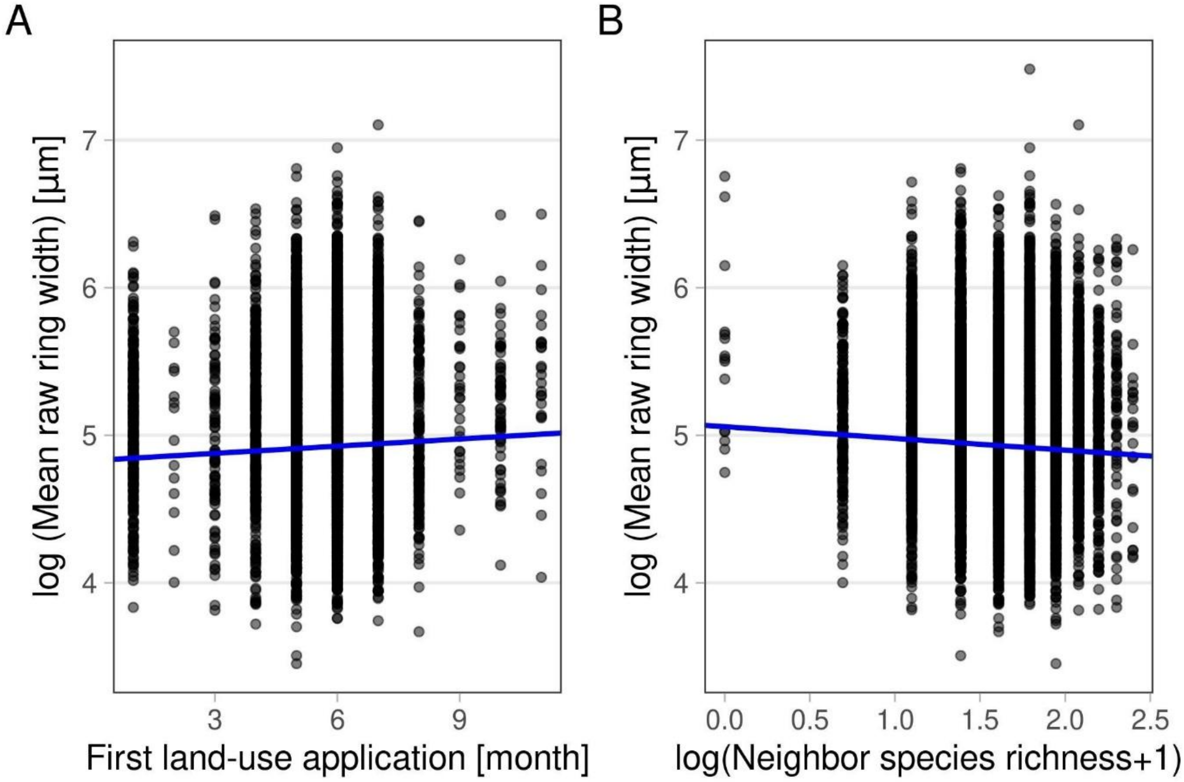

Figure 4. Log-transformed mean ring width in relation to A) first application time of land-use (the numbers are modelled ordinally) ($R^2_m = 0.03$, $R^2_c = 0.52$), B) log transformed neighbour species richness ($R^2_m = 0.02$, $R^2_c = 0.52$). The blue lines represent a significant regression line derived from fits to the model with age. The data shown represent the years 2009-2019.

Table 1. Results of the stepwise forward modelling process resulting in the final model. All models had the response variable log transformed ring width, the random effect sample nested in plot nested in region and the temporal autocorrelation structure of order 1 with the grouping factor sample nested in plot nested in region. The base model included plant age modelled as a smoothed term to allow for non-linear ontogenetic effects of age. Model performance was evaluated based on a combination of delta loglikelihood values, significant p-values and AICs. [a] log transformed data. Significant effects ($P < 0.05$) are printed in bold, marginally significant effects ($P < 0.1$) are printed in italic.

Step	Term added	ΔLogLik	P	AIC
0	Base model with plant age	-	-	8595.05
1	First land-use application	113.35	**<0.001**	8302.29
2	Summer soil temperature	49.11	**<0.001**	8175.82
3	Spring soil temperature	18.88	**<0.001**	8117.36
4	Summer air temperature	4.67	**<0.001**	8090.93
5	Summer soil moisture	2.91	*0.06*	8089.41
6	Neighbour species richness [a]	0.0	**0.016**	8085.59

Table 2. Results of the final generalized additive mixed model combining the fixed factors which were significant after stepwise forward modelling starting with the best performing (based on AIC) model of the single factor models (Table S3 in Supplementary Information). Age was added using a smoothing term to account for a nonlinear ontogenetic effect. The response variable was log transformed ring width, random effects of the final model were sample nested in plot nested in region and a temporal autocorrelation structure of order 1 with the grouping factor sample nested in plot nested in region. The upper part of the table presents the parametric model results with estimates, standard error (SE) and t-value. The lower part of the table presents the results of the smoothed term with estimated degrees of freedom (edf), reference degrees of freedom (Ref. df) and F-value. [a] log transformed data. Significant effects ($P < 0.05$) are printed in bold, marginally significant effects ($P < 0.1$) are printed in italic.

	Across regions			
	Estimate	SE	t-value	P
Intercept	5.012	0.173	29.055	**<0.001**
First land-use application	0.016	0.005	3.524	**<0.001**
Summer soil temperature	0.001	0.007	0.192	0.848
Spring soil temperature	0.001	0.004	0.126	0.900
Summer air temperature	0.005	0.008	0.587	0.587
Summer soil moisture	0.002	0.001	1.517	*0.055*
Neighbour species richness [a]	-0.080	0.032	-2.843	**0.013**
	edf	Ref. df	F	P
s(Age)	7.094	7.904	92.88	**<0.001**