Running title: Regrowth ability in grassland herbs

Variation in regrowth ability in relation to land use intensity and predictability in three common grassland herbs

Anna Kirschbaum[1*], Oliver Bossdorf[1], JF Scheepens[1, 2]

[1] University Tübingen, Plant Evolutionary Ecology, Institute of Evolution and Ecology, Auf der Morgenstelle 5, 72076 Tübingen

[2] Goethe University Frankfurt, Plant Evolutionary Ecology, Institute of Ecology, Evolution and Diversity, Max-von-Laue-Str. 13, 60438 Frankfurt am Main

[*]Correspondence: Anna Kirschbaum
anna.kirschbaum@student.uni-tuebingen.de
Phone: +49 7071 2972669

Abstract

Aims

Plant populations in managed grasslands are subject to strong selection exerted by grazing, mowing and fertilization. Many previous studies showed that this can cause evolutionary changes in mean trait values, but little is known about the evolution of adaptive plant phenotypic plasticity in response to grassland management.

Methods

We conducted an outdoor common garden experiment to test if plants from more intensively mown and grazed sites showed an increased ability to regrow after biomass removal. We worked with three common plant species from temperate European grasslands, with seed material from 58 – 68 populations along gradients of land-use intensity, ranging from extensive (only light grazing) to very intensive use (up to four cuts per year).

Important findings

In two species, we found strong population-level variation in regrowth ability of fitness-related traits in response to a clipping treatment, which could reflect adaptation to land-use intensity. While the regrowth ability was unrelated to the land-use intensity of populations of origin, we found a relationship with the predictability of grassland management in *P. lanceolata* where plants experiencing more stable environmental conditions over the last 11 years showed stronger regrowth in reproductive biomass after clipping. In summary, grazing and mowing intensity apparently did not select for regrowth ability, but in some species, predictable heterogeneous environmental conditions created by land use may have caused its evolution.

Keywords: environmental heterogeneity, grazing, mowing, predictability, phenotypic plasticity

Introduction

Around 26 % of the earth's land surface is currently used as grassland in an agricultural context (Foley *et al.* 2011). These managed grasslands provide multiple ecosystem services from provisioning of food and habitat to water purification and recreation (Carlier *et al.* 2009; Gossner *et al.* 2016). In the European temperate zone, grasslands are mainly used as pastures for livestock grazing, as meadows for haymaking or as a combination of both. Thus, they have a high economic and societal value (Heidenreich 2009).

A major aspect of environmental variation in managed grasslands are the recurring disturbances exerted by mowing or grazing. Plants are sessile, and therefore, in order to survive, they need to be able to adapt to these disturbances. The effects of land-use intensity on the evolution of plant traits have received increasing attention during the last decades, and previous studies have repeatedly demonstrated genetically based trait changes in morphology, physiology and phenology in response to grassland management. In general, grazing and mowing appear to select for decreased plant size, tolerance to damage, and phenological shifts (Warwick and Briggs 1979; Louault *et al.* 2005; Díaz *et al.* 2007; Völler *et al.* 2017). Dwarf morphology and prostrate growth are generally thought to reflect avoidance strategies that reduce the proportion of biomass accessible to livestock or above mowing height (Kotanen and Bergelson 2000), whereas shifts in flowering phenology, oftentimes advanced flowering, are interpreted as "escapes in time" that ensure reproduction (Reisch and Poschlod 2009; Völler *et al.* 2017) despite recurrent biomass losses. Other strategies to avoid or tolerate grazing in semi-natural grasslands include decreased plant palatability, or changes in functional traits related to regrowth ability (Louault *et al.* 2005).

Common land-use practices not only change the means of environmental conditions, but they also create spatiotemporal heterogeneity (Suzuki 2008). Grazing and mowing in managed grasslands often vary in intensity, timing, duration and frequency (Wang *et al.* 2017) and can create spatially and temporally heterogeneous conditions. Grazing, in particular, creates spatial heterogeneity through selective grazing, trampling and patchy dung deposition. One mechanism for plants to respond to such heterogeneous habitat conditions is through phenotypic plasticity (Schlichting and Levin 1986; Valladares *et al.* 2007), the ability of a genotype to produce multiple phenotypes depending on the environmental conditions (Bradshaw 1965). Phenotypic plasticity could not only buffer against the effects of common land-use practices, but as a genetically controlled trait itself (Pigliucci 2005) it might also evolve when the strengths and patterns of environmental heterogeneity (i.e. land-use practices) differ among populations (Suzuki 2008). Generally, if phenotypic plasticity improves plant performance (i.e. fitness) across environments, it is considered adaptive and expected to evolve given sufficient genetic variation for plasticity (Relyea and Morin 2002; Van Kleunen and Fischer 2005; Pigliucci 2005). In heterogeneous environments where plants need to rapidly adjust their morphology, physiology or reproduction to maintain or improve fitness, plasticity of functional traits should be adaptive and thus evolve (Matesanz *et al.* 2010; Gianoli and Valladares 2012; Scheiner 2013). Many empirical studies have confirmed the importance of phenotypic plasticity for organisms to cope with environmental heterogeneity and ultimately global change (Matesanz *et al.* 2010). In homogeneous environments, in contrast, plasticity should not evolve, or should even be lost if greater plasticity is associated with fitness costs for an organism (Van Kleunen and Fischer 2005).

The ability of a plant to maintain relatively constant fitness under stressful conditions is called plant tolerance (Rejmánek 2000; Simms 2000; Barton 2013). In managed grasslands, a key tolerance trait is the ability to (partially) compensate for biomass loss through regrowth (Strauss and Agrawal 1999). Plants with a high tolerance to damage would show a rather flat reaction norm of fitness over a range of damage intensities (Rejmánek 2000; Simms 2000; Richards *et al.* 2006).This will likely be achieved through plasticity in some morphological or physiological traits that affect regrowth and thus fitness (Bradshaw 1965; Strauss and Agrawal 1999; Tiffin 2000), such as increased photosynthetic rates after stress (McNaughton 1979; Sultan *et al.* 1998; Strauss and Agrawal 1999) or the use of storage compounds for regrowth after damage (Oesterheld and McNaughton 1988; Strauss and Agrawal 1999).

So far, only few studies explored the extent of genetic variation in and evolution of plasticity in plant responses to grazing and mowing. Moreover, most previous studies compared only two contrasting environments, and they often remained limited in their levels of replication. For instance, Carman & Briske (1985) found that regrowth ability after clipping of plants from three grazed sites was greater than in plants from three non-grazed sites, suggesting selection of increased regrowth ability under recurrent biomass removal. Oesterheld & McNaughton (1988) found population differentiation in growth rate, tillering frequency and leaf morphology along a gradient of three grazing intensities in *Themeda triandra* in response to a clipping treatment. Yet**,** other studies found no differences in plasticity between land-use origins, e.g. Rotundo and Aguiar (2007) studied three *Poa ligularis* populations with different grazing intensity and history and found no differences in their responses to clipping. Similarly, Suzuki (2008) showed that *Persicaria longiseta* plants from one grazed population did not respond differently to clipping than those from two ungrazed populations. Given such studies with contrasting results and low population replication, larger and better replicated studies across multiple species are needed for more powerful tests of the effects of grassland management on the evolution of plasticity and its adaptive value.

Many previous studies on the evolution of phenotypic plasticity compared only two contrasting habitats. This however, might be misleading as intermediate environmental states are not considered (Kreyling *et al.* 2018). To overcome this, one could look at plastic responses along a gradient of an environmental condition. In the case of the Biodiversity Exploratories (see methods), grazing and mowing intensities have been measured continuously over the past 14 years on 150 grassland plots and is therefore ideally suited to investigate plant responses along a land-use gradient. As increasing management intensity represents increasing environmental heterogeneity, we would expect the strength of phenotypic plasticity to increase with increasing land-use intensity.

Here, we studied the evolution of regrowth ability in relation to grassland management in three temperate grassland plants. In a common garden experiment, we subjected plants from a broad range of land-use intensities to a standardized clipping treatment. Specifically, we asked the following questions: 1) Is there genetic variation for regrowth ability in the three studied plant species? If yes, is variation in regrowth ability associated with 2) land-use intensity, in particular with grazing and mowing intensity? and 3) inter-annual temporal variation in these land-use practices?

Material and methods

Study area

We worked in a system of grasslands plots located in three regions in Germany, embedded in the framework of the Biodiversity Exploratories, a large-scale and long-term project investigating the relationship between land use, biodiversity and ecosystem functioning (https://www.biodiversity-exploratories.de). The three regions – UNESCO Biosphere Reserve Schorfheide-Chorin, Hainich National Park and surrounding areas and UNESCO Biosphere Area Schwäbische Alb – span a north-south transect in Germany and are approximately 300 km apart from each other. In each of the three regions, there are 50 grassland plots at distances of a few hundred meters to 30-40 km (mean distance 13.4 km) (Fischer *et al.* 2010; Völler *et al.* 2017). The grassland plots, each with an area of 50 × 50 m, cover a land-use gradient from extensive to very intensive management based on different types (grazing, mowing, fertilization) and intensities of land use. For each plot, annual inventories record the mowing intensity as the number of cuts per year, and grazing intensity as livestock units per hectare, multiplied with the grazing period and weighted by livestock type (cattle, sheep, horse, goat) (Blüthgen *et al.* 2012; Vogt *et al.* 2019). To integrate land-use history and inter-annual variability, we used a long-term measure of grazing and mowing intensity from 2006-2016, i.e all available data before 2017, the year of our seed sampling.

Study species and seed collection

Between May and September 2017, we collected seeds from three common grassland species (Table 1) – *Achillea millefolium* L., *Plantago lanceolata* L., *Bromus hordeaceus* L. – from the plots of the Biodiversity Exploratories. We selected the species based on their frequent occurrence and high abundance in the plots. As the species differ in their timing of seed maturity, we visited all 150 grassland plots several times and collected ripe seeds from a maximum of 12 plant individuals on each plot where the species occurred. However, current land use, especially mowing, might have prevented sampling on every plot where the species occurred. We finally collected seeds of *A. millefolium* from 58 plots, of *B. hordeaceus* from 68 plots and of *P. lanceolata* from 63 plots. We generally chose individuals randomly but with at least 1 m distance between each. In the remainder of this paper, the seeds from one individual are referred to as seed families and all collected individuals from one plot as population. We dried all seeds at room temperature and stored them in paper bags at 4°C in the dark until further use.

Common garden experiment

In April 2018 we sowed seeds from seven seed families per population per species in cultivation trays (PL, TK series, Pöppelmann GmbH & Co KG Kunststoff-Werkzeugbau, Lohne) filled with a standard potting soil (Topferde CL T, Classic, Einheitserdewerke Werkverband e.V., Sinntal-Altengronau) and placed them in a shade house at the experimental station of the University of Tübingen. In the case of *P. lanceolata*, we stratified seeds prior to germination for two weeks at 4°C in the dark. Three weeks (five weeks for *P. lanceolata*) after sowing, we transplanted two seedlings from each of five to seven seed families, depending on germination success, per population in 1L pots (Ø 13 cm, Hermann Meyer KG, Langenau) filled with a sand-soil-mixture (2:1:1, Rheinsand, 0 - 2mm, Flammer Bauunternehmung GmbH & Co. KG, Mössingen : Pro Start, Geb. Brill Substrate GmbH & Co KG, Georgsdorf : Topferde CL T, Classic, Einheitserdewerke Werkverband e.V., Sinntal-Altengronau) and added an equivalent of 60 kg N ha^{-1} of a NPK slow release fertilizer (Osmocote Pro 5-6 M, 19-9-10 + 2MgO + SP, Herman Mayer KG, Langenau). We placed all pots on an individual saucer (Ø 15 cm, Herman Mayer KG, Langenau) in completely randomized order on an experimental field (Fig. S1) covered with weed-control fabric (PPX® 100 g/m^2 Ground Cover, Hermann Mayer KG, Langenau). After four weeks of growth, during which the plants were watered as needed but at least two times per week, we clipped half of the plants (one seedling per seed family) with pruning shears above the soil surface. After the clipping treatment, all plants grew for another 16 weeks with the same watering regime. In September 2018 we harvested the aboveground biomass of all plants, separated it into reproductive and vegetative biomass, dried it for four days at 70°C and weighed all samples. Reproductive biomass was defined as flowering stems and infloresences for *P. lanceolata*, flowering stems (without leaves) and inflorescences for *B. hordeaceus*, and only inflorescences for *A. millefolium*.

Statistical analyses

To balance our data for the statistical analyses, we only included seed families where both clipped and unclipped plants had survived the experiment. Therefore, the sample sizes for the statistical analyses were smaller than those in the experiment were (Table 1). To test for genetic variation in regrowth ability and treatment effects on plant performance, we fitted linear mixed effects models that included region, population, treatment and the interaction between population and treatment as fixed effects and seed family as a random effect. We analyzed each species separately, with total biomass at harvest – the sum of reproductive and vegetative biomass at harvest – as response variable for all three species, and additionally reproductive biomass at harvest for *P. lanceolata*.

To test for the effects of land use on regrowth ability, we first calculated an index of plasticity for each seed family as the log response ratio (LRR) of total biomass between clipped and unclipped plants. Because of zeroes in the reproductive biomass, we could not calculate an LRR of reproductive biomass for seed families in *P. lanceolata*, and we therefore first calculated the population means of clipped and unclipped plants and then the LRR of these two. Two populations of *P. lanceolata* where only clipped plants had reproduced were excluded from these analyses. To analyze the variation in LRR of total biomass, we fitted linear mixed models with region and land use as fixed effects and population as random effect. Land use in these models was either mowing intensity, or grazing intensity, or a compound variable of both further called total damage intensity, averaged across 11 years. Total damage intensity is calculated as the square-root of the sum of the standardized grazing and mowing intensities. Following the calculation of the land-use index by Blüthgen et al. (2012), we applied square-root transformation to minimize the effects of outliers and balance the distribution. The variation in LRR of reproductive biomass of *P. lanceolata* was analyzed with a simpler linear model with only region and land use as fixed effects.

In order to test for relationships between regrowth ability and temporal heterogeneity in land use, we first calculated the interannual variability of mowing and grazing intensity as the standard deviation of these land-use factors from 2006 to 2016, i.e. all recorded years before the year of our seed sampling. We calculated temporal heterogeneity of total damage intensity as the square root of the sum of the standard deviations of both mowing and grazing from 2006 to 2016. To test for effects of temporal heterogeneity on regrowth ability, we fitted linear mixed models with the LRR of total biomass as response variable, region and one of the measures of temporal heterogeneity in land use as fixed effects, and population as random effect. Again, the LRR of reproductive biomass of *P. lanceolata* was analyzed with simpler linear models that included only region and one of the measures of temporal heterogeneity in land use.

To ensure normality and homoscedasticity of model residuals we log-transformed total biomass and square-root-transformed reproductive biomass for all biomass analyses. For the analyses of LRR, residuals were generally normally distributed and homoscedastic without transformation.

All statistical analyses were done in R version 3.6.1 (R Core Team 2019), using in particular the *lme4* (Bates *et al.* 2015) and *lmerTest* (Kuznetsova *et al.* 2017) packages. When analyzing multiple species and land-use factors, we generally adjusted false discovery rates (FDR) following Benjamini-Hochberg (Benjamini and Hochberg 1995).

Results

Population differentiation

Populations of *A. millefolium*, *B. hordeaceus* and *P. lanceolata* significantly differed in their mean biomass (Table 2, Fig. 1). Moreover, populations of *A. millefolium* also differed in their regrowth response to the clipping treatment (population-by-treatment interaction in Table 2; Fig. 1a), suggesting genetic variation in regrowth ability. In contrast, we found no significant population by treatment interactions for *B. hordeaceus* and *P. lanceolata* (Table 2, Fig. 1b and c), indicating a lack of genetic variation in regrowth ability. However, populations of *P. lanceolata* differed significantly in their production of reproductive biomass as well as in the responses of their reproductive biomass to the clipping treatment (Table 2, Fig. 1d), indicating substantial genetic variation in regrowth ability.

Regrowth ability in response to land use

Regrowth ability in response to clipping, estimated as the log response ratio (LRR) of total biomass, or as the LRR of reproductive biomass in *P. lanceolata*, was uncorrelated to mowing intensity, grazing intensity, and total damage intensity in all three studied species (Table 3). The LRRs of total biomass were also unrelated to temporal heterogeneity of land use, but we found that the LRR of *P. lanceolata* reproductive biomass significantly correlated with temporal heterogeneity in mowing intensity (Table 3). With temporally more variable mowing regimes, the LRR of *P. lanceolata* reproductive biomass was significantly lower, i.e. populations were responding less plastically to the clipping treatment in our experiment (Fig. 2).

Discussion

Temperate grassland plants are regularly subjected to land-use practices such as mowing and grazing. Plenty of studies investigated the ecological und evolutionary effects of land use on mean trait variation. However, as grazing and mowing change the environmental conditions throughout the year and create spatially and temporal heterogeneous habitats, plants may also adapt via plasticity. Furthermore, land-use practices might also change between years, creating temporal variability, which could favor plasticity as well. In this study, we investigated if populations of three common European grassland species along a gradient of grassland management show differences in plasticity, specifically in their regrowth ability after biomass loss, and if these differences are related to mean grazing and mowing intensities or their temporal variability across years.

Population differentiation in regrowth ability

We found that grassland populations of *A. millefolium* exhibited genetic variation in regrowth of total biomass in response to a clipping treatment. This population differentiation in the ability to regrow could be caused by the land-use differences between populations and might be facilitated by insect pollination and short seed dispersal distances *of A. millefolium*, limiting gene flow among populations. We found no significant population differences in the total biomass responses of *B. hordeaceus* and *P. lanceolata* to the clipping treatment, indicating a lack of genetic variation in regrowth ability in these two species. In contrast, Bergelson and Crawley (1992) found site-specific differences in individual performance in *Ipomopsis aggregata* populations after a clipping treatment. Population differentiation in response to a clipping treatment was also found for root/shoot ratio and reproductive allocation in three tallgrass prairie species (Damhoureyeh and Hartnett 2002). However, three populations of *Persicaria longiseta* responded similarly to a clipping treatment in several traits, suggesting no variation in plasticity among those populations (Suzuki 2008).

In contrast to the lack of variation in regrowth ability of total biomass, we found significant population differences in regrowth of reproductive biomass in *P. lanceolata*. This observation of genetic variation for plasticity is rather unexpected, as *P. lanceolata* is an obligate outcrosser and wind-pollinated which together should lead to strong gene flow between populations and thus reduce the potential for population differentiation. However, several previous studies, including some from the same study regions, already demonstrated population differentiation in this species. Comparing *P. lanceolata* plants from a late- and early-mown meadow and a pasture, Van Tienderen (1991) showed local adaptation for seed yield, onset of flowering and growth habit. Genetically based population differentiation was also found for reproductive effort and components controlling seed yield between eight *P. lanceolata* populations in North Carolina (Primack and Antonovics 1981, 1982). Additionally, Gáspár et al. (2019) found significant genetic and epigenetic differentiation among populations of *P. lanceolata* from the Biodiversity Exploratories. Thus, genetic and phenotypic differentiation in geographically close populations of *P. lanceolata* are possible and may reflect adaptation to local environmental conditions.

Regrowth ability and mean land use intensities

Although we found heritable variation in regrowth ability in *A. millefolium* and *P. lanceolata* after biomass removal, there were no relationships between this variation and the mean mowing or grazing intensities of the populations of origin. Despite our prediction that increasing land-use intensity would result in the evolution of increased ability to regrow after biomass removal, we found no relationship between grazing or mowing and regrowth ability in this particular system. Similarly, *Persicaria longiseta*, with a long history of grazing, exhibited adaptation in mean values of fitness-related traits but did not show evolution of phenotypic plasticity in response to clipping in these traits (Suzuki 2008). In contrast, comparing grazed or mown with unmanaged populations of *Gentianella campestris*, increased regrowth ability evolved only in managed habitats (Lennartsson *et al.* 1997), suggesting selective potential of land use. Likewise, comparing populations of three tallgrass prairie species differing in grazing history, population differentiation in grazing tolerance in response to clipping was related to grazing history (Damhoureyeh and Hartnett 2002). In our experiment, management intensities varied from no mowing at all to around three times mowing per year and from no grazing to a year-round permanent pasture (averaged across 11 years), i.e. our study populations encompassed a rather broad range of management intensities in these types of temperate grasslands. However, looking at total damage, there are no populations that are not subject to recurring biomass removal, through either grazing or mowing. Although we are missing a true zero-point, we would have expected that the strength of the land-use gradient and the heterogeneous conditions this creates within a single growing season would have exerted a selective pressure strong enough to affect regrowth ability of our study species differentially. However, a recorded history of 11 years of land use might not be representative of historic management of these populations and might not represent the relevant evolutionary time scale. Nevertheless, as we found population variation in regrowth ability for some traits and species but no relationship with mean land use, other factors may have driven this population differentiation.

Regrowth ability and inter-annual land-use variation

Grassland management regimes might change across years, for instance when farmers alter the frequency of mowing or the stocking densities on pastures, creating not only heterogeneous environmental conditions within one year but also temporal heterogeneity in land use across years. We expected that regrowth ability of plant populations would increase with increasing temporal heterogeneity, but to our surprise, we found the opposite: regrowth ability for reproductive biomass of *Plantago lanceolata* was higher under temporally less variable mowing conditions. Generally, plasticity evolves under heterogeneous environmental conditions (Scheiner 2013) but is thought to be more advantageous in more predictable environments as plants need to be able to forecast future conditions accurately to benefit from plasticity (Scheiner 1993; Stuefer 1996; Alpert and Simms 2002; Lande 2009; Reed *et al.* 2010). In our study system, low temporal heterogeneity in mowing intensity means that farmers maintained the numbers of cuts per year over the last 11 years compared to high temporal heterogeneity where mowing intensity changed across years. Hence, a temporally less variable mowing regime creates a heterogenous but more predictable environment.

While the importance of environmental predictability for the evolution of plasticity has been well-demonstrated in theoretical models (Scheiner 1993; Jong 1999; Lande 2009), empirical tests are more scarce. For instance, plastic responses in allocation and fitness traits to nutrient stress were strongest in *Hordeum spontaneum* plants from a predictable Mediterranean habitat characterized by low inter-annual variation in precipitation (Volis *et al.* 2002). Concluding, theoretical and empirical results, including those from our study on land-use management, highlight the importance of heterogeneous but predictable environmental conditions for the evolution of plasticity.

The predictability of environmental cues may also help to explain our different results regarding mowing versus grazing. Mowing usually affects the whole population equally and creates spatially predictable environmental conditions. It might therefore be a reliable cue for initializing regrowth at the population level. Grazing, in contrast, is spatially patchy and does not affect the population equally (Völler *et al.* 2017) but usually creates spatial heterogeneity and might therefore be much less predictable at the population level. In our study, only temporal heterogeneity in mowing intensity (but not grazing intensity) was related to differential regrowth ability after biomass loss. Hence, unpredictable environmental conditions, either within one year or across several years could hamper selection on regrowth ability in response to a land-use gradient. Furthermore, in our study system some grassland plots are managed with a combination of both mowing and grazing, which might have added additional complexity, as the different cues might interfere with each other and create unpredictable conditions. Altogether, we propose that the predictability of biomass removal may be a key factor determining whether population differentiation in regrowth ability will evolve in response to land use, because only reliable cues allow to infer future environmental conditions from current ones (Reed *et al.* 2010).

In general, reproductive biomass is a more representative fitness measure than vegetative biomass. Therefore, it might not be surprising that we only found a correlation of one land-use element – mowing – with reproductive biomass of *P. lanceolata* as this might be the biomass component selection acts on. To lose unripe seeds, that have received a substantial amount of resources but are still unable to germinate, in a mowing or grazing event is much more costly for a plant than losing only a part of its vegetative biomass. Unfortunately, the other two species produced too few flowering individuals to perform statistical analysis on their reproductive output. Since *B. hordeaceus* is an annual species, the experiment apparently did not provide minimal conditions to fulfil its life cycle. This result therefore reflects a discrepancy between field and experimental conditions, which may also have affected plant responses to clipping.

Conclusions

Land use in grasslands creates environmental heterogeneity, which should affect the evolution of phenotypic plasticity in plants. So far, to our knowledge, no previous study explored the effects of inter- and intra-annual heterogeneity in land-use intensity on the evolution of plasticity. We studied plasticity in response to biomass removal in three common grassland plants from 58 – 68 populations and found genetic variation in regrowth ability in two of the three species. While the land-use gradient *per se* was unrelated to variation in regrowth ability, we showed that inter-annual temporal predictability was significantly related to regrowth ability in one of the studied species. Thus, our data suggest that inter- rather than intra-annual variability affected the evolution of regrowth ability in this system. We need more long-term data on temporal land-use variation together with common garden comparisons of the resident populations to test this hypothesis more broadly.

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Table 1: The three plant species used in our study, with numbers of individuals and populations in the experiment and in the final statistical analyses.

	Achillea millefolium	*Bromus hordeaceus*	*Plantago lanceolata*
Plant family	Asteraceae	Poaceae	Plantaginaceae
Pollination type	Insects	Wind	Wind
Life cycle	Perennial	Annual	Perennial
# Individuals (experiment/analyses)	812 / 736	952 / 832	882 / 772
# Populations (experiment/analyses)	58 / 58	68 / 67	63 / 63

Table 2: Results of linear mixed effects models testing effects of region of origin, population, clipping treatment and the interaction of the latter two on the total biomass of *Achillea millefolium*, *Bromus hordeaceus* and *Plantago lanceolata* as well as the reproductive biomass of *P. lanceolata*. Bold *P*-values indicate effects that are significant ($P < 0.05$) after FDR correction.

	A. millefolium				*B. hordeaceus*				*P. lanceolata*				*P. lanceolata*			
	Total biomass				Total biomass				Total biomass				Reprod. biomass			
	Num d.f.	Den d.f.	F	P	Num d.f.	Den d.f.	F	P	Num d.f.	Den d.f.	F	P	Num d.f.	Den d.f.	F	P
Region	2	596	2.55	0.079	2	682	0.11	0.897	2	634	1.60	0.202	2	615	10.68	**<0.001**
Population	55	309	2.26	**<0.001**	64	348	1.89	**<0.001**	60	323	1.53	**0.011**	60	323	1.90	**<0.001**
Treatment	1	308	284.02	**<0.001**	1	347	414.46	**<0.001**	1	322	338.86	**<0.001**	1	323	6.67	**0.010**
Pop:Treat	57	308	1.37	**0.049**	66	347	0.83	0.823	62	322	1.04	0.414	62	322	1.95	**<0.001**

Table 3: Results of linear mixed effects models testing for relationships between land-use intensities (mowing, grazing, or both processes combined) and their temporal heterogeneities, and the regrowth abilities, i.e. phenotypic plasticity in response to clipping, of *Achillea millefolium*, *Bromus hordeaceus* and *Plantago lanceolata*. We estimated plasticity as the log response ratios (LRR) of total biomass for all three species, and as the LRR of reproductive biomass for *P. lanceolata*. All land-use intensities and their temporal heterogeneities are based on 11 years of data (2006-2016), with temporal heterogeneity calculated as the standard deviations of mowing and grazing intensity, respectively. Bold *P*-values indicate values significant at $P<0.05$) after FDR correction.

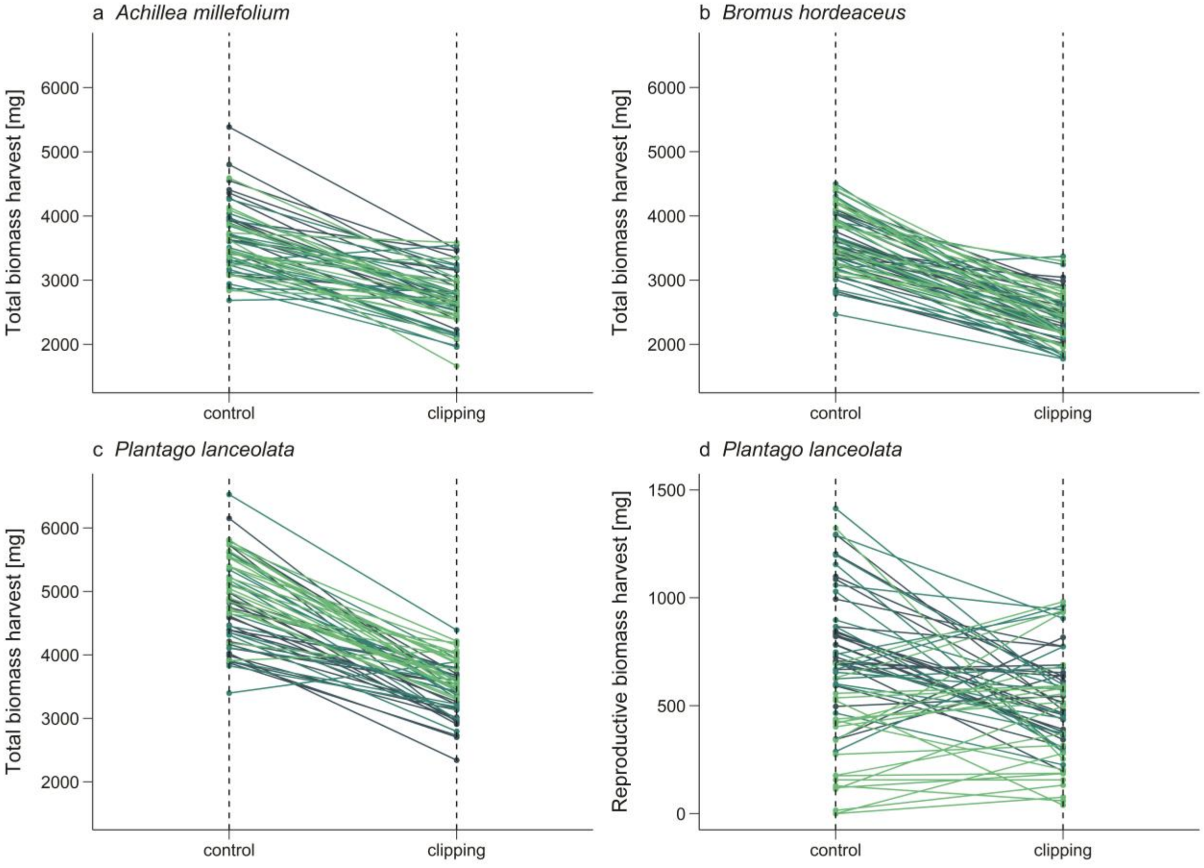
		A. millefolium LRR of total biomass			*B. hordeaceus* LRR of total biomass			*P. lanceolata* LRR of total biomass			*P. lanceolata* LRR of reprod. biomass	
	Num d.f.	Den d.f.	F	P	Den d.f.	F	P	Den d.f.	F	P	F	P
Region	2	54	1.80	0.175	400	1.91	0.149	376	1.06	0.349	3.51	0.036
Mowing intensity	1	55	0.10	0.753	400	0.82	0.365	376	0.07	0.791	0.05	0.817
Region	2	54	1.96	0.150	400	1.80	0.167	376	1.34	0.263	3.53	0.036
Grazing intensity	1	53	0.45	0.503	400	0.72	0.397	376	0.54	0.463	0.34	0.562
Region	2	54	1.85	0.167	400	1.49	0.226	376	1.20	0.301	3.52	0.036
Total damage intensity	1	53	0.46	0.500	400	0.24	0.621	376	0.40	0.529	0.23	0.631
Region	2	53	1.80	0.175	400	1.33	0.288	376	1.23	0.293	3.92	0.026
Temporal variation in mowing intensity	1	56	0.20	0.653	400	0.45	0.480	376	0.99	0.320	6.63	0.013
Region	2	54	2.50	0.091	400	1.77	0.172	376	1.40	0.248	3.55	0.035
Temporal variation in grazing intensity	1	52	2.75	0.103	400	0.66	0.419	376	0.81	0.369	0.60	0.442
Region	2	54	2.36	0.105	400	1.73	0.179	376	1.80	0.167	3.71	0.031
Temporal variation in total damage intensity	1	52	1.48	0.230	400	0.41	0.523	376	1.91	0.168	3.27	0.076

Figure legends

Figure 1: Reaction norms for total biomass of *Achillea millefolium* (a), *Bromus hordeaceus* (b) and *Plantago lanceolata* (c) and for reproductive biomass of *P. lanceolata* (d) in response to clipping (Schwäbische Alb in dark blue, Hainich in cyan, Schorfheide-Chorin in light green). Each reaction norm represents the mean values of five to seven seed families per population.

Figure 2: Relationship between the temporal land-use heterogeneity, calculated as the standard deviation of mowing frequencies across 11 years, and the plastic regrowth ability of 58 *Plantago lanceolata* populations, quantified as the log response ratio (LRR) of their reproductive biomass to experimental clipping (Schwäbische Alb in dark blue, Hainich in cyan, Schorfheide-Chorin in light green).

Figure 1: Reaction norms for total biomass of *Achillea millefolium* (A), *Bromus hordeaceus* (B) and *Plantago lanceolata* (C) and for reproductive biomass of *P. lanceolata* (D) in response to clipping (Schwäbische Alb in dark blue, Hainich in cyan, Schorfheide-Chorin in light green). Each reaction norm represents the mean values of five to seven seed families per population.



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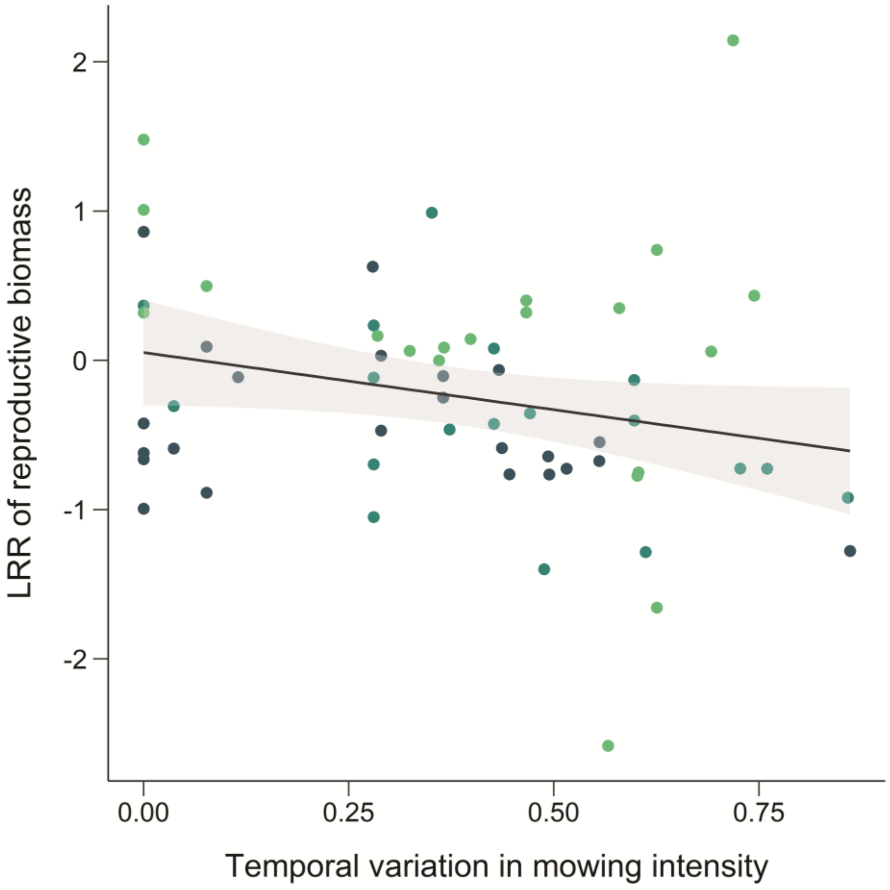

Supplementary material

Figure S1: Common garden at the experimental station of the University Tübingen. We put the plants on individual saucers in a complete randomized order on weed control fabric. We watered the plants as needed but at least two times per week.