1   Original Article

2

3   # Intraspecific variation in land use-related functional traits in

4   # *Plantago lanceolata*

5

6   Bence Gáspár[1,2]*, Oliver Bossdorf[1], Madalin Parepa[1]

7

8   *[1] Institute of Evolution and Ecology, University of Tübingen, Auf der Morgenstelle 5, 72070*

9   *Tübingen, Germany*

10   *[2] Department Community Ecology, Helmholtz-Centre for Environmental Research – UFZ,*

11   *Theodor-Lieser-Str. 4, 06120 Halle, Germany*

12

13   **Author for correspondence. Email: bence.gaspar@uni-tuebingen.de*

14

Abstract

16   • **Background and aims** Intraspecific variation in functional traits is essential for the evolutionary success of organisms. The co-variation between trait variation and environment, as well as between different traits, can help us to understand which ecological factors drive habitat adaptation, and to what extent adaptation may be constrained by trait correlations and trade-offs. In managed grasslands, plants experience a combination of competition, recurrent biomass removal and nutrient pulses. Each of these ecological challenges requires specific plant tolerances, and populations should locally adapt if intraspecific variation exists in these traits.

24   • **Methods** We studied variation in land use-related traits in the common grassland plant *Plantago lanceolata*. In a common environment, we quantified the competitive ability ($R*$), clipping tolerance and responses to a nitrogen pulse of plants from 54 populations with different land use intensities across Germany.

28   • **Key results** We found significant population differentiation in competitive ability but there was little evidence that trait variation was related to land use intensity. There was a positive relationship between competitive ability and clipping tolerance at the population level, indicating a genetic, and possibly functional, link between these two traits. In contrast, clipping tolerance and nitrogen responses were negatively correlated at the levels of plant individuals, indicating a physiological trade-off between plant responses to these two land-use processes.

35   • **Conclusions** Our results show that there is substantial intraspecific variation in some of the key functional traits for plant success in managed grasslands, and that rapid evolution and adaptation is therefore possible in these traits.

38

Introduction

40 Understanding evolution in response to land use in grassland plants is of great interest

41 because of the wide distribution and economic importance of these ecosystems, and because

42 land use change is the strongest driver of global change (Foley *et al.*, 2005, Díaz *et al.*, 2019).

43 Already from the early 20th century, grassland researchers showed that different management

44 regimes resulted in rapid evolutionary changes in a range of grassland species. For instance,

45 in a common-garden collection of over 400 *Dactylis glomerata* ecotypes, Stapledon (1928)

46 found that there were persistent growth form differences between plants from different kinds

47 of pastures and meadows. Later, Warwick and Briggs, in their classic studies on the

48 "genecology of lawn weeds", found similar results for several grassland species, e.g. dwarf,

49 prostrate morphotypes originating from frequently mown lawns, and more erect ones in

50 neighbouring populations that lacked the frequent mowing (Warwick & Briggs 1978, 1979).

51 Evolution in response to land use was also found in the famous long-term Park Grass

52 Experiment where Snaydon and Davies (1976) demonstrated local adaptation of *Antoxanthum*

53 *odoratum* to different fertilisation and liming treatments (see also Davies & Snaydon 1973,

54 1976). In all of these classic studies, however, researchers compared simple categories of land

55 use such as pastures versus meadows, or different types of fertilisation regimes, whereas

56 finer-resolution analyses of land use processes are still rare. Moreover, previous studies

57 usually focused on traits relevant for agriculture, such as yield, growth form and phenology,

58 whereas other ecologically relevant functional traits received less attention.

59 From a plant eye's view, three of the key processes in grasslands are (1) competition

60 with neighbouring plants, (2) the temporary nutrient pulses created by animal droppings or

61 fertilisation, and (3) the regular disturbance and biomass removal imposed by mowing or

62 grazing. The abilities of plants to compete with neighbours, exploit nutrient pulses, and

63 tolerate biomass removal are thus important functional traits in grassland plants.

64   Competition ultimately reduces the survival, growth or reproduction of an individual

65   plant (Aarsen & Keogh, 2002), and plant species differ in the degree to which they they are

66   impacted by neighbours (e.g. Keddy, 1990; Aarssen, 1992; Tokeshi, 2009). Plant competitive

67   ability can be quantified in different ways (Aarssen & Keogh, 2002), and at the species level

68   it appears to be particularly the ability to persist at low nutrient levels that makes some plant

69   species outcompete others (resource ratio hypothesis; Tilman 1985). The significance of the

70   so-called R^* value of species – the lowest resource level that allows persistence – has been

71   proven by many species-level experimental studies (Wilson *et al*., 2007). At the intraspecific

72   level, a number of previous studies demonstrated genetically-based variation in competitive

73   ability and the selective agency of neighbouring plants (Cheplick, 2015), but intraspecific

74   variation in R^* has so far not been examined.

75   The second key process are nutrient pulses. Many ecosystems experience fluctuating

76   resource availability, e.g. because of snowmelt, seasonal weather events or fires (Ostfeld &

77   Keesing, 2000). Human activity is especially associated with such pulses, either indirectly

78   through causing extreme climatic events (Coumou & Rahmstorf, 2012), or more directly

79   through intentional nutrient deposition in agricultural landscapes. Resource pulses can impact

80   population dynamics across communities and trophic networks (Gratton & Denno, 2003;

81   Yang *et al*., 2008) as well as across generations (Miao *et al*., 1991), and they tend to promote

82   particular plant species over others (Bilbrough & Caldwell, 1997), or even the spread of plant

83   invaders (Parepa *et al*., 2013). However, to our knowledge no previous study has investigated

84   plant responses to nutrient pulses at the intraspecific level.

85   The third key process in grasslands is recurrent biomass removal. While strong mowing

86   or grazing generally reduce fitness, plants possess the ability to regrow and to some extent

87   compensate for such damage. Because of this, some species are able to maintain their fitness

88   or even overcompensate and increase it in response to moderate levels of herbivory

89   (McNaughton, 1983; Strauss & Agrawal, 1999). Plant tolerance to biomass damage has been

4

90   extensively researched, and previous studies have repeatedly demonstrated not only species

91   differences but also heritable variation within and among natural populations (e.g. Bergelson

92   & Crawley, 1992; Agrawal, 1998; Strauss & Agrawal, 1999; Johnson, 2011), although rarely

93   in relation to land use (but see Lennartsson *et al*., 1997, 1998).

94   While all of the three described functional traits are expected to be important for

95   success in managed grasslands, it seems unlikely that plants can evolutionarily improve all of

96   them simultaneously. Increased competitive ability (= lower R^*) requires greater resource-

97   efficiency, while stronger responses to nutrient pulses are only possible if plants are on the

98   faster (= less resource-efficient) side of the fast-slow plant economy spectrum (Reich, 2014).

99   Tolerance to biomass removal is usually based on belowground storage of resources, which

100   means that some resources are not available for other purposes anymore. In general, we

101   should expect evolutionary trade-offs (Agrawal *et al*., 2010) between the three functional

102   traits, and that the specific phenotypes evolving in different grasslands depend on the local

103   intensities of fertilisation versus mowing and grazing damage.

104   We addressed these questions in the framework of the Biodiversity Exploratories

105   project (www.biodiversity-exploratories.de), a large-scale and long-term network of

106   ecological study sites for understanding relationships between land use, biodiversity and

107   ecosystem functioning. The project includes 150 grassland plots across Germany (Fischer *et*

108   *al*., 2010), with 50 plots in each of the three regions Schorfheide-Chorin (northern Germany),

109   Hainich-Dün (central Germany) and Schwäbische Alb (southwest Germany). Within each

110   region, the plots cover a broad range of land use types and intensities. The detailed land use

111   information available for these plots, with precise data on mowing frequencies, livestock

112   densities and amounts of fertilisation, obtained from annual surveys (Blüthgen *et al*., 2012), is

113   a unique feature of the Biodiversity Exploratories project and, together with the large number

114   of plots, makes it a powerful system for studying evolution in managed grasslands.

115   There is already evidence from the Biodiversity Exploratories that the phenotypes of

116   several grassland species evolve in response to land use (Kloss *et al*., 2011; Völler *et al*.,

117   2013, 2017). We built on these studies and examined 54 populations of the common perennial

118   *Plantago lanceolata*. Unlike the previous studies, which only conducted simple phenotyping

119   in a common environment, we carried out a greenhouse experiment with a series of treatments

120   (Fig. 1) which allowed us to quantify the $R*$ values of plants, as well as their nutrient pulse

121   responses and clipping tolerances. Specifically, we asked the following questions: (1) Is there

122   intraspecific variation in the three functional traits in *P*. *lanceolata*? (2) What is the

123   relationship between land use and the variation in these traits? (3) Are there trade-offs

124   between the three traits, and are these trade-offs influenced by land use intensity?

125

Materials and Methods

Study species and experimental design

128   To test the questions outlined above, we worked with *Plantago lanceolata* L.

129   (Plantaginaceae), a short-lived perennial rosette herb that is very common in European

130   grasslands and grows under a wide range of environmental conditions. *P*. *lanceolata* is also

131   one of the most common plant species in the Biodiversity Exploratories, occurring on over

132   100 of the 150 grassland plots. In September 2015, we collected ripe seeds of *P*. *lanceolata* in

133   each of the three regions, and from the broadest possible land-use gradient in each

134   (Supplementary Table S2). Altogether, we sampled seeds from 54 plots, with 5–12 individual

135   plants per plot.

136   We stratified the seeds at 5°C under moist and dark conditions for three weeks (Pons,

137   1992) and transplanted the germinated seedlings to 1-L pots filled with a 7:1.5:1 mixture of

138   nutrient-poor soil, vermiculite and sand, with 5–12 individuals per population and a total of

139   540 plants (Supplementary Table S2). The pots were placed in a climate-controlled

140   greenhouse with temperature set to 21°C/15°C at a 16h/8h day/night cycle. After six weeks,

6

141 we rearranged all pots into a randomised block design, and we let the plants grow for another

142 seven weeks to ensure strong nutrient depletion in all pots (Fig. 1). At this point, we took a 5

143 cm^3 soil sample from each pot that was later analysed for total nitrogen content with a

144 EuroEA Elemental Analyser (HEKAtech, Wegberg , Germany) at the Soil Biogeochemistry

145 Lab at Karlsruhe Institute of Technology, and we measured the chlorophyll content of two

146 leaves on each plant with a SPAD 502 chlorophyll meter (Konica-Minolta, Tokyo, Japan).

147 After that, we fertilised each plant with 10 ml of liquid NPK fertiliser (Wuxal

148 Universaldünger; Hauert MANNA Düngerwerke GmbH, Nürnberg, Germany) at a

149 concentration equivalent to 50 kg N/ha. Ten days later, we measured chlorophyll content

150 again on two newly grown leaves of each plant. Two weeks after adding the fertiliser, we

151 clipped all plants one centimetre above ground. After another three weeks, we harvested the

152 aboveground biomass of all plants, dried it at 70°C for three days, and weighed it.

153

154 *Data Analysis*

155 Our data analyses generally focused on three variables: (1) the competitive ability of each

156 plant, estimated as *1-R** (Tilman, 1985) where *R** was the fraction of total nitrogen in the

157 potting soil left after 11 weeks of growth, (2) the pulse response as the ratio between the leaf

158 chlorophyll contents after and before the fertilisation, with higher values indicating more

159 successful utilisation of the added nitrogen, and (3) the clipping tolerance of plants, calculated

160 as the ratio between their aboveground biomass from the second and first harvest, again with

161 higher values indicating faster recovery from clipping damage.

162 Prior to the main analyses, we simplified our data by removing sources of variation that

163 were not relevant to our study questions. We fitted linear models with the three regions of the

164 Biodiversity Exploratories and the blocks in the greenhouse as fixed factors to each dependent

165 variable, and we used the residuals from these models for all subsequent analyses (Manning *et*

7

166 *al*., 2015; Soliveres *et al*., 2016). To improve the normality of error distributions, the data for

167 pulse response and clipping tolerance were additionally log-transformed.

168 First, we tested for intraspecific variation in the three focus traits with mixed-effect

169 models that included populations as fixed factors and maternal seed families nested within

170 populations as random factors (Zuur *et al*., 2009; see Supplementary Information Table S1 for

171 model formulas). Second, we tested for relationships between land use and the three traits by

172 fitting separate mixed models for each combination of land use intensities (mowing,

173 fertilisation, grazing) and trait (competitive ability, pulse response, clipping tolerance), with

174 each model including one of the land use intensities as explanatory variable plus population

175 and maternal seed families nested within populations as random factors (Table S1). Next, we

176 tested for trade-offs between the three focus traits by examining their statistical relationships

177 at the level of individuals, seed families and populations. At the individual-level, we fitted

178 mixed models with random intercept and slope that included the respective other trait as

179 explanatory variable, plus population and family nested within population as random factors.

180 At the family level, we analysed family means and included only population as random factor,

181 and at the population level, we used simple linear models regressing the population means of

182 two traits against each other. In the cases where we found significant relationships between

183 the traits, we proceeded to the final step in our analyses where we tested the effects of land

184 use on trait relationships. We did this through a series of mixed models with random

185 intercepts and slopes that included the respective other trait, one of the three land use

186 intensities, and their interactions, as fixed factors, plus populations and families nested within

187 populations as random factors. All statistical analyses were done in R (R Development Core

188 Team, 2008). We corrected all *P*-values for false discovery rates (FDR).

189

Results

191 We found significant heritable variation, both at the population and seed family level, for

192 competitive ability, but only marginally significant family-level variation in clipping

193 tolerance, and no significant variation at all in pulse response (Table 1 and Figure 2). There

194 were no significant relationships between land use intensity and the three studied functional

195 traits (Table 2). When we tested for relationships between competitive ability, pulse response

196 and clipping tolerance, we found significant negative relationships between pulse response

197 and clipping tolerance at the level of individuals and seed families, and a significant positive

198 relationship between competitive ability and clipping tolerance at the population level (Table

199 3 and Fig 3). Furthermore, we found a significant effect of mowing on the individual-level

200 relationship between pulse response and clipping tolerance ($F = 9.08$, $P = 0.025$ for mowing x

201 pulse response interaction), with the negative relationship between the two traits disappearing

202 at higher mowing intensities (Fig 4). There were no other significant land use effects on trait

203 relationships.

204

Discussion

206 To understand plant intraspecific variation in relation to land use, we studied 54 grassland

207 populations of *Plantago lanceolata* that strongly differed in their intensities of mowing,

208 grazing and fertilisation. We specifically examined three functional traits that we expected to

209 be important for plant survival in grasslands: competitive ability, clipping tolerance and the

210 ability of plants to quickly respond to nutrient pulses. We found substantial intraspecific

211 variation in competitive ability (R^*) but not in the other two traits, and there was no evidence

212 for population-level relationships between traits and land-use intensity. However, there were

213 several positive or negative relationships between functional traits at the levels of individuals,

214 families or populations, indicating physiological or evolutionary links between these traits.

215 Below, we discuss the results in detail, and attempt to place them into a broader context.

9

216

Intraspecific variation

A necessary prerequisite for genetic differentiation and local adaptation in the examined traits is that our study system harbours significant intraspecific variation. We did not find any significant family- or population-level variation in clipping tolerance and plant responses to a nutrient pulse, but there was substantial intraspecific variation in $R*$ competitive ability, both at the level of seed families and populations. To our knowledge, this is the first time that intraspecific variation, and thus microevolution, in this aspect of competitive ability has been studied and demonstrated in plants.

We were surprised to not find population differentiation in clipping tolerance because intraspecific variation has been repeatedly shown in other plant species (e.g. Agrawal *et al*., 1999; Johnson, 2011; Juenger & Bergelson, 2000; Strauss & Agrawal, 1999; Deng *et al*. unpublished). We also found no population differentiation in pulse response, and there are no previous studies on intraspecific variation in this trait. With 54 populations and 199 seed families, a lack of statistical power is an unlikely explanation in our case. Instead, we think that it may have been a combination of weak true patterns and high signal-to-noise ratio. First, since *Plantago lanceolata* is wind-pollinated and self-incompatible (Kuiper & Bos, 1992), there is generally strong gene flow and relatively weak population differentiation in this species (Gáspár *et al*. 2019). Second, we worked with an F_1 generation that had random fathers (from the field) but that, unlike under field conditions, was not experiencing strong natural selection. This likely further increased variation among individuals and therefore lowered the signal-to-noise ratio in our system. Finally, clipping tolerance and pulse response are both derived traits based on several, error-prone measurements, and thus error propagation could have further added to this problem. However, in spite of all this, we did find significant family- and population-level variation in $R*$, which underlines the ecological and evolutionary significance of this result.

10

242

243 *No relationships with land use*

244 We found no relationships between the land-use intensities recorded in the Biodiversity

245 Exploratories and the three studied functional traits. This contrasts with previous studies in

246 the Biodiversity Exploratories (Völler *et al*., 2013, 2017) as well as in other systems that

247 demonstrated land use-related phenotypic changes in plants (e.g. Aarssen & Turkington,

248 1985a, b, c, 1987; Lennartsson *et al*., 1997; Briggs, 2009). In principle, there are three

249 possible explanations: (1) a true pattern could not be detected because of statistical or

250 methodological shortcomings, (2) there was no pattern yet because the land use has not been

251 acting long enough yet in our system, or (3) there is no pattern. As already explained above,

252 our study did not lack statistical power, and it covered a broad range of land use intensities,

253 also compared to previous studies. Moreover, although there is some interannual variation in

254 land use in the Biodiversity Exploratories (Blüthgen *et al*., 2012; Allan *et al*., 2014), which

255 could potentially impede the impacts of natural selection, previous studies already found land

256 use-related differentiation of plant phenotypes in our system (Völler *et al*., 2013, 2017). It is

257 also known from other studies that that a couple of years can be enough for stable shifts in

258 plant phenotypes between differential management (Briggs, 2009). Therefore, explanations

259 (1) and (2) both appear unlikely, and we need to consider the third option that there might

260 simply be no relationships between land use and the three studied functional traits; possibly

261 because of the derived nature of the traits, or evolutionary constraints particular to these traits

262 and land use in this system.

263

264 *Correlations between three functional traits*

265 Besides quantifying intraspecific variation in the three functional traits and their relationships

266 with land use, we also tested for interrelationships between traits, and we did this at three

267 levels: plant individuals, maternal seed families and populations. Each of these levels

11

268   provides us with different answers: at the level of individuals, trait correlations are most likely

269   related to functional-physiological constraints or necessities, whereas at the level of seed

270   families they reflect underlying genetic correlations, and at the level of populations they

271   rather indicate trait syndromes associated with habitat adaptation.

272   We found no relationships between competitive ability and pulse response at any of

273   these levels. This was surprising as lower R^* values (i.e. better competitive ability) should be

274   coupled to a resource-conservative plant economy, whereas strong responses to nutrient

275   pulses require a large metabolic capacity. We therefore expected a trade-off between the two

276   traits. However, our results suggest that competitive ability evolves independently. The only

277   observed trait correlation involving competitive ability was a positive population-level

278   correlation between competitive ability and clipping tolerance, indicating both traits might be

279   beneficial in the same environments. Resprouting in *Plantago lanceolata* is based on

280   belowground resource storage (Latzel & Klimesová, 2009; Latzel *et al*., 2014). Thus, in

281   contrast to pulse response, both clipping tolerance and R^* competitive ability are resource-

282   conservative, and therefore both traits should be beneficial in the less nutrient-rich pastures or

283   meadows which make up part of the grassland plots in the Biodiversity Exploratories.

284   However, the two traits were not significantly correlated at the level of individuals or seed

285   families, indicating that they are not physiologically or genetically linked. Another potential

286   explanation for the lack of a family-level relationship could be the inflated genetic variation in

287   the F_1 generation already explained above (see also Gáspár *et al*., 2019). However, while F_1

288   plants from the same mother may have many different fathers, these most likely come from

289   the same population (Kuiper & Bos, 1992, p. 226), so that population-level differences may

290   have been maintained, and could thus be detected, in our study.

291   Surprisingly, plant responses to nutrient pulses were negatively correlated to clipping

292   tolerance at the levels of individuals and maternal seed families. Together with the lack of a

293   population differentiation in these traits, this indicates physiological and/or genetic links

294   between them. Again, resource economy appears to be the best explanation. Clipping

295   tolerance is generally thought to be more prevalent in species or genotypes with a more

296   conservative metabolism and more root-, non-structural carbohydrate reserves, whereas a

297   stronger response to a nutrient pulse should requires higher metabolic rate and less storage

298   (Strauss & Agrawal, 1999; Reich, 2014). Thus, there could be a classic resource allocation

299   trade-off between the two traits. The explanation is further supported by the fact that we

300   found the negative correlation mainly in plants from plots with less than one mowing event

301   per year, whereas the relationship tended to disappear at higher mowing frequencies. In the

302   Biodiversity Exploratories, frequent mowing is usually associated with strong fertilisation

303   (Blüthgen *et al*., 2012). Thus, the resource trade-off disappears when resources become less

304   limiting (Agrawal *et al*., 2010).

305

Acknowledgements

14

Literature Cited

Aarssen LW. 1992. Causes and consequences of variation in competitive ability in plant communities. *Journal of Vegetation Science* 3: 165–174.

Aarssen LW. 2005. On size, fecundity, and fitness in competing plants. In: Reekie EG, Bazzaz FA, eds. *Reproductive Allocation in Plants*. Academic Press, 215–244.

Aarssen L, Keogh T. 2002. Conundrums of competitive ability in plants: what to measure? *Oikos* 96: 531–542.

Aarssen LW, Turkington R. 1985a. Within-species diversity in natural populations of *Holcus lanatus*, *Lolium perenne* and *Trifolium repens* from four different-aged pastures. *The Journal of Ecology* 73: 869–886.

Aarssen LW, Turkington R. 1985b. Biotic specialization between neighbouring genotypes in *Lolium perenne* and *Trifolium repens* from a permanent pasture. *The Journal of Ecology* 73: 605–614.

Aarssen LW, Turkington R. 1985c. Competitive relations among species from pastures of different ages. *Canadian Journal of Botany* 63: 2319–2325.

Aarssen LW, Turkington R. 1987. Responses to defoliation in *Holcus lanatus*, *Lolium perenne*, and *Trifolium repens* from three different-aged pastures. *Canadian Journal of Botany* 65: 1364–1370.

Agrawal AA. 1998. Induced responses to herbivory and increased plant performance. *Science* 279: 1201–1202.

Agrawal AA, Conner JK, Rasmann S. 2010. Tradeoffs and negative correlations in evolutionary ecology. In: M. A. Bell, W. F. Eanes, D. J. Futuyma, and J. S. Levinton, ed. *Evolution After Darwin: The First 150 Years*. 243–268.

Agrawal AA, Strauss SY, Stout MJ. 1999. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution* 53: 1093–1104.

Allan E, Bossdorf O, Dormann CF, Prati D, Gossner MM, Tscharntke T, Blüthgen N, Bellach

349   M, Birkhofer K, Boch S, *et al*. 2014. Interannual variation in land-use intensity enhances

350   grassland multidiversity. *Proceedings of the National Academy of Sciences of the United*

351   *States of America* 111: 308–313.

352   Bergelson J, Crawley MJ. 1992. The effects of grazers on the performance of individuals and

353   populations of scarlet gilia, *Ipomopsis aggregata*. *Oecologia* 90: 435–444.

354   Bilbrough CJ, Caldwell MM. 1997. Exploitation of springtime ephemeral N pulses by six

355   Great Basin plant species. *Ecology* 78: 231–243.

356   Blüthgen N, Dormann CF, Prati D, Klaus VH, Kleinebecker T, Hölzel N, Alt F, Boch S,

357   Gockel S, Hemp A, *et al*. 2012. A quantitative index of land-use intensity in grasslands:

358   Integrating mowing, grazing and fertilization. *Basic and Applied Ecology* 13: 207–220.

359   Briggs D. 2009. *Plant Microevolution and Conservation in Human-influenced Ecosystems*.

360   Cambridge University Press.

361   Coumou D, Rahmstorf S. 2012. A decade of weather extremes. *Nature Climate Change* 2:

362   491–496.

363   Davies, M.S. & Snaydon, R.W. 1973. Physiological differences among populations of

364   *Anthoxanthum odoratum* L. on the Park Grass Experiment, Rothamsted. I. Response to

365   calcium. *Journal of Applied Ecology* 10: 33– 45.

366   Davies, M.S. & Snaydon, R.W. 1976. Rapid population differentiation in a mosaic

367   environment. III. Measures of selection pressures. *Heredity* 36: 59–66.

368   Díaz S, Settele J, Brondízio E, Ngo H, Guèze M, Agard J, Arneth A, Balvanera P, Brauman

369   K, Butchart S, *et al*. 2019. Summary for policymakers of the global assessment report on

370   biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on

371   Biodiversity and Ecosystem Services.

372   Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, Korte G, Nieschulze

373   J, Pfeiffer S, Prati D, *et al*. 2010. Implementing large-scale and long-term functional

374   biodiversity research: The Biodiversity Exploratories. *Basic and Applied Ecology* 11: 473–

16

485.

Foley JA, Defries R, Asner GP, Barford C, Bonan G, Carpenter SR, Chapin FS, Coe MT, Daily GC, Gibbs HK, *et al*. 2005. Global consequences of land use. *Science* 309: 570–574.

Gáspár B, Bossdorf O, Durka W. 2019. Structure, stability and ecological significance of natural epigenetic variation: a large-scale survey in *Plantago lanceolata*. *The New Phytologist* 221: 1585–1596.

Gratton C, Denno RF. 2003. Inter-year carryover effects of a nutrient pulse on *Spartina* plants, herbivores, and natural enemies. *Ecology* 84: 2692–2707.

Johnson MTJ. 2011. Evolutionary ecology of plant defenses against herbivores. *Functional Ecology* 25: 305–311.

Juenger T, Bergelson J. 2000. The evolution of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*: herbivore-imposed natural selection and the quantitative genetics of tolerance. *Evolution* 54: 764–777.

Keddy PA. 1990. Competitive hierarchies and centrifugal organization in plant communities. In: Grace JB, Tilman D, eds. *Perspectives on Plant Competition*. Academic Press, 265–290.

Kloss L, Fischer M, Durka W. 2011. Land-use effects on genetic structure of a common grassland herb: A matter of scale. *Basic and Applied Ecology* 12: 440–448.

Kuiper PJC, Bos M. 1992. *Plantago: A Multidisciplinary Study*. Springer.

Latzel V, Klimešová J. 2009. Fitness of resprouters versus seeders in relation to nutrient availability in two *Plantago* species. *Acta Oecologica* 35: 541–547.

Latzel V, Janeček Š, Hájek T, Klimešová J. 2014. Biomass and stored carbohydrate compensation after above-ground biomass removal in a perennial herb: does environmental productivity play a role? *Folia Geobotanica* 49: 17–29.

Lennartsson T, Nilsson P, Tuomi J. 1998. Induction of overcompensation in the field gentian: *Gentianella campestris*. *Ecology* 79: 1061–1072.

17

401   Lennartsson T, Tuomi J, Nilsson P. 1997. Evidence for an evolutionary history of
402   overcompensation in the grassland biennial *Gentianella campestris* (Gentianaceae). *The*
403   *American Naturalist* 149: 1147–1155.

404   Manning P, Gossner MM, Bossdorf O, Allan E, Zhang Y-Y, Prati D, Blüthgen N, Boch S,
405   Böhm S, Börschig C, *et al*. 2015. Grassland management intensification weakens the
406   associations among the diversities of multiple plant and animal taxa. *Ecology* 96: 1492–
407   1501.

408   McNaughton SJ. 1983. Compensatory plant growth as a response to herbivory. *Oikos* 40:
409   329–336.

410   Miao SL, Bazzaz FA, Primack RB. 1991. Persistence of maternal nutrient effects in *Plantago*
411   *major*: the third generation. *Ecology* 72: 1634–1642.

412   Ostfeld RS, Keesing F. 2000. Pulsed resources and community dynamics of consumers in
413   terrestrial ecosystems. *Trends in Ecology & Evolution* 15: 232–237.

414   Parepa M, Fischer M, Bossdorf O. 2013. Environmental variability promotes plant invasion.
415   *Nature Communications* 4: 1604.

416   Pons TL. 1992. Seed germination of Plantago major ssp. major and *Plantago lanceolata*. In:
417   *Plantago: A Multidisciplinary Study*. Springer, 161–169.

418   R Development Core Team. 2008. R: A Language and Environment for Statistical
419   Computing.

420   Reich PB. 2014. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto.
421   *The Journal of Ecology* 102: 275–301.

422   Snaydon RW, Davies MS. 1976. Rapid population differentiation in a mosaic environment.
423   IV. Populations of *Anthoxanthum odoratum* at sharp boundaries. *Heredity* 37: 9-27.

424   Soliveres S, van der Plas F, Manning P, Prati D, Gossner MM, Renner SC, Alt F, Arndt H,
425   Baumgartner V, Binkenstein J, *et al*. 2016. Biodiversity at multiple trophic levels is needed
426   for ecosystem multifunctionality. *Nature* 536: 456–459.

18

427   Stapledon RG. 1928. Cocksfoot grass (*Dactylis Glomerata* L.): Ecotypes in relation to the

428   biotic factor. *The Journal of Ecology* 16: 71–104.

429   Strauss SY, Agrawal AA. 1999. The ecology and evolution of plant tolerance to herbivory.

430   *Trends in Ecology & Evolution* 14: 179–185.

431   Tilman D. 1985. The resource-ratio hypothesis of plant succession. *The American Naturalist*

432   125: 827–852.

433   Tokeshi M. 2009. *Species Coexistence: Ecological and Evolutionary Perspectives*. John

434   Wiley & Sons.

435   Völler E, Auge H, Bossdorf O, Prati D. 2013. Land use causes genetic differentiation of life-

436   history traits in Bromus hordeaceus. *Global Change Biology* 19: 892–899.

437   Völler E, Bossdorf O, Prati D, Auge H. 2017. Evolutionary responses to land use in eight

438   common grassland plants. *The Journal of Ecology* 105: 1290–1297.

439   Warwick SI, Briggs D. 1978. The Genecology of Lawn Weeds. I. Population differentiation

440   in *Poa annua* L. in a mosaic environment of bowling green lawns and flower beds. *The*

441   *New Phytologist* 81: 711–723.

442   Warwick SI, Briggs D. 1979. The Genecology of Lawn Weeds. III. Cultivation experiments

443   with *Achillea millefolium* L., *Bellis perennis* L., *Plantago lanceolata* L., *Plantago major* L.

444   and *Prunella vulgaris* L. collected from lawns and contrasting grassland habitats. *The New*

445   *Phytologist* 83: 509–536.

446   Weiner J. 1988. The influence of competition on plant reproduction. In: Lovett Doust J,

447   Lovett Doust L, eds. *Plant reproductive ecology: patterns and strategies*. Oxford

448   University Press, 228–245.

449   Wilson JB, Spijkerman E, Huisman J. 2007. Is there really insufficient support for Tilman's

450   *R** concept? A comment on Miller *et al*. *The American Naturalist* 169: 700–706.

451   Yang LH, Bastow JL, Spence KO, Wright AN. 2008. What can we learn from resource

452   pulses? *Ecology* 89: 621–634.

453   Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009. *Mixed effects models and*

454   *extensions in ecology with R*. Springer.

455

456   **Table 1.** Results of mixed models testing for heritable variation in three functional traits in

457   *Plantago lanceolata*, with populations and seed families as fixed and random factors,

458   respectively. LRT = likelihood-ratio test. All *P*-values are FDR-corrected.

	Population		Seed family	
	F	*P*	*LRT*	*P*
Competitive ability	1.60	**0.049**	16.82	**0.000**
Pulse response	1.01	0.463	0.04	0.835
Clipping tolerance	1.30	0.169	4.05	0.066

459

460 **Table 2.** Results of mixed models testing for the effects of mowing, fertilisation and grazing

461 on three functional traits in *Plantago lanceolata*, with populations and seed families included

462 as random factors. Est. = slope estimate of the models. All *P*-values are FDR-corrected.

	Mowing			Fertilisation			Grazing		
	Est.	*F*	*P*	Est.	*F*	*P*	Est.	*F*	*P*
Competitive ability	0.142	4.31	0.387	0.050	1.51	0.504	-0.071	1.68	0.504
Pulse response	0.014	0.08	0.838	0.001	0.04	0.838	0.009	0.06	0.838
Clipping tolerance	0.007	1.85	0.838	0.011	1.60	0.504	0.007	0.39	0.838

463

464 **Table 3.** Results of random slope and intercept mixed effects models testing for relationships

465 between competitive ability (CA), pulse response (PR) and clipping tolerance (CT) in

466 *Plantago lanceolata*, with populations and seed families included as random factors. Est. =

467 slope estimate of the models. All *P*-values are FDR-corrected, and *P*<0.05 are in bold.

	Individuals			Families			Populations		
	Est.	*F*	*P*	Est.	*F*	*P*	Est.	*F*	*P*
CA ~ PR	0.07	3.26	0.162	0.12	1.31	0.336	0.01	0.00	0.967
CA ~ CT	-0.01	0.01	0.967	0.11	1.33	0.336	0.44	6.21	**0.048**
PR ~ CT	-0.05	11.47	**0.009**	-0.05	8.81	**0.018**	-0.05	2.52	0.212

468

469

23

470   **Figure 1.** Schematic of the sequence and duration of experimental treatments used to estimate

471   competitive ability (R^*), pulse response and clipping tolerance in *Plantago lanceolata* plants

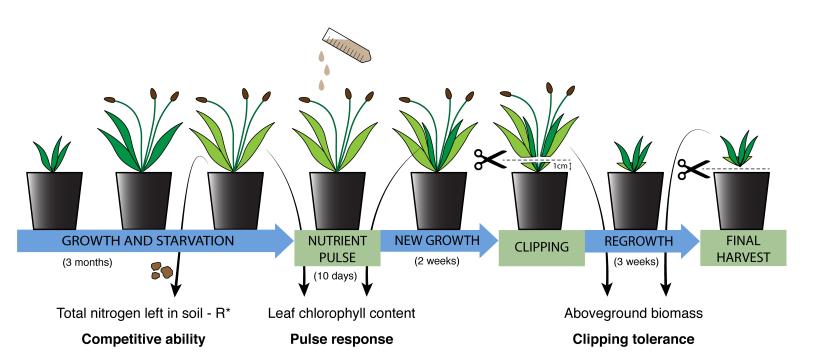472   from 54 grasslands of different land-use intensities.

473

474   **Figure 2.** Variation among populations (boxplots) and seed families (black dots within

475   boxplots) in three functional traits in *Plantago lanceolata*. The boxplots are based on all

476   individuals per population and indicate medians, 25th/75th percentiles, and the 1.5 x

477   interquartile ranges. For each trait, populations are ordered by their median values.
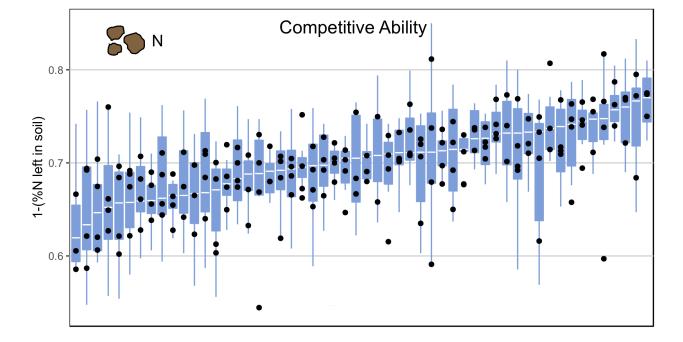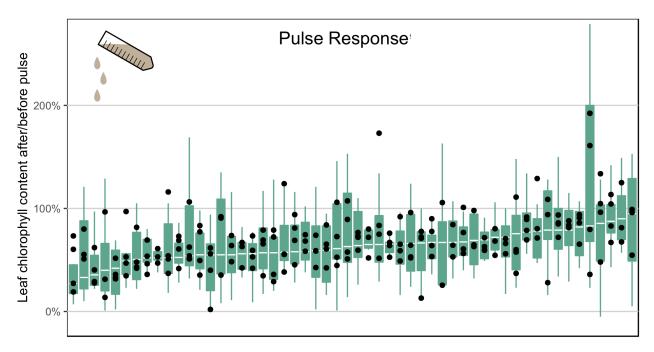
478

479   **Figure 3.** Relationships between the three studied functional traits of *Plantago lanceolata* at

480   the levels of individuals, seed families and populations. Solid and dashed line plots indicate

481   the fitted models for significant and non-significant relationships, respectively, with their 95%
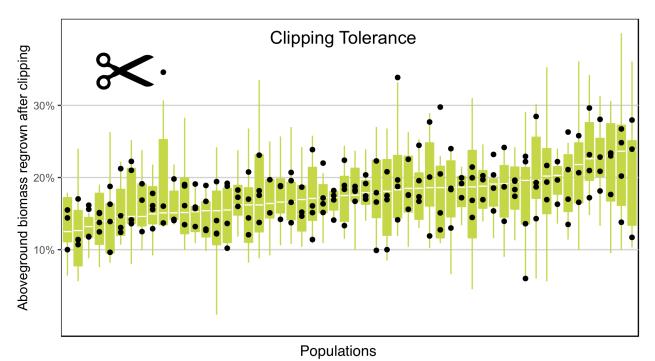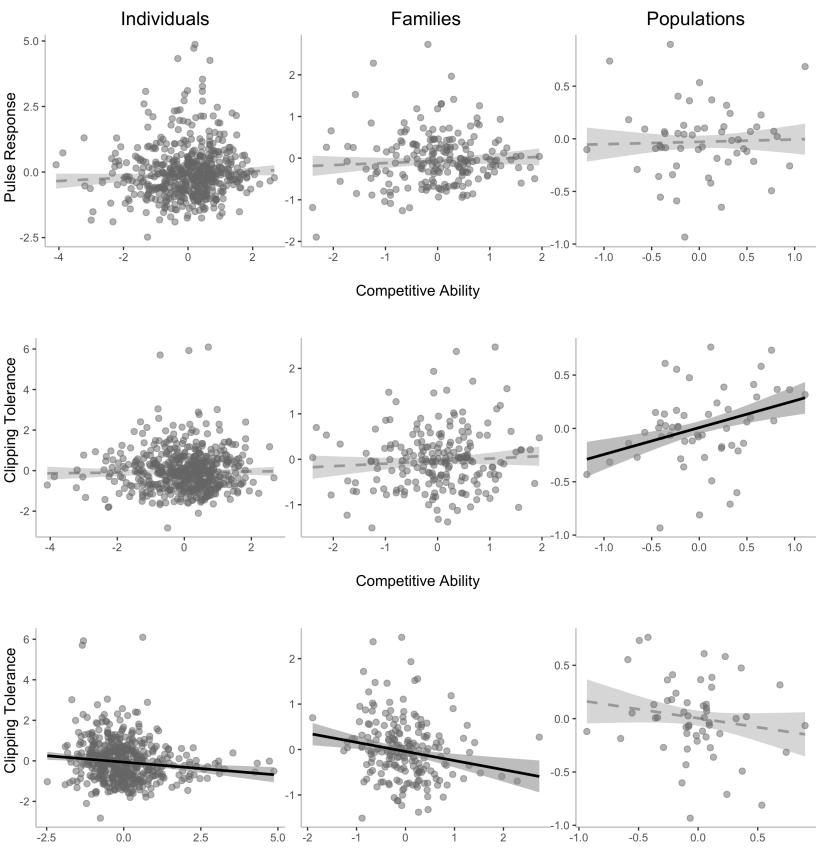
482   confidence intervals.

483

484   **Figure 4.** The mowing intensity of their grasslands of origin affects functional trait

485   correlations in *Plantago lanceolata*. Each dot represents a plant individual grown in a

486   common environment.

GROWTH AND STARVATION
(3 months)

NUTRIENT PULSE
(10 days)

NEW GROWTH
(2 weeks)

CLIPPING

1cm

REGROWTH
(3 weeks)

FINAL HARVEST

Total nitrogen left in soil - R*

Competitive ability

Leaf chlorophyll content

Pulse response

Aboveground biomass

Clipping tolerance

Competitive Ability

Pulse Response

Clipping Tolerance