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## **Context matters: the landscape matrix determines the population genetic structure of temperate forest herbs across Europe**

Tobias Naaf, Jannis Till Feigs, Siyu Huang, Jörg Brunet, Sara Cousins, Guillaume Decocq, Pieter de Frenne, Martin Diekmann, Sanne Govaert, Per-Ola Hedwall, et al.

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1 **Context matters: the landscape matrix determines the population genetic structure of temperate**  
2 **forest herbs across Europe**

3

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24 **Abstract**

25 Context. Plant populations in agricultural landscapes are mostly fragmented and their functional  
26 connectivity often depends on seed and pollen dispersal by animals. However, little is known about  
27 how the interactions of seed and pollen dispersers with the agricultural matrix translate into gene  
28 flow among plant populations.

29 Objectives. We aimed to identify effects of the landscape structure on the genetic diversity within,  
30 and the genetic differentiation among, spatially isolated populations of three temperate forest  
31 herbs. We asked, whether different arable crops have different effects, and whether the orientation  
32 of linear landscape elements relative to the gene dispersal direction matters.

33 Methods. We analysed the species' population genetic structures in seven agricultural landscapes  
34 across temperate Europe using microsatellite markers. These were modelled as a function of  
35 landscape composition and configuration, which we quantified in buffer zones around, and in  
36 rectangular landscape strips between, plant populations.

37 Results. Landscape effects were diverse and often contrasting between species, reflecting their  
38 association with different pollen- or seed dispersal vectors. Differentiating crop types rather than  
39 lumping them together yielded higher proportions of explained variation. Some linear landscape  
40 elements had both a channelling and hampering effect on gene flow, depending on their  
41 orientation.

42 Conclusions. Landscape structure is a more important determinant of the species' population  
43 genetic structure than habitat loss and fragmentation *per se*. Landscape planning with the aim to  
44 enhance the functional connectivity among spatially isolated plant populations should consider that  
45 even species of the same ecological guild might show distinct responses to the landscape structure.

46

47

48 **Keywords**

49 arable crops; dispersal vectors; functional connectivity; genetic differentiation; genetic diversity;  
50 linear landscape elements

51 **Declarations**

52

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62

63 **Conflicts of interest**

64 We have no conflict of interest to declare.

65

66 **Ethics approval**

67 We have no ethical concerns to declare.

68

69 **Consent to participate**

70 All authors contributed to this study and this manuscript of their own accord.

71

72 **Consent for publication**

73 All authors agree with the content of this manuscript and its publication in Landscape Ecology.

74

75 **Availability of data and material**

76 The microsatellite allele tables for all species and populations as well as population locations are  
77 available on DRYAD (<https://doi.org/10.5061/dryad.tb2rbp00k>). The population genetic variables at

78 the node level are published in Naaf et al. 2021 (<https://doi.org/10.1007/s10980-021-01292-w>). The  
79 population genetic variables at the link level and all landscape metrics (node and link level) are  
80 available on DRYAD as well (<https://doi.org/10.5061/dryad.h70rxwdf>).

81

## 82 **Authors' contributions**

83 TN, SIJH and KK conceived and designed the study. All authors except JLe and JP were involved in site  
84 selection, field work and sampling. JTF, SH, TN and KK performed the molecular lab work and did the  
85 allele scoring. TN analyzed the data and wrote the first draft of the manuscript with contributions of  
86 JLe, JP, JTF, KK, SH, and SIJH. All authors contributed to revisions and gave final approval for  
87 publication.

88

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95

96 **1. Introduction**

97

98 In many regions on earth, the progressive occupation of land by humans for settlements and  
99 agriculture has forced wildlife and wildflowers to live in small remaining fragments of once  
100 contiguous natural habitats (Kennedy et al. 2019). Surviving in a system of habitat fragments only  
101 succeeds if local populations are functionally connected through the regular exchange of individuals  
102 or diaspores. Plants with a limited dispersal potential, low seed production, a transient seed bank  
103 and a high age of first sexual reproduction appear poorly equipped to establish such regional  
104 population dynamics (Eriksson 1996). Typical temperate forest herbs belong to this group of plants  
105 (Whigham 2004) as they evolved within landscapes that used to be covered by forest to a much  
106 greater extent (Honnay et al. 2005). Numerous population genetic studies revealed that the  
107 functional connectivity among temperate forest herb populations may be strongly reduced in  
108 landscapes with a high degree of forest fragmentation. For instance, small, spatially isolated  
109 populations often exhibit a reduced allelic richness (Vellend 2004; Jacquemyn et al. 2006; Vandepitte  
110 et al. 2007; Kolb and Durka 2013; Naaf et al. 2021) and are strongly genetically differentiated from  
111 each other (Jacquemyn et al. 2006; Schmidt et al. 2009; Gentili et al. 2018). However, local  
112 populations may maintain a high level of genetic diversity (Culley et al. 2007; Toma et al. 2015) and a  
113 low level of genetic differentiation among them (Van Rossum et al. 2002; Tomimatsu and Ohara  
114 2003; Jacquemyn et al. 2009) if they are functionally connected by steady gene flow.

115 Gene flow in plants depends on abiotic or biotic vectors that transport seeds or pollen  
116 between populations. For many plants, these vectors are animals that actively cross the landscape  
117 matrix, i.e. the non-habitat part of the landscape (Murphy and Lovett-Doust 2004). We can therefore  
118 assume that the structure of the matrix has a significant impact on gene flow in plants and thus their  
119 susceptibility to detrimental effects of habitat loss and fragmentation. Nevertheless, population  
120 genetic studies in general (Holderegger et al. 2010) and on forest herbs in particular (e.g., Tomimatsu  
121 and Ohara 2003; Jacquemyn et al. 2006; Vandepitte et al. 2007; Kolb and Durka 2013; Gentili et al.  
122 2018; but see Westerberg and Saura 1994 and Schmidt et al. 2009) largely ignored the matrix.

123           Several mechanisms of how the matrix may influence the seed and pollen transport among  
124 plant populations are conceivable. First, different land-use types may exhibit different degrees of  
125 resistance for seed-dispersing animals or pollinators. In general, the landscape permeability for large  
126 mammals, such as deer, wild boar or carnivores, increases with forest cover (Coulon et al. 2004;  
127 Herrera et al. 2016), while pollinators seem to prefer open landscapes and transport pollen further,  
128 when forest cover is low (Kreyer et al. 2004; Kamm et al. 2010). Many mammals avoid proximity to  
129 settlements and roads (Trombulak and Frissell 2000; Bonnot et al. 2013). Grasslands and arable fields  
130 are avoided by deer and wild boar after harvest when they provide no shelter and little forage  
131 biomass (Thurfjell et al. 2009; Morellet et al. 2011). However, maize fields with their specific  
132 phenology and structure provide shelter even during late summer and autumn (Keuling et al. 2009;  
133 Tillmann 2011) and thus enhance landscape permeability for mammals at a time, when seeds of  
134 many forest herb-layer plants are ripe (Heinken and Raudnitschka 2002).

135           Second, land-use types serving as forage or nesting habitat may affect animals' abundance  
136 and behaviour. The chance for seeds to be dispersed by birds is directly linked to bird abundance  
137 (Garcia et al. 2010) and the abundance of woodland birds in agricultural landscapes increases with  
138 increasing forest cover (Heikkinen et al. 2004; Radford and Bennett 2007). Mass-flowering crops such  
139 as oilseed rape have been shown to enhance the abundance of bumblebee workers (Westphal et al.  
140 2003) and solitary bees (Holzschuh et al. 2013) at the landscape scale. However, whether such an  
141 attractive resource pulse results in spill-over to semi-natural habitats and enhanced pollination  
142 service to wild plants (Kovacs-Hostyanszki et al. 2013; Ekroos et al. 2015) or a dilution of pollinators  
143 and reduced pollination service (Holzschuh et al. 2011, 2016; Riedinger et al. 2015; Proesmans,  
144 Smagghe, et al. 2019) needs further investigation. More continuous floral resources in semi-natural  
145 habitats such as grasslands or hedgerows may sustain a high pollinator richness and abundance in  
146 the long term (Kovacs-Hostyanszki et al. 2013; Riedinger et al. 2015; Bartual et al. 2019). The  
147 proximity to such semi-natural habitats has been found to enhance the seed-set of insect-pollinated  
148 wild plants in agricultural landscapes (Cussans et al. 2010; Jakobsson and Ågren 2014; Chateil and  
149 Porcher 2015; Lindgren et al. 2018). Apart from floral resources, pollinators may depend on further



150 food resources. Larva of aphidophagous hoverflies, for instance, whose preferred adult habitat might  
151 be deciduous forest, find their prey mostly in arable fields (Meyer et al. 2009). They can profit from  
152 high densities of aphids in cereals as well as in oilseed rape (Haenke et al. 2014).

153 Third, linear landscape elements influence the movement behaviour of seed and pollen  
154 dispersal vectors. Roe deer and wild boar, for instance, move preferably along edges such as  
155 hedgerows, forest edges and ditches (Saïd and Servanty 2005; Thurfjell et al. 2009; Morellet et al.  
156 2011). Bumblebees and honey bees fly preferably along hedgerows or other linear landscape  
157 elements (Cranmer et al. 2012; Collett and Graham 2015). However, hedgerows may also act as  
158 relative barriers to pollinator movement when their orientation crosses the flight direction (Wratten  
159 et al. 2003; Klaus et al. 2015). Busy roads may act as barrier to the movement of large mammals  
160 (Trombulak and Frissell 2000; Breyne et al. 2014), and may also restrict bumblebee or hoverfly  
161 movement (Lövei et al. 1998; Bhattacharya et al. 2003; Fitch and Vaidya 2021).

162 Although it is evident that the agricultural landscape matrix influences the behaviour of  
163 pollen- and seed dispersal vectors, it is unclear how this translates into seed and pollen dispersal  
164 rates, and thus functional connectivity, among forest herb populations. Here, we therefore  
165 quantified the landscape composition and configuration in terms of both area-based land-use types  
166 and linear landscape elements in seven agricultural landscapes across north-western Europe to study  
167 their effects on the population genetic structure of three common temperate forest herb species:  
168 *Anemone nemorosa* L., *Oxalis acetosella* L. and *Polygonatum multiflorum* (L.) All. All three species are  
169 typical, slow-colonizing forest specialists, but differ in their reproduction strategy and associated  
170 pollen and seed dispersal vectors. Therefore, we expect them to respond differently to the landscape  
171 structure (Table 1). In our understanding, the landscape-scale population genetic structure comprises  
172 both the genetic diversity within and the genetic differentiation among local populations. We use it  
173 here as an indirect measure of functional connectivity among local plant populations (Aavik et al.  
174 2014) and tested the following main hypotheses:

175 H1 Landscape effects on the population genetic structure differ among the three forest herbs  
176 because of their association with different pollen and/or seed dispersal vectors.

177 H2 Different arable crops (oilseed rape, maize, other cereals) have different effects on the forest  
178 herbs' population genetic structure due to their differential effect on the associated pollen  
179 and/or seed dispersal vectors.

180 H3 Linear landscape elements may have a channelling or impeding effect on gene flow  
181 depending on their orientation in relation to gene dispersal pathways.

182

183

## 184 **2. Material and Methods**

185

### 186 *2.1. Study species*

187 The three studied forest herbs were selected for being typical, common slow-colonizing forest  
188 specialists (Verheyen et al. 2003; Schmidt et al. 2014). They all exhibit strong clonal growth, but also  
189 regular seedling recruitment (Holderegger et al. 1998; Berg 2002; Kosiński 2012). They flower in  
190 spring and are pollinated by insects (Klotz et al. 2002). However, they differ in their reproduction  
191 strategy. *Oxalis acetosella* has been found to produce most of its seeds from cleistogamous flowers  
192 (Berg and Redbo-Torstensson 1998), thus it is less dependent on insect pollinators for sexual  
193 reproduction. According to our own research, however, *O. acetosella* is mainly outcrossing (Naaf et  
194 al. 2021). *Anemone nemorosa* and *Polygonatum multiflorum* are mainly and strictly outcrossing,  
195 respectively (Müller et al. 2000; Kosiński 2012). *Oxalis acetosella* and *A. nemorosa* are pollinated by a  
196 wide range of pollinators, including hoverflies, wild bees and honey bees (Shirreffs 1985; Redbo-  
197 Torstensson and Berg 1995; Stehlik and Holderegger 2000; Naaf et al. 2021). In contrast,  
198 *P. multiflorum* is mostly pollinated by long-tongued bumblebees (Kosiński 2012; Naaf et al. 2021). All  
199 three species have a low seed-dispersal potential and are classified as autochorous (Müller-Schneider  
200 1986). In addition, seeds of *A. nemorosa* are dispersed by some short-distance vectors such as ants  
201 and slugs (Türke et al. 2012). Seeds of *O. acetosella* were found in the fur of wild boar by several  
202 independent studies, though at low quantities (Mrotzek et al. 1999; Heinken and Raudnitschka  
203 2002). The fleshy berries of *P. multiflorum* may suggest endozoochorous dispersal (Müller-Schneider

204 1986). In fact, however, they are toxic and probably rarely dispersed by birds and mid-sized  
205 carnivores such as martens (Ehrlén and Eriksson 1993; Schaumann and Heinken 2002), while short-  
206 distance dispersal by small rodents might occur more often (Ehrlén and Eriksson 1993).

207

208

## 209 2.2. Population genetic structure

210 We compiled population genetic data from seven  $5 \times 5$  km<sup>2</sup> landscape windows spread across north-  
211 western Europe from North France, over Belgium, West Germany, East Germany and South Sweden  
212 up to Central Sweden and Estonia (Figure 1a, b). All landscape windows represent typical agricultural  
213 landscapes, in which forest fragments are embedded in an agricultural matrix interfused by small  
214 settlement areas and roads (see land-use maps in Supp. Inf. S1). We studied up to six forest herb  
215 populations from each species in each landscape windows. *Oxalis acetosella* had too few occurrences  
216 in Belgium to be included in the analysis of this landscape window. *Polygonatum multiflorum* did not  
217 occur in the landscape window of Central Sweden. The final number of surveyed populations was  
218 therefore 42, 34 and 36 for *A. nemorosa*, *O. acetosella* and *P. multiflorum*, respectively. Population  
219 sizes varied by several orders of magnitude both within and among species ranging from 15 flowering  
220 shoots in the smallest *P. multiflorum* population up to  $>12 \times 10^6$  flowering shoots in the largest  
221 *A. nemorosa* population (Table S2). Geographic distances among populations within landscape  
222 windows ranged between 214 and 5518 m and were similarly distributed for each species (Table S2).  
223 The population genetic data for these populations comprised four measures of within-population  
224 genetic diversity, i.e. allelic richness ( $A_r$ ), expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ )  
225 and the inbreeding coefficient  $F = 1 - H_o/H_e$ , as well as two measures of among-population genetic  
226 differentiation for each pair of populations within landscapes, i.e.  $G''_{ST}$  and  $D_{PS}$  (Table S2). While  $G''_{ST}$   
227 is the recommended genetic differentiation measure with microsatellite markers (Meirmans and  
228 Hedrick 2011),  $D_{PS}$  equals 1 minus the proportion of shared alleles and therefore facilitates an  
229 intuitive interpretation. The genetic data were based on species-specific sets of nuclear microsatellite  
230 markers, which comprised six, nine and six markers with a total number of 102, 61 and 149 alleles for

231 *A. nemorosa*, *O. acetosella* and *P. multiflorum*, respectively (Supp. Inf. S3). While *O. acetosella* and  
232 *P. multiflorum* are diploid, *A. nemorosa* was treated as tetraploid (Stehlik and Holderegger 2000). For  
233 details on genetic analyses and the calculation of population genetic variables see Naaf et al. (2021),  
234 in which we studied the effects of habitat loss and fragmentation *per se*.

235

236

### 237 *2.3. Landscape metrics*

238 As a basis for our landscape analysis, we created digital land-use maps for all landscape windows  
239 based on recent orthophotos and additional, region-specific data (Supp. Inf. S1). Moreover, for all  
240 arable fields, we determined the dominance of three different crop types, i.e. oilseed rape, maize  
241 and other cereals, over the preceding decade (2008 – 2017) based on data generated within the  
242 European Integrated Administration and Control System (IACS) (European Commission 2020; Supp.  
243 Inf. S4). To quantify the composition and configuration of area-based and linear landscape elements,  
244 we calculated a set of landscape metrics (Table 1) in (a) buffer zones around each herb population  
245 (node-level analysis, Figure 1c, e.g. Schmidt et al. 2009) and (b) rectangular landscape strips  
246 connecting the centres of each pair of herb populations within landscape windows (link-level  
247 analysis, Figure 1d, e.g. Braunisch et al. 2010). Several buffer distances were chosen to reflect range  
248 sizes and forage distances of potential seed and pollen dispersal vectors (Table 1): 125 m, 250 m, 500  
249 m, 1000 m and 2000 m. Similarly, we chose several width-to-length ratios for the landscape strips  
250 connecting the herb populations to account for the fact that different pollen and seed dispersal  
251 vectors have different sight distances for their orientation and thus will move more or less linearly  
252 through the landscape: 1:7, 1:5, 1:3, 1:2 and 2:3. For each buffer zone and landscape strip, we  
253 calculated the percent cover of different area-based land-use types, the relative length of different  
254 linear landscape elements (= total length divided by buffer or strip area, respectively) and two index  
255 measures, i.e. the Shannon diversity of land-use types and the density of all land-use patch edges  
256 (Table 1). Since the effect that a linear landscape element exerts on gene dispersal might depend on  
257 its orientation relative to the movement direction of vectors (orthogonal vs. parallel), we calculated

258 also the orthogonal and parallel length component of each linear landscape element (Figure 1c, d). In  
259 buffer zones, the parallel direction corresponds to the direction from the midpoint of the linear  
260 element to the population centre. In landscape strips, the parallel direction corresponds to the  
261 connection line between population centres. The orthogonal-to-parallel length ratio was then used  
262 as conditioning variable in statistical models (see below). Moreover, the effect of settlement areas on  
263 gene dispersal vectors might depend on the relative proportion of sealed or built-up area vs.  
264 unsealed green areas, such as gardens. The latter might serve as forage habitat for pollinators,  
265 particularly, when many fruit trees or ornamental shrubs can be found there (Cussans et al. 2010;  
266 Goulson et al. 2010; Nakamura and Kudo 2019). Therefore, we used also the proportion of green  
267 settlement area as conditioning variable in statistical models.

268

269

#### 270 *2.4. Data analysis*

271 To study the effects of landscape metrics on genetic diversity (node level) and pairwise genetic  
272 differentiation (link level), we used linear mixed-effects models (LMMs) separately for each species  
273 with landscape window as random intercept. We fitted these models with the function `lme` of the R  
274 package `nlme` (Pinheiro et al. 2019). At the link level, we took the correlation among population pairs  
275 including a common population into account by defining a correlation structure within the `lme`  
276 function using the function `corMLPE` (Pope 2020; maximum likelihood population-effects models  
277 sensu Clarke et al. 2002). Prior to modelling, all variables were Box-Cox-transformed to increase the  
278 symmetry of their distribution and then centred and scaled to yield standardized regression  
279 coefficients. We were interested here in marginal effects of the landscape metrics that they exert on  
280 genetic variables in addition to those exerted by some basic population genetic determinants, which  
281 had been studied earlier (Naaf et al. 2021). At the node level, these basic determinants were  
282 population size, i.e. the total number of flowering shoots in a population, and the degree of spatial  
283 isolation measured by Hanski's (1994) incidence function model (see Naaf et al. 2021 for details). At  
284 the link level, the basic determinant was edge-to-edge geographical distance. Therefore, all models

285 included population size and isolation or geographic distance, respectively, as fixed effects that could  
286 not be removed during model selection. At the link level, we also allowed for interactions between  
287 basic determinant and landscape metrics, which was not possible at the node level due to the limited  
288 sample size (node level 30-42 vs. link level 78-104; Supp. Inf. S6).

289 To identify the most important predictors among the large number of landscape metrics and  
290 to avoid collinearity, our statistical modelling followed three steps. First, we identified for each  
291 landscape metric (Table 1) its most influential buffer distance (node level) or width-to-length ratio  
292 (link level) as the one yielding the lowest  $AIC_c$  in LMMs containing only one landscape metric at a  
293 time. To account for curvilinear or unimodal relationships these models contained also a quadratic  
294 term if this lowered  $AIC_c$ . Models for linear landscape elements and settlement area included also  
295 the interaction with a corresponding conditioning variable (Table 1). Models at the link level included  
296 also an interaction term with geographic distance if this lowered  $AIC_c$ . In the following steps, we  
297 considered only those most influential landscape metrics that showed a significant effect in the  
298 single-metric models at a level of  $\alpha = 0.15$  based on a likelihood ratio test against the reduced model  
299 without the landscape metric in question.

300 Second, we checked for collinearity among the remaining landscape metrics. Any correlations  
301 with  $|r| \geq 0.7$  were not tolerated. In case of intrinsic collinearity, we used principal components  
302 analysis to calculate a principal component of the collinear variables. In case of collinearity among  
303 variables that are not obviously ecologically related, we chose the one yielding the highest  
304 importance value in the final average model (see below) and excluded the other one (keeping the  
305 collinearity in mind for our interpretation). In the special case of percent cover of arable land, we  
306 allowed either the percent cover of different crop types (RAPE, MAIZE, CEREAL; Table 1) or the  
307 percent cover of arable land in general (ARABLE) to be included to see which version explained a  
308 higher proportion of variation in the final average model (see below).

309 Third, all remaining metrics entered the global model, which was then used for model  
310 selection followed by multi-model inference with the R package MuMIn (Barton 2019). In this step,  
311 we fitted models using ML estimation for all subsets of predictors allowing for a maximum of four

312 and nine landscape metric terms in models at the node and link level, respectively, given the limited  
313 sample size. All models with a  $\Delta AIC_C < 2$  were refit with REML estimation and then subjected to full  
314 model averaging (Grueber et al. 2011). For each term in the average model, we calculated an  
315 importance value as the sum of the Akaike weights over all component models, in which the term  
316 appeared. This importance value ranges between 0 and 1 with a value of 1 indicating that the  
317 corresponding term occurs in all component models. Any term with an importance value  $\geq 0.5$  will be  
318 reported and interpreted. To quantify the amount of variation in genetic variables explained by the  
319 landscape metrics, we calculated the difference between the marginal  $R^2$  for the average model and  
320 the marginal  $R^2$  for the basic model including only the basic population genetic determinants. For the  
321 visualization of any important effects (i.e., those with an importance value  $\geq 0.5$ ), we used the single  
322 best model that included all important terms.

323

324

### 325 **3. Results**

326

#### 327 *3.1. Landscape effects at the node level*

328 Out of the 16 landscape metrics considered, 15 were involved in at least one important landscape  
329 effect (i.e., with an importance value  $\geq 0.5$ ) on a genetic diversity variable (Table 2). The proportion  
330 of variation explained uniquely by the landscape effects was mostly larger than the proportion  
331 explained by the basic population genetic determinants, population size and spatial isolation  
332 (Table 2).

333 Some of the landscape metrics had contrasting effects for different species. With increasing  
334 cover of arable land, allelic richness of *A. nemorosa* increased, but decreased for *P. multiflorum*  
335 (Figures 2a and k). Expected heterozygosity of *A. nemorosa* increased with increasing maize cover in  
336 the landscape, whereas for *O. acetosella*, expected heterozygosity was highest at a lower-than-  
337 average maize cover (Figures 2d and h). Expected and observed heterozygosity of *A. nemorosa* also  
338 appeared to benefit from a high settlement cover in the landscape, while expected and observed

339 heterozygosity of *P. multiflorum* decreased with increasing settlement cover (Figures 2c, e, m, and  
340 n).

341 The differentiation of crop types resulted mostly in higher marginal  $R^2$ -values than  
342 considering arable land in general, even though often only a single crop type yielded importance  
343 (Table 2a, b) or several crop types were highly correlated and were thus united in a principal  
344 component (Table 2c). All crop types had unique effects. The inbreeding coefficient of *A. nemorosa*  
345 populations was highest with an oilseed rape cover slightly above the mean (Figure 2f). As stated  
346 above, maize cover affected the expected heterozygosity of *A. nemorosa* and *O. acetosella*  
347 differently. Allelic richness and expected heterozygosity of *P. multiflorum* populations decreased  
348 with increasing cover of cereals and oilseed rape, which was, however, at the same time negatively  
349 correlated with grassland cover (Table 2, Figure 2k).

350 Linear landscape elements affected genetic diversity mostly independent of their prevalent  
351 orientation relative to the gene dispersal direction, with one exception (Figure 3g): water courses  
352 orthogonal to the gene flow direction reduced observed heterozygosity in *P. multiflorum*  
353 populations, while water courses pointing towards the populations enhanced observed  
354 heterozygosity.

355 The spatial scale, at which the species' genetic diversity responded to the landscape  
356 structure, was variable both across genetic diversity variables and landscape metrics (Table 2).  
357 Often, landscape effects were similarly high at several buffer distances (results not shown). For  
358 *P. multiflorum*, the majority (75%) of important landscape effects were most pronounced at a buffer  
359 distance  $\geq 1000$  m. No clear pattern occurred for the other two species.

360

361

### 362 3.2. Landscape effects at the link level

363 Twelve landscape metrics were involved in at least one important landscape effect on a genetic  
364 differentiation measure (Table 3). The proportion of variation explained uniquely by the landscape  
365 effects generally exceeded 90% of the total variation explained by fixed effects. This means that



366 geographic distance alone had little explanatory power. It determined, however, the magnitude and  
367 direction of several landscape effects (Figure 3).

368 Two landscape metrics had contrasting effects for the different species, forest cover and  
369 arable land. Genetic differentiation among *A. nemorosa* populations measured by  $D_{PS}$  was highest  
370 with an intermediate forest cover (Figure 2g), while for *O. acetosella*,  $D_{PS}$  was highest with a high  
371 forest cover (Figure 3d). When distance between populations was short, a high forest cover reduced  
372  $G''_{ST}$  among both *O. acetosella* and *P. multiflorum* populations. However, when distance between  
373 population was far, a high forest cover enhanced  $G''_{ST}$  among *O. acetosella* populations, but reduced  
374 it among *P. multiflorum* populations (Figures 3c and h). A high cover of arable land generally  
375 increased  $D_{PS}$  between *O. acetosella* populations (Figure 2j), whereas for *A. nemorosa*, it either  
376 increased or decreased  $D_{PS}$  depending on whether the distance between populations was short or  
377 far, respectively (Figure 3b).

378 Of the different crop types, only oilseed rape had a unique effect (Figure 3e). For short  
379 distances between *O. acetosella* populations,  $G''_{ST}$  was highest, when oilseed rape cover was high.  
380 With far distances between *O. acetosella* populations,  $G''_{ST}$  was highest, when oilseed rape cover  
381 was intermediate, but lowest when oilseed rape cover was either very high or low.

382 Two of the eight effects of linear landscape elements depended on the orientation of the  
383 landscape elements relative to the landscape strip (Table 3). For *O. acetosella*, woody linear elements  
384 running parallel to the landscape strip had little effect, but those running orthogonal to the  
385 landscape strip enhanced  $D_{PS}$  among populations (Figure 3f). For *P. multiflorum*, roads running  
386 parallel to the landscape strip reduced  $G''_{ST}$  among populations, whereas those running orthogonal  
387 to the landscape strip enhanced  $G''_{ST}$  (Figure 3i).

388 The width-to-length ratio of the landscape strips, at which genetic differentiation among  
389 populations was influenced most, was variable (Table 3). There was no clear difference among  
390 species. Most landscape effects (81%) were most pronounced at an intermediate width-to-length  
391 ratio (1:5 to 1:2).

392

393

#### 394 4. Discussion

395

396 Our results show that gene flow among spatially isolated forest herb populations in agricultural  
397 landscapes is influenced by a multitude of different land-use types and landscape elements that act  
398 at different spatial scales. The composition and configuration of the landscape prove here to be more  
399 important determinants of the forest herbs' landscape-scale population genetic structure than the  
400 size of local populations and their geographic distance to each other.

401

##### 402 4.1. Forest herbs respond differently to the landscape structure (H1)

403 Each species not only responded to a different set of landscape metrics, but also showed contrasting  
404 responses when affected by the same landscape metric (Tables 2 and 3). These contrasting responses  
405 were most pronounced for the cover of forest, arable land and settlements. Forest cover in  
406 landscape strips among populations affected the genetic differentiation among populations of all  
407 three species. For *O. acetosella*, a high forest cover in the landscape increased genetic differentiation  
408 among populations, particularly when distances among populations were far (Figures 3c and d),  
409 indicating that forest hampers far-distance gene dispersal. While forests do not represent  
410 insurmountable barriers for bees, they appear to enhance landscape resistance for forage flights  
411 (Kreyer et al. 2004; Goulson et al. 2010; Kamm et al. 2010; Zurbuchen et al. 2010). In contrast,  
412 *P. multiflorum* showed the lowest genetic differentiation among populations, when forest cover was  
413 high (Figure 3h). This stands in contrast to the observation that forest increases landscape resistance  
414 to bumblebee flights (Kreyer et al. 2004; Goulson et al. 2010). In fact, one of the most important  
415 pollinators of *P. multiflorum*, *Bombus pascuorum* (Naaf et al. 2021), was found to use floral resources  
416 in forests and open habitats at similar rates and at similar distances from their nests (Kreyer et al.  
417 2004). Thus, for this bumblebee species, forests do not represent barriers but even allow *B.*  
418 *pascuorum* to practice its typical trap line behaviour and to visit several *P. multiflorum* populations  
419 on a single forage flight (Kreyer et al. 2004). Moreover, there is ample evidence that seed dispersal

420 by both woodland birds (Heikkinen et al. 2004; Garcia et al. 2010) and carnivores (Herrera et al.  
421 2016) is enhanced with a high forest cover. Finally, genetic differentiation among *A. nemorosa*  
422 populations was highest with an intermediate forest cover in the landscape (Table 3, Figure 2g). This  
423 relationship might reflect a trade-off between effects that limit and promote gene flow. As  
424 mentioned above, a high forest cover might restrict bee movement through the landscape. However,  
425 it allows also a high richness and abundance of forest-dwelling hoverflies (Meyer et al. 2009;  
426 Proesmans, Bonte, et al. 2019; Schirmel et al. 2018) and a short distance to other *A. nemorosa*  
427 populations.

428 Besides forest, the cover of arable land affected the species' population genetic structure  
429 differently. While a high cover of arable land appeared to facilitate gene flow among *A. nemorosa*  
430 populations (Figure 2a and 3b), it apparently restricted gene flow among populations of *O. acetosella*  
431 (Figure 2j) and *P. multiflorum* (Figure 2k). One gene-dispersal vector for *A. nemorosa* that benefits  
432 from arable land are aphidophagous hoverflies. Many hoverflies, which prefer forests and hedgerows  
433 as their adult habitat, prefer cropland as their larval habitat, where they feed on aphid colonies  
434 (Meyer et al. 2009). In fact, several studies found positive relationships between the abundance and  
435 species richness of aphidophagous hoverflies and the proportion of arable land in the landscape  
436 (Jauker et al. 2009; Meyer et al. 2009; Haenke et al. 2014). Thus, high abundances of aphidophagous  
437 hoverfly species, such as *Melanostoma scalare*, *Platycheirus albimanus* or *Syrphus ribesii*, which are  
438 important pollinators of *A. nemorosa* (personal observations), might be responsible for the positive  
439 effect of arable land on allelic richness and its negative effect on genetic differentiation among  
440 *A. nemorosa* populations. This is apparently not true for *O. acetosella*, for which genetic  
441 differentiation among populations increased with increasing cover of arable land in the landscape.  
442 Since the most dominant crop types, i.e. cereals and maize (Supp. Inf. S4), do not provide any floral  
443 resources during spring, they occur at the cost of more valuable habitats for pollinators, such as  
444 grasslands (Jakobsson and Ågren 2014; Bartual et al. 2019). The trade-off between arable land and  
445 grassland was even clearer for *P. multiflorum*, for which allelic richness and expected heterogeneity  
446 decreased with increasing cover of arable land at the cost of grassland (Table 2, Figure 2k). Many

447 bumblebee species benefit from a high grassland cover in the landscape because grasslands may  
448 provide both nesting sites and floral resources (Goulson et al. 2010; Vray et al. 2019). Also, the  
449 occupancy of forest patches by woodland birds appears to be positively affected by the amount of  
450 grassland in contrast to the amount of arable fields in the surrounding landscape (Radford and  
451 Bennett 2007; Montague-Drake et al. 2009).

452           Moreover, settlements and roads affected the forest herbs differently. Close to villages, the  
453 road network is particularly dense. Therefore, the effects of settlement area and road density were  
454 often confounded (Table 2). For *P. multiflorum*, the lower genetic diversity in populations less than  
455 1 km away from settlements (Figures 2l-n) implies that here, the inflow of new alleles via pollen or  
456 seeds occurs at lower rates than at far distance from settlements. Two mechanisms, corresponding  
457 to different gene dispersal vectors, might explain this pattern. First, woodland birds might avoid  
458 landscapes with more settlements and roads (Dunford and Freemark 2005; Rüdissler et al. 2015) and  
459 therefore transport *P. multiflorum* seeds less often into nearby forest patches. However, woodland  
460 birds were found to respond most strongly to landscape composition at a smaller spatial scale of 100  
461 to 500 m (Rüdissler et al. 2015). Second, settlements in our agricultural landscapes consist mostly of  
462 rural villages with many gardens. Gardens are important forage habitats for bumblebees in rural  
463 landscapes (Goulson et al. 2010; Nakamura and Kudo 2019). The flowering period of fruit trees such  
464 as *Malus* spp., *Pyrus* spp. or *Prunus* spp. as well as ornamental shrubs such as *Syringa vulgaris*  
465 overlaps with that of *P. multiflorum*. These ample floral resources might attract bumblebees and  
466 reduce bumblebee abundance and their pollination service in nearby forest patches (Nakamura and  
467 Kudo 2019; Proesmans, Smagghe, et al. 2019). Interestingly, at a smaller spatial scale (250 m), the  
468 inbreeding coefficient for *P. multiflorum* was negatively related to settlement area (Figure 2o),  
469 indicating that *P. multiflorum* populations very close to villages might benefit from bumblebee  
470 spill-over (Cussans et al. 2010). In contrast to *P. multiflorum*, *A. nemorosa* appeared to benefit from  
471 settlement areas in the landscape (250-500 m), at least in terms of expected and observed  
472 heterozygosity (Figures 2c and e). Close to villages, different foraging, resting or nesting habitats for  
473 pollinators, such as gardens and hedgerows, have a relatively high density. They might sustain a high

474 abundance of pollinators (Cussans et al. 2010; Garratt et al. 2017; Schirmel et al. 2018; Bartual et al.  
475 2019), which then spill over to nearby forest patches to feed on pollen of *A. nemorosa* in early spring.

476 In general, there was no clear difference in the spatial scale, at which the three species  
477 responded to the landscape structure. The prevalent buffer distances  $\geq 1000$  m for *P. multiflorum*  
478 reflect its association with far-flying bumblebees. Important pollinator species such as *Bombus*  
479 *pascuorum* and *B. pratorum* (Naaf et al. 2021) may forage over distances  $> 1800$  m (Redhead et al.  
480 2016) and  $> 670$  m (Knight et al. 2005), respectively.

481

#### 482 4.2. Distinguishing crop types matters (H2)

483 In nine out of 11 landscape effects, in which arable crops were involved (Tables 2 and 3), a  
484 differentiation of crop types resulted in higher proportions of explained variation than merging the  
485 different crop types into arable land in general. In particular oilseed rape and maize had distinct  
486 effects, which is remarkable considering that both crop types represent relatively young elements in  
487 European landscapes that were more or less absent seven decades ago (Knoema 2020). There was  
488 thus limited time for them to leave their imprint in the forest herbs' population genetic structure. We  
489 had expected to find any effects of oilseed rape or maize most likely for *O. acetosella* given its  
490 shorter generation time compared to *A. nemorosa* and *P. multiflorum* (Naaf et al. 2021). Indeed,  
491 oilseed rape affected the genetic differentiation among populations of *O. acetosella* (Figure 3e),  
492 while maize influenced genetic diversity within its populations (Figures 2h and i). The high genetic  
493 differentiation among *O. acetosella* populations separated by a matrix of high oilseed rape cover  
494 might reflect a dilution effect (Holzschuh et al. 2011 2016). The flowering periods of oilseed rape and  
495 *O. acetosella* greatly overlap and oilseed rape is highly attractive for various pollinators (Haenke et al.  
496 2014; Riedinger et al. 2015). The attraction of pollinators by oilseed rape might lead to diluted  
497 pollinators in adjacent forests (Holzschuh et al. 2011; Van Reeth et al. 2019), where *O. acetosella*  
498 plants might receive little compatible pollen from other forest patches. However, when oilseed rape  
499 cover in the landscape strip was very high and distances between populations were  $> 2300$  m, we  
500 observed a reduced genetic differentiation among *O. acetosella* populations (Figure 3e). In this case,

501 the landscape strip crossed large parts of the landscape window. A permanently high oilseed rape  
502 cover across the landscape window might result in positive population growth rates of pollinators  
503 and thus higher abundances in the long term. For instance, the abundance of solitary bees was found  
504 to be enhanced by a high oilseed rape cover in the preceding year (Riedinger et al. 2015).

505 The negative effect of maize cover on expected heterozygosity of *O. acetosella* populations  
506 (Figure 2h) indicates that a high cover of maize reduces the inflow of new alleles. Also the inbreeding  
507 coefficient of *O. acetosella* populations increased with maize cover, potentially reflecting pollen  
508 limitation (Figure 2i). However, this increase levelled off and changed into a decrease when maize  
509 cover was very high. Since pollen limitation is unlikely to decrease, when maize cover is very high,  
510 enhanced seed dispersal might prevent inbreeding under these circumstances. Maize fields are a  
511 very attractive forage and shelter habitat for wild boar during summer and autumn (Keuling et al.  
512 2009; Tillmann 2011), when the cleistogamous seeds of *O. acetosella* are ripe (Berg and Redbo-  
513 Torstensson 1998). Some of these animals appear to move regularly between the maize fields and  
514 forests (Keuling et al. 2009) and might thus disperse seeds of *O. acetosella*.

515 Despite its longer generation time, also *A. nemorosa* showed some responses to oilseed rape  
516 and maize. Populations with a high oilseed rape cover in the surrounding landscape showed an  
517 increased inbreeding signal (Figure 2f). Although *A. nemorosa* starts flowering earlier than oilseed  
518 rape, there might be an overlapping flowering period of one or two weeks. Thus, as with  
519 *O. acetosella*, the increased inbreeding signal might result from pollinator dilution (Holzschuh et al.  
520 2011; Van Reeth et al. 2019). The positive effect of maize cover on expected heterozygosity of  
521 *A. nemorosa* populations (Figure 2d) was unexpected (Table 1) and difficult to explain. Given the  
522 young history of maize in our landscapes in relation to the long generation time of *A. nemorosa*, this  
523 result should be considered with caution.

524

525

526 *4.3. The orientation of linear landscape elements matters (H3)*

527 Our results show that linear landscape elements, including woody line elements, water courses,  
528 herbaceous fringes and roads, influence the movement of gene dispersal vectors across the  
529 landscape. The orientation of the linear landscape elements was important in three out of 16 effects  
530 (Tables 2 and 3). Here, the linear landscape elements appeared to have a promoting or hampering  
531 effect on gene flow as hypothesized, depending on whether they were predominantly oriented  
532 parallel or orthogonal to the gene dispersal pathways (Figures 3f, g, and i). These results confirm the  
533 observation that linear landscape elements may not just channel animal movements, but may also  
534 act as relative barrier (Wratten et al. 2003; Levey et al. 2005; Saïd and Servanty 2005; Krewenka et al.  
535 2011; Klaus et al. 2015; Fitch and Vaidya 2021).

536 At large spatial scales (2000 m radius around populations), orthogonal and parallel  
537 orientations of linear landscape elements can be expected to be balanced. For this situation, we  
538 found a strong positive effect of road density on the inbreeding coefficient of *P. multiflorum*  
539 populations, indicating that roads restrict outbreeding (Table 2, Figure 2p). Whether this effect is  
540 mediated through pollen or seed dispersers or both remains unclear. Bumblebees as well as many  
541 rodents seem to be reluctant to cross roads (Trombulak and Frissell 2000; Bhattacharya 2003).

542 Also at the 2000 m scale, there was a strong unimodal response of allelic richness in  
543 *A. nemorosa* populations to the density of woody linear elements (Table 2, Figure 2b). These  
544 appeared to promote allelic richness up to an above-average density in the landscape. This effect  
545 might result from a higher abundance of pollinators in landscapes with a high hedgerow density and  
546 spill-over from hedgerows to adjacent forests. Hedgerows represent important resting and foraging  
547 habitats for both hoverflies (Haenke et al. 2014; Garratt et al. 2017; Schirmel et al. 2018) and bees  
548 (Garratt et al. 2017; Bartual et al. 2019). The subsequent decrease in allelic richness at an even higher  
549 density of woody linear elements was mainly due to the French populations (Figure 2b). On the one  
550 hand, this decrease could result from an increasing resistance that woody linear elements constitute  
551 for pollinator movements (Wratten et al. 2003; Krewenka et al. 2011; Klaus et al. 2015). This  
552 interpretation would be analogous to that of the unimodal effect of forest cover on genetic  
553 differentiation among *A. nemorosa* populations (see above, Figure 2g). On the other hand, the high

554 hedgerow density in the French landscape window is rather recent and established only in the 19<sup>th</sup>  
555 century (Jamoneau et al. 2012). Thus, the time passed since then might not suffice for the hedgerows  
556 to leave their imprint in the genetic diversity of *A. nemorosa* populations.

557

558

## 559 **5. Conclusions**

560

561 Our study shows that, more than habitat loss and fragmentation *per se*, the composition and  
562 configuration of the agricultural landscape matrix exerts significant control over the population  
563 genetic structure, and thus the functional connectivity, in fragmented temperate forest herb  
564 populations. Although we could only discuss rather than reveal the underlying mechanisms of the  
565 observed landscape effects, it became obvious that a multitude of mechanisms are at work in our  
566 landscapes. In this respect, our study has generated many hypotheses, which deserve to be tested in  
567 depth. The observed landscape effects turned out to be rather species-specific. Nevertheless, they  
568 are general in the sense that they were consistent across multiple agricultural landscapes across  
569 Europe.

570         Landscape planning with the aim to enhance the functional connectivity among spatially  
571 isolated plant populations in agricultural landscapes should consider that (a) species of the same  
572 ecological guild (e.g., forest herbs) might respond quite differently to the landscape structure, if they  
573 are associated with different pollen or seed dispersal vectors; (b) it may be worth to differentiate  
574 crop types rather than merging them into arable crops in general given their distinct effects on  
575 functional connectivity; and (c) linear landscape elements that are mostly perceived as relative  
576 barriers for animals, such as roads, may also have a channelling effect on their movement and thus  
577 promote gene flow, whereas linear landscape elements that are mostly perceived as connecting  
578 corridors, such as hedgerows, may also act as barrier for gene dispersal vectors.

579

580



581

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857

859 **Table 1** Landscape metrics used to study the effects of the landscape composition and configuration on the population genetic structure of the temperate forest herbs  
 860 *Anemone nemorosa*, *Oxalis acetosella*, and *Polygonatum multiflorum*. For each landscape metric, the columns provide the definition and the expected effects on gene flow based  
 861 on available literature (references indicated by superscripts are provided in Table S5.1). The minimum, median and maximum value of each landscape metric across all populations  
 862 for a 1000 m buffer zone is given in parentheses. The potential mechanisms via pollen- or seed-dispersal vectors behind expected effects are detailed in Table S5.2

Landscape metric	Definition (min, median, max)	Expected effect on gene flow among populations <sup>a</sup>			
		Species: Vector:	<i>Anemone nemorosa</i> <i>Oxalis acetosella</i> hoverflies, bees	<i>Oxalis acetosella</i> wild boar, deer	<i>Polygonatum multiflorum</i> bumblebees
<b>Area-based metrics</b>					
	Percent cover of ...				
FOREST	deciduous forest (2.5; 8; 23.6)	- 40, 46, 81, 84, <b>0</b> 51, 86, + 30, 50, 54, 59, 60, 70	+ 55, 56	- 22, 40, 46, 81, 84, <b>0</b> 51	+ 5, 9, 20, 29, 31, 34, 38, 63, 67, 78
GRASS	grassland in general (2.9; 18.7; 78.9)	<b>0+</b> 3, 11, 39, 45, 66, 72, 73	<b>0+</b> 2, 71	<b>0+</b> 22, 37, 80	<b>0+</b> 5, 23, 52, 63
SEM-NAT-GRASS	semi-natural grassland (0; 1.3; 7.3)	+ 3, 11, 39, 45, 66, 72, 73	<b>0+</b> 2, 71	- 64, + 11, 17, 22, 61, 80	+ 5, 23, 52, 63
SEM-NAT-VEG	other semi-natural vegetation (0; 0.7; 8.9)	+ 3, 11, 39, 66, 72	<b>0</b>	- 64, + 11, 17, 61	+ 23
ARABLE	arable land in general (10.6; 58.8; 81.9)	- 27, -+ 25, 50, 74	+ 2, 58, 71	- 27	- 5, 29, 63, 67, 68
RAPE	arable land cultivated with oilseed rape (0; 4; 17.3)	- 35, 36, 53, 79, -+ 44, + 7, 25, 65	<b>0</b>	- 17, 35, 36, <b>0</b> 22, -+ 44, 83, + 26, 33, 43, 82	- 5, 29, 63, 67, 68
MAIZE	arable land cultivated with maize (0; 8.1; 43.4)	- 27	+ 2, 8, 32, 41, 71, 76	- 27	- 5, 29, 63, 67, 68
CEREAL	arable land cultivated with other cereals (2; 21.4; 52.4)	-+ 25, 50, 74	+ 2, 58, 71	<b>0</b>	- 5, 29, 63, 67, 68
ORCHARD	traditional grassland orchards (0; 0.1; 3)	- 35, + 3, 11, 39, 66, 72	<b>0</b>	- 61	<b>0</b>
SETTLE	settlement area (0; 4.3; 19)	<b>0</b>	- 8, 13	- 57, -+ 15, 22	- 5, 13, 67
	+ conditioning variable: PROPGREEN = Prop. of green urban areas				
<b>Linear landscape elements</b>					
	Relative length [m ha <sup>-1</sup> ] of ...				
LWOOD	hedgerows and tree lines (0.4; 27.3; 135.3)	- 85, -+ 12, 42, 45, + 1, 16, 21, 25, 59, 70	-+ 69, 75	-+ 12, 14, 21, 42	<b>0+</b> 18, 24, 47, + 4, 5, 20, 34, 38
	+ conditioning variable: O:P_LWOOD = Orthogonal-to-parallel ratio				
LWATER	water courses (incl. draining ditches) (0.9; 11.8; 37.3)	-+ 12, + 1	-+ 69, 75	-+ 12	<b>0</b>
	+ conditioning variable: O:P_LWATER = Orthogonal-to-parallel ratio				
LFRINGE	broad herbaceous fringes (0; 3.3; 21.3)	-+ 12,	-+ 69, 75	-+ 12, 43,	<b>0+</b> 5, 52, 63
	+ conditioning variable: O:P_LFRINGE = Orthogonal-to-parallel ratio	+ 3, 11, 39, 45, 66, 72, 73		+ 11, 17, 22, 37, 61	
LROAD	roads (0.7; 15.6; 36.2)	- 48, -+ 12	-+ 10, 13, 69, 75, 77	-+ 6, 12	- 5, 13, 19
	+ conditioning variable: O:P_LROAD = Orthogonal-to-parallel ratio				
<b>Index metrics</b>					
SHANNON	Diversity of land-use types (0.9; 1.7; 2.1)	+ 11, 72	<b>0</b>	+ 11, 72	+ 29, 67
EDGEDEN	Land-use parcel edge density [m ha <sup>-1</sup> ] (55; 131; 294)	-+ 49, + 28, 72	- 62, 69	+ 72	- 69, + 67, 72

863 <sup>a</sup> Effect codes: - negative, + positive, -+ negative or positive depending on conditions, 0 no effect, 0+ no or positive effect depending on conditions

864 **Table 2** Summary of landscape effects on allelic richness ( $A_r$ ), expected ( $H_e$ ) and observed heterozygosity ( $H_o$ ) and  
865 the inbreeding coefficient ( $F$ ) as resulting from linear mixed-effects models at the node level, separately for (a)  
866 *Anemone nemorosa*, (b) *Oxalis acetosella*, and (c) *Polygonatum multiflorum*. All important effects (importance  
867 value  $\geq 0.5$ , see main text), are symbolized as follows: / positive effect, \ negative effect, X interactive effect (see  
868 Figure 3),  $\cap$  and  $\cup$  unimodal effect with maximum and minimum, respectively, at an intermediate level of the  
869 landscape metric, / $\cap$ , \ $\cap$  and / $\cup$  asymmetric unimodal effects with positive or negative trend, respectively. In  
870 addition,  $R^2$  values for each model are provided: marginal  $R^2$  (variation explained by fixed effects, i.e. jointly by  
871 basic population genetic determinants and landscape metrics), landscape  $R^2$  (variation explained uniquely by  
872 landscape metrics) and the percentage of the marginal  $R^2$  that can be uniquely attributed to landscape effects  
873 (%Landscape). See Table 1 for explanations on variable names. Numbers added to the variable names correspond  
874 to the most influential buffer distance in meters. See Table S6.1 for complete model results. Visualizations of the  
875 effects are presented in Figures 2, 3, and S6.1 to S6.3

Landscape metrics	$A_r$	$H_e$	$H_o$	$F$
<b>(a) <i>Anemone nemorosa</i></b>				
FOREST_2000			\	
ARABLE_2000	/			
RAPE_500				/ $\cap$
MAIZE_1000		/		
pcSETTLE_250 <sup>a</sup>			/ $\cup$	
pcSETTLE_500 <sup>b</sup>		/		
LROAD_250				\ $\cap$
LWOOD_2000	/ $\cap$			
LFRINGE_500	\			
Marginal $R^2$	0.74	0.38	0.60	0.48
Landscape $R^2$	0.56	0.29	0.58	0.41
% Landscape	76.4	74.9	97.7	84.7
<b>(b) <i>Oxalis acetosella</i></b>				
SEMSTATGRASS_1000			$\cup$	
SEMSTATVEG_500		/ $\cap$	/	
MAIZE_250		\ $\cap$		
MAIZE_1000				/ $\cap$
pcLWOODGRASS_1000 <sup>c</sup>				/
LFRINGE_2000				\
LROAD_500	$\cap$			
EDGEEDEN_125			\	
Marginal $R^2$	0.42	0.57	0.42	0.47
Landscape $R^2$	0.08	0.30	0.19	0.47
% Landscape	19.5	53.0	45.2	99.5
<b>(c) <i>Polygonatum multiflorum</i></b>				
FOREST_2000				/
SEMSTATGRASS_250		$\cap$		
pcARABvsGRASS_2000 <sup>d</sup>	\	\		
SETTLE_250				\
SETTLE_1000	\	\		
pcSETTLE_1000 <sup>e</sup>			\	
LWATER_1000			X	
LWATER_2000	/			
SHANNON_250	/			
LROAD_2000				/
Marginal $R^2$	0.76	0.38	0.19	0.58

Landscape $R^2$	0.42	0.24	0.01	0.42
% Landscape	55.9	63.6	4.1	72.3

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876 <sup>a</sup> Principal component from SETTLE\_250 ( $r = 0.94$ ) and LROAD\_250 ( $r = 0.94$ )

877 <sup>b</sup> Principal component from SETTLE\_500 ( $r = 0.93$ ), LROAD\_500 ( $r = 0.89$ ) and EDGEDEN\_500 ( $r = 0.90$ )

878 <sup>c</sup> Principal component from GRASS\_1000 ( $r = 0.94$ ) and LWOOD\_1000 ( $r = 0.94$ )

879 <sup>d</sup> Principal component from CEREAL\_2000 ( $r = 0.97$ ), RAPE\_2000 ( $r = 0.90$ ), and GRASS\_2000 ( $r = -0.95$ )

880 <sup>e</sup> Principal component from SETTLE\_1000 ( $r = 0.95$ ) and LROAD\_1000 ( $r = 0.95$ )

881

882 **Table 3** Summary of landscape effects on measures of pairwise genetic differentiation ( $G''_{ST}$  and  $D_{PS}$ ) as resulting  
883 from MLPE models at the link level, separately for (a) *Anemone nemorosa*, (b) *Oxalis acetosella*, and (c)  
884 *Polygonatum multiflorum*. All important effects, i.e. those with an importance value  $\geq 0.5$  (see main text), are  
885 symbolized as follows: / positive effect, \ negative effect, X interactive effect (see Figure 3),  $\cap$  and U unimodal  
886 effect with maximum and minimum, respectively, at an intermediate level of the landscape metric, / $\cap$ , \ $\cap$  and  
887 /U asymmetric unimodal effects with positive or negative trend, respectively. In addition,  $R^2$  values for each  
888 model are provided: marginal  $R^2$  (variation explained by fixed effects, i.e. jointly by basic population genetic  
889 determinants and landscape metrics), landscape  $R^2$  (variation explained uniquely by landscape metrics) and the  
890 percentage of the marginal  $R^2$  that can be uniquely attributed to landscape effects (%Landscape). See Table 1 for  
891 explanations on variable names. The ratio added to each variable name corresponds to the most influential  
892 width-to-length ratio of the landscape strips. See Table S6.2 for complete model results. Visualizations of the  
893 effects are presented in Figures 2, 3, and S6.1 to S6.3

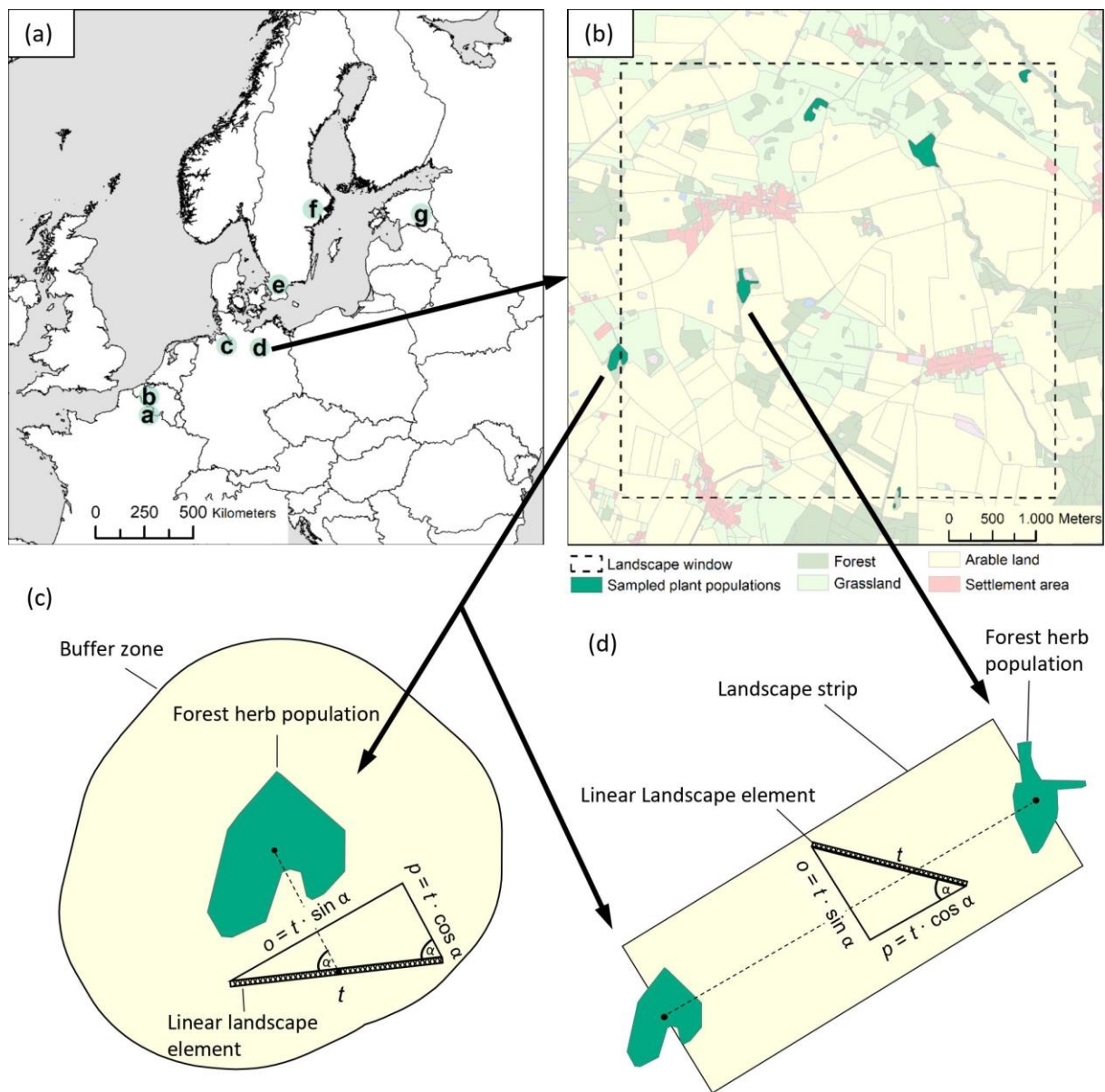
894

Landscape metrics	$G''_{ST}$	$D_{PS}$
<b>(a) <i>Anemone nemorosa</i></b>		
FOREST_1:3	/	$\cap$
pcARABvsGRASS_1:3 <sup>a</sup>	X	
pcARABvsGRASS_1:2 <sup>b</sup>		X
LFRINGE_1:3	/	/
Marginal $R^2$	0.16	0.25
Landscape $R^2$	0.16	0.25
% Landscape	99.2	100.0
<b>(b) <i>Oxalis acetosella</i></b>		
FOREST_1:3	X	X
ARABLE_1:3		/
RAPE_1:2	X	
LWOOD_1:7		X
LWATER_1:3	\	
LROAD_1:5	\	
SHANNON_2:3		\
Marginal $R^2$	0.11	0.10
Landscape $R^2$	0.11	0.09
% Landscape	99.9	99.4
<b>(c) <i>Polygonatum multiflorum</i></b>		
FOREST_1:2	X	
SEMSTATGRASS_1:3	/U	/
ORCHARD_2:3		/
LWOOD_1:5		\
LROAD_1:7	X	
LROAD_1:2		\
Marginal $R^2$	0.18	0.33
Landscape $R^2$	0.17	0.30
% Landscape	95.7	91.4

895 <sup>a</sup> Principal component from CEREAL\_1:3 ( $r = 0.95$ ), RAPE\_1:3 ( $r = 0.90$ ) and GRASS\_1:3 ( $r = -0.92$ )

896 <sup>b</sup> Principal component from CEREAL\_1:2 ( $r = 0.96$ ), RAPE\_1:2 ( $r = 0.90$ ) and GRASS\_1:2 ( $r = -0.92$ )

897

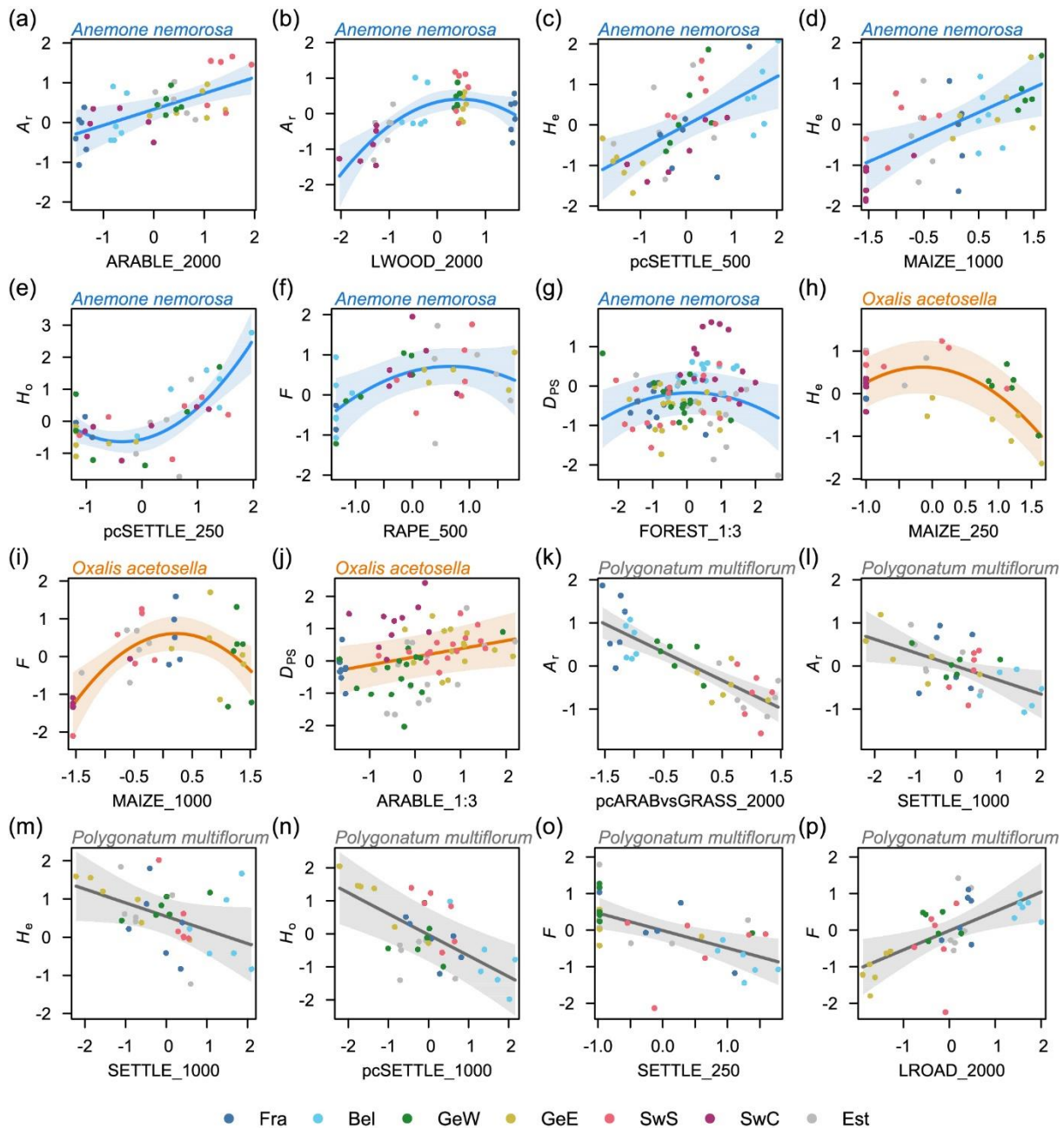


900

901 **Fig. 1** Overview on the study design. The seven  $5 \times 5 \text{ km}^2$  landscape windows (a) are spread across  
 902 temperate Europe from North France (A) over Belgium (B), West Germany (C), East Germany (D), and  
 903 South Sweden (E) up to Central Sweden (F) and Estonia (G). In each agricultural landscape, we  
 904 surveyed up to six populations of each species, here exemplified for *Polygonatum multiflorum* in East  
 905 Germany (b). See Supp. Inf. 1 for detailed land-use maps of each landscape window, including  
 906 locations of all sampled populations. The landscape surrounding the populations was analysed at the  
 907 node level, i.e., in buffer zones of distances between 125 and 2000 m (c), and at the link level, i.e. in



908 landscape strips between populations with width-to-length ratios between 1:7 and 2:3 (d). For linear  
909 landscape elements within buffer zones and landscape strips, we calculated not only the total length  
910 ( $t$ ), but also the parallel ( $p$ ) and orthogonal ( $o$ ) length component in relation to the gene dispersal  
911 direction  
912  
913



914

915 **Fig. 2** Visualization of landscape effects (cf. Tables 2 and 3) on population genetic variables of

916 *Anemone nemorosa* (*A. nem.*), *Oxalis acetosella* (*O. ace.*) and *Polygonatum multiflorum* (*P. mul.*).

917 Shown are those effects, which are directly related to our hypotheses and therefore discussed in the

918 main text. Panels display the partial slopes and residuals as well as the 95% confidence band. All

919 variables are scaled in standard deviation units. Colours of partial residuals represent the different

920 landscape windows: France (Fra), Belgium (Be), West Germany (GeW), East Germany (GeE), South

921 Sweden (SwS), Central Sweden (SwC), and Estonia (Est). Population genetic variables are allelic

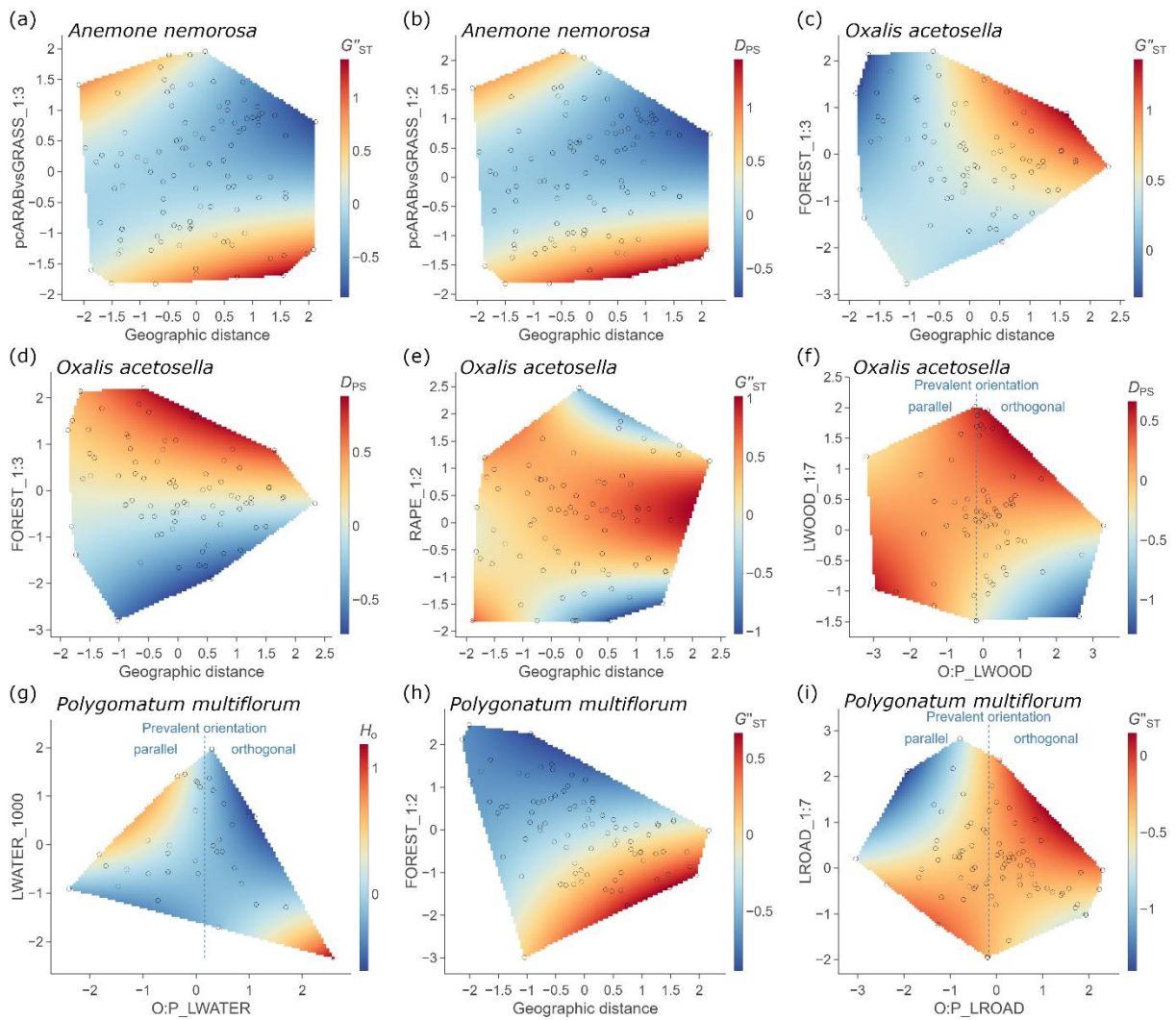
922 richness ( $A_r$ ), expected ( $H_e$ ) and observed heterozygosity ( $H_o$ ), inbreeding index ( $F$ ), and genetic

923 differentiation ( $D_{ps}$ ). The landscape metrics ARABLE, FOREST, MAIZE, RAPE, and SETTLE refer to the  
924 percent cover of arable land, deciduous forest, maize, oilseed rape and settlement area, respectively.  
925 LWOOD and LROAD refer to the relative length of hedgerows/tree lines and roads, respectively.  
926 pcSETTLE is a principal component reflecting settlement area, road density and edge density (cf.  
927 Table 2). pcARABvsGRASS is a principal component reflecting the trade-off between arable land  
928 (cereals and oilseed rape) on the one hand and grassland on the other hand (cf. Table 2). Numbers or  
929 ratios added to the variable names correspond to the most influential buffer distance in meters or  
930 the most influential width-to-length ratio of the landscape strips, respectively

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**Fig. 3** Visualizations of interactive effects (cf. Tables 2 and 3) of landscape metrics (x- and y-axis) on

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population genetic variables of *Anemone nemorosa* (*A. nem.*), *Oxalis acetosella* (*O. ace.*) and

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*Polygonatum multiflorum* (*P. mul.*) as heatmap. Only the area covered by the values of both

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explanatory variables (overlaid as points) is plotted. All variables are scaled in standard deviation

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units. Population genetic variables are measures of genetic differentiation ( $D_{PS}$  and  $G''_{ST}$ ) and

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observed heterozygosity ( $H_0$ ). The landscape metrics FOREST and RAPE refer to the percent cover of

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deciduous forest and oilseed rape, respectively. LROAD, LWATER and LWOOD refer to the relative

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length of roads, water courses and hedgerows/treelines, respectively. The prefix O:P refers to

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orthogonal-to-parallel length ratio. pcARABvsGRASS is a principal component reflecting the trade-off

944

between arable land (cereals and oilseed rape) on the one hand and grassland on the other hand (cf.

945 Table 3). Numbers or ratios added to the variable names correspond to the most influential buffer  
946 distance in meters or the most influential width-to-length ratio of the landscape strips, respectively  
947  
948