

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

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# 1 Context matters: the landscape matrix determines the population genetic structure of temperate

## 2 forest herbs across Europe

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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 enhance the functional connectivity among spatially isolated plant populations should consider that 44 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

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52

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- 81

### 82 Authors' contributions

TN, SIJH and KK conceived and designed the study. All authors except JLe and JP were involved in site

selection, field work and sampling. JTF, SH, TN and KK performed the molecular lab work and did the

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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96 1. Introduction

97

98 In many regions on earth, the progressive occupation of land by humans for settlements and agriculture has forced wildlife and wildflowers to live in small remaining fragments of once 99 100 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 wild plants in agricultural landscapes (Cussans et al. 2010; Jakobsson and Ågren 2014; Chateil and 148 149 Porcher 2015; Lindgren et al. 2018). Apart from floral resources, pollinators may depend on further

food resources. Larva of aphidophagous hoverflies, for instance, whose preferred adult habitat might
be deciduous forest, find their prey mostly in arable fields (Meyer et al. 2009). They can profit from
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 rates, and thus functional connectivity, among forest herb populations. Here, we therefore 164 165 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. 2014) and tested the following main hypotheses: 174

H1 Landscape effects on the population genetic structure differ among the three forest herbs
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.
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184	2. M	aterial and Methods
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186	2.1.St	udy species
187	The th	ee studied forest herbs were selected for being typical, common slow-colonizing forest
188	special	ists (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	strateg	y. Oxalis acetosella has been found to produce most of its seeds from cleistogamous flowers
192	(Berg a	nd Redbo-Torstensson 1998), thus it is less dependent on insect pollinators for sexual
193	reprod	uction. According to our own research, however, O. acetosella is mainly outcrossing (Naaf et
194	al. 202	1). Anemone nemorosa and Polygonatum multiflorum are mainly and strictly outcrossing,
195	respec	tively (Müller et al. 2000; Kosiński 2012). Oxalis acetosella and A. nemorosa are pollinated by a
196	wide ra	ange of pollinators, including hoverflies, wild bees and honey bees (Shirreffs 1985; Redbo-
197	Torster	nsson and Berg 1995; Stehlik and Holderegger 2000; Naaf et al. 2021). In contrast,
198	P. mult	iflorum is mostly pollinated by long-tongued bumblebees (Kosiński 2012; Naaf et al. 2021). All
199	three s	pecies 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 slu	gs (Türke et al. 2012). Seeds of <i>O. acetosella</i> were found in the fur of wild boar by several
202	indepe	ndent studies, though at low quantities (Mrotzek et al. 1999; Heinken and Raudnitschka
203	2002).	The fleshy berries of <i>P. multiflorum</i> 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).

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#### 209 2.2. Population genetic structure

210 We compiled population genetic data from seven 5 × 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  $>12*10^6$  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  $F = 1 - H_0 / H_e$ , 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<sub>PS</sub> 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

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

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#### 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 measures, i.e. the Shannon diversity of land-use types and the density of all land-use patch edges 255 256 (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.

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#### 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

included population size and isolation or geographic distance, respectively, as fixed effects that could
not be removed during model selection. At the link level, we also allowed for interactions between
basic determinant and landscape metrics, which was not possible at the node level due to the limited
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  $AIC_{\rm c}$  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<sub>c</sub>. 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<sub>c</sub>. 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  $|r| \ge 0.7$  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).

Third, all remaining metrics entered the global model, which was then used for model selection followed by multi-model inference with the R package MuMIn (Barton 2019). In this step, 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 $\Delta A/C_{\rm C}$ < 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.
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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

heterozygosity of *P. multiflorum* decreased with increasing settlement cover (Figures 2c, e, m, andn).

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 <i>P. multiflorum</i>
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 and367 direction of several landscape effects (Figure 3).

Two landscape metrics had contrasting effects for the different species, forest cover and 368 369 arable land. Genetic differentiation among A. nemorosa populations measured by D<sub>PS</sub> was highest 370 with an intermediate forest cover (Figure 2g), while for O. acetosella, D<sub>PS</sub> 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<sub>PS</sub> between O. acetosella populations (Figure 2j), whereas for A. nemorosa, it either 376 increased or decreased D<sub>PS</sub> depending on whether the distance between populations was short or 377 far, respectively (Figure 3b).

Of the different crop types, only oilseed rape had a unique effect (Figure 3e). For short distances between *O. acetosella* populations, *G*<sup>"</sup><sub>ST</sub> was highest, when oilseed rape cover was high. With far distances between *O. acetosella* populations, *G*<sup>"</sup><sub>ST</sub> was highest, when oilseed rape cover was intermediate, but lowest when oilseed rape cover was either very high or low.

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

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

### **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

to bumblebee flights (Kreyer et al. 2004; Goulson et al. 2010). In fact, one of the most important

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 grasslands (Jakobsson and Ågren 2014; Bartual et al. 2019). The trade-off between arable land and 444 445 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

bumblebee species benefit from a high grassland cover in the landscape because grasslands may provide both nesting sites and floral resources (Goulson et al. 2010; Vray et al. 2019). Also, the occupancy of forest patches by woodland birds appears to be positively affected by the amount of grassland in contrast to the amount of arable fields in the surrounding landscape (Radford and 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 2I-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; Rüdisser 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 (Rüdisser 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 settlement areas in the landscape (250-500 m), at least in terms of expected and observed 471 472 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

abundance of pollinators (Cussans et al. 2010; Garratt et al. 2017; Schirmel et al. 2018; Bartual et al.
2019), which then spill over to nearby forest patches to feed on pollen of *A. nemorosa* in early spring.
In general, there was no clear difference in the spatial scale, at which the three species
responded to the landscape structure. The prevalent buffer distances ≥ 1000 m for *P. multiflorum*reflect its association with far-flying bumblebees. Important pollinator species such as *Bombus pascuorum* and *B. pratorum* (Naaf et al. 2021) may forage over distances > 1800 m (Redhead et al.
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,

the landscape strip crossed large parts of the landscape window. A permanently high oilseed rape
cover across the landscape window might result in positive population growth rates of pollinators
and thus higher abundances in the long term. For instance, the abundance of solitary bees was found
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 rape, there might be an overlapping flowering period of one or two weeks. Thus, as with 518

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

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 <i>A. nemorosa</i> populations.
557	

#### 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.

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## 858 Tables

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

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

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  $\ge 0.5$ , see main text), are symbolized as follows: / positive effect, \ negative effect, X interactive effect (see 868 Figure 3),  $\Pi$  and U unimodal effect with maximum and minimum, respectively, at an intermediate level of the 869 landscape metric,  $/\Omega$ ,  $\backslash\Omega$  and /U 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<sup>2</sup> 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 to the most influential buffer distance in meters. See Table S6.1 for complete model results. Visualizations of the
- 874
- 875 effects are presented in Figures 2, 3, and S6.1 to S6.3

Landscape metrics	Ar	He	Ho	F
(a) Anemone nemorosa FOREST_2000			١	
ARABLE_2000 RAPE_500	/			/∩
MAIZE_1000 pcSETTLE_250 ª		/	/U	
pcSETTLE_500 <sup>b</sup> LROAD_250	1-	/		\∩
LWOOD_2000 LFRINGE_500	\ \			
Marginal R <sup>2</sup>	0.74	0.38	0.60	0.48
% Landscape	76.4	74.9	97.7	84.7
(b) Oxalis acetosella				
SEMNATGRASS_1000 SEMNATVEG_500		/∩	U /	
MAIZE_250 MAIZE_1000		λΠ		/N /
LFRINGE_2000 LROAD 500	Π			$\backslash$
EDGEDEN_125			١	
Marginal R <sup>2</sup>	0.42	0.57	0.42	0.47
Kandscape R <sup>2</sup> % Landscape	0.08 19.5	0.30 53.0	0.19 45.2	0.47 99.5
(c) Polygonatum multiflorum				
FOREST_2000 SEMNATGRASS_250		n		/
pcARABvsGRASS_2000 <sup>a</sup> SETTLE_250	\	\		١
SETTLE_1000 pcSETTLE_1000 °	١	١	$\langle \rangle$	
LWATER_1000 LWATER_2000 SHANNON 250	/		^	
LROAD_2000	/			/

	Marginal R <sup>2</sup>	0.76	0.38	0.19
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0.58

Landscape R <sup>2</sup>	0.42	0.24	0.01	0.42
% Landscape	55.9	63.6	4.1	72.3

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)

879 880 <sup>d</sup> Principal component from CEREAL\_2000 (*r* = 0.97), RAPE\_2000 (*r* = 0.90), and GRASS\_2000 (*r* = -0.95)

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

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), N and U unimodal 886 effect with maximum and minimum, respectively, at an intermediate level of the landscape metric,  $/\Omega$ ,  $\backslash\Omega$  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<sup>2</sup> 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 radio 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

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Landscape metrics	<b>G</b> ′′ <sub>st</sub>	D <sub>PS</sub>
(a) Anemone nemorosa		
FOREST_1:3	/	Π
pcARABvsGRASS_1:3 a	х	
pcARABvsGRASS_1:2 <sup>b</sup>		Х
LFRINGE_1:3	/	/
Marginal R <sup>2</sup>	0.16	0.25
Landscape R <sup>2</sup>	0.16	0.25
% Landscape	99.2	100.0
(b) Oxalis acetosella		
FOREST 1:3	х	Х
ARABLE 1:3		/
RAPE 1:2	х	,
LWOOD 1:7		Х
LWATER 1:3	\	
LROAD 1:5	Ň	
SHANNON_2:3		١
Marginal R <sup>2</sup>	0.11	0.10
Landscape R <sup>2</sup>	0.11	0.09
% Landscape	99.9	99.4
(c) Polvaonatum multiflorum		
FOREST 1:2	х	
SEMNATGRASS 1:3	/U	/
ORCHARD 2:3		
LWOOD 1:5		Ň
LROAD 1:7	х	
LROAD_1:2		١
Marginal R <sup>2</sup>	0.18	0.33
Landscape R <sup>2</sup>	0.17	0.30
% Landscape	95.7	91.4

<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)

<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)







Fig. 1 Overview on the study design. The seven 5 × 5 km<sup>2</sup> landscape windows (a) are spread across
temperate Europe from North France (A) over Belgium (B), West Germany (C), East Germany (D), and
South Sweden (E) up to Central Sweden (F) and Estonia (G). In each agricultural landscape, we
surveyed up to six populations of each species, here exemplified for *Polygonatum multiflorum* in East
Germany (b). See Supp. Inf. 1 for detailed land-use maps of each landscape window, including
locations of all sampled populations. The landscape surrounding the populations was analysed at the
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





Fig. 2 Visualization of landscape effects (cf. Tables 2 and 3) on population genetic variables of 915 916 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 radio of the landscape strips, respectively
931	
932	



Fig. 3 Visualizations of interactive effects (cf. Tables 2 and 3) of landscape metrics (x- and y-axis) on 935 936 population genetic variables of Anemone nemorosa (A. nem.), Oxalis acetosella (O. ace.) and 937 Polygonatum multiflorum (P. mul.) as heatmap. Only the area covered by the values of both 938 explanatory variables (overlaid as points) is plotted. All variables are scaled in standard deviation 939 units. Population genetic variables are measures of genetic differentiation ( $D_{PS}$  and  $G''_{ST}$ ) and 940 observed heterozygosity (H<sub>0</sub>). The landscape metrics FOREST and RAPE refer to the percent cover of 941 deciduous forest and oilseed rape, respectively. LROAD, LWATER and LWOOD refer to the relative 942 length of roads, water courses and hedgerows/treelines, respectively. The prefix O:P refers to orthogonal-to-parallel length ratio. pcARABvsGRASS is a principal component reflecting the trade-off 943 944 between arable land (cereals and oilseed rape) on the one hand and grassland on the other hand (cf.

- 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 radio of the landscape strips, respectively