

Basic and Applied Ecology

Microclimate influences plant reproductive performance via an antagonistic interaction

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	micro-climatic variation; plant reproduction; plant-animal interactions; predispersal seed predation; butterflies; <i>Gentiana pneumonanthe</i> ; <i>Phengaris alcon</i> ; <i>Myrmica</i> ; environmental variation; environmental context
Corresponding Author:	Alicia Valdés Stockholm University: Stockholms Universitet SWEDEN
First Author:	Alicia Valdés
Order of Authors:	Alicia Valdés Ehrlén Johan, Professor
Manuscript Region of Origin:	Europe
Abstract:	<p>Climatic conditions can influence plant reproduction directly, but also via changes in plant traits, interactions with animals, and the surrounding environment. Such indirect effects can often be complex and involve multiple steps. While the joint effects of climatic variation and indirect effects in terms of plant-animal interactions have sometimes been assessed at larger spatial scales, little is known about how microclimatic variation affects within-population variation in reproductive performance. Here, we studied the direct and indirect effects of microclimate on among-individual variation in reproductive performance of the plant <i>Gentiana pneumonanthe</i> in presence of the butterfly seed predator <i>Phengaris alcon</i>. We found that microclimatic effects on plant performance were mainly indirect, and that effects of temperature and moisture were interactive. The number of seeds per flower of <i>G. pneumonanthe</i> decreased in cold and moist microsites, and these effects were mediated by an increased oviposition by <i>P. alcon</i> in these microsites. The effects of soil temperature and moisture on the incidence of oviposition and plant performance were mediated by effects on plant phenology, density and phenology of neighbouring host plants, and host ant abundance. Plants that flowered earlier and where host ants were more abundant, and especially plants surrounded by fewer and later-flowering neighbours, produced fewer seeds per flower because of a higher incidence of oviposition. Our results demonstrate that effects of microclimatic variation on plant reproductive performance are mostly indirect and largely mediated by species interactions. These findings highlight that among-individual variation in small-scale environmental conditions within populations can cause variation in individual plant performance through multiple pathways.</p>
Suggested Reviewers:	<p>Nathan P. Lemoine Marquette University nathan.lemoine@marquette.edu Expert in terrestrial plant-insect ecology</p> <p>Irma Wynhoff Dutch Butterfly Conservation irma.wynhoff@vlinderstichting.nl Expert in butterfly conservation and <i>Phengaris</i> butterflies</p> <p>David R. Nash University of Copenhagen: Københavns Universitet drnash@bio.ku.dk Expert on <i>Phengaris</i> butterflies and their <i>Myrmica</i> ant hosts</p>

Dear Editor,

Please find attached the manuscript entitled “Microclimate influences plant reproductive performance via an antagonistic interaction” to be considered for publication in Basic and Applied Ecology.

Variation in plant reproductive performance is widespread both among and within natural plant populations, and identifying the environmental causes of this variation is necessary to understand the ecological and evolutionary dynamics of populations. Sexual plant reproduction is particularly sensitive to climatic variation and effects can be both direct and indirect, mediated by changes in plant traits, plant interactions with animals, and the environmental context of plants. Although some of these indirect effects have been assessed at large scales, we still lack a comprehensive knowledge of how climatic variability results in within-population variation in plant performance through different pathways.

In this study, we examined how microclimate influences reproductive performance of a plant through effects on oviposition by a seed-predator butterfly, plant phenology, plant density, and the distribution of ants serving as second hosts of the butterfly. We show that microclimatic effects on plant reproductive performance were mainly indirect and mediated by the interaction with the antagonist. We also show that microclimate indirectly affects plant reproduction not only by directly influencing the intensity of the antagonistic interaction, but also by affecting plant phenology and the plant context, which in turn influence the intensity of the interaction. Such complex indirect effects of microclimate on within-population differences in plant performance have not been explored previously.

Our results are exciting and novel because they illustrate the important role that species interactions play in mediating effects of climate on plant reproductive performance. A broader implication of the results is that variation in environmental conditions over spatial scales of only a few meters can cause variation in individual plant performance within a population through multiple and complex pathways.

The attached work has not been published or accepted for publication elsewhere, and is not under consideration for publication in any other journal or book. Its submission for publication has been approved by both authors, and all persons entitled to authorship have been so named.

We thank you in advance for your consideration of our manuscript.

Yours sincerely,

Alicia Valdés, on behalf of both authors

1 TITLE: Microclimate influences plant reproductive performance via an antagonistic interaction

2

3 Valdés, Alicia*, ^{a, b} and Ehrlén, Johan ^{a, b}

4 ^a Department of Ecology, Environment and Plant Sciences, Stockholm University, SE-106 91

5 Stockholm, Sweden

6 ^b Bolin Centre for Climate Research, Stockholm University, Stockholm, Sweden

7 ORCID IDs: <https://orcid.org/0000-0001-9281-2871>, <https://orcid.org/0000-0001-8539-8967>

8 E-mail addresses: alicia.valdes@su.se, johan.ehrlen@su.se

9 *Corresponding author

10 ABSTRACT

11 Climatic conditions can influence plant reproduction directly, but also via changes in plant traits,
12 interactions with animals, and the surrounding environment. Such indirect effects can often be
13 complex and involve multiple steps. While the joint effects of climatic variation and indirect effects
14 in terms of plant-animal interactions have sometimes been assessed at larger spatial scales, little is
15 known about how microclimatic variation affects within-population variation in reproductive
16 performance. Here, we studied the direct and indirect effects of microclimate on among-individual
17 variation in reproductive performance of the plant *Gentiana pneumonanthe* in presence of the
18 butterfly seed predator *Phengaris alcon*. We found that microclimatic effects on plant performance
19 were mainly indirect, and that effects of temperature and moisture were interactive. The number of
20 seeds per flower of *G. pneumonanthe* decreased in cold and moist microsites, and these effects were
21 mediated by an increased oviposition by *P. alcon* in these microsites. The effects of soil temperature
22 and moisture on the incidence of oviposition and plant performance were mediated by effects on
23 plant phenology, density and phenology of neighbouring host plants, and host ant abundance. Plants
24 that flowered earlier and where host ants were more abundant, and especially plants surrounded by
25 fewer and later-flowering neighbours, produced fewer seeds per flower because of a higher
26 incidence of oviposition. Our results demonstrate that effects of microclimatic variation on plant
27 reproductive performance are mostly indirect and largely mediated by species interactions. These
28 findings highlight that among-individual variation in small-scale environmental conditions within
29 populations can cause variation in individual plant performance through multiple pathways.

30

31 KEYWORDS: micro-climatic variation, plant reproduction, plant-animal interactions, predispersal
32 seed predation, butterflies, *Gentiana pneumonanthe*, *Phengaris alcon*, *Myrmica*, environmental
33 variation, environmental context

34

35 INTRODUCTION

36 Climate is a main driver of plant distributions, and differences in climatic conditions over many
37 different spatial scales might be important for plant performance (Woodward & Williams, 1987).
38 Sexual plant reproduction is particularly sensitive to climatic variations (Hedhly et al., 2009), and
39 several stages of the reproductive process, from flower development to pollination, fertilization and
40 seed and fruit maturation, might be affected (Bykova et al., 2012). Experimental evidence also
41 shows that the production of plant reproductive structures might depend on temperature and water
42 availability. For example, warming can increase seed biomass (Klady et al., 2011), and drought may
43 reduce seed set due to resource limitation (del Cacho et al., 2013). Understanding how climatic
44 variation over different spatial scales influences the reproductive performance of plants is thus
45 important to understand differences in performance among and within natural plant populations.
46 While the effects of climatic variability on plant performance are relatively well-studied at
47 moderate and large spatial scales (e.g. Dainese, 2011; De Frenne et al., 2009, 2010), they have
48 rarely been explored at smaller spatial scales, although it is well-known that the local environment
49 is important for many organisms (Geiger et al., 2009).

50 The influence of climatic variability on plant reproductive performance might not only be
51 direct but also indirect, and mediated by changes in plant traits, such as reproductive phenology
52 (Inouye, 2008), or interactions with other species (Memmott et al., 2007). Changes in interactions
53 are likely to play an important role, as they have been shown to often strongly influence plant
54 fitness (Agrawal et al., 2012; Ågren et al., 2013), and as many animals interacting with plants are
55 sensitive to the environmental context of the plant (Chamberlain et al., 2014). Moreover, such
56 animal-mediated effects of climate on plant performance might often involve multiple steps. For
57 example, climatic variation might induce changes in plant phenology, which in turn influences the
58 intensity of mutualistic or antagonistic interactions (Elzinga et al., 2007; Valdés & Ehrlén, 2017).
59 Climate might also affect the context of plant-animal interactions through effects on vegetation

60 height (Valdés & Ehrlén, 2018), canopy cover (Kolb & Ehrlén, 2010), community composition
61 (Biere & Tack, 2013; Wynhoff & van Langevelde, 2017), and density and quality of conspecific or
62 heterospecific plant neighbours (Hambäck et al., 2014; Valdés & Ehrlén, 2019). For example, small-
63 scale variation in soil temperature or water availability may condition plant density, and differences
64 in plant densities might in turn lead to differences in intensity of herbivory and in plant reproductive
65 output (Shea et al., 2000). Taken together, this means that identifying both the direct and complex
66 indirect effects of variation in microclimate is important to understand within-population variation
67 in plant reproductive performance.

68 In this study, we investigated the effects of microclimate on reproductive performance of the
69 plant *Gentiana pneumonanthe* in presence of an antagonist, the specialized seed-predator butterfly
70 *Phengaris alcon*. The caterpillars of these butterflies live inside the fruits of *G. pneumonanthe*
71 during their first instars, and parasitize the nests of ants (*Myrmica* spp.) during later stages. It has
72 previously been shown that the effects of plant phenology and ant presence on oviposition patterns
73 are interdependent: butterflies oviposit preferentially on early-flowering plants where host ants are
74 abundant (Valdés & Ehrlén, 2019). The density and phenology of neighbouring host plants also
75 influences oviposition patterns, with higher oviposition probability in plants with few, late-
76 flowering neighbours (Valdés & Ehrlén, 2019). Here, we examine how microclimate may affect
77 plant performance of *G. pneumonanthe*, through effects on butterfly oviposition, plant phenology,
78 plant density, and the distribution of ant hosts. We addressed the following more specific questions:
79 1) Does microclimate have direct effects on plant reproductive performance?, 2) Does microclimate
80 influence plant reproductive performance indirectly, through changes in plant phenology or
81 oviposition by *P. alcon*?, and 3) Can indirect effects of microclimate on plant reproductive
82 performance be mediated in two steps, by effects via plant phenology or the plant context on
83 oviposition by *P. alcon*?

84

85 MATERIAL AND METHODS

86 **Study system**

87 The marsh gentian (*Gentiana pneumonanthe* L.) is a rare, long-lived perennial herb occurring in
88 open habitats, such as wet heathlands and grasslands (Simmonds, 1946), with one to many shoots
89 up to 45 cm in height. It is self-compatible and flowers in July-August in the study area (SW
90 Sweden), producing deep blue, bumblebee-pollinated flowers. Fruits are capsules containing many
91 small, wind-dispersed seeds (mean seed number per fruit \pm SD = 490 ± 243 , mean seed weight =
92 0.041 mg, authors' unpublished data). *G. pneumonanthe* is the host plant of the Alcon Blue butterfly
93 (*Phengaris alcon*), a specialist predispersal seed predator. Adult females oviposit on young flower
94 buds in July and August (Appelqvist et al., 2007), usually laying more than one egg on the same
95 plant or even on the same bud, and not avoiding plants that already carry eggs (Van Dyck &
96 Regniers, 2010). Caterpillars feed inside the fruit, strongly reducing seed production (mean \pm SD =
97 13.8 ± 57.8 for preyed fruits vs. 493.1 ± 208.6 for intact fruits, calculated from a sample of 459
98 plants). After their fourth instar, the caterpillars drop to the ground and are picked up by *Myrmica*
99 ants and carried to their nest, where they are fed and live as brood parasites for the rest of their
100 larval period. *Phengaris* females detect host ants through chemicals produced by ants or by plants in
101 response to root tissue damage caused by ants (Patricelli et al., 2015; Wynhoff & van Langevelde,
102 2017). *Myrmica rubra* is considered to be the most common host ant species in Sweden (Elmes et
103 al., 1994).

104 **Data collection**

105 Field work was carried out between 9 July and 3 September 2016 in a population of *G.*
106 *pneumonanthe*, where *P. alcon* was present, located in a fenced pasture grazed by cattle in late
107 summer every year (Tånga Hed nature reserve, Västra Götaland, SW Sweden, 58°01'40.0"N
108 12°49'47.0"E). A study plot of 69 m \times 45 m was established in the pasture, and subdivided into a
109 grid of 345 3 m \times 3 m subplots with corners marked by poles (see Valdés & Ehrlén, 2019, Fig. 1).

110 In 154 of the subplots that were occupied by *G. pneumonanthe*, all reproductive shoots were
 111 mapped, and the phenology and the number of *P. alcon* eggs on each shoot were recorded between
 112 29 July and 5 August (18 additional occupied subplots were not surveyed due to time constraints).
 113 Shoots were mapped instead of plant individuals) because we judged that individual shoots are the
 114 most relevant unit for butterfly females to assess the food resource quality, and act as the unit of
 115 attraction for butterflies. Shoot phenology was recorded as the developmental stage of the most
 116 advanced bud, with six ordinal categories: (1) sepals covering bud completely, (2) bud becoming
 117 visible, (3) bud growing over the sepals, (4) bud turning blue, (5) flower opening, and (6) flower
 118 wilting (Valdés & Ehrlén, 2017, 2018, 2019). Higher values of shoot phenology thus indicate a
 119 more advanced floral development at the day of recording and an earlier flowering, with a one-unit
 120 increase roughly corresponding to one week earlier development (Valdés & Ehrlén, 2017, 2018,
 121 2019).

122 Five *G. pneumonanthe* shoots belonging to five different plants (fewer if fewer plants were
 123 available) were permanently marked into each subplot to measure plant reproductive performance.
 124 In these shoots, we recorded flower number once per shoot between 29 July and 5 August, and
 125 counted the number of intact and preyed fruits once per shoot between 30 August and 1 September.
 126 We also took a sample of one intact and preyed fruit (when available) per shoot, and counted the
 127 number of developed seeds in these fruits in the lab. For each marked shoot, we calculated the
 128 number of seeds per flower as a measure of plant reproductive performance, as:

$$129 \quad n_{seedsperflower} = \frac{n_{seedsintactfruit} + n_{seedspreyedfruit}}{n_{flowers}}$$

130 The ant community was sampled using sugar baits at each corner of all occupied subplots
 131 (254 points, Valdés & Ehrlén, 2019, Fig. 1) at two occasions, 14 July and 3 September. Ants
 132 collected were transferred to 70° ethanol and the number of ants of different *Myrmica* species were
 133 counted in the lab (using Douwes et al., 2012 for identification).

Two microclimatic variables (soil temperature and soil moisture) were recorded at the same 254 points. At each point, an iButton data logger was buried at 5 cm depth in mid-July 2016, and recorded temperature every third hour from 14 July to 2 September. We calculated the average of daily mean temperatures over the recording period (hereafter, soil temperature). While it is true that daily mean temperature in the soil might not accurately represent the microclimate experienced by the flowers, this measure integrates temperature conditions over a long time, and relative differences among plants in soil temperature are likely to be similar to relative differences in mean air temperature. Moreover, soil temperature might be more related to some of the variables representing the plant context (e.g. host ant abundance, plant density, see below) than air temperature. We measured soil moisture (%; average of three measurements) at each point with a Theta Probe sensor connected to a HH2 moisture meter (Delta-T Devices, Cambridge, UK) on 30 July. To get maximum resolution in moisture measurements, we chose a date when the conditions were semi-dry (i.e. 5 days after the last rain). Although moisture values might vary a lot from one day to another, we expect relative differences to remain similar over the flowering season.

Data preparation

Field maps were digitized using ArcGIS 10.3.1, where we assigned relative coordinates to each recorded shoot. We defined a buffer area with a 3-m radius, centered at each shoot (see Valdés & Ehrlén, 2019, Fig. 1), and calculated the number of *G. pneumonanthe* reproductive shoots per m² within this buffer area (hereafter, “neighbour density”) as well as the mean shoot phenology (hereafter, “neighbour phenology”), excluding in each case the focal shoot.

The locations of the 254 points where the ant community and microclimatic variables were sampled were also incorporated into the GIS. We used inverse distance weighted interpolation (Pebesma, 2004) based on the values at the sampling points with a maximum distance of 3 m to generate values of *Myrmica rubra* abundance over the surface of all occupied subplots, and

158 extracted values for each of the mapped shoots (hereafter, “ant abundance”, see Valdés & Ehrlén,
159 2019 for details on the choice of ant species and maximum distance).

160 We used an ordinary kriging interpolation (autoKrige function of the R package automap,
161 Hiemstra et al., 2009) based on the values of soil temperature and soil moisture at the sampling
162 points to generate values of these two microclimatic variables over the surface of all occupied
163 subplots, and extracted values of soil temperature and soil moisture for each of the mapped shoots.

164 **Statistical analyses**

165 First, in order to evaluate the overall relationship between *G. pneumonanthe* reproductive
166 performance and microclimate, we performed univariate linear regressions of number of seeds per
167 flower against soil temperature and soil moisture. As residual spatial autocorrelation could lead to
168 biased model estimates and invalid statistical inference (Dormann et al., 2007), we checked for
169 spatial autocorrelation in the residuals of these regressions by calculating global Moran's I with a
170 permutation test (1000 random permutations), based on a connectivity matrix of pairwise Euclidean
171 distances among shoots up to 30 m. We found significant autocorrelation in the residuals of both
172 regressions (Appendix A). We therefore applied Moran's eigenvector mapping (MEM, Thayn &
173 Simanis, 2013) to both regressions. MEM translates the spatial arrangement of data points into
174 eigenvectors that capture spatial effects. Refitting the regressions including, in each case, one
175 eigenvector issued from MEM as spatial predictor removed the residual spatial autocorrelation
176 (Appendix A).

177 Second, in order to decompose the overall relationship into different pathways, we applied
178 piecewise structural equation modelling (piecewise SEM) to assess the relationships between
179 microclimate, plant phenology and context (in terms of host ant presence, density and phenology of
180 neighbouring host plants), occurrence of oviposition by *P. alcon*, and the number of seeds per
181 flower. We chose a piecewise approach, rather than the traditional variance-covariance based SEM,
182 because it allows to fit multiple separate linear models with non-normal distributions. We

183 considered both direct effects of microclimate on number of seeds per flower, indirect effects
184 mediated by oviposition occurrence or plant phenology, and indirect effects mediated by effects of
185 changes in plant phenology and plant context, on oviposition occurrence.

186 The piecewise SEM consisted of six component models: 1) a linear model (LM) with plant
187 phenology as response and soil temperature, soil moisture and their interaction as predictors; 2) a
188 generalized linear model (GLM) with a negative binomial error distribution with ant abundance as
189 response and soil temperature, soil moisture and their interaction as predictors; 3) a LM with
190 neighbour density as response and soil temperature, soil moisture and their interaction as predictors;
191 4) a LM with neighbour phenology as response and soil temperature, soil moisture and their
192 interaction as predictors; 5) a GLM with a binomial error distribution with a binomial variable “egg
193 occurrence” (being 1 when the shoot had at least one egg, and 0 otherwise) as response and plant
194 phenology, ant abundance, the interaction plant phenology \times ant abundance, neighbour density,
195 neighbour phenology, the interaction neighbour density \times neighbour phenology, soil temperature,
196 soil moisture and their interaction as predictors; and 6) a LM with number of seeds per flower as
197 response and plant phenology, egg occurrence, soil temperature, soil moisture and their interaction
198 as predictors. We used a LM for plant phenology, treating phenology as a numeric rather than an
199 ordinal variable, because a one-unit increase in phenology roughly corresponds to one week earlier
200 development, and a LM for neighbour phenology because it could take any values between 1 and 6.
201 We use egg occurrence as a variable accounting for the existence of a butterfly-plant interaction, but
202 not as a measure of presence or absence of predation, as some shoots with eggs might finally not
203 suffer from seed predation (e.g. if eggs do not hatch). One might thus argue that egg presence is a
204 better estimate of the butterfly-plant interaction intensity, while seed predation is more of an
205 estimate of effects on plant fitness. For the model of egg occurrence, we included only interactions
206 that were biologically plausible based on our knowledge of the study system and previous evidence
207 (Valdés & Ehrlén, 2019). In all component models, we considered the possibility of interactive

208 effects of both microclimatic variables by testing for effects of the interaction soil temperature ×
209 soil moisture, but removed the interaction term when it was not significant to allow for simpler
210 models.

211 Overall fit of the model was assessed using Shipley's test of direct separation (d-separation,
212 Shipley, 2009), which evaluates the probability that none of the paths missing from the
213 hypothesized causal network contain useful information. This test yields the Fisher's C statistic that
214 is χ^2 -distributed, and a value below the significance level ($p < 0.05$) indicates that the model is
215 inconsistent with the data, and should be rejected. The test of d-separation suggested several
216 missing paths between variables, which we modelled as correlated errors, because we were
217 uncertain regarding the direction of any potential underlying causal relationships between them (see
218 Appendix B). Finally, obtaining standardized coefficients for component model 2) was not possible
219 because of transformation via the link function of the GLM. Therefore, we repeated the piecewise
220 SEM using a LM for component model 2) in order to obtain standardized coefficients for this
221 model.

222 We checked for spatial autocorrelation in the residuals of the six component models of the
223 SEM as explained previously. We found significant autocorrelation in the residuals of component
224 models 1-5 (Appendix C), and thus applied MEM to these models. Each of the models was then
225 refitted including, in each case, one or several (up to five) eigenvectors issued from MEM as spatial
226 predictors, and this removed the residual spatial autocorrelation (Appendix C).

227 Statistical analyses were carried out in R version 4.1.0 (R Core Team, 2021) using (apart
228 from the above mentioned) the packages MASS (Venables & Ripley, 2002), spdep (Bivand et al.,
229 2013), adespatial (Dray et al., 2021) and piecewiseSEM (Lefcheck, 2016).

230

231 RESULTS

232 There was considerable variation in the microclimate experienced by *G. pneumonanthe* plants; soil
233 temperature ranged from 14.7 to 17.2 °C (mean \pm SD = 16.2 ± 0.5 °C), and soil moisture ranged
234 from 35.4 to 80.4 % (mean \pm SD = 66.1 ± 10.8 %). Plant reproductive performance did not
235 significantly change with soil temperature after correcting for residual spatial autocorrelation (Table
236 1A) but decreased with increasing soil moisture (Table 1B).

237 The piecewise SEM model had a reasonable fit to the data after including the missing paths
238 as correlated errors (Fisher's C = 10.86, $p = 0.093$). There was no evidence of significant direct
239 effects of microclimate on plant reproductive performance. Instead, microclimate affected
240 reproductive performance indirectly through multiple pathways (Fig. 1, Table 2, Appendix B).
241 Oviposition by *P. alcon* occurred more often in plants located at warmer microsites, resulting in a
242 reduced number of seeds per flower. There were no significant indirect effects of microclimate on
243 reproductive performance mediated only by changes in plant phenology, as plant phenology did not
244 have a significant direct effect on reproductive performance.

245 Indirect effects of microclimate on plant reproductive performance also acted through
246 pathways involving multiple steps (Fig. 1, Table 2, Appendix B). Microclimate was related to plant
247 phenology and to the plant context, and these two parameters influenced oviposition and
248 reproductive performance. Soil temperature and soil moisture showed significant interactive effects
249 on plant phenology, ant abundance, neighbour density and neighbour phenology (Fig. 1-2,
250 Appendix B). Plants flowered earlier in colder and drier microsites (Fig. 2A). Ant abundance, in
251 turn, decreased with temperature in dry microsites, but increased with temperature in wet microsites
252 (Fig. 2B). Early-flowering plants where host ants were abundant showed a higher occurrence of
253 oviposition, as evidenced by the significant effect of the interaction plant phenology \times ant
254 abundance on egg occurrence (Fig. 3A), and therefore produced less seeds per flower (Fig. 1,
255 Appendix B). Also pathways involving conspecific neighbours mediated effects of microclimate on
256 plant performance, and their effects were the most important, especially for soil temperature (Table

257 2). Neighbour density decreased with temperature in dry microsites, but increased with temperature
258 in wet microsites (Fig. 2C), and neighbours flowered earlier in colder and drier microsites (Fig.
259 2D). Plants surrounded by few, late-flowering neighbours showed a higher egg occurrence, as
260 evidenced by the significant effect of the interaction neighbour density \times neighbour phenology on
261 egg occurrence (Fig. 3B), and therefore produced less seeds per flower (Fig. 1, Appendix 2).

262

263 DISCUSSION

264 Our results show that microclimatic effects on reproductive performance of the plant *G.*
265 *pneumonanthe* were mainly indirect and involved multiple steps. Microclimate, in terms of soil
266 temperature and moisture, was related to oviposition by the butterfly *P. alcon*, which had a strong
267 negative effect on plant performance. Microclimate also influenced plant phenology, host ant
268 abundance and density and phenology of neighbouring host plants, which in turn affected
269 oviposition and plant reproductive output.

270 We found no direct effects of microclimate on reproductive performance of *G.*
271 *pneumonanthe*. Direct effects of climatic variation might be more important at larger spatial scales,
272 although the role of direct effects in previous larger-scale studies reporting climatic influences on
273 plant reproduction is difficult to assess because these studies do not separate direct and indirect
274 effects (Dainese, 2011; De Frenne et al., 2009, 2010). Experimental studies have found effects of
275 small-scale warming and drought on seed production (del Cacho et al., 2013; Klady et al., 2011).
276 These studies also did not discriminate between direct and indirect effects, but suggested that the
277 observed responses to experimental treatments might partly be due to effects of warming on plant
278 phenology (Klady et al., 2011), or due to effects of drought on plant size (del Cacho et al., 2013).
279 Although the range of variation, at least in soil temperature, was larger in our study than in these
280 previous studies, we did not detect direct effects of microclimate on plant performance.

281 In contrast to the lack of direct effects, we detected several indirect effects of microclimate
282 on reproductive performance of *G. pneumonanthe*. The overall impact of these indirect effects was
283 that the number of seeds per flower increased with soil temperature and decreased with soil
284 moisture, the effect of temperature being more important than the effect of moisture (total effects,
285 Table 2). The occurrence of oviposition by *P. alcon* was higher in warm microsites, but was not
286 related to soil moisture after correcting for residual spatial autocorrelation. Microclimatic effects on
287 the intensity of plant-animal interactions, such as seed predation, might be due to effects on larval
288 growth and survival, as females select oviposition sites that maximize development and survival of
289 larvae (Bonebrake et al., 2010). Our results agree with previous studies showing an increased
290 incidence of butterfly oviposition in warmer microclimates, particularly near their geographical
291 range margins (Eilers et al., 2013; Fourcade & Öckinger, 2016). The increased incidence of eggs in
292 *G. pneumonanthe* individuals in warmer microsites was associated with a reduced reproductive
293 output. Our results therefore show that antagonistic interactions play an important role in mediating
294 effects of microclimate on local-scale environmental variation on plant performance.

295 Microclimate also affected oviposition by *P. alcon* and the reproductive performance of *G.*
296 *pneumonanthe* individuals indirectly via effects on plant phenology, host ant abundance, and
297 neighbouring conspecific plants, and the effects of soil temperature and moisture were interactive.
298 Plants flowered latest in warm and wet microsites. In contrast, the few other studies that have
299 documented small-scale variation in plant phenology in relation to soil temperature have found
300 earlier flowering in warmer microsites (Dahlgren et al., 2007; Toftegaard, 2016). Our analyses also
301 suggested that the effects of moisture on plant phenology were more important than those of
302 temperature, due to the larger range of variation in soil moisture in the study population (Appendix
303 D). Earlier flowering has also been related to higher nutrient levels (Dahlgren et al., 2007), and one
304 possibility is that the later flowering of *G. pneumonanthe* in warm and moist microsites is due to
305 that moister microsites are poorer in nutrients.

306 Soil temperature and moisture were also correlated with host ant abundance. When soils
307 were dry, *M. rubra* were more abundant in cold microsites, but when moisture was high it was more
308 abundant in warm microsites. *Myrmica* ants occupy different ecological niches with respect to
309 vegetation and soil characteristics (Elmes et al., 1998). Specifically, *M. rubra* shows preferences for
310 intermediate soil temperature and moisture (Elmes et al., 1998), and might thus avoid very warm
311 and dry microsites, where there might be a risk of dessication, as well as very cold and wet
312 microsites, which might be too damp. Lastly, neighbour density was the highest in warm and moist
313 microsites. This might be the result of that these microsites can support higher plant densities due to
314 a higher water availability in the soil and a low cover of grasses resulting from low nutrient
315 concentrations (Oostermeijer et al., 1998).

316 Effects of microclimate on neighbour density and phenology influenced oviposition by *P.*
317 *alcon* and reproductive performance of *G. pneumonanthe*, oviposition incidence being highest, and
318 reproductive performance lowest, in cold and moist microsites. Microclimate also affected plant
319 phenology and ant abundance via oviposition, but these effects were less important. A previous
320 study with this species found that *P. alcon* egg incidence increased with an earlier plant phenology,
321 and that the preference for early-flowering host plants was stronger where host ants were abundant
322 (Valdés & Ehrlén, 2019). Moreover, plants with neighbours that were less preferred by *P. alcon* for
323 oviposition had a higher probability of receiving eggs. As a result of these interactive effects, plants
324 that flowered earlier and where host ants were more abundant, and plants surrounded by fewer and
325 later-flowering neighbours produced fewer seeds per flower. The fact that both plant phenology,
326 host ant abundance and density and phenology of neighbouring conspecific plants were related to
327 microclimate thus implies that microclimate indirectly affects plant reproduction by modifying
328 plant phenology and the plant context, which in turn influence interactions. Although indirect
329 effects of climate on plant reproduction through altered species interactions have been investigated
330 before (e.g. Lemoine et al., 2017; Richman et al., 2020), the type of complex indirect effects of

331 microclimate on within-population differences in plant reproductive performance found in this
332 study have not previously been explored.

333 **Conclusions**

334 Our results illustrate the complexity of the relationship between local-scale environmental
335 variation and plant performance, and highlight that species interactions can play an important role
336 in mediating effects of climate. We simultaneously assessed the effects of microclimatic variation
337 via multiple pathways, including direct effects of microclimate on plant performance, effects via
338 plant-animal interactions, as well as effects of microclimate on plant traits and the environmental
339 context that influence these interactions. We also showed that the effects of temperature and
340 moisture are interactive. The approach used enables a more comprehensive understanding of the
341 many and complex ways in which among-individual variation in small-scale climatic conditions
342 within populations can affect individual plant performance. Approaches considering multiple
343 pathways, such as the one used in this study, provide a powerful way to identify the plausible
344 environmental causes of differences in individual performance over small spatial scales, which is
345 necessary to understand the ecological and evolutionary dynamics of plant populations.

346

347 **ACKNOWLEDGEMENTS**

348 We thank Annelie Jörgensen for helping with fieldwork and Daniela Guasconi for laboratory
349 assistance.

350

351 Funding: This work was supported by the Swedish Research Council (VR) [grant number 2011-
352 03710 to JE] and by the “Clarín” postdoctoral program (FICYT, Gobierno del Principado de
353 Asturias, Spain, and Marie Curie-Cofund Actions, EU) [grant number AC A14-02 to AV].

354

355 **REFERENCES**

- Agrawal, A. A., Hastings, A. P., Johnson, M. T. J., Maron, J. L., & Salminen, J.-P. (2012). Insect herbivores drive real-time ecological and evolutionary change in plant populations. *Science*, 338(6103), 113–116. <https://doi.org/10.1126/science.1225977>
- Ågren, J., Hellström, F., Toräng, P., & Ehrlén, J. (2013). Mutualists and antagonists drive among-population variation in selection and evolution of floral display in a perennial herb. *Proceedings of the National Academy of Sciences*, 110(45), 18202–18207. <https://doi.org/10.1073/pnas.1301421110>
- Appelqvist, T., Bengtsson, O., Sverige, & Naturvårdsverket. (2007). *Åtgärdsprogram för alkonblåvinge och klockgentiana 2007-2011: Maculinea alcon och Gentiana pneumonanthe*. Naturvårdsverket. <http://www.naturvardsverket.se/Documents/publikationer/620-5686-7.pdf>
- Biere, A., & Tack, A. J. M. (2013). Evolutionary adaptation in three-way interactions between plants, microbes and arthropods. *Functional Ecology*, 27(3), 646–660. <https://doi.org/10.1111/1365-2435.12096>
- Bivand, R. S., Pebesma, E., & Gómez-Rubio, V. (2013). *Applied Spatial Data Analysis with R*. Springer New York. <https://doi.org/10.1007/978-1-4614-7618-4>
- Bonebrake, T. C., Boggs, C. L., McNally, J. M., Ranganathan, J., & Ehrlich, P. R. (2010). Oviposition behavior and offspring performance in herbivorous insects: Consequences of climatic and habitat heterogeneity. *Oikos*, 119(6), 927–934. <https://doi.org/10.1111/j.1600-0706.2009.17759.x>
- Bykova, O., Chuine, I., Morin, X., & Higgins, S. I. (2012). Temperature dependence of the reproduction niche and its relevance for plant species distributions. *Journal of Biogeography*, 39(12), 2191–2200. <https://doi.org/10.1111/j.1365-2699.2012.02764.x>
- Chamberlain, S. A., Bronstein, J. L., & Rudgers, J. A. (2014). How context dependent are species interactions? *Ecology Letters*, 17(7), 881–890. <https://doi.org/10.1111/ele.12279>

- Dahlgren, J. P., Zeipel, H. von, & Ehrlén, J. (2007). Variation in vegetative and flowering phenology in a forest herb caused by environmental heterogeneity. *American Journal of Botany*, 94(9), 1570–1576. <https://doi.org/10.3732/ajb.94.9.1570>
- Dainese, M. (2011). Impact of land use intensity and temperature on the reproductive performance of *Dactylis glomerata* populations in the southeastern Alps. *Plant Ecology*, 212(4), 651–661. <https://doi.org/10.1007/s11258-011-9902-6>
- De Frenne, P., Graae, B. J., Kolb, A., Brunet, J., Chabrierie, O., Cousins, S. A. O., Decocq, G., Dhondt, R., Diekmann, M., Eriksson, O., Heinken, T., Hermy, M., Jõgar, Ü., Saguez, R., Shevtsova, A., Stanton, S., Zindel, R., Zobel, M., & Verheyen, K. (2010). Significant effects of temperature on the reproductive output of the forest herb *Anemone nemorosa* L. *Forest Ecology and Management*, 259(4), 809–817. <https://doi.org/10.1016/j.foreco.2009.04.038>
- De Frenne, P., Kolb, A., Verheyen, K., Brunet, J., Chabrierie, O., Decocq, G., Diekmann, M., Eriksson, O., Heinken, T., Hermy, M., & others. (2009). Unravelling the effects of temperature, latitude and local environment on the reproduction of forest herbs. *Global Ecology and Biogeography*, 18(6), 641–651.
- del Cacho, M., Peñuelas, J., & Lloret, F. (2013). Reproductive output in Mediterranean shrubs under climate change experimentally induced by drought and warming. *Perspectives in Plant Ecology, Evolution and Systematics*, 15(6), 319–327. <https://doi.org/10.1016/j.ppees.2013.07.001>
- Dormann, C. F., M. McPherson, J., B. Araújo, M., Bivand, R., Bolliger, J., Carl, G., G. Davies, R., Hirzel, A., Jetz, W., Daniel Kissling, W., Kühn, I., Ohlemüller, R., R. Peres-Neto, P., Reineking, B., Schröder, B., M. Schurr, F., & Wilson, R. (2007). Methods to account for spatial autocorrelation in the analysis of species distributional data: A review. *Ecography*, 30(5), 609–628. <https://doi.org/10.1111/j.2007.0906-7590.05171.x>

- Douwes, P., Abenius, J., Cederberg, B., & Wahlstedt, U. (2012). *Steklar: Myror-getingar: Hymenoptera: Formicidae-Vespidae*. ArtDatabanken, Sveriges lantbruksuniversitet.
- Dray, S., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Jombart, T., Larocque, G., Legendre, P., Madi, N., & Wagner, H. H. (2021). *adespatial: Multivariate Multiscale Spatial Analysis* (0.3-14) [Computer software]. <https://CRAN.R-project.org/package=adespatial>
- Eilers, S., Pettersson, L. B., & Öckinger, E. (2013). Micro-climate determines oviposition site selection and abundance in the butterfly *Pyrgus armoricanus* at its northern range margin. *Ecological Entomology*, 38(2), 183–192. <https://doi.org/10.1111/een.12008>
- Elmes, G. W., Thomas, J. A., Hammarstedt, O., Munguira, M. L., Martin, J., & van der Made, J. G. (1994). Differences in host-ant specificity between Spanish, Dutch and Swedish populations of the endangered butterfly, *Maculinea alcon* (Denis et Schiff.)(Lepidoptera). *Memorabilia Zoologica*, 48, 55–68.
- Elmes, G. W., Thomas, J. A., Wardlaw, J. C., Hochberg, M. E., Clarke, R. T., & Simcox, D. J. (1998). The ecology of *Myrmica* ants in relation to the conservation of *Maculinea* butterflies. *Journal of Insect Conservation*, 2(1), 67–78. <https://doi.org/10.1023/A:1009696823965>
- Elzinga, J. A., Atlan, A., Biere, A., Gigord, L., Weis, A. E., & Bernasconi, G. (2007). Time after time: Flowering phenology and biotic interactions. *Trends in Ecology & Evolution*, 22(8), 432–439. <https://doi.org/10.1016/j.tree.2007.05.006>
- Fourcade, Y., & Öckinger, E. (2016). Host plant density and patch isolation drive occupancy and abundance at a butterfly's northern range margin. *Ecology and Evolution*, 7(1), 331–345. <https://doi.org/10.1002/ece3.2597>
- Geiger, R., Aron, R. H., & Todhunter, P. (2009). *The climate near the ground* (Seventh Edition edition). Rowman & Littlefield Publishers.

- Hambäck, P. A., Inouye, B. D., Andersson, P., & Underwood, N. (2014). Effects of plant neighborhoods on plant–herbivore interactions: Resource dilution and associational effects. *Ecology*, *95*(5), 1370–1383. <https://doi.org/10.1890/13-0793.1>
- Hedhly, A., Hormaza, J. I., & Herrero, M. (2009). Global warming and sexual plant reproduction. *Trends in Plant Science*, *14*(1), 30–36. <https://doi.org/10.1016/j.tplants.2008.11.001>
- Hiemstra, P. H., Pebesma, E. J., Twenhöfel, C. J. W., & Heuvelink, G. B. M. (2009). Real-time automatic interpolation of ambient gamma dose rates from the Dutch radioactivity monitoring network. *Computers & Geosciences*, *35*(8), 1711–1721. <https://doi.org/10.1016/j.cageo.2008.10.011>
- Inouye, D. W. (2008). Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology*, *89*(2), 353–362. <https://doi.org/10.1890/06-2128.1>
- Klady, R. A., Henry, G. H. R., & Lemay, V. (2011). Changes in high arctic tundra plant reproduction in response to long-term experimental warming. *Global Change Biology*, *17*(4), 1611–1624. <https://doi.org/10.1111/j.1365-2486.2010.02319.x>
- Kolb, A., & Ehrlén, J. (2010). Environmental context drives seed predator-mediated selection on a floral display trait. *Evolutionary Ecology*, *24*(2), 433–445. <https://doi.org/10.1007/s10682-009-9316-2>
- Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in r for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, *7*(5), 573–579. <https://doi.org/10.1111/2041-210X.12512>
- Lemoine, N. P., Doublet, D., Salminen, J.-P., Burkepile, D. E., & Parker, J. D. (2017). Responses of plant phenology, growth, defense, and reproduction to interactive effects of warming and insect herbivory. *Ecology*, *98*(7), 1817–1828. <https://doi.org/10.1002/ecy.1855>
- Memmott, J., Craze, P. G., Waser, N. M., & Price, M. V. (2007). Global warming and the disruption of plant–pollinator interactions. *Ecology Letters*, *10*(8), 710–717.

- Oostermeijer, J. G. B., Luijten, S. H., Křenová, Z. V., & Den Nijs, H. C. M. (1998). Relationships between population and habitat characteristics and reproduction of the rare *Gentiana pneumonanthe* L. *Conservation Biology*, 12(5), 1042–1053. <https://doi.org/10.1046/j.1523-1739.1998.97090.x>
- Patricelli, D., Barbero, F., Occhipinti, A., Berteà, C. M., Bonelli, S., Casacci, L. P., Zebelo, S. A., Crocoll, C., Gershenzon, J., Maffei, M. E., Thomas, J. A., & Balletto, E. (2015). Plant defences against ants provide a pathway to social parasitism in butterflies. *Proc. R. Soc. B*, 282(1811), 20151111. <https://doi.org/10.1098/rspb.2015.1111>
- Pebesma, E. J. (2004). Multivariable geostatistics in S: The gstat package. *Computers & Geosciences*, 30(7), 683–691.
- R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Richman, S. K., Levine, J. M., Stefan, L., & Johnson, C. A. (2020). Asynchronous range shifts drive alpine plant–pollinator interactions and reduce plant fitness. *Global Change Biology*, 26(5), 3052–3064. <https://doi.org/10.1111/gcb.15041>
- Shea, K., Smyth, M., Sheppard, A., Morton, R., & Chalimbaud, J. (2000). Effect of patch size and plant density of Paterson’s curse (*Echium plantagineum*) on the oviposition of a specialist weevil, *Mogulones larvatus*. *Oecologia*, 124, 615–621.
- Shipley, B. (2009). Confirmatory path analysis in a generalized multilevel context. *Ecology*, 90(2), 363–368. <https://doi.org/10.1890/08-1034.1>
- Simmonds, N. W. (1946). *Gentiana pneumonanthe* L. *Journal of Ecology*, 33(2), 295–307.
- Thayn, J. B., & Simanis, J. M. (2013). Accounting for spatial autocorrelation in linear regression models using spatial filtering with eigenvectors. *Annals of the Association of American Geographers*, 103(1), 47–66. <https://doi.org/10.1080/00045608.2012.685048>

- Toftegaard, T. (2016). *Temperature and the synchrony of plant-insect interactions* [Department of Ecology, Environment and Plant Sciences, Stockholm University]. <http://www.diva-portal.org/smash/record.jsf?pid=diva2:937496>
- Valdés, A., & Ehrlén, J. (2017). Caterpillar seed predators mediate shifts in selection on flowering phenology in their host plant. *Ecology*, 98(1), 228–238. <https://doi.org/10.1002/ecy.1633>
- Valdés, A., & Ehrlén, J. (2018). Direct and plant trait-mediated effects of the local environmental context on butterfly oviposition patterns. *Oikos*, 127(6), 825–833. <https://doi.org/10.1111/oik.04909>
- Valdés, A., & Ehrlén, J. (2019). Resource overlap and dilution effects shape host plant use in a myrmecophilous butterfly. *Journal of Animal Ecology*, 88(4), 649–658. <https://doi.org/10.1111/1365-2656.12952>
- Van Dyck, H., & Regniers, S. (2010). Egg spreading in the ant-parasitic butterfly, *Maculinea alcon*: From individual behaviour to egg distribution pattern. *Animal Behaviour*, 80(4), 621–627. <https://doi.org/10.1016/j.anbehav.2010.06.021>
- Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th Edition). Springer.
- Woodward, F. I., & Williams, B. G. (1987). Climate and plant distribution at global and local scales. *Vegetatio*, 69(1–3), 189–197. <https://doi.org/10.1007/BF00038700>
- Wynhoff, I., & van Langevelde, F. (2017). *Phengaris (Maculinea) teleius* butterflies select host plants close to *Myrmica* ants for oviposition, but *P. nausithous* do not. *Entomologia Experimentalis et Applicata*, 165(1), 9–18. <https://doi.org/10.1111/eea.12624>

FIGURE LEGENDS

Figure 1: Results of a piecewise structural equation model (piecewise SEM) examining the relationships among microclimate (soil temperature = T and soil moisture = M), *G. pneumonanthe* phenology and context (host ant abundance, density and phenology of neighbouring host plants), occurrence of *P. alcon* oviposition (egg occurrence), and *G. pneumonanthe* reproductive performance (number of seeds per flower). Values shown are standardized coefficients from the piecewise SEM. Non-significant relationships (according to component models fitted including spatial vectors) are shown with dotted arrows. Correlated errors have been omitted, and soil temperature and soil moisture are depicted several times in the diagram for clarity.

Figure 2: Interactive effects of microclimatic variables on *G. pneumonanthe* phenology and context. Plots show the effects of soil temperature on A) plant phenology, B) ant abundance, C) neighbour density, and D) neighbour phenology at different levels of soil moisture (mean \pm 1 SD). Lines show predicted marginal effects (i.e. effects of the focal variables when holding the non-focal variables constant at their means) and 95% confidence intervals from component models 1-4 of the piecewise SEM including spatial predictors.

Figure 3: Interactive effects of A) plant phenology and ant abundance, and B) neighbour density and neighbour phenology, on the probability of occurrence of *P. alcon* oviposition on *G. pneumonanthe*. In A), the effect of plant phenology on the probability of egg occurrence is shown at different levels of ant abundance (from minimum to maximum abundance, at 10-unit intervals). In B), the effect of neighbour density is shown at different levels of neighbour phenology (mean \pm 1 SD). Lines show predicted marginal effects (i.e. effects of the focal variables when holding the non-focal variables constant at their means) and 95% confidence intervals from component model 5 of the piecewise SEM including spatial predictors.

Figure 1

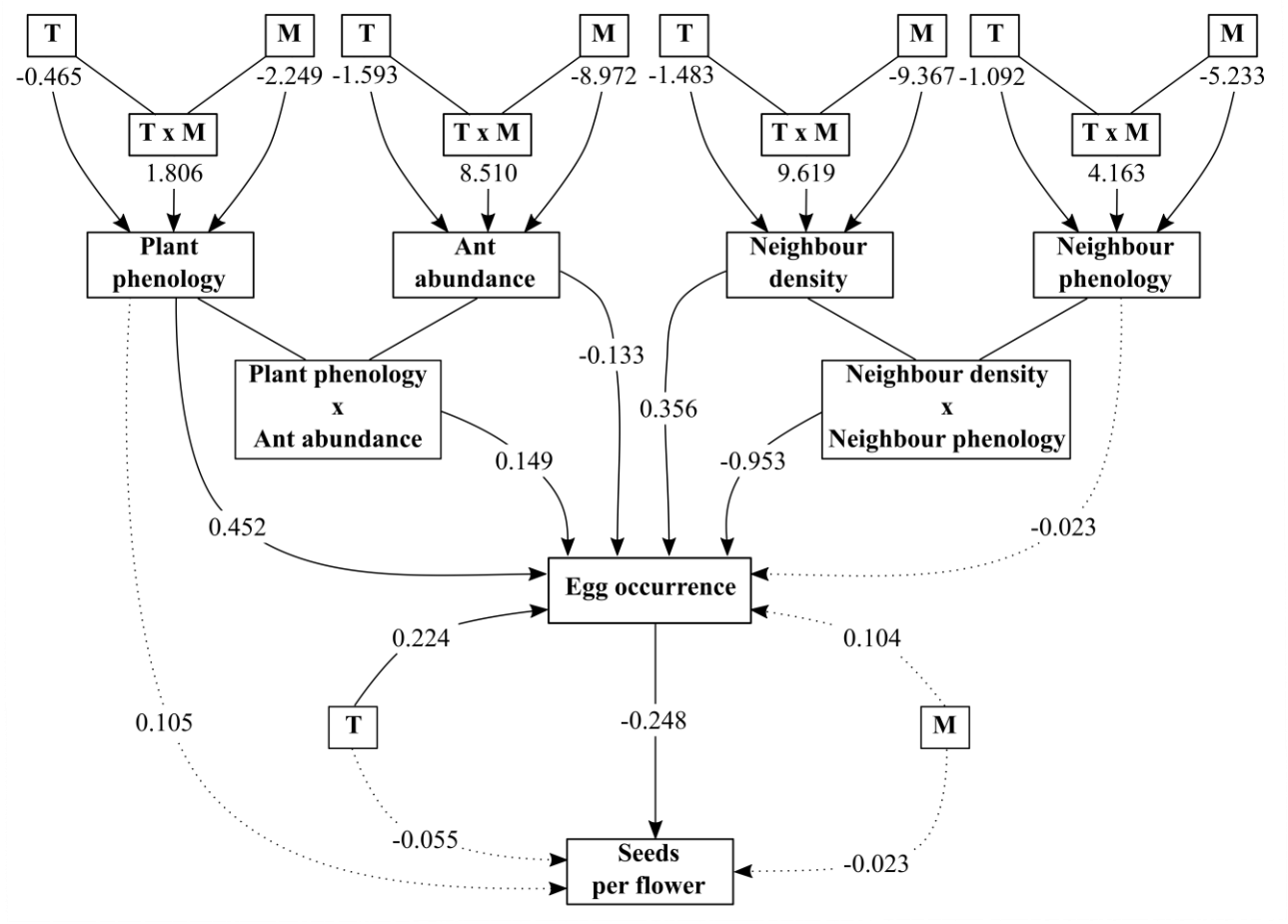


Figure 2

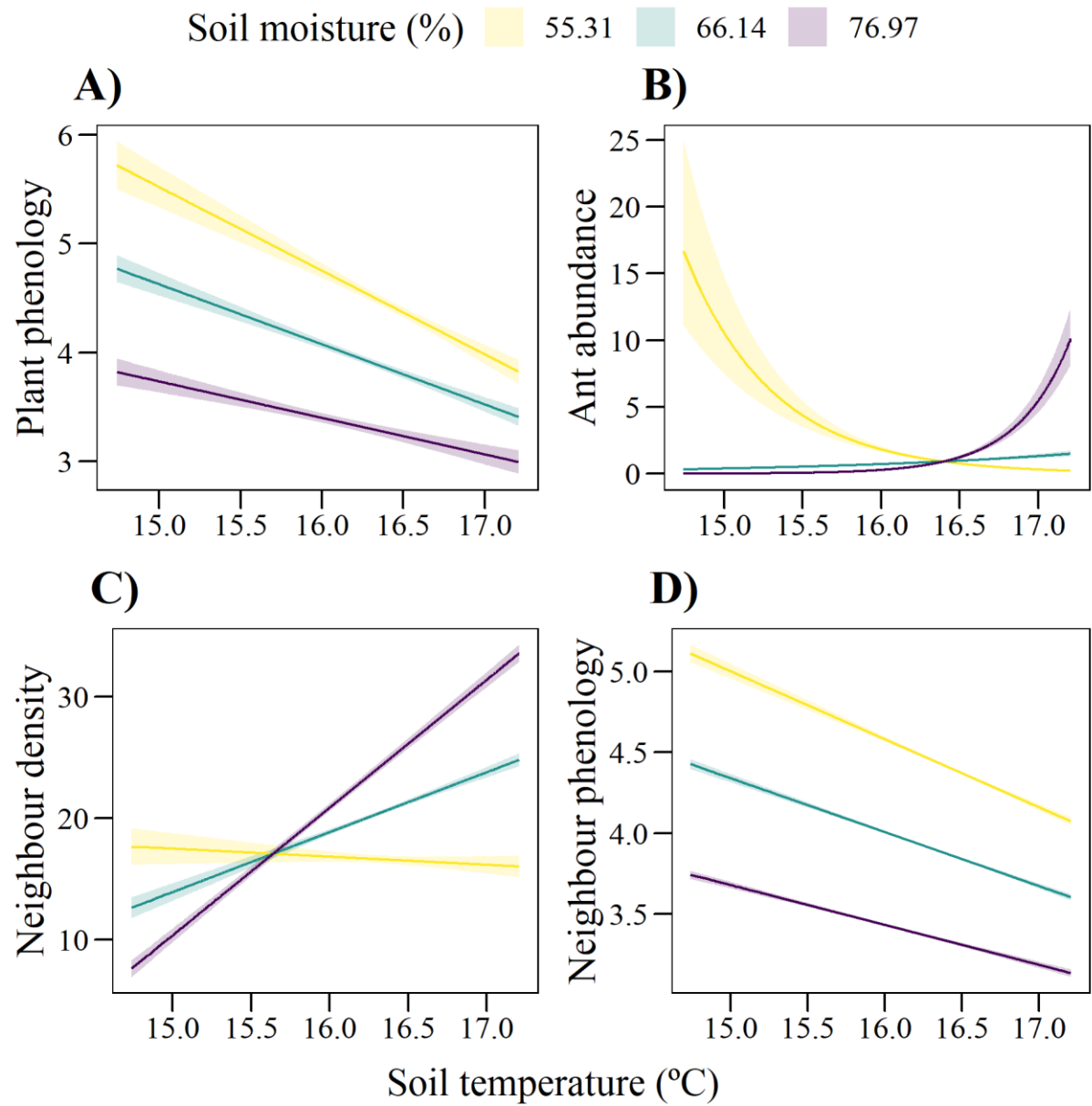
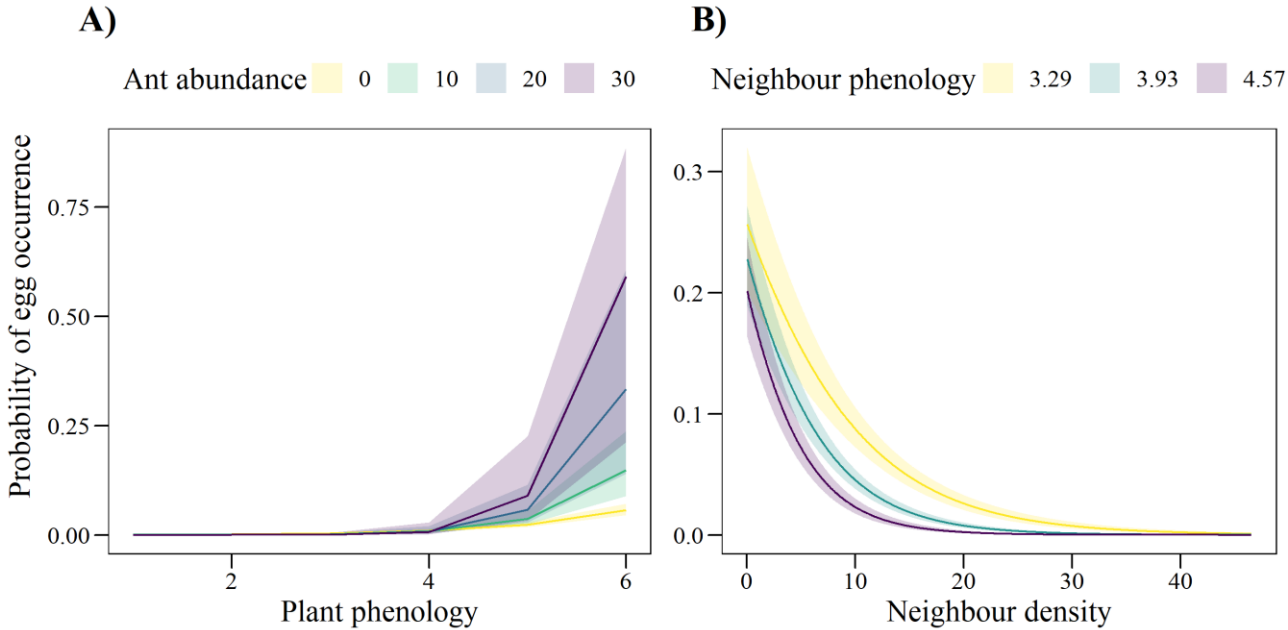


Figure 3



TABLES

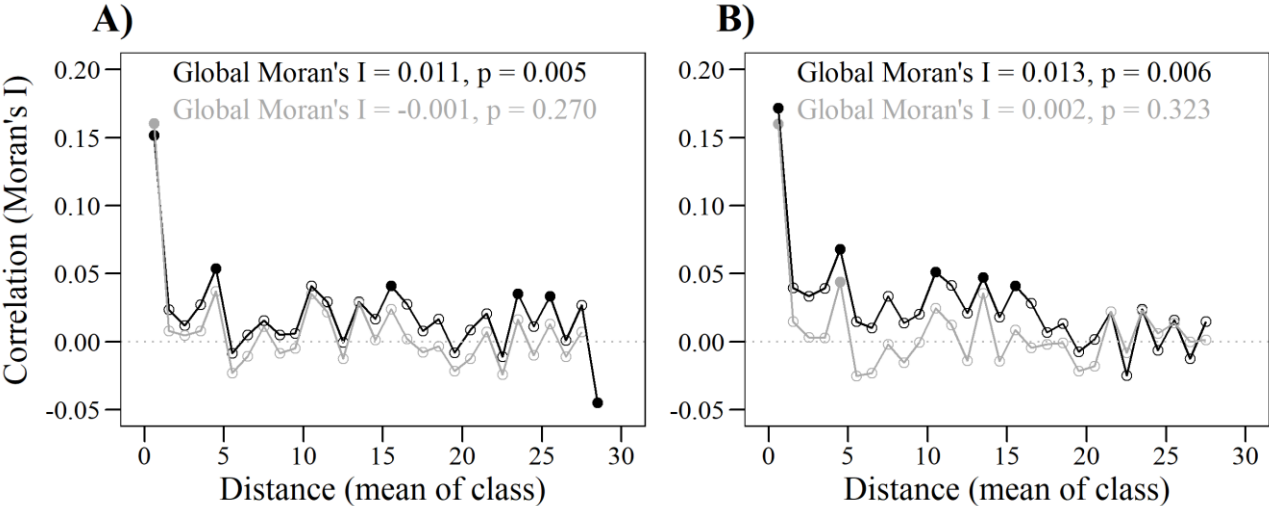
Table 1: Relationships among the number of seeds per flower in *Gentiana pneumonanthe* and A) soil temperature and B) soil moisture. Models shown are univariate linear regressions (n = 454 plants for which seed data was available) without spatial predictors, and including, in each case, one eigenvector issued from MEM as spatial predictor.

		Without spatial predictor				With spatial predictor			
		Estimate	SE	t	P	Estimate	SE	t	P
A)	Soil temperature	-66.406	21.000	-3.162	0.002	-33.306	23.930	-1.392	0.165
	MEM vector					-698.550	248.240	-2.814	0.005
B)	Soil moisture	1.534	0.810	1.894	0.059	-3.299	1.383	-2.385	0.018
	MEM vector					-1599.569	374.628	-4.270	<0.001

Table 2: Effects of microclimate (soil temperature and soil moisture) on plant reproductive performance (number of seeds per flower) of *G. pneumonanthe*, calculated based on standardized coefficients from the piecewise SEM (Fig. 1). Total, direct and indirect effects are shown, and indirect effects are divided into different pathways.

Type of effect on number of seeds per flower	Microclimatic variable	
	Soil temperature	Soil moisture
Total effects	1.746	-0.250
Direct effects	-0.055	-0.023
Indirect effects	1.801	-0.227
Through changes in oviposition occurrence	-0.061	-0.026
Through changes in phenology	0.141	-0.047
Through effects of changes in plant phenology and ant abundance on oviposition occurrence	-0.227	0.068
Through effects of changes in neighbour density and phenology on oviposition occurrence	1.948	-0.222

356 Appendices
 357 Appendix A: Spatial correlogram of the residuals of the univariate linear regressions of number of
 358 seeds per flower against soil temperature (A) and soil moisture (B) before (black) and after (grey)
 359 including one eigenvector issued from Moran's eigenvector mapping in each case. Filled circles
 360 indicate significant values of Moran's I, while hollow circles indicate non-significant values. Global
 361 Moran's I and p-value are shown in each case.



362

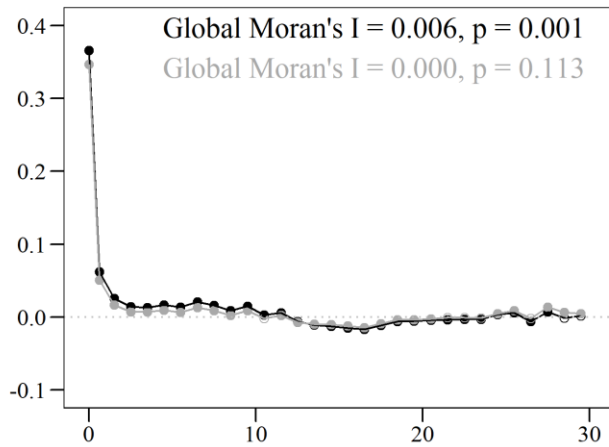
Appendix B: Results of A) a piecewise structural equation model (piecewise SEM) examining the relationships among microclimate (soil temperature and soil moisture), *G. pneumonanthe* phenology and context (host ant abundance, density and phenology of neighbouring host plants), occurrence of seed predation by *P. alcon* (egg occurrence), and *G. pneumonanthe* reproductive performance (number of seeds per flower), and B) component models of the piecewise SEM including spatial predictors. In A), both unstandardized and standardized coefficients are shown (note that standardized coefficients for component model 2) were calculated using a LM instead of a GLM, see main text), and pairs of variables with correlated errors (i.e. those not considered causal but which had a significant correlation) are denoted by \sim . In B), coefficients from spatial predictors are not shown. SE = standard error, P = p-value, N vectors = number of eigenvectors included as spatial predictors.

Model	Response	Predictor	A) Piecewise SEM				B) Component models including spatial predictors			
			Unstd. coef.	Std. coef.	SE	P	Unstd. coef.	SE	P	N vectors
1	Plant phenology	Soil temperature	-1.583	-0.465	0.252	<0.001	-1.874	0.254	<0.001	2
		Soil moisture	-0.325	-2.249	0.058	<0.001	-0.382	0.058	<0.001	
		Soil temperature \times Soil moisture	0.017	1.806	0.004	<0.001	0.020	0.004	<0.001	
2	Ant abundance	Soil temperature	-13.821	-1.593	0.558	<0.001	-13.820	0.549	<0.001	1
		Soil moisture	-3.590	-8.972	0.133	<0.001	-3.577	0.131	<0.001	
		Soil temperature \times Soil moisture	0.219	8.510	0.008	<0.001	0.218	0.008	<0.001	
3	Neighbour density	Soil temperature	-38.368	-1.483	1.467	<0.001	-29.172	1.768	<0.001	2
		Soil moisture	-10.293	-9.367	0.339	<0.001	-8.061	0.392	<0.001	
		Soil temperature \times Soil moisture	0.676	9.619	0.021	<0.001	0.515	0.024	<0.001	
4	Neighbour phenology	Soil temperature	-1.522	-1.092	0.062	<0.001	-0.86	0.057	<0.001	5
		Soil moisture	-0.310	-5.233	0.014	<0.001	-0.181	0.013	<0.001	
		Soil temperature \times Soil moisture	0.016	4.163	0.001	<0.001	0.008	0.001	<0.001	
5	Egg occurrence	Plant phenology	0.865	0.452	0.051	<0.001	0.918	0.053	<0.001	4
		Ant abundance	-0.184	-0.133	0.095	0.050	-0.247	0.099	0.012	
		Neighbour density	0.090	0.356	0.045	0.032	0.152	0.045	0.001	

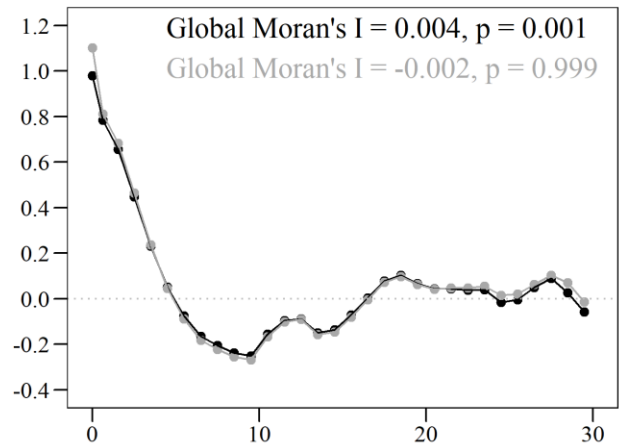
		Neighbour phenology	-0.105	-0.023	0.120	0.419	-0.242	0.129	0.061	
		Soil temperature	1.461	0.224	0.129	<0.001	1.173	0.138	<0.001	
		Soil moisture	0.029	0.104	0.006	<0.001	0.009	0.007	0.186	
		Plant phenology × Ant abundance	0.049	0.149	0.019	0.011	0.059	0.020	0.003	
		Neighbour density × Neighbour phenology	-0.070	-0.953	0.012	<0.001	-0.085	0.012	<0.001	
6	Seeds per flower	Plant phenology	14.770	0.105	9.603	0.125	-	-	-	0
		Egg occurrence	-197.745	-0.248	20.692	<0.001	-	-	-	
		Soil temperature	-26.391	-0.055	23.910	0.270	-	-	-	
		Soil moisture	-0.463	-0.023	0.905	0.609	-	-	-	
	~~ Neighbour phenology	~~ Plant phenology	0.230	0.230	-	<0.001	-	-	-	-
	~~ Neighbour phenology	~~ Neighbour density	-0.221	-0.221	-	<0.001	-	-	-	-
	~~ Neighbour phenology	~~ Ant abundance	-0.090	-0.090	-	<0.001	-	-	-	-
	~~ Neighbour density	~~ Ant abundance	-0.050	-0.050	-	<0.001	-	-	-	-
	~~ Ant abundance	~~ Plant phenology	-0.031	-0.031	-	0.002	-	-	-	-
	~~ Neighbour density	~~ Plant phenology	-0.060	-0.060	-	<0.001	-	-	-	-

371 Appendix C: Spatial correlograms of the residuals of the six component models of the piecewise
372 SEM before (black) and after (grey) including eigenvectors issued from Moran's eigenvector
373 mapping (MEM) in each case. Filled circles indicate significant values of Moran's I, while hollow
374 circles indicate non-significant values. Global Moran's I and p-value are shown in each case. For
375 component model 6, MEM was not applied. Note the different scales of the y-axis.

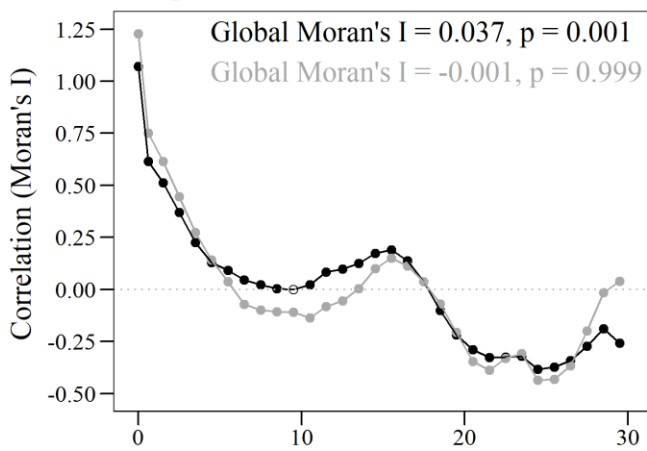
Component model 1



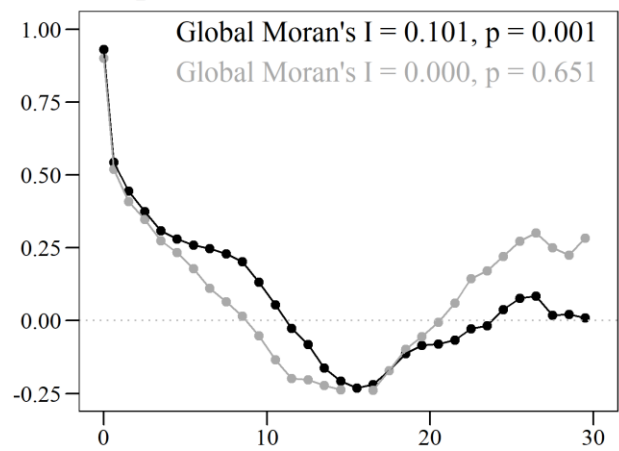
Component model 2



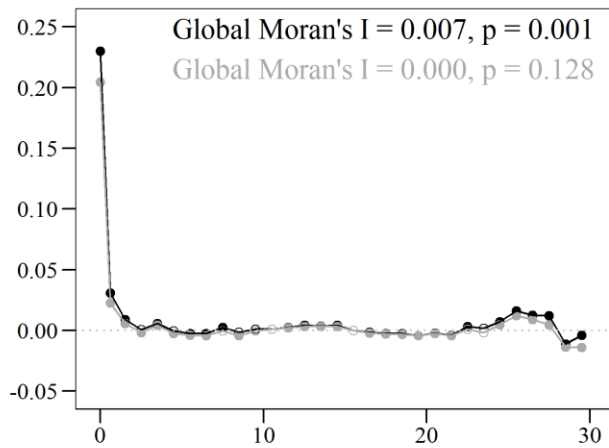
Component model 3



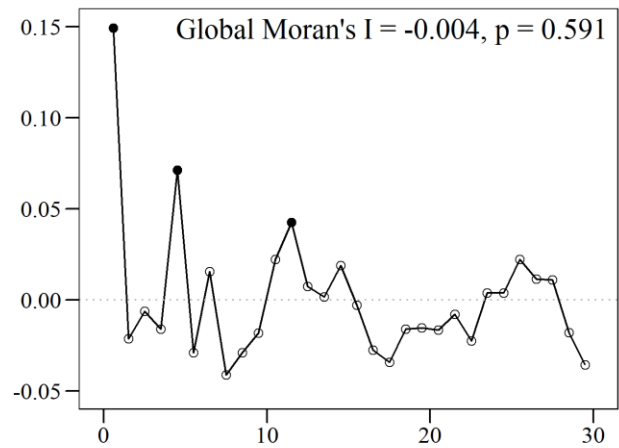
Component model 4



Component model 5



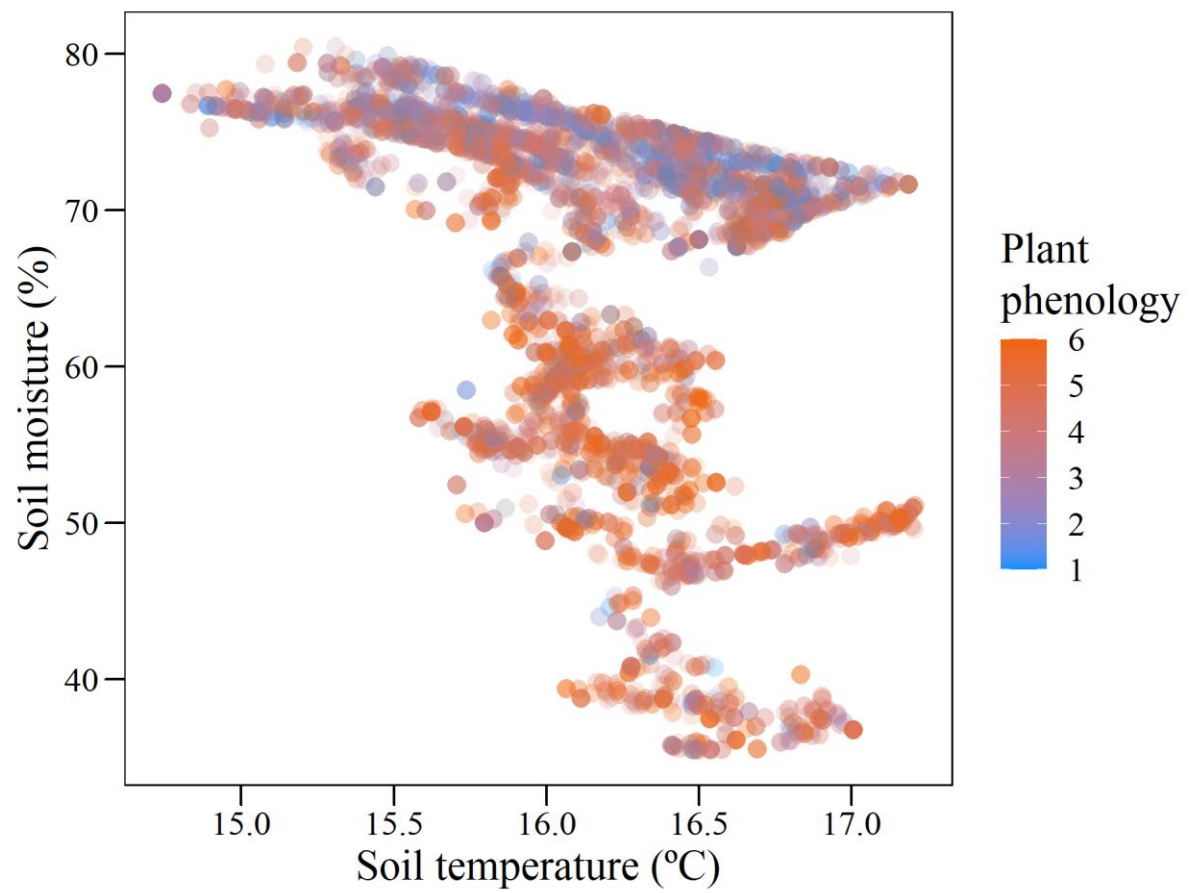
Component model 6



Distance (mean of class)

377 Appendix D: Scatterplot of soil moisture against soil temperature (Pearson's $r = -0.369$, $p < 0.001$).

378 Points are coloured according to plant phenology.



Declaration of interests

X The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: