Impacts of soil temperature, phenology and plant community composition on invertebrate herbivory in a natural warming experiment

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Abstract

Species and community-level responses to warming are well documented, with plants and invertebrates known to alter their range, phenology, or composition as temperature increases. The effects of warming on biotic interactions are less clearly understood, but can have consequences that cascade through ecological networks. Here, we used a natural soil temperature gradient of 5-35 °C in the Hengill geothermal valley, Iceland, to investigate the effects of temperature on plant community composition and plant-invertebrate interactions. We quantified the level of invertebrate herbivory on the plant community across the temperature gradient and the interactive effects of temperature, plant phenology (*i.e.* development stage), and vegetation community composition on the probability of herbivory for three ubiquitous plant species, *Cardamine pratensis*, *Cerastium fontanum* and *Viola palustris*. We found that the percentage cover of graminoids and forbs increased, while the amount of litter decreased, with increasing soil temperature. Invertebrate herbivory also increased with soil temperature at the plant community level, but this was underpinned by different effects of temperature on herbivory for individual plant species, mediated by the seasonal development of plants and the composition of the surrounding vegetation. This illustrates the importance of considering the development stage of organisms in climate change research given the variable effects of temperature on susceptibility to herbivory at different ontogenetic stages.

Introduction

Mean global surface temperatures are projected to rise by at least 1.5 °C by 2100, with the fastest rates of warming in the Arctic region (IPCC 2014). Species-level responses to warming over the last century have been comprehensively documented, such as altered geographical ranges and phenology, *i.e.* the seasonal timing of life-cycle events (Visser & Holleman 2001, Parmesan 2006). These changes reflect a combination of direct physiological responses to warming and indirect responses mediated by changes in interspecific interactions. But there is limited understanding of how species interactions will respond to climate change, even though disruption of interactions has the potential to cause cascading effects through ecological networks (Tylianakis et al. 2008, Van der Putten et al. 2010, Kharouba et al. 2018). Antagonistic interactions could be exacerbated or mutualistic interactions disrupted, especially if interacting partners display dissimilar responses to changing climatic cues (Durant et al. 2007, Walther 2010).

Experimental warming of plant communities at northern latitudes has led to increased growth rates (Arft et al. 1999, Klein et al. 2008) and biomass production (Richardson et al. 2002, Brooker & van der Wal 2003), although responses often depend on the type of vegetation (Jónsdóttir et al. 2005, Walker et al. 2006). Warming also leads to changes in plant community composition that can have consequences for higher trophic levels (Richardson et al. 2002, Brooker & van der Wal 2003, Walker et al. 2006). The effects of warming on plants may even be mediated by higher trophic levels (Walther 2010), *e.g.* the response of an Arctic plant community to warming depended on the presence of herbivores within the system (Post & Pedersen 2008). Direct responses to temperature at one trophic level can also propagate through ecological networks, *e.g.* warming in Greenland reduced caribou reproductive success because the timing of calving no longer coincided with the onset of plant growth (Post & Forchhammer 2008). Therefore, it is vital to understand how trophic interactions will change with warming

49 in order to predict how species and communities will respond to climate change (Gilman et al.

50 2010).

51   Terrestrial invertebrate herbivory is important in structuring plant communities (Wardle

52 & Barker 1997, Allan & Crawley 2011), but climate change is likely to alter the strength of

53 herbivory and the extent and frequency of invertebrate herbivore outbreaks (Bale et al. 2002,

54 Wolf et al. 2008). Evidence from the fossil record shows greater damage to plant tissues during

55 climate warming from the late-Palaeocene to mid-Eocene, suggesting that the activity of insect

56 herbivores increases with temperature (Wilf & Labandeira 1999). Dynamic modelling also

57 suggests that climate change could intensify levels of invertebrate herbivory on vegetation

58 (Wolf et al. 2008). The response of plant-herbivore interactions to future warming is difficult

59 to predict, however, with previous studies emphasising the complexity of the expected response

60 through variable, and often species-specific, effects of temperature on invertebrate herbivory

61 (Kingsolver & Woods 1998, Richardson et al. 2002, Roy et al. 2004, Lemoine et al. 2013,

62 Leckey et al. 2014, Barrio et al. 2016, Birkemoe et al. 2016, Barrio et al. 2017).

63   Herbivorous invertebrates rely on the presence of plant material of a specific age at key

64 points in their life-cycle, therefore synchronous plant and invertebrate responses to altered

65 climatic cues are crucial (Memmott et al. 2007, Van der Putten et al. 2010). There are species-

66 specific examples of strong selection on the timing of insect emergence, ensuring coordination

67 with availability of the required food plant (Visser & Holleman 2001, van Asch et al. 2007).

68 Shifting climatic patterns might disrupt the coincidence of insect emergence and food plant

69 availability, influencing the quality and quantity of food available (van Asch et al. 2007). At

70 larger scales, climate warming mediates insect herbivore outbreaks with ecosystem-level

71 impacts (Karlsson et al. 2004, Jepsen et al. 2008). Climate change could thus facilitate

72 invertebrate range expansions, but phenological mismatches could result in lower fitness and

73 impacts that propagate through an ecosystem (Kharouba et al. 2018).

74   The effect of temperature on plant-invertebrate interactions will often have consequences

75   for plant fitness, depending on whether plants suffer increased or decreased exposure to

76   herbivory (Bale et al. 2002, Jepsen et al. 2008). For example, changes in the onset of plant

77   growth can alter exposure to herbivores (Post et al. 2009). Advanced onset of growth in

78   common St John's wort (*Hypericum perforatum*) due to experimental warming led to decreased

79   damage from insect herbivores (Fox et al. 1999). Conversely, with earlier onset of growth in

80   high Arctic plant communities due to earlier snowmelt, the simultaneous earlier emergence of

81   moths resulted in greater herbivory on mountain avens flowers (Berg et al. 2008).

82   Natural temperature gradients have been proposed as model systems that substitute space

83   for time in the study of climate warming impacts (Dunne et al. 2003, O'Gorman et al. 2014).

84   They simulate some of the control provided by temperature manipulations in a laboratory

85   setting, whilst maintaining all the processes and variability of natural environments. Here, we

86   used a natural soil temperature gradient of 5-35 °C in a low Arctic community, dominated by

87   herbaceous plants and bryophytes to investigate the response of plants and invertebrates to

88   environmental warming at high latitudes. We hypothesised (H1) that vegetation community

89   composition would change with increasing soil temperature, with a greater percentage cover

90   of graminoids and litter and a reduction in bryophytes and lichens (Brooker & van der Wal

91   2003, Walker et al. 2006). We hypothesised (H2) that invertebrate herbivory would increase

92   with increasing soil temperature at both the plant community and species levels (Wilf &

93   Labandeira 1999, Wolf et al. 2008). Finally, we hypothesised (H3) that plant phenology would

94   mediate temperature effects on plant-invertebrate interactions, with less damage from

95   invertebrate herbivores at later stages of development (Fox et al. 1999).

96   **Methods**

97   *Study site*

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The study was conducted from May to July 2017 in the Hengill Valley, Iceland (64° 3'
N, 21° 18' W, 350 – 420 m above sea level), 40 km east of Reykjavík (Figure S1). Geothermal
activity generates a natural gradient of soil temperatures at a fine spatial scale, facilitating the
exploration of thermal effects on the terrestrial community without confounding factors such
as atmospheric conditions, biogeography, and dispersal constraints (O'Gorman et al. 2014,
Robinson et al. 2018). Previous research in the study area has shown changes in plant
phenology and reductions in the overall diversity of the plant and invertebrate communities as
soil temperature increases, although total plant cover and invertebrate biomass are largely
unchanged across the temperature gradient (Robinson et al. 2018, Valdés et al. 2019). The
vegetation is representative of a low Arctic community, dominated by herbaceous perennial
flowering plants and bryophytes, with a flowering season from May to September. The
terrestrial fauna is dominated by invertebrates, thus while the area is grazed by sheep from June
to September, only invertebrate herbivory was considered in this study. Key invertebrate
herbivores at the site include aphids, springtails, scale insects, slugs, moth larvae, leafhoppers,
and weevils (see Table S1). Survey work conducted during the same time of year as this study
showed a similar species richness and abundance of invertebrate herbivores across the soil
temperature gradient in Hengill (see Figure S2).

Herbivory assessments were made at the plant community and species levels. We focused
on three plant species with a widespread occurrence across the temperature gradient:
cuckooflower (*Cardamine pratensis*, Linnaeus), common mouse-ear (*Cerastium fontanum*,
Baumgerten), and marsh violet (*Viola palustris*, Linnaeus). The three species have different
growth forms and expected susceptibilities to invertebrate herbivory (Turcotte et al. 2014).
Cardamine pratensis has pinnate leaves with multiple leaflets up to 1 cm wide and individuals
in our study had up to 20 flowering stems and 30 basal leaves (Figure S3a-b). *Cerastium*
fontanum is a densely hairy herb with runners, erect flowering shoots, and paired leaves that

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123   are up to 1 cm wide, while individuals in our study had up to 30 runners and 250 leaves (Figure

124   S3c-d). *Viola palustris* has hairless kidney-shaped leaves that are 1–4 cm wide and individuals

125   in our study had up to 14 leaves (Figure S3e).

126   *Experimental design*

127   Fourteen experimental plots measuring 66-210 m^2 were established within a 1 km^2 area

128   of the Hengill valley in May 2017 (Figure S1). Plot locations were chosen to ensure that

129   individuals of the focal plant species were distributed evenly across a gradient of soil

130   temperatures, whilst also aiming for within-plot variation in temperature where possible (Table

131   S2). Thirty individuals per species of *C. pratensis*, *C. fontanum*, and *V. palustris* were marked

132   in each of ten plots (Table S2), using a stratified random sampling method where individuals

133   were randomly selected, but the full range of within-plot soil temperatures was represented.

134   For the community-level herbivory assessments, five 50 × 50 cm quadrats (hereafter

135   community-level quadrats) were marked at random points in eight of the plots that best

136   captured the full temperature gradient (Table S2).

137   *Environmental variables*

138   Soil temperature was recorded at 12 cm depth at five points within each community-level

139   herbivory quadrat and during each herbivory survey at every marked individual of *C. pratensis*,

140   *C. fontanum*, and *V. palustris*, using a soil temperature probe. Percentage soil moisture was

141   recorded in each community-level herbivory quadrat using an ML3 ThetaProbe and HH2

142   Handheld Readout Unit (Delta-T Devices Ltd). To quantify soil pH and nutrient content, five

143   soil cores were taken in each community-level herbivory quadrat, using a 2 cm diameter soil

144   auger, sampling 2-10 cm deep, and homogenising the five cores prior to analysis. Nitrate and

145   ammonium were extracted from fresh soil using 2M KCl solution with a 5:1 soil-to-solution

146   ratio (Blakemore et al. 1987). Phosphate was extracted from fresh soil using an ammonium

147   lactate-acetic acid buffer (pH 3.75) with a 10:1 soil-to-solution ratio (Egnér et al. 1960).

148   Nutrient concentrations were assessed colourimetrically, with detection limits of 0.17 mg kg^{-1}

149   for nitrate and ammonium, and 0.30 mg kg^{-1} for phosphate. The remainder of the soil was dried

150   for 13 hours at 80 °C, after which time 10 g was added to 25 ml deionised water, shaken

151   vigorously, and left for 12 hours before measurement with a pH probe (Blakemore et al. 1987).

152   *Quantifying plant phenology and vegetation communities*

153   The floral development stage of each marked individual of *C. pratensis*, *C. fontanum*,

154   and *V. palustris* was estimated weekly throughout the study as a measure of plant phenology.

155   The development stages were denoted as follows: (1) only vegetative growth, plant <2 cm; (2)

156   only vegetative growth, plant >2 cm; (3) buds just starting to form, very small, close to leaves;

157   (4) buds at medium size; (5) buds large, but still completely closed; (6) buds large and starting

158   to open; (7) buds large and opening more, petals > sepals; (8) at least one flower opened; (9)

159   all flowers opened, none yet wilted; and (10) flowers starting to wilt. The aboveground

160   vegetation community was also surveyed in association with each community-level quadrat

161   from 6th to 7th June, and each individual marked plant (*i.e.* species-level quadrats) from 20th to

162   27th May, using 50 × 50 cm quadrats. Percentage cover of functional groups of vegetation

163   (bryophytes, forbs, graminoids, lichens, litter, and bare ground) was visually estimated at

164   ground level as the midpoint of the following cover classes: 0-1%, 1-2%, 2-5%, 5-10%,

165   10-25%, 25-50%, 50-75%, 75-95%, and 95-100% (after Peet et al. 1998).

166   *Quantifying herbivory*

167   Leaf herbivory was quantified using a 'standing' measure of damage to leaves at each

168   sampling time-point (Turcotte et al. 2014). The community-level herbivory assessment was

169   conducted on 19th June. The number of damaged plants was recorded out of 100 random

170   individuals, selected using a 10 × 10 grid within each 50 × 50 cm quadrat. For the species-level

171   herbivory assessment, individual marked plants were surveyed for signs of invertebrate

172   herbivory every two weeks from 30th May to 2nd July, generating three time-points per species.

173   At each survey, all marked individuals for each species were assessed within a 48-hour period.

174   Plants were recorded as damaged or not damaged by invertebrate herbivores at each time-point.

175   We only saw evidence for defoliation due to biting, with no leaf mining or galling. The

176   percentage of plants exhibiting damage ranged from 30–60% for each plant species at each

177   sampling time-point, so our measure of herbivory was never in danger of saturating. The

178   assessment of herbivore damage was only recorded on healthy, fully expanded leaves; evidence

179   of damage on wilted leaves was not included in the overall estimation of herbivore damage, as

180   damage to these leaves could not be exclusively attributed to invertebrate herbivory. To avoid

181   overestimation of the level of invertebrate herbivory, loss of entire leaves or leaflets was only

182   categorised as herbivore damage if part of the petiole was still present with invertebrate bite

183   marks evident (Turcotte et al. 2014).

184   *Statistical analysis*

185   All statistical analyses were carried out in R 4.0.2. Plant data with corresponding soil

186   temperatures >35 °C were excluded because these extreme temperatures are less relevant in a

187   climate change context. This excluded one of 40 community-level quadrats and 20 of 984

188   species-level quadrats. A Mantel test was used to check for spatial structure in the soil

189   temperature data by comparing pairwise distances between experimental plots with pairwise

190   temperature differences after taking the average of all the soil temperatures recorded in each

191   plot ('*mantel*' function in the '*vegan*' package with '*method = "spearman"*' to account for

192   non-normality of the distance data).

193   Associations between temperature and other measured environmental variables (*i.e.* soil

194   moisture, pH, and concentrations of nitrate, ammonium, and phosphate) were tested using

195 Pearson correlations and the false discovery rate correction for multiple testing ('*cor.test*' and

196 '*p.adjust*' functions with '*method = "fdr"*' in the '*stats*' package). Note that the false discovery

197 rate is not as conservative as the Bonferroni correction and thus less likely to discard significant

198 effects when correcting for multiple testing (Benjamini & Hochberg 1995). Associations

199 between environmental variables and the percentage cover for each vegetation group sampled

200 in the community-level quadrats ($n = 39$) were also explored using Pearson correlations.

201   The relationship between soil temperature and percentage cover for each vegetation

202 group in the species-level quadrats ($n = 964$) was assessed using linear mixed effects models

203 (LMM), with soil temperature as a continuous fixed effect and a random intercept for

204 experimental plot ('*lme*' function in the '*nlme*' package). The midpoints of the percentage

205 cover data were converted to proportions and logit transformed to meet the assumption of

206 normality of the model residuals. Detrended Correspondence Analysis (DCA) was also used to

207 produce a measure of vegetation community composition for both the community- and species-

208 level quadrats ('*decorana*' function in the '*vegan*' package). The input was a site by vegetation

209 matrix, based on the midpoints of the cover classes for each vegetation group, and the first

210 DCA axis was used as a measure of vegetation community composition in subsequent analyses.

211   The major drivers of herbivory at the community level were explored using LMM with

212 a random intercept for experimental plot. We explored the main and interactive effects of soil

213 temperature and vegetation community composition as continuous fixed effects to explain the

214 proportion of plants damaged per quadrat, dropping the interaction term from the model if it

215 was not significant (Crawley 2012).

216   The major drivers of herbivory at the species level were explored using generalised linear

217 mixed effects models (GLMM) for each species with a random intercept for plant identity

218 nested within experimental plot nested within sampling time-point, and a binomial error

219 structure and logit link ('*glmer*' function in the '*lme4*' package). We explored the main effects

220   of soil temperature (a continuous fixed effect), plant phenology (a discrete fixed effect with

221   integer values for each development stage), and vegetation community composition (a

222   continuous fixed effect), plus the interactive effects of temperature:phenology and

223   temperature:composition. We also included a second-order polynomial term for phenology to

224   account for possible reductions in herbivory in later stages of development. We dropped any

225   non-significant second-order polynomial or interaction terms from the model, starting with the

226   least significant higher-order term (Crawley 2012). Note that we used the measure of plant

227   phenology that was closest in time to our measure of herbivory in these analyses.

228   **Results**

229   *Environmental variables*

230   There was no significant correlation between pairwise distance and temperature

231   difference between plots (Mantel test: $r = 0.15$; $p = 0.103$). There was also no significant

232   correlation between temperature and soil moisture (Pearson correlation: $r = -0.15$; $p = 0.533$),

233   pH (Pearson correlation: $r = 0.20$; $p = 0.580$), nitrate (Pearson correlation: $r = 0.02$; $p = 0.907$),

234   ammonium (Pearson correlation: $r = -0.10$; $p = 0.664$), or phosphate (Pearson correlation: $r =$

235   0.35; $p = 0.143$).

236   *Vegetation community*

237   There was no significant relationship between temperature and the percentage cover of

238   bryophytes (LMM: $t = -0.98$; $p = 0.329$; Figure 1a) or lichens (LMM: $t = -1.53$; $p = 0.127$;

239   Figure 1d) in the species-level quadrats. There was a significant increase in the percentage

240   cover of forbs (LMM: $t = 6.88$; $p < 0.001$; marginal $r^2 = 0.10$; conditional $r^2 = 0.48$; Figure 1b),

241   graminoids (LMM: $t = 6.32$; $p < 0.001$; marginal $r^2 = 0.09$; conditional $r^2 = 0.46$; Figure 1c),

242   and bare ground (LMM: $t = 2.54$; $p = 0.011$; marginal $r^2 = 0.02$; conditional $r^2 = 0.09$; Figure

243   1f), and a significant decrease in the percentage cover of litter (LMM: $t = -5.86$; $p < 0.001$;

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marginal $r^2 = 0.06$; conditional $r^2 = 0.62$; Figure 1e) with increasing soil temperature. Soil moisture was positively correlated with graminoids and negatively correlated with forbs in the community-level quadrats, whilst phosphate was negatively correlated with bryophytes (Figure S4).

For both the community- and species-level quadrats, negative values of DCA axis 1 corresponded to communities dominated by bryophytes, lichens, and forbs, while positive values of DCA axis 1 corresponded to communities dominated by litter (Figure S5). There was only a significant (but weak) effect of temperature on vegetation community composition in the species-level quadrats ($p < 0.001$, $r^2 = 0.02$), with no effect of temperature in the community-level quadrats ($p = 0.228$, $r^2 = 0.08$; Figure S5b).

Herbivory

In the community-level quadrats, $24 \pm 11\%$ (mean ± standard deviation, SD) of plants exhibited damage by invertebrate herbivores. The optimal model describing the proportion of herbivory at the plant community-level included the main effects of soil temperature and plant community composition only (marginal $r^2 = 0.21$; conditional $r^2 = 0.21$). There was a significant increase in the proportion of plants exhibiting damage by herbivores with increasing soil temperature (LMM: $t = 3.06$; $p = 0.005$; Figure 2), with no significant effect of plant community composition (LMM: $t = 1.58$; $p = 0.126$).

For *C. pratensis*, $44 \pm 3\%$ (mean ± SD) of plants exhibited damage by invertebrate herbivores across all sampling time-points. The optimal model describing the probability of herbivory for *C. pratensis* included the main effects of temperature, phenology, and plant community composition only (marginal $r^2 = 0.06$; conditional $r^2 = 0.21$). There was a significant increase in the probability of damage to *C. pratensis* by herbivores with increasing soil temperature (GLMM: $t = 2.14$; $p = 0.033$; Figure 3a), more advanced development stage

268   (GLMM: $t = 2.79$; $p = 0.005$; Figure 3b), and as the vegetation community became increasingly

269   dominated by litter (GLMM: $t = 3.01$; $p = 0.003$; Figure 3c).

270   For *C. fontanum*, $33 \pm 3\%$ (mean $\pm$ SD) of plants exhibited damage by invertebrate

271   herbivores across all sampling time-points. The optimal model describing the probability of

272   herbivory for *C. fontanum* included the main effects of temperature, phenology (first- and

273   second-order polynomial terms), and plant community composition only (marginal $r^2 = 0.09$;

274   conditional $r^2 = 0.17$). There was a significant reduction in the probability of damage to *C.*

275   *fontanum* by herbivores with increasing soil temperature (GLMM: $t = -3.48$; $p < 0.001$; Figure

276   3d). There was an increase in the probability of damage to *C. fontanum* by herbivores up to

277   development stage 7 (GLMM first-order polynomial term: $t = 3.00$; $p = 0.003$), with a reduction

278   in later stages (GLMM second-order polynomial term: $t = -2.61$; $p = 0.009$; Figure 3e). There

279   was also an increase in the probability of damage to *C. fontanum* by herbivores as the

280   vegetation community became increasingly dominated by litter (GLMM: $t = 2.56$; $p = 0.011$;

281   Figure 3f).

282   For *V. palustris*, $52 \pm 10\%$ (mean $\pm$ SD) of plants exhibited damage by invertebrate

283   herbivores across all sampling time-points. The optimal model describing the probability of

284   herbivory for *V. palustris* included the main effects of temperature, phenology, and plant

285   community composition, and the interactive effect of temperature $\times$ phenology (marginal $r^2 =$

286   0.06; conditional $r^2 = 0.12$). There was a significant increase in the probability of damage to *V.*

287   *palustris* by herbivores with increasing soil temperature overall (GLMM: $t = 4.22$; $p < 0.001$;

288   Figure 3g) and more advanced development stage (GLMM: $t = 2.62$; $p = 0.009$; Figure 3h),

289   with a marginally non-significant increase as the vegetation community became increasingly

290   dominated by litter (GLMM: $t = 1.78$; $p = 0.075$; Figure 3i). The significant interactive effect

291   of temperature and phenology (GLMM: $t = -2.20$; $p = 0.028$) was driven by an increase in the

292   probability of damage to *V. palustris* by herbivores with increasing soil temperature in the early

293 stages of development, but a decline in the probability of damage in the later stages of

294 development (Figure 4).

295 **Discussion**

296   We considered the impact of soil temperature on plant communities and plant-

297 invertebrate interactions. We found that soil temperature altered plant community composition,

298 increasing the dominance of forbs and graminoids, and reducing the amount of litter. We found

299 general support for stronger plant-invertebrate interactions at the community level in warmer

300 environments, but with variable effects depending on individual plant species. Invertebrate

301 herbivory increased with soil temperature for *C. pratensis* and *V. palustris*, but decreased with

302 temperature for *C. fontanum*. All three species were also more susceptible to herbivory as they

303 developed (though *C. fontanum* was less susceptible in the later stages of development) and

304 when there was a higher proportion of litter in the surrounding environment. Plant phenology

305 played a crucial role in determining the thermal sensitivity of herbivory in *V. palustris*, with

306 contrasting responses at early and late stages of development. This illustrates the importance

307 of considering the entire life-cycle of organisms in climate change research, given their variable

308 susceptibility to herbivory at different ontogenetic stages.

309   There was partial support for our first hypothesis, with an increase in the percentage

310 cover of graminoids with increasing soil temperature. Grasses, which dominate the graminoids

311 in our study system, have also been shown to increase with experimental warming in the Arctic

312 region (Brooker & van der Wal 2003, Walker et al. 2006). There was no effect of soil

313 temperature on the percentage cover of bryophytes or lichens, however, which is in contrast to

314 Walker et al. (2006) who found a reduction in the cover of both groups following warming at

315 high latitudes. The increase in percentage cover of forbs with increasing temperature may be

316 driven by increased growth rates, with forbs exhibiting the strongest increases in vegetation

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growth in warmed tundra communities (Arft et al. 1999, Walker et al. 2006, Winkler et al. 2016). Given that bryophytes were the dominant vegetation class throughout the study site (Figure 2), an increase in the percentage cover of forbs in warmer areas should increase habitat availability and food quality for invertebrate herbivores (Lawton 1983, Southwood et al. 1986). This could be an important factor in attracting insect herbivores to warmer habitats, as a potential indirect effect of temperature on stronger plant-invertebrate interactions (Moise & Henry 2010). The reduction in litter with increasing soil temperatures was likely due to faster decomposition rates at higher temperatures, as previously shown in tundra ecosystems (Hobbie 1996). Lower quantities of litter are associated with higher germination rates (Xiong & Nilsson 1999), but may increase the risk of invertebrate herbivory for individual plants (Figure 3c,f,i).

Invertebrate herbivory increased with increasing temperature at the plant community level, in support of our second hypothesis. Strengthening of consumer-resource interactions with increasing temperature has been widely observed in functional response experiments (Rall et al. 2012), due to the higher metabolic demands of life in warmer environments (Brown et al. 2004). Stronger plant-herbivore interactions at higher temperatures may also be driven by changes in the invertebrate community, but we found no evidence for altered abundance or species richness of invertebrate herbivores across the temperature gradient here (Figure S2). This suggests that changes in the energy requirements and feeding rates of invertebrates should be the major factor driving these results, although changes in the species identity and relative abundance of insect herbivores across the soil temperature gradient could also be a factor. Stronger plant-herbivore interactions can reverse the positive effects of temperature on plant growth (O'Connor 2009), disrupt temperature effects on plant community composition (Post & Pedersen 2008), and shape plant productivity responses to warming (Post et al. 2009). Vegetation community composition may also form a feedback loop by influencing invertebrate feeding preferences (Loranger et al. 2013), whereby desirable plant species attract herbivores

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342 and less attractive vegetation masks the detection of desirable plants (Hambäck et al. 2000,

343 Finch & Collier 2012). Nevertheless, we found no independent effects of the surrounding

344 vegetation community composition on invertebrate herbivory at the community-level here,

345 suggesting mainly indirect effects on invertebrate herbivory mediated by temperature.

346   There were contrasting effects of temperature on the probability of herbivory for each

347 plant species, with inconsistent effects of warming on invertebrate herbivory at the species-

348 level also shown across previous research at high latitudes (Leckey et al. 2014, Birkemoe et al.

349 2016, Barrio et al. 2017). This may be driven by individual plant traits, with life history,

350 morphology, and physical defence known to be correlated with resistance to herbivory (Wardle

351 et al. 1998, Carmona et al. 2011). For example, *C. fontanum* has densely hairy leaves (Figure

352 S3b), which may limit its appeal to invertebrate herbivores (Fordyce & Agrawal 2001, Hanley

353 et al. 2007) and contribute to the lower risk of herbivory that was observed at higher

354 temperatures compared to the other smooth-leaved species. In contrast, the susceptibility of *C.*

355 *pratensis* to invertebrate herbivores has been demonstrated experimentally, with lower

356 percentage cover in their presence compared to their absence, whereas *C. fontanum* actually

357 increased in percentage cover in the presence of invertebrate herbivores (Stein et al. 2010).

358   Probability of damage increased with advancing development over the flowering period

359 for both *C. pratensis* and *V. palustris*, suggesting that the plants became more susceptible to

360 herbivory as they developed. This contrasts with other research showing greater invertebrate

361 herbivore damage on young plants due to their weaker defences (Boege & Marquis 2005). Our

362 finding could reflect a change in the visibility of the plants to herbivores as they grow or a

363 higher abundance of invertebrates at the study site as the season develops. Greater invertebrate

364 damage has been demonstrated at later stages of development in other *Cardamine* species,

365 accompanied by negative effects on vegetative propagation (Collinge & Louda 1988), which

366 highlights the potential consequences of increased invertebrate herbivory on plant fitness. The

367 reduction in herbivore damage at later development stages for *C. fontanum* (Figure 3e) could

368 suggest greater rates of plant tissue regeneration or lower invertebrate attack rates later in the

369 season or as flowers begin to wilt. The nutritional quality of *C. fontanum* decreases with

370 maturity (Kramberger & Klemenčič 2003), which lends some support for a lower preference

371 of invertebrates for this species over time.

372   In support of our third hypothesis, plant development stage influenced temperature

373 effects on herbivory for *V. palustris*. Here, plants were more vulnerable to invertebrate

374 herbivores in warmer soils at earlier development stages, whereas they were more vulnerable

375 in cooler soils at later stages. Warming has been shown to reduce the nutritional quality of

376 leaves, by decreasing the amount of leaf biomass relative to stems (Veteli et al. 2002). Leaves

377 from warmer regions also tend to be tougher, with less nitrogen content, and more tannins and

378 alkaloids (Coley 1998). A similar response in *V. palustris* may make the plant less appealing

379 to herbivores at higher temperatures as it grows. Interactive effects of temperature and

380 herbivory have been shown on plant phenology, with herbivores altering impacts of warming

381 on plant height and seed production (Lemoine et al. 2017). We believe our study is the first

382 demonstration of a feedback response, such that plant phenology also alters the effect of

383 temperature on herbivory. This illustrates the complex and reciprocal nature of plant-herbivore

384 interactions and the potential for phenological mismatches in a warmer climate to alter the

385 dynamics of both plant and invertebrate communities (de Sassi et al. 2012). Note that our study

386 most likely quantified accumulated damage by herbivores across development stages and so

387 future research should test for different rates of herbivory at each stage for a deeper

388 understanding of how phenology might alter temperature effects on herbivory.

389   We have shown that increasing temperature has different effects on plant-invertebrate

390 interactions at high latitudes depending on the plant species, but the overall response at the

391 community level was greater invertebrate herbivory in warmer environments. In tropical

392 ecosystems, where invertebrate herbivores play an even stronger role in structuring plant

393 communities, and are much closer to their thermal limits, temperature-induced changes in

394 plant-invertebrate interactions could have more severe community-level consequences (Coley

395 & Barone 1996, Deutsch et al. 2008). Furthermore, future warming will be accompanied by

396 changes in other climatic variables, such as precipitation, CO_2 levels, and the frequency of

397 extreme events, eliciting physiological and behavioural responses in plants and invertebrates

398 that may modulate the overall response to warming (Bale et al. 2002). As research into the

399 impacts of climate change broadens, it is important to consider that responses can vary at

400 different life-cycle stages of the individual organisms involved. Future research should also

401 aim to disentangle the complex feedbacks between temperature effects on plant communities

402 and invertebrate herbivores by quantifying changes in the structure of ecological networks.

403 **Data Availability**

404 All data will be archived with the NERC Environmental Information Data Centre.

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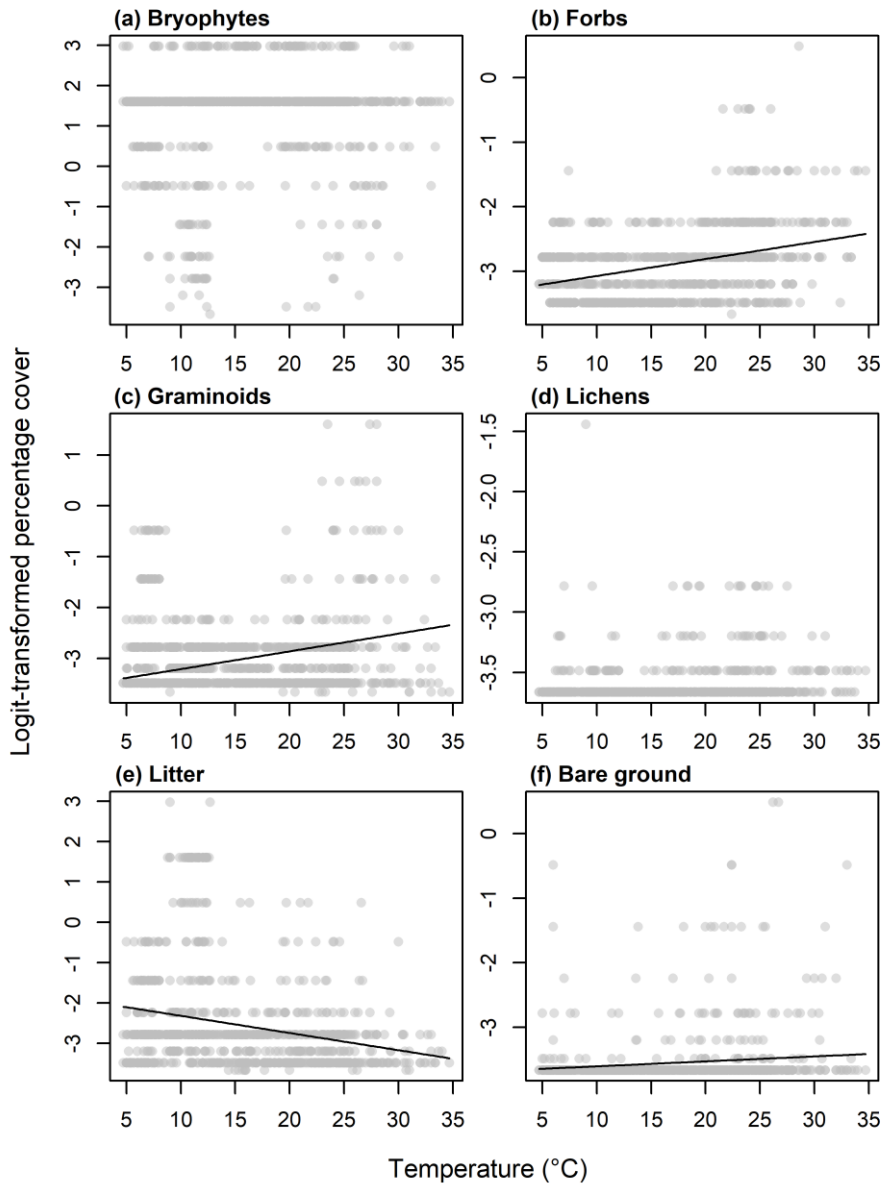
603   **Figure Legends**

604   **Figure 1.** Relationships between temperature and the percentage cover of (a) bryophytes, (b)

605   forbs, (c) graminoids, (d) lichens, (e) litter, and (f) bare ground. Logit-transformed midpoints

606   of percentage cover classes are shown on the *y*-axis and only significant trendlines for forbs (*y*

607   = 0.026*x* – 3.34; marginal r^2 = 0.10; conditional r^2 = 0.48), graminoids (*y* = 0.035*x* – 3.56;

608   marginal r^2 = 0.09; conditional r^2 = 0.46), litter (*y* = -0.043*x* – 1.89; marginal r^2 = 0.02;

609   conditional r^2 = 0.62), and bare ground (*y* = -0.008*x* – 3.68; marginal r^2 = 0.06; conditional r^2

610   = 0.09) are visualised in the figure.

611   **Figure 2.** Relationship between temperature and the proportion of plants in the community-

612   level quadrats exhibiting damage by invertebrate herbivores (*y* = 0.0077*x* + 0.1014; marginal

613   r^2 = 0.21; conditional r^2 = 0.21).

614   **Figure 3.** The main effects of temperature, phenology, and vegetation community composition

615   on the probability of damage to *Cardamine pratensis* (a-c), *Cerastium fontanum* (d-f), and

616   *Viola palustris* (g-i) by invertebrate herbivores. Phenology is shown as increasing stages of

617   development from small vegetative (stage 1) to the wilting of flowers (stage 10). Vegetation

618   community composition is shown as the first DCA axis of the species-level quadrats, with

619   negative or smaller values indicating communities dominated by bryophytes, lichens, and forbs

620   and larger positive values indicating communities dominated by litter (see Figure S5b).

621   Trendlines are visualised at the median values for other explanatory variables in the model.

622   **Figure 4.** The interactive effect of temperature and phenology on the probability of damage to

623   *V. palustris* by invertebrate herbivores. Temperature effects are visualised at the (a) first, (b)

624   middle, and (c) last stage of development observed during the current study. See Figure S6 for

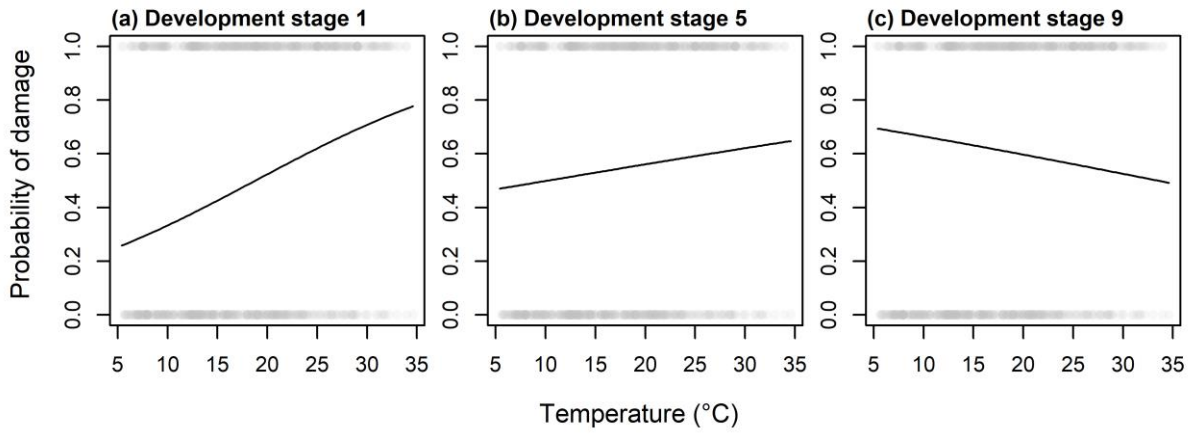625   temperature effects at all development stages.

26

Figure 1

628   **Figure 2**

629

Figure 3

632　**Figure 4**

(a) Development stage 1　　(b) Development stage 5　　(c) Development stage 9

Probability of damage
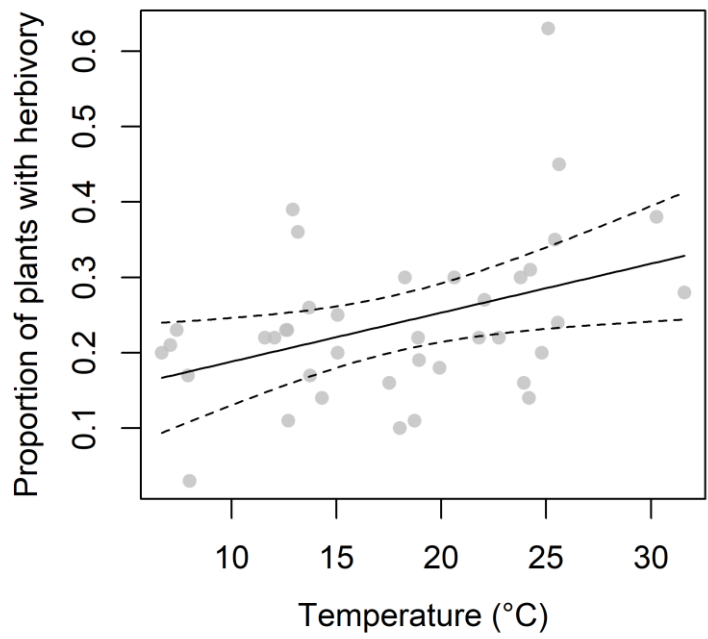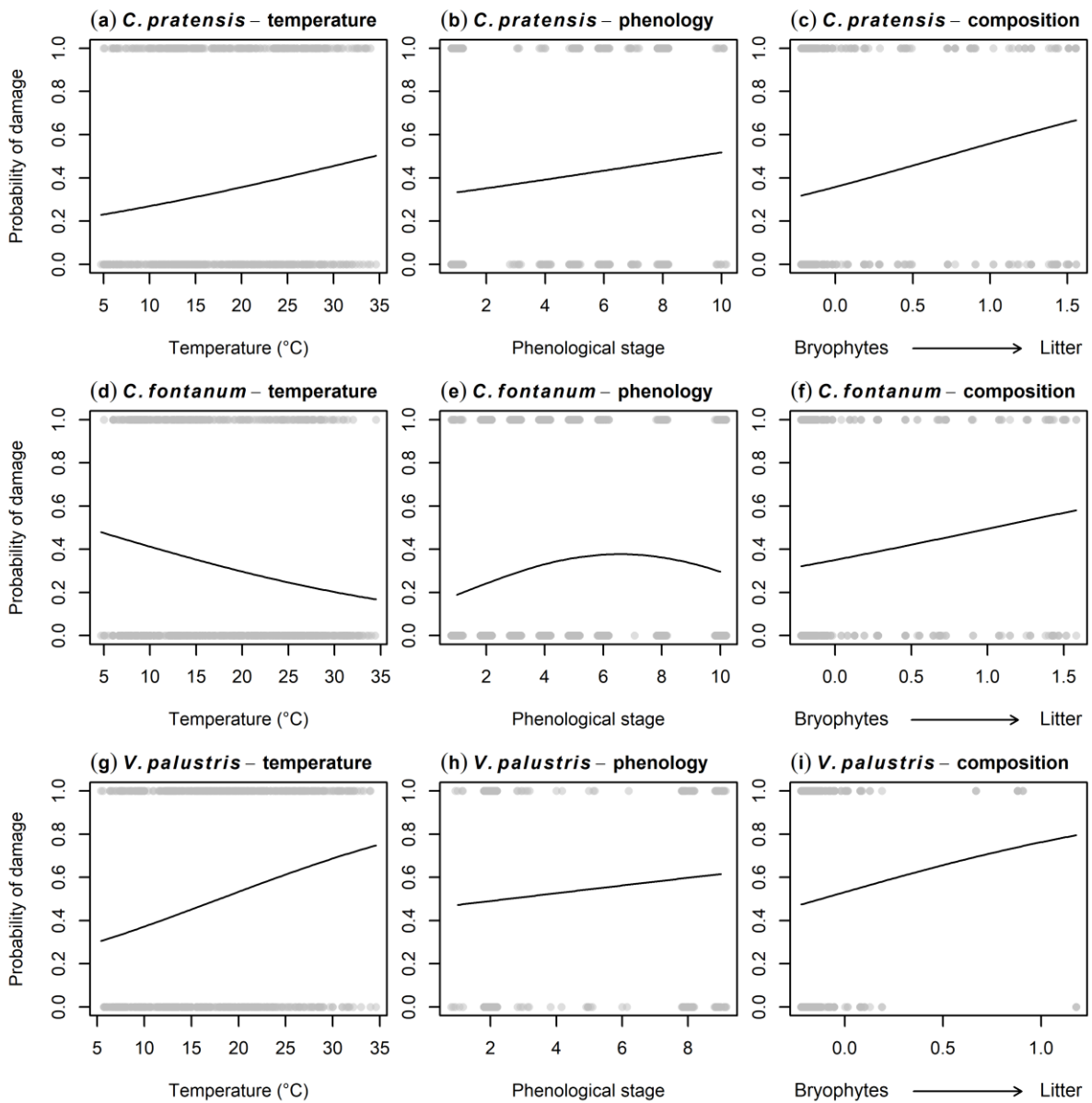
Temperature (°C)

633

Supporting Information:

Impacts of soil temperature, phenology, and plant community composition on invertebrate herbivory in a natural warming experiment

**Figure S1.** Map of the study site. (a) Location of the Hengill valley in Iceland. (b) Aerial view of the study area from Google Earth, with red boxes indicating the location of the experimental plots in (c) the geothermally influenced area and (d) the ambient area of the valley. The black outlines in (c) and (d) indicate the location of the experimental plots relative to the location of the streams in the valley (blue lines), with labels corresponding to the information in Table S2.
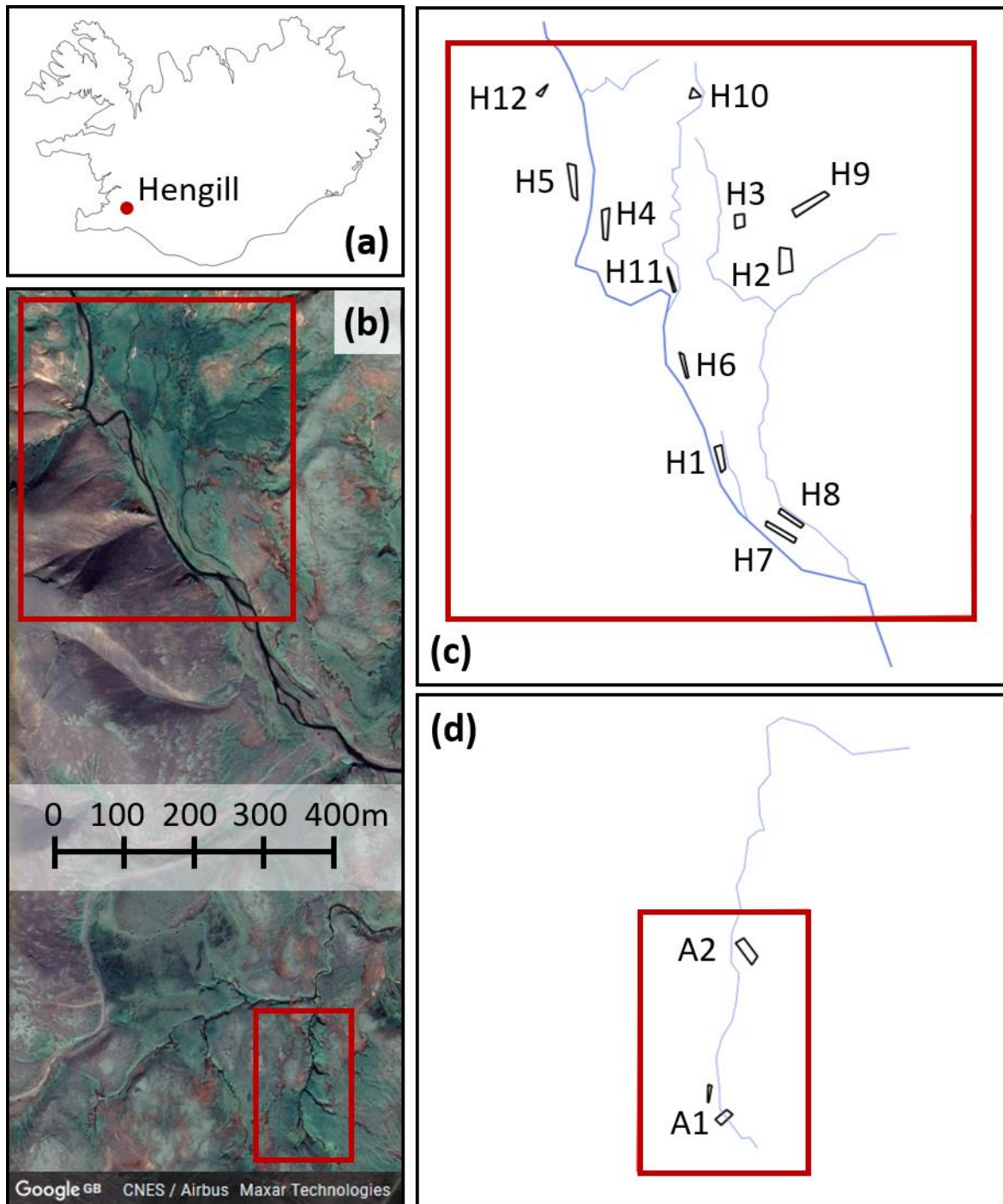
Figure S2. (a) Species richness and (b) abundance of invertebrate herbivores sampled in the Hengill valley from 19th May to 5th July 2015, using pitfall traps placed in 50 sites across the soil temperature gradient. There was no significant relationship between soil temperature and either species richness (Linear regression: $F_{1,48} = 1.024$, $p = 0.317$) or abundance (Linear regression: $F_{1,48} = 0.265$, $p = 0.609$).
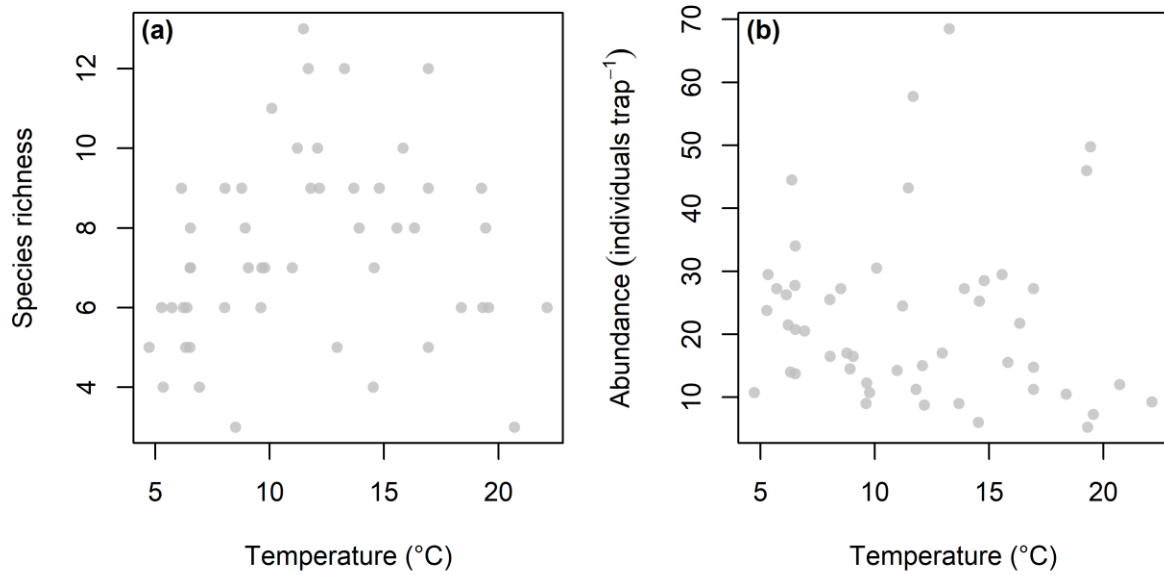
Figure S3. Photographs of the three plant species: (a-b) *Cerastium fontanum*, whole plant (https://upload.wikimedia.org/wikipedia/commons/d/dd/20130503Cerastium_fontanum2.jpg) and leaves (https://upload.wikimedia.org/wikipedia/commons/4/4a/Cerastium_fontanum_ subsp._vulgare_kz03.jpg), (c-d) *Cardamine pratensis*, whole plant (https://commons. wikimedia.org/wiki/File:Cardamine_pratensis_Pinksterbloem.jpg) and leaves (https://upload. wikimedia.org/wikipedia/commons/thumb/8/81/Cardamine_pratensis_leaf_kz.jpg/800px-Car damine_pratensis_leaf_kz.jpg), and (e) *Viola palustris* (https://upload.wikimedia.org/ wikipedia/commons/d/de/Moerasviooltje_in_het_Aamsveen.jpg).
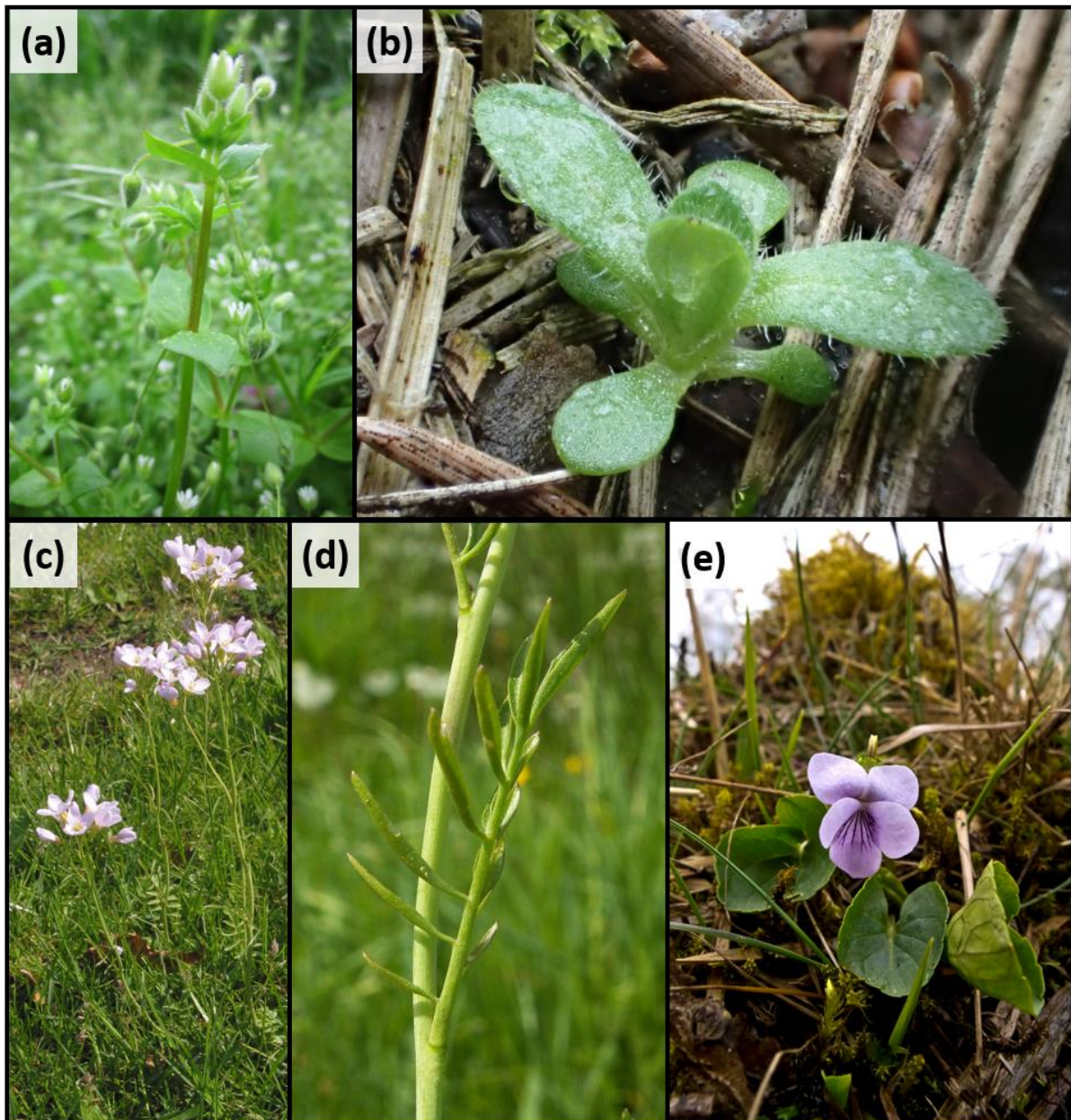
Figure S4. Associations between environmental variables and the percentage cover of vegetation groups in the community-level quadrats ($n = 39$). The figure was created using the '*pairs.panels*' function in the '*psych*' package of R and provides bivariate scatter plots below the diagonal, histograms on the diagonal, and the Pearson correlation above the diagonal. Lichens, graminoids, forbs, litter, nitrate, ammonia, and phosphate were $\log_{10}$-transformed to help meet the assumptions of normality. Asterisks indicate significant correlations (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$), though note that there is no correction for multiple tests.
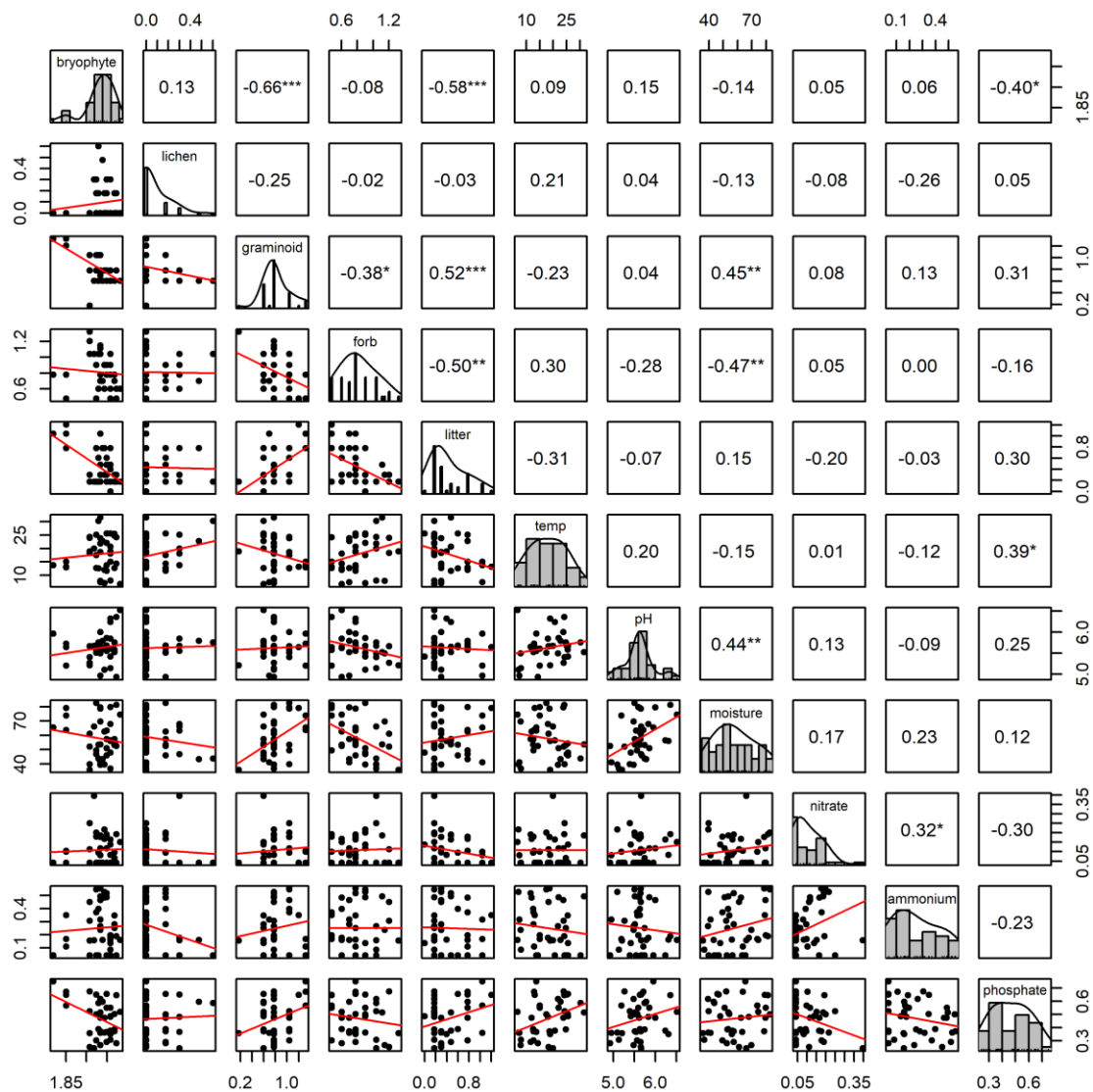
Figure S5. Detrended Correspondence Analysis (DCA) plots, showing the correlation between vegetation groups and the first DCA axis, which was used as a measure of vegetation community composition in the herbivory analyses. Note that there was only a significant (but weak) effect of temperature (temp) on vegetation community composition in the species-level quadrats ($p < 0.001$, $r^2 = 0.02$), not the community-level quadrats ($p = 0.228$, $r^2 = 0.08$).
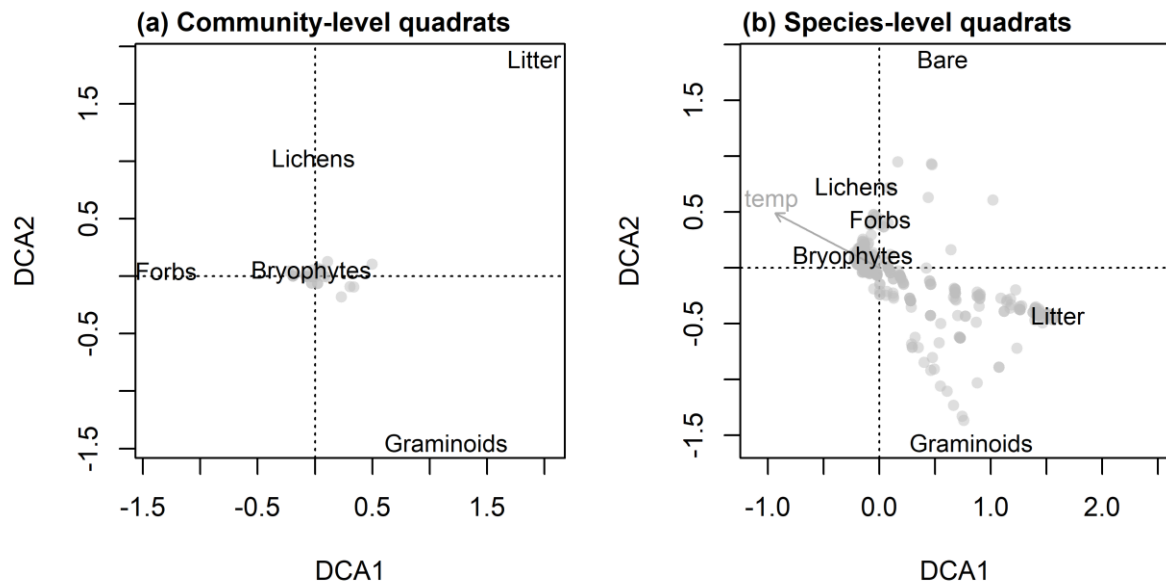
Figure S6. The interactive effect of temperature and phenology on the probability of damage to *Viola palustris* by invertebrate herbivores for all development stages observed in the current study (note that stages are explained in the main text).
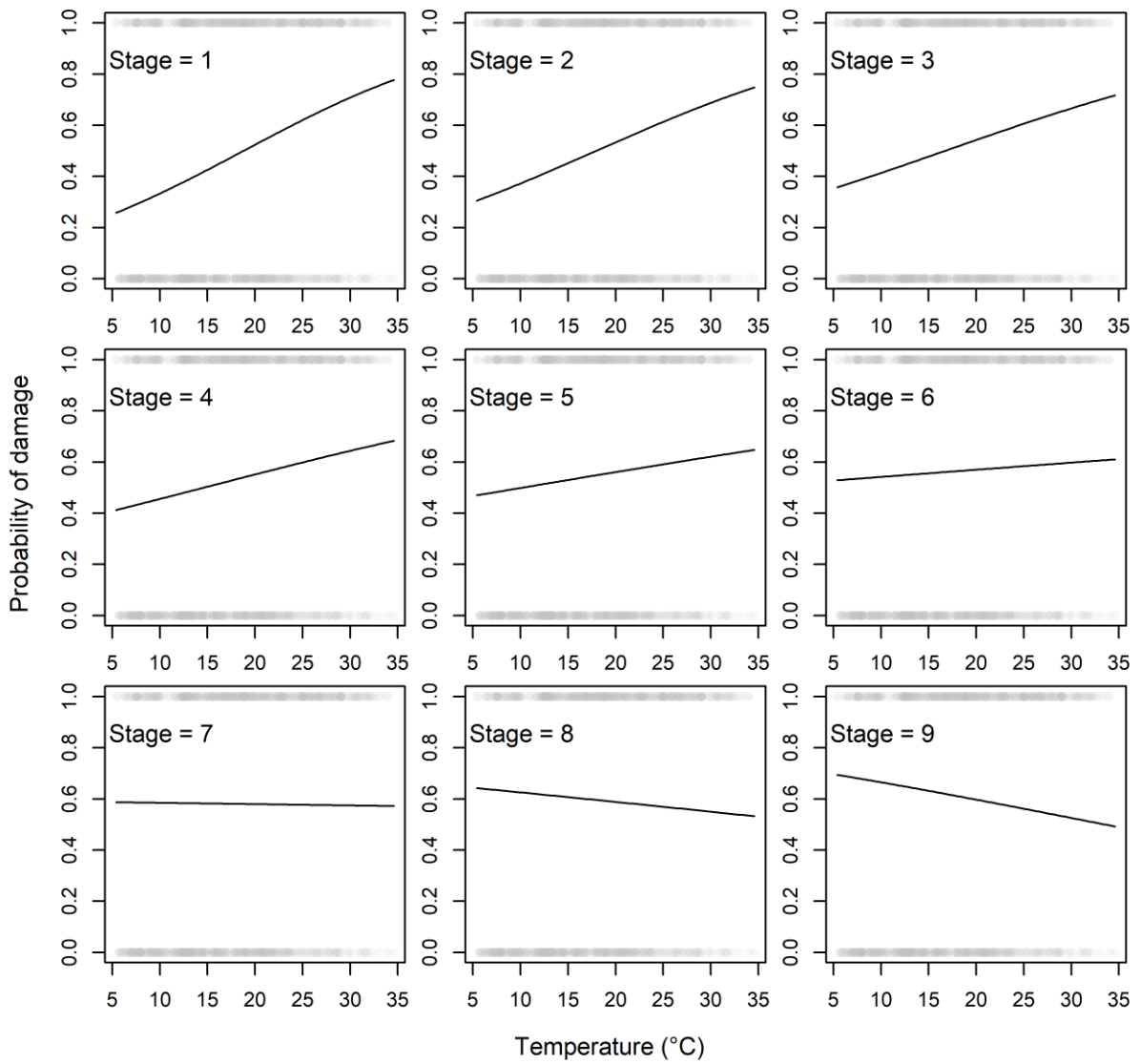
Table S1. List of invertebrate herbivores sampled in the Hengill valley from 19th May to 5th July 2015, using pitfall traps placed in 50 sites across the soil temperature gradient. The average abundance of individuals per trap for each species is also shown.

Species	Group	Abundance
Aphididae spp.	aphid	3.0
Arctorthezia cataphracta	scale insect	47.5
Arion spp.	slug	24.0
Byrrhus fasciatus	pill beetle	0.5
Cerapteryx graminis larva	moth	4.3
Entomobryomorpha spp.	springtail	97.5
Cytilus sericeus	pill beetle	22.0
Deroceras spp.	slug	12.3
Eana osseana larva	moth	1.0
Hemiptera spp.	true bug	0.3
Hemiptera spp. nymph	true bug	15.5
Hypnoidus riparius	click beetle	50.8
Jassargus distinguendus	leafhopper	3.3
Jassargus distinguendus nymph	leafhopper	0.5
Lepidoptera spp. larva	moth	0.8
Macrosteles laevis nymph	leafhopper	1.3
Microlophium spp.	aphid	6.3
Otiorhynchus nodosus	weevil	3.5
Phratora polaris	leaf beetle	0.3
Poduromorpha spp.	springtail	576.3
Psilidae spp.	rust fly	1.3
Symphypleona spp.	springtail	233.3
Thysanoptera spp.	thrip	0.5
Thysanoptera spp. nymph	thrip	4.5
Zonitoides spp.	snail	0.3

Table S2. Details of the experimental plots used in the study. Plot codes correspond to those listed in Figure S1. The total area of each plot is provided, along with the minimum and maximum temperature recorded within each plot over the study duration, the number of marked individuals for *Cardamine pratensis*, *Cerastium fontanum*, and *Viola palustris* included in the final analysis, and the number of community-level quadrats (CLQ) conducted. Note that 300 individual plants of each species were marked at the outset of the study (30 plants in each of 10 plots), however, some plants were lost (*e.g.* trampled) and plants at soil temperatures exceeding 30 °C were excluded from the analysis.

Plot	Area (m²)	Min (°C)	Max (°C)	Number of marked individuals			CLQ
				C. pratensis	*C. fontanum*	*V. palustris*	
H1	154	16.0	35.0	28	30	31	4
H2	180	10.5	25.4	30	30	30	5
H3	88	8.8	12.7	26	28	-	5
H4	120	11.6	48.7	-	-	-	-
H5	168	14.7	41.1	-	29	28	5
H6	66	9.4	26.6	29	-	30	-
H7	108	13.0	27.6	-	-	30	-
H8	80	10.9	20.0	29	30	30	5
H9	210	8.5	9.8	-	30	30	5
H10	120	10.8	29.3	30	30	30	5
H11	133	8.7	11.7	30	-	-	-
H12	210	16.8	30.5	27	-	-	-
A1	120	4.7	9.6	30	30	30	-
A2	200	4.7	8.0	29	30	30	5