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1 Survival in the understorey: testing direct and indirect effects of
2 microclimatological changes on *Ixodes ricinus*.

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13 Highlights

- 14 • Warming and lighting changes herb layer biomass and understorey microclimate.
- 15 • Direct warming did not significantly alter tick survival during summer.
- 16 • Changes in herb layer biomass did not alter summer tick survival either.

17

18 Abstract

19 The distribution of ticks in the *Ixodes ricinus* species complex is partly driven by climate, with
20 temperature and relative humidity affecting survival. These variables are driven by macroclimate, but
21 vary locally due to microclimate buffering. This buffering has been suggested to be one of the driving
22 forces behind variation in tick survival and density in time and space. In order to understand the role
23 of the herb layer with respect to this variation, we deployed *I. ricinus* within an existing experimental
24 setup studying the response of forest understorey to micrometeorological changes. This allowed for
25 the analysis of both direct effects of warming on tick survival in controlled field conditions, as well as
26 indirect effects through changes in herb layer biomass. Herb layer biomass estimates were observed

27 to be higher in plots that had been experimentally warmed, with a trend towards higher survival in
28 these warmed plots. This marginal increase in survival rate may be due to increased microclimate
29 buffering. Comparing our results to literature implies that canopy and shrub layer vegetation have a
30 larger effect on climate buffering, and therefore also on tick survival. Since the herb layer biomass is
31 expected to increase due to global warming and increased frequency of disturbance-induced canopy
32 gaps, survival in forested habitats may increase in the future. This would increase the difference in
33 survival compared to that in open habitats.

34 Keywords:

35 *Ixodes ricinus*, *Ectoparasites*, *Survival*, *Global Warming*, *Microclimate Buffering*

36

37 Introduction

38 Over the last decades, tick-borne diseases (“TBDs”) have seen an increase in prevalence,
39 distribution and diversity throughout the northern hemisphere (Hofhuis et al., 2015; Kugeler
40 et al., 2015; Rizzoli et al., 2011). The most prevalent vectors causing tick-borne infections in
41 temperate climate zones are ticks of the *Ixodes ricinus* species complex (henceforth “tick(s)”)
42 (Keirans et al., 1999). The sheep tick (*Ixodes ricinus*, Linnaeus 1758) is the most abundant
43 species in Western Europe, whereas the most abundant vector in Northern America is the
44 ecologically and physiologically closely related species, the blacklegged tick (*Ixodes scapularis*,
45 Say 1821) (Piesman and Gern, 2004). In Asia, the taiga tick (*Ixodes persulcatus*, Schulze 1930)
46 is the most abundant vector of TBD (Gray, 1998). The life cycle of these ticks is characterised
47 by three parasitic life stages: the larva, the nymph and the adult, of which the nymph is most
48 often responsible for transmitting tick-borne pathogens to humans (Braks et al., 2016; Ostfeld
49 et al., 2006). Each stage attaches to a vertebrate host, takes a single bloodmeal and detaches,

50 after which larvae and nymphs will molt into the next life stage and adult females that have
51 copulated will produce a single egg mass before dying. Because of this dependence on
52 vertebrate hosts, their distribution is limited to areas that support sufficient host
53 communities: the presence of a tick population has been proven dependant on the availability
54 of propagation hosts, with the density of hosts for larval and nymphal ticks further regulating
55 population sizes (Gilbert et al., 2012; Gray, 1998; Hofmeester et al., 2017).

56 Other than the distribution and local density of hosts, the off-host survival of ticks determines
57 their population dynamics and distribution. In this regard, varying survival in field conditions
58 has recently been outlined as a prominent knowledge gap in the modelling of its life cycle
59 (Gray et al., 2021). Climate correlates with tick distribution and activity, with temperature and
60 humidity both playing a pivotal role. It has been established that extremely low temperatures
61 and contact with ice decrease over-winter tick survival, especially for engorged larvae,
62 although the significance of over-winter mortality in temperate regions has been questioned
63 (Brunner et al., 2012; Burtis et al., 2019; Dautel et al., 2016; Gray et al., 2021; Knülle and
64 Dautel, 1997). Furthermore, low temperature increases the development time between
65 subsequent life stages, reducing population growth (Gern et al., 2008; Jaenson and Lindgren,
66 2011). The questing activity of ticks has also been shown to be diminished by low
67 temperatures, thereby reducing the chance of finding a suitable host and completing the life
68 cycle (Perret et al., 2000). High temperatures, on the other hand, may also hamper survival,
69 as water loss due to integument damage increases above 30 °C (Sonenshine, 1991).
70 Accordingly, desiccation is a prominent cause of tick mortality at all temperatures, with large
71 vapour pressure deficits, driven by low relative humidity and/or high temperatures,
72 correlated with high mortality and a decrease in questing activity (Perret et al., 2004;
73 Tagliapietra et al., 2011). The optimal relative humidity for the survival of ticks has been found

74 to be 80-85% RH (Kahl and Knülle, 1988; MacLeod, 1935). These climatic drivers are frequently
75 utilized for course-scale modelling of habitat suitability for ticks of the *I. ricinus* complex, both
76 under current conditions and global change scenarios (Alkishe et al., 2017; Estrada-Peña,
77 2002, 2001; Ginsberg et al., 2017).

78 However, temperature and relative humidity often vary on a micro-geographic scale, being
79 key components of the microclimate. The latter has been defined as “fine-scale climate
80 variations which are, at least temporarily, decoupled from the background atmosphere”
81 (Bramer et al., 2018). The decoupling of microclimate from the background macroclimate is
82 established through the mediating effects of landscape composition, topography, vegetation
83 and soil characteristics (Dobrowski, 2011; Geiger et al., 2009; Kraus, 1911). In forested
84 landscapes, vegetation is the main driver of the decoupling effect from macroclimate, as
85 canopy buffers variation in macroclimate (De Frenne et al., 2019). This can lead to steep
86 gradients of climatic variables on a micro-geographic scale that are amplified during extreme
87 weather events (Chen et al., 1993; Honnay et al., 2005; Suggitt et al., 2011; Vanneste et al.,
88 2020).

89 Besides the abovementioned effect of macroclimate on tick distribution, microclimate has
90 been suggested to drive tick survival and distribution on finer scales. Forest canopy not only
91 buffers climatic extremes detrimental to tick survival, but also produces a litter layer which
92 creates a humid microhabitat for ticks to replenish water reserves between bouts of questing
93 activity (Knülle and Rudolph, 1982). A number of studies have, indeed, found a positive effect
94 of litter presence and depth on the survival and density of ticks (Burtis et al., 2019; Linske et
95 al., 2019; Medlock et al., 2012; Schulze and Jordan, 2005). Besides the tree layer, the
96 complexity and density of the shrub layer has been shown to correlate positively with the

97 density of ticks, suggesting a role in the buffering of microclimate (Lubelczyk et al., 2004; Tack
98 et al., 2013, 2012; Van Gestel et al., 2021). This all suggests that vegetation-driven fluctuations
99 in microclimate may have an effect on the survival of ticks of the *I. ricinus* species complex,
100 as suggested by a correlative study where higher tick mortality was observed in edge habitat,
101 compared to the associated forest stands (Bertrand and Wilson, 1996). In addition to canopy-
102 and shrub-layer vegetation, further buffering of the climatic conditions to which ticks are
103 exposed is also regulated by the understorey. Based on this observation, it has been
104 suggested that the forest understorey is an important component of the ticks' microhabitat
105 (Clow et al., 2017).

106 This understorey has been shown to react to micrometeorological changes in forests, with
107 lighting and warming leading to taller herb communities (Blondeel et al., 2020; Maes et al.,
108 2020). This process is sped up when there is a high availability of light (De Frenne et al., 2015;
109 Govaert et al., 2021). Therefore, we hypothesise that changes in microclimatic conditions,
110 more specifically a temperature increase, affect tick survival directly, as well as indirectly
111 through changes in the buffering capacity of the herb layer. We aim to study these drivers in
112 a controlled, but natural environment by conducting an exposure experiment within an
113 existing setup studying the effects of warming and lighting on the herb layer community
114 within a single deciduous forest plot (Blondeel et al., 2020). This allows us to examine
115 variation in microclimate (driven by treatments as well herb layer biomass) in parallel with
116 off-host survival of *I. ricinus* nymphs. The variation in microclimate under different treatment
117 levels is relevant in the light of the ongoing climate warming. In addition, the treatments
118 exemplify spatial microclimatic gradients, such as can be found at forest edges or clearings. It
119 is to be expected that warming reduces survival, whereas increasing herb layer biomass
120 benefits survival of ticks through the limitation of climatic extremes such as drought.

121 Materials and Methods

122 Site and plot description

123 This study was performed in the Aelmoeseneie forest, Gontrode, Belgium within a long-term
124 ecological research site (LTER) (50°58'30" N, 3°48'16" E, 20 m a.s.l). This temperate mixed
125 deciduous forest has an Atlantic maritime climate. The tree canopy composition consists
126 largely of *Fagus sylvatica*, *Quercus robur*, *Acer pseudoplatanus*, *Fraxinus excelsior* and *Larix*
127 *decidua*. The mean annual temperature is 10.6 °C and the mean annual precipitation 786 mm
128 (DEIMS-SDR Database). The experimental site utilised in this experiment was situated within
129 a 1.8 ha fenced area within the forest.

130 Our setup was nested within the experiment described in Blondeel et al. (2020). In the latter,
131 drivers of herbaceous plant community trajectories were studied in a multifactor global
132 change experiment within a temperate deciduous forest. Soil samples of varying land use
133 history (henceforth "subplots") were introduced to 96 randomly distributed plots each
134 containing four subplots. A pool of 15 herb species commonly found in temperate European
135 forests was defined, after which plant communities consisting of five species selected from
136 this pool were planted in each subplot in April 2016. The four-sample plots were subjected to
137 three two-level environmental treatments: Nitrogen enrichment, experimental warming and
138 lighting. The temperature treatment was realised with an open-top climate chamber,
139 whereas the light treatment consisted of two 18 W fluorescent tubes suspended 75 cm above
140 ground level (see the rightmost panel of Figure 1). These tubes follow the natural photoperiod
141 throughout the year and do not significantly affect air temperatures within the plots (De
142 Frenne et al., 2015). With exception of these treatments, the plots were left undisturbed. For
143 this experiment, only the light and temperature treatments were taken into consideration,

144 resulting in four treatment types: a combined treatment with both temperature and artificial
145 lighting, two singular treatments (either temperature or lighting) and a control treatment
146 where neither were present.

147 The four subplots studied by Blondeel et al. (2020) were kept in rectangular containers,
148 organised in a squared shape within each plot (Fig.1). This resulted in a central location with
149 access to the original forest soil, where ticks were introduced based on the method described
150 by Brunner et al. (2012). The *I. ricinus* used in this experiment were captured on 22th of June
151 2020 and stored in groups of 20 in 40 ml plastic tubes with a perforated lid overnight. They
152 were captured by flag dragging at De Makegemse bossen, a forested location 6.3 km from the
153 Aelmoeseneie forest, at which time species identification was done visually. A soil sample
154 (diameter: 75 mm; hight: 150 mm) was taken at the central location of a subset of 88 plots
155 (22 for each treatment type) and enclosed within a wire mesh bag. The number of surviving
156 ticks in a single tube were counted and introduced to the soil sample on the 23rd of June 2020
157 (between 14 and 25 per plot, with a mean of 17.8). A total of 1569 nymphs were introduced.
158 Three wooden skewers were placed upright to keep the bag distended, providing similar
159 questing opportunities in all plots. Leaf litter present at the sampling site was introduced to
160 half of the bags before sealing them with sealing clips. In this way it was possible to study the
161 effect of this microrefugium on tick survival. Seven bags were left empty in order to evaluate
162 whether nymphs were likely to already be present before experimental introduction.

163 Tick extraction

164 In order to assess tick survival, ticks were extracted from the wire mesh bags after removal
165 from the forest plots as follows: bags were opened and fixed in plastic containers with
166 adhesive tape protruding inward over the edge to avoid ticks escaping. Ticks crawling on the

167 sides of the bag or the wooden skewers were collected with tweezers, after which the skewers
168 were removed. The plastic containers were then placed in larger trays, which were filled with
169 water which creates a favourable, humid microenvironment for the ticks to quest. Soap was
170 added to the water to reduce surface tension and prevent ticks from escaping. The enclosures
171 were checked every second day for questing ticks. Both the adhesive tape and water
172 containers were checked in parallel, as ticks may attempt to move through the moisture
173 gradient towards the water, in which case they would be trapped in the water or on the
174 adhesive tape (Jones et al., 2015). Initial tick extraction was carried out on the 10th of July
175 2020. During these extractions low mortality (32.4%) was observed. In order to maximize
176 statistical power, and approach 50% mortality, it was decided that only 32 out of 88
177 containers would be extracted at this time (8 plots from each treatment combination,
178 henceforth “early extraction”). Ticks were extracted from the remaining 64 containers on the
179 27th of July 2020 (“Late extraction”). Visual searches for ticks in the containers, on the tape
180 and in the water were carried out until the 14th of July for plots included in the early
181 extraction and until the 10th of August for plots included in the late extraction.

182 Environmental measurements

183 Macroclimatic conditions were comparatively mild during our experiment. The mean
184 temperature during the 6-week study was 17.8 °C, with weekly averages ranging from 16 to
185 20.8 °C. A total of 99.0 mm of precipitation was recorded during the same period (Weather
186 Underground).

187 In a subset of 18 plots, temperature and relative humidity were logged every hour using
188 IButton microclimate loggers from the 29th of June onward: 5 in both the combined and
189 control treatments and 4 in each of the singular treatments. Loggers were attached to the

190 underside of a small plastic cap that was fixated 5 cm above ground level using wooden
191 skewers. These loggers were added to the central part of the plot, adjacent to the tick
192 enclosure. Deployment lasted until the late extraction. These data were transformed to daily
193 ranges of temperature and humidity in order to quantify the buffering capacity of the herb
194 layer.

195 Since the treatment to which plots were subjected since 2016 has been proven to have an
196 effect on plant community (Blondeel et al., 2020), it may also effect the water content of the
197 ticks' direct environment during our experiment. Therefore, water content of the containers
198 was measured after final tick extraction. The outside of the plastic containers was dried
199 thoroughly before weighing it. Subsequently, it was placed in a drying oven at 105° C and
200 dried for 24 hours. The dry weight was measured and the water content of each tick container
201 was obtained by dividing the difference by the total wet weight.

202 Herb layer biomass estimates

203 The biomass of the herb layer on all four subplots surrounding the bags was estimated. This
204 was done non-destructively through the use of the PhytoCalc model (equation 1) (Bolte et al.,
205 2009; Heinrichs et al., 2010).

206 With this model, an estimate is attained for each species (i) through the use of its cover
207 percentage within the considered area (C), its shoot length (S) and growth form specific
208 empirical values (a_g , b_g and c_g). For each subplot, the cover of each occurring species was
209 estimated visually and the mean height of the overall vegetation was measured. The plant
210 species were assigned to a morphological growth class (Table S1, supplementary
211 information). For species designated as "small", a stem height of 10 cm was used, whereas
212 the measured mean height per subplot was used for all other species. For all calculations

213 described below, a total plot-level biomass value was used, obtained by adding up individual
214 species values.

215 Statistical analysis

216 Statistical analyses were performed in R version 3.5.2 (2018-12-20, The R Foundation for Statistical
217 Computing, Austria, www.r-project.org). Generalised linear models (GLMs) describing tick survival on
218 plot level using a quasibinomial error distribution was drafted using the glm function of the stats
219 library (part of R version 3.6.2). In the first model, both the light and temperature treatment were
220 included, as well as the presence of litter within the plot. All two-sided interactions between these
221 factors were taken into account. In addition, a second GLM describing tick survival was drafted, with
222 the biomass of the herb layer and the water content of the enclosure as fixed variables, as well as their
223 interaction. A linear model (lm) was built to predict herb layer biomass estimates on plot level, using
224 the lm function of the stats library. Temperature increase, lighting and their interaction term were
225 included as explanatory factors. For both models, F-statistics and p-values were obtained through
226 analysis of variance using the anova function, whereas estimates were obtained with the summary
227 function. Linear models were drafted with both absolute values and daily variation in temperature
228 and humidity as response variables and the factorial treatments as explanatory variables. These
229 models were constructed using the lm function in order to quantify differences in microclimate
230 between treatments using analysis of variance and TukeyHSD post-hoc testing.

231

232 Results

233 (a) Treatment effects on microclimate conditions

234 A significant effect of treatment on both relative humidity and air temperature was observed
235 ($p < 0.01$). For relative humidity, significant differences were observed between all
236 treatments, with mean values for all four treatments above 93% relative humidity during the

237 experiment. The lighting treatment was the only treatment differing significantly from the
238 others with respect to air temperature (summarised in Table 1). We also observed a
239 significant effect of the temperature and lighting treatments on the daily range of both air
240 temperature and relative humidity (Table 2). For both microclimate variables, control plots
241 were subject to significantly larger daily variation than plots subjected to the temperature or
242 light treatment. The interaction term between treatments indicates that their effects are not
243 additive. In addition, there was more variation in relative air humidity in lighted plots,
244 compared to both plots within the temperature treatment and plots subjected to the
245 combined treatment (Figure 1S).

246

247 The model describing herb layer biomass estimates revealed no significantly higher biomass
248 in lighted or warmed plots ($p > 0.1$). However, we did observed a trend for higher biomass in
249 plots exposed to the combined treatment of warming and lighting ($p = 0.058$) (Table 3) which
250 is in line with previous studies using the same setup (Blondeel et al., 2020; Depauw et al.,
251 2020; Govaert et al., 2021).

252 The linear model on water content revealed that plots subjected to the temperature
253 treatment contained more water ($p = 0.020$). A similar positive correlation was observed with
254 litter ($p = 0.029$). Plots that were both lighted and heated were dryer than would be expected
255 by considering these factors separately, as was the case for plots where lighting was
256 combined with a litter layer ($p = 0.026$ and 0.038 , respectively) (Table 4). Plots that were
257 lighted, heated and provided with litter yielded disproportionately humid containers ($p =$
258 0.012) (Figure 2).

259

260 (b) Treatment effects on tick survival

261 Following extraction 388 ticks were recovered from the early extraction and 686 from the late
262 extraction. This translates to an overall survival rate of 68.4%, with survival rates of 65.6% and
263 68.8% for the early and late extraction, respectively (Figure 3).

264 In the GLM describing tick survival we observed no significant treatment effects: all observed
265 p-values were above 0.10, with the exception of a trend for higher tick survival in warmed
266 plots ($p = 0.071$, Table 5).

267 The glm exploring herb layer biomass and water content of the tick containers as explanatory
268 variables to nymph survival yielded no significant terms, nor a significant interaction (all $p >$
269 0.1)(Table 6).

270 Discussion

271 We observed an increase in plant biomass in plots that were both warmed and lighted, which is in line
272 with past studies investigating the response of the herb layer to global change drivers (Blondeel et al.,
273 2020; Depauw et al., 2020; Vockenhuber et al., 2011). This supports the hypothesis that the lighting
274 of warmed plots invokes a transition to a taller herb community (De Frenne et al., 2015; Govaert et
275 al., 2021; Maes et al., 2020). The lack of significance in the current analyses may be due to the single
276 timepoint we observed, compared to the continuous data collection in the cited references (e.g. April
277 2016 to August 2018 in Blondeel et al., 2020). Differences in herb layer biomass likely drive the
278 variation in microclimate buffering, with open-top climate chambers further increasing buffering in
279 warmed plots (Table 2, Figure 1S). This may in turn drive variation in water content of the plots, as
280 increased buffering reduces evaporation from the soil. The same is presumably true for litter, with the
281 added effect of the litter itself containing water.

282

283 Our setup has been adjusted from a previously published setup studying overwinter survival of *I.*
284 *scapularis* (Brunner et al., 2012). This setup allows the ticks access to microrefugia in the soil, litter
285 and living vegetation. Doing so mimics natural conditions and avoids overestimation of mortality due
286 to suboptimal conditions (Bertrand and Wilson, 1996). We opted for a different extraction method in
287 order to minimize the chances of overlooking surviving ticks by human error. The high survival rate of
288 ticks we observed indicates that this technique is adequate for the retrieval of nymphs. Furthermore,
289 this technique depends on the ability of ticks to quest, omitting ticks that are no longer able to do so
290 and are, therefore, ecologically irrelevant. The practicality and reliability of this setup makes it very
291 suitable for further investigation of the drivers of tick survival, both on macro- and micro-geographical
292 scales. We did not observe a significant difference in survival rate between the plots extracted on the
293 11th of July and the 27th of July 2021. This implies that a large portion of mortality occurs early after
294 deployment, possibly due to activity and stress related to the handling of ticks. The same observation
295 has been made by Bertrand and Wilson (1996).

296 The models analysing variation in tick survival revealed only small effect sizes of the established
297 treatments, none of which were significant. Additionally, given the non-significant or (in the case of
298 the lighting treatment) very small temperature variation between treatments, we cannot reliably
299 discuss the direct effects of consistent warming on tick survival. The model describing the response in
300 survival to treatment-induced changes in microclimate also yielded small effect sizes and insignificant
301 p-values. This indicates that the indirect effect of the treatments through changes in biomass,
302 microclimate buffering and soil moisture was very limited, if present at all, within our experiment. This
303 may be due to the short duration of our experiment resulting in relatively high survival rates. A longer
304 deployment may lead to increased variation in survival. In addition, these observations were made
305 within a forest stand, under a single continuous canopy. In this setting, the canopy is the main driver
306 of microclimate buffering, although a notable treatment-related variation in microclimate buffering
307 was observed (De Frenne et al., 2019; Pringle et al., 2003). We furthermore suggest that the
308 differences in microclimate under this canopy are not large enough to cause spatial variation in

309 survival within the timeframe of this study. However, weather conditions were mild during our
310 experiment, with relative air humidity at ground level rarely dropping below 80%. Mean temperature
311 at ground level was slightly below the average for July in the province of Eastern Flanders (18 to 18.5
312 °C) and 99 mm of precipitation is slightly more than was to be expected (75-80 mm, Klimaatatlas KMI).
313 The additional microclimate buffering provided by the herb layer may be of bigger importance to the
314 survival of ticks during less favourable conditions, such as extreme weather events like heat waves or
315 droughts, which present short periods of challenging macroclimatic conditions. The additional
316 microclimate offset a well-developed herb layer can establish, based on our data, may be of larger
317 importance to tick survival at such times. In this respect, the role of the herb layer may also be of more
318 importance in forest edges, where less buffering is provided by the canopy layer.

319 Given the predictions that global change (warming and an increase in disturbance-induced canopy
320 gaps) will lead to taller plant communities in the herb layer, it is likely that the buffering capacity of
321 the herb layer will increase slightly. This would lead to an increased microclimate offset between
322 forest and nearby (semi-)open landscapes (Maes et al., 2020; Vanneste et al., 2020). As the buffering
323 effect of vegetation is more pronounced when ambient temperature is extremely high or low, the
324 offset will be even larger during extreme temperature events (Chen et al., 1993; Honnay et al., 2005;
325 Suggitt et al., 2011). It is universally expected that such extreme weather events (e.g. heatwaves,
326 droughts and floods) will occur more frequently in the future, due to global change (Blenkinsop and
327 Fowler, 2007; Fisher and Knutti, 2014; Spinoni et al., 2019). Given the ticks' vulnerability to climatic
328 extremes, this could increase the variation in tick survival between forested and more open habitats
329 as observed under current climate circumstances. Whether these predicted increases in survival
330 variability will lead to changes in relative tick abundance remains to be seen: many factors other than
331 survival influence the local density of ticks, such as the abundance and habitat use of ticks' hosts.
332 These factors may also be affected by global change, further complicating any predictions in this
333 regard.

334 Based on our observations and the available literature, we conclude that herb layer vegetation is of
335 lesser importance to nymphal tick survival compared to shrub- and canopy-layer vegetation. This can
336 be attributed to the latter having a larger buffering effect on the microclimate at ground level.
337 However, given the predicted transition to a taller herb community and the associated increase in
338 temperature- and humidity offsets between forested and open habitats, we expect differences in tick
339 survival between such habitats to increase.

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527 2022.

528 Tables and Figures

529

530 *Figure 1 – Visualisation of a single plot, with a cloth mesh bag tick container surrounded by four sub-plots with varying*
 531 *vegetation characteristics. The pictured full plot (right) illustrates both warming (by open-top climate chamber) and lighting*
 532 *treatments.*



533

534 *Equation 1 – Formula used to estimate a relative value for herb layer biomass surrounding each tick container*

$$\text{biomass} \left[\frac{\text{g}}{\text{m}^2} \right] = \sum_{i=1}^n a_g \cdot C_i^{b_g} S_i^{c_g}$$

535

536 *Table 1 - Overview of measured microclimate variables for each treatment group.*

Treatment	Temperature: mean	SD	Relative humidity: mean	SD
Control	16.2 °C	2.48	93.0%	10.2

Lighting	16.4 °C	1.98	95.5%	8.52
Temperature	16.2 °C	2.00	99.6%	6.01
Combined treatment	16.2 °C	2.10	97.0%	6.92

537

538

539 *Table 2 - Overview of the variables within the linear models with daily ranges of microclimate variables (air temperature and*
540 *relative air humidity) as response variable and two factorial treatments (temperature, lighting) as fixed factors, as well as*
541 *their interactions. Values of non-significant fixed effects are those before exclusion. The bold p-value indicates the factor*
542 *included in the final model.*

Response: Daily range in temperature	F-statistic	Estimate ± SE	p-value
Intercept		6.37 ± 0.24	
Lighting	F _{1,86} = 7.08	-1.51 ± 0.24	< 0.01
Temperature	F _{1,86} = 7.04	-1.59 ± 0.36	< 0.01
Lighting * Temperature	F _{1,84} = 13.12	1.83 ± 0.51	< 0.01

Response: Daily range in relative Humidity	F-statistic	Estimate ± SE	p-value
Intercept		20.10 ± 0.84	
Lighting	F _{1,86} = 13.52	-5.63 ± 1.26	< 0.01
Temperature	F _{1,86} = 50.41	-9.41 ± 1.26	< 0.01
Lighting * Temperature	F _{1,84} = 11.86	6.15 ± 1.79	< 0.01

543

544

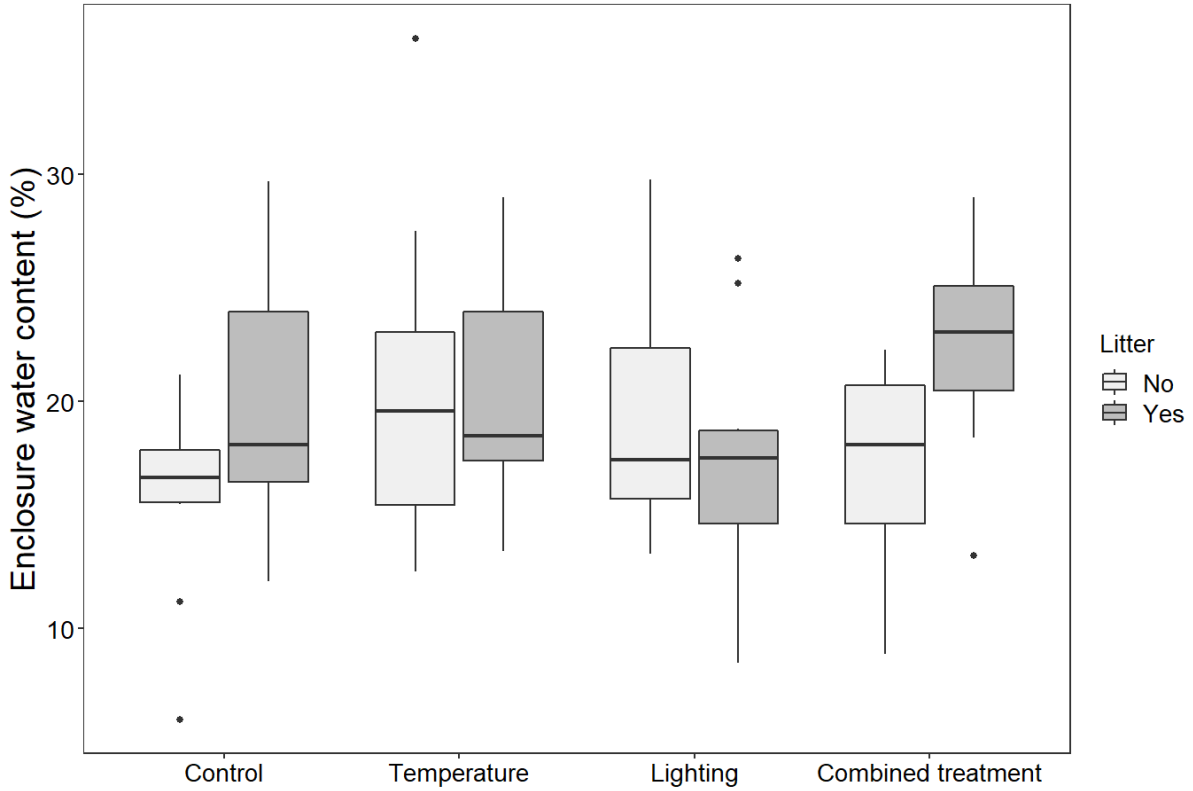
545 *Table 3 - Overview of the variables within the linear model with estimated biomass in the litter layer on plot level as response*
546 *variable and two factorial treatments (temperature, lighting) as fixed factors, as well as their interactions. Values of non-*
547 *significant fixed effects are those before exclusion. The bold p-value indicates the factor included in the final model.*

Response: Biomass estimate	F-statistic	Estimate ± SE	p-value
Intercept		109.49 ± 11.06	
Lighting	F _{1,86} = 9.83	13.42 ± 15.65	0.39
Temperature	F _{1,86} = 2.65	-3.27 ± 15.65	0.84
Lighting * Temperature	F _{1,84} = 3.70	42.54 ± 22.13	0.058

548

549 *Figure 2 – Water content (%) of enclosures in all considered treatments, both with and without litter.*

550



551

552 *Table 4 - Overview of the variables within the linear model with enclosure water percentage as response variable*

553 *and the treatments (temperature, lighting and litter) as fixe2d factors, as well as their interactions. Values of non-significant*

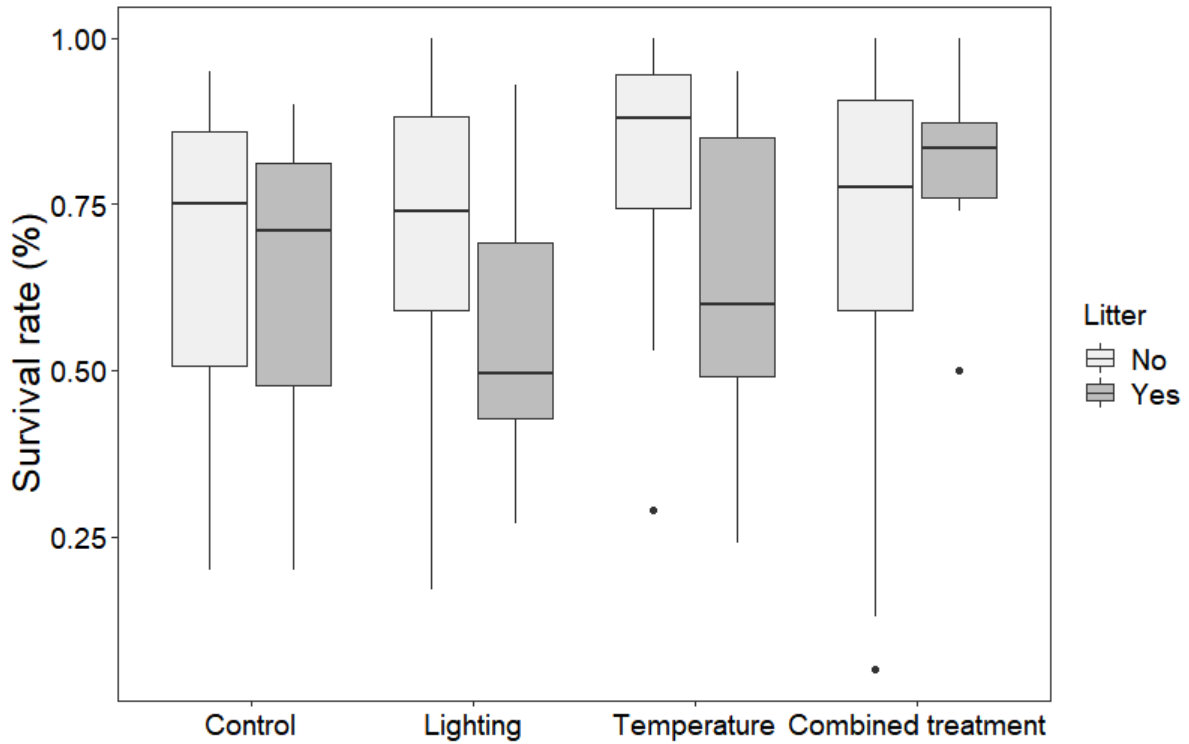
554 *fixed effects are those before exclusion. The bold p-value indicates the significant factors included in the final model.*

Response: Enclosure water %	<i>F-statistic</i>	<i>Estimate ± SE</i>	<i>p-value-value</i>
Intercept		14.600 ± 1.77	
Lighting	F _{1,86} = 0.04	4.725 ± 2.391	0.0516
Temperature	F _{1,86} = 3.00	5.800 ± 2.440	0.0198
Litter	F _{1,86} = 2.89	5.308 ± 2.391	0.0292
Lighting * Temperature	F _{1,84} = 0.44	-7.575 ± 3.339	0.0260
Lighting * Litter	F _{1,84} = 0.17	-7.153 ± 3.382	0.0375
Temperature * Litter	F _{1,84} = 0.07	-5.508 ± 3.375	0.1065
Temperature * Litter * Lighting	F _{1,80} = 6.62	12.303 ± 4.777	0.0119

555

556

557 *Figure 3 - Survival rates of ticks in all considered treatments, for both enclosures with and without litter.*



558

559 *Table 5 - Overview of the variables within the general linear model with the survival of nymphal I. ricinus as response variable*
 560 *and the factorial treatments (temperature, lighting and litter) as fixed factors, as well as their interactions. Values of non-*
 561 *significant fixed effects are those before exclusion. The bold p-value indicates the factor included in the final model.*

Response: Tick survival _ Treatment effects	<i>F-statistic</i>	<i>Estimate ± SE</i>	<i>p-value</i>
Intercept		0.686 ± 0.354	
Lighting	$F_{1,86} = 0.03$	0.054 ± 0.247	0.874
Temperature	$F_{1,86} = 3.36$	0.443 ± 0.244	0.071
Litter	$F_{1,86} = 0.67$	-0.201 ± 0.245	0.415
Interaction terms			
Lighting * Temperature	$F_{1,84} = 0.20$	0.263 ± 0.502	0.654
Lighting * Litter	$F_{1,84} = 0.84$	-0.452 ± 0.495	0.361
Temperature * Litter	$F_{1,84} = 0.28$	-0.260 ± 0.496	0.601

562

563 Table 6 - Overview of the variables within the general linear model with the survival of nymphal *I. ricinus* as response variable
 564 the biomass of herb layer vegetation on plot level and the **water** percentage of the tick enclosure as fixed factors, as well
 565 as their interactions. Values of non-significant fixed effects are those before exclusion. The bold *p*-value indicates the factor
 566 included in the final model.

Response: Tick survival _ indirect effects	<i>F</i>-statistic	<i>Estimate</i> ± SE	<i>p</i>-value
intercept		0.322 ± 1.14	
Biomass estimate	$F_{1,85} = 0.42$	0.001 ± 0.00217	0.516
water percentage	$F_{1,86} = 0.19$	-0.010 ± 0.02346	0.664
Biomass estimate * water percentage	$F_{1,84} = 0.63$	-0.0003 ± 0.0004	0.431

567

568

569 Supplementary information

570

571 Table S1 - Overview of plant species present within the experimental plots and their morphological growth class within the
 572 PhytoCalc model. For tree saplings, determination was done on genus level (noted as 'sp.')

Scientific name	Common name	Morphological growth class
<i>Aegopodium podagraria</i>	Ground elder	Tall_Herb
<i>Ajuga reptans</i>	Common bulge	Small_Herb
<i>Anemona nemerosa</i>	Wood anemone	Small_Herb
<i>Carex sylvatica</i>	Wood sedge	Tall_Herb
<i>Galium adorum</i>	Sweetscented bedstraw	Small_Herb
<i>Geranium robertianum</i>	Herb-Robert	Tall_Herb
<i>Glechoma hederacea</i>	Ground ivy	Small_Herb
<i>Hedera Helix</i>	European ivy	Dwarf_Shrub
<i>Hyacinthoides non-significantus</i>	Common bluebell	Small_Herb
<i>Poa nemoralis</i>	Wood bluegrass	Middle_Grass
<i>Poa trivialis</i>	Rough meadow-grass	Middle_Grass
<i>Polygonatum multiflorum</i>	Solomon's seal	Tall_Herb
<i>Ranunculus ficaria</i>	Lesser celandine	Small_Herb
<i>Urtica dioica</i>	Stinging nettle	Tall_Herb
<i>Vinca minor</i>	Lesser periwinkle	Dwarf_Shrub
<i>Acer sp.</i>	Maple	Beech
<i>Fraxinus sp.</i>	Ash	Ash

<i>Quercus sp.</i>	Oak	Oak
<i>Fagus sp.</i>	Beech	Beech
<i>Castanea sp.</i>	Chestnut	Oak
<i>Oxalis sp.</i>	Wood sorrels	Small_Herb
<i>Rubus sp.</i>	Bramble	Tall_Herb
<i>Veronica montana</i>	Speedwell	Small_Herb
<i>Ilex aquifolium</i>	Common holly	Dwarf_Shrub
<i>Dryopteris carthusiana</i>	Narrow buckler-fern	Fern

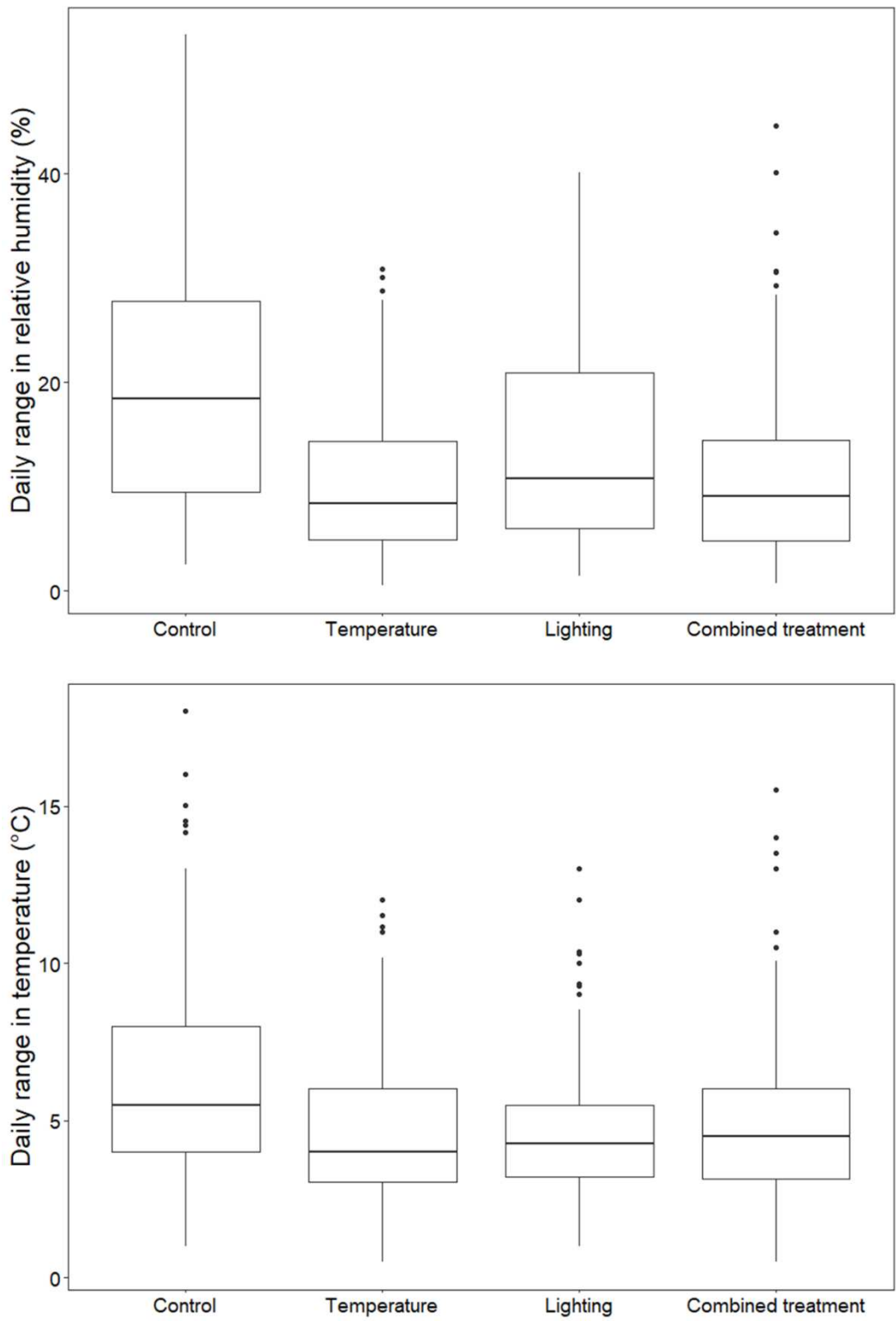
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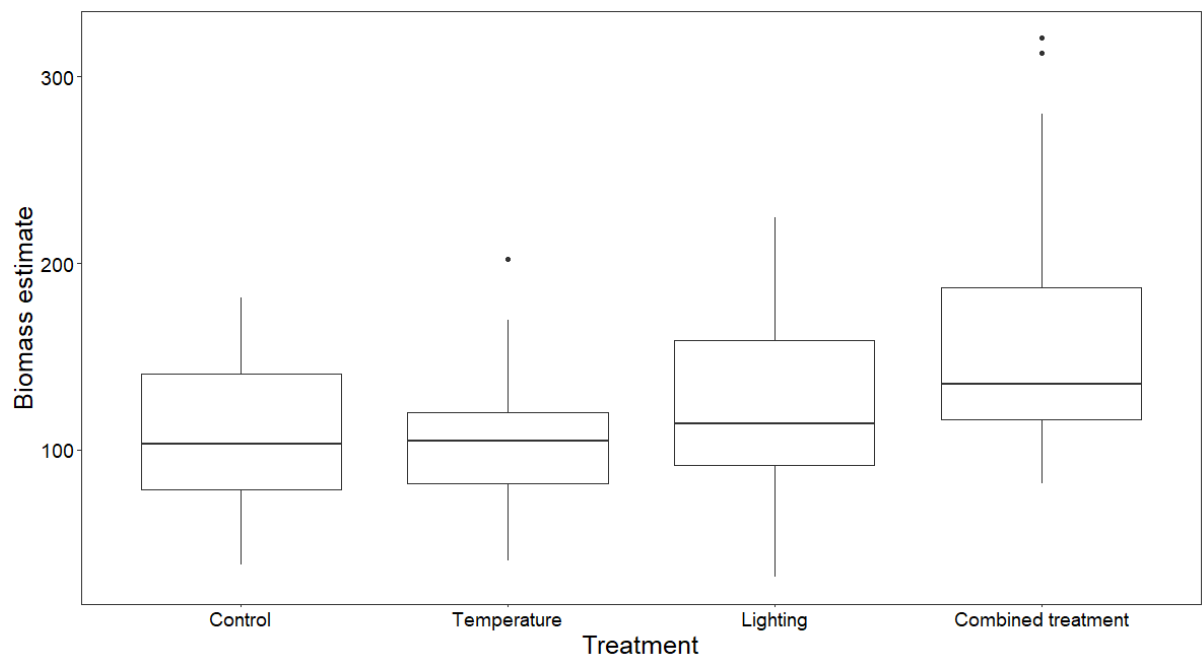
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580

581 *Figure S2 – Biomass estimates for plots of all considered treatments.*



582