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

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Highlights

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

Abstract

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

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

Keywords:

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

Introduction

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

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

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

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

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

Materials and Methods

Site and plot description

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

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

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

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

Tick extraction

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

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

Environmental measurements

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

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

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

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

Herb layer biomass estimates

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

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

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

Statistical analysis

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

Results

(a) Treatment effects on microclimate conditions

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

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

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

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

(b) Treatment effects on tick survival

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

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

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

Discussion

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

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

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

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

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

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

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528 Tables and Figures

529

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



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

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

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

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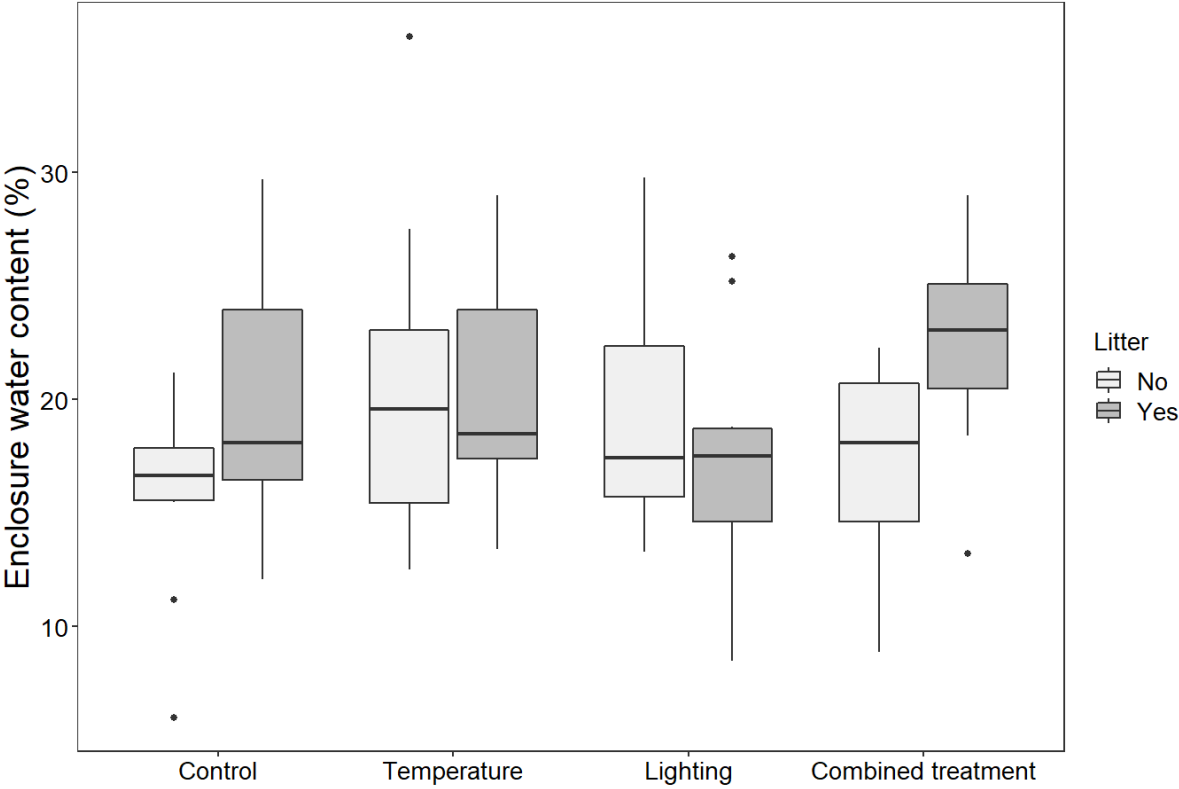
545 *Table 3 - Overview of the variables within the linear model with estimated biomass in the litter layer on plot level as response*
546 *variable and two factorial treatments (temperature, lighting) as fixed factors, as well as their interactions. Values of non-*
547 *significant fixed effects are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

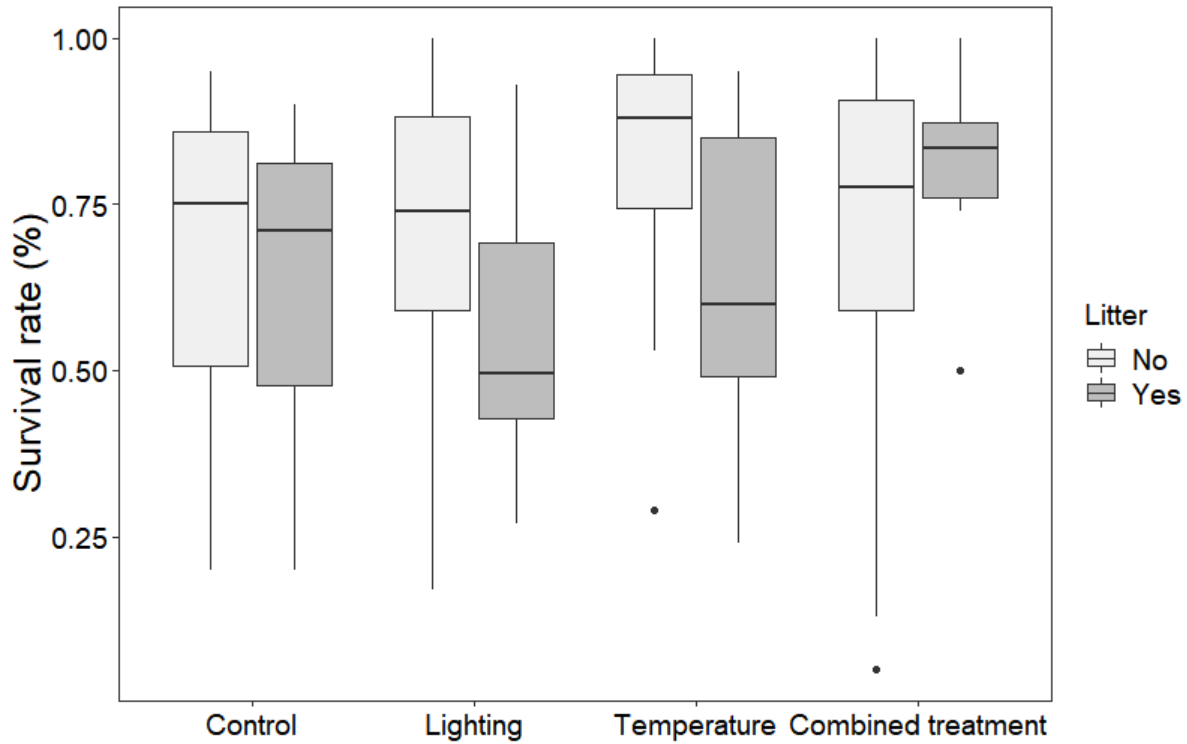
554 *fixed effects are those before exclusion. The bold p-value indicates the significant factors included in the final model.*

Response: Enclosure water %	F-statistic	Estimate ± SE	p-value-value
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</i>-statistic	<i>Estimate</i> ± SE	<i>p</i>-value
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

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

Response: Tick survival _ indirect effects	<i>F</i> -statistic	Estimate ± 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

Supplementary information

Table S1 - Overview of plant species present within the experimental plots and their morphological growth class within the 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 odoratum</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 nonsignificatus</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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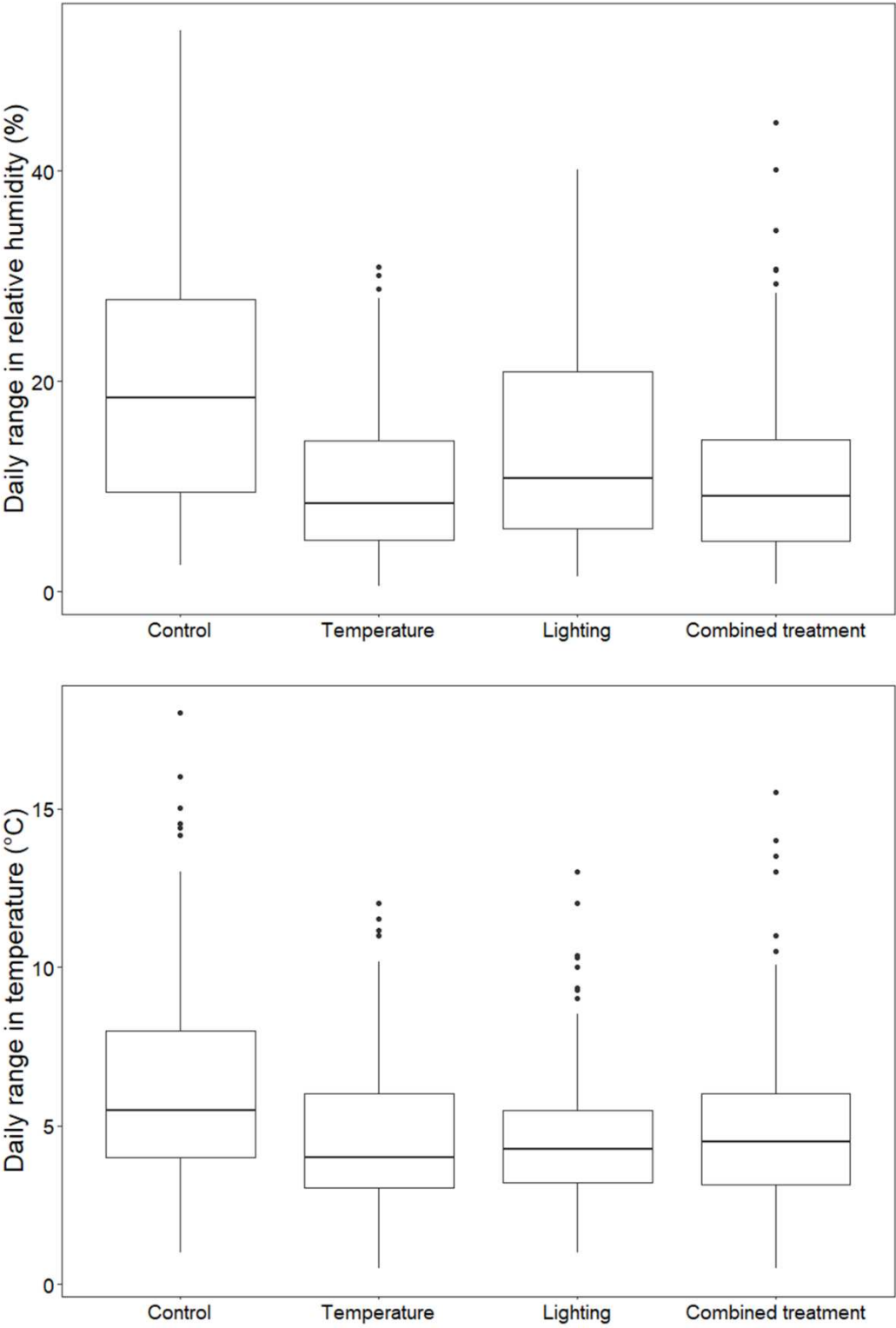
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577 *Figure S1 - Daily range of relative humidity and temperature of the air at 5 cm above the soil.*

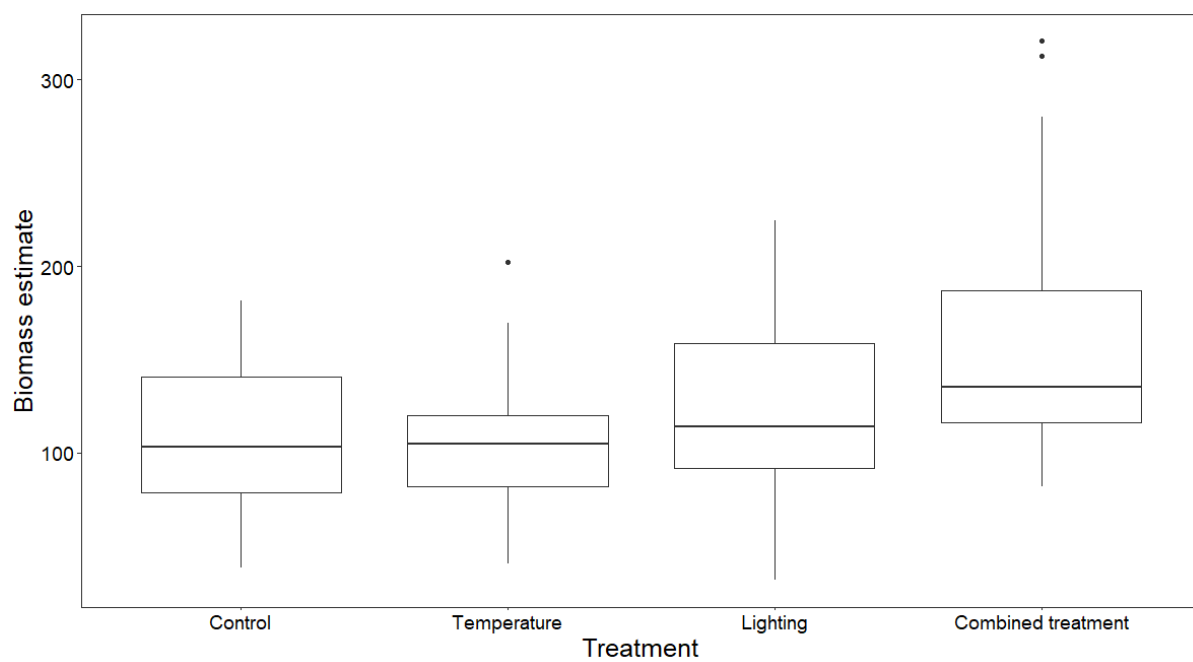
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581 *Figure S2 – Biomass estimates for plots of all considered treatments.*



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