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Microclimatic buffering in forest, agricultural and urban landscapes through the lens of a grass-feeding insect

Authors:

Simon Braem[1*], Michel Crucifix[2], Caroline Nieberding[3] and Hans Van Dyck[1]

[1]Behavioural Ecology & Conservation Group, Earth & Life Institute, UCLouvain, Louvain-la-Neuve, Belgium

[2]Georges Lemaître Centre for Earth and Climate Research, Earth & Life Institute, Earth & Life Institute, UCLouvain, Louvain-la-Neuve, Belgium

[3] Evolutionary Ecology and Genetics group, Earth & Life Institute, UCLouvain, Louvain-la-Neuve, Belgium

ORCID:

Simon Braem – https://orcid.org/0000-0002-9520-9741

Michel Crucifix – https://orcid.org/0000-0002-3437-4911

Caroline Nieberding – https://orcid.org/0000-0002-2654-3287

Hans Van Dyck – https://orcid.org/0000-0002-2013-6824

Corresponding author:

S. Braem

Earth & Life Institute, UCLouvain

Croix du Sud 4-5, Box L7.07.04

1347 Louvain-la-Neuve

Belgium

simon.braem@uclouvain.be

Open Research Statement:

Upon acceptance, data and R-code that support the findings will be permanently available in Dryad data repository via the following link: [hyperlink to dataset will be provided here]. No novel code was used for statistical analysis. If deemed necessary, Subject-matter Editor may request the data earlier.

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ABSTRACT

For small, ectotherm species, dealing with microclimatic variation during sensitive stages of their development is a critical component of their ecological interactions within and among different landscape settings. However, it is often unknown whether the use of habitats in different landscape settings may affect microclimatic conditions at the level of microhabitats (e.g. at host plants for herbivorous insects). As opposed to standard ecological monitoring experiments, we aim to identify the microclimatic offsets that accurately represents the environment in which a small arthropod spend most of its life. As a case study, we selected grassy sites that corresponded to the microhabitat of grass-feeding insects in general, and larvae of the butterfly *Pararge aegeria* in particular as this insect recently expanded its habitat use from forest (edges) to agricultural and urban environments. We tested to what extent local microclimates and microclimatic buffering capacity differed between tufts of grass in forest and in two anthropogenic (i.e. agricultural and urban) landscape settings by measuring microclimatic variables with sensors at the level of the grasses. We compared temperature, relative humidity and vapour pressure deficit (VPD) during an exceptionally warm and dry summer period with parallel data from weather stations and tested for differences between microclimatic profiles among the three landscape settings. Microclimatic conditions corresponded only partly to variables based on weather station data. Differences were particularly significant for relative humidity and VPD, but not for day and night temperature. Effects of canopy openness on the variability of microclimatic variables were landscape-specific. Host plants in agricultural settings experienced strong microclimatic fluctuations and low short-term variability in VPD. In urban settings, urban heat island effects increased night temperature, but also showed microclimatic buffering effects similar to the ones in forest settings. Our findings stress the functional implications of landscape-specific microclimatic profiles at the appropriate organism-centred scale. We discuss the significance of such

microclimatic profiles for ecological studies dealing with ectotherms under climate and land use change.

INTRODUCTION

Structural habitats (e.g. particular woodland types) have often been assumed to be functional habitats for particular species based on the simple presence of the species. Environmental correlates of presence-only distribution data help our understanding of the complex nature of a species' habitat. Hence, such information has frequently been used to make future range predictions under different scenarios of climate change. However, the very same structural habitat may provide different thermal conditions at different latitudes (or under different levels of climate change). Thermophilous ectotherms may occupy, for example, sandy or rocky habitats in the north of Europe, grassy or shrubby habitats at intermediate latitude and forest habitats in the south of Europe (e.g., Grayling butterfly *Hipparchia semele*; Tropek et al. 2017). Hence, the organism may find its functional habitat in different structural habitats under different macroclimatic conditions (Van Dyck 2012). Moreover, although niche conservatism (i.e., the tendency of species to retain ancestral ecological characteristics) has often been assumed for species with a specific affiliation with a particular habitat type (Wiens & Graham 2005), modifications in resource-use and in habitat-use have been documented in species that deal successfully with rapidly changing anthropogenic landscapes. Examples include *Turdus merula* blackbirds that expanded their use of woodland habitat into urban environments (Evans et al. 2009) and *Aedes aegypti* mosquitoes that expanded their habitat use from African forests into domestic habitats throughout the world (Xia et al. 2021). In the latter case, females switched their larval sites from natural water containing tree holes to artificial containers like clay pots, which provides multiple opportunities for range expansion.

Understanding how living conditions of species alter in response to human-induced rapid environmental change requires the study of the combined effects of climate and landscape-scale habitat availability (Wilson et al. 2010). The majority of animal species are small-sized terrestrial insects (Wilson, 1992), which have complex life cycles and whose larvae develop in close interaction with the (thermal) environment on a small scale (Dillon et al., 2016; Pincebourde et al., 2016; Pincebourde & Woods, 2012, 2020). Although the role of microclimates for understanding the ecology of insects have long been acknowledged (Willmer, 1982), few studies have looked at how dominant structural habitats at the landscape scale may affect temperature and humidity experienced by such organisms at fine resolution (e.g. on their host plants).

A bottom-up, resource-based approach to identify a species' functional habitat within and across different structural habitat types (i.e. land cover types) has been shown to be a much more appropriate way to conceive habitat in insects, than the much more widespread, top-down structural habitat or biotope-based approach (Dennis et al. 2003; Turlure et al. 2019). This is true for both fundamental and applied ecological studies that need to understand and delineate a species' habitat (Van Dyck 2012). This matter has been particularly addressed for species of conservation interest (e.g. glacial relict species that rely as larva on microclimatic buffering of specific components of the vegetation within structural habitat types; Turlure et al. 2010). We argue that it may be equally important for understanding how successful, rapidly spreading species are able to deal with microclimatic conditions in different structural habitats. As has been shown for forest species in bees, dung beetles and dipterans, light and microclimate conditions provided by local woodlots may indicate suitable habitat, independent of landscape composition of the wider landscape (Williams & Winfree, 2013; Righi et al., 2018).

Grass-feeding insects that make use of a number of widely distributed grass species that occur under a range of environmental conditions (i.e. both within and outside forest), may be such an example. For species with such an ecological profile, structural habitat-based approaches do not grasp well their functional habitat. Here, we address the issue of microclimatic variation at the level of grasses that grow under a range of environmental conditions within and outside forest. This approach is of general interest to larger communities of grass-feeding insects. Here, we are particularly interested in testing its significance within the context of an insect that recently expanded its ecological niche and habitat use from forest to different types of more open, anthropogenic environments. We tested for microclimatic differences between ancestral habitat (i.e. forest) and two recently colonized environments (i.e., agricultural and urban landscape settings) in the case of the Speckled Wood (*Pararge aegeria*).

We focus on microclimate variables, i.e. at the level of the host grass species that grow in the grass layer accompanied by nearby canopy. On the one hand, nearby woodlots may provide microclimatic conditions that protect larval stages and grass blades from heat stress, regardless of the nature of the wider landscape. At the level of the host plant, even small nearby woodlots could provide shade, block wind and retain outgoing radiation (Geiger et al., 2003; Süle et al, 2020). However, on a landscape scale, forest, agricultural and urban climatic conditions differ significantly: forests typically buffer fluctuations in temperature and humidity with effects gradually diminishing towards the forest edges (Lin et al., 2020) and cause sub-canopy cooling during summer (Haesen et al., 2021). Agricultural and urban landscapes are characterized by more open, early succession environments and smaller, dispersed woodlots. The corresponding conditions in these landscapes are generally warmer, drier and less buffered (Geiger et al., 2003; Boggs & McNulty, 2010; Ewers & Banks-Leite, 2013; von Arx et al., 2013; Middel et al., 2015).

Urban climates are, particularly in temperate-zone regions, generally warmer and dryer than surrounding areas because of 1) heat-absorption by building materials with a high heat retention capacity, 2) reduction in evaporation and heat-dissipating capacity due to a reduced vegetation cover, 3) restricted air circulation because of buildings and 4) ‘waste heat’ caused by engines (i.e. Urban Heat Island effect; Arnfield, 2003; Larsen, 2015). This can affect an organism in various ways. Relatively warmer conditions in urban habitats may increase metabolic rate and, indirectly, available food abundance (Lowe et al., 2014; Serruys & Van Dyck, 2014; Merckx et al., 2018; Chick et al., 2021). Urban conditions may also affect heat stress risks (Kaiser et al., 2016; Pincebourde et al., 2016). For example, mosquito larvae (*Aedes albopictus*) growing in areas that are more exposed to urban heat effects during fall, survived better when compared to growing conditions in rural microclimates. During a hot summer, however, the effect is reversed (Murdock et al., 2017).

Although the measurement of VPD has gained much more attention in studies on plant microhabitats (Davies-Colley et al., 2000; von Arx et al., 2013; Süle et al., 2020; Aguirre et al., 2021), it may also matter for microclimates that affect small organisms like insects. VPD is exponentially related to changes in temperature under constant humidity and is regarded as the 'drying power' of the air on wet surfaces (Monteith & Unsworth, 2013). Measuring such 'drying power' may have significance for understanding how desiccation stress affects growth and survival in drought-sensitive ectotherms, either directly (Tarrant & McCoy, 1989; Shipp & Gillespie, 1993; Rickards & Boulding, 2015) or indirectly via decreased plant growth (Bauerfeind & Fischer, 2013; Massmann et al., 2019). As temporal variation in microclimatic conditions may affect the development of traits and life-history decisions in microclimate-sensitive ectotherms (Doody et al., 2006; Pincebourde & Woods, 2012; Parmentier et al., 2017), we introduced two novel variables other than standard deviation that reflect different rates of temporal variation (i.e., short-term variability and day-to-day variability of VPD). These variables are assumed to reflect the way caterpillars or other grass-feeding insects experience predictability of drought conditions over time. At least in *P. aegeria*, larvae are known to use microclimatic information to change developmental strategies accordingly (Shreeve, 1986a).

In this paper, we measured microclimatic conditions at the level of tufts of grasses during extremely warm summer. We compared these with standardized measures gathered at nearby weather stations and tested whether these differences (i.e. microclimatic effects) differed between landscape types. Because all sampled sites had a similar vegetation configuration (i.e. grass layer with nearby canopy), we predict that the climatic differences from weather station data are larger than the microclimatic differences measured between landscape types. Since all weather stations were located in flat, open fields, we also predict that, even when controlling for canopy openness, the agricultural microclimate is most similar to the weather station measures.

Secondly, we tested whether the raw climatic measures of a grass habitat in agricultural and urban landscape types are different from those of forest habitats, and whether measures gathered from these two anthropogenic landscape types differed from each other. We predict more extreme temporal variation in temperature and humidity in agricultural settings than in forest settings. Levels of temporal variation in urban locations are predicted to be higher compared to forest settings (i.e. reduced buffering effect of large-scale canopy cover), but lower than agricultural settings (i.e. higher night temperatures in urban landscapes due to the urban heat island effect).

MATERIALS AND METHODS

Sample sites

We selected four study areas consisting each of an urban, an agricultural and a forest location (latitudinal range: 50° 51' 0" N ± 6' 0"; study areas: Aalst, Brussels, Leuven and Tienen; see Fig. 1). Urban built area of the cities ranges from 13.6 km^2 (Tienen) to 128.86 km^2 (Brussels). Each of the 12 sample locations were chosen at a 61.8 acres (25 hectares) grid area and were characterized by their dominant land cover type (i.e. i) buildings and other impervious surfaces, ii) meadows and pastures or iii) tree cover; land cover of interest > 70% at a grain size of 500 m^2). Tree types within the sample locations all consisted of deciduous trees. In each of these locations, *P. aegeria* females have been repeatedly observed since 2016 (S. Braem, A. Kaiser, C. Turlure and T. Merckx, personal observations). Urban sample locations were at two city parks (Aalst and Leuven), a cemetery (Brussels) and a small woodland patch near the city centre (Tienen). Agricultural locations consisted of small woodlots, hedgerows and sunken lanes along meadows and cultivated fields. Locations differed with an altitudinal range of 80 m at most (see description Fig. 1) and showed usually limited topographic variation within location (maximum altitude difference between two sample sites never exceeded 20m, except in the agricultural site in Leuven where the maximum difference was 42m).

Climatic measures

Microclimatic measures were taken during a period of six weeks from the 13th of June until the 25th of July 2018. This period covers the start of summer conditions during which the species spends its life in large parts as egg or larva (i.e., in between two peaks of adult stages, which represent the first two annual generations) and may be particularly vulnerable to microclimatic conditions (Schweiger et al., 2006; Oliver et al., 2015; Pateman et al. 2016). Our Belgian study system experienced exceptionally warm and dry weather at that time (average temperature: 20.5°C during June & July 2018 vs. 17.3°C June & July 1830-2010; precipitation: 19 mm/m^2 vs. 100 mm/m^2 for the same 42-day period averaged for 1830 - 2010; Belgian Meteorological Institute KMI-RMI Brussels-Ukkel).

Within each location we measured temperature and relative humidity (RH) in two sample sites, using climatic sensors connected to a data logger (HOBO U23 v2; temperature accuracy of ± 0.2°C from 0° to 50°C and a RH accuracy of ± 2.5% from 10% to 90% and ±5% below

10% and above 90%; Onset Computer Corporation, 2010). The sensors measured relative humidity and temperature every 30 minutes. Every week for six weeks, we randomized sample sites by moving the two HOBO sensors to a different sample site, positioning them within a 100m perimeter of the previous sampling site (see Fig. 1b-d as example).

Although several herbivorous insects can be found on grasses, we selected a number of grassy microhabitats that corresponded to the oviposition site search profile of *Pararge* aegeria as an instructive case. During summer, females prefer tufts of grass in a humid and thermally buffered, semi-canopy-shaded microhabitat (Shreeve, 1986b, Schweiger et al., 2006; Oliver et al., 2015; Pateman et al. 2016). We positioned the microclimatic sensors at tufts of host grass at 12 cm above ground and in partial or nearly full shade of the surrounding canopy layer, which were always deciduous. We did not use any casing to shield off the HOBO climatic sensors to better simulate biologically realistic conditions and thus increase sensitivity to variation in wind and direct exposure to solar radiation (Terando et al., 2017). However, to avoid direct heating through the semi-transparent lid of the HOBO sensor, sensors were provided with a green plastic roof (∅ 90 mm), positioned at 15 mm above the sensor (see Fig. 1e). Nine of the 144 data logger series were lost due to either issues with the HOBO climatic sensors or failure of data transfer from the logger.

Degree of canopy openness was recorded for each sample site by taking a picture while poisoning the camera 12 cm above ground and facing a fisheye lens camera skywards with the horizontal part of the frame in an east-west orientation (full-frame Fisheye Converter FCON-T01 with a diagonal 130° angle of view; Olympus Tough TG-4 camera). Afterwards, we corrected for the southward position of the sun (at the highest point of the sun path, solar zenith angle ranged between 27° and 32°) by using only the 75% southside-segment of the picture. This procedure resulted in canopy segments with a zenithal angle of 41.8° and an azimuthal angle of 74.3° with the centre point 7.0° tilted to the south. We used ImageJ freeware (https://imagej.nih.gov/ij/) to transform pictures into black and white format and lowered brightness thresholds manually until mostly sky-obstructing vegetation was turned black and sky was turned white. Canopy openness was expressed as the percentage of white on the cropped pictures. Canopy openness was lacking for the three sample locations around Tienen.

Hourly macroclimatic measures were recorded by The Royal Meteorological Institute of Belgium (KMI-RMI). Unfortunately, we only obtained measures over the course of a four week period and therefore had to deal with a lower sample size when testing for microclimatic differences (see below). We selected three of their nearby weather stations to retrieve these data: Bauvechevain (for all sites near Tienen and the forest site in Leuven), Melle (for all sites near Aalst), and Zaventem (for all sites near Brussels and the agricultural and urban site from Leuven; Fig. 1). All three weather stations were located in flat and open fields. At these stations, ambient temperature and relative humidity were measured 2 m above ground under standardized measuring conditions (i.e. casings with ‘Stevenson screens’ preventing the sensors from being directly exposed to sunlight or wind).

Offset variables and microclimatic effects

We obtained temperature and relative humidity (RH) data directly from HOBO climatic sensors and we derived Vapour Pressure Deficit (VPD) from the measurements of temperature and RH (Tetens 1930; Monteith & Unsworth 2013; for details on the calculation, see von Arx et al., 2013). To quantify the microclimatic conditions at the level of the host plant in different landscapes relative to the data from the weather stations (i.e. surrounding macroclimate), we used so-called offset microclimatic variables (De Frenne et al., 2019; Haesen et al., 2021). Microclimatic offset variables were calculated for each of the 135 weeklong sample series by subtracting average and standard deviation of local measures of temperature, RH and VPD of a single weeklong sample series with the corresponding averages and standard deviations from the nearby weather stations data. The selected nine offset variables included offset day average (7:00 – 21:00 GMT+1), offset night average (21:00 – 7:00 GMT+1) and offset standard deviation of T, RH and VPD. In all statistical models, each of these calculated variables per sample series are treated as single replicates. Correlation analysis showed a strong correlation between standard deviation and range (i.e. maximum – minimum). However, standard deviation is less sensitive for short-lasting but extreme measures.

Other variables to tests for landscape type differences

We used the microclimatic data to test for differences in microclimate relative to landscape type. For each of the 135 weeklong time series, we used day mean, night mean, their standard deviation, range, minimum and maximum value of temperature, RH and VPD. These 18 intercorrelated variables were standardized and integrated by a principal component analysis (PCA) using R function ‘prcomp’ (R Core Team 2018).

Next, we introduced two variables reflecting the variability of VPD that aims to exclude variation caused by repeated diurnal oscillations or by long-term trends over multiple days. These variables were interpreted as negative correlates of predictability. For each of the weeklong time series, short-term variability of VPD was calculated by taking the mean of the residual variation (see ‘random’ in Appendix S1: Figure S1), after decomposition of time-series-wide trends (see ‘trend’ in Appendix S1: Figure S1) and day-night variation (see ‘cyclical’ in Appendix S1: Figure S1), using a decomposition algorithm that relies on moving averages (i.e. ‘decompose’ function in R; Hyndman & Athanasopoulos, 2021). ‘Day-to-day variability of VPD' represents how the standard deviation of VPD differs, on average, from day to day and was calculated as follows: for one 7-day sequence of data, the difference in standard deviation was calculated between measures of day 2 and day 3, day 3 and day 4, day 4 and day 5, and day 5 and day 6. Then, the average of those four values represented the 'day-to-day variability'. Day 1 and day 7 were omitted from the calculation.

Statistical modelling

Offset climatic variables to test for differences with weather station data for each landscape type

All statistics was performed using R 3.5.0 (R Core Team 2018). The effect of landscape type on offset variables of T, RH and VPD was analysed with a linear mixed model with week as random effect. Due to a limited amount of categories in our random factor (i.e. four weeks for offset variables), we used Bayesian models using the brms package from R Studio (Bürkner, 2017). For every model, non-flat, weakly informative priors were specified (Appendix S1: Section S3). 'Landscape type' and 'canopy openness' showed high collinearity as sites in agricultural landscape were usually less enclosed by overhanging vegetation, while forest sample sites had most overhanging vegetation (Appendix S1: Section S2, Fig. S2). Including 'canopy openness' as a categorical random variable (10 categories: 0-10%, 10-20%, …, 90-100%) resulted in very similar results as models without this random variable. Therefore, we excluded 'canopy openness' as a fixed effect and tested each of our nine variables against 'canopy openness' as a fixed effect for each landscape in separate general linear models.

For the Bayesian model output (in R syntax: 'offset variable ~ landscape type + (1|week)') of each of the nine microclimatic offset variables tested, we conducted two tests. First, we derived the means of the microclimatic variables from the posterior samples for each landscape type, and tested the deviance from zero with distribution boundaries calculated at a 95% credibility interval (CI). Second, we tested the difference of the microclimatic variables between landscape types. For this, we performed a non-linear hypothesis testing for each landscape type pair (agri vs. urban, agri vs. forest and urban vs. forest). We applied Bonferroni corrections to the threshold confidence interval.

Landscape differences in microclimate by PCA

Between-landscape differences were tested with the two first principal components that together explained 77% of the variation (PC1 explained 59 % and PC2 19%; Table 1) using a linear mixed model with week (N=6) as a random effect (Bates et al., 2015). In R syntax: ‘PC1 or PC2 ~ landscape type + (1|week)’. A pairwise comparison was made among the least square means of the three landscape type, using linear contrasts. Bonferroni corrections were applied. We also present the results of the two variables that had the highest loadings in each of the two principal components, separately. These four variables, short-term variability of VPD and day-to-day variability of VPD were modelled similarly as PC1 and PC2.

RESULTS

Offset variables show landscape-related microclimatic differences

Output of the Bayesian mixed linear models are summarized in Fig. 2 and presented in Appendix S1: Table S2 ; between-landscape differences are presented in Appendix S1: Table S3. Offset RH was higher than zero in all three landscape types, indicating consistently more humid conditions near the grass layer. Humidity offset variables were highest during the day (day: 10-20% higher; night: 5-15% higher). Diurnal averages were highest in forest with an offset day RH +17.69% (95% CI: 14.29 – 20.83). Derived from the offset standard deviation, sample locations from agricultural settings showed higher temperature variability (i.e. +1.17

offset standard deviation of T; 95% CI: 0.48 – 1.77), but had equal variability for humidity. Urban and forest landscape settings showed lower variability in humidity compared to weather stations (urban: -1.81 offset standard deviation of RH; 95% CI: -3.21 - -0.46; forest: -2.62 offset standard deviation of RH; 95% CI: -4.01 - -1.23), but not in temperature and VPD. For offset day and offset night T, there was little deviation from zero, indicating that average temperature was similar to weather station data.

Agriculture settings scored highest in offset standard deviation (i.e. least climatically buffered) of temperature, RH and VPD compared to the other landscape types (Fig. 2). Offset day T was significantly higher when compared with the forest setting and offset day VPD was significantly higher than the other two landscape types (Fig 2). Offset night T was higher in urban than in forest sites (+0.85 offset day T; value tested against lies outside 98.3% interval).

Effect of canopy openness on microclimate depends on landscape type

Average canopy openness differed strongly between landscape types (mean canopy openness in agricultural settings ± SE: 50 ± 5%; urban settings: 37 ± 5%; forest settings: 24 ± 5%). Once the dataset was subdivided in agricultural, urban and forest landscape types, distinct patterns on how canopy openness affects temperature and humidity offset variables became clear (Appendix S1: Table S1). For offset day T, RH and VPD in agricultural settings, we did not find an effect of canopy openness, whereas during the night offset T and offset VPD decreased and offset RH increased with canopy openness. In urban and forest settings, offset day T significantly increased with canopy openness while offset night T decreased with canopy openness. Offset night RH increases with canopy openness in urban (estimate = 0.18; $F_{1,22} = 10.43$, $p < 0.01$) and in agricultural settings (estimate back-transformed from a log-transformation = 0.13; $F_{1,21} = 0.39$, $p < 0.01$), while in forests there was no such an effect (estimate = 0.039; $F_{1,20} = 0.39$, $p = 0.54$). In agricultural settings, offset standard deviation of T increased with canopy openness (estimate = 0.027; $F_{1,21} = 6.97$, $p < 0.05$). In urban settings, standard deviation of the three different offset variables was not affected by canopy openness. In forest settings, offset standard deviation of T and VPD increased with canopy openness (offset standard deviation of T: estimate = 0.023; $F_{1,20} = 13.654$, $p < 0.01$; offset standard deviation of VPD: estimate = 0.002; $F_{1,20} = 13.654$, $p < 0.01$).

Hotter and dryer conditions in agricultural settings, warmer nights in urban settings

A high PC1-score corresponded mainly with dry and hot day values and overall with more variable conditions (i.e., high standard deviation and range). A high PC2 corresponded with colder and more humid night conditions (see Table 1). For PC1, agricultural settings had significantly higher values compared to forest settings, while for PC2, agricultural settings had significantly higher values compared to both urban and forest settings, indicating colder and more humid nights (Fig. 3 and Appendix S1: Table S4).

The two highest contributing variables for PC1 were VPD standard deviation and day VPD. These showed similar trends as their corresponding offset variables in Figure 2, with higher day VPD and higher VPD fluctuations in agricultural settings compared to the other landscape types (Table 2). The two highest contributing variables for PC2 were night temperature and minimum temperature. On average, we observed 0.7°C higher night temperature in urban settings compared to the other two landscape types and 1.2°C higher minimum temperature in urban settings when compared to forests.

Our novel variables of VPD variability as a proxy measure of temporal predictability of microclimate had similar effects standard deviation of VPD: agricultural settings showed stronger day-to-day and short-term variability compared to forest settings. Short-term VPD variability in agricultural settings was also higher than in urban settings (Table 2).

DISCUSSION

Our study tested for differences in microclimatic profiles at the level of tufts of host grass that grow in woodland, agricultural land and urban environments, respectively. The study of microclimates has recently regained growing interest in the context of biodiversity responses to ongoing climate change (De Frenne et al. 2021), which is particularly significant for insects (Ma et al. 2021). There is a large community of grass-feeding insects, which interact on this small scale with their (thermal) environment. These insects include habitat generalists that can make use of similar grasses that occur in different structural habitat types (e.g. *Popillia japonica*; Lemoine et al. 2013), but also forest specialists that have recently expanded their habitat and host plant use beyond forest (e.g. *P. aegeria*; Vandewoestijne & Van Dyck 2010). Our results confirm that there is a significant difference between weather station data and microclimatic data measured at the level of the grasses, but here we show that such differences vary among landscape types. Microclimates at the level of the grass layer were better buffered under forest conditions than under non-forest conditions in anthropogenic landscapes. However, some of the microclimatic variables under urban conditions showed more resemblance to forest conditions than to the other non-forest landscape type, i.e. agricultural landscape. This clear difference between the two non-forest landscape categories opens new avenues for exploring microclimatic opportunities and undervalued resources of grass-feeding insects in anthropogenic landscapes.

Microclimate measures of grass habitat nearby trees deviates from weather station data

The ecological relevance of using readily available weather station data to interpret species-environment interactions at the microclimatic level has been criticized lately (Haesen et al., 2021; Lembrechts & Nijs, 2020). Our case study analysed this issue for microclimates around tufts of grass growing in different landscape settings, i.e. in forest, agricultural and urban landscape. Our study demonstrated that weather station measures taken with shielded sensors 2 m above ground in open field do not correspond well with the habitat-specific microclimatic conditions at the level of the grass layer. Consequently, we endorse the plea for a more biologically realistic positioning of climatic sensors when studying organismal responses to climate (Ashcroft, 2018; Suggitt et al., 2011).

As predicted, microclimatic conditions deviated significantly from time-matched measures of nearby weather station, particularly in terms of higher relative humidity and the related VPD. This is in accordance with well-established work on near-ground microclimate (Geiger et al., 2003) showing that RH can increase with 10% or more at ground surface, when compared to 2 m above ground. In contrast with the overall opposite trends at bare ground, increasing humidity closer to the ground is only observed under the presence of vegetation layers such as grasses (Geiger et al., 2003). Surrounding tree canopy and understorey vegetation at each sample site likely contributed as well to higher humidity and buffering of humidity levels in comparison with open field conditions (De Frenne et al., 2013; Geiger et al., 2003). Overall, offset values showed that average day and night temperatures at the height of the grass corresponded well with those measured 2 m above ground and behind Stevenson screens. .This effect was opposite to what one would expect from near-ground microclimates in dense grass vegetation (and other vegetation near ground), which ensures better thermal buffering: surface layers receive less direct solar radiation during the day and receive relatively higher infrared ground radiation during the night (Geiger et al., 2003; Morecroft et al., 1998; von Arx et al., 2013). However, other mechanisms may counter this higher-buffering-near-ground effect. For example, in an open field with little ground vegetation, thermal buffering capacity at 12 cm above ground is generally lower than that at 2 m height. This is caused by the relatively cooler ground surface at night due to convective cooling, and a relatively decreased surface cooling during the day due to wind-generated boundary layer effects (wind speed is usually lower during the night; Geiger et al., 2003; Munro, 1987). This also confirms why we only found higher thermal variability near-ground-offset values in agricultural but not in forest and urban landscape types. (Geiger et al., 2003; Morecroft et al., 1998; von Arx et al., 2013).

Microclimate of grass habitat nearby trees is affected by landscape

In agricultural landscapes, offset variables of standard deviation of temperature, day-to-day variability in VPD and short-term variability in VPD all showed higher values, when compared to urban and forest conditions. Therefore, as predicted, agricultural settings were characterized by stronger fluctuations in both temperature and humidity. Agricultural sites were more exposed to sun radiation, drought and heat, which creates harsher microclimates for drought-sensitive insects. Nevertheless, variation of canopy openness did not exert microclimatic changes in day temperature for grasses on agricultural sites, while there was a positive effect for grasses in the other two landscape types. Small-scale effects by wind and soil humidity, which were not measured in our current study, may also contribute to the observed microclimate effects. During summers in the temperate-zone region, urban areas typically have drier soils and lower wind speeds (Larsen, 2015), open fields have drier soils and stronger winds (von Arx et al., 2013) and forests wetter soils and lower wind speeds (Boggs & McNulty, 2010; Morecroft et al., 1998; von Arx et al., 2013). Hence, woodlots in agricultural sites are likely to experience more wind, partly dampening solar heating at solar-exposed sample sites, making these microsites in a way more similar to canopy-shaded microsites.

Forest conditions provided higher buffering capacity in grassy sites for both temperature and humidity. However, lower canopy openness is not likely to be the only mechanism, as our results showed that contributing effects of canopy openness were relatively low; the effects may interact with other factors such as soil moisture and wind speed (Boggs & McNulty, 2010; von Arx et al., 2013). Assuming higher soil humidity, as typically observed for forest soils, our results on RH and VPD are in agreement with those of von Arx et al. (2013): wet soils maintain high above ground humidity levels, which will dampen the effects of canopy cover on near-ground VPD. The humidity effect of canopy cover was much more pronounced for grasses growing in urban and agricultural sites (i.e., on relatively drier soils). For temperature, the forest canopy effect was more pronounced (Appendix S1: Table S1). However, we need to be cautious with this comparison, as von Arx et al. (2013) retrieved data measured at 2 m above surface. Higher above surface heat is disseminated more strongly than air moisture (Geiger et al., 2003), which may explain why canopy cover and soil moisture interact with temperature in a different way at different heights above ground.

A second reason why landscape effects may overshadow local effects (e.g., canopy cover) relates to the higher-order thermodynamic effects of large forests. Large forests become more thermodynamically stable in terms of evapotranspiration and temperature with increasing vegetation abundance per surface unit (Whittaker, 1975; H. Zhang & Wu, 2002). Increased vegetation complexity increases the capacity to capture and dissipate solar energy, making landscapes where such vegetation is dominant, more resilient to deal with external fluxes, which can be detected at both the macro- (Ehbrecht et al., 2021; Norris et al., 2011; Schneider & Kay, 1994) and micro-climatic level (Redmond et al., 2018; von Arx et al., 2013).

We detected Urban Heat Island effects as night temperature was on average 0.7°C higher around grass tufts in urban sites than around grasses in agricultural and forest sites. These urban heat effects were expected to be mild for two reasons. First, built-up surface areas of most urban areas were relatively small. Larger cities, such as Birmingham (229.8 km^2), generated 0.7°C to 3.4°C higher night temperatures during the summer (Azevedo et al., 2016). Hence, the increase we observed was at the lower end of this range. Second, woody and grassy vegetation that surrounded a tuft of grass will help dampen local Urban Heat Island effects, especially during the day (Yan, 2019; Y. Zhang, 2017). This has also been argued by Kaiser et al. (2016), showing 1°C higher average day temperature – but not night temperature – in urban sites compared to agricultural sites. Kaiser et al. (2016) analysed the effect of urbanisation on larval microclimate in *P. aegeria* and took measurements at 1 m height in late summer to early spring. Although such an experimental set-up does not allow direct comparison with our findings, it does stress the importance of season- and height-dependent microclimatic conditions within a species' functional habitat (Ashcroft, 2018; Suggitt et al., 2011; Vermunt et al., 2012). As predicted, relatively higher night temperatures, but not higher day temperatures, contributed to higher microclimatic buffering capacity in urban sites compared to agricultural sites.

Microclimatic properties of host grasses out of forest: an insect's viewpoint

Egg and larval development

The landscape-specific effects on local microclimates around grass tufts provide insight on how grass-feeding insects are exposed to different microclimatic conditions. To the butterfly *P. aegeria*, which expanded its habitat use from forest to agricultural and urban environments over the last decades, agricultural microclimates pose a significant challenge for early development. Later larval stages are, to some extent, able to tolerate a larger thermal range as they are able to move to the cooler and more humid centre of the grass sward under high temperatures (Berger et al., 2011; Kaiser et al., 2016). For example, Kaiser et al. (2016) found no differences in larval survival when – from second instar onwards – being exposed to dryer environments or when comparing agricultural vs. woodland ecotypes (however, their experiment was not performed during periods of extended drought and heat, thereby mitigating the risks of desiccation in more exposed environments). In contrast, the egg and first instar stages are more likely to be affected by heat stress, as has been shown in several satyrine butterfly species and other Lepidoptera (Bergman, 1999; Clark & Faeth, 1998; Karlsson & Wiklund, 1985; Klockmann et al., 2017; Potter et al., 2011; Zhang et al., 2015). At 30% relative humidity and 20°C (VPD: 1.63 kPa), Karlsson & Wiklund (1985) showed *P. aegeria* egg survival to be at 29% only. 3% of our obtained values for mean day VPD exceeded 1.63 kPa in forest, 10% in urban areas and 23% in agricultural areas (Appendix S1: Fig. S3). Hence, during hot summers, our measurements suggest that oviposition sites with low risk of desiccation are less frequently available in agricultural landscape settings. Urban habitats could provide microclimatic conditions for eggs and larvae that are far more similar to those found in forest habitat. Additionally, higher urban night temperatures may benefit larval development as it increases foraging rate overnight and induces overall faster larval growth (Berger et al., 2011; Chown & Nicolson, 2004). So, at the smaller spatial scale, immobile egg stages may benefit from microhabitats that are sufficiently hygrothermally buffered against climatic extremes (e.g., semi-shaded conditions in the grass, 12 cm above ground and near larger, dense vegetation). However, at the larger spatial scale of a few meters, more mobile and larger life stages may benefit from both patches of elevated solar radiation used for basking and patches that are more buffered against climatic extremes (Anthes et al., 2008; Woods, 2013; Pincebourde et al. 2021). This shows that microclimatic mosaics at various spatial scales – from egg microhabitats to the structural habitat in a landscape – need to be taken into account when studying how microclimate affects an insect's survival in times of climatic change.

Microclimate and environmental predictability

Short-term and day-to-day variability in VPD reflect variation in desiccation risks over the span of hours or days, respectively. These values were again higher in grasses in agricultural landscape, where surrounding vegetation was lower and wind exposure higher. On the short-term, a canopy-open spot is more exposed to the fluctuating conditions of cloudiness that may or may not let sunbeams pass, but a spot that is covered by canopy is never exposed whether sunny or not. On a day-to-day resolution, uncertainties about the microclimate may impose constraints on larval foraging behaviour (e.g., decisions to move to the base of the plant, or to increase foraging). These levels of environmental predictability at different temporal resolutions open new perspectives for studies on how organisms deal with life-history strategies, ranging from (ir)reversible plasticity, learning to bet hedging (Botero et al., 2015; Joschinski & Bonte, 2020). In sticklebacks, for example, higher thermal uncertainty on a week-to-week temporal resolution has been shown to induce bet-hedging strategies for egg size (Shama, 2015).

Microhabitat and adult behaviour

The spatial patterns of the microclimatic variations are experienced differently for flying adults – where microclimates significantly fluctuate both horizontally (Montejo-Kovacevich et al., 2020) and vertically (Pryke et al., 2012) – when compared to mobile larvae and even more when compared to the eggs or the little mobile first instar larvae. Our results imply that oviposition sites with a hydrothermally buffered microclimate are differently distributed and possibly harder to find in microhabitats in agricultural landscape than in urban and forest landscapes. Such differences in spatial configuration are likely to have fitness consequences for searching females that need to balance energetic costs with microclimatic preferences during oviposition (García-Barros & Fartmann, 2009; Resetarits, 1996; Shreeve, 1986b). Microclimatic preference and site selection strategies are therefore expected to differ relative to the type of environment. For example, Braem et al. (2021) showed that ovipositing butterflies of agricultural landscape origin were more willing to accept locally available oviposition sites than butterflies of forest origin, even when microclimatic conditions were not optimal. In a common garden laboratory experiment, females of the agricultural ecotype laid more and heavier eggs under high ambient temperatures compared to conspecific females of the forest ecotype (Karlsson & Van Dyck, 2005). Therefore, mapping the spatial configuration of microclimates could benefit future research on movement and reproductive ecology of small ectotherm organisms, especially those that colonize areas undergoing rapid environmental change (Fagan et al., 2013).

In conclusion, we showed that agricultural, but not urban, habitat settings reduced the microclimatic buffering of host plants in space and time, when compared to microhabitat settings in forest landscapes. We emphasize the possible implications of these differences for grass feeding butterflies and insects occupying similar niches, in particular during immobile stages of higher hydrothermal sensitivity and during extreme weather conditions. Although this study was initially inspired by the specific resource-based habitat point of view of the larval stages of *P. aegeria*, the significance of our results is much broader as they allow to better understand how microclimatic conditions around and on grasses are shaped in different anthropogenic environments.

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AUTHOR CONTRIBUTIONS

S.B., C.N. and H.V.D. co-designed the study and prepared the experiment. S.B. collected and analyzed the data in interaction with M.C, C.N. and H.V.D. S.B. and H.V.D. wrote the first draft of the paper. All authors discussed the results and contributed critically to the final manuscript. All authors gave final approval for publication.

CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interests to disclose.

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TABLES

Table 1. Variable contributions to the principal components PC1 and PC2. Loadings are indicated as percentages relative to PC1 and PC2. Rows are ordered along descending ratio of % contributing to PC1 relative to % contributing to PC2. In grey are contributions of >20%. T: temperature; RH: relative humidity, VPD: vapour pressure deficit.

	PC1 (59 %)	*PC2 (19 %)*
Minimum RH	**88 % (-)**	0 % (-)
Day mean VPD	**92 %**	1 % (-)
Day mean T	**79 %**	1 % (-)
Range RH	**83 %**	1 %
Standard deviation RH	**77 %**	3 %
Standard deviation VPD	**91 %**	4 %
Maximum VPD	**83 %**	7 %
Range VPD	**83 %**	8 %
Day mean RH	**77 % (-)**	7 %
Maximum T	**72 %**	10 %
Standard deviation T	**61 %**	**26 %**
Range T	**44 %**	**41 %**
Night mean VPD	**42 %**	**46 % (-)**
Night mean RH	**39 % (-)**	**44 %**
Night mean T	**27 %**	**47 % (-)**
Minimum VPD	6 %	**21 % (-)**
Maximum RH	4 % (-)	**16 %**
Minimum T	11 %	**50 % (-)**

Table 2. Landscape type differences for the two variables that contributed to most of the variation of PC1 and PC2, respectively, and for two proxy measures of microclimatic predictability. Relative contributions to PC1 or PC2 are shown between brackets. The table presents a pairwise comparison among least square means among the three landscape types, based on linear mixed model output with week (N=6) as a random effect. Numerator and denominator degrees of freedom for all estimates are 2 and 127 respectively. '*': logarithmically transformed in the linear mixed model.

Main variables PC1

	Standard deviation VPD* (91.06%)				Day VPD* (91.80%)			
Predictors	*Estimates*	*SE*	*t.ratio*	*p*	*Estimates*	*SE*	*t.ratio*	*p*
agri - urban	**0.19**	**0.068**	**2.78**	**0.019**	0.15	0.072	2.037	0.13
agri - forest	**0.27**	**0.072**	**3.81**	**0.0006**	**0.26**	**0.076**	**3.46**	**0.002**
urban - forest	0.083	0.071	1.17	0.73	0.12	0.076	1.53	0.39

Main variables PC2

	Minimum night temperature (50.23%)				Night temperature (47.03%)			
Predictors	*Estimates*	*SE*	*t.ratio*	*p*	*Estimates*	*SE*	*t.ratio*	*p*
agri - urban	**-1.12**	**0.278**	**-4.038**	**0.0003**	**-0.72**	**0.23**	**-3.18**	**0.006**
agri - forest	-0.51	0.29	-1.73	0.26	-0.051	0.24	-0.21	1
urban - forest	0.62	0.29	2.13	0.11	**0.67**	**0.24**	**2.83**	**0.016**

VPD predictability

	Day-to-day VPD variability				Short-term VPD variability			
Predictors	*Estimates*	*SE*	*t.ratio*	*p*	*Estimates*	*SE*	*t.ratio*	*p*
agri - urban	0.19	0.095	1.98	0.15	**0.19**	**0.073**	**2.55**	**0.035**
agri - forest	**0.29**	**0.1**	**2.9**	**0.013**	**0.29**	**0.076**	**3.75**	**0.0008**
urban - forest	0.1	0.099	1.015	0.94	0.1	0.076	1.32	0.57

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FIGURE LEGEND

Fig. 1: Overview of the sample sites. a: Twelve sample locations in and around four Belgian cities that are characterized by agricultural (square), urban (triangle) or woodland (circle) landscape settings. Crosses represent nearby weather stations from The Royal Meteorological Institute of Belgium in Melle (M), Zaventem (Z) and Bauvechevain (B). Altitude of all weather locations Grey lines depict Belgian borders. White coloration in the upper left represents the North Sea and Easter Scheldt region. Altitude (in meter above sea level) for all sample locations and weather stations are (from West to East): 15, 8, 18, 62, 60, 73, 61, 45, 55, 49, 86, 87, 59, 33, 101. b,c & d: examples of sample locations for forest, urban and agricultural landscape types, respectively. Green shading represents woodland cover. Diamond marks indicate sample sites and numbers below the marks represent the week during which measures were made, with week 1 starting on the 13th of June 2018. **e**: sample site with HOBO U23 v2 climatic sensor placed 12 cm above ground in the grass layer.

Fig. 2. Posterior distribution of the landscape type-related estimates of the nine microclimatic variables. Thick lines mark the posterior 66%-credible interval around the mean. Thin lines mark the posterior 95%-credibility interval around the mean. When this line crosses the dashed line at zero, there is no difference between the HOBO sensor data and the weather station data within the 95%-credibility interval. ‘u’: urban; ‘f’: forest; ‘a’: agricultural (intercept). Horizontal lines with asterisks indicate differences in which the value tested against lies outside the 98.33% credibility interval.

Fig. 3. Mean value (± SE) of the first two principal components representing climatic variability relative to landscape type. A high PC1-score (explained 59 %) corresponds to variables that relate to hot and dry daytime conditions and stronger fluctuations during the day. A high PC2-score (explained 19%) corresponds to colder and more humid night

conditions. a: agriculture; u: urban; f: forest. Horizontal lines with asterisks indicate differences with Bonferroni-corrected p-values lower than 0.02 (*) or 0.002 (**).

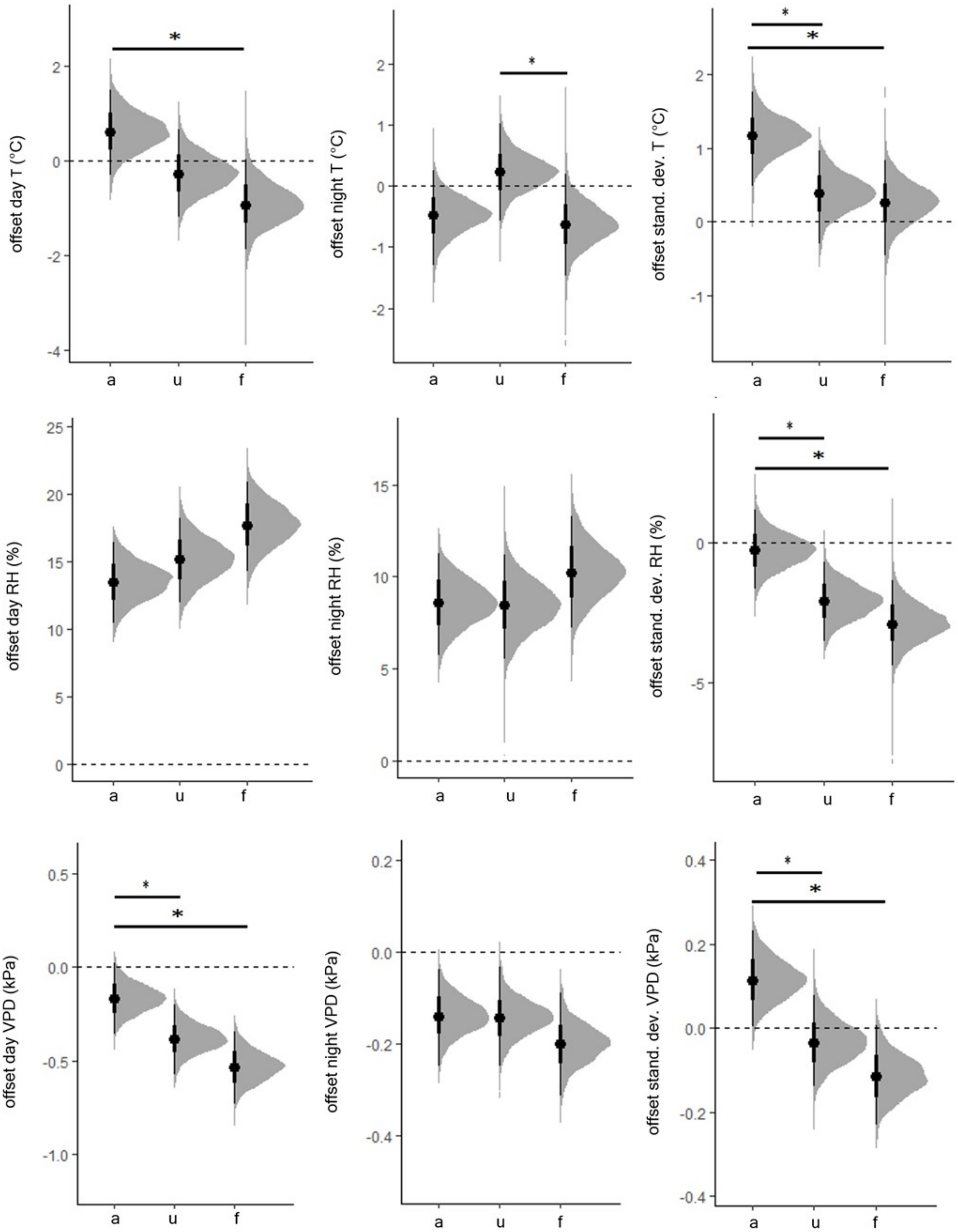
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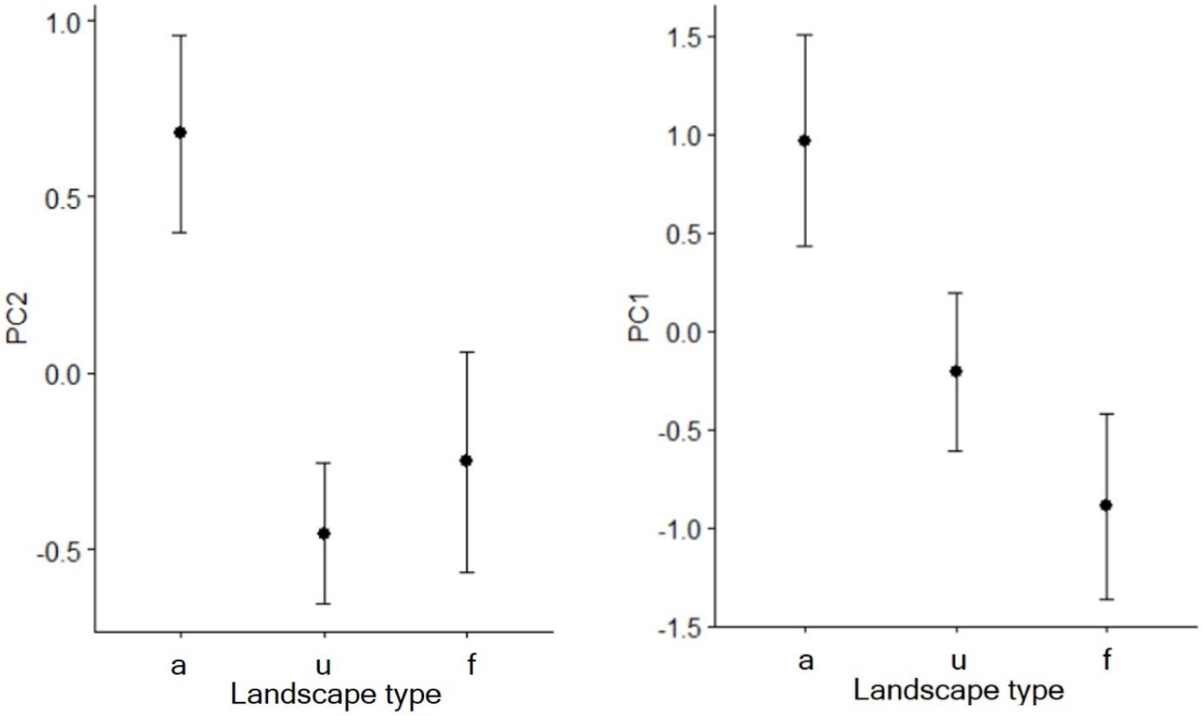
Figure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