Title: Climate, latitude, and land cover predict flying insect biomass across a German malaise trap network

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ABSTRACT

We examine the effects of climate and land cover on flying insect biomass using an ecological gradients approach and data from the first year of a new, ongoing, standardized German Malaise Trap Program coordinated by the German Long-Term Ecological Research network. We hypothesized that biomass would display a unimodal relationship with temperature, consistent with thermal performance theory. We additionally predicted biomass would decrease with precipitation due to reduced flying activity, and would decrease in areas with more heavily human-modified land cover. Flying insect biomass was quantified from malaise traps at 84 locations across Germany throughout the 2019 growing season. We used an Akaike information criterion approach to parse drivers of temperature, deviation in 2019 temperature from long-term averages, precipitation, land cover, geographic coordinates, elevation, and sampling period. We further examined how effects of temperature on insect biomass change across space by testing for interactions between temperature and latitude. Flying insect biomass increased linearly with monthly temperature across all samples. However, positive effects of temperature on flying insect biomass declined with latitude, suggesting the warm 2019 summer temperatures in southern Germany may have exceeded local insect optimums, and highlighting the spatial variation in climate change-driven impacts on insect communities. Land cover explained less variation in insect biomass, with the largest effect being lower biomass in forested sites. Large-scale, standardized biodiversity monitoring like the German Malaise Trap Program are critical to meet the challenge of disentangling the roles of multiple drivers on insect communities.

Keywords: ecological gradient, climate change, insect monitoring, observational, distributed, malaise, pollinator, land use, thermal performance, LTER

INTRODUCTION

Amidst burgeoning evidence of widespread insect declines, there has been an increasing call for high quality, standardized, and large scale insect monitoring to improve estimates of trends, and identify drivers (Didham et al. 2020, Wagner 2020). Insects, and flying insects in particular, are hyper-diverse, and insect activity and life supports many other taxa both directly and indirectly. Insects pollinate of both the majority of wild plants and 75% of crop species (Losey and Vaughan 2006, Klein et al. 2007, Vanbergen and Insect Pollinators Initiative 2013, Reilly et al. 2020). Insect biomass is a key component of energy flows for many food webs (Stepanian et al. 2020). Biomass is a useful indicator of ecosystem function (Dangles et al. 2011, Barnes et al. 2016), and is representative of whole insect communities (Shortall et al. 2009, Hallmann et al. 2017). Climate change and anthropogenically-altered land cover are among the most commonly cited drivers of insect declines (Habel et al. 2019), but we have yet to understand their full impacts on insect biomass.

The effects of climate change are geographically pervasive (Wilson and Fox 2020) and may explain insect decline in natural areas (Rada et al. 2019, Janzen and Hallwachs 2019, Baranov et al. 2020, Welti et al. 2020a). Some insect taxa are currently benefiting from rising temperatures, which can increase local populations (Kaspari et al. 2019, Baker et al. 2021), diversity (Hofmann et al. 2018), and species' range sizes (Termaat et al. 2019). However, as temperatures continue to rise and increase more rapidly, temperature is expected to decrease insect productivity (Warren et al. 2018). Thermal performance theory captures this non-linearity in predicting a unimodal relationship between temperature and insect fitness, as measured by biomass or other performance indicators (Kingsolver and Huey 2008, Kühsel and Blüthgen 2015, Sinclair et al. 2016, Kaspari et al. 2019).

The predicted response of precipitation regimes to climate change varies with region, but forecasts generally suggest increased frequency of both extreme events including both heavy precipitation events and droughts (Myhre et al. 2019). Precipitation may directly and indirectly affect flying insects. High precipitation may directly cause insect mortality, reducing population sizes, but may also decrease the period of time insects are flying, reducing trap catch but not necessarily reflecting true community biomass (Totland 1994). Indirect effects of precipitation on flying insects mediated by plants (e.g. altering plant phenology or plant nutrition) are context-dependent but increasing rainfall in average to wet climates is often detrimental (Lawson and Rands 2019).

Changing land cover due to human activities is also a major threat to insects (Wagner 2020), with wide-ranging impacts from loss of resources and nesting locations at local scales, to fragmented habitats at larger scales (Newbold et al. 2020). Heavily human modified landscapes come with associated pressures, such as eutrophication and pesticide use with agricultural intensification (Goulson et al. 2018, Carvalheiro et al. 2020), and light pollution from urban environments (Owens et al. 2020). Urban land cover can result in declines of both insect diversity (Fenoglio et al. 2020, Piano et al. 2020), and biomass (Macgregor et al. 2019, Svenningsen et al. 2020).

Using data from the first year of monitoring (2019) of the German Malaise Trap Program, we examine the effects of climate and surrounding land cover on flying insect biomass across both the growing season (April-October) and a regional geographic gradient (ranging over 7° latitude, from the German Alps to the Baltic and North Sea). A recent study (Hallmann et al. 2017) reported flying insect biomass from 63 German locations over 27 years. However, not all locations were sampled across years (the most sampling within an individual year comprised 23

locations), and the majority of sampling locations (58 out of 63) were clustered in central-west Germany; thus the sites do not have representative coverage of Germany or represent an extensive latitudinal gradient (coverage of 2° latitude). While our sampling does not yet have multi-year coverage, the high number trap locations and broad spatial coverage allows us to examine drivers of flying insect biomass using a macroecological gradients approach (Pianka 1966, Halbritter et al. 2013, Welti and Joern 2015, Peters et al. 2019).

Here we introduce the German Malaise Trap Program and provide a first examination from this network of the drivers of variation in flying insect biomass. We hypothesize **(H1)** that the effect of temperature on insect biomass will become less positive with increasing temperatures, consistent with thermal performance theory. Spatially, we predict **(H2)** greater increases in flying insect biomass with increasing temperature in colder sites and weaker to negative effects of temperature on flying insect biomass at warmer sites. As elevation tends to increase from northern to southern Germany and may affect temperature, we additionally test for the effects of elevation on flying insect biomass. We hypothesize **(H3)** that flying insect biomass will decline with increasing precipitation due to reduced flying activity. Finally, we predict **(H4)** lower insect biomass in areas surrounded by more heavily human-modified land cover types such as urban and agricultural areas.

MATERIALS AND METHODS

The German Malaise Trap Program

The German Malaise Trap Program currently comprises 31 LTER-D and National Natural Landscape sites (biosphere reserves and national parks) (https://www.ufz.de/lter-

d/index.php?de=46285). The program was established in early 2019 with the aim of investigating long-term trends in flying insect biomass and species composition using DNA metabarcoding. In each site, one to six locations were selected and one malaise trap was installed per location. For this study, we used the 2019 biomass data retrieved from 25 of the 31 sites; the remaining six sites began sampling in 2020. To fill in spatial gaps, we included 8 sites in Bavaria from an additional project but using the same malaise trap type and measurement methods. Overall, this study includes 1039 samples from 84 malaise trap locations and 33 participating sites widely distributed across Germany (Fig. 1). All traps were identically constructed with an opening area of 1.16 m^2 on each of the two trap sides (Fig. S1). Traps predominantly ran from early April to late October 2019 and were usually emptied every two weeks (14.03 days ± 0.06 SE; but ranging 7 - 29 days). Several samples were lost due to animal or wind damage. By sampling across all times of day for the duration of the growing season, these data provide representative measures of each location's flying insect community.

Lab procedures

Insect biomass was weighed while wet to preserve samples for future identification. Samples were placed in a stainless steel sieve (0.8 mm mesh width) to remove excess alcohol, following the weighing procedure in Hallmann et al. (2017), with one modification: instead of waiting until filtered alcohol drops occurred >10 seconds apart, each sample was filtered for a standard five minutes. Subsequently, insects were weighted to the nearest 0.01g.

Climate

Monthly means of maximum and minimum temperatures, and monthly cumulative precipitation from 2019 were extracted from each sampling location using the Terraclimate dataset (Abatzoglou et al. 2018). To compare 2019 with long-term climate averages, we extracted monthly means of maximum and minimum temperature, and monthly cumulative precipitation from 1960-2018 from each trap location using the CRU-TS 4.03 dataset (Harris et al. 2014) downscaled with WorldClim 2.1 (Fick and Hijmans 2017). Data from both time periods (2019 and 1960-2018) were not available from either dataset alone. Both the Terraclimate and CRU-TS 4.03/ WorldClim 2.1 datasets have spatial resolutions of 2.5 arc minutes (~21 km^2) with our 84 trap locations occurring in 72 separate climate grid cells.

Maximum and minimum monthly temperatures in 2019 were generally higher than 1960-2018 averages, especially during the hottest months (Supporting Information: Fig. S2a). While one study suggested that minimum nightly temperatures are warming at a faster rate than maximum temperatures, potentially limiting the ability of insects to recover from hot daytime periods (Speights *et al.*, 2017), trap locations had slightly greater warming in their 2019 maximum monthly temperatures (2.062°C ± 0.047 SE), than minimum monthly temperatures (1.938°C ± 0.048 SE) in comparison to 1960-2018 averages. As mean maximum and minimum monthly temperatures were highly correlated ($F_{(1,512)}$=4442, R^2=0.9, P<0.001), we used only mean maximum temperatures in our analyses. Cumulative annual precipitation was lower in 2019 (784 mm ± 32 SE) compared to the long-term 1960-2018 average (842 mm ± 32 SE). There was no consistent difference between monthly cumulative precipitation in 2019 and the long-term average, but the mid-summer months tended to be drier in 2019 (Supporting Information: Fig. S2b). Both 2019 mean monthly maximum temperatures and precipitation

across our trap locations tended to decline with increasing latitude (Supporting Information: Fig. S3).

Land cover

Land-cover categories in a 1-km buffer around each trap location were extracted using the CORINE Land cover data from 2018 (European Union, Copernicus Land Monitoring Service 2018). A 1-km buffer was selected based on previous work suggesting land cover effects on insect communities declined at larger scales (Seibold et al. 2019). Extracted land cover data was comprised of 30 CORINE land cover categories, which we pooled into eight categories: urban (7.5% of surrounding land cover), intensive agriculture (2.3%), non-irrigated agriculture (15.9%), pasture/orchard (12.7%), forest (44.7%), grassland/shrubland (12.1%), freshwater (3.9%), and saltwater (0.9%). For data visualization, trap locations were additionally categorized by the dominant land cover within the 1-km buffer: urban (n=6), non-irrigated agriculture (n=16), pasture/orchard (n=6), forest (n=44), grassland/shrubland (n=9), freshwater (n=2), and saltwater (n=1).

Elevation

Elevation (m above sea level) was extracted using the Digital Terrain Model with 200-m grid widths (DGM200) from the German Federal Agency for Cartography and Geodesy (GeoBasis-DE / BKG 2013). Locations varied from elevations of 0 m on a barrier island in northeast Germany, to 1413 m in the German Alps. Elevation across the 84 locations is negatively correlated with latitude (Supporting Information: Fig. S4).

All GIS data extraction was conducted in QGIS ver. 3.14 (QGIS.org 2020).

AIC_c analysis

To identify drivers of insect biomass, we used an Akaike Information Criterion corrected for small sample sizes (AIC_c) modelling framework (Burnham and Anderson 2003); first building an *a priori* full model, and then comparing all possible reduced models using the *dredge* function in the R package MuMIn (Bartoń 2016). Mixed models were fit using the R package lme4 (Bates et al. 2015). All analyses were conducted in R ver. 4.0.3 (R Core Team 2020). To reduce variance inflation due to land cover categories being percentages, we sequentially removed land cover categories from the model starting with the least common until the variance inflation factor was <10; this removed the land cover types freshwater, intensive agriculture, and saltwater. Initial analyses substituting the Land Use Index (LUI; Büttner 2014) for land cover percentages resulted in no top models containing LUI; thus we opted to use individual land cover categories. The 2nd degree polynomial of the sampling period was included in the model to capture the hump-shaped pattern of flying insect biomass across the growing season. Sampling period refers to the half-month period most overlapping the days of sampling, and is numerical (e.g. first half of April = sampling period 1, second half of April = sampling period 2). Monthly temperature and precipitation predictors correspond to the month in which the majority of sampling days occurred. Monthly temperature was included as a 2nd degree polynomial to test our prediction of a unimodal relationship. One approach to examine our hypothesis that temperature effects varied spatially would be to include the interaction between temperature variables and latitude; however, we did not include this interaction as it caused variance inflation. Precipitation and elevation were scaled by dividing by 100. We did not include 2019 precipitation minus the long-

term average in our model because it caused inflated variance and 2019 precipitation was not consistently different from the long-term average (Fig. S2b).

The full model contained the response variable of log10(x+1) transformed mg/day biomass, the fixed predictors of the 2nd degree polynomial of 2019 average monthly maximum temperature (poly(tmax,2)), the difference between 2019 average monthly maximum temperature and the long-term average temperature (Δtemp), monthly cumulative precipitation (precip), latitude, longitude, elevation, % cover of the five most dominant land cover categories within a 1-km radius, a 2nd degree polynomial of sampling period to account for seasonality (poly(period,2)), and a random effect of trap identity (trap) to account for repeated observations. The form of the mixed model was:

log10(mg/day+1) ~ poly(tmax,2) + Δtemp + precip + latitude + longitude + elevation + %forest + %grass/shrubland + %pasture/orchard + %non-irrigated crop + %urban + poly(period,2) + (1|trap)

Temperature effects across latitudes

While variance inflation prohibited a temperature by latitude interaction term in the mixed model, we wished to further examine our hypotheses that the effects of temperature on flying insect biomass would decrease in the lower latitudes which had the highest temperatures in 2019. For this analysis, we reduced the dataset to one average biomass value (in mg/day) for each location and month. Calculating monthly values reduces complexity due to variation in when samples are collected across locations, and was the first step towards calculating one value of biomass across the growing season for each sampling location. We estimated the daily biomass

collected within each month, assuming traps caught the same amount of biomass each day within a sample. We pooled samples within the same month and, for samples spanning more than one month, we allocated the proportion of biomass from the sample to each month based on the number of sampling days occurring within the month. With these assumptions, the average biomass B*ij* (mg/day) of location *i* in month *j* in location is a weighted average of the n samples occurring in the month according to the following formula:

Eq. 1

$$B_{i,j} = \frac{\sum_{k=1}^{n}(b_{ijk} \times D_{k,j} \div D_k)}{\sum_{k=1}^{n} D_{k,j}}$$

Where b_{ijk} = the total biomass (mg) at location *i* occurring at least partially in month *j* for a sample *k*, n= the total number of samples occurring at least partially in month *j* for location *i*, $D_{k,j}$ = the number of sampling days occurring in month *j* for a given sample *k*, D_k = the total number of sampling days for a given sample *k*

To examine how the effect of temperature changed over latitude, we averaged $B_{i,j}$ across the seven sampling months (Apr. - Oct.) to calculate one monthly average of mg/day per location. We used only the 48 locations which conducted sampling in all seven sampling months. We calculated average monthly maximum temperature across the same period (Apr. - Oct. 2019), henceforth “growing season temperature”. Then we examined the linear relationship between monthly averages of mg/day and the interaction between growing season temperature and latitude. We visualized the interaction between temperature and latitude using the R package “effects” (Fox and Weisberg 2019).

Dominant land cover categories

To visualize changes in flying insect biomass in the dominant land-cover categories and across the growing season, we plotted flying insect biomass/day over median sampling day (numerical day of the year halfway between trap start and end day) for locations corresponding to each dominant land cover. We used Welch's t-tests, which do not assume equal variance, to identify significant differences between log10(x +1) transformed $B_{i,j}$ of sites dominated by forest, grassland, pasture/orchard, non-irrigated agriculture, and urban landscapes, and averages of all samples within each month. The locations with surrounding land cover dominated by saltwater (n=1) and freshwater (n=2) were excluded due to low replication.

RESULTS

Mean flying insect biomass was 2,329 mg/day ± 79 SE across all sampling periods and trap locations and varied from 0 to 17,543 mg/day. On average across locations, biomass increased from 734 mg/day ± 98 SE in early April, to a peak of 5,356 mg/day ± 401 SE in late June, declining to 568 mg/day ± 111 SE in late October. Flying insect biomass was strongly predicted by temperature, latitude, precipitation, percent forest cover, and sampling period (Table 1). AIC_c model comparison selected three top models which always included the 2nd degree polynomial of sampling period, the 2nd degree polynomial of average monthly maximum 2019 temperature, and negative effects of Δ temperature from the long-term average, latitude, monthly precipitation, and percent forest cover (Supporting Information: Table S1). The second and third top models additionally included a negative effect of percent urban cover and a positive effect of percent pasture/orchard cover (Supporting Information: Table S2). However, these models did not

greatly improve R^2 or log-likelihood; thus these two land cover types provided only weak additional explanatory power (Arnold 2010). Elevation did not appear in any top models (Supporting Information: Table S1). The top model explained 51% of the variance in flying insect biomass without location information (marginal R^2) and 74% of flying insect biomass was accounted for when including the random effect of location identity (conditional R^2; Supporting Information: Table S1).

Climate and latitude

Flying insect biomass increased with mean monthly maximum temperature within 2019 sampling (Table 1, Fig. 2a), and declined with increasing latitude (Table 1, Fig. 2b) and monthly cumulative precipitation (Table 1, Fig. 2c). While 2019 mean monthly maximum temperature was included in all top models, only the first order polynomial term was significant, suggesting the effect was linear (Table 1; Supporting Information: Table S2). When included in the same model as 2019 monthly maximum temperature, the Δ temperature had a negative effect on flying insect biomass (Table 1; Fig. 2d). Across the 48 sites in which sampling occurred in all seven months (Apr. - Oct.), an interaction between growing season temperature and latitude was predictive of flying insect biomass (Supporting Information: Table S3; $F_{3,44} = 4.3$, $R^2 = 0.23$, $P = 0.01$). The effect of growing season temperature on flying insect biomass increased from a negative temperature effect at lower latitudes to a positive effect at higher latitudes (Fig. 3).

Land cover

Flying insect biomass declined with increasing percent forest cover and tended to decrease with percent urban cover and increase with percent pasture/orchard (Supporting Information: Table S2). Categorizing trap locations by dominant land cover showed grassland/shrubland locations to generally have the highest biomass in the early-mid growing season (Fig. 4c), and a moderate increase in biomass in non-irrigated cropland across the growing season (Fig. 4e). However, neither percent grassland/shrubland nor percent non-irrigated cropland explained variation in biomass after accounting for seasonality and temperature (Supporting Information: Table S1). Urban-dominated sites tended to have slightly higher catches of biomass within months (Fig. 4f), but this may be due to the six urban-dominated sites all being located in southern Germany (Fig. 1). When included in the full model with temperature variables, flying insect biomass was weakly negatively correlated with percent urban cover (Supporting Information: Table S2a).

DISCUSSION

In this first outcome of the German Malaise Trap Program, we found flying insects to be extremely sensitive to temperature, with both a positive effect of monthly temperature and a negative effect of 2019 temperatures exceeding long-term averages predicting daily sampled biomass. Across all sampling, flying insect biomass increased linearly with temperature in contrast to the unimodal relationship predicted by our first hypothesis (H1). However, in support of H2, the relationship between temperature and flying insect biomass shifted from positive in colder locations in northern Germany to negative in warmer locations in southern Germany. Additionally, precipitation had a negative relationship with flying insect biomass, as predicted by H3. Finally, we found mixed support for our H4 prediction that more heavily-human impacted land use types would have the lowest biomass, with locations surrounded by pasture/orchard,

grassland, and non-irrigated agriculture tending to have higher insect biomass than those surrounded by forests or urban areas.

Climate

Temperature effects on ectothermic insects can manifest directly on individuals' metabolism, growth, reproduction, immunity, survival, behavior, and dispersal (Sinclair et al. 2016, Harvey et al. 2020), and through effects on other trophic levels such as through altering plant biomass, plant quality, or active time-periods of natural enemies (Laws and Joern 2013, Kwon et al. 2019, Thakur 2020). Our results suggest an overall positive linear effect of temperature on flying insect biomass across Germany. The lack of a unimodal relationship between temperature and insect biomass may be a result of the coarse taxonomic (flying insects caught in malaise traps) and temporal (two week) resolution of our samples in comparison to other studies (e.g. Kühsel and Blüthgen 2015). However, we detected a negative effect of the Δ temperature (2019 temperatures minus the colder long-term average). Additionally, the slope of the relationship between temperature and flying insect biomass declined towards the lower latitudes, which tended to be the warmest locations in 2019. Hallmann *et al.* (2017) found a positive effect of temperature on insect biomass; however, biomass loss over time was greatest in the middle of the growing season, when temperatures are highest. A decelerating benefit of temperature in regions with greater increases in temperature is consistent with a study of two surveys of ant communities across North America that were conducted 20 years apart, which found that sites with the largest increases in average temperature had the largest declines in colony density (Kaspari et al. 2019). We predict future monitoring will detect increasingly negative effects of temperature due to

ongoing climate warming, as temperature begins to exceed species' optimum temperature ranges.

Climate change forecasts for Germany suggest slight increases in cumulative annual precipitation but also shifts in the timing of rainfall with longer dry periods, especially in summer (Bender et al. 2017). The 2019 growing season matched this prediction with June, July and August being much drier than the long-term average and with the wettest month of the study period being October. We detected an overall negative effect of precipitation on flying insect biomass. As our study covered only one growing season, our result likely reflects direct insect mortality from heavy rain or reduced flight activity. Insects can detect changes in barometric pressure and will stop flying if they sense storms approaching (Pellegrino et al. 2013).

Land cover

With ~75% of global land significantly altered by human activities (IPBES 2019), land cover change and land use intensification is a major contributor to insect decline (Potts et al. 2010, Winfree et al. 2011, Díaz et al. 2019). However, we detected only weak effects of urban and agricultural land cover on flying insect biomass. This may in part be due to a minority of heavily human-impacted land cover surrounding our sites, especially with regards to intensive agriculture. While percentage urban area had only a slight negative effect on flying insect biomass, urban environments may affect insect communities in other ways, such as through altering species composition (Theodorou et al. 2020). Higher temperatures in urban areas compared to rural surroundings may partly compensate for lower habitat quality and explain the

above average biomass in spring and late summer/autumn in the urban areas investigated in our study, while also making insects in urban areas more vulnerable to future warming.

Even within land-cover types, large variability exists in habitat quality. This is particularly true of urban areas and agricultural land, which range from large paved urban expanses and areas with intensive pesticide use to urban gardens and low intensity organic farms, offering a wide range of habitat quality for insects (Bengtsson et al. 2005, Hausmann et al. 2020). While moderately impacted by human activity, non-irrigated agricultural areas, pasture land, and orchards in this study tended to support higher biomass, suggesting these land use types may provide suitable habitats for Germany's flying insects. Alternatively, fertilization and the prevalence of monoculture on conventional farms may increase insect biomass through alleviating nutrient limitation and providing high concentrations of host plants, while not benefiting insect biodiversity (Root 1973, Haddad et al. 2000).

We detected reduced insect biomass in trap locations surrounded predominantly by forests. This result is in contrast to previous work, albeit using different sampling methods, showing high Lepidoptera biomass coming to light traps in forests compared to other land use types in the UK (Macgregor et al. 2019), and higher flying insect biomass with increasing forest cover in a study using car-mounted nets in Denmark (Svenningsen et al. 2020). Our malaise-trapping methods are more directed toward Hymenoptera and Diptera, and towards the lower canopy, potentially contributing to this discrepancy. Forests may also provide fewer floral resources than open fields (Jachuła et al. 2017). Alternatively, the vegetation structure in forests may limit insect movement through the landscape, reducing trap catch in comparison to more open systems like grasslands (Cranmer et al. 2012).

Comparison with Hallmann et al. 2017

Average insect biomass captured in malaise traps of Hallmann *et al.* (2017) varied from 9,192 mg/day in 1989 to 2,531 mg/day in 2016 (mean of within-month sample means for May-Sept; April excluded as no April 1989 sampling was conducted). In comparison, our traps collected a monthly average of 2,404 mg/day in May-Sept. However, the malaise traps used in Hallmann *et al.* (2017) were ~51% larger (Hallmann *et al.* [2017] trap opening dimensions per side: 1.79 m^2, traps used in this study: 1.16 m^2). If trap opening has an appreciable positive effect on trap catch, this suggests higher trap catch in this study compared to the last sampling year in Hallmann *et al.* (2017). This discrepancy is most likely due to the difference in sampling locations between the studies, but we cannot rule out an increase in biomass of flying insects in Germany.

Caveats

Insect biomass is a common currency ecosystem-level measure of insect productivity and is an index of energy availability for higher trophic levels. Nonetheless, we cannot differentiate variation in abundance, body size, species diversity, or dominance from biomass data alone. High temperatures may reduce average insect body size either by causing smaller adult body sizes within species (Atkinson 1994, Klockmann et al. 2017, Polidori et al. 2020) or by favoring smaller species (Bergmann 1848, Daufresne et al. 2009, Merckx et al. 2018). While one long-term study of flying insects in the Netherlands found no evidence of higher rates of decline in larger species over the past two decades (Hallmann et al. 2020), larger-bodied species may have become rare earlier in the last century (Seibold et al. 2015). Climate and land-cover change may otherwise alter insect communities by favoring particular trophic levels (Agosta et al. 2018,

Welti et al. 2020b), invasive species (Ju et al. 2017), or pest species with outbreak dynamics (Bernal and Medina 2018). Furthermore, insect responses to climate and land cover may vary with microhabitat preference (Suggitt et al. 2018). For example, flying insects may be more affected by rising temperatures than other insects as they cannot buffer high temperatures by burrowing in soil or plant tissue (Baudier et al. 2015, Wagner 2020).

Future directions for insect monitoring

Limited large-scale, long-term standardized insect monitoring is one reason for low power in our ability to disentangle different potential drivers of insect decline, and understand how this varies with region and taxa. Empirical studies of insect communities often lack the spatial coverage needed to be broadly representative or to test for variation due to land cover types (but see Jeliazkov et al. 2016, Wepprich et al. 2019). Meta-analyses have large spatial coverage, but can be subject to site and/or publishing bias and must reckon with variation in research goals and methodology (Gurevitch and Mengersen 2010). Spatially distributed observational monitoring efforts of multiple biological taxa have tended to target plants and vertebrates but not insects (Eggleton 2020). However, several notable exceptions include mosquito and ground beetle monitoring by the US National Ecological Observation Network (Thorpe et al. 2016, Blair et al. 2020), and regional-scale monitoring programs of butterflies and macro-moths (e.g. Kühn et al. 2008, Dennis et al. 2019, van Swaay et al. 2019). Additionally, several large scale malaise trap programs including the Global Malaise Trap program operating since 2012 (http://biodiversitygenomics.net/projects/gmp/) and the Swedish Malaise Trap Program (operational from 2003-2006; Karlsson et al. 2020) are taxonomic treasure troves. However, neither measure insect biomass. More distributed, standardized, and continuous insect

monitoring programs are needed to fill gaps in current knowledge of temporal insect population and community dynamics (Cardoso and Leather 2019).

The German Malaise Trap Program helps to fill this gap for Germany through both continued monitoring of flying insect biomass and recently begun DNA metabarcoding. This program provides a blueprint for standardized long-term biodiversity monitoring across Europe (Haase et al. 2018), with malaise traps currently being considered as a standard component of European insect biodiversity surveys. European LTER network (eLTER RI; Mirtl et al. 2018) was recently accepted by the EU as part of the European Research Infrastructure Roadmap (ESFRI), and the more than 400 European LTER sites are currently working to establish stronger linkages and comparable monitoring schemes. Continued long-term monitoring by the German Malaise Trap Program will advance our understanding of trends on spatial-temporal scales that are lacking in previous studies on insects (Didham et al. 2020), and in productivity/biodiversity trends more generally (McGill et al. 2015). As highlighted by the recent insect decline debate (e.g. Wagner et al. 2021), comprehensive and standardized monitoring is critical to meet the challenge of unravelling insect trends and drivers in the Anthropocene.

Data Archiving Statement

Flying insect biomass and trap location characteristics will be uploaded to the B2SHARE depository upon acceptance of this manuscript.

References

Abatzoglou, J. T. et al. 2018. TerraClimate, a high-resolution global dataset of monthly climate and climatic water balance from 1958–2015. - Scientific Data 5: 170191.
Agosta, S. J. et al. 2018. Upper thermal limits differ among and within component species in a tritrophic host-parasitoid-hyperparasitoid system. - PLOS ONE 13: e0198803.
Arnold, T. W. 2010. Uninformative parameters and model selection using Akaike's Information Criterion. - J. Wildl. Manage. 74: 1175–1178.
Atkinson, D. 1994. Temperature and organism size—A biological law for ectotherms? - In: Begon, M. and Fitter, A. H. (eds), Advances in Ecological Research. Academic Press, pp. 1–58.
Baker, N. J. et al. 2021. Drivers of long-term changes in aquatic macroinvertebrate communities within a near pristine mountainous stream. - Science of The Total Environment 785: 14685.
Baranov, V. et al. 2020. Complex and nonlinear climate-driven changes in freshwater insect communities over 42 years. - Conservation Biology 34: 1241–1251.
Barnes, A. D. et al. 2016. Species richness and biomass explain spatial turnover in ecosystem functioning across tropical and temperate ecosystems. - Philosophical Transactions of the Royal Society B: Biological Sciences 371: 20150279.
Bartoń, K. 2016. MuMIn: Multi-Model Inference.
Bates, D. et al. 2015. Fitting linear mixed-effects models using lme4. - Journal of Statistical Software 67: 1–48.
Baudier, K. M. et al. 2015. Microhabitat and body size effects on heat tolerance: implications for responses to climate change (army ants: Formicidae, Ecitoninae). - J Anim Ecol 84: 1322–1330.
Bender, S. et al. 2017. Einfluss des Klimawandels auf die terrestrischen Wassersysteme in Deutschland. Eine Analyse ausgesuchter Studien der Jahre 2009 bis 2013.
Bengtsson, J. et al. 2005. The effects of organic agriculture on biodiversity and abundance: a meta-analysis. - Journal of Applied Ecology 42: 261–269.
Bergmann, C. 1848. Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Größe.
Bernal, J. S. and Medina, R. F. 2018. Agriculture sows pests: how crop domestication, host shifts, and agricultural intensification can create insect pests from herbivores. - Current Opinion in Insect Science 26: 76–81.
Blair, J. et al. 2020. Robust and simplified machine learning identification of pitfall trap-collected ground beetles at the continental scale. - Ecology and Evolution in press.
Burnham, K. P. and Anderson, D. R. 2003. Model selection and multimodel inference: A practical information-theoretic approach. - Springer-Verlag.
Büttner, G. 2014. CORINE land cover and land cover change products. - In: Manakos, I. and Braun, M. (eds), Land Use and Land Cover Mapping in Europe: Practices & Trends. Remote Sensing and Digital Image Processing. Springer Netherlands, pp. 55–74.
Cardoso, P. and Leather, S. R. 2019. Predicting a global insect apocalypse. - Insect Conservation and Diversity 12: 263–267.
Carvalheiro, L. G. et al. 2020. Soil eutrophication shaped the composition of pollinator assemblages during the past century. - Ecography 43: 209–221.
Cranmer, L. et al. 2012. Landscape structure influences pollinator movements and directly affects plant reproductive success. - Oikos 121: 562–568.
Dangles, O. et al. 2011. Predicting richness effects on ecosystem function in natural communities: insights from high-elevation streams. - Ecology 92: 733–743.

Daufresne, M. et al. 2009. Global warming benefits the small in aquatic ecosystems. - PNAS 106: 12788–12793.
Dennis, E. B. et al. 2019. Trends and indicators for quantifying moth abundance and occupancy in Scotland. - J Insect Conserv 23: 369–380.
Díaz, S. et al. 2019. Pervasive human-driven decline of life on Earth points to the need for transformative change. - Science in press.
Didham, R. K. et al. 2020. Interpreting insect declines: seven challenges and a way forward. - Insect Conservation and Diversity 13: 103–114.
Eggleton, P. 2020. The state of the world's insects. - Annual Review of Environment and Resources 45: 61–82.
European Union, Copernicus Land Monitoring Service 2018. European Environment Agency (EEA). in press.
Fenoglio, M. S. et al. 2020. Negative effects of urbanization on terrestrial arthropod communities: A meta-analysis. - Global Ecology and Biogeography 29: 1412–1429.
Fick, S. E. and Hijmans, R. J. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. - International Journal of Climatology 37: 4302–4315.
Fox, J. and Weisberg, S. 2019. An R Companion to Applied Regression. - Sage Publications, Inc.
GeoBasis-DE / BKG 2013. Digital Terrain Model grid wisth 200 m (DGM200).
Goulson, D. et al. 2018. Rapid rise in toxic load for bees revealed by analysis of pesticide use in Great Britain. - PeerJ 6: e5255.
Gurevitch, J. and Mengersen, K. 2010. A statistical view of synthesizing patterns of species richness along productivity gradients: devils, forests, and trees. - Ecology 91: 2553–2560.
Haase, P. et al. 2018. The next generation of site-based long-term ecological monitoring: Linking essential biodiversity variables and ecosystem integrity. - Science of The Total Environment 613–614: 1376–1384.
Habel, J. C. et al. 2019. Mitigating the precipitous decline of terrestrial European insects: Requirements for a new strategy. - Biodivers. Conserv. 28: 1343–1360.
Haddad, N. M. et al. 2000. The effects of long-term nitrogen loading on grassland insect communities. - Oecologia 124: 73–84.
Halbritter, A. H. et al. 2013. How comparable are species distributions along elevational and latitudinal climate gradients? - Global Ecology and Biogeography 22: 1228–1237.
Hallmann, C. A. et al. 2017. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. - PLoS One 12: e0185809.
Hallmann, C. A. et al. 2020. Declining abundance of beetles, moths and caddisflies in the Netherlands. - Insect Conservation and Diversity 13: 127–139.
Harris, I. et al. 2014. Updated high-resolution grids of monthly climatic observations - the CRU TS3.10 Dataset. - Int. J. Climatol. 34: 623–642.
Harvey, J. A. et al. 2020. Climate change-mediated temperature extremes and insects: From outbreaks to breakdowns. - Global Change Biology in press.
Hausmann, A. et al. 2020. Toward a standardized quantitative and qualitative insect monitoring scheme. - Ecology and Evolution 10: 4009–4020.
Hofmann, M. M. et al. 2018. Changes in the bee fauna of a German botanical garden between 1997 and 2017, attributable to climate warming, not other parameters. - Oecologia 187: 701–706.

IPBES 2019. Summary for policymakers of the global assessment report on biodiversity and ecosystem services. S. Díaz, J. Settele, E. S. Brondízio E.S., H. T. Ngo, M. Guèze, J. Agard, A. Arneth, P. Balvanera, K. A. Brauman, S. H. M. Butchart, K. M. A. Chan, L. A. Garibaldi, K. Ichii, J. Liu, S. M. Subramanian, G. F. Midgley, P. Miloslavich, Z. Molnár, D. Obura, A. Pfaff, S. Polasky, A. Purvis, J. Razzaque, B. Reyers, R. Roy Chowdhury, Y. J. Shin, I. J. Visseren-Hamakers, K. J. Willis, and C. N. Zayas (eds.).: 11.

Jachuła, J. et al. 2017. Validation of floral food resources for pollinators in agricultural landscape in SE Poland: Validation of floral food resources for pollinators. - Journal of the Science of Food and Agriculture in press.

Janzen, D. H. and Hallwachs, W. 2019. Perspective: Where might be many tropical insects? - Biological Conservation 233: 102–108.

Jeliazkov, A. et al. 2016. Large-scale semi-automated acoustic monitoring allows to detect temporal decline of bush-crickets. - Global Ecology and Conservation 6: 208–218.

Ju, R.-T. et al. 2017. Spring warming increases the abundance of an invasive specialist insect: links to phenology and life history. - Scientific Reports 7: 14805.

Karlsson, D. et al. 2020. The data of the Swedish Malaise Trap Project, a countrywide inventory of Sweden's insect fauna. - Biodiversity Data Journal 8: e56286.

Kaspari, M. et al. 2019. Species energy and Thermal Performance Theory predict 20-yr changes in ant community abundance and richness. - Ecology 100: e02888.

Kingsolver, J. and Huey, R. 2008. Size, temperature, and fitness: Three rules. - Evolutionary Ecology Research 10: 251–268.

Klein, A.-M. et al. 2007. Importance of pollinators in changing landscapes for world crops. - Proceedings of the Royal Society B: Biological Sciences 274: 303–313.

Klockmann, M. et al. 2017. Carried over: Heat stress in the egg stage reduces subsequent performance in a butterfly. - PLOS ONE 12: e0180968.

Kühn, E. et al. 2008. Getting the public involved in butterfly conservation: Lessons learned from a new monitoring scheme in Germany. - Israel Journal of Ecology and Evolution 54: 89–103.

Kühsel, S. and Blüthgen, N. 2015. High diversity stabilizes the thermal resilience of pollinator communities in intensively managed grasslands. - Nature Communications 6: 7989.

Kwon, E. et al. 2019. Geographic variation in the intensity of warming and phenological mismatch between Arctic shorebirds and invertebrates. - Ecological Monographs 89: e01383.

Laws, A. N. and Joern, A. 2013. Predator–prey interactions in a grassland food chain vary with temperature and food quality. - Oikos 122: 977–986.

Lawson, D. A. and Rands, S. A. 2019. The effects of rainfall on plant–pollinator interactions. - Arthropod-Plant Interactions 13: 561–569.

Losey, J. E. and Vaughan, M. 2006. The economic value of ecological services provided by insects. - BioScience 56: 311–323.

Macgregor, C. J. et al. 2019. Moth biomass increases and decreases over 50 years in Britain. - Nature Ecology and Evolution in press.

McGill, B. J. et al. 2015. Fifteen forms of biodiversity trend in the Anthropocene. - Trends in Ecology & Evolution 30: 104–113.

Merckx, T. et al. 2018. Body-size shifts in aquatic and terrestrial urban communities. - Nature 558: 113–116.

Mirtl, M. et al. 2018. Genesis, goals and achievements of Long-Term Ecological Research at the global scale: A critical review of ILTER and future directions. - Science of The Total Environment 626: 1439–1462.
Myhre, G. et al. 2019. Frequency of extreme precipitation increases extensively with event rareness under global warming. - Scientific Reports 9: 16063.
Newbold, T. et al. 2020. Global effects of land use on biodiversity differ among functional groups. - Functional Ecology 34: 684–693.
Owens, A. C. S. et al. 2020. Light pollution is a driver of insect declines. - Biological Conservation 241: 108259.
Pellegrino, A. et al. 2013. Weather forecasting by insects: Modified sexual behaviour in response to atmospheric pressure changes. - PloS one 8: e75004.
Peters, M. K. et al. 2019. Climate–land-use interactions shape tropical mountain biodiversity and ecosystem functions. - Nature 568: 88–92.
Pianka, E. R. 1966. Latitudinal gradients in species diversity: A review of concepts. - The American Naturalist 100: 33–46.
Piano, E. et al. 2020. Urbanization drives cross-taxon declines in abundance and diversity at multiple spatial scales. - Glob Chang Biol 26: 1196–1211.
Polidori, C. et al. 2020. Climate change-driven body size shrinking in a social wasp. - Ecological Entomology 45: 130–141.
Potts, S. G. et al. 2010. Global pollinator declines: trends, impacts and drivers. - Trends in Ecology & Evolution 25: 345–353.
QGIS.org 2020. QGIS Geographic Information System. Open Source Geospatial Foundation Project.
R Core Team 2020. R: A language and environment for statistical computing. - R Foundation for Statistical Computing.
Rada, S. et al. 2019. Protected areas do not mitigate biodiversity declines: A case study on butterflies. - Diversity and Distributions 25: 217–224.
Reilly, J. R. et al. 2020. Crop production in the USA is frequently limited by a lack of pollinators. - Proceedings of the Royal Society B: Biological Sciences 287: 20200922.
Root, R. B. 1973. Organization of a plant-arthropod association in simple and diverse habitats: The fauna of collards (Brassica oleracea). - Ecological Monographs 43: 95–124.
Seibold, S. et al. 2015. Association of extinction risk of saproxylic beetles with ecological degradation of forests in Europe. - Conservation Biology 29: 382–390.
Seibold, S. et al. 2019. Arthropod decline in grasslands and forests is associated with landscape-level drivers. - Nature 574: 671–674.
Shortall, C. R. et al. 2009. Long-term changes in the abundance of flying insects. - Insect Conservation and Diversity 2: 251–260.
Sinclair, B. J. et al. 2016. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? - Ecology Letters 19: 1372–1385.
Stepanian, P. M. et al. 2020. Declines in an abundant aquatic insect, the burrowing mayfly, across major North American waterways. - PNAS 117: 2987–2992.
Suggitt, A. et al. 2018. Extinction risk from climate change is reduced by microclimatic buffering. - Nature Climate Change 8: 713–717.
Svenningsen, C. S. et al. 2020. Contrasting impacts of urban and farmland cover on flying insect biomass. - bioRxiv: 2020.09.16.299404.

Termaat, T. et al. 2019. Distribution trends of European dragonflies under climate change. - Diversity and Distributions 25: 936–950.
Thakur, M. P. 2020. Climate warming and trophic mismatches in terrestrial ecosystems: the green–brown imbalance hypothesis. - Biology Letters 16: 20190770.
Theodorou, P. et al. 2020. Urban areas as hotspots for bees and pollination but not a panacea for all insects. - Nature Communications 11: 576.
Thorpe, A. S. et al. 2016. Introduction to the sampling designs of the National Ecological Observatory Network Terrestrial Observation System. - Ecosphere 7: e01627.
Totland, Ö. 1994. Intraseasonal variation in pollination intensity and seed set in an alpine population of Ranunculus acris in southwestern Norway. - Ecography 17: 159–165.
van Swaay, C. et al. 2019. The EU butterfly indicator for grassland species: 1990-2017.
Vanbergen, A. J. and Insect Pollinators Initiative 2013. Threats to an ecosystem service: pressures on pollinators. - Frontiers in Ecology and the Environment 11: 251–259.
Wagner, D. L. 2020. Insect declines in the Anthropocene. - Annual Review of Entomology 65: 457–480.
Wagner, D. L. et al. 2021. Insect decline in the Anthropocene: Death by a thousand cuts. - PNAS in press.
Warren, R. et al. 2018. The projected effect on insects, vertebrates, and plants of limiting global warming to 1.5°C rather than 2°C. - Science 360: 791–795.
Welti, E. A. R. and Joern, A. 2015. Structure of trophic and mutualistic networks across broad environmental gradients. - Ecology and evolution 5: 326–334.
Welti, E. A. R. et al. 2020a. Nutrient dilution and climate cycles underlie declines in a dominant insect herbivore. - Proc. Natl. Acad. Sci. U. S. A. 117: 7271–7275.
Welti, E. A. R. et al. 2020b. Salty, mild, and low plant biomass grasslands increase top-heaviness of invertebrate trophic pyramids. - Global Ecology and Biogeography 29: 1474–1485.
Wepprich, T. et al. 2019. Butterfly abundance declines over 20 years of systematic monitoring in Ohio, USA. - PLOS ONE 14: e0216270.
Wilson, R. J. and Fox, R. 2020. Insect responses to global change offer signposts for biodiversity and conservation. - Ecological Entomology in press.
Winfree, R. et al. 2011. Native pollinators in Anthropogenic habitats. - Annual Review of Ecology, Evolution, and Systematics 42: 1–22.

Table

Table 1. Top AIC_c model of predictors of flying insect biomass. Model includes the random variable of trap location. T-tests use Satterthwaite's method. Poly(X,1) indicates the first-order term of the 2nd degree polynomial for variable X, while poly(X,2) indicates the second-order term of the 2nd degree polynomial. Predictor variables are defined in the Materials and Methods. Model characteristics include estimate (Est), standard error (SE), degrees of freedom (df), t-value, and p-value (*P*).

	Est	**SE**	**df**	**t-value**	***P***
Intercept	7.84	0.95	89.46	8.24	< 0.001
%forest	-0.33	0.10	87.6	-3.31	0.001
latitude	-0.09	0.02	87.51	-4.79	< 0.001
poly(period,1)	-3.30	0.39	981.7	-8.56	< 0.001
poly(period,2)	-4.02	0.81	1011	-4.94	< 0.001
poly(tmax,1)	8.05	0.97	1017	8.34	< 0.001
poly(tmax,2)	0.21	0.35	961	0.59	0.55
precip	-0.13	0.04	1006	-2.96	0.003
Δtemp	-0.01	0.003	990.5	-3.81	< 0.001

Figures

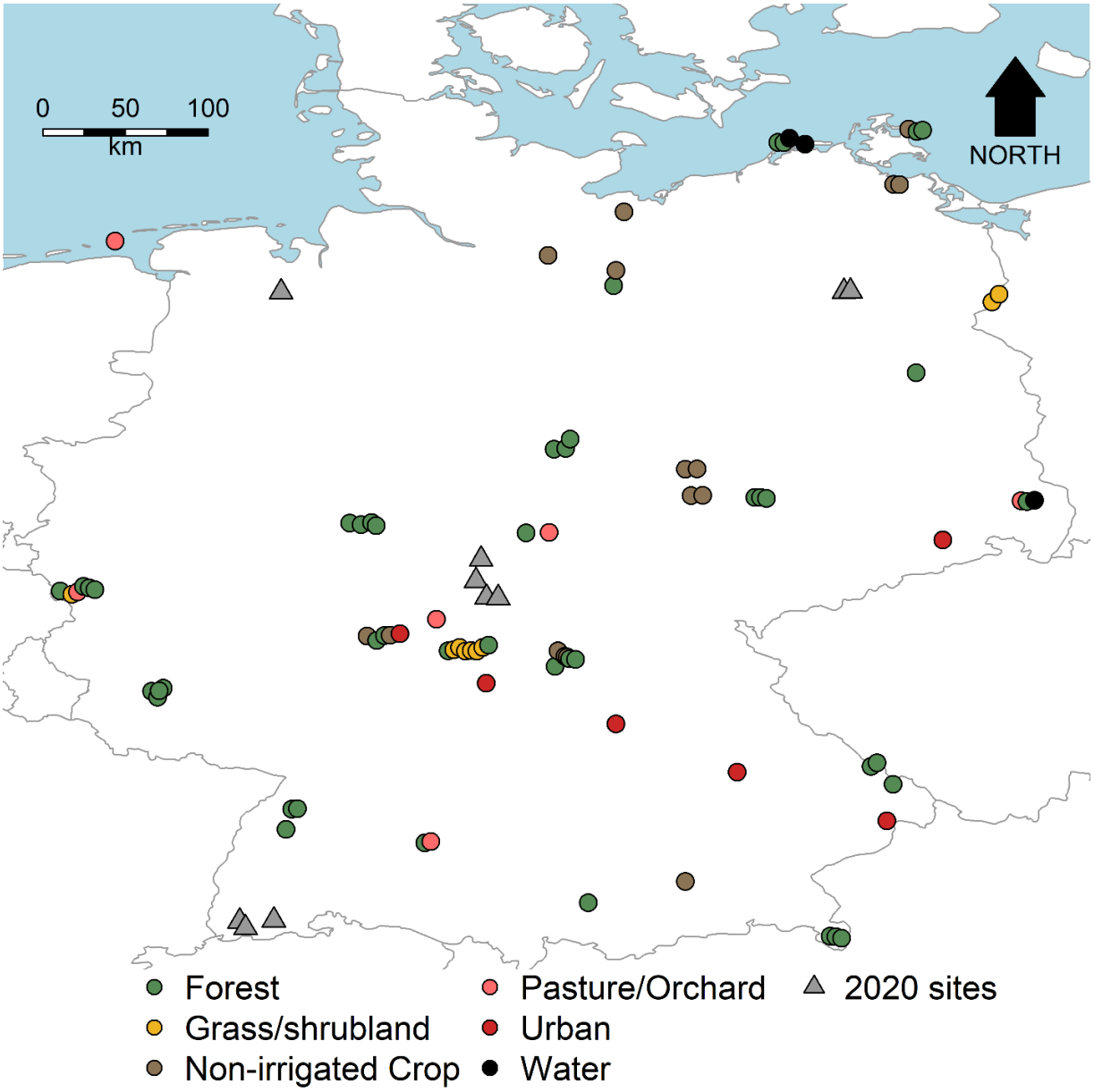

Figure 1. Map of sampling locations. Malaise trap locations where samples were collected beginning in 2019 are depicted as filled circles and color-coded by the dominant land cover in the surrounding 1 km. Points coded as grey triangles indicate trap locations at which sampling began in 2020 and are not included in the analyses. Overlapping locations were jittered longitudinally to improve visualization.

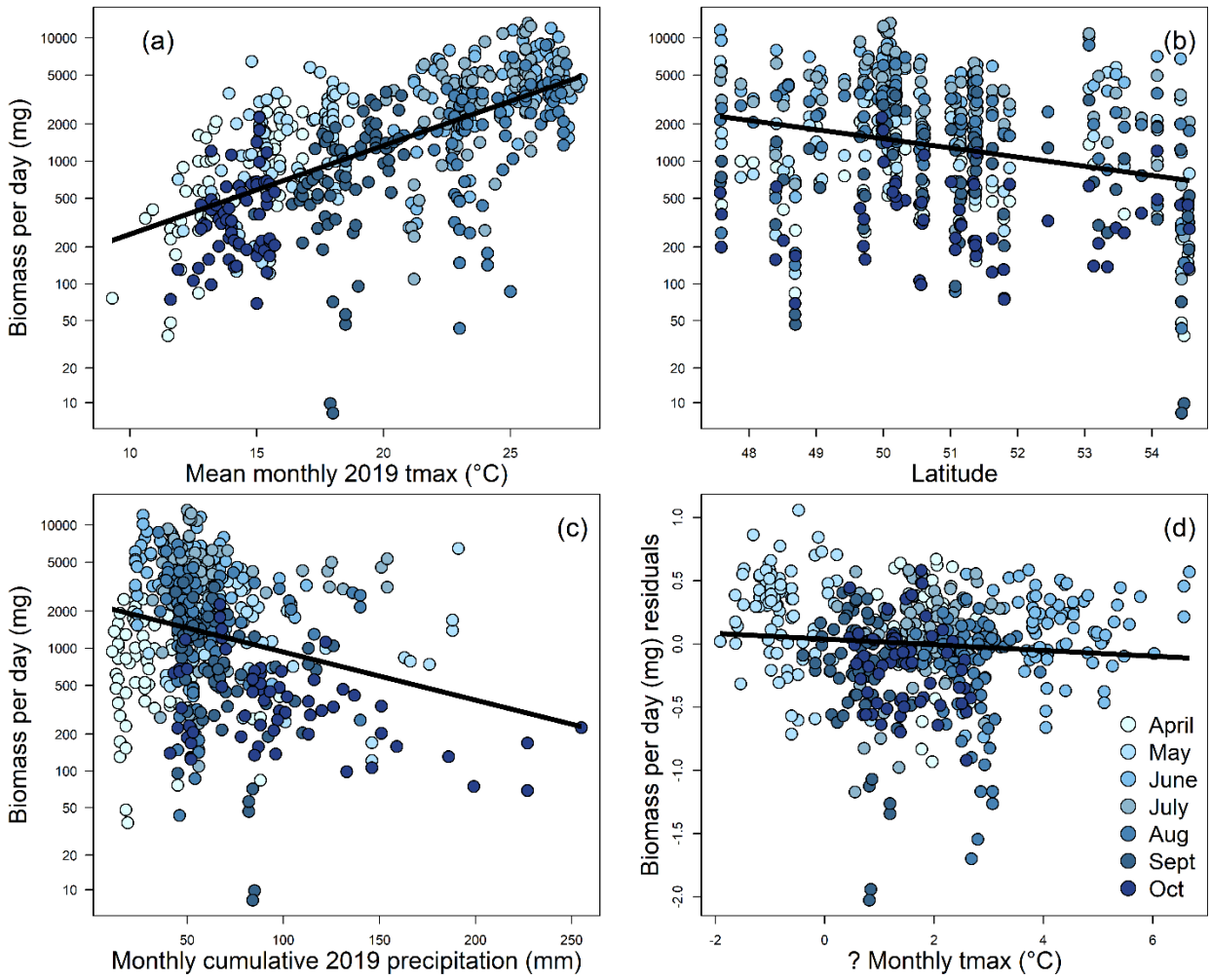

Figure 2. Responses of flying insect biomass to the climate variables and latitude. Each point represents the biomass at each site within each month ($B_{i,j}$ as calculated by Eq. 1). Across all months and site combinations, flying insect biomass increased with mean monthly 2019 maximum temperature (a), and decreased with latitude (c) and cumulative monthly 2019 precipitation (c). The response of flying insect biomass to Δtemp shifted from positive when examined alone to a negative when the effect of 2019 tmax was included in the model. We therefore used the residuals of the relationship between biomass and 2019 tmax as our response variable to show the negative relationship between insect biomass and Δtemp evident in the top AIC_c model (Table 1) (d).

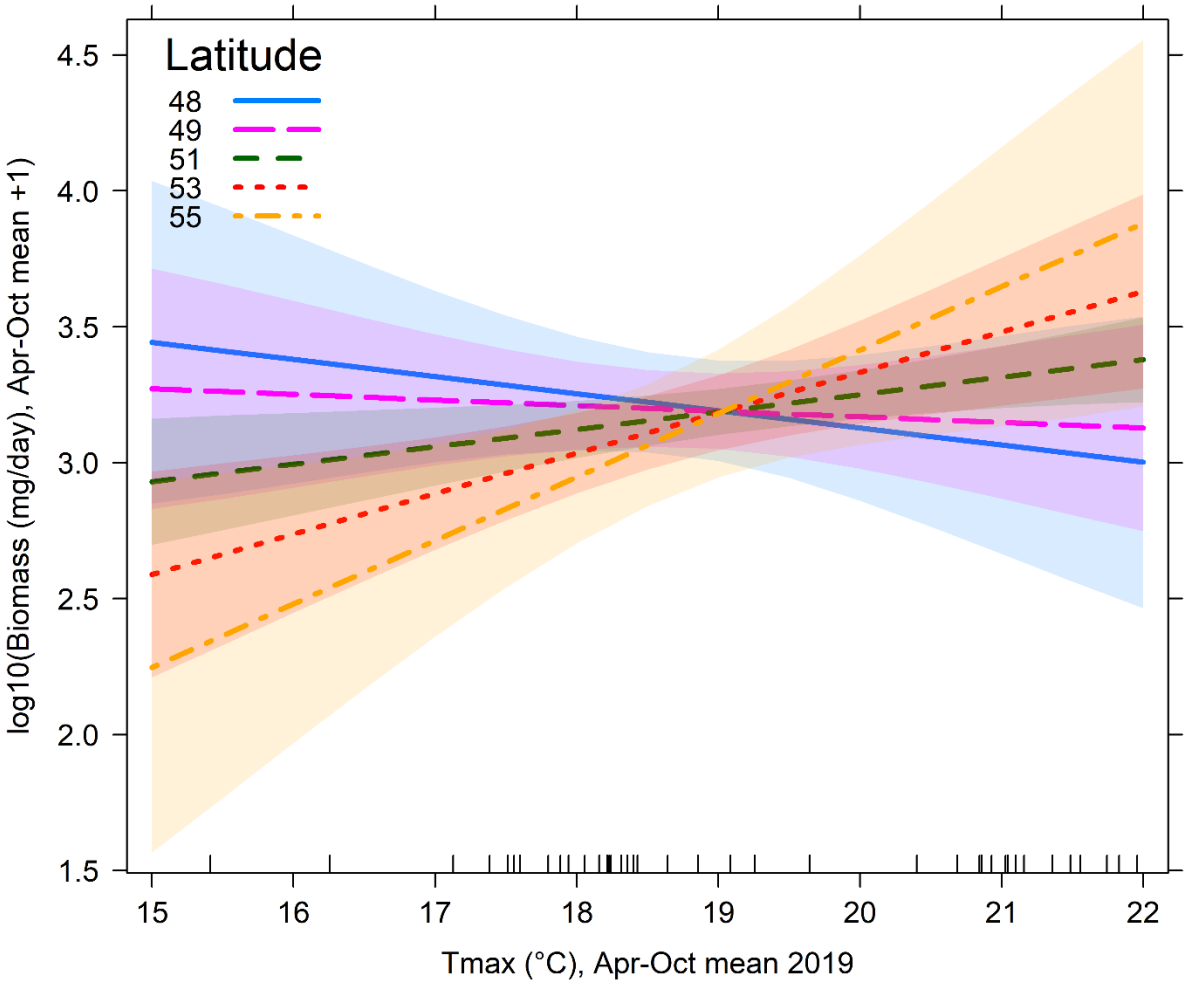

Figure 3. The effect of temperature on flying insect biomass across latitude. The effect of temperature shifted from negative to positive with increasing latitude across the 48 sites with sampling in all 7 months (Apr.-Oct. 2019). Model coefficients are provided in Table S2.

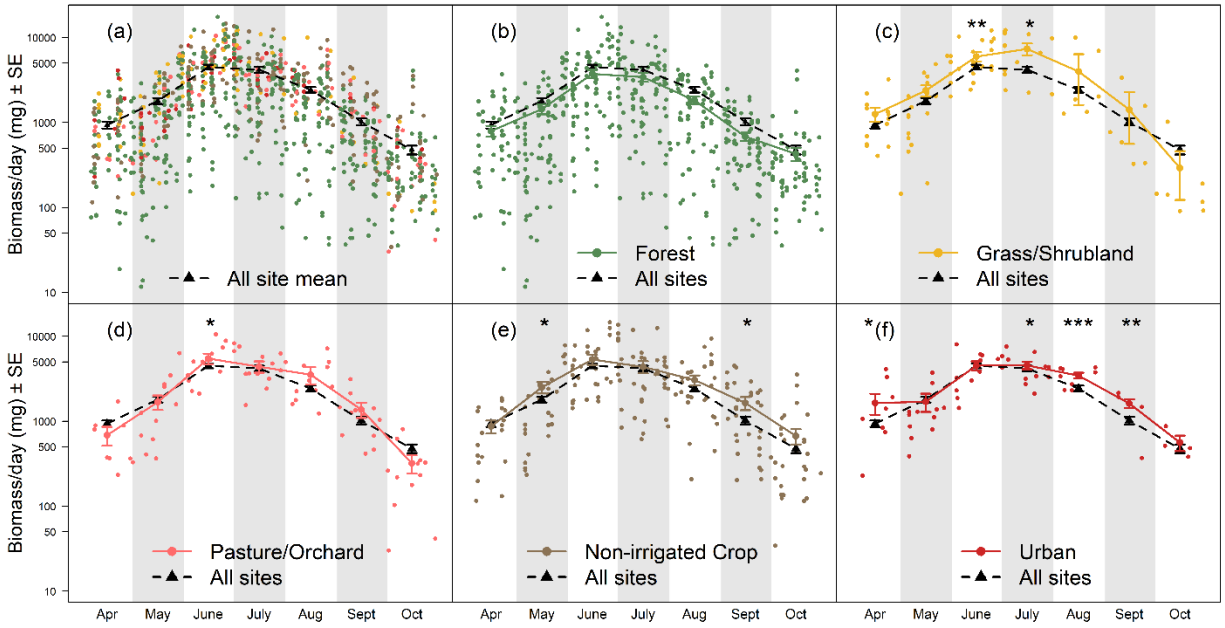

Figure 4. Flying insect biomass over the growing season by dominant land cover. Biomass (mg/day) over the median sampling day across all 84 trap locations (a), and comparisons between all locations and locations with surroundings dominated by forests (b), grassland/shrubland (c), pasture/orchard (d), non-irrigated cropland (e), and urban environments (f). Point colors in panel (d) match the dominant land category following colors in panels B-F. Mean and standard error are provided for biomass within each land cover category and month. Stars indicate significant differences within each month based on Welch's t-tests between biomass from dominant land cover categories and all site averages (* = 0.05 > P > 0.01, ** = 0.01 > P > 0.001, *** = P < 0.001).

Supporting Information for:

Climate, latitude, and land cover predict flying insect biomass across a German malaise trap network

Contents:

Table S1-S3

Fig. S1-S4

Table S1. Top AIC models ($\Delta AIC_c<2$) of predictors of flying insect biomass. All models included the random variable of trap identity. Int=intercept, poly(X,1) indicates the first-order term of the 2nd degree polynomial for variable X, while poly(X,2) indicates the second-order term of the 2nd degree polynomial. Predictor variables are defined in the Methods. Marg R^2= marginal R^2 or the percent variance explained by the fixed effects, Cond R^2= conditional R^2 or the percent variance explained by the fixed effects plus the random effect of trap, df= degrees of freedom, logLik= log likelihood, $\Delta = \Delta AIC_c$, w= model weight. For summary tables of the top model, see Table 1. For summary tables of the second and third top models, see Appendix 1: Table S2.

Int	latitude	poly (period,2)	poly (tmax,2)	Δtemp	precip	%forest	%urban	%pasture/ orchard	Marg R^2	Cond R^2	df	logLik	AICc	Δ	w
7.84	-0.088	+	+	-0.0124	-0.131	-0.328			0.51	0.74	11	-364.35	751	0	0.147
8.26	-0.095	+	+	-0.0126	-0.133	-0.402	-0.405		0.52	0.74	12	-363.55	751.4	0.44	0.118
7.81	-0.089	+	+	-0.0125	-0.131	-0.292		0.253	0.51	0.74	12	-364.3	752.9	1.94	0.056

Table S2. Summary tables of the second (a) and third (b) mixed effects models included in top AIC_c models (Table S1). A summary table of the top model is provided in Table 1. All models contain the random variable of trap identity. T-tests use Satterthwaite's method. See Methods and Table 1 caption for variable explanations. Model characteristics include estimate (Est), standard error (SE), degrees of freedom (df), t-value, and p-value (*P*).

	Est	SE	df	t-value	*P*
a.) Model 2					
Intercept	8.26	0.98	87.66	8.43	< 0.001
%forest	-0.40	0.11	83.87	-3.7	< 0.001
latitude	-0.10	0.02	86.01	-5.07	< 0.001
poly(period,1)	-3.30	0.39	982.1	-8.55	< 0.001
poly(period,2)	-3.99	0.81	1011	-4.91	< 0.001
poly(tmax,1)	8.10	0.97	1016	8.39	< 0.001
poly(tmax,2)	0.22	0.35	960.2	0.63	0.53
precip	-0.13	0.04	1006	-2.99	0.003
Δtemp	-0.01	0.003	989.9	-3.86	< 0.001
%urban	-0.40	0.26	84.52	-1.59	0.12
b.) Model 3					
Intercept	7.81	0.95	88.53	8.21	< 0.001
%forest	-0.29	0.10	87.4	-2.80	0.006
latitude	-0.09	0.02	86.39	-4.81	< 0.001
%pasture/orchard	0.25	0.23	80.44	1.09	0.28
poly(period,1)	-3.32	0.39	979.9	-8.59	< 0.001
poly(period,2)	-3.98	0.81	1009	-4.89	< 0.001
poly(tmax,1)	8.10	0.97	1015	8.38	< 0.001
poly(tmax,2)	0.20	0.35	961	0.59	0.56
precip	-0.13	0.04	1006	-2.94	0.003
Δtemp	-0.01	0.003	989.5	-3.84	< 0.001

Table S3. Model coefficients for the interaction between monthly temperature and latitude (Fig. 3). While individual model coefficients were all marginally significant, the full model predicted 23% of the variation in average biomass (mg/day) averaged across the seven sampling months for the 48 sites with sampling in all months ($F_{3,44} = 4.3$, $R^2 = 0.23$, $P = 0.01$). GST= growing season temperature, the average temperature for each site from Apr-Oct 2019. Model characteristics include estimate (Est), standard error (SE), degrees of freedom (df), t-value, and p-value (*P*).

	Est	**SE**	**t-value**	***P***
Intercept	43.1	21.65	1.99	0.053
GST	-2.1	1.17	-1.8	0.079
Latitude	-0.81	0.42	-1.91	0.063
GST * Latitude	0.04	0.02	1.86	0.07

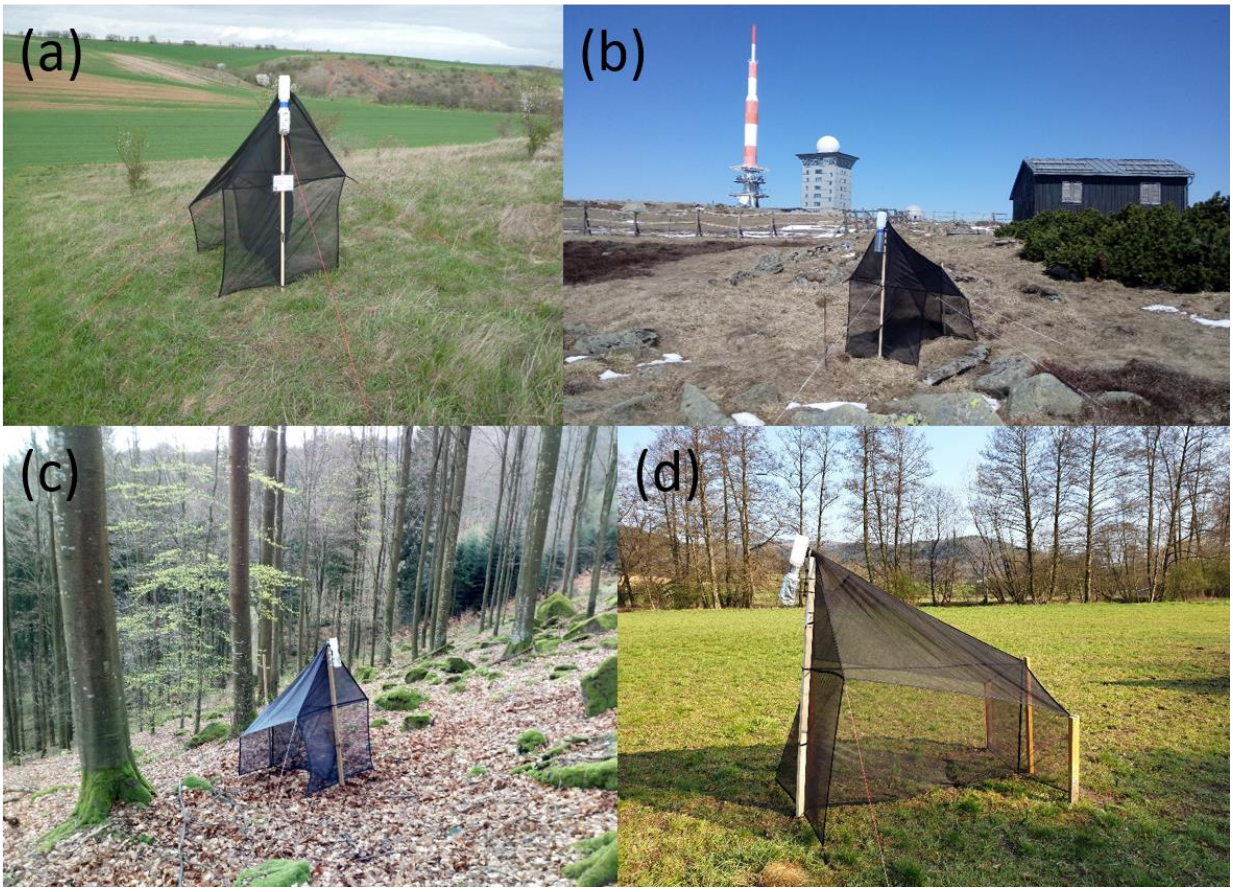

Figure S1. Examples of traps running in 2019 as part of the German Malaise Trap Program. Photos show traps at the LTER site Tereno- Friedeburg (a; photo credit: Mark Frenzel), at the Harz National Park (b; photo credit: Andreas Marten), at the Black Forest National Park (c; photo credit: Jörn Buse), and at the LTER site Rhine-Main-Observatory (d; photo credit: Peter Haase).

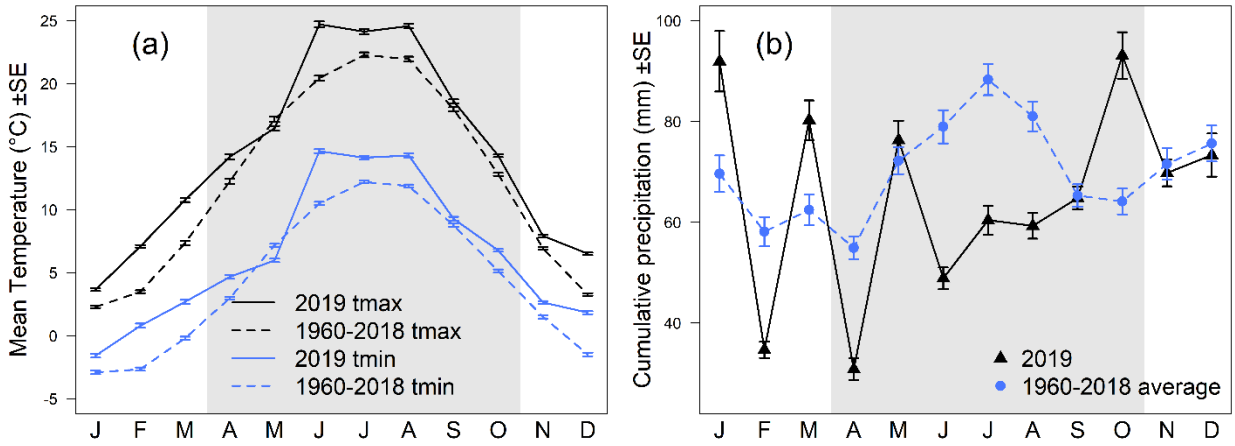

Figure S2. Comparison of climate at the 84 trap locations between 2019 and long-term average (1960-2018) including the average maximum monthly temperatures (tmax) and minimum monthly temperatures (tmin) in °C ± standard error (a) and cumulative monthly precipitation in mm ± standard error (b). Period of the year in which malaise trap sampling occurred is shaded in grey.

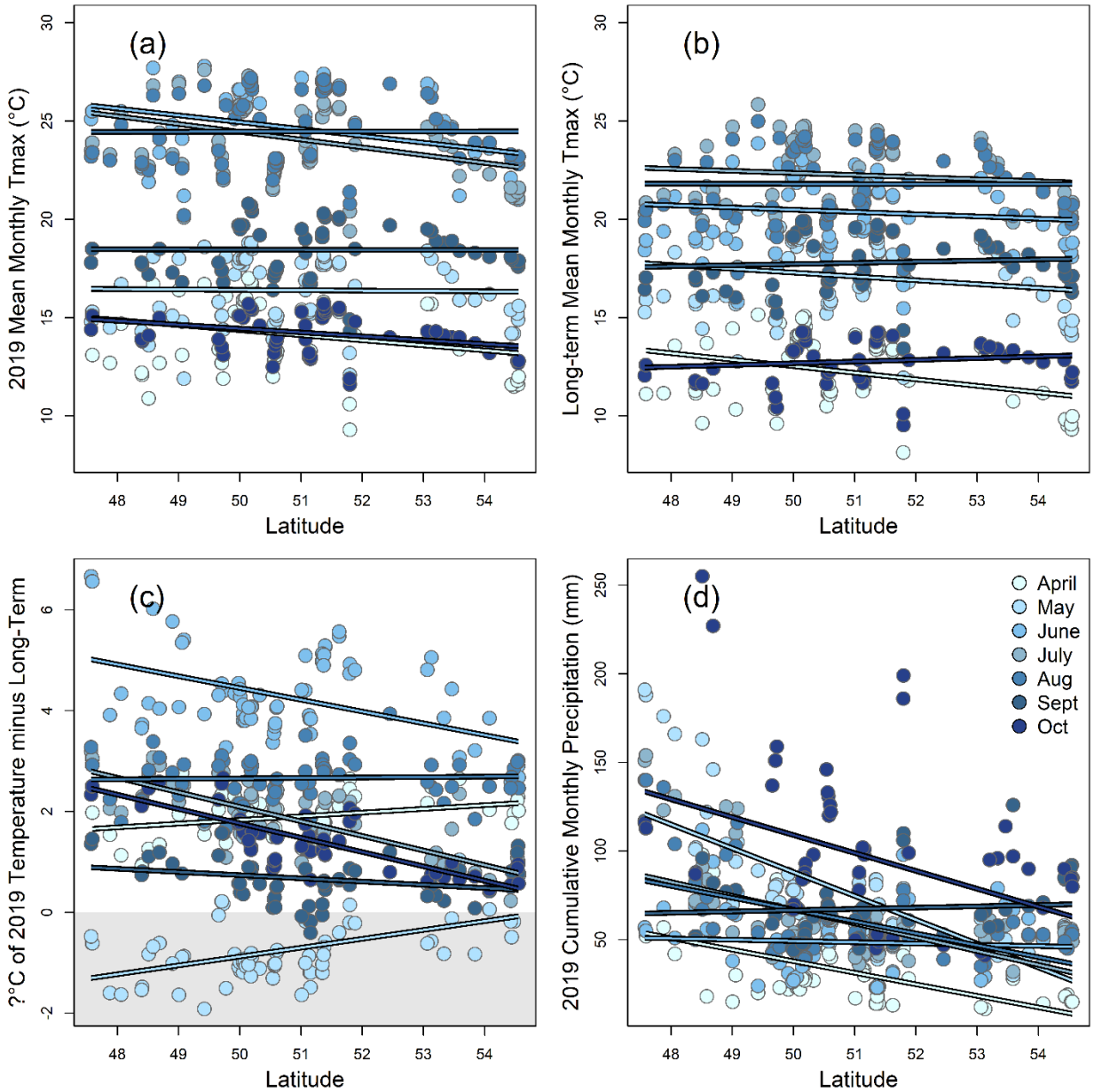

Figure S3. Changes with latitude across our 84 trap locations in 2019 mean monthly maximum temperature (a), the 1960-2018 long-term average monthly maximum temperature (b), the change in 2019 mean monthly maximum temperature minus the 1960-2018 long-term average (c), and 2019 cumulative monthly precipitation (d). Each point represents one month at one location, and only month/location combinations from which flying insect biomass data were collected are included. Across all months, 2019 mean monthly maximum temperature showed a weak trend to decrease with latitude (a; $F_{1,520} = 3.6$, $R^2 = 0.01$, $P = 0.06$), while the 1960-2018 long-term average monthly maximum temperature did not vary with latitude (b; $F_{1,520} = 1.6$, $R^2 < 0.01$, $P = 0.21$). While varying with month, the average Δ °C of 2019 maximum temperature over the long-term average decreased with latitude (c; $F_{1,520} = 6$, $R^2 = 0.01$, $P = 0.01$), as did cumulative monthly precipitation (d; $F_{1,520} = 60.6$, $R^2 = 0.1$, $P < 0.001$).

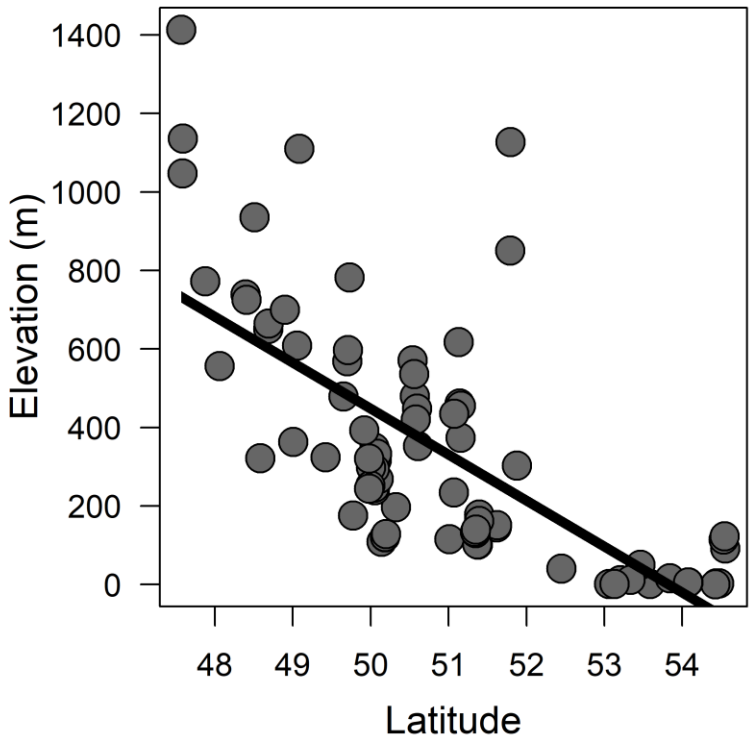

Figure S4. Elevation declined with increasing latitude across our 84 trap locations ($F_{1,82} = 74.5$, $R^2 = 0.48$, $P < 0.001$).