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

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ABSTRACT

Using data from the first year of a new, long-term, standardized German Malaise Trap Program coordinated by the German Long-Term Ecological Research network, we apply an ecological gradients approach to examine the effects of climate and land cover on flying insect biomass. We hypothesized that biomass would display a unimodal relationship with temperature, consistent with thermal performance theory, 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 AIC_c 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. Future work from this newly begun German Malaise Trap Program will add a multi-year dimension to this large-scale, distributed sampling network, with the aim of disentangling the roles of multiple drivers on flying insect communities.

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

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INTRODUCTION

Amidst growing 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 provide critical ecological services, including pollination of both wild species and 75% of crop plants, decomposition, and pest control (Losey & Vaughan, 2006; Klein *et al.*, 2007; Vanbergen & Insect Pollinators Initiative, 2013; Reilly *et al.*, 2020). Additionally, 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 & Fox, 2020) and may explain recent reports of insect decline in natural areas (Janzen & Hallwachs, 2019; Rada *et al.*, 2019; Baranov *et al.*, 2020; Welti *et al.*, 2020b). 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 negatively affect 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 & Huey, 2008; Kühsel & Blüthgen, 2015; Sinclair *et al.*, 2016; Kaspari *et al.*, 2019).

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Precipitation may directly and indirectly affect flying insect biomass. High precipitation may directly cause insect mortality, reducing population sizes, but may also decrease the period of time insects are flying, reducing trap catch while not 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 & Rands, 2019). The predicted response of precipitation regimes to climate change varies with region, with forecasts for Germany suggesting slight increases in cumulative annual precipitation but shifts in the timing of rainfall with longer dry periods, especially in summer (Bender *et al.*, 2017).

Changing land cover due to human activities has been described as the largest threat to insect biodiversity (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 also 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).

In early 2019, a collaboration between the German Long-Term Ecological Research network (LTER-D; e.g. Haase *et al.*, 2016) and the German National Natural Landscapes (Nationale Naturlandschaften; biosphere reserves and national parks) established the German Malaise Trap Program, a new long-term, standardized, flying insect monitoring initiative of malaise traps distributed across Germany (https://www.ufz.de/lter-d/index.php?de=46285). Following the first year of monitoring (2019), we examine the effects of climate and surrounding

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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 locations in Germany over 27 years. However, 58 locations were from central-west Germany and 5 were from central-east Germany; thus the sites do not have representative coverage of Germany or represent an extensive latitudinal gradient (coverage of 2° latitude). Additionally, the individual year with the most sampling in Hallmann *et al.* (2017) comprised 23 locations. While our sampling does not yet have the multi-year coverage of Hallmann *et al.* (2017), the higher number of within year trap locations and broader spatial coverage allows us to examine drivers of flying insect biomass using a macroecological gradients approach (Pianka, 1966; Halbritter *et al.*, 2013; Welti & 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 **(1)** that the effect of temperature on insect biomass will become less positive with increasing temperatures, consistent with thermal performance theory. Spatially, we predict **(2)** 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 **(3)** that flying insect biomass will decline with increasing precipitation due to reduced flying activity. Finally, we predict **(4)** lower insect biomass in areas surrounded by more heavily human-modified land cover types such as urban and agricultural areas (Ricketts *et al.*, 2008; Leather, 2018).

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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) (Fig. 1). 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 added data from another project comprising 8 sites in Bavaria using the same malaise trap type and measurement methods. Overall, 1039 samples from 84 malaise trap locations and 33 participating sites widely distributed across Germany (Fig. 1) were included in this study. 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. Traps were usually emptied every two weeks (14.03 days ± 0.06 SE; range 7 - 29 days). Several samples were lost due to animal or wind damage. As trap runs varied in length, we use biomass/day as the response variable for all analyses. 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. Following the procedure in Hallmann et al. (2017), samples were placed in a stainless steel sieve (0.8 mm mesh width) to remove excess alcohol. Instead of waiting until filtered alcohol drops

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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 & Hijmans, 2017). Two datasets were used because the 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 temperatures in 2019 were generally higher than 1960-2018 averages, especially during the hottest months (Appendix 1: Fig. S2). While one study suggested that minimum nightly temperatures are warming at a faster rate than maximum temperatures which may limit the ability of insects to recover from hot daytime periods (Speights *et al.*, 2017), we found 2019 maximum monthly temperatures across our trap locations were on average slightly higher than 1960-2018 averages (2.062°C ± 0.047 SE), than were minimum monthly temperatures (1.938°C ± 0.048 SE). 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

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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 (Fig. S2). Both 2019 mean monthly maximum temperatures and precipitation across our trap locations tended to decline with increasing latitude (Appendix 1: Fig. S3).

Land cover and elevation

Land-cover categories in a 1-km buffer around each sampling location were extracted using the CORINE Land cover data from 2018 (European Union, Copernicus Land Monitoring Service, 2018). A buffer of 1 km was selected based on previous studies of insect communities that found land cover effects 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 purposes, trap locations were additionally categorized by the dominant land cover within the 1-km buffer: urban (n=6), non-irrigated agriculture (16), pasture/orchard (6), forest (44), grassland/shrubland (9), freshwater (2), and saltwater (1).

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

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northeast Germany, to 1413 m in the German Alps. Elevation across our 84 sampling locations was negatively correlated with latitude (Appendix 1: 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 & 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 percent values 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 corresponded 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

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temperature effects varied spatially would be to include the interaction between temperature variables and latitude; however, this interaction caused variance inflation and thus was not included. Precipitation and elevation were scaled by dividing by 100. We did not include 2019 precipitation minus the long-term average in our model, as we did for temperature, because it caused inflated variance and 2019 precipitation was not consistently different from the long-term average (Fig. S2).

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 around the trap location, 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 warmer lower latitudes. For this analysis, we reduced the

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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 (e.g. for a trap run with 1 day in month A and 13 days in month B we assumed 1/14th of the biomass was collected in month A and 13/14^{ths} was in month B). 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 for each site. We used only the 48 sites 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

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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 & Weisberg, 2019).

Dominant land cover categories

To further visualize and explore 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 traps, 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

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negative effects of Δ temperature from the long-term average, latitude, monthly precipitation, and percent forest cover (Appendix 1: 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 (Appendix 1: 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 (Appendix 1: 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; Appendix 1: 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; Appendix 1: 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 (Appendix 1: 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).

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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 (Appendix 1: 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 (Appendix 1: 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 (Appendix 1: 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. However, in support of our second hypothesis, the relationship between temperature and flying insect biomass shifted from positive in colder locations in northern Germany to negative in warmer locations in

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southern Germany. Additionally, precipitation had a negative relationship with flying insect biomass, in support of our third hypothesis. Finally, we did not find strong support for our fourth hypothesis 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.

Temperature

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 & 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 & Blüthgen, 2015). However, we additionally detected a negative effect of the Δ temperature (2019 temperatures minus the colder long-term average), and the slope of the relationship between temperature and flying insect biomass declined as temperatures declined at 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

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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.

Precipitation

We detected a 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). Future investigations of the German Malaise Trap Program can examine long-termer effects of changing precipitation regimes on insect populations which may manifest through indirect paths, including through altering plant biomass (Prather *et al.*, 2020), plant phenology (Jamieson *et al.*, 2012), nutrient availability in host plants and nectar (Eisikowitch & Woodell, 1975; Welti *et al.*, 2020b), pollen availability (Ortega *et al.*, 2007), the efficacy of chemical signals used by insects (Lawson & Rands, 2019), and the moisture and other properties of soils where many insects overwinter (Yang *et al.*, 2018).

Land cover

Land cover change and intensification of human land use, resulting in loss and reduced quality of habitat, is likely a major contributor to insect decline (Potts *et al.*, 2010; Winfree *et al.*, 2011; Díaz *et al.*, 2019) with ~75% of global land significantly altered by human activities (IPBES, 2019). In contrast to our hypothesis, we detected only weak effects of urban and agricultural land

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cover on flying insect biomass. This may be due in part to a minority of heavily human-impacted land cover surrounding our sites, especially with regards to intensive agriculture. While the percent urban area surrounding the trap location had only a slight negative effect on flying insect biomass, urban areas may alter insect communities in other ways, such as through species composition (Theodorou *et al.*, 2020). Higher temperatures in urban areas compared to their 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.

Even within one land-cover type, large variability exists in habitat quality. This is particularly true of urban areas and agricultural land, which range from large paved urban areas and areas with intensive pesticide use to urban gardens and low intensity organic farms which can provide high quality habitat 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. Forests may 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). However, 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

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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.

Comparison with Hallmann et al. 2017

Average flying insect biomass captured in 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

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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.*, 2020a), invasive species (Ju *et al.*, 2017), or pest species with outbreak dynamics (Bernal & 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

With several notable exceptions including 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; Wepprich *et al.*, 2019), spatially distributed observational monitoring efforts of multiple biological taxa have tended to target plants and vertebrates but not insects (Eggleton, 2020). 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. 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

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research goals and methodology (Gurevitch & Mengersen, 2010). Thus more distributed, standardized, and continuous insect monitoring programs are needed to fill gaps in current knowledge of temporal insect population and community dynamics (Cardoso & 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. A major advantage of using LTER and National Natural Landscape sites is that they are well established and managed by personnel with excellent knowledge of the respective site. As the European LTER network (eLTER RI; Mirtl *et al.*, 2018) was recently accepted by the EU as part of the European Research Infrastructure Roadmap (ESFRI), stronger linkages between the more than 400 European LTER sites are currently being established. One major goal is to standardize and harmonize long-term biodiversity monitoring across Europe (Haase *et al.*, 2018) with malaise traps currently being considered as a potential European biodiversity standard observation, and the German Malaise Trap Program providing a blueprint. Future work aims to answer how flying insects will respond to climate and land cover change over the long-term. Finally, continued 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).

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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

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Figures

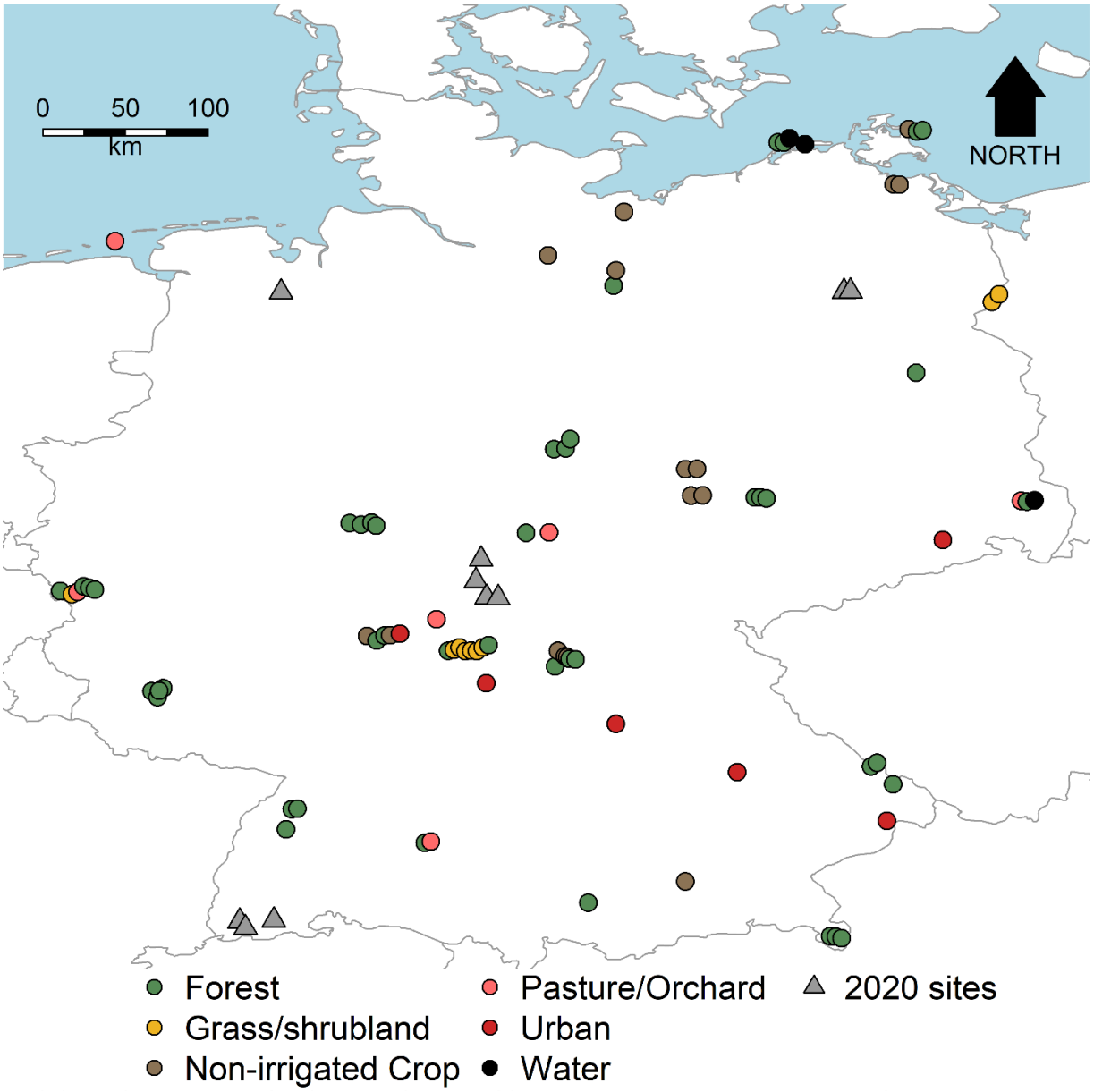

Figure 1. Map of sampling locations. Malaise trap locations where samples were collected beginning in 2019 are depicted as filled circles and color-coded by the dominant land cover in the surrounding 1 km. Points coded as grey triangles indicate trap locations at which sampling began in 2020 and are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

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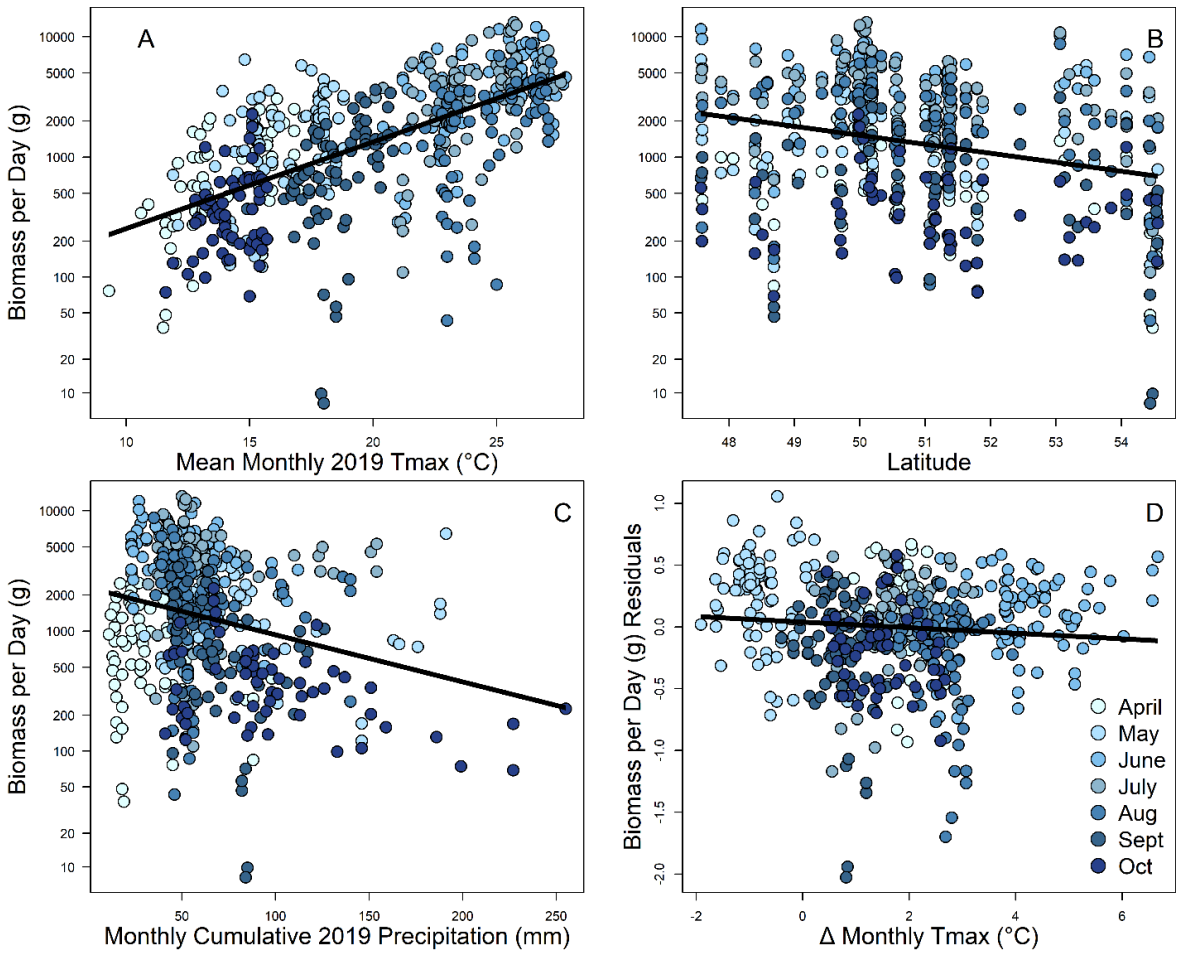

Figure 2. Responses of flying insect biomass to the climate variables and latitude. Each point represents the biomass at each site within each month ($B_{i,j}$ as calculated by Eq. 1). Across all months and site combinations, flying insect biomass increased with mean monthly 2019 maximum temperature (A), and decreased with latitude (B) 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).

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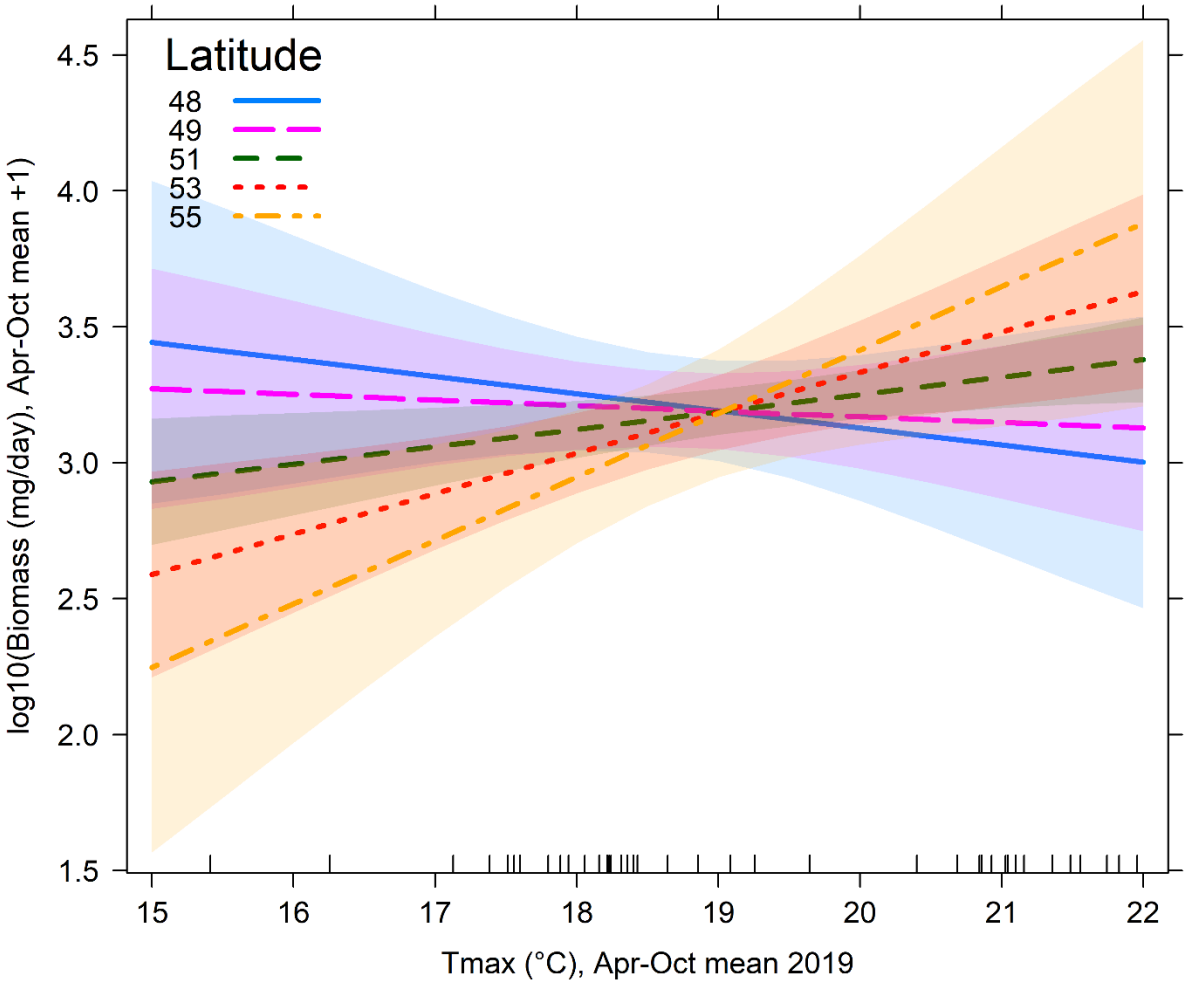

Figure 3. The effect of temperature on flying insect biomass across latitude. The effect of temperature shifted from negative to positive with increasing latitude across the 48 sites with sampling in all 7 months (Apr.-Oct. 2019). Model coefficients are provided in Table S2.

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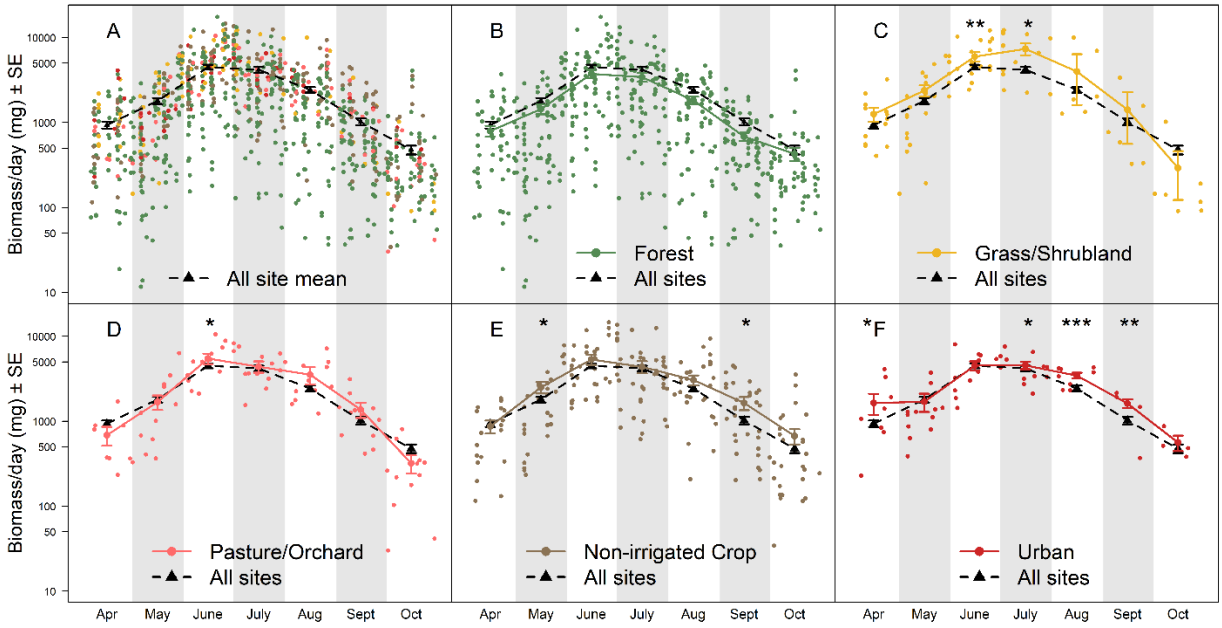

850

851 **Figure 4. Flying insect biomass over the growing season by dominant land cover.** Biomass
852 (mg/day) over the median sampling day across all 84 trap locations (A), and comparisons
853 between all locations and locations with surroundings dominated by forests (B),
854 grassland/shrubland (C), pasture/orchard (D), non-irrigated cropland (E), and urban
855 environments (F). Point colors in panel A match the dominant land category following colors in
856 panels B-F. Mean and standard error are provided for biomass within each land cover category
857 and month. Stars indicate significant differences within each month based on Welch's t-tests
858 between biomass from dominant land cover categories and all site averages (* = $0.05 > P > 0.01$,
859 ** = $0.01 > P > 0.001$, *** = $P < 0.001$).

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Appendix 1: 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 (ΔAIC_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, Δ= ΔAIC_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

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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

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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

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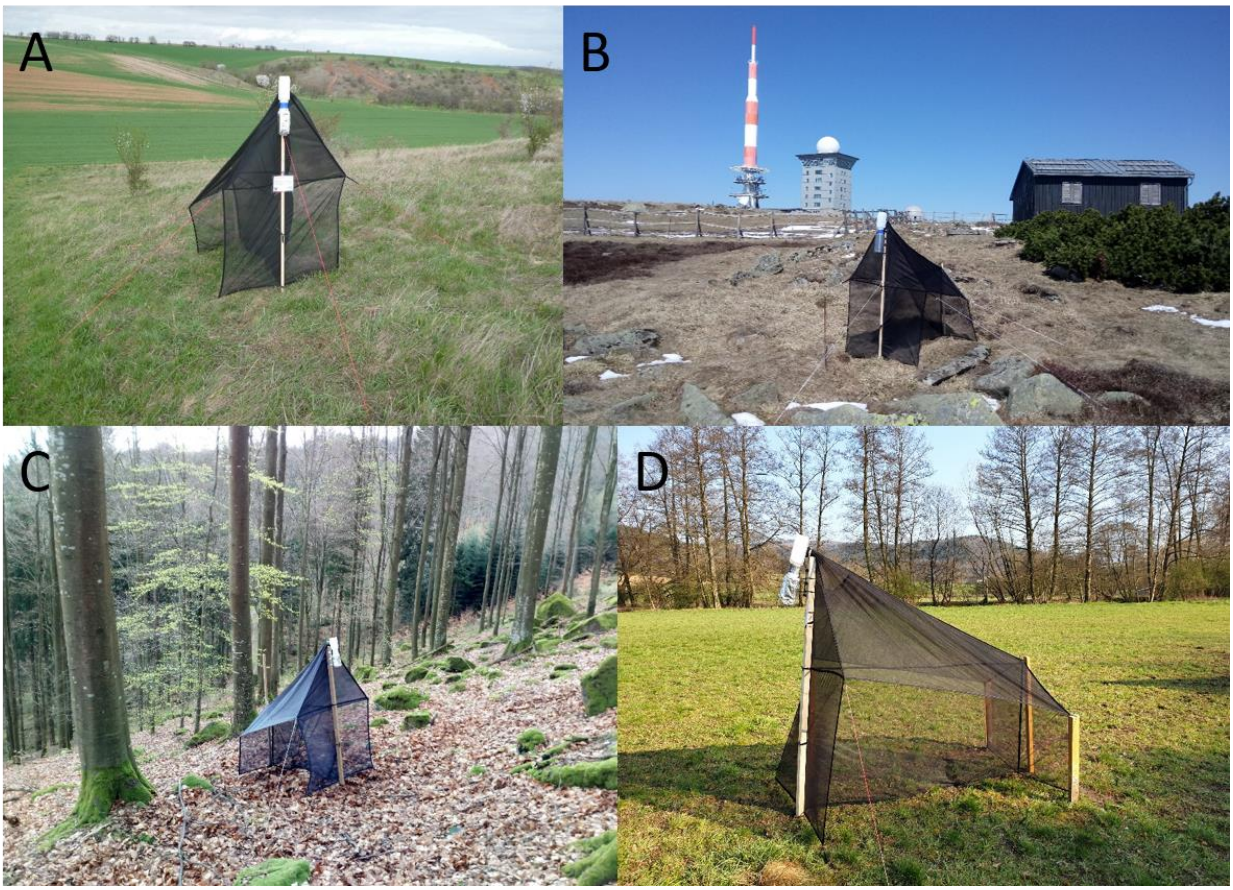

Figure S1. Examples of traps running in 2019 as part of the German Malaise Trap Program. Photos show traps at the LTER site Tereno- Friedeburg (A; photo credit: Mark Frenzel), at the Harz National Park (B; photo credit: Andreas Marten), at the Black Forest National Park (C; photo credit: Jörn Buse),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

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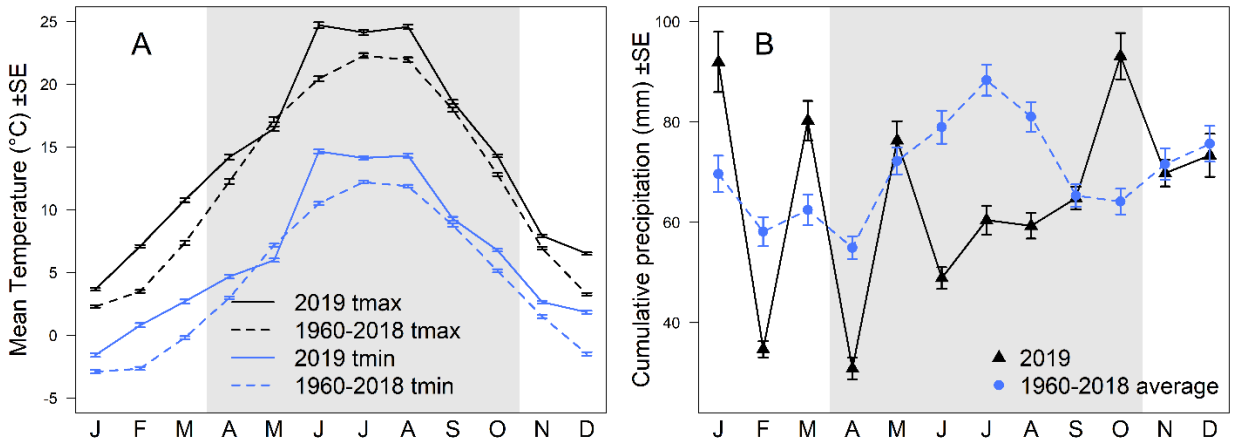

Figure S2. Comparison of climate at the 84 trap locations between 2019 and long-term average (1960-2018) including the average maximum monthly temperatures (tmax) and minimum monthly temperatures (tmin) in °C ± standard error (A) and cumulative monthly precipitation in mm ± standard error (B). Period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

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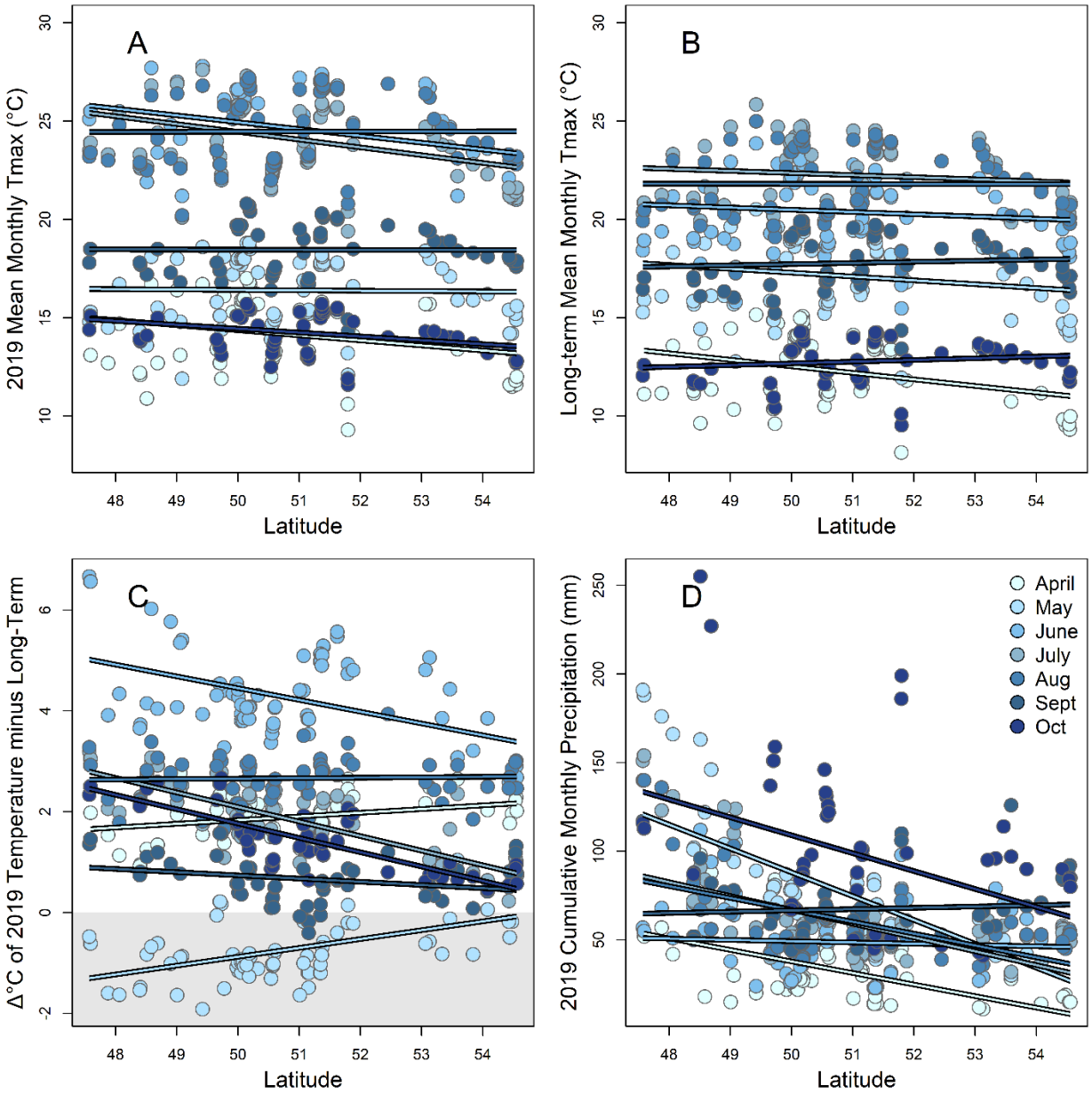

Figure S3. Changes with latitude across our 84 trap locations in 2019 mean monthly maximum temperature (A), the 1960-2018 long-term average monthly maximum temperature (B), the change in 2019 mean monthly maximum temperature minus the 1960-2018 long-term average (C), and 2019 cumulative monthly precipitation (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; $F_{1,520} = 60.6$, $R^2 = 0.1$, $P < 0.001$).

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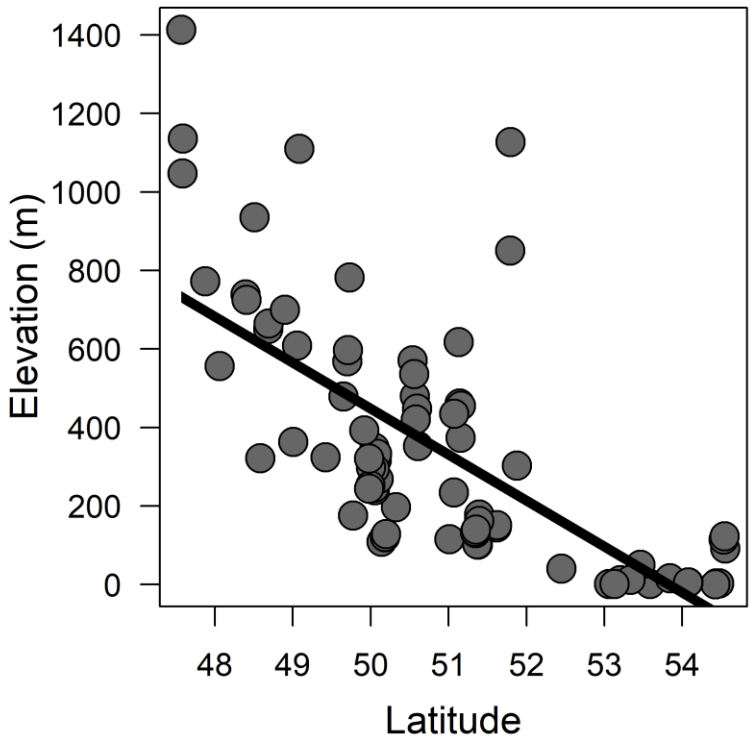

Figure S4. Elevation declined with increasing latitude across our 84 trap locations ($F_{1,82} = 74.5$, $R^2 = 0.48$, $P < 0.001$).