



## Diversity of Insects in Nature protected Areas (DINA): an interdisciplinary German research project

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### Abstract

Insect declines and biodiversity loss have attracted much attention in recent years, but lack of comprehensive data, conflicting interests among stakeholders and insufficient policy guidance hinder progress in preserving biodiversity. The project DINA (Diversity of Insects in Nature protected Areas) investigates insect communities in 21 nature reserves in Germany. All selected conservation sites border arable land, with agricultural practices assumed to influence insect populations. We taught citizen scientists how to manage Malaise traps for insect collection, and subsequently used a DNA metabarcoding approach for species identification. Vegetation surveys, plant metabarcoding as well as geospatial and ecotoxicological analyses will help to unravel contributing factors for the deterioration of insect communities. As a pioneering research project in this field, DINA includes a transdisciplinary dialogue involving relevant stakeholders such as local authorities, policymakers, and farmers, which aims at a shared understanding of conservation goals and action pathways. Stakeholder engagement combined with scientific results will support the development of sound policy recommendations to improve legal frameworks, landscape planning, land use, and conservation strategies. With this transdisciplinary approach, we aim to provide the background knowledge to implement policy strategies that will halt further decline of insects in German protected areas.

**Keywords** Insect diversity · Metabarcoding · Pesticides · Societal dialogues · Spatial analysis · Stakeholder analysis

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## Introduction

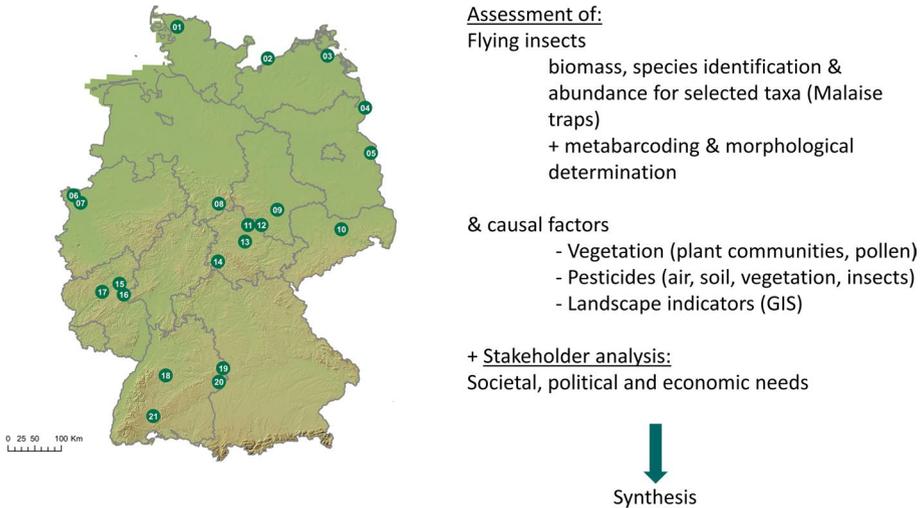
Insects are key organisms for terrestrial ecosystems and serious losses in insect diversity lead to a decline in insectivorous species, affect ecological functions like pollination and alter vegetation and community composition (IPBES 2019). An increasing number of publications show the gradual loss in insect species diversity, including biomass and abundance (summarized in Sánchez-Bayo and Wyckhuys 2019, 2021; van Klink et al. 2020; Wagner 2020). Most alarmingly, loss of insect biodiversity is not only observed on arable land but also within various types of nature conservation areas (Hallmann et al. 2017, 2021; Rada et al. 2019). An observed biomass reduction in insects of almost 80% over 27 years in German nature conservation areas (Hallman et al. 2017) has received widespread attention in the scientific community, the public and German policy makers (Bundestag 2017) and the authors called for a transdisciplinary approach analysing the current state of insect diversity.

## The project DINA—Diversity of Insects in Nature protected Areas

The project (DINA 2021), developed by a consortium of eight partners, implements an interdisciplinary approach of comprehensive assessment of insect and associated plant diversity and pesticide exposure in combination with intensive stakeholder analyses and exchange in German nature protected areas. Combined with the scientific results, DINA aims for full integration into societal knowledge, as effective solutions can only be implemented through cooperation between stakeholders.

Sample sites were selected following geospatial analysis of all 8,836 nature protected areas with a total surface of around 15,843 km<sup>2</sup> (as of 2018). Based on GIS analysis, landscape indicators were evaluated and sites that fulfilled our requirements of grassland dominated habitat types with adjacent or integrated arable land, representing all German nature regions, were preselected. An initial analysis using the land cover model Germany LBM-DE 2018 (BKG 2018; Meinel and Reiter 2019) to identify the arable land shows how tightly these protected areas are interconnected with arable land: Germany's nature conservation areas contain around 450 km<sup>2</sup> of arable land inside their borders and arable land is in direct contact with nature conservation areas along a common borderline of 10,850 km, possibly affecting nature reserves and strictly protected habitats by range effects. If a buffer zone of 100 m around the sites is considered, the arable area increases to around 1,960 km<sup>2</sup>, or one eighth of the protected area itself (Meinel et al. unpubl. data). After the preselection process we evaluated the potential for cooperation of authorities and landowners, finally selecting 21 sites spread all over Germany (Fig. 1, Supplementary Table 1). This number still allows us to manage the incoming samples and is comprehensive enough to generate insights for other nature protected areas.

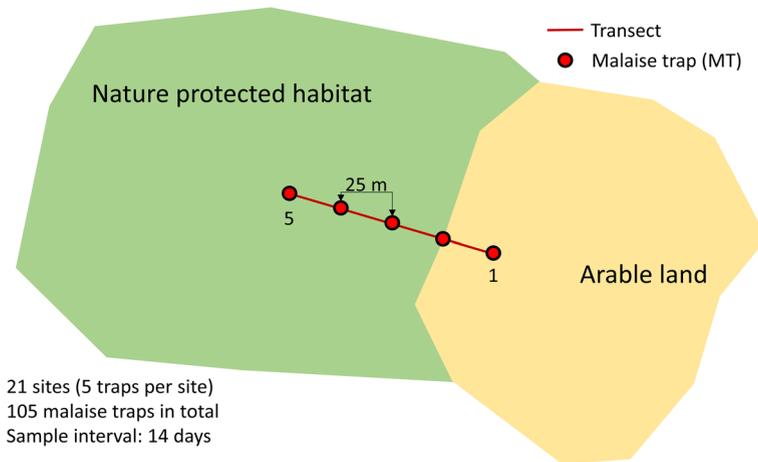
For insect bulk sampling Malaise traps are used, building upon the experience and recommendations of previous research (Sorg et al. 2019) for which first results have already been published, including insect biomass declines (Hallmann et al. 2017) and data on hoverflies (Diptera: Syrphidae, Hallmann et al. 2021). The Malaise trap is a well-established method for collecting flying insects (Ssymank et al. 2018; Skvarla et al. 2021) used in several projects worldwide such as the well-known Swedish Malaise trap project (Karlsson et al. 2020), theILTER network across Europe (Mirtl et al. 2018), and recent and ongoing



**Fig. 1** Transdisciplinary approach in DINA and sampling sites across Germany

studies from Germany (Hardaluk et al. 2020; Hausmann et al. 2020; Welti et al. 2021). To allow data compatibility with previous studies (Hallmann et al. 2017, 2021), we use a standardized sampling design for Malaise traps and insect biodiversity assessment (Schwan et al. 1993; Ssymank et al. 2018). The advantage of Malaise traps over other methods is that they capture continuously, are semiquantitative and have low selectivity (overview in Ssymank et al. 2018). There are 33,466 estimated insect species in Germany (Klausnitzer 2003) of which more than 90 percent can fly. Therefore, Malaise traps cover the most species rich orders (such as Diptera), for which they are the recommended method (Brown 2021). Equally well represented in Malaise traps are the Hymenoptera (Prado et al. 2017; Ssymank et al. 2018) and Coleoptera (especially the small species) (Ulyshen et al. 2005), but ground dwelling and heavier insects may be underrepresented (Stork and Grimbacher 2006; Ssymank et al. 2018; Montgomery et al. 2021; Skvarla et al. 2021). Consequently, our approach comes close to an all-taxa biodiversity inventory (ATBI: Eymann et al. 2010) and represents the overall biodiversity better than studies of single insect orders, which dominate the research landscape (overviews in Sánchez-Bayo and Wyckhuys 2019, 2021). In addition, not only do we analyse caught insects, but also plant traces, to correlate insect activity with plant visitation and usage.

The traps operate continuously over the season, with a two-week collection interval, providing phenological data and the potential to detect species with short flying seasons. To reveal edge and geographical contour effects and to investigate whether the diversity of flying insects changes significantly along a gradient between the arable land and the nature reserve, we simultaneously positioned five traps along a transect 25 m apart (Fig. 2.). Transect sampling is useful for analysing heterogeneity within areas, and despite the long-established application for aquatic insects (e.g., Petersen et al. 2004) most Malaise trap designs are restricted to single traps per area (Sheikh et al. 2016). With this design, we can analyse insect biodiversity along the transects within and across sites, stratified for trap locality and correlated with geospatial parameters, vegetation parameters and pesticide residues (see Fig. 1). The spacing of 25 m between traps was chosen based on previous experience (Ssymank et al. 2018). Indeed, support comes



**Fig. 2** Schematic illustration of our sampling design with a transect of traps reaching from arable land (MT1) into a nature protected habitat (MT2-5)

from recent experiments that found that species co-occurrence and biodiversity similarity decrease in Malaise traps further than 15 m apart (Steinke et al. 2020). The first trap was erected inside arable land, a second one directly at the border to a strictly protected habitat type (according to the EU habitats directive) and the other three further within the protected area (Fig. 2). With 21 areas and five traps per transect we deploy 105 traps in parallel, which is to our knowledge the largest number of Malaise traps in any past or ongoing program; for comparison Sweden was covered by 55 traps (Karlsson et al. 2020), and the LTER operates 79 traps in Germany (Welti et al. 2021). In total, our 105 traps with a two-week sampling interval sum up to around 1500 samples per year.

Our project goals were not limited to the scientific debate of insect declines, but to promote a transition towards a more sustainable nature protection policy. Citizen Science provides opportunities to engage people into ecological research (Silvertown 2009; MacPhail and Colla 2020; Sommerwerk et al. 2021), educate participants (Schleicher and Schmidt 2020) and enforce sustainability transitions (Sauermaun et al. 2020). As the largest and oldest nature protection organization in Germany the Nature and Biodiversity Conservation Union (NABU) has recruited Citizen Scientists through its countrywide network. These volunteers are trained to manage the Malaise traps throughout the season, several also engage in pesticide sampling following protocols and video training. With our Citizen Science approach, we create regional support and the possibility to give people a sense of ownership of "their" nature protected area, and connect our research project with the larger community as an opportunity to democratize science (Alder et al. 2020).

In agreement with the standard protocol of the Entomological Society of Krefeld (Hallmann et al. 2017), all samples are weighed for biomass of the total insects. Total biomass represents the whole insect community (Shortall et al. 2009) and can be used as an indicator of ecosystem processes (Yang and Gratton 2014) and ecosystem function (Barnes et al. 2016). Despite its power as a bioindicator, especially for energy flow and impacts on higher trophic levels (Stepanian et al. 2020; Shaftel et al. 2021), biomass alone might not always be a reliable predictor of biodiversity (Vereecken et al. 2021).

DNA metabarcoding is used for taxonomic identification up to species level. This molecular approach is best suited for large-scale biodiversity assessments that would otherwise not be feasible with morphological identification methods due to time and cost constraints (Taberlet et al. 2012; Yu et al. 2012; Elbrecht et al. 2017). In contrast to single-specimen DNA barcoding based on Sanger sequencing, metabarcoding allows the analysis of bulk samples comprising hundreds of taxonomically diverse specimens through high throughput sequencing (Yu et al. 2012; Taberlet et al. 2012; reviewed in Liu et al. 2020). Hundreds of samples can be pooled and sequenced in one single run yielding millions of sequences, depending on the platform used. Through a specific indexing system and bioinformatic pipelines, sequences can subsequently be assigned back to their sample of origin, molecular units are defined, and the taxa contained in the sample are identified by comparison with DNA barcodes deposited in reference databases. DNA metabarcoding thus considerably up-scales diversity assessments of bulk samples, and accuracy is continuously improved through the refinement of molecular approaches and the expansion of reference libraries (BOLD, Ratnasingham and Hebert 2007; GBOL, Geiger et al. 2016a). Results from German Malaise trapping programs prove the strength of this approach for biodiversity assessments (Morinière et al. 2016; Geiger et al. 2016b; Hausmann et al. 2020; Hardulak et al. 2020).

However, amplification biases (Lamb et al. 2019; Krehenwinkel et al. 2017; Piñol et al. 2019) still prevent reliable estimates of species abundances using DNA metabarcoding. To compensate for this limitation, Diptera, Hymenoptera and Coleoptera from peak biomass samples will be morphologically identified and individuals counted. The combination of presence/ absence data for the entire range of species contained in bulk samples and species abundances for selected taxonomic groups will result in a hitherto unprecedented assessment of flying insect diversity in German protected areas. A key hypothesis is that intensive agriculture reduces insect diversity not only on farmland, but also affects populations in adjacent protected habitats via source-sink dynamics (Furrer and Pasinelli 2016).

The diversity and abundance of insect taxa in an area are highly dependent on available vegetation that provides structure and biological functions (Shinohara & Yoshida 2020). For this reason, an integral part of this project comprises vegetation surveys in the area surrounding the Malaise traps. To extend our understanding of the insects' local and temporal use of the surrounding vegetation, we will implement DNA metabarcoding of the plant traces found in the preservative ethanol of Malaise trap bottles. These traces represent pollen or plant fragments directly carried into the traps on the insect bodies or digested plant material expelled from the digestive tract. Although it will not be possible to directly link the insects with their particular plant species in this mixed sample approach, through information from the vegetation surveys and knowledge of the crops planted on the arable land, we will be able to determine whether insects in the protected areas travel to and from the arable land, thus increasing their exposure to pesticides.

Among other anthropogenic influencing factors, we investigate pesticide contamination in- and outside the nature protected areas. Because of the broad spectrum of toxicity of pesticides and their extensive use in agriculture, contamination of environmental matrices with pesticide residues from multiple applications is a critical issue (Brühl and Zaller 2019; IPBES IPBES). Only a few studies exist on terrestrial pesticide exposure in wildflowers (Botías et al. 2015, 2017; David et al. 2016), agricultural soils (Hvězdová et al. 2018; Silva et al. 2019) or entire landscapes (Humann-Guilleminot et al. 2019). Pesticide transport from cropping areas into adjacent non-target areas was measured in a playground in Southern Tyrol (Linhardt et al. 2019, 2021). By collecting environmental samples of soil and vegetation along a transect, we measure residue concentrations of

realistic current use pesticide mixtures and are able to evaluate contamination in central parts of nature conservation areas. Additionally, we also measure the pesticide residues on insects captured by the malaise traps. Passive movement of pesticides from cropping areas by wind can then be compared to active movement of insects, integrating pesticide use over a larger area around the conservation areas. These current use pesticide measurements are complemented by tree bark sampling to analyse aerial contaminants, including also legacy compounds, over a two-year time span.

To translate our research results into evidence-based cross-sectoral policy recommendations for an effective insect conservation in nature reserves, we correlate insect data with anthropogenic stressors. While different stakeholders in conservation and agriculture generally seem to agree on the finding of a nationwide insect decline, they may have fundamentally different opinions about the causes of biodiversity loss (Fickel et al. 2020). In general, gaps in knowledge and action prevent effective biodiversity protection (Mehring et al. 2017). Transdisciplinary approaches seek integration of stakeholders' knowledge into research to develop relevant and applicable recommendations for decision making at different scales from local to national and across stakeholder groups. Thus, within the framework of the stakeholder analysis, a semi-structured questionnaire as well as literature and media analysis and discourse field analysis are mainly used. Built on this, stakeholder knowledge is gathered via expert interviews and dialogue workshop series in the context of agricultural activities along nature protected areas. Due to this approach, research becomes valuable at local level and fosters knowledge transfer. With the inclusion of data of further nature protected areas, the data compiled up to that point will be supplemented by a subsequent quantitative survey on a much broader scale. This provides a solid basis for further dialogue and ensures the transferability of the results to other nature protected areas and neighbouring regions. Additionally, a national discussion forum between stakeholders from agriculture, administration, nature conservation, and science should vitalize networks among stakeholders, and between basic science and practical applications, and opens new management options for insect biodiversity conservation.

## Expected results

The project DINA measures insect biodiversity at 21 representative nature protected areas in Germany. Results on insect diversity will include vegetation and spatial characteristics, correlated with agricultural practices in and around nature reserves, and risk assessment of pesticide exposure to evaluate possible drivers of biodiversity loss. Equally ambitious as the scientific endeavour, a societal exchange among stakeholders will be fostered, enabling mutual conflict resolution strategies to provide political and practical recommendations for evidence-based optimization of protected area planning and land use.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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