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ORIGINAL ARTICLE

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Transect walks and malaise traps differ in temperature sensitivity but reveal consistent drivers of pollinator richness

Transektläufe und Malaisefallen unterscheiden sich in ihrer Temperaturempfindlichkeit, zeigen jedoch konsistente Treiber der Artenvielfalt von Bestäubern

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Abstract

1. While transect walks have long been the preferred monitoring method for many flying insect taxa, malaise traps combined with DNA metabarcoding have gained growing prominence. However, it remains unclear whether both methods reveal comparable species richness and the same ecological drivers along environmental gradients.
2. We selected three groups of pollinators (wild bees, hoverflies and butterflies) and one group of herbivores (grasshoppers) as functionally important and conservation-relevant model groups, comparing results of both methods along an elevational gradient in the German Alps.
3. Across the study region, both methods detected a similarly high species richness of pollinators with ~50% overlap of species pools, but transect walks revealed more species per site, especially in higher elevations and under low temperatures. Body size spectra differed between methods, with on average more large butterfly and more small bee species in transect walks. Nevertheless, temperature and flower richness were consistent drivers of pollinator richness, independent of the sampling method. Grasshopper richness from transect walks was considerably higher than from malaise traps. Both methods identified temperature and only malaise traps also identified management as drivers of grasshopper richness.

4. We conclude that malaise traps are principally suitable substitutes for the more time-consuming pollinator transect walks. However, the effectiveness of these passive traps is more susceptible to changes in sampling temperature, and in some pollinator groups, body size classes are presented differently, which is important to consider during analyses. For grasshoppers, transect walks appear to be more suitable to assess species richness, as considerably more species can be monitored.

KEYWORDS
Apoidea, arthropods, flying insects, insect monitoring, insect trap, Lepidoptera, mountain biodiversity, Orthoptera, sweep netting, Syrphidae

INTRODUCTION

The monitoring of insects has a long history in Central Europe. However, in the past, it was mainly focused on single popular species groups like butterflies or bees (Habel et al., 2016; Sánchez-Bayo & Wyckhuys, 2019), which were typically sampled along standardised transects by well-versed ecologists and naturalists. The observation of insect communities as a whole drew more widespread public attention recently, after enormous declines in insect biomass were reported from protected areas in Germany (Hallmann et al., 2017). An exponentially increasing number of research items, especially during the last five years (Clarivate Analytics, 2022), emphasised the severity of the situation for the persistence of the insect fauna, not only considering biomass but also abundance and species richness and both on a local (Hallmann et al., 2021; Seibold et al., 2019) as well as on a global scale (Cardoso et al., 2020; Didham et al., 2020; Habel et al., 2019; Raven & Wagner, 2021).

Working on large spatial or temporal scales favoured the use of passive sampling methods in recent insect monitoring programmes, mainly malaise traps (Adams et al., 2020; Hausmann et al., 2022), which are independent of observer experience and cover a wide range of different, mostly flying insect taxa without targeting a specific group (such as flower visitors). Combined with subsequent DNA metabarcoding of insect bulk samples, such traps have become a popular technique for standardised large-scale studies (Piper et al., 2019), as they eliminate the need for time-consuming sorting processes and the dependence on specialised taxonomists, who are becoming increasingly rare (Hochkirch et al., 2022; Ssymank et al., 2018). While this workflow has undoubtedly transformed insect biodiversity research and is certainly useful in many contexts (e.g., determining changes in biomass, recording biodiversity across different taxa), it is still debated to what extent it can keep up with traditional assessments in terms of trustworthiness of species richness data in different environments and when it comes to asking which environmental factors drive species richness of certain groups.

One problem that affects basically all insect sampling methods is that the activity of insects, and with this the efficiency of those methods, strongly depends on temperature during sampling (Wikström et al., 2009). Traditional transect walks are thus typically conducted during more or less standardised weather conditions, for example, only above a certain temperature threshold (Pollard, 1977). With passive sampling methods like malaise traps, it is assumed that sampling temperature is not as critical for sampling efficiency, as traps are usually active for several days or even weeks, reducing the risk that weather conditions are always unsuitable for insect catching. However, when observing insects along elevational or latitudinal gradients, temperature changes systematically—with large impact on insect communities, which typically have smaller populations and less species in higher latitudes and elevations (Classen et al., 2015; Jones et al., 2022; Timms et al., 2016). While both transect walks (Fontana et al., 2020) and malaise traps (Uhler et al., 2021) are generally capable of tracking such changes in insect communities, discrepancies in temperature-dependent sampling efficacy of methods could affect the detectable degree of insect responses to climatic changes along broad environmental gradients and related interpretations. Knowing about such method-dependent specificities in monitoring data is important, as spatial temperature gradients such as along elevation are a frequently used useful tool to derive predictions about the reactions of insects to climate warming (Blüthgen et al., 2022; Verheyen et al., 2019).

Previous studies identified climate and land use change as concurrent major drivers of the current insect decline (Didham et al., 2020; Sánchez-Bayo & Wyckhuys, 2019), and responses of insects to changing temperatures and management at the same time are a major topic in current research (Neff et al., 2022; Outhwaite et al., 2022; Uhler et al., 2021). Therefore, comparing malaise traps and transect walks regarding their suitability to assess temperature effects on insects, while at the same time also considering possible impacts by management might deliver valuable insights for current monitoring practice. Further, including resource availability might improve the validity of detected temperature effects, as this may play an important role for many species in how they react to different aspects of global change (Rafferty, 2017). Additionally, knowing about method-specific peculiarities in species data derived by DNA metabarcoded malaise trap samples as a new state-of-the-art technique enables a better interpretation of its results and facilitates the comparison with previous studies that used different approaches. This is the case, for example, when studying the effects of climate change over time, as there are no data from some decades or longer ago where the combination of malaise traps and DNA metabarcoding was used. However, standardisation of DNA metabarcoding has still a long way to go until reliable comparisons over time and between different projects are possible (Förster et al., 2023).

It is well known that each method captures only subsets of species communities. Still, if species are randomly picked from the species pool, it should be possible to identify the same environmental drivers that shape communities, regardless of the method. However, methods that differ systematically in detected species subgroups may also reveal no consistent ecological drivers of species richness. For example, insect body size could possibly introduce such a bias, as very small species might be overseen by transect walkers and/or the occurrence of very big species might be reduced in malaise trap sampling by the limited size of the entrance to the trapping bottle. As insect body size exhibits pronounced patterns along environmental gradients, for example, temperature (König et al., 2024; Maihoff et al., 2023; Zeuss et al., 2017), the absence of a certain size spectrum in the collected richness data may impact results systematically. Further, when using DNA metabarcoding for species identification, no reliable abundance data can be obtained (Elbrecht & Leese, 2015). Nevertheless, abundance can be an important mediating variable for temperature effects on insect richness (Laiolo et al., 2018; Maihoff et al., 2023), so the method-specific availability of these data might lead to differing results in defining the drivers of species richness. Knowledge about such sampling differences is thus of major importance for the development of effective conservation strategies.

Here, we illustrate the comparability of traditional and modern monitoring methods by comparing data obtained from DNA metabarcoded malaise trap samples and from simultaneously performed transect walks via morphological species identification by taxa specialists. We focus our comparison on three groups of pollinators (wild bees, hoverflies and butterflies) and one important group of herbivores (grasshoppers), which all have relevance for conservation. All these groups have been studied with malaise traps before (Campbell & Hanula, 2007; Ganuza et al., 2022; Uhler et al., 2021), even though malaise traps are mainly proven to be effective for Diptera and Hymenoptera (Montgomery et al., 2021; Skvarla et al., 2021). We sampled insect groups with both methods along a steep 1.4-km-long elevational gradient in the German Alps, including managed (mowing, extensive grazing) and unmanaged grassland habitats. The gradient naturally shows a large variation in temperature and plant resource availability, the ideal setting to study the following research questions:

- Are malaise traps combined with DNA-based species identification and transect walks using classical taxonomical species identification equally well suited to depict patterns of richness along environmental gradients or are there method-specific biases, for example, introduced by insect body size?
- Does the data from each sampling method allow for equivalent identification of environmental drivers of richness?

MATERIALS AND METHODS

Study area

The study area was located in Berchtesgaden National Park and its surroundings, situated in the south-easternmost corner of Germany as part of the Berchtesgaden Alps, belonging to the Northern Limestone Alps. The national park is characterised by high mountains and steep valleys, where elevation ranges around 600–2713 m a.s.l. Mean annual temperature varies between −0.8 and 9.7°C and mean annual precipitation between 1240 and 2881 mm, depending on elevation (averaged yearly means over the last 10 years, extracted from 1 × 1 km grid data from the German Meteorological Service (DWD)). Above the timberline, alpine meadows and bare rock dominate the landscape and below, coniferous forests interspersed with pastures. Alpine pastoral use has a centuries-old tradition in this area and is still traditionally practiced on some meadows, while others have been abandoned.

We selected 31 grassland sites, arranged along five transects, keeping approx. 250 m in elevation between sites within a transect (Figure 1). We covered an elevational gradient around 600–2000 m a.s.l., which was the highest elevation that still had a closed herb layer. While 28 sites were located inside the national park, the lowest three were in close vicinity to extent the elevational gradient towards lower elevations. All sites were either unmanaged (13 sites) or extensively managed by grazing or one cut during late summer (18 sites).

Data collection

Malaise trap sampling

We installed one malaise trap per site, thus 31 in total, sampling flying insects between 14 June and 1 September 2019, covering the peak time of insect activity in this area. Within this period, each trap was active three times for one week each, in parallel to a manual transect walk assessment of major pollinator groups (butterflies, wild bees and hoverflies) and one herbivore group (grasshoppers), as described below. Study sites were visited in random order, sampling each plot once per month in June, July and August. Due to a shorter season duration, the first sample in higher elevations had to be taken in the beginning of July instead of June. In pastures, traps were roughly fenced to protect them from grazer damage, and at all sites, the grass around the traps was cut after some time to keep the flying corridor open. The trap design followed the Townes model and was similar in colour and measurements to Uhler et al. (2021) with black walls and roof and the top with the collection bottle was always oriented south-facing (Figure S1). Each collection bottle was wrapped in aluminium foil to protect the samples from UV radiation and contained a 70% alcohol dilution, which was filled up with pure 99% ethanol after sample collection.

DNA metabarcoding

Malaise trap samples were sent to biome-id (Emsstraße 20, 26,382 Wilhelmshaven, Germany) for DNA metabarcoding and subsequent bioinformatics. Each sample was fractionated into two size classes: small (<6.5 mm) and big (>6.5 mm), which were separately

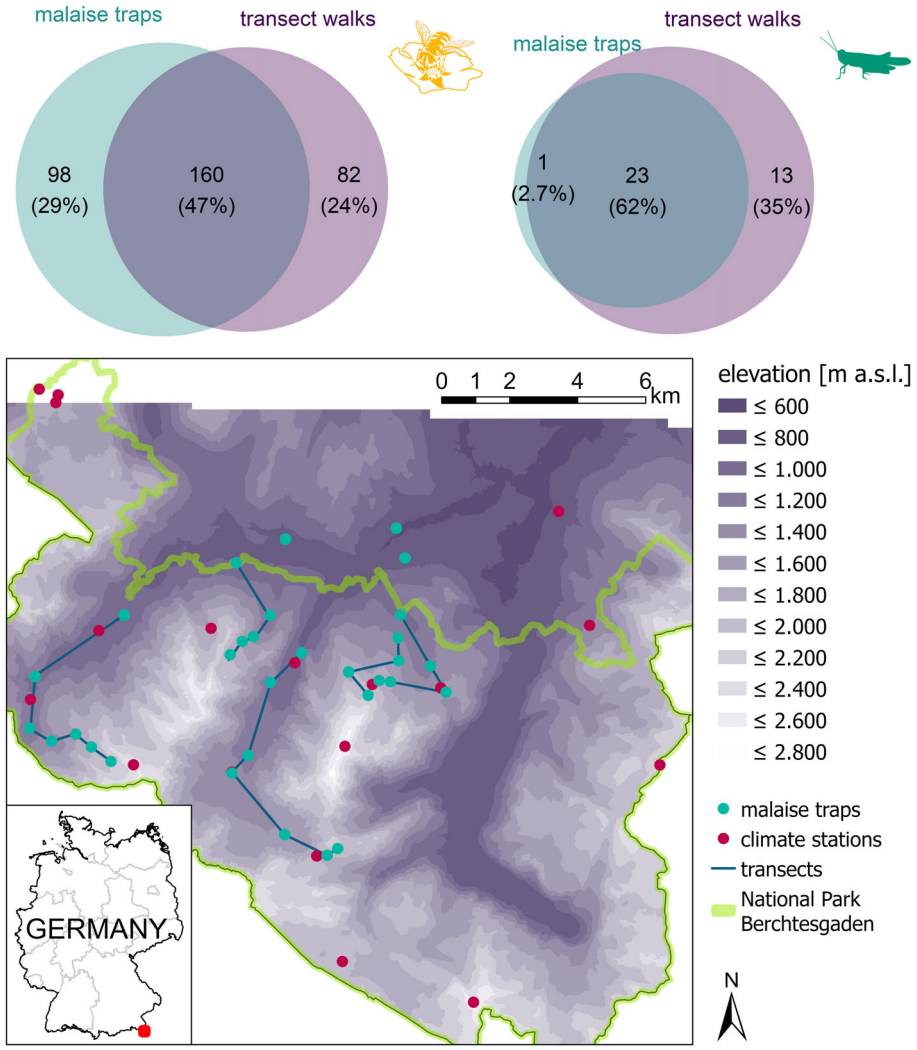

FIGURE 1 **Top**: Species richness of pollinators (left) and grasshoppers (right) caught by malaise traps (turquoise circle), transect walks (purple circle) or both (overlap). **Bottom:** Study area in the south-easternmost corner of Germany in the Berchtesgaden National Park (green) and its surroundings. Study sites (turquoise dots) were located along five elevational transects (blue lines) plus three valley plots in lower elevations. Climate stations used for temperature modelling (red dots) were distributed throughout the national park and its surroundings. The base layer shows elevation in 200-m intervals, ranging from 400 (dark) to 2800 m a.s.l. (bright) (based on a digital elevation model with 1-m grid cell width, provided by the national park administration).

homogenised and then with similar volume shares reunited. Doing so was proven to be an efficient method to control for the higher amount of DNA provided by individuals from bigger species, which would otherwise impede the detection of smaller species with a lower amount of DNA in the sample (Elbrecht et al., 2021). The homogenised composite sample was the base material for the DNA extraction in a lysis volume of 5 mL. After DNA extraction, a 313 bp fragment of the mitochondrial cytochrome c oxidase gene (Geller et al., 2013; Leray et al., 2013) was amplified. The prepared libraries were pooled at equimolar ratio and sequenced on an Illumina® MiSeq platform using v3 chemistry. Vsearch (Rognes et al., 2016) was used to join paired ends, discard short sequences, filter chimeric sequences and cluster the remaining into operational taxonomic units (OTUs) with 97% similarity threshold. We filtered OTUs with less than 0.01% of the total reads per sample. Taxonomy was assigned by mapping against available reference sequences from the BOLD database. Details of the sequencing and bioinformatic pipeline are described in the Supplementary Material.

Species richness

Species richness was assessed taking only OTUs into account that were determined to species level, summing up the detected species of all three samples per site. We classified taxa as potential pollinators on family level (Table S1), using the literature and supporting online material, similar to Ganuza et al. (2022). Pollinators were considered as such if they were either proven to transport pollen and/or to feed exclusively on pollen and/or nectar, thus spending a lot of their time visiting flowers according to the information of the scanned literature

(among others: Banza et al., 2019; Hall & Reboud, 2019; Larson et al., 2001; Macgregor et al., 2015; Zemenick et al., 2019). To compare richness between methods, we filtered species richness to only consider pollinator families assessed by sweep netting, which included butterflies (Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, Pieridae, Riodinidae), wild bees (Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae) and hoverflies (Syrphidae). Additionally, we recorded the richness of herbivores, using Orthoptera as a representative group (Acrididae, Tetrigidae, Tettigoniidae; hereafter referred to as grasshoppers). The species lists of the four chosen groups were checked for plausibility by experts (Table S2 & S3) and implausible entries were either reassigned to occurring species where possible or excluded from analyses (Table S4).

Transect walk surveys

We sampled each species group by doing transect walks, visiting plots in random order and activated the malaise traps the same day when finishing the assessment. We conducted transect walks between 9 a.m. and 6 p.m. when the sun was shining or when temperature under cloudy conditions reached at least 17°C in the valley. On each site, we sampled inside a fixed 60 × 60 m plot, with sampling lengths adjusted to the different taxa. Butterflies were sampled for 32 min, subdivided into 8 × 4 min (Kerner et al., 2023), while we jointly sampled wild bees and hoverflies for 50 min, subdivided into 10 × 5 min (Maihoff et al., 2023). Abundances were assessed as the number of recorded individuals summed up over all three sampling rounds. Grasshopper transect walks were also conducted for 50 min, subdivided into 5 × 10 min intervals (König et al., 2022). Grasshopper recordings did not exactly match the pollinator assessments by date but were conducted in the same time period. Grasshopper abundance was measured by averaging the number of individuals of the three sampling rounds, as they are quite sedentary in this setting and live several months, so the same individuals were probably sampled several times.

Body size

To evaluate whether insect body size causes a systematic bias in method-specific species pools, we extracted body sizes for each recorded species from the literature. For butterflies, body size is approximated as wing length taking the median of the given range in Paolucci (2013). For bees, the female body length of each species was extracted from Hofmann et al. (2019), Maihoff et al. (2023) and Westrich (2019), using the worker caste for bumble bees. Body size ranges for each hoverfly species were compiled from a set of references (Barkalov & Ståhls, 1997; Bartsch, Binkiewicz, Klintbjer, et al., 2009; Bartsch, Binkiewicz, Rådén, & Nasibov, 2009; Bot & Van de Meutter, 2019; Bot & Van de Meutter, 2023; Claussen, 1998; Sack, 1930; Skevington et al., 2019) and complemented by own measurements (*Eumerus consimilis*, *Melanostoma* cf. *certum*, *Merodon cinereus*, *Platycheirus naso*, *Platycheirus tatricus*, *Sphaerophoria estebani*, *Sphaerophoria infuscata*, *Syrphocheilosia claviventris*) from which we took the median. Grasshopper body size was determined using means of female body length ranges, as given in Harz (1969) and Harz (1975). We calculated the community mean of body size per site and plotted size distributions along elevation for each group based on presence–absence data, thus not weighted by abundance within a site, as DNA metabarcoding does not provide reliable abundance estimates.

Vegetation assessment

To mirror the actual resource availability for pollinators, cover and richness of flowering plants were assessed in parallel to every transect walk or activation of the malaise traps, respectively. We randomly distributed ten 1-m^2 squares in each plot and recorded all vascular plant species flowering at the time of data collection. We averaged flower cover and summed up the species records of all squares from the three sequenced malaise trap sampling rounds to assess flowering plant species richness per site. During analysis, only flower richness turned out to have explanatory power in the models, so the flower cover was discarded. For grasshoppers, resource availability was measured as the number of all vascular plant species per site, combining species records of an early (June–July) and late season (August–October) vegetation assessment to cover the whole available species spectrum. Details are described in Kerner et al. (2023).

As a measure for primary productivity, we collected the aboveground plant biomass of four randomly distributed 0.25-m^2 squares per site, cutting all vascular plant material inside directly above the ground. At the sites with cattle, we took two samples from the grazed part and two from the fenced area around the malaise traps, whereby we only considered the latter for primary productivity. The two from the grazed part were used to determine effective biomass in pastures, which however showed no explanatory value and was thus discarded during analysis. All samples were dried at 81°C for 48 h and weighted afterwards to obtain final data [g/m^2], averaging the weight of all samples per site.

Temperature modelling

We modelled hourly temperatures for the year 2019 for every site based on recorded temperatures from 16 nearby climate stations (for locations, see Figure 1). The modelling process equals Kerner et al. (2023). In a nutshell, annual temperature represents the mean of the whole year, while sampling temperature of malaise traps includes the daytime average (9 AM–6 PM) of all days the trap was activated. For transect walk data, sampling temperature was defined as the mean of the next full hour after sampling started. All exact sampling times of malaise traps and transect walks are listed in Table S5. We averaged the sampling temperature of all three sample rounds per site, so that results do not reflect the temperature of the single sampling event per se but comprise the whole sampling at a site.

Statistical analyses

We performed all analyses using R V. 4.2.3 (R Core Team, 2023). To assure the representativity of the results from the selected groups for the whole pollinator community, we showed that the richness of all pollinators and only of the selected groups are highly correlated (Figure S2). Further, we used the package mgcv V. 1.8–42 (Wood, 2011) to calculate generalised additive models (GAMs) from the malaise trap data, one including all pollinator species and one for the selected groups to ensure that elevational patterns were similar (Figure S2).

To check whether sampling method efficiency—determined by the number of detected species—depends on the temperature regime (elevation as a surrogate for mean annual temperature (cor = −0.99) or temperature during sampling (malaise traps: cor = −0.79; transect walks: cor = −0.47; malaise traps-transect walks: cor = 0.36), respectively (Figure S3)), we fitted generalised additive mixed models (GAMMs) using the package mgcv V. 1.8–42 (Wood, 2011), including sampling method as a factor (malaise trap, transect walks), a smoothing function of elevation or sampling temperature respectively and a smoothing function of the interaction between the two, adding study site as a random term. Additionally, we included a smoothing function of elevation in the models for sampling temperature to account for the systematic climatic effect of the elevational gradient. We set the basis dimension k of the smoothing parameters to 4 to avoid overfitting.

We used the same model set-up replacing richness with the community mean of body size to evaluate whether there are systematic patterns of insect body size along elevation and/or sampling temperature and whether there is a systematic bias between sampling methods.

To check whether the two sampling methods detect the same ecological drivers, we calculated separate piecewise structural equation models (SEMs)—based on linear models—for richness data from malaise traps and transect walks, once for pollinators and once for grasshoppers, using the package piecewiseSEM V. 2.1.0 (Lefcheck, 2016). We included annual temperature, sampling temperature, primary productivity, management (binary, extensively managed yes/no) and resource availability in the form of flower richness (pollinators) or plant richness (grasshoppers) as potential drivers (hypothesized causal structure see Figure S4). Further, we repeated the SEM calculation for pollinator richness from transect walks, adding pollinator abundance to test its potential mediating role to better explain the impact of flowering plant richness as well as of annual and sampling temperature on pollinator richness.

RESULTS

Overall, we recorded 340 pollinator species: 173 hoverfly species, 102 wild bee species and 65 butterfly species. Species pools from both methods were similarly large (258 malaise trap (mt) vs. 242 transect walk (tw) species), with a species overlap of 47% (160 species, Figure 1, top left). However, shares differed between species groups (Figure S5). While more hoverfly species were present in malaise traps (140 (mt) vs. 95 (tw) species), butterflies (50 (mt) vs. 64 (tw) species) and wild bees (68 (mt) vs. 83 (tw) species) showed a higher species richness in transect walks. Further, we found a total of 37 grasshopper species, of which a considerably bigger share was detected by transect walks (24 (mt) vs. 36 (tw) species, Figure 1, top right). All recorded species are listed in Tables S6–S9.

Differing temperature sensitivity between sampling methods

The elevational pattern of pollinator species richness did not differ between survey methods (GAMM, interaction elevation: method $p \leq 0.01$, Table 1) but transect walks detected a higher species richness (GAMM, difference between methods $p \leq 0.001$, Table 1), especially in higher elevations (Figure 2a). When dividing the elevational gradient into three sections (sub-montane–montane (644–999 m), montane–high montane (1000–1499 m), sub-alpine–alpine (1500–2034 m)) and testing species richness per method against each other within sections, only the high elevations showed a method-specific difference (t-tests, sub-montane–montane: $t = -0.0637$, $df = 9.598$, $p = 0.9505$, montane–high montane: $t = -1.6059$, $df = 20.585$, $p = 0.1235$, sub-alpine–alpine: $t = -2.0806$, $df = 16.757$, $p = 0.0531$). Similarly, the elevational pattern of grasshopper richness did not differ between methods (GAMM, interaction elevation: method $p > 0.05$, Table 1), but more grasshopper species were recorded by transect walks (GAMM, difference between sampling methods $p \leq 0.001$, Table 1, Figure 2b).

In contrast to the elevational pattern, the pollinator response pattern to sampling temperature differed between methods (GAMM, interaction sampling temperature: method $p \leq 0.001$, Figure 2c, Table 1), showing a strong effect by sampling temperature in malaise traps but not in transect walks (linear models richness ~ sampling temperature, malaise traps: adj. $R^2 \approx 0.47$, $p \leq 0.001$, transect walks: adj. $R^2 \approx -0.02$, $p > 0.1$). The impact of sampling temperature on grasshopper richness did not differ between methods and was generally not significant (GAMM, interaction sampling temperature: method $p > 0.05$; sampling temperature $p > 0.05$, Figure 2d, Table 1).

Strong differences in elevational patterns and temperature sensitivity between single pollinator groups

The elevational pattern of hoverfly richness differed between methods (GAMM, interaction elevation: method $p \leq 0.001$, Table S10), with more species in malaise traps (GAMM, difference between methods $p \leq 0.001$, Table S10), especially in lower elevations (Figure S6). In contrast, wild bee richness was higher in transect walks along the whole elevational gradient (GAMM, difference between methods $p \leq 0.001$, Table S10) and generally decreased towards higher elevations (GAMM, elevation $p \leq 0.001$, Table S10). Butterflies showed no elevational pattern (GAMM, elevation $p > 0.05$, Table S10) but also a constantly higher species richness in transect walks (GAMM, difference between methods $p \leq 0.001$, Table S10).

TABLE 1 Summary statistics of generalised additive mixed models (GAMMs) evaluating pollinator and grasshopper richness regarding method-specific differences in elevational and sampling temperature patterns in the Berchtesgaden National Park.

Response variable	Predictor	Estimate	Std. error	Edf	Ref. df	*p*-value	Adj. R^2	Dev. Expl.
Pollinator richness	(Intercept)	3.575	0.047			<0.001 ***	0.538	73.9%
	Sampling method-transect walks	0.130	0.030			<0.001 ***		
	Elevation			1.798	1.881	0.009 **		
	Elevation: sampling method-transect walks			1.000	1.000	0.002 **		
	Study site (random term)			22.301	29.000	<0.001 ***		
Pollinator richness	(Intercept)	3.606	0.041			<0.001 ***	0.689	81.9%
	Sampling method-transect walks	0.036	0.034			0.283 n.s.		
	Sampling temperature			1.000	1.000	<0.001 ***		
	Sampling temperature: sampling method-transect walks			1.000	1.000	<0.001 ***		
	Elevation			1.793	1.906	0.233 n.s.		
	Study site (random term)			20.126	29.000	<0.001 ***		
Grasshopper richness	(Intercept)	1.734	0.066			<0.001 ***	0.842	80.3%
	Sampling method-transect walks	0.991	0.089			<0.001 ***		
	Elevation			1.733	2.046	<0.001 ***		
	Elevation: sampling method-transect walks			1.000	1.000	0.567 n.s.		
	Study site (random term)			4.192	29.000	0.22 n.s.		
Grasshopper richness	(Intercept)	1.688	0.073			<0.001 ***	0.848	81.8%
	Sampling method-transect walks	1.087	0.101			<0.001 ***		
	Sampling temperature			1.635	1.941	0.094		
	Sampling temperature: sampling method-transect walks			1.000	1.000	0.083		
	Elevation			1.432	1.682	<0.001 ***		
	Study site (random term)			4.167	29.000	0.205 n.s.		

Note: Each model was fitted with sampling method as a factor (malaise trap, transect walk), a smoothing function of elevation or sampling temperature, respectively, and a smoothing function of the interaction between sampling method and elevation or sampling temperature, respectively, including study site as a random term. Additionally, a smoothing function of elevation was added in the models for sampling temperature to account for the systematic climatic effect of the elevational gradient. For all models, the basis dimension k of the smoothing parameter was set to 4 to avoid overfitting ($N = 62$).
Signif. codes: 0 < = '***' < 0.001 < '**' < 0.01 < '*' < 0.05 < '.' < 0.1 < 'n.s.' < 1.

For both hoverflies and wild bees, the increase in species richness under higher sampling temperatures was method-dependent (GAMMs, interaction elevation: method $p \leq 0.001$ (hoverflies)/≤ 0.01 (wild bees), Table S10), with a stronger increase of richness in malaise traps (Figure S7). For butterfly richness, there was no effect of sampling temperature (GAMM, sampling temperature $p > 0.05$, Table S10).

Bee and butterfly body size differed systematically between methods

Hoverfly and wild bee communities contained more big species in higher elevations, but there was no effect of sampling temperature (GAMMs, elevation $p \leq 0.05$ (hoverflies)/$p \leq 0.001$ (wild bees)). Further, community mean body size of wild bees was higher in malaise traps than in transect walks (GAMM, difference between methods $p \leq 0.001$), due to less small species in malaise traps (Figure S10). Body size in butterfly communities was neither impacted by elevation nor sampling temperature, but malaise traps included less big species (GAMMs, difference between methods $p \leq 0.01$ (elevation), $p \leq 0.001$ (sampling temperature)) (Figure S10). For grasshoppers, the community mean of body size showed no pattern at all (GAMMs, elevation $p > 0.05$, difference between methods $p > 0.05$, sampling temperature $p > 0.05$, difference between methods $p > 0.05$). Detailed model results and figures are presented in the Supplementary Material (Table S11, Figures S8 and S9).

Congruent drivers of pollinator richness but not grasshopper richness across sampling methods

The best fitting path models of pollinator richness (malaise traps: Fisher's $C = 9.198$, $df = 8$, $p = 0.326$, $N = 31$; transect walks: Fisher's

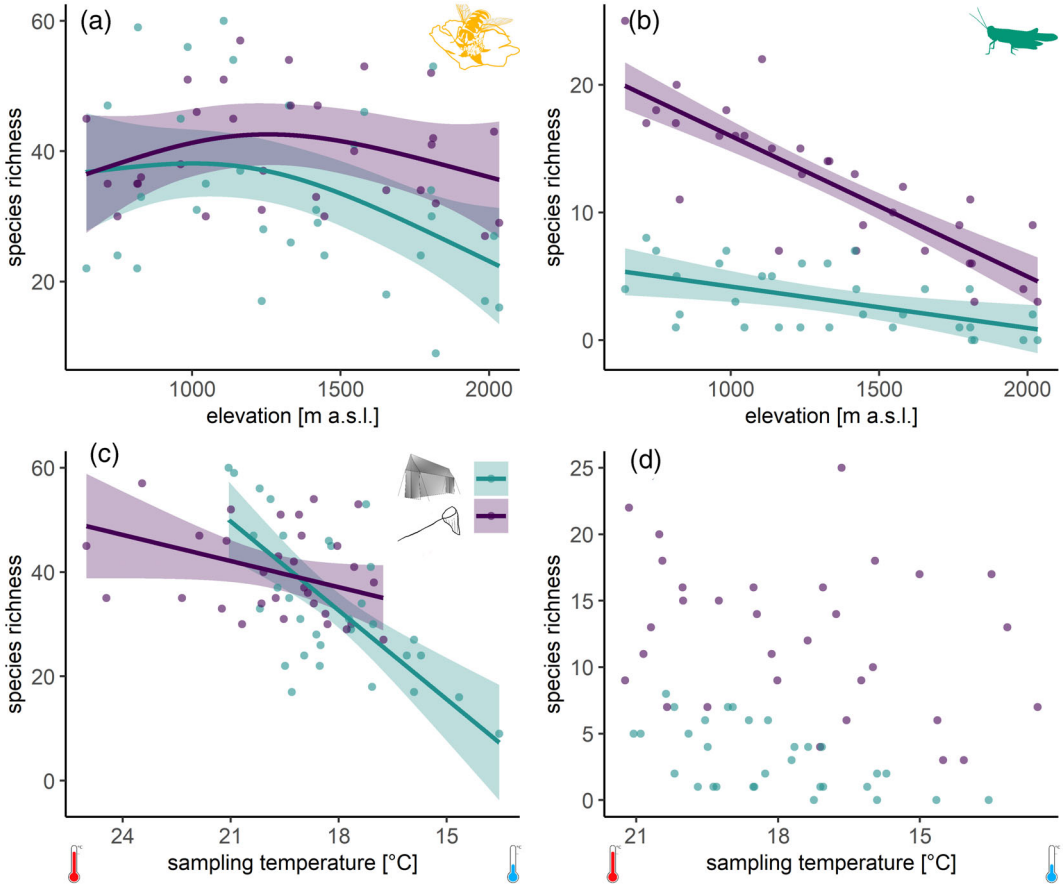

FIGURE 2 Patterns along elevation (top) and sampling temperature (bottom) of species richness of pollinators (left) and grasshoppers (right) assessed by malaise traps (blue, solid line) and transect walks (purple, dashed line) in the Berchtesgaden National Park. Sampling temperature is presented on a reversed scale (high → low) to match with the direction of the elevational gradient. (a) For pollinators, there was no significant difference in elevational patterns between methods but transect walks detected a higher species richness, especially in higher elevations. (c) Temperature sensitivity of pollinators differed significantly between methods, showing a strong effect of sampling temperature on malaise trap results but not transect walk data. (b) and (d) For grasshoppers, elevational patterns (b) did not differ significantly between methods and sampling temperature (d) showed no effect at all. However, in both cases, species richness from transect walks was significantly higher.

C = 6.354, df = 8, $p = 0.608$, N = 31) included the same explanatory variables for both methods: sampling temperature and flower richness (Figure 3a,b). Compared with the malaise trap model, the transect walk model explained a lower proportion of pollinator richness ($R^2 \approx 0.58$ (malaise traps)/0.27 (transect walks)). However, the explanatory value of transect walk data increased to ~0.44 when including abundance in the path model, showing that both the effect of sampling temperature and flower richness on pollinator richness are mediated by pollinator abundances (Figure 3c). Further, annual temperature, primary productivity and management presented indirect drivers of pollinator richness in all three models (malaise traps, transect walks without and with abundance), as they explained flower richness ($R^2 \approx 0.57$).

The best fitting path models of grasshopper richness (malaise traps: Fisher's $C = 1.048$, df = 2, $p = 0.592$, $N = 31$; transect walks: Fisher's $C = 2.003$, df = 6, $p = 0.919$, $N = 31$) identified different drivers of richness depending on the survey method without any impact by plant richness as a surrogate for resource availability (Figure 4). In malaise traps, we detected annual temperature and management as drivers of grasshopper richness, while annual temperature was the only driver in transect walks, with a higher explanatory value of grasshopper richness ($R^2 \approx 0.5$ (malaise traps)/0.69 (transect walks)).

DISCUSSION

Detected pollinator and grasshopper species richness differs between sampling methods

Transect walks and malaise traps detected similar total numbers of pollinator species in the investigated region. Nevertheless, transect walks detected on average a higher α-diversity, indicating that community species composition recorded by malaise traps differed more between sites than in transect walks, which was confirmed when looking at β-diversity (Figure S11). Malaise traps might have detected a smaller share of the species community per site and could possibly record a higher species richness per site when the sampling period

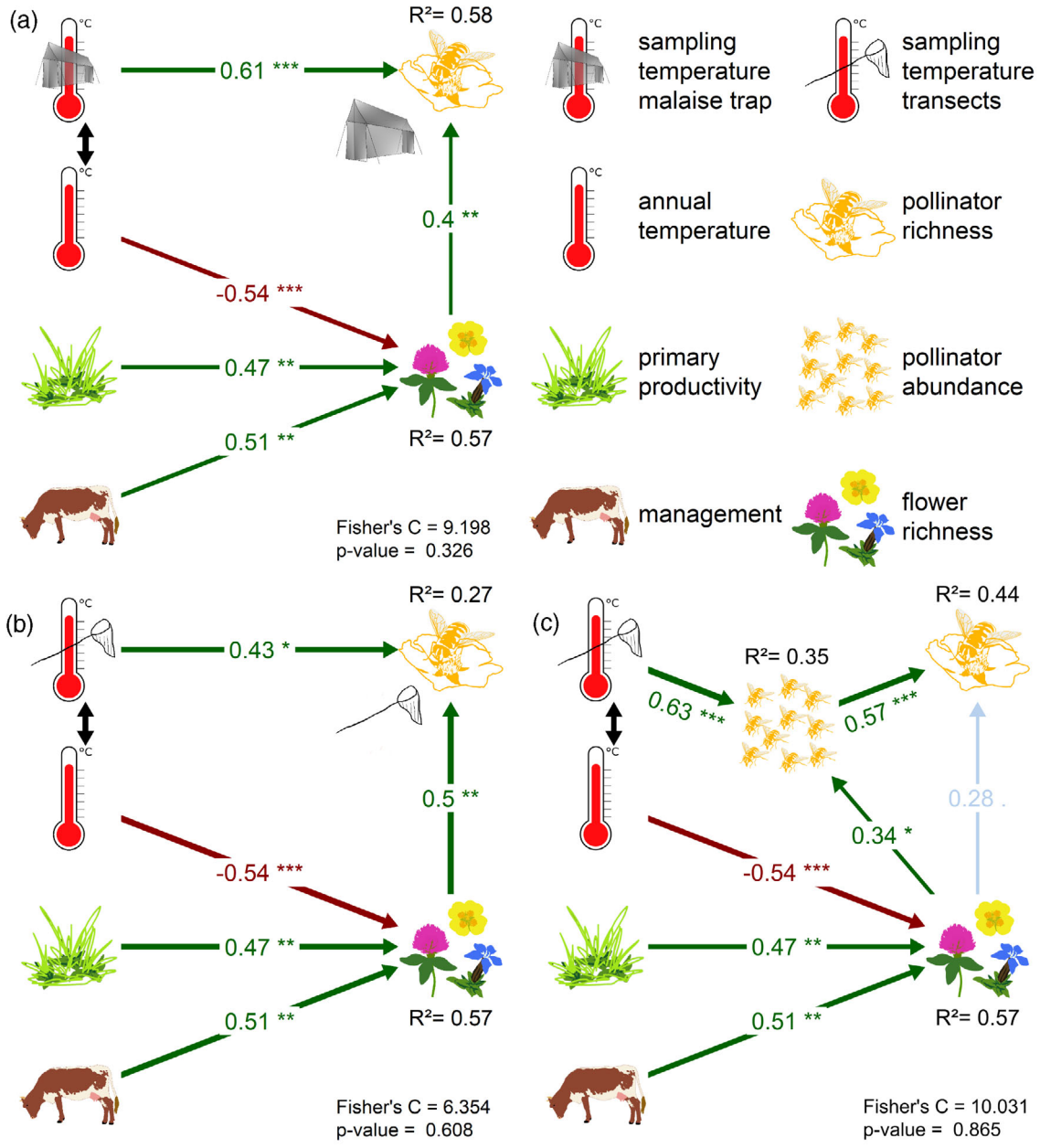

FIGURE 3 Best fitting path models (based on the Akaike information criterion with a correction for small sample sizes = AICc, $N = 31$) to explain pollinator richness assessed by different sampling methods in the Berchtesgaden National Park. Both pollinator richness from malaise traps (a) and transect walks (b) were explained by sampling temperature and flower richness with an indirect impact of annual temperature, primary productivity and management via flower richness. (c) When adding pollinator abundance to the transect walk model, the indirect effects stayed the same, but the direct effect of sampling temperature and partly also the one of flower richness were mediated by abundance, increasing the explanatory value for pollinator species richness. The standardised path coefficients, their statistical significance ($p \leq 0.1$ (.), $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***)) and the conditional coefficients of determination (R^2) are given. Paths with a significance of $p \leq 0.05$ are presented in green (positive correlation) or red (negative correlation) and higher values in grey. Annual temperature was log-transformed prior to analysis.

was prolonged from one week to longer sampling intervals. However, community composition in malaise traps was most heterogeneous in low elevations, so the strongest increase of species richness by longer sampling periods would be expected there, while the strongest difference in species richness between methods was observed in high elevations. Thus, while malaise traps might be able to detect even more species in the lowlands, the better performance of transect walks in detecting pollinator richness in high elevations seems to be persistent. Further, we found that this methodological difference was systematically biased, as small wild bee species were missing in mid- and high elevations in malaise traps (Figure S10), where these temperature-sensitive insects are less active and therefore get caught less, in contrast to the more cold-adapted hoverflies. Using DNA metabarcoding for species identification of malaise traps might have intensified that

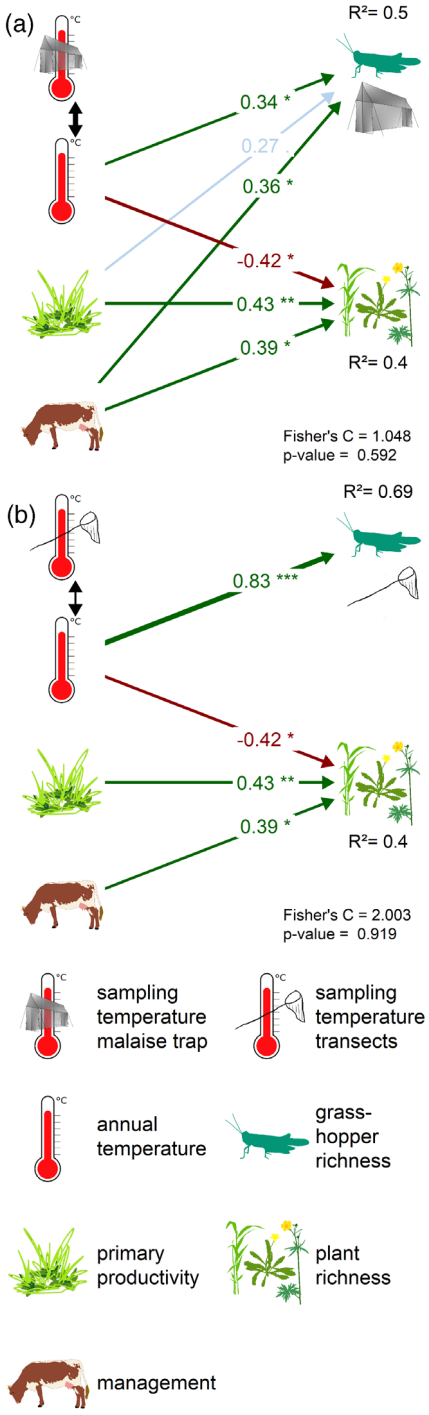

FIGURE 4 Best fitting path models (based on the Akaike information criterion with a correction for small sample sizes = AICc, N = 31) to explain grasshopper richness assessed by different sampling methods in the Berchtesgaden National Park. (a) While grasshopper richness from malaise traps was mainly explained by management and marginally by primary productivity and annual temperature, (b) richness assessed by transect walks was solely driven by annual temperature. In both cases, there was no relationship between plant richness and grasshopper richness. The standardised path coefficients, their statistical significance ($p \leq 0.1$ (.), $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***)) and the conditional coefficients of determination (R^2) are given. Paths with a significance of $p \leq 0.05$ are presented in green (positive correlation) or red (negative correlation) and higher values in grey. Annual temperature was log-transformed prior to analysis.

effect, as it was shown that bee and hoverfly species with low abundances are not always detected by this method (Remmel et al., 2024) and abundances are generally rather low in alpine environments. Additionally, big butterfly species were missing in malaise traps (Figure S10), probably due to the limited size of the trap entry potentially hindering very big species from entering. This might have also contributed to the weaker sampling performance of malaise traps in high elevations and under low temperatures, as those conditions favour the occurrence of bigger species (Leingärtner et al., 2014). However, malaise traps tend to sample species that are locally attracted or present due to small differences in microhabitats, while transect walks cover a wider area. Therefore, using several malaise traps per site might capture a wider range of species.

Grasshopper richness showed similar elevational patterns and no effect of sampling temperature in both methods, indicating that both seem to be able to depict elevational patterns of grasshopper richness similarly. Nevertheless, in line with previous assessments (Guevara & Avilés, 2009; Montgomery et al., 2021), malaise traps consistently detected a lower share of the species richness per site so that malaise trap trends already reached zero species at the highest and coolest sites. Therefore, the general usability of malaise traps for grasshopper assessments is limited to areas with higher grasshopper abundance, while observers can detect rare specimens during transect walks, taking advantage of direct search in suitable microhabitats and identification based on species-specific stridulation.

This illustrates that malaise traps—and likely also other passive sampling methods—depend much more on the activity of insects, which is reduced under low temperatures and strong wind in higher elevations (Mellanby, 1939), so sampling efficiency is highly dependent on suitable weather conditions. The underestimation of species richness via malaise traps is thus not equally distributed along climatic gradients but pronounced in cool environments, in line with the observed limited suitability of malaise traps for shaded, north-facing slopes (Ssymank et al., 2018). A transect walker is less dependent on insect activity and thus less biased by sampling temperature, as also resting individuals can be detected in the surrounding. However, in contrast to malaise traps, transect walk assessments might possibly be biased by the person collecting the data, so the observer should be keept in mind as a possible random factor, especially in long-term and large-scale datasets where it is likely that many different persons were involved in data collection.

It might appear as if in our study pollinator transect walks were conducted under more favourable conditions than the malaise trap sampling (Figure 2), biasing results in favour of transect walks. However, the differences in final average temperatures during sampling arise from different sampling durations. Malaise trap sampling started under the same conditions as transect walks on the same day but also includes conditions from subsequent days. Further, inherent to these differences in sampling lengths, malaise trap sampling temperature is more strongly correlated with annual temperature than transect walk samplings (Figure S3), which could also lead to a stronger impact of sampling temperature on malaise trap data. However, we included elevation as an explanatory variable in the sampling temperature

models to control for this and obtained similar results for pollinator richness between models including and excluding elevation.

Detected species richness and temperature sensitivity differ between pollinator groups

Method-dependent differences in recorded species pools, elevational patterns and temperature sensitivity varied between the included pollinator groups (Figures S5–S7), which should be taken into account when focusing on surveying a certain group of pollinators. In line with previous observations (Guevara & Avilés, 2009; Montgomery et al., 2021), the detectability of butterfly richness was generally better in transect walks and a higher total number of species was recorded, while a notable share of the species pool was missing in malaise traps. Nevertheless, butterfly richness showed similar, weak elevational patterns and temperature sensitivity in both methods, so they seemed to be equally suitable to assess temperature patterns. For wild bees, detectability of richness and total number of species were also considerably higher in transect walks, as expected (Montgomery et al., 2021). However, in contrast to butterflies, elevational patterns and temperature sensitivity differed significantly between methods, suggesting that transect walks performed particularly better in detecting wild bee richness in cooler or high-elevation environments respectively, and were less biased by sampling temperature. Yet, there was still an effect of sampling temperature in the transect walk data that should be kept in mind when analysing temperature effects. In contrast to the other two groups and in line with previous assessments (Guevara & Avilés, 2009; Montgomery et al., 2021), we found a higher total number of hoverfly species and a better detectability of richness in malaise traps, although not equally distributed along the elevational gradient but with a peak in mid-elevations (Figure S6). This hump-shaped pattern underlines the strong impact of sampling temperature on the detectability of hoverfly richness from malaise traps, as sampling temperature followed a similar pattern along elevation. In transect walks, this activity effect could be compensated (Figure S7), enabling to obtain less biased results although with possibly lower species richness in warmer or low-elevation environments, respectively.

That malaise traps performed generally better in trapping hoverflies than butterflies or bees could origin from differences in flight behaviour, assuming that adult butterflies and bees might navigate more specifically towards their floral resources, as trapping success of those groups by malaise traps could be improved by installing coloured patches on the middle wall of the trap (Campbell & Hanula, 2007), showing that there is potential to improve trapping success.

Temperature and resource availability are consistent joint drivers of pollinator richness, mediated by abundance, while grasshoppers show method-specific drivers

The main aim of biodiversity research is not only to assess species richness but also to define the major drivers of richness patterns to be able to derive effective measures for biodiversity conservation (Williams et al., 2020). Both our sampling methods congruently identified temperature and resource availability as joint drivers of pollinator richness, despite varying species compositions. We found that even with a lower sampling intensity and thus less impact on the studied ecosystem than most malaise trap studies (Ganuza et al., 2022; Uhler et al., 2021; Welti et al., 2022), consistent drivers of pollinator richness can be identified. That sampling temperature was also identified as a driver of pollinator richness in transect walks, although we found no correlation with sampling temperature in the previous analysis (Figure 2c, Table 1), might indicate that there is also an effect in transect walks after all, but considerably weaker than in malaise traps. It could also illustrate that it is necessary to account for food resource availability, which is also impacted by climate change and might show interacting effects with temperature changes that need to be disentangled (Rafferty, 2017).

Further, we found abundance to play a mediating role between the identified environmental drivers and pollinator species richness from transect walks (Maihoff et al., 2023; Prather et al., 2020; Sassi et al., 2012). We would expect equal results from malaise traps, but could not evaluate it, as no reliable abundance estimates can be derived from DNA metabarcoding data so far (Liu et al., 2020), so transect walks currently still allow a more detailed analysis in this regard. However, the recent development of an insect sample fractionizer for DNA metabarcoding samples (Hörren et al., 2022) opens the way for detection of abundance classes via metabarcoding of different subsamples.

Although it is known that malaise traps are not a very effective method to capture grasshoppers in comparison with transect walks or isolation quadrats (Gardiner et al., 2005; Montgomery et al., 2021), they appear as a study group in current malaise trap studies focusing on climate change (e.g., Uhler et al., 2021). Therefore, we also assessed the drivers of grasshopper richness and compared the results between methods. While transect walks reflect the overall pattern of species richness along the elevational gradient by showing annual temperature as the sole main driver, malaise trap data identified management as an additional driver of richness, indicating a strong influence on the results by the sampling method itself. Managed sites have a lower vegetation height, facilitating mobility and thereby an encounter of individuals with the trap so that more species might get caught than at an unmanaged site, although species richness was actually similarly high at both sites. Thus, when trying to identify drivers of grasshopper richness, conclusions drawn from malaise trap data are probably questionable.

Accuracy of species identification by DNA metabarcoding

When choosing DNA metabarcoding to analyse bulk samples instead of time-consuming manual sorting of specimens, it needs to be considered that this analysis method has its own pitfalls, affecting the obtained results. One important point to keep in mind when choosing to work with species identities and not BINs is that, although

taxonomic assignment to recorded DNA sequences of insects using BOLD is already well developed, there is still a loss of accuracy from the family level down to the species level (Hleap et al., 2021; Somervuo et al., 2017). Thus, there might be a share of species that was present in the sample but does not appear in the final species list and vice versa, that some species appear erroneously in the results without having been present (Förster et al., 2023), so final species lists from DNA metabarcoding need careful examination. As individual information is lost during the metabarcoding process and normally no manual sorting has been done previously, the accuracy of taxonomic assignments cannot be checked definitively but the plausibility of the results can be assessed, insofar that it can be reviewed whether detected species are likely to occur in the studied area. Checking our species identifications from metabarcoding by experts revealed relatively reliable species identifications for butterflies and hoverflies with only 4 out of 54 and 5 out of 145 species being very unlikely to occur in the research area (Table S3). Species identifications for wild bees were also highly plausible, that is, all listed species are likely to occur in the area (Table S3), equal to previous comparisons with barcoding results (Herrera-Mesías et al., 2022; Schmidt et al., 2015). However, the most abundant bee species in transect walks, the honey bee (*Apis mellifera*) and common carder bee (*Bombus pascuorum*), were missing from the barcoding results. As other bee species were detected, it did not seem to be a general problem of the bioinformatic pipeline. Nevertheless, there seems to be a weakness of the applied COI marker in the quality of Hymenoptera species identifications (Marquina et al., 2019). Further, while the selected primers are generally well suited to detect a high share of all the different species groups present in a bulk sample (Brandon-Mong et al., 2015), they potentially have a problem with unintended amplification, for example, of nuclear mitochondrial pseudogenes (*numts*) (Bensasson et al., 2001), which appear exceptionally often in honey bees (Pamilo et al., 2007). Additionally, among others, the frequent presence of *numts* in association with COI markers was also identified as an issue for species identification of grasshoppers (Hawlitschek et al., 2017; Song et al., 2008), of which 15 out of 39 identified species were not likely to occur in the study area, thus showing a considerably higher share of misidentifications than the considered pollinator groups (Table S3). Hence, while taxonomic identification of insects to the species level by DNA metabarcoding is already well developed to detect a considerable share of the contained species richness, it still shows weaknesses in accurately identifying some species or species groups, respectively. Therefore, DNA metabarcoding in its current state seems to be a good match for time- and resource-saving analysis of insect bulk samples when targeting richness patterns of insect communities as a whole but might have limited utility for some other research questions with a species-specific focus.

CONCLUSIONS

Both malaise traps with a short exposure time and transect walks seem to be generally suitable to monitor pollinator richness and analyse its ecological drivers. However, species pools differed between methods, so it could make sense to combine both monitoring methods if assessing the species pool as completely as possible is the main aim. In most settings, malaise traps present a more standardised and less time-consuming sampling approach for long-term and large-scale assessments. However, we found them to be more susceptible to changes in sampling temperature than transect walks and small bee as well as big butterfly species might be underrepresented, so the obtained data need to be handled and interpreted with care. Further, abundance had a mediating role between environmental impacts and pollinator species richness, making it a useful early indicator of long-term changes before they become visible in species richness. However, when choosing DNA metabarcoding to analyse species richness from malaise trap samples, this information is currently lost, so its preservation should be an aim in future method refinement. For grasshoppers, we found both methods to be able to depict richness patterns along the temperature gradient and have no effect on sampling temperature or body size. However, species richness in malaise traps was considerably lower and identified ecological drivers differed between methods, indicating a method-specific bias. Thus, transect walks appear to be the more suitable method for grasshopper monitoring, as a better coverage of the present species pool can be obtained and the species identification was more reliable. For all considered insect groups, species lists from DNA metabarcoding need careful examination, as species identifications might yet have limited validity, including false positives and false negatives, restricting the explanatory power of presence/absence analyses or conservation-relevant assessments.

AUTHOR CONTRIBUTIONS

Janika M. Kerner: Visualization; writing – original draft; conceptualization; investigation; validation. **Sebastian König:** Writing – review and editing; investigation; validation. **Fabienne Maihoff:** Writing – review and editing; investigation; validation. **Lukas Bofinger:** Investigation; writing – review and editing. **Nikki Sauer:** Investigation; writing – review and editing. **Axel Ssymank:** Writing – review and editing; validation. **Peter Väth:** Investigation; writing – review and editing. **Alice Classen:** Writing – review and editing; supervision; conceptualization; investigation; validation.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data and code supporting this study are openly available in Zenodo under https://zenodo.org/doi/10.5281/zenodo.10998249.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supporting Information.

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