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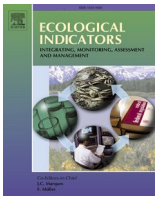

Evaluation of four different methods for assessing bee diversity as ecological indicators of agro-ecosystems

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ABSTRACT

Monitoring of wild bees is becoming more and more popular in nature conservation because of the high indicator value of this insect group. However, uncertainties about the sampling performance of different methods still exist, especially in areas of limited accessibility. We therefore compared four commonly applied sampling methods across vineyard fallows in a species diverse study area over two successive years: hand netting along variable transects (HN), pan traps (PT), trap nests (TN) and Malaise traps (MT). The chosen method significantly affects the number of sampled bee species and individuals, with PT sampling by far the largest number of species and individuals, and HN sampling the most diverse bee fauna. HN samples contained a significantly higher proportion of males, red-listed, large and social *Bombus* species than PT, but HN and PT samples contained a significantly lower proportion of male individuals compared to MT and TN. PT colour had a significant effect on the number of sampled individuals and species, with yellow PT sampling the largest numbers, while blue PT sampled the largest number of individuals of social *Bombus* species. The HN sampling results of an experienced and a less-experienced observer differed remarkably, with the turnover component of the Jaccard distance being significantly higher compared to the nestedness component. Our findings indicate that PT was the most efficient method for sampling bees in our study system. Due to species-specific differences in attractivity, sets of different PT colours should be used. However, if the study focus is on red-listed species or male individuals, HN represents a more efficient method. When HN is applied, observer bias should be considered as much as possible, especially with regard to differences in sampling experience. Due to different shortcomings, MT and TN cannot be seen as appropriate methods for standard monitoring of bees.

1. Introduction

A growing number of observations indicates a global decline of insect pollinator populations (Potts et al., 2010; Hallmann et al., 2017; Seibold et al., 2019; for review see Wagner, 2020), a fact that also applies to wild bees (Nieto et al., 2014; Vanbergen and The Insect Pollinators Initiative, 2013; Goulson et al., 2015). The greatest threat to European wild bee diversity comes from the intensification and expansion of agriculture (Nieto et al., 2014), and Rotherham (2015) suspects that the abandonment of traditional management forms poses the greatest threat to the conservation of natural resources. In this context, Agnoletti and Rotherham (2015) see the necessity to rethink current approaches and the need to revise current strategies to protect biodiversity. This is particularly important as the maintenance of ecological services in agricultural ecosystems, such as plant protection and pollination, depends on safeguarding biological diversity (Altieri, 1999). In agro-ecosystems, the conservation of the economically important wild bee diversity is safeguarded both by the preservation of high-quality habitats in the vicinity of cultivated areas and by the use of farming methods that compensate for the influence of intensive cultivation and monocultures (Kennedy et al., 2013). Consequently, wild bees are an important indicator group for the biodiversity in agro-ecosystems, and the acquisition of reliable information is crucial.

However, the analysis of the extent of population decline in species

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communities of wild bees requires monitoring over several years (Patiny et al., 2009; Lebuhn et al., 2013, 2015). In this context, the direct observation of biodiversity represent one research approach among several whose integration is necessary for predicting and managing climate-change-related consequences for biodiversity (Dawson et al., 2011). Thus, Westrich (1990) describes sighting as the method of choice for recording wild bees, as it includes the possibility of observing behaviour. However, the recording of wild bees in areas of difficult accessibility, like wine-growing areas, by hand netting is beset with difficulties. Thus, problems can be caused by the steep slope inclination, especially in connection with slippery substrate and partly dense growth of herbaceous and woody vegetation. A constant speed of the observer's movement cannot be guaranteed under these conditions so that sweep netting catches cannot be standardized. A high heterogeneity of the vegetation structure counteracts the comparability of catch results for sweep netting, as different vertical extensions of the flowering horizons lead to different proportions of individuals surveyed in a sampled population (Southwood and Henderson, 2000). A large number of targeted wild bees probably elude sightings because the observer cannot overcome the escape distance quickly enough. The number of wild bee individuals captured by sight catches on steep slopes is therefore probably significantly lower than in more easily accessible habitats with comparable individual density. In addition, any transect inspection on steep slopes, as e.g. in vineyards, is associated with a considerable risk of injury to the observer, which can be avoided by other methods.

For the reasons given above, it seems reasonable to evaluate alternative methods to hand netting, especially in preparation for long-term monitoring of wild bees in poorly accessible areas like vineyards. In addition to the hand netting method, various other methods are used to record pollinating insects (Dafni, 1992). In contrast to manual sampling methods, these are not carried out by the action of an observer, but by the bee individual to be sampled (Southwood and Henderson, 2000). These methods include pan traps (PT), Malaise traps (MT) and trap nests (TN), all of which are common methods for recording wild bees (Moericke, 1951; Aerts, 1960; Townes, 1962; Southwood and Henderson, 2000). In general, there is a need for knowledge regarding standardized and economic methods for monitoring pollinator insects (Dicks et al., 2013).

In this study, we therefore compared three different trap types (PT, MT and TN) against hand netting (HN) with regard to the wild bee communities recorded by these methods. Hereby, we first address the question whether the detection results differ between the methods. If so, we further investigate the degree of difference between the methods. Third, we evaluate which individual method is achieving the highest detection rates. Although several comparisons of wild bee assessment methods already exist (e.g. Westphal et al., 2008; Wilson et al., 2008; Nielsen et al., 2011; Grundel et al., 2011; Larsen et al., 2014; Gibbs et al., 2017; McCravy and Ruholl, 2017; Templ et al., 2019), none of them is comparing all these methods together in a similar standardised way and none of them addresses steep slope vineyards, which are regarded as hotspots of bee diversity (e.g. Krahner et al., 2018). With these results we therefore want to contribute to an evidence-based improvement of the monitoring concept for wild bees and thus to a better implementation of monitoring, especially in habitats with limited accessibility. The improvements in monitoring schemes are regarded as a new methodological step towards an optimised indicator system for management practices in steep-slope vineyards.

2. Materials and methods

2.1. Study area

The investigated area is located in the wine-growing area of the central Moselle Valley in the federal state of Rhineland-Palatinate, south-western Germany. The sampling sites are in the vicinity of the villages Kesten, Maring-Noviand and Bernkastel-Kues (municipality of Bernkastel-Kues, district of Bernkastel-Wittlich) and belong to the 'Osann-Veldenzer Umlaufberge' (LUWG, 2009). This region is geologically characterised by clay and siltstone with small intercalations of sandstone (LGB, 2003). The study sites extend over steep slopes used for viticulture, which consist of clay slates and greywacke and are overlaid by raw and skeleton soils of low thickness, and terraces, which are mainly composed of sandy and gravelly loams (MU & LUG, 1995). The vineyard management was mainly characterised by small-structured plots until the 1950s. As a consequence of land consolidation measures, vineyard management changed to larger plots, accompanied by a loss of biotopes for xerothermophile organisms. Today, lines of plants of vineyards on steep slopes are predominantly oriented parallel to the slope line.

2.2. Study organisms

The group of bees (Apiformes) is delimited as suggested by Michener (2007). All members of the Apiformes, excluding the honey bee (*Apis mellifera*), are hereafter called wild bees. The nomenclature of wild bee species is based on Westrich et al. (2011). A mention of the first describing authors is omitted, in favour of better readability. All individuals were determined to species level, unless necessary identification characters were missing or unrecognizable. Wild bee species which could not be reliably identified were merged into species groups (aggregates, agg. for short), which are treated as morphospecies in this paper: i.e. *Andrena ovatula*, *A. wilkella*, *A. intermedia*, *A. similis* and *A. gelriae* as the *Andrena ovatula* agg.; *Bombus cryptarum*, *B. lucorum*, *B. magnus* and *B. terrestris* as the *Bombus terrestris* agg.; *Bombus hortorum* and *B. ruderatus* as the *Bombus hortorum* agg.; *Lasioglossum nitidulum* and *L. smeathmanellum* as the *Lasioglossum smeathmanellum* agg.; *Halictus eurygnathus*, *H. langobardicus* and *H. simplex* as the *Halictus simplex* agg.

2.3. Sampling

Sampling was carried out from mid-April to early September 2013 and from early April to early October 2014 (Appendix: Table A1).

A total of four methods were applied for sampling: 1) hand netting along variable line transects (HN), 2) pan traps (PT), 3) trap nests (TN) and 4) Malaise traps (MT). HN took place under favourable weather conditions (no or little wind, cloud cover 50% or less, temperatures $\geq$15 °C), from 10 am to 5 pm, around midsummer from 9 am to 6 pm. Intervals between HN sampling events were irregular (Table A1), since unfavourable weather conditions prevented regular intervals. Within each sampling event, each transect was walked once. On average, about 13 transects were walked per day (about 15 in 2013 and 11 in 2014). The sampling sequence of transects was randomised for each sampling day so that the individual transects were sampled at different times of the day, with the restriction that sampling along shaded transects was avoided. A transect was 1 m wide; it was located within a delimited plot, which was walked as completely as possible while maintaining a minimum distance of 2 m from the edge of the plot. Transect walks were standardized to 10 min of sampling time per transect (excluding handling of caught individuals), and sampling motivation was to sample as many species and individuals as possible within the given time. In case of high bee abundances, for example on highly attractive flower patches, the sampling protocol prioritised the collection of different morphospecies over the collection of all sighted individuals. All transect walks were undertaken by the first author, except for some of those used to investigate the effect of sampling experience on HN samples (see below). All sampled individuals were killed with ethyl acetate and taken to the laboratory for species identification.

Pan traps (PT) were manufactured in modification of the trap type developed by Moericke (1951). A sampling unit consisted of three yellow bowls, each with a diameter of 30 cm (Rondo-Gelbfangschale, Temmen GmbH, Hattersheim), the inside of which had been painted with blue, white and yellow UV-reflecting paints after white priming

(Sparvar Leuchtfarbe, Spray-Color GmbH, Merzenich; item numbers 3107, 3108, 3104 and 1315, respectively). The three differently coloured PT were placed 5 m from each other, forming an equilateral triangle around the plot centre. This distance was chosen because the findings by Droege et al. (2010) indicate that PT positioned at the selected distance do not influence each other with respect to sampling results. Since bees seem to collect flower resources within horizontal strata (Chauvin and Roth, 1966; Levin and Kerster, 1973; Waddington, 1979; Gumbert and Kunze, 1999), PT were placed at the level of the surrounding flower horizon (Rühl, 1978; Pellmyr, 1989; Vega et al., 1990; Tuell and Isaacs, 2009). PT contained water with a small amount of detergent and remained in the field 2–4 days during each sampling event (Table A1).

The construction of trap nests (TN) largely corresponded to Steffan-Dewenter (1998) and consisted of 20 cm long sections of *Phragmites australis*, which were bundled into plastic tubes of 11 cm diameter and 20 cm length. The diameter of the reed stalks varied between 2 and 7 mm, the number of stalks was about 150 per plastic tube. A sampling unit consisted of two TN, which were attached to a wooden post at heights of 0.5 and 1.5 m, respectively, with one opening pointing southeast. The TN were exposed in the field from 12 or 13 April to 22 November 2013. After exposure, they were stored at outside temperature. Then, TN units from some plots were randomly selected, nest structures were dissected, and the corresponding individuals were isolated in hatching vials. The remaining TN units were incubated with trapping vessels at both openings. From February to October 2014, TN and hatching vials were stored in the shade and sheltered against rain at outside temperatures and were inspected daily for hatched animals. The imagines were treated similarly to the HN catches.

A total of three black Malaise traps (MT; bioform Dr. J. Schmidl e.K., Nuremberg; type “Bartak”, item number A84b), modified according to Townes (1962) and Townes (1972), were set up. MT sampled three days on most sampling events (Table A1). PT and MT were activated when the weather forecast predicted favourable conditions for the sampling period. The permission required for the collection of bees was granted by the responsible authority (Struktur- und Genehmigungsdirektion Nord, Koblenz).

2.4. Study design

We used an existing study design created for the investigation of different treatments for managing vineyard fallows on steep slopes (Krahner, 2017). A total of 7 vineyard fallows served as study areas (Fig. 1). Fallow areas were divided into four areas of equal size, on which two experimental treatments were carried out according to a 2*2 full factorial experimental plan. Treatments consisted of sowing an

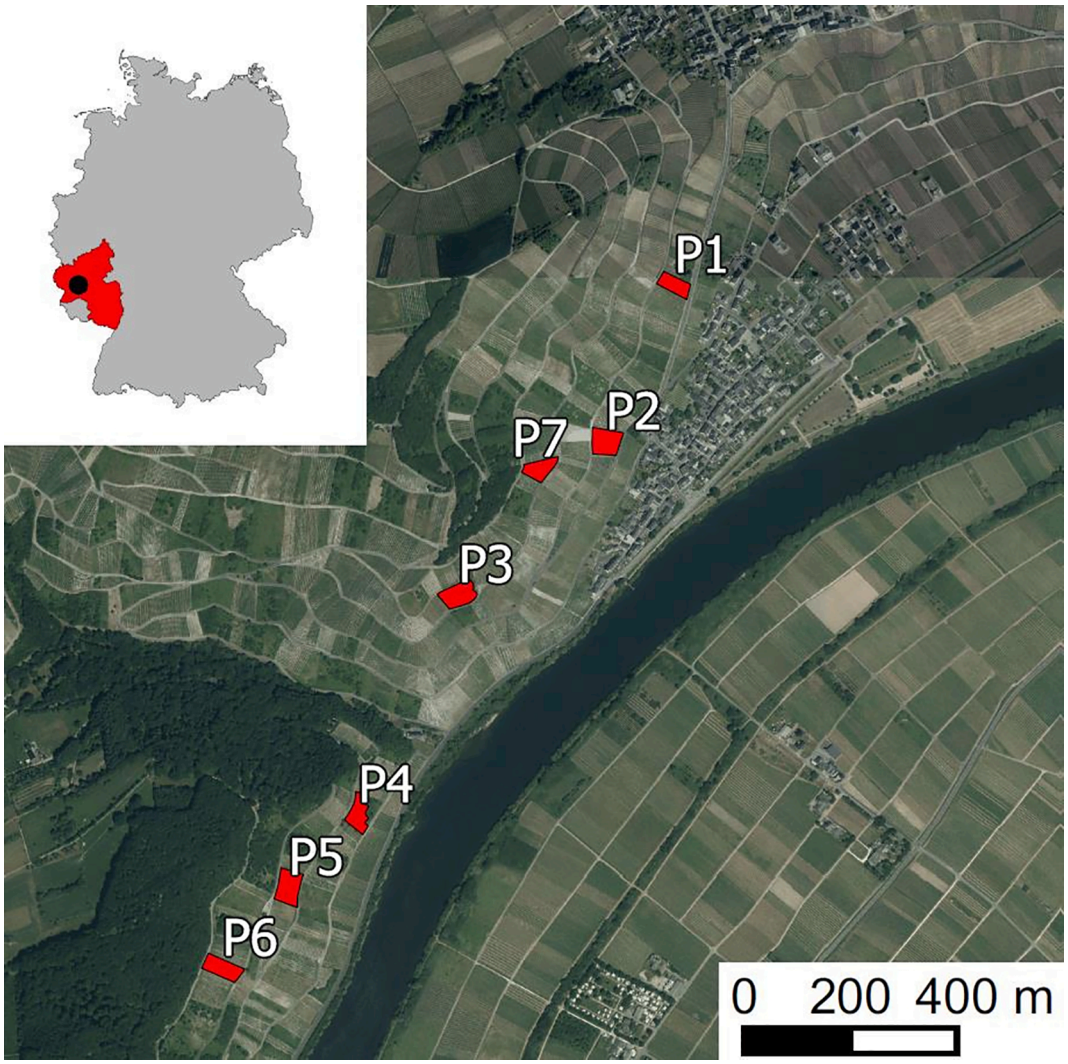

Fig. 1. Location of sampled vineyard fallows (P1-P7), inset graphic showing the location of the study site (black dot) within Rhineland-Palatinate (red) and Germany (grey). Aerial photographs: LVermGeo (2016). Geodata for Germany and Rhineland-Palatinate: modified from Hijmans et al. (2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

autochthonous wild herb mixture and yearly mulching in autumn; one quarter of each vineyard fallow was not treated. Plot 7 (P7) remained entirely untreated and served as control.

Each plot within the treated vineyard fallows and the control plot P7 served as an individual variable transect (resulting in a total of 25 variable transects) and were equipped with PT and TN (one sampling unit each) in the centre of each plot. P2 and P7 were additionally equipped with two and one MT, respectively. In P2, MT were installed within the sown and unsown area. The number of plots and the number of sampling events (Table A1) resulted in a total of 225 transect walks on these plots, while the number of PT sampling units and the number of sampling days resulted in 1950 trap days. For the MT, the number of traps and the sum of days in operation (Table A1) result in a total of 78 trap days.

2.5. Statistical analysis

To compare different sampling methods, we used only data from study plots on which all respective methods were applied. Compared to the other methods, a much lower number of MT were replicated. Therefore, a separate dataset was created for comparison with MT. Further, only samples taken in the same time period were used for comparisons (same sampling event; Table A1). Since the total sampling period and the set of applied methods differed between years, data were analysed separately for each study year. Data were collected on six vineyard fallows and comprise PT and HN samples from 2013 and 2014 and TN samples from 2013 (P1-P6; Fig. 1). For comparison between MT and the other methods, data gathered from two vineyard fallows in 2013 and 2014 were used (P2 and P7, Fig. 1).

Statistical analyses were performed in the R environment (R Core Team, 2014). To assess the increase in sampled species with increasing spatial sampling intensity, rarefaction curves based on cross-section samples were calculated (Krauss et al., 2003; Westphal et al., 2008), using EstimateS (Colwell, 2013). Four cross-section samples were created for each method by pooling the sampling units for each variant plot over all six vineyard fallows, hence minimizing the impact of seasonal species turnover (Westphal et al., 2008), over all sampling events in one year. MT were excluded from this analysis due to the lower replication compared to the other methods.

For assessing the efficiency of different methods (ratio of sampled species to sampled individuals), individual-based rarefaction and extrapolation curves (Colwell et al., 2012) and asymptotic estimators for species number (Chao, 1984, 1987) were calculated with the R package iNEXT (Hsieh et al., 2015). Rarefaction was used to compare the methods by correlating the sampled species number with a certain number of sampled individuals, i.e. the minimum number of individuals sampled over all methods. Asymptotic estimators for species numbers were used to compare the potential number of sampled species in a scenario of maximised sampling intensity.

To investigate the effect of temporal sampling intensity on PT sampling results, two datasets per year were generated and compared using rarefaction curves based on cross-section samples: 1) the complete dataset, containing all monthly sampling events (May–September 2013; April–July 2014); and 2) a reduced dataset containing only two sampling events in May and July of each year.

To assess the sampling effectiveness of the different methods, the composition of the sampled fauna was compared using the R-package 'iNEXT' (Hsieh et al., 2015). Exponential Shannon diversity was calculated (Colwell et al., 2012; Chao et al., 2014) based on the same number of sampled individuals. An asymptotic estimator of Shannon diversity was calculated (Chao et al., 2013), in order to compare the methods in a scenario of maximised sampling intensity.

The proportion of individuals belonging to certain groups and the total number of individuals sampled with one method was compared with the other methods using Fisher's exact test, in order to detect whether these groups were differently represented in the sampling results yielded by the different methods. Likewise, the proportions of certain groups within the sampling results from different PT colours were compared. Analysed groups included: small species according to Amiet (1996), Amiet et al. (1999), Amiet et al. (2001), Amiet et al. (2004), Amiet et al. (2007) and Amiet et al. (2010), i.e. each analysed dataset was split into small and large individuals so that the number of species per group was as equal as possible, the resulting cut-off value defines species with female body length < 9 mm as small species; parasitic species according to Westrich (1990); Red-Listed species (Westrich et al., 2011); and male individuals. Social non-parasitic bumblebees (*Bombus* spp.) were excluded from group analyses to avoid bias due to their high numbers that would dominate all other taxa (Hirsch, 2003). Species pooled to aggregations due to determination problems (see above) were only considered in these analyses if all species within one aggregation were assigned to the same group.

Fisher's exact test was also used to determine whether there was a difference between the various sampling methods and between the PT colours for species that were frequently detected (at least 20 individuals per year). This subjective frequency criterion was chosen according to Rühl (1978), who applied this criterion in the comparison of different pan trapping methods. Fisher's exact tests were performed using the R package 'RVAideMemoire' (Hervé, 2018), applying p value correction for multiple testing (Benjamini and Hochberg, 1995).

To investigate the effect of sampling experience on HN, rarefaction curves based on cross-section samples were compared. These were calculated from HN sampling results obtained by two investigators with different experience among three full cycles of transect walk within comparable time periods in 2014 (1st cycle: 13 April to 4 May and 13 April to 5 May, respectively; 2nd cycle: 2 to 7 July and 14 to 23 July, respectively; 3rd cycle: 2 to 12 September and 3 September to 3 October). The first author ("experienced investigator") had already more than two years of field experience in sampling bees, while the second investigator ("inexperienced") had no prior experience at the beginning of the sampling season.

For each vineyard fallow, Jaccard distances for all pairings of faunas sampled through HN by investigators differing in HN experience were calculated, and the means and standard deviations over all vineyard fallows were calculated. Jaccard distances were partitioned into their nestedness and turnover components (Baselga, 2012) to assess their impact on Jaccard distances, using the R-package 'betapart' (Baselga and Orme, 2012). For comparison of the turnover and nestedness component, the Wilcoxon-signed-rank test for paired samples was used.

The effect of the methods HN, PT and TN on the numbers of sampled individual and species was analysed with Poisson-GLMM (log link; function 'glmer' in package 'lme4'; Bates et al., 2015). Full models included two random effects: vineyard fallow to account for spatial correlation of neighbouring transects/sampling units; and one observational level random effect to account for overdispersion (Anderson et al., 2013). Fixed effects included sampling method, treatment variant and the interaction between treatment variants. Using likelihood ratio tests (LRT), non-significant fixed effect terms were successively removed from the model (backwards selection; Sydenham et al., 2016).

3. Results

The comparison between PT, TN and HN is based on a total of 10,330 individuals of 134 species. The dataset for the comparison of MT with the other methods comprises a total of 2225 individuals representing 99 wild bee species. A significant effect of the method on the number of species and individuals is observed in both study years (Fig. 2, Table 1, Table A2). PT sampled by far the largest number of individuals and species, including unique species sampled by only this method, in both study years; HN sampled the second largest number of species; TN the lowest (Fig. 2, Table 1). The difference between HN and TN is less pronounced with regard to the number of individuals sampled, but overall the fewest individuals were sampled by HN (Fig. 2A, Table 1). HN sampled a more diverse fauna compared to the other methods

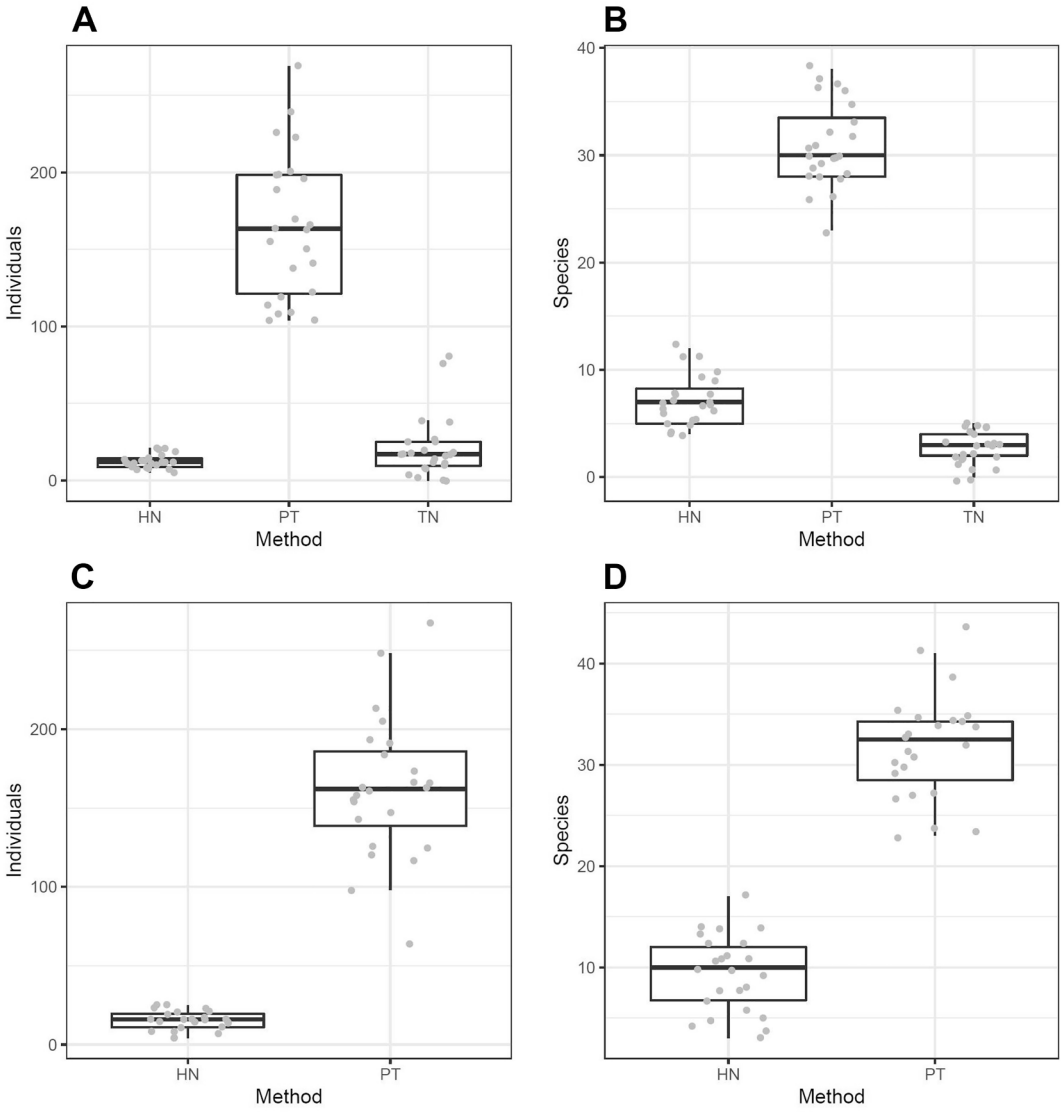

Fig. 2. Sampling results of different methods used on six vineyard fallows, each separated into 4 sampling plots, in 2013 (A, B) and 2014 (C, D). Dots represent results for each sampling unit (dots jittered along both axes for improved readability). HN = hand netting, PT = pan traps, TN = trap nests.

(Shannon H'; Table 1). MT resulted in fewer species than PT, but more species compared to the other methods, this also applied to the number of unique species sampled by only one method (Table 2). MT also ranked behind PT and before HN in terms of the number of individuals sampled, while there were no marked differences between MT and TN in this regard. Shannon diversity of the fauna sampled by MT was less diverse compared to the other methods, except for TN.

3.1. Rarefaction and extrapolation

With regard to sample based rarefaction curves, PT sampled 100 [95% confidence interval: 95.2; 105.8] species in 2013 and 77 [72.9; 81.1] species in 2014 (Fig. 3), corresponding to 88.5% [84.3%; 92.7%] and 78.8% [74.6%; 82.9%] of all recorded species per study year, respectively. Reducing the number of PT to one set per vineyard fallow (by sample-based rarefaction) results in a sampling coverage of over 50% of all recorded species in both study years, i.e. 69 [62.1; 76.0] species in 2013 and 55 [49.3; 60.7] species in 2014. For HN, the sampling coverage is 38.1% [33.8%; 42.3%] of all species sampled in 2013 and 48.7% [42.7%; 54.6%] of all species sampled in 2014, while sampling coverage for the TN is 8.0% [4.8%; 11.1%] in 2013. Reduction in temporal sampling intensity for PT resulted in a sampling coverage of 63.7% [58.5%; 69.0%] and 68.1% [64.5%; 71.8%] of all sampled species in 2013 and 2014, respectively. A combined reduction in temporal sampling intensity and spatial sampling intensity (one PT set per vineyard fallow) led to a sample coverage of 37.4% [31.9%; 42.9%] and 48.7% [43.7%; 53.7%] of all species sampled in 2013 and 2014, respectively (Fig. 3).

A similar ranking of the methods was observed with regard to species richness from individual-based rarefaction and extrapolation (Table 1 and 2; Appendix A, Fig. A1-A2). The highest asymptotic species-richness estimator is calculated for PT, followed by HN, while the values for TN were considerably lower (Table 1). MT has a higher asymptotic species-richness estimator than TN, but does not differ markedly from the other methods (Table 2).

3.2. Composition of the sampled fauna

In both years, HN samples contained a significantly higher proportion of males and red-listed species as well as a higher proportion of individuals belonging to the social *Bombus* spp. and belonging to the larger-sized species, compared to PT (Fig. 4). In 2014, HN samples also contained a significantly higher proportion of individuals belonging to parasitic species compared to PT. TN sampled a significantly higher

Table 1
Sampling results, inter- and extrapolated estimators for species richness and diversity (Shannon H′, exponential form) of the fauna sampled by hand netting (HN), pan traps (PT) and trap nests (TN; 95% confidence intervals in square brackets; different letters mark significant differences between methods in one year based on non-overlapping confidence intervals; α = 0.05).

Observation

Year	Method	Individuals	Species	Unique species[1]	Shannon H'
2013	HN	310	53	6	20.2
	PT	4088	108	54	18.1
	TN	532	12	–	6.3
2014	HN	404	65	8	33.8
	PT	4996	109	49	15.9

Interpolation (individual-based rarefaction)

Year	Method	Individuals	Species		Shannon H'	
2013	HN	310	53.0	[47.3; 58.7][a]	20.2	[17.3; 23.1][a]
	PT	310	46.7	[45.3; 48.2][a]	15.9	[15.1; 16.6][b]
	TN	310	11.8	[11.2; 12.4][b]	6.2	[5.8; 6.7][c]
2014	HN	404	65.0	[59.4;70.6][a]	33.8	[29.8; 37.7][a]
	PT	404	51.9	[50.1; 53.7][b]	14.3	[13.7; 14.8][b]

Extrapolation (asymptotic estimators)

Year	Method	Species		Shannon H'	
2013	HN	73.0	[60.0; 110.0][a]	23.1	[20.2; 26.7][a]
	PT	128.8	[115.9; 162.7][b]	18.4	[18.1; 19.4][b]
	TN	12.0	[12.0; 12.8][c]	6.4	[6.3; 6.9][c]
2014	HN	81.9	[71.0; 113.0][a]	37.8	[33.8; 42.3][a]
	PT	135.2	[118.7; 180.0][b]	16.1	[15.9; 16.9][b]

[1] Species sampled by only one method in one year.

proportion of male individuals than HN and PT, and a significantly higher proportion of larger-sized, parasitic and red-listed species compared to PT. Furthermore, the proportion of individuals of red-listed species sampled by TN was significantly lower compared to HN. In both years, MT samples contained a significantly lower proportion of *Bombus* individuals and a significantly higher proportion of male individuals compared to HN and PT. Only in 2014, MT samples contained a significantly higher proportion of small species and a significantly lower proportion of parasitic species compared to HN and PT. Furthermore, MT samples contained a significantly higher proportion of small species and a significantly lower proportion of parasitic and red-listed species compared to TN.

Nineteen of the frequently sampled species showed a consistently significant difference in the proportion of individuals, at least for one pairing of methods (Table A3). There was a set of species over-represented in the TN samples compared to one or more other methods, consisting of species belonging to the genera *Coelioxys*, *Hylaeus*, *Megachile* and *Osmia*.

With regard to the PT colours, there was a significant difference in sampled individuals in 2013 and 2014 (Table A4), yellow PT sampled the highest number of individuals and white PT sampled the lowest number. Yellow PT contained a significantly lower proportion of social *Bombus* ssp. and a significantly higher proportion of red-listed species compared to blue PT in both years (Fig. 5). Moreover, white PT samples contained a lower proportion of social *Bombus* ssp. and a higher number of red-listed species compared to blue PT in both years. Only in 2013, white PT samples contained a significantly higher proportion of social *Bombus* spp. and a significantly lower proportion of red-listed species compared to yellow PT samples, while blue PT samples contained a

Table 2
Sampling results, inter- and extrapolated estimators for species richness and diversity (Shannon H′, exponential form) of the fauna sampled by Malaise traps (MT), hand netting (HN), pan traps (PT) and trap nests (TN) (95% confidence intervals in square brackets; * significant differences between MT and the marked method in one year based on non-overlapping confidence intervals; α = 0.05).

Observation

Year	Method	Individuals	Species	Unique species[1]	Shannon H'
2013	**MT**	**111**	**31**	**5**	**10.8**
	HN	61	21	2	13.2
	PT	660	63	29	17.9
	TN	103	7	1	5.4
2014	**MT**	**343**	**39**	**8**	**5.2**
	HN	79	26	2	17.0
	PT	868	72	35	21.3

Interpolation (individual-based rarefaction)

Year	Method	Individuals	Species		Shannon H'	
2013	**MT**	**61**	**21.2**	**[17.6; 24.8]**	**9.3**	**[6.6; 11.9]**
	HN	61	21.0	[16.2; 25.8]	13.2	[9.2; 17.3]
	PT	61	21.2	[19.7; 22.7]	12.5	[11.3; 13.6]
	TN	61	6.6	[5.8; 7.3]*	5.3	[4.8; 5.8]*
2014	**MT**	**79**	**17.0**	**[14.2; 19.8]**	**4.3**	**[3.4; 5.1]**
	HN	79	26.0	[22.0; 30.0]*	17.0	[13.2; 20.8]*
	PT	79	26.9	[25.5; 28.4]*	15.0	[13.7; 16.4]*

Extrapolation (asymptotic estimators)

Year	Method	Species		Shannon H'	
2013	**MT**	**66.8**	**[42.0; 147.6]**	**14.7**	**[10.8; 20.2]**
	HN	40.8	[25.6; 106.8]	18.6	[13.2; 25.5]
	PT	92.3	[73.5; 145.3]	19.3	[17.9; 21.5]
	TN	7.0	[7.0; 8.4]*	5.6	[5.4; 6.3]*
2014	**MT**	**59.2**	**[45.5; 101.7]**	**5.7**	**[5.2; 6.8]**
	HN	31.5	[27.3; 48.8]	21.3	[17.0; 26.4]*
	PT	80.0	[74.5; 98.0]	22.4	[21.3; 24.4]*

[1] Species sampled by only one method in one year.

significantly higher proportion of parasitic species compared to yellow PT samples only in 2014.

Fiveteen frequently sampled species showed a consistently significant difference in the proportion of individuals, at least for one pairing of PT colours (Table A5). Among these, 8 species showed a preference towards yellow-coloured PT.

3.3. Hand netting expertise

Based on sample-based rarefaction curves, hand netting resulted in a lower number of sampled species for the inexperienced compared to the expert observer, irrespective of spatial sampling intensity (Fig. 6). The rarefaction curve for the inexperienced observer reached an asymptote, unlike the one for the experienced observer. Moreover, the turnover component of the Jaccard distance was significantly higher compared to the nestedness component.

4. Discussion

In general, comparisons between studies are complicated by differences in the studied habitats and the local bee fauna, as well as

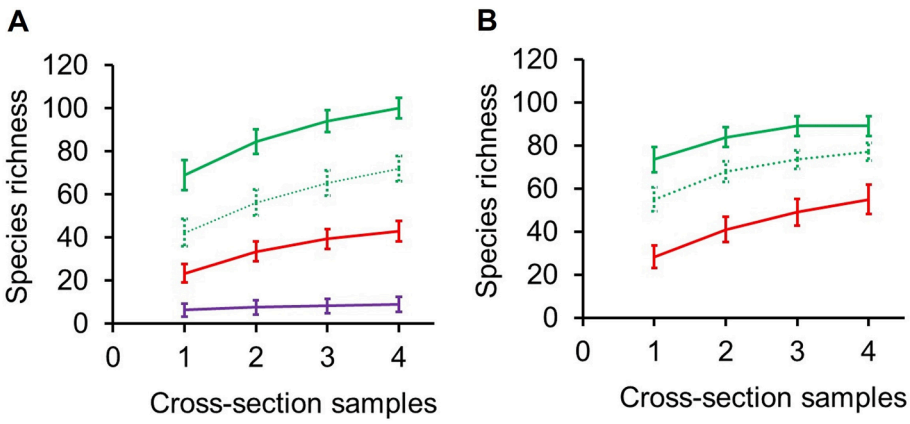

Fig. 3. Rarefaction curves based on cross-section samples in 2013 (A) and 2014 (B). ■ Hand netting (HN); ■ pan trap (PT); ■ trap nest (TN). Error bars represent 95% confidence intervals. One cross-section sample represents species richness obtained from PT, TN and HN in one treatment variant across all experimental study plots and season. For PT, two different scenarios for temporal sampling effort are shown (continuous line: monthly sampling from May-September (A) and from April-July (B), respectively; dotted line: reduced sampling effort, i.e. one sampling event in May and July each year). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

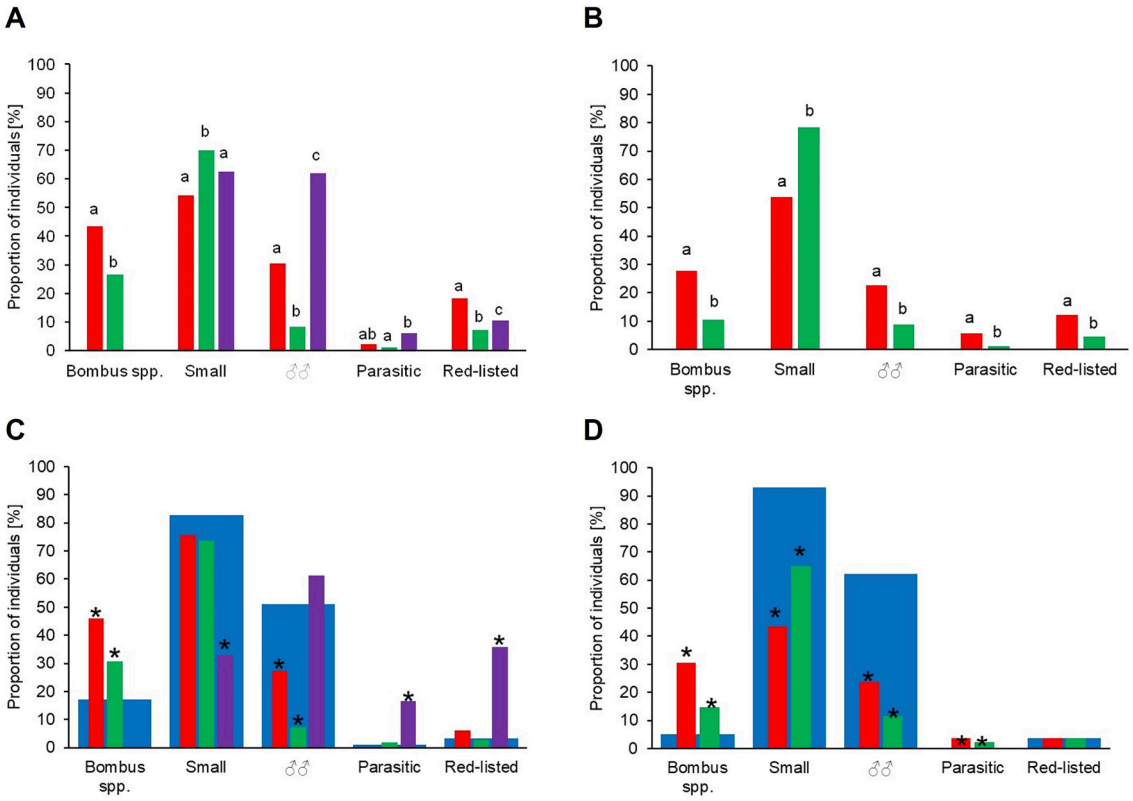

Fig. 4. Proportion of individuals sampled in 2013 (A, C) and 2014 (B, D) belonging to social *Bombus* and other bee species; for the latter, the following is analysed: proportion of small (body length < 9 mm), parasitic and red-listed (Westrich et al., 2011) species as well as male individuals; ■ hand netting; ■ pan trap; ■ trap nest; ■ Malaise trap. A-B: Comparison of hand netting, pan trap and trap nest; different letters mark significant differences between methods (Fisher's exact test, $p \leq 0.05$). C-D: Comparison of Malaise traps and other sampling methods; * = significant differences between Malaise traps and respective method (Fisher's exact test, $p \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

differences in the sampling protocols underlying these studies. Therefore, disentangling these effects from each other and from principle effects of a certain method on the sampling results is often difficult, hampering general conclusions independent from the study system and the specific monitoring protocol. The general discussion of the performance of different sampling methods has to be regarded indicative at most. An assessment of the sampling strategy, focused on the research question and the specific study system, is highly suggested and has been recommended repeatedly before (e.g. Cane et al., 2000; Westphal et al., 2008).

The recording method had a significant effect on the number of sampled individuals and species of wild bees, with PT being the most effective method in terms of observed species richness and asymptotic estimators for species richness. While Westphal et al. (2008) also observed the highest number of sampled species in PT, they sometimes found a relatively higher sample coverage for HN compared to the present work. This might be due to the better accessibility of the sites studied by Westphal et al. (2008) compared to the steep slopes in the present study. Moreover, PT used by Westphal et al. (2008) were much smaller compared to the present study, and smaller PT collect less bee specimens (Wilson et al., 2016). Cane et al. (2000) also sampled a higher number of bee species by HN compared to PT. However, Westphal et al.

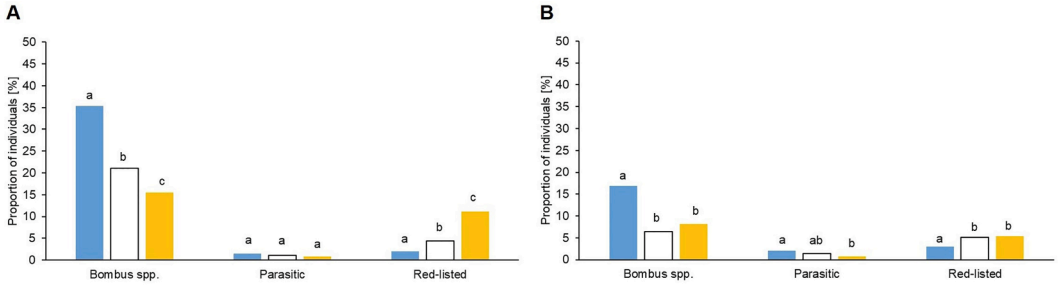

Fig. 5. Proportion of individuals sampled with pan traps in 2013 (A) and 2014 (B). Shown are individuals belonging to social *Bombus* and other bee species, for the latter, only parasitic and red-listed (Westrich et al., 2011) species are presented. Colours represent the different pan trap colours, different letters mark significant differences between colours (Fisher's exact test, $p \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

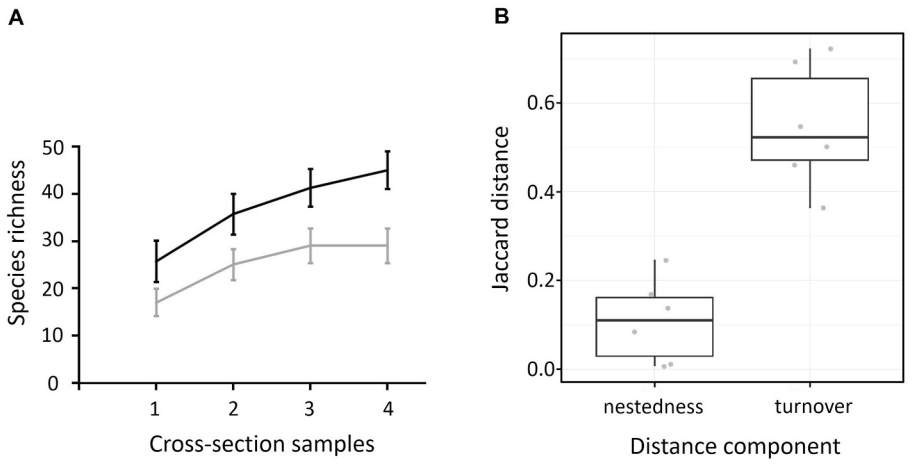

Fig. 6. Hand-netting sampling results for two differently experienced observers. A: Rarefaction curves based on cross-section samples; ■ experienced observer; ■ inexperienced observer. Error bars represent 95% confidence intervals. B: Nestedness and turnover component of the Jaccard distance between sampled faunas, differing significantly (Wilcoxon signed-rank test for paired samples, $n = 6$, $P \leq 0.05$).

(2008) argue that these findings might have resulted from placing the PT below the level of floral resources and from the great expertise in the HN observers with regard to the habitat type analysed. Roulston et al. (2007) also sampled fewer species with PT compared to HN, but it is argued that this resulted from a too short exposure of pan traps, i.e. for only 9 h (Westphal et al., 2008). In another study, PT sampled more bee morphospecies than sweep-netting (Spafford and Lortie, 2013).

In our approaches, the fewest species were recorded by TN, supporting the results of Westphal et al. (2008). Similar findings were also made by Rubene et al. (2015), who sampled more Aculeata and pollen-collecting species of wild bees with PT than with TN. The number of species sampled by TN in our study is similar to that obtained by Steffan-Dewenter (1998) in calcareous grasslands in Germany over several years. The number of species detected by TN is limited by its specific design, which offers a suitable nesting substrate for only a subset of above-ground nesting wild bee species.

There is a tendency towards higher rarefied species richness for HN compared to PT, supporting the findings by Grundel et al. (2011). However, as the difference in rarefied species richness between HN and PT is small, there is no strong reason, based on our results, to prefer one method over the other. Ethical reasoning might favour HN, because this only samples the target organisms without producing by-catch. However, the conclusion by Gezon et al. (2015) that continued PT has no long-term impact on population development, supports the contention that this method is appropriate for long-term monitoring of wild bees.

With regard to the diversity of the sampled fauna, higher values are observed for HN compared to PT and TN. Comparing HN and PT, this result is in contrast to the sampled species richness, which could be due to a bias toward rare species in HN. This is especially the case during mass occurrences of certain bee species (Schmid-Egger, 1995), such as social *Bombus* spp., because the HN objective is to sample as many different species as possible. In general, HN should have a negative bias towards smaller species and quick flyers as these are more difficult to sample as also revealed by our data.

Rarefaction curves based on cross-section samples for HN and PT are largely in parallel, indicating that the increase in spatial sampling intensity leads to a relatively similar increase in sampled species richness in both methods. Westphal et al. (2008) obtained similar results for PT and HN along variable transects, but studying more accessible habitats than in our study. Even with a reduced number of only two sampling events per year, PT sampled considerably more species compared to the high sampling frequency approach with HN. Consequently, PT should be preferred over HN when field work resources are limited and the objective is to sample the bee fauna at a study site as completely as possible, even if HN is done by experienced surveyors. Similar to our data, the sample-based rarefaction curve for TN obtained by Westphal et al. (2008) is almost flat, indicating that the increase in the sampled species richness obtained by increasing spatial sampling intensity is rather limited for this sampling method.

For HN, Banaszak et al. (2014) detected a maximum in sampling coverage (in a much smaller study area, but applying a temporally much denser design) at sampling intervals of 7–10 days. However, the method comparisons from our study are likely to hold also at higher sampling intervals, because sampling success will probably increase to the same extent in all methods. A similar increase for PT and HN is likely also for our data because of the almost parallel rarefaction curves obtained for

these two methods.

As expected due to their size, conspicuousness and short escape distance, the proportion of social bumblebee species sampled was higher in HN samples than in PT and MT samples. Likewise, small species not belonging to the social *Bombus* species are relatively underrepresented in HN compared to PT samples. This result is also reflected in the lower proportions of individuals of the large species *Andrena dorsata*, *Bombus rupestris*, *Colletes similis* in PT, and in the higher proportions of the small species *Lasioglossum morio* and *L. smeathmanellum* agg. These findings match observations by Toler et al. (2005) and Templ et al. (2019) comparing PT and HN. They might be (at least partly) explained by the better visibility of larger species making them easier to catch, but also by a higher escape rate of such species from PT. However, successful escapes from PT were never observed in our study. Although PT collected lower numbers of social *Bombus* species than HN, the proportion of individuals sampled is not nearly as low as observed for *Bombus* species by Droege et al. (2010) in the US, which might be explained by differences in PT design and the faunal context. The relatively large diameter of PT used in this study compared to other studies (Gonzalez et al., 2020) might reduce the escape rate even for larger individuals. Further investigations with regard to the effect of PT size on the sampling result could clarify this issue, considering that Gonzalez et al. (2020) compared PT sizes only at the lower end of the applied spectrum (i.e. between 4 and 12 cm in diameter).

At least in one year, the proportion of individuals belonging to large species is lower in MT compared to PT and HN. Likewise, MT were negatively associated with body length and escapes from MT have been considered as an explanation (McGravy et al., 2019) as well as one reason for the low number of sampled bee individuals in MT (Campbell and Hanula, 2007).

Parasitic species are sampled in higher proportions by TN compared to MT and PT. However, this result is mainly driven by a single species, *Coelioxys inermis*. HN tended to sample relatively more individuals of parasitic species compared to PT, which could be due to the lacking pollen collection in these species, resulting in a lower attractiveness of flower-mimicking PT, but no such negative bias by HN. In addition, HN might result in relatively high individual numbers of parasitic species, because HN by experienced collectors usually includes an active search for parasitic species, which are often seen on the ground searching for the nests of their hosts.

By design, TN samples reflect the true proportion of male individuals. While the proportion of males in MT samples was not significantly different from TN, PT and HN sampled comparatively lower proportions, with PT samples containing the lowest of all methods. As for the parasitic species compared to nest building species, males could be attracted to PT less than females, due to lacking pollen collecting behaviour of the former.

HN sampled more individuals of red-listed species compared to PT and TN, the latter however sampling more such individuals than PT and MT. For TN, this proportion is mainly driven by a single species, *Megachile centuncularis*, but not so for the other methods. These results let us argue that, if the research focus is set on the red-listed species at a study site, HN outperforms TN and PT and should be the method of choice.

Matching the results by Leong and Thorp (1999) and Grundel et al. (2011), in our study PT colours differed in the number of sampled individuals, and several wild bee species also showed a clear colour preference. Overall, most of the frequently sampled species in our study showed a preference for yellow-coloured PT compared to one or both other colours tested. In contrast to our results, Stephen and Rao (2005) observed higher sampling rates for blue compared to yellow traps, but using a different trap design (vane traps) and investigating a fundamentally different bee fauna. Also Campbell and Hanula (2007), using non-UV-reflecting PT, sampled more halictid individuals in blue PT compared to white and yellow PT. They considered the proximity of the wavelengths reflected by the blue PT to the UV spectrum to be a potential explanation. Therefore, differences in UV reflectance might explain these differing findings.

As expected, the more experienced surveyor sampled more species by HN compared to the less experienced. However, the higher turnover component compared to the nestedness component of the Jaccard distance indicates that both surveyors sampled particular species lacking in the samples collected by the other surveyor. Thus, it seems that sampling experience can increase the number of species sampled by the surveyor through HN, but that a high degree of sampling bias remains using this method, despite growing sampling experience.

5. Conclusion

A comparison of the four investigated sampling methods for wild bees in steep-slope vineyards revealed striking differences between them. Pan traps (PT) and hand netting (HN) are particularly suitable for a comprehensive recording of the species. In both cases, a high proportion of the existing wild bee species is reliably recorded. PT proved to be particularly suitable if the greatest possible comparability of the samples is to be achieved, because in this case differences in the sample locations (e.g. due to different vegetation, terrain conditions) and differences in the experience of the observer do not have a major impact. However, it must be ensured that the traps are exposed for a sufficiently long time ($\geq$2 days), are sufficiently large (>12 cm diameter) and are optimally placed in the habitat (i.e. at the level of the flower horizon). The colour of the traps is also important; three colours (yellow, white, blue) are best used together, but alone yellow is most efficient; UV reflecting colours should be used. HN is particularly suitable where species numbers are to be maximised, especially with regard to rare species and parasitic taxa; there is also no by-catch, so this method has the least negative impact on populations. However, of all the methods, HN is most dependent on the experience of the observer, and the labour input is high. Malaise traps (MT) sampled fewer species than the previous two methods. Due to the extremely high by-catch of MT, they are not very practicable in terms of work load and are also regarded by conservation experts as the most problematic of the methods tested. Trap nests (TN) only record a specific segment of the species specialised on these nesting conditions, which is why they are the most incomplete method of recording species communities and are therefore only suitable for specific research questions.

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CRediT authorship contribution statement

André Krahner: Conceptualization, Methodology, Formal analysis, Investigation, Interpretation and Discussion of results, Resources, Writing - original draft, Writing - review & editing, Project administration. **Juliane Schmidt:** Writing - original draft, Writing - review & editing, Project administration. **Michael Maixner:** Conceptualization, Supervision, Funding acquisition, Writing - review & editing. **Matthias Porten:** Conceptualization, Interpretation and Discussion of results, Funding acquisition. **Thomas Schmitt:** Conceptualization, Funding acquisition, Writing - review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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