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MEASURING BEE DIVERSITY IN DIFFERENT EUROPEAN HABITATS AND BIOGEOGRAPHICAL REGIONS

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Abstract. Bee pollinators are currently recorded with many different sampling methods. However, the relative performances of these methods have not been systematically evaluated and compared. In response to the strong need to record ongoing shifts in pollinator diversity and abundance, global and regional pollinator initiatives must adopt standardized sampling protocols when developing large-scale and long-term monitoring schemes.

We systematically evaluated the performance of six sampling methods (observation plots, pan traps, standardized and variable transect walks, trap nests with reed internodes or paper tubes) that are commonly used across a wide range of geographical regions in Europe and in two habitat types (agricultural and seminatural). We focused on bees since they represent the most important pollinator group worldwide. Several characteristics of the methods were considered in order to evaluate their performance in assessing bee diversity: sample coverage, observed species richness, species richness estimators, collector biases (identified by subunit-based rarefaction curves), species composition of the samples, and the indication of overall bee species richness (estimated from combined total samples).

The most efficient method in all geographical regions, in both the agricultural and seminatural habitats, was the pan trap method. It had the highest sample coverage, collected the highest number of species, showed negligible collector bias, detected similar species as the transect methods, and was the best indicator of overall bee species richness. The transect methods were also relatively efficient, but they had a significant collector bias. The observation plots showed poor performance. As trap nests are restricted to cavity-nesting bee species, they had a naturally low sample coverage. However, both trap nest types detected additional species that were not recorded by any of the other methods.

For large-scale and long-term monitoring schemes with surveyors with different experience levels, we recommend pan traps as the most efficient, unbiased, and cost-effective method for sampling bee diversity. Trap nests with reed internodes could be used as a complementary sampling method to maximize the numbers of collected species. Transect walks are the principal method for detailed studies focusing on plant–pollinator associations. Moreover, they can be used in monitoring schemes after training the surveyors to standardize their collection skills.

Key words: Abundance-based Coverage Estimator (ACE); agricultural and seminatural habitats; Hymenoptera, Apiformes; indicator method; pan traps; pollinator initiatives; standardized monitoring schemes; subunit-based rarefaction curve; transect walks; trap nests; unbalanced data.

Introduction

Pollination is an important ecosystem service (Daily 1997), which is essential for the production of entomophilous crops (Free 1993, Delaplane and Mayer 2000, Klein et al. 2007) and the conservation of biodiversity, as pollinators ensure the reproduction of many wild plants (Neff and Simpson 1993, Allen-Wardell et al. 1998, Fontaine et al. 2006). Over the last decade increasing concern has been raised about the declines and losses of pollinators and the deterioration of the ecosystem service they provide (Jennersten 1988, Kearns et al. 1998, Cunningham 2000, Donaldson et al. 2002, Committee on the Status of Pollinators in North America 2007). However, direct evidence for perceived

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ongoing declines of pollinators is scarce (Ghazoul 2005, Steffan-Dewenter et al. 2005; but see Williams 1982) and difficult to measure, as comparable records over long time spans are needed (Committee on the Status of Pollinators in North America 2007). Currently, most entomological records are sparsely scattered and idiosyncratically collected, allowing analyses only in exceptionally well-studied areas. Thus, even in well-studied countries such as the United Kingdom and The Netherlands, evidence for pollinator declines (Biesmeijer et al. 2006) must rely on the slow accumulation of haphazard records.

In this study, we focused on bee pollinators (Hymenoptera: Apiformes) since they represent the most important pollinator groups worldwide (Neff and Simpson 1993, Kearns et al. 1998, Michener 2000). Bees occur in a wide range of biogeographical regions and habitat types, where both sufficient floral resources and suitable nesting sites and materials are available (Michener 2000). Hence, in structurally complex and flower-rich seminatural habitats the most diverse bee communities can be found (e.g., Wcislo and Cane 1996, Steffan-Dewenter et al. 2002, Potts et al. 2003, Grixti and Packer 2006). In agricultural landscapes, seminatural habitats are often small and fragmented, so that bees have to travel between their nesting sites and different foraging habitats (Westrich 1996). In such landscapes, mass-flowering entomophilous crops may represent highly rewarding additional foraging habitats that are, however, only temporarily available (Banaszak 1996, Westphal et al. 2003, 2006*b*). Solitary bees have shorter life cycles than social bee species. For this reason, solitary species can only benefit from flowering crops when the bloom falls into their active period.

Given the great importance of pollinators and the accumulating evidence for declines, the International Initiative for the Conservation and Sustainable Use of Pollinators was established within the framework of the Convention on Biological Diversity (*available online*).[11] A primary objective of this global initiative is the establishment of systematic and long-term pollinator monitoring in order to identify relationships between changes in pollinator diversity and abundance and the putative causes of these changes (São Paulo Declaration on Pollinators 1999). The global pollinator initiative is complemented by several regional initiatives that also prioritize long-term and large-scale monitoring schemes to quantify pollinator loss (see Ghazoul [2005] for an overview). However, changes in pollinator assemblages can only be identified efficiently if pollinator abundance and diversity are recorded with standardized, replicated, and repeated sampling protocols, allowing the direct comparison of records across space and time (Williams et al. 2001, Committee on the Status of Pollinators in North America 2007).

[11] ⟨http://www.biodiv.org/programmes/areas/agro/pollinators.asp⟩

Historically, several different sampling methods have been used to assess pollinator diversity and abundance (Kearns and Inouye 1993, Sutherland 1996, Southwood and Henderson 2000, Dafni et al. 2005), among which census methods (e.g., transect walks or observation plots) are the ones most often employed (e.g., Banaszak 1980, Steffan-Dewenter et al. 2002, Cane et al. 2006, Westphal et al. 2006*a*). Commonly used passive sampling methods are pan traps (water traps; e.g., Aizen and Feinsinger 1994, Cane et al. 2000, Thomas 2005) and trap nests (e.g., Frankie et al. 1998, Steffan-Dewenter 2003, Tylianakis et al. 2005, Buschini 2006). Census methods are relatively time consuming and require experienced surveyors, but have the benefit that floral associations can also be investigated (Cane 2001). In contrast, passive sampling methods are generally less time consuming to implement, but may be subject to taxonomic biases. For instance, pan traps preferentially catch small-bodied bees and may under sample larger bee species (Cane et al. 2000, Cane 2001, Roulston et al. 2007), and trap nests are naturally limited to cavity-nesting bee species (Tscharntke et al. 1998, Dafni et al. 2005).

Although a scientifically sound evaluation of sampling methods is the first crucial step towards standardized long-term pollinator monitoring, the performance of different sampling methods has not been systematically tested and evaluated across a wide range of habitats and geographical regions. Detailed knowledge of the performance of different sampling methods will also allow more profound comparisons between historical observations and current surveys. As one main objective of the European Union's Sixth Framework Integrated Project ALARM (Assessing large-scale environmental risks for biodiversity with tested methods; Settele et al. 2005; *available online*),[12] we evaluated the performance of six commonly used sampling methods with respect to their efficiency in assessing bee diversity. The methods were tested at a continental scale in two different habitat types in each of five countries that represented different biogeographical regions. The following three questions, which are relevant for designing long-term monitoring schemes for pollinators, were addressed. (1) Do the tested methods differ in their efficiency (i.e., sample coverage) in detecting bee species richness? (2) Is the efficiency of the tested methods in detecting bee species richness affected by collector biases? (3) Are there differences in the species composition of the samples taken with the tested methods? Since long-term monitoring schemes are often limited by the availability of manpower and financial resources, we also aimed at the identification of the methods that would be most effective for detecting a defined proportion of pollinator communities and thereby providing an efficient and cheap indicator tool to assess overall bee diversity.

[12] ⟨http://www.alarmproject.net⟩

FIG. 1. Overview of the study regions (UK, United Kingdom; F, France; D, Germany; PL, Poland; and S, Sweden). The coordinates of the study sites are listed in Appendix A: Table A1.

METHODS

Study regions and sites

The study was conducted in five European countries representing different biogeographical regions in Sweden, Poland, the United Kingdom, Germany, and France (Fig. 1, Appendix A). We selected a total of eight study sites per country, which represented two contrasting habitat types. Four study sites in each country represented intensively managed agricultural habitats with mass-flowering bee-visited annual crops, and the other four study sites represented seminatural habitats with low-level agricultural management. We chose crops and seminatural habitats that were characteristic for the respective countries to allow the evaluation of the efficiency of the methods across a wide range of different habitat types (Table 1). In two of the countries only one of the two habitat types was included (seminatural in Sweden and agricultural in France). Thus, in total, the methods were tested in four sets of agricultural fields with three different entomophilous crops (Klein et al. 2007), and in four sets of flower-rich seminatural habitats that represent important nesting and foraging habitats for pollinators and included some Special Areas of Conservation (SACs; European Commission 2003).

Sampling methods and experimental design

We analyzed the performance of six commonly used sampling methods: (1) observation plots, (2) standardized transect walks, (3) variable transect walks, (4) pan traps, (5) trap nests with reed internodes, and (6) trap nests with paper tubes of different sizes. The methods were tested within a highly standardized experimental setup in the center of the study sites covering locally typical vegetation (Fig. 2). The sampling took place during suitable weather conditions for pollinators (minimum of 15°C, low wind, no rain, and dry vegetation) from mid-April to September 2004 (see Appendix B for exact dates). The time of the transect walks and plot observations was varied in consecutive surveys to account for diurnal patterns of bee activity.

In each study site, 10 rectangular quadrats of 1×2 m were established as observation plots to analyze the value of small-scale observations for predicting pollinator diversity in larger areas (Banaszak 1980). During 6-min observational periods, all flower-visiting bees were recorded. Species that could not be identified in the field were caught for identification. Ten sets of observations were made throughout the main flowering period in the seminatural habitats (exceptions listed at the end of this section). Because of the short flowering period of annual crops, only four recordings were performed in the agricultural habitats.

For the standardized transect walks, a permanently marked corridor was established on the study sites (Dafni et al. 2005). The 250 m long $\times$ 4 m wide transect was divided into 10 equal subunits (Fig. 2). The bees within the corridor were collected for each subunit separately during a 5-min walk covering the length of the subunit (i.e., 50-min recording time for the whole standardized transect). Species that could not be identified in the field were kept for identification later. Individuals that could be identified were released. The likelihood of counting released individuals twice was relatively small because the collectors were moving in one direction and the released individuals tended to fly out of the transect area (presumably to escape or for orientation). Ten standardized transect walks were conducted in the seminatural habitats (exceptions explained below) and four were conducted in the

TABLE 1. Countries and habitat types that were selected for the method testing.

Study region within country	Agricultural habitats	Seminatural habitats
Uppsala, Uppland, Sweden		semidry pasture
Krakow, Małopolska, Poland	*Fagopyrum esculentum* (buckwheat)	wet meadow
Reading, England, United Kingdom	*Brassica napus* (oilseed rape)	chalk grassland
Göttingen, Lower Saxony, Germany	*Brassica napus* (oilseed rape)	calcareous grassland
Avignon, Provence, France	*Cucumis melo* (cantaloupe)	

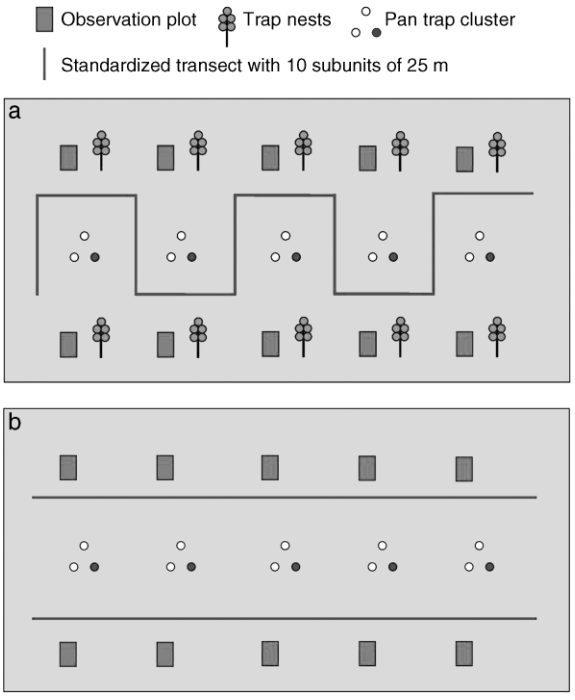

FIG. 2. Standardized experimental setup for the method testing in (a) seminatural habitats and (b) agricultural habitats. The 1-ha plot for the variable transects in the seminatural habitats is not shown. Each pan trap cluster contained one UV-bright blue, one yellow, and one white pan. A color version of this figure is available in Appendix G.

agricultural habitats. The transect walks effectively covered the main flowering period in both habitat types.

As bee faunas and floral resources are highly variable in space and time (Michener 2000, Williams et al. 2001), a fixed transect corridor might not represent the full temporal and spatial foraging and nesting area of a study site, and so capture only a small fraction of the entire pollinator community. Direct searching along variable transect corridors, which cover the most attractive resource patches, might minimize this problem (Sutherland 1996, Dafni et al. 2005). For the variable transect walks, a 1-ha plot was established at the seminatural study sites adjacent to the standard plots. Within this plot the surveyors were not restricted to a fixed transect line; instead they walked at slow speed among any potentially attractive resource patches and collected bees during an observational period of 30 minutes. Owing to the much more homogeneous distribution of flowers in mass-flowering annual crops, variable transect walks were not performed in the agricultural habitats. Ten variable transect walks were carried out per seminatural site (exceptions explained below).

Pan traps are a common passive sampling method for bees (Kearns and Inouye 1993, Southwood and Henderson 2000, Dafni et al. 2005; see also the sampling protocol in The Bee Inventory Plot, *available online*).[13] We set up yellow, white, and blue pan traps, which represented prevailing floral colors in our study regions, to account for different color preferences of bee species (Kirk 1984, Leong and Thorp 1999, Toler et al. 2005, Campbell et al. 2007). Since pan traps have a higher efficiency when they are UV-bright (Stephen and Rao 2005, Droege 2006), we painted 500-mL plastic soup bowls (Pro-Pac, Vechta, Germany) with UV-bright yellow, white, and blue paint (Sparvar Leuchtfarbe, Spray-Color GmbH, Merzenich, Germany). Fifteen pan traps were established in five clusters (each containing one of the three colors at a distance of five meters) at each study site (Fig. 2, Plate 1). The clusters were separated by 15 meters. The pan traps were mounted on a wooden pole at vegetation height, filled with 400 mL of water and a drop of detergent, and left active for 48 hours. During the season, six surveys were conducted at regular intervals (21–28 days) in the seminatural habitats and three surveys were conducted at regular intervals (7–10 days) in the agricultural habitats, because of the short flowering period of mass-flowering crops. The collected specimens were temporarily stored in 70% ethanol until pinned for identification.

A commonly used method to sample cavity-nesting bees is the introduction of artificial nesting substrates known as trap nests (Krombein 1967, Dafni et al. 2005, Cane et al. 2007). Although cavity-nesting bees represent only a minor fraction of bee communities, trap nests have been shown to be a good indicator of bee diversity (Tscharntke et al. 1998, Gathmann and Tscharntke 1999). Ten poles with trap nests were established in the seminatural habitats to investigate whether the overall bee species richness was related to the number of trap-nesting species (Fig. 2, Plate 1). We set up two different types of trap nests: (1) traps with common reed (*Phragmites australis*) internodes (~150 stems per trap) with diameters between 2 mm and 10 mm (Gathmann et al. 1994) and 15–20 cm in length; and (2) trap nests filled with paper tubes. Each pole carried two trap nests with reed internodes and three paper tube nests filled with tubes of distinct diameters (6.5, 8, and 10 mm, respectively), which were provided by the Oxford Bee Company (CJ WildBird Foods, Shrewsbury, UK). Trap nests were not established in agricultural habitats due to the frequent disturbances in crops (i.e., applications of fertilizers or pesticides and harvesting activities). The trap nests were exposed in the field from early spring to autumn. After collection from the field, the reed internodes and paper tubes with occupied nests were set aside. These tubes were dissected and the cocoons containing larvae were reared after diapause (minimum of three months at 4°C). The hatched adult bees were pinned and identified to species.

Because of unusually adverse weather conditions during the flowering period of red clover (*Trifolium*

[13] ⟨http://online.sfsu.edu/~beeplot/⟩

PLATE 1. Experimental setup on a calcareous grassland in Germany (Huhnsberg, Scheden). The UV-bright pan traps (shown in the foreground) represented the most effective method in detecting bee species richness and thus can be recommended for pollinator monitoring schemes. Trap nests (shown in the background) can be used as a complementary method to detect additional cavity-nesting bee species. Photo credit: C. Westphal.

pratense), the Swedish agricultural habitats could not be sampled. Moreover, the bad weather forced a reduced sampling effort in the Swedish seminatural habitats, where only six instead of 10 surveys were carried out for the observation plots and transect methods. Unfortunately, the seminatural study sites in France could not be surveyed throughout the entire flowering season. The project started so late that bees flying at the beginning of the season could not be sampled. Furthermore, the study sites could not be accessed during the second half of the flowering period due to a severe drought and high fire risk. Because of this incomplete sampling, we excluded the French seminatural sites from the analysis. Prior to the last survey, one seminatural study site in Germany was devastated due to intensive grazing. On this site, only nine, instead of 10, surveys were conducted for the observation plots and both transect methods.

All collected specimens were identified to species, except for *Stelis breviuscula* and *Stelis phaeoptera*, and for *Bombus lucorum* and *Bombus terrestris*. Each of these species pairs was aggregated (Appendix C). Overall, 2.5% of the collected specimens could not be identified to species level (mainly due to damage) and were excluded from the statistical analyses. The excluded specimens belonged to a wide variety of different bee genera. Generic classification follows Michener (2000); species names follow the ALARM Bee Database (held by Stuart P. M. Roberts, Reading, UK). The reference collections of voucher specimens are held by the Universities of Uppsala (Agricultural Sciences), Krakow, Reading, and Bayreuth, and by INRA in Avignon.

Data analysis

We used a variety of approaches to analyze the relative performance of different methods in order to estimate the effects of sampling intensity, to explore similarities in species composition, and to identify methods that indicate bee species richness.

Efficiency of the tested methods.—Linear mixed-effects models were used for the statistical analysis of differences in the efficiency of the methods. Within the nested design, we included country as a random effect to account for the variation between countries, and habitat type as a fixed effect (Pinheiro and Bates 2000). To standardize regional and habitat-specific differences in bee species richness, we analyzed the relative performance of the methods with respect to sample coverage.

Sample coverage was defined as the number of species that were detected per individual method divided by the cumulative number of species per study site (i.e., the total number of species that was detected with all methods on a site combined). Sample coverage is expressed as a percentage. Additional analyses for the different methods were based on the absolute numbers of species and of individuals that were detected. We also used the Abundance-based Coverage Estimator of species richness (ACE; see Magurran [2004] for an overview) as dependent variable in the statistical models. ACE was calculated for each method separately with EstimateS (Colwell 2005). ACE values indicate the extrapolated numbers of species that might have been detected with ideal sampling intensity and represent asymptotic species accumulation curves. We calculated the ACE for cross-section samples to avoid adverse effects of the species turnover throughout the season (a definition of cross-section samples and a detailed description of the calculation are given in the following section, *Subunit-based rarefaction curves*). All statistical analyses were carried out with R, Version 2.4.1 for Windows (R Development Core Team 2006). The numbers of detected species and individuals, and the ACE values were log-transformed, $\log(n+1)$, to achieve normal distribution of the residuals (Crawley 2002).

Subunit-based rarefaction curves.—The sampling effort, which is necessary to detect a representative fraction of bee species richness, was assessed with rarefaction curves based on cross-sections of the collected samples. In temperate regions, bee species have different phenologies, and thus new species can be found throughout the season. To minimize the effects of newly occurring bee species on the calculation of the rarefaction curves, we used cross-section samples, which contained bee species that occurred from the beginning until the end of the season. The sampling of this study was therefore arranged in subunits that were represented by: (1) individual observation plots, (2) pan trap clusters, (3) 5-min intervals of the standardized transect walks, (4) single trap nests with reed internodes, or (5) the clusters of trap nests with paper tubes. The variable transects could not be subdivided into subunits, therefore we used each individual variable transect through the season as a sampling unit in the rarefaction curves. For a cross-section sample, we pooled the first subunits of the respective methods, then the second subunits, and so forth (Krauss et al. 2003*a*, *b*). Thus, a cross-section sample represents the species that were detected in a defined subunit during all surveys per study site that were conducted for the different methods. For example, all species that were collected with pan trap cluster #1 during the three surveys in an agricultural site made up the first cross-section sample. Similarly, the cumulative species richness that was collected during the 10 surveys in the first five minutes of a standardized transect walk in a seminatural site also represented a cross-section sample. We calculated the number of expected species (Mao Tau; Colwell et al. 2004) for the rarefied number of subunits with EstimateS (100 runs; Colwell 2005). Based on these estimates, we calculated the sample coverage (%) as number of expected species (Mao Tau) divided by the total number of detected species per site. Hence, the subunit-based rarefaction curves provide information about the numbers of observation plots, pan trap clusters, and trap nests, and the duration of standardized transect walks that are necessary to achieve a certain level of sample coverage. The subunit-based rarefaction curves show the mean sample coverage ($\pm$SE) for all study sites per country ($N = 4$ sites).

Arbitrarily, we chose a sample coverage of 50% of the total bee community as a standard to judge the various methods tested here. When 50% of the bee species that occur on a study site are detected with any method, the data will comprise most of the common species but also a representative fraction of the rarer species. Hence, the relative species richness of a site should certainly be indicated. However, this critical value of sample coverage should be adapted to the objective of a study. For instance, it should be much higher in studies aiming at the absolute quantification of bee species richness of a study site.

Complementarity of methods.—To assess the species richness of bees as comprehensively as possible, methods detecting complementary species assemblages could be used in monitoring schemes or short-term surveys (Vane-Wright et al. 1991, Colwell and Coddington 1994). Complementarity, which is defined as dissimilarity in species composition of the samples, can be estimated with similarity indices (Magurran 2004). We used the novel Chao-Sørensen abundance-based estimator as a measure of similarity, because in comparative studies this estimator proved to be less biased than classic indices of similarity (Chao et al. 2005, 2006). For the calculations of the Chao-Sørensen abundance-based estimator, we pooled the abundance of each recorded species for each method and study site. The pairwise comparisons of the similarities of samples were performed separately for each study site with EstimateS (Colwell 2005). Based on the site-specific similarity values, we calculated the mean similarity values ($\pm$SE) to assess the complementarity of the methods ($N = 16$ replicates).

In addition to the evaluation of the complementarity of the methods based on the Chao-Sørensen abundance-based estimator, we calculated the relative contribution of each method to the total numbers of species that were detected per site. First, we identified the most efficient method that detected the largest percentage of the total number of species per study site (this value is identical to the method's sample coverage). Then we identified the second-most efficient method and calculated the percentage of additional species that was detected by this method. We then calculated the percentages of additional species for all other methods in descending order.

TABLE 2. Bee species richness in agricultural and seminatural habitats for the study regions in five European countries.

Study region within country	Agricultural habitats			Seminatural habitats		
	Individual bees (*N*)	Bee genera (*N*)	Bee species (*N*)	Individual bees (*N*)	Bee genera (*N*)	Bee species (*N*)
Uppsala, Uppland, Sweden				1220	20	73
Krakow, Małopolska, Poland	4106	13	46	2253	23	99
Reading, England, United Kingdom	843	6	26	2886	13	70
Göttingen, Lower Saxony, Germany	1746	9	27	8813	25	122
Avignon, Provence, France	4341	26	104			

If two methods detected the same numbers of additional species, we defined the method as more efficient that detected larger numbers of individuals. These calculations were done for all study sites separately. The mean percentages of additionally detected species (±SE) were used to identify complementary methods that can be combined in monitoring schemes.

Identification of indicator methods for assessing bee species richness.—Comparisons between studies employing a variety of sampling techniques can be made and used for the documentation of shifts in pollinators if the relationship between the numbers of species sampled with different methods is known. Furthermore, studies and long-term monitoring schemes are often limited by the availability of manpower and financial resources. Hence, cost-effective and less labor-intensive methods that detect defined proportions of bee species richness might be useful as indicator methods. For this reason, we analyzed the correlations between the numbers of species that were detected among individual methods (Zar 1984). To identify methods that measure bee species richness accurately, we correlated the numbers of species that were detected with the different methods with the total number of species that were detected with all methods per site combined. However, these correlations must be interpreted with caution, as the variables are not statistically independent (Zar 1984). Nevertheless, the correlation analysis was, in our opinion, the most appropriate procedure to evaluate the indication potential of the tested methods.

RESULTS

Bee species richness

In total, 278 bee species were identified from the 26 208 specimens detected in this study. The highest bee richness was recorded in the calcareous grasslands in Germany (122 species) and the cantaloupe fields in France (104 species). The lowest bee richness was detected for the oilseed rape fields in the United Kingdom (26 species) and Germany (27 species; Table 2). Overall, 59 species were recorded as singletons and 31 species as doubletons. In the three countries where both habitat types were sampled (Poland, Germany, and the United Kingdom) we found almost three times as many singletons and doubletons in the seminatural habitats (across all countries, 91.3 ± 15.3 [mean ± SE]) as in the agricultural ones (30.7 ± 5.7). The numbers of detected bees were comparable in the agricultural and seminatural sites, with 11 036 and 15 172 individuals, respectively. However, a large fraction of the individuals in the agricultural habitats can be attributed to foraging honey bees (*Apis mellifera*) that were attracted by the mass-flowering crops (across all sites, 65.7% ± 13.3% [mean ± SE]; minimum, 28.2%; maximum, 91.0%). Far fewer honey bees were recorded in the seminatural habitats (10.0% ± 3.7%; minimum, 4.0%; maximum, 20.9%), which had lower overall densities but much higher diversity of food plants.

Performance of the tested methods

We found significant differences between the sample coverage of the three methods that were tested in both habitat types (i.e., observation plots, standardized transect walks, and pan traps; Table 3). The most efficient method for detecting bee species richness was the pan trap method, followed by the standardized transect walks; the observation plots performed poorly (Fig. 3). The sample coverage of the three methods was significantly higher in the agricultural habitats than in the seminatural habitats (Fig. 3, Table 3). Moreover, there was a significant interaction (method × habitat type): the observation plots and pan traps had higher sample coverage in the agricultural habitats; whereas the standardized transect walks had almost the same sample coverage in both habitat types (Fig. 3, Table 3).

The numbers of detected bee species differed significantly between methods and habitat types. Again, the pan traps were the most powerful method for detecting bee species richness, followed by the standardized transect walks and the observation plots (Fig. 4, Table 4). More bee species were detected in seminatural compared to agricultural habitats (Fig. 4). However,

TABLE 3. Results of the mixed-effects model testing for differences in the sample coverage between methods (observation plots, standardized transects, pan traps) and habitat types ($N = 96$ observations).

Source of variation	df	*F*	*P*
Method	2, 86	262.98	<0.0001
Habitat type	1, 86	10.96	0.0014
Method × habitat type	2, 86	4.61	0.0125

Note: Country was included as a random effect.

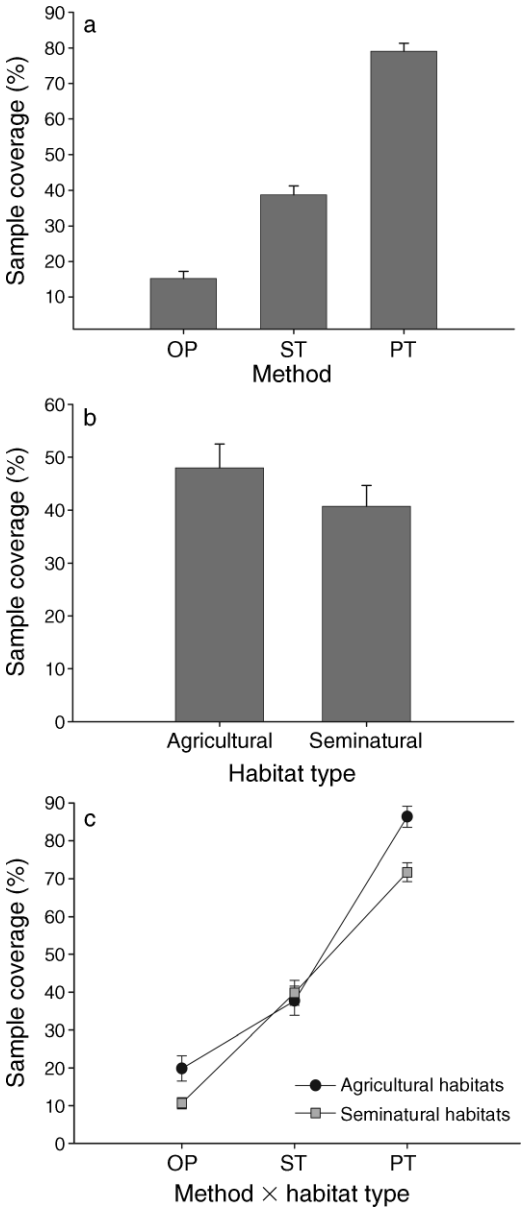

FIG. 3. Differences in sample coverage between (a) the observation plots (OP), standardized transect walks (ST), and pan traps (PT), and (b) the agricultural and seminatural habitats. (c) The interaction between the two factors method and habitat type with respect to sample coverage (mean ± SE). For statistical details see Table 3.

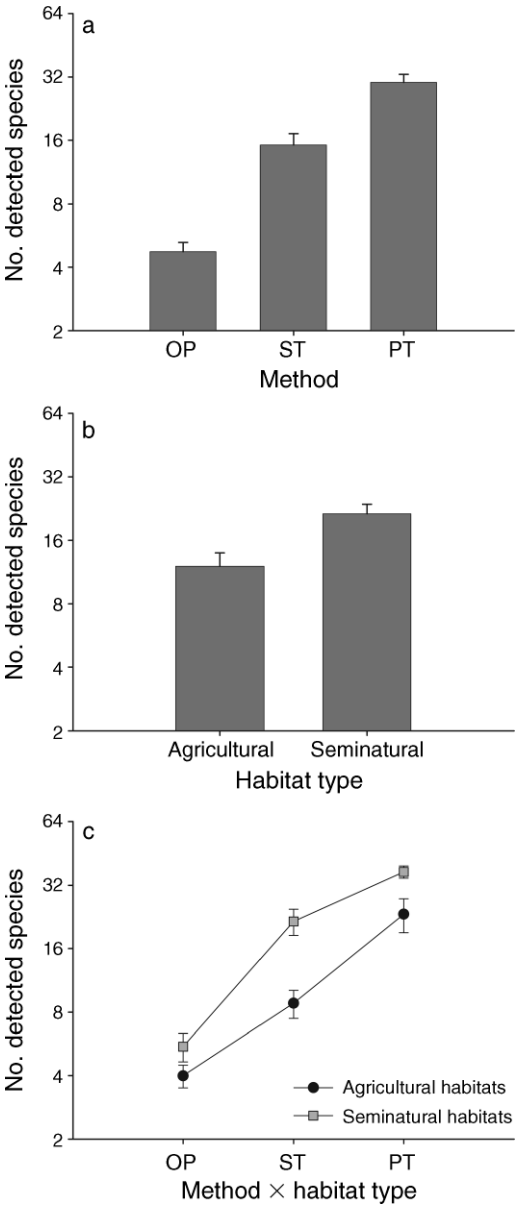

FIG. 4. Differences in the numbers of detected bee species between (a) the observation plots (OP), standardized transect walks (ST), and pan traps (PT), and (b) the agricultural and seminatural habitats. (c) The interaction between the two factors method and habitat type with respect to the numbers of detected bee species (mean ± SE). For statistical details see Table 4.

the difference in species richness between the two habitat types was insufficiently revealed by the observation plots, which detected, on average, only two additional species in the seminatural habitats. The more efficient standardized transect walks and pan traps revealed the difference in species richness between the habitat types more adequately (Fig. 4). This discrepancy in method performance might be attributed to the relatively low sampling effort for each observation plot. One observation plot was only examined for six minutes per site and survey, whereas one standardized transect walk lasted 50 minutes and a variable transect walk lasted 30 minutes. The pan trap clusters were even installed for 48 hours (2880 minutes). However, one should note that bees were only caught during their active periods during the day (likely a maximum of 24 hours for most species during the two days of catching). Likewise, the area that was sampled with the observation plots was much smaller (20 m^2) compared to the area sampled during a

TABLE 4. Results of the mixed-effects model testing for differences in the detected bee species richness between methods (observation plots, standardized transects, pan traps) and habitat types ($N = 96$ observations).

Source of variation	df	F	P
Method	2, 86	130.76	<0.0001
Habitat type	1, 86	64.52	<0.0001
Method × habitat type	2, 86	4.46	0.0143

Note: Country was included as random effect.

standardized transect walk (1000 m^2). Discrepancies in sampling effort might also explain significant differences in the numbers of individuals that were detected with the various methods (Appendix D; Gotelli and Colwell 2001). However, the mixed-effects models with the method-specific Abundance-based Coverage Estimator (ACE) values as dependent variable indicated that there were true differences in the efficiencies of the tested methods irrespective of deviations in sampling effort and different numbers of detected individuals (Appendix D).

We found significant differences in sample coverage among methods, comparing the performance of the observation plots, standardized transect walks, and pan traps with the three additional methods that were only tested in the seminatural habitats (i.e., variable transect walks, trap nests with reed internodes, and trap nests with paper tubes; Fig. 5). Again, colored pan traps were the most efficient method. The second most efficient were the transect methods, among which the variable transects performed better than the standardized ones. Among the methods that can detect a wide range of bee species, the observation plots had the lowest sample coverage. In comparison with the observation plots, the trap nests with reed internodes performed relatively well despite being restricted to sampling cavity-nesting bees only. The lowest sample coverage of all tested methods was recorded for the trap nests with paper tubes.

Likewise, we found significant differences between the numbers of bee species that were detected with the six methods in the seminatural habitats. The samples from the trap nests and observation plots had the lowest species richness, whereas the samples from the transect walks and pan traps had much higher species numbers (Fig. 5). There were also significant differences in the numbers of detected individuals (Appendix E). But again, the analysis of the method-specific ACE values showed the same ranking of method performance. Hence, differences in the efficiencies of the methods cannot merely be attributed to inequality of sampling effort and numbers of detected individuals.

Effects of sampling intensity

We examined the relationships between sampling intensity (measured as number of surveyed subunits) and sample coverage separately for the five countries in order to explain some of the variation that was attributed to the biogeographical regions. For the methods tested in the agricultural habitats, we found distinct subunit-based rarefaction curves for each country (Fig. 6). The subunit-based rarefaction curves of the pan traps increased steeply with sampling intensity, but did not reach an asymptote in any country. Nevertheless, the sampling effort of five subunits (clusters) in three surveys seemed to be sufficient for assessing bee species richness, as the pan traps detected considerable proportions of the total numbers of species (all methods combined; means ranging from 71% to 94% for the different countries).

Compared to the pan traps, the initial increases of the subunit-based rarefaction curves for the standardized transect walks and observation plots in the agricultural habitats were less steep. The curves tended to level off after approximately five subunits. There was only a slight increase in the sample coverage with increasing numbers of subunits for both the standardized transect walks and the observation plots in Poland. Similarly, the sample coverage for these two methods increased only marginally with sampling effort in France, indicating that additional effort using these methods would not

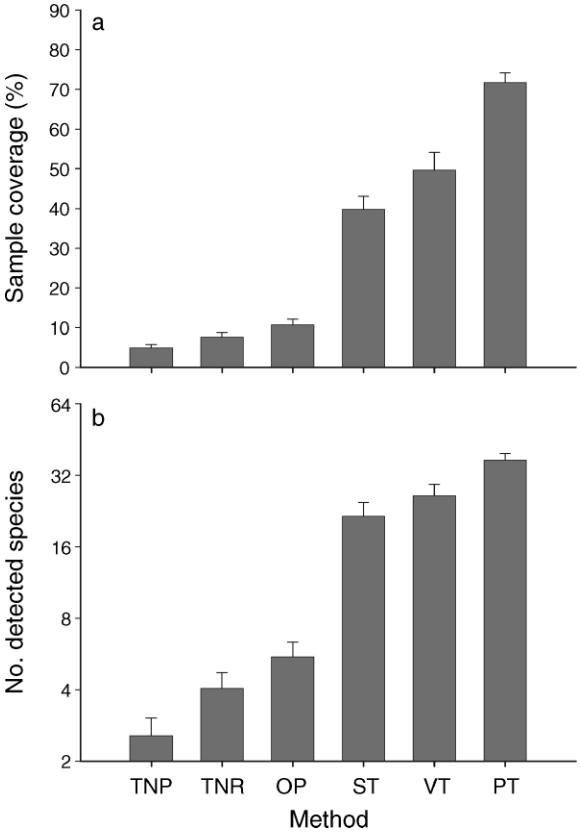

FIG. 5. Differences between (a) the sample coverage and (b) the numbers of detected bee species among the methods that were tested in the seminatural habitats: trap nests with paper tubes (TNP) or reed internodes (TNR), observation plots (OP), standardized (ST) or variable (VT) transect walks, and pan traps (PT). Values are means ± SE. Linear mixed-effects models: (a) $F_{5,87} = 124.36$, $P < 0.0001$, (b) $F_{5,87} = 90.30$, $P < 0.0001$; $N = 96$ observations.

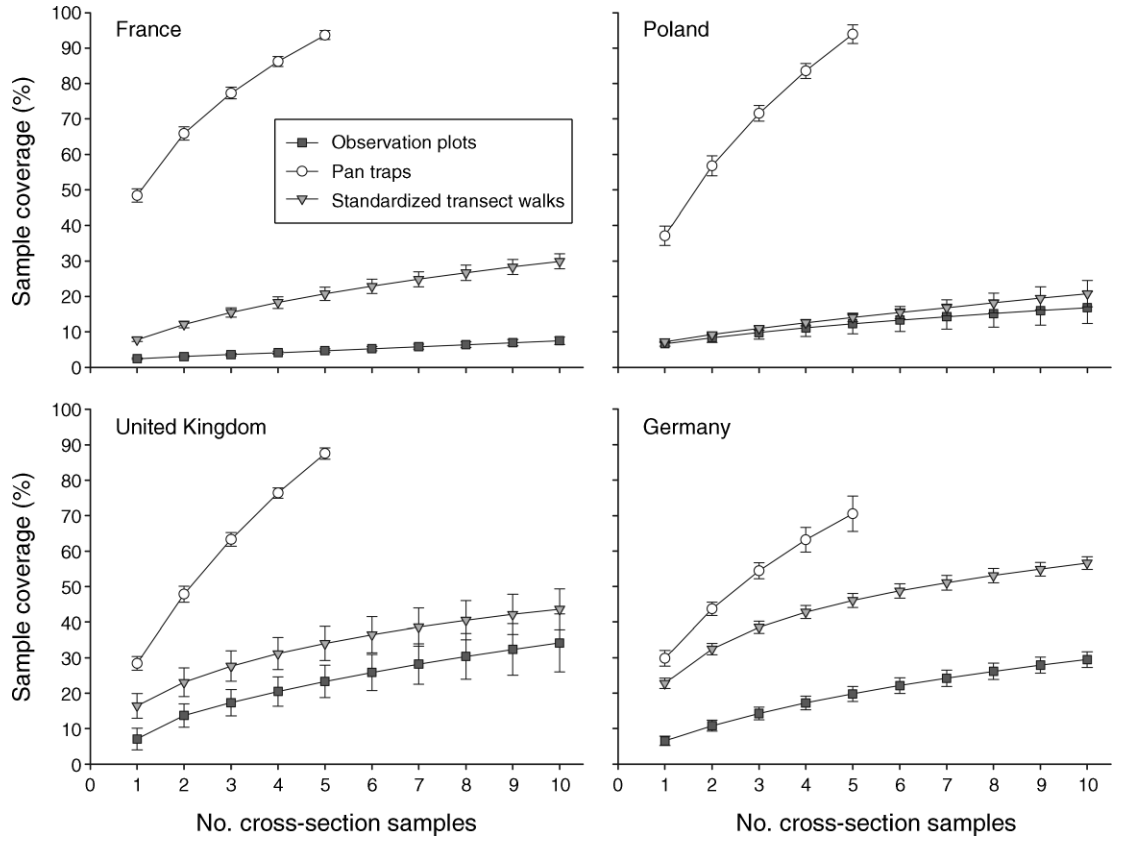

FIG. 6. Subunit-based rarefaction curves for the observation plots, pan traps, and standardized transect walks that were tested in the agricultural study sites ($N = 4$ sites) of four European countries. The sample coverage of rarefied cross-section samples is given (mean ± SE). One cross-section sample represents the cumulated species numbers found during all surveys in one specific subunit (i.e., specific plot, 5-min interval, or pan trap cluster).

result in an adequate estimate of bee species richness with a sample coverage of a minimum of 50%. Even if the steeper rarefaction curves for the British and German observation plots were extrapolated, a minimum of 50% sample coverage of bee species richness would only be reached by doubling (United Kingdom) or tripling (Germany) the sampling effort. For the standardized transect walks, 50% sample coverage was reached with seven subunits (i.e., 35 minutes of sampling) in Germany, whereas longer transect walks seemed to be necessary in the United Kingdom, where only 44% sample coverage was achieved during 50 minutes.

For the methods that were tested in the seminatural habitats, we found comparable subunit-based rarefaction curves for the different countries (Fig. 7). On average, the pan traps achieved a minimum of 67% and a maximum of 83% sample coverage in the different countries (Fig. 7). Hence, five subunits (clusters) in six surveys seemed to be an adequate sampling effort to assess bee species richness for all countries, even though the curves did not level off.

In contrast, the sampling effort that was needed to achieve 50% sample coverage varied considerably between the transect methods in the seminatural habitats. In Germany, both transect methods detected >50% of the species after seven subunits. Thus, seven 30-min walks for the variable transects and 10 35-min walks for the standardized transects could be considered as minimum sampling effort in the German seminatural habitats. With the variable transect walks in the United Kingdom, 50% of the species richness was already detected after four surveys. However, for the standardized transects somewhat longer walks would be needed to finally reach 50% sample coverage with 10 surveys in the United Kingdom. In the Polish seminatural habitats, >15 surveys for the variable and >110 minutes of sampling for the standardized transect walks (with 10 surveys) would be needed to detect 50% of the species. If extrapolated, the rarefaction curves indicate that >11 surveys with the variable transects are needed, and that six standardized transects should last longer than 110 minutes to reach the 50% mark in Sweden. The distinct patterns of the subunit-based rarefaction curves between countries suggest that the transect methods were particularly biased and may be highly influenced by the experience of the surveyors. The least sampling effort was necessary in the United Kingdom and in

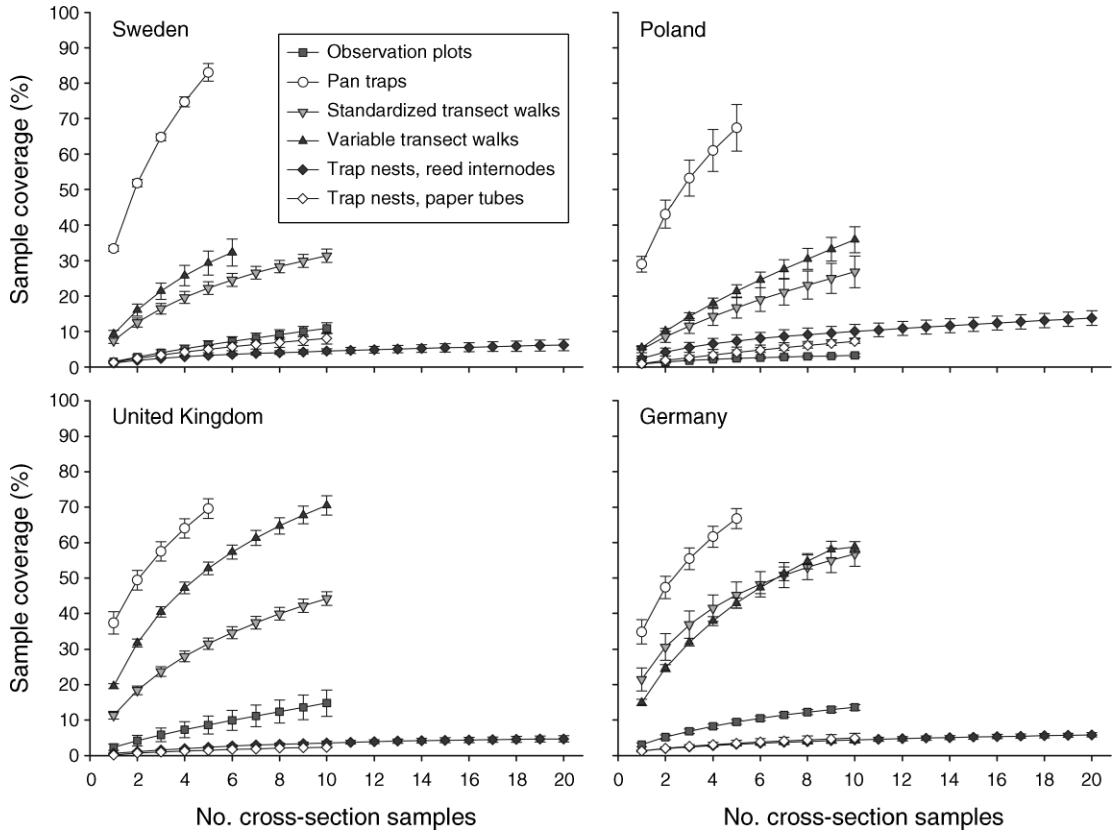

FIG. 7. Subunit-based rarefaction curves for the observation plots, pan traps, standardized and variable transect walks, and trap nests with reed internodes and paper tubes that were tested in the seminatural study sites ($N = 4$ sites) of four European countries. The sample coverage of rarefied cross-section samples is given (mean ± SE). One cross-section sample represents the cumulated species numbers found during all surveys in one specific subunit (i.e., specific plot, 5-min interval, pan trap cluster, or trap). Only for the variable transects were the species cumulated over the numbers of surveys, because the sampling was not based on subunits.

Germany, where very experienced surveyors performed the transect walks. In contrast, sampling effort had to be extended substantially to reach the suggested sample coverage of 50% with the less experienced surveyors in Poland and Sweden.

The sample coverage of the observation plots in the seminatural habitats increased only marginally with sampling effort in all countries, indicating that their effectiveness was largely independent of the surveyors' experience. This pattern shows that sampling effort must be at least quadrupled (United Kingdom) to result in adequate sample coverage of bee species richness, which is also indicative of the short periods of observation employed here.

As the trap nests detect only cavity-nesting bees, this method cannot reach 50% sample coverage (in the seminatural habitats only 8.63% of the detected species were cavity nesters). The maximum sample coverage for the trap nests (14%) was achieved in Poland with reed internodes. The best performance of the trap nests with paper tubes was recorded in Sweden with 8% sample coverage, which was higher than the 6% sample coverage of the Swedish trap nests with reed internodes. The sampling effort for both trap nest types might be reduced while achieving similar results. For instance, a reduction to 15 trap nests with reed internodes and six clusters of trap nests with paper tubes would only decrease the sample coverage between 1% and 2%.

Species composition and complementarity

The species composition of the samples collected with the tested methods varied considerably (Tables 5 and 6). In agricultural habitats, the greatest overlap of species

TABLE 5. Pairwise similarity of species assemblages that were sampled with the observation plots, pan traps, and standardized transect walks in the agricultural habitats.

Sampling method	Observation plots	Pan traps
Pan traps	0.523 ± 0.074	
Standardized transect walks	0.858 ± 0.066	0.758 ± 0.053

Notes: The similarity of the samples was estimated as the proportion of shared species according to the Chao-Sørensen abundance-based estimator (Chao et al. 2005). Mean values (±SE) are given, based on separate calculations of the pairwise similarity of the species assemblages for all study sites ($N = 16$ sites).

TABLE 6. Pairwise similarity of species assemblages that were sampled with the observation plots, pan traps, standardized and variable transect walks, trap nests with paper tubes, and trap nests with reed internodes in the seminatural habitats.

Sampling method	Observation plots	Pan traps	Standardized transects	Variable transects	Trap nests, paper
Pan traps	0.507 ± 0.072				
Standardized transects	0.816 ± 0.039	0.851 ± 0.039			
Variable transects	0.772 ± 0.054	0.835 ± 0.039	0.926 ± 0.015		
Trap nests, paper tubes	0	0.062 ± 0.018	0.009 ± 0.004	0.009 ± 0.004	
Trap nests, reed internodes	0	0.074 ± 0.023	0.009 ± 0.004	0.017 ± 0.007	0.488 ± 0.106

Notes: The similarity of the samples was estimated as the proportion of shared species according to the Chao-Sørensen abundance-based estimator (Chao et al. 2005). Mean values (±SE) are given, based on separate calculations of the pairwise similarity of the species assemblages for all study sites ($N = 16$ sites).

was found between the samples coming from the standardized transect walks and the observation plots. The samples with both methods had large proportions of *Apis mellifera* in common (>90%), whereas the most dominant species in the pan trap samples belonged to the genus *Lasioglossum* (29%), with *Apis mellifera* (13%) representing the second largest fraction in the pan trap samples. Because of the differences in species richness (Fig. 4) and relative abundance, we found the greatest dissimilarity in species composition between the samples of the pan traps and the observation plots. The samples taken with the pan traps and the standardized transect walks had a relatively large overlap in species composition (Table 5).

The species composition of the samples that were collected in the seminatural habitats contrasted even more among methods (Table 6). We found great dissimilarities in species composition between the samples from the trap nests compared to the other methods, which detected only minor fractions of trap-nesting bee species. The most dominant trap-nesting species was *Osmia rufa* representing 62% and 73% of the individuals in the trap nests with reed internodes and paper tubes, respectively. In the samples using other methods, <1% *O. rufa* was recorded. In total, nine species were exclusively detected with the trap nests (three of them were parasites of cavity-nesting bee species). Overall, we found 20 species in the trap nests with reed internodes and 16 species in the trap nests with paper tubes. On average, the species richness was much greater in the trap nests with reed internodes (Fig. 5), which explains the relatively small overlap in species composition between the two trap nest types.

The greatest similarity in species composition was found for the samples coming from the standardized and variable transect walks. We also found a considerable overlap in the species composition of the samples originating from the pan traps and transect walks. Differences in species composition were caused by greater species richness in the pan trap samples compared to the standardized and variable transect walks (Fig. 5). Moreover, the relative abundance of the most dominant genera differed. In pan traps, the largest fractions of the sampled individuals represented species belonging to the genera *Lasioglossum* (41%), *Bombus* (14%), and *Andrena* (13%). In contrast, *Bombus* species were the dominant species group in the standardized and variable transect walks (33% and 32%, respectively), followed by *Lasioglossum* species (18% and 21%, respectively), and *Apis mellifera* (17% and 18%, respectively). The pan traps seemed to undersample *Apis mellifera*, which only made up 4% of the specimens. All three methods sampled *Halictus* species in similar proportions ranging from 11% to 13% of the collected specimens.

The samples taken with the observation plots and both transect methods were fairly similar in species composition, irrespective of the differences in species richness (Fig. 5). This similarity was caused by their equivalent dominance structure. As for the transect methods, *Bombus* species (44%), *Apis mellifera* (29%), and *Lasioglossum* species (12%) represented the largest fractions of the specimens that were sampled with the observation plots. The species compositions of the samples taken with the observation plots and pan traps were more dissimilar, because of large differences in species richness and relative abundance.

For the combined use of two or more methods in monitoring schemes or other studies on pollinator diversity, the selected methods should be able to detect different species out of the species pool of a study site. For this reason, the composition of the samples should be dissimilar and large proportions of additional species should be detected. In the agricultural habitats the samples of the observation plots and pan traps were quite dissimilar (Table 5). Yet, the percentage of species that was additionally detected by the observation plots was rather small (2.8% ± 1.2% [mean ± SE]; Appendix

TABLE 7. Correlations between the number of bee species that were detected with the observation plots, pan traps, and standardized transect walks, and the total number of bee species per study site in the agricultural habitats.

Sampling method	Observation plots	Pan traps	Standardized transect walks
Pan traps	NS		
Standardized transect walks	NS	0.828***	
Site total	NS	0.994***	0.870***

Note: Pearson correlation coefficients (R) are given ($N = 16$ observations).

*** $P < 0.0001$; NS = not significant.

TABLE 8. Correlations between the number of bee species that were detected with the observation plots, pan traps, standardized and variable transect walks, trap nests with paper tubes and reed internodes, and the total number of bee species per study site in the seminatural habitats.

Sampling method	Observation plots	Pan traps	Transect walks		Trap nests	
			Standardized	Variable	Paper tubes	Reed internodes
Pan traps	NS					
Standardized transect walks	0.766**	0.638*				
Variable transect walks	0.783**	0.497†	0.896***			
Trap nests, paper tubes	NS	NS	NS	NS		
Trap nests, reed internodes	NS	NS	NS	NS	0.554*	
Site total	0.471†	0.885***	0.788**	0.729*	NS	0.476†

Note: Pearson correlation coefficients (R) are given ($N = 16$ observations).
† $P < 0.1$; * $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$; NS = not significant.

F). In the seminatural habitats, the samples originating from the trap nests were very dissimilar to the ones from the pan traps (Table 6). Moreover, the relative contribution of the trap nests with reed internodes in detecting additional species was quite large (4.1% ± 1.1%; Appendix F), particularly when considering that this method can only detect the small fraction of cavity-nesting bee species. Because of the lower efficiency of the trap nests with paper tubes, their relative contribution in detecting additional species was comparably small (1.4% ± 0.5%; Appendix F). No additional species were detected in the observation plots of the seminatural habitats than were collected with just pan traps (Appendix F).

Indicator methods for assessing bee species richness

Correlation analyses were performed to assess the correspondence among methods in capturing bee species richness, and to identify potential indicator methods. To be able to identify habitat-specific indicator methods, we performed separate analyses for the agricultural and seminatural habitats, which vary considerably in the temporal and spatial availability of floral resources. For the agricultural habitats, we found a strong correlation between the numbers of species that were sampled with the pan traps and standardized transect walks (Table 7). There was also a positive correlation between the total numbers of species per site and the numbers of species that were sampled with the pan traps and standardized transect walks, respectively. These consistent findings suggest that both pan traps and standardized transect walks are suitable methods to assess overall bee species richness in agricultural habitats. The numbers of species that were detected with the observation plots did not correlate with either the numbers of species that were detected with the other methods, or with the total numbers of bee species per site (Table 7).

In contrast to the agricultural habitats, we found significant correlations between the numbers of species that were detected with the observation plots and both transect methods in the seminatural habitats (Table 8). The correlation between the numbers of species that were detected with the pan traps and the standardized transect walks was weaker in the seminatural habitats than in the agricultural ones. The correlation between the numbers of species in the samples coming from the pan traps and variable transect walks was only marginally significant. As expected, the numbers of species that were sampled with similar methods (i.e., both transect types and both trap nest types) were highly correlated (Table 8).

The correlation analyses between the numbers of species that were detected with the different methods and the overall numbers of species per study site revealed that the pan traps were the best overall method, but both transect methods were also good indicators of overall species richness. Even methods that detected only minor fractions of the overall bee species richness (i.e., the observation plots and trap nests with reed internodes) could also indicate bee richness effectively (Table 8).

DISCUSSION

Performance of the tested methods

There is a clear need for standardized methods that can be used to monitor pollinator shifts, as highlighted by the International Initiative for the Conservation and Sustainable Use of Pollinators (São Paulo Declaration on Pollinators 1999), the report on the Status of North American Pollinators (Committee on the Status of Pollinators in North America 2007), the Ecosystems and human well-being assessment (Millennium Ecosystem Assessment 2005), and many researchers studying changes in the diversity and abundance of pollinators and potential consequences for pollination services (e.g., Ghazoul 2005, Biesmeijer et al. 2006, Klein et al. 2007). Our study provides a scientifically sound assessment of the relative performance of six commonly used sampling methods and uses this to underpin recommendations for developing large-scale pollinator monitoring schemes.

Despite the common use of transect methods to detect pollinator richness (e.g., Dicks et al. 2002, Potts et al. 2003, Kremen et al. 2004, Cane et al. 2006), we found that pan traps were the superior method for detecting bee species richness in both agricultural and seminatural grasslands. The clusters of UV-bright yellow, white, and blue pans had the highest sample coverage, caught the

largest numbers of species, and the most individuals. Moreover, the species composition of the pan trap samples was very similar to the species composition of the samples that were collected during the transect walks, and the pan traps represented the overall bee species richness of the study sites very well. Only *Apis mellifera* seemed to be under represented in the pan trap samples from both habitat types (see also Aizen and Feinsinger 1994, Roulston et al. 2007). These findings contrast with the results of Cane et al. (2000), who found that samples from transect walks represented the bee fauna of creosote bush (*Larrea tridentata*) much better than pan trap samples, which were lacking many abundant and specialized bee species. The deviations between the studies are presumably due to different sampling protocols: Cane et al. (2000) placed their pan traps on the ground and not at vegetation height. UV-bright colors and the placement of the pan traps at vegetation height seemed to improve the results considerably (Stephen and Rao 2005). Moreover, the Cane et al. (2000) team are experts in the bee fauna of the region and very familiar with their study sites. For this reason, they would be expected to find more bees in transects than with any other method. In contrast, we did not specialize on a single habitat, but used a wide variety of habitats and countries for our study, and sampling was conducted by less experienced surveyors. Another study also reported great differences between the outcomes of pan traps and net collections (Roulston et al. 2007). However, in this study the pan traps were only active for one single day. Hence, this low sampling intensity might have caused the relatively bad performance of the pan traps (but see Carboni and LeBuhn 2003).

The greatest advantage of the pan trap method is that there is no collector bias, which is very important for large-scale and long-term monitoring schemes using surveyors with variable experience to conduct field work (Dicks et al. 2002, Dafni et al. 2005). By pooling the samples from the differently colored pan traps, we could also avoid bias due to color preferences of different bee taxa (Kirk 1984, Leong and Thorp 1999, Toler et al. 2005). Apparently, the UV-bright pan traps had distinctive color signals that bees could easily detect against the background colors (mainly green, but also different colors of other flowers; Spaethe et al. 2001, Dyer and Chittka 2004). Together these findings suggest that studies addressing questions of pollinator diversity should ideally use pan traps with UV-bright colors representing the prevailing colors of the flowers in the study site (see also Droege 2006). Other practical benefits of the pan traps are: (1) they are good at catching small bee species that are often missed during transect walks; (2) they account for the diurnal activity patterns of bees; (3) they are fairly low in cost, reliable, and simple to use; (4) they can be used to attract pollinators in the absence of bloom; and (5) they provide data from sampling effort that is easy to quantify (G. Frankie, *personal communication*; see also Cane et al. [2000], and The Bee Inventory Plot [see footnote 13]). Moreover, they are good at capturing cleptoparasites that spend little time on flowers (T. Griswold, *personal communication*). Disadvantages of the pan traps are that they (1) provide no information on floral associations, (2) do not only collect bees but also other flower-visiting insects and other animals that accidentally drown in the water, (3) do not measure bee abundance, since bees are not sampled within a defined area, (4) have a (color-dependent) taxonomic bias, and (5) might undersample larger bees that possibly can escape more easily (Toler et al. 2005; T. Griswold, *personal communication*). Also (6) their efficiency is affected by the composition and richness of the local floral community (Dafni et al. 2005; G. Frankie, *personal communication*). Another important disadvantage of pan traps is the high post-sampling processing time involved in preparing the bees for identification. In comparison to the net captures from the transect walks and observations plots, a considerable amount of extra time is needed to sort the specimens and dry them before pinning (see "Guidelines for processing wet specimens" in The Bee Inventory Plot [see footnote 13]). Some time is also needed for manufacturing the pans and poles. However, this time investment is relatively small, considering the large amount of time that is invested in field work for the transect walks. Moreover, the pans and poles can be used many times.

Either the standardized or variable transect walk proved to be the second most efficient method with respect to sample coverage and numbers of collected bee species and individuals. The samples that were taken with the transect methods represented the overall bee species richness of the study sites very well, and their species composition was similar to that from all other methods except for the trap nests. These findings confirm that both transect methods are suitable for recording pollinator communities in different habitat types and biogeographical regions, as suggested earlier (Banaszak 1980, Cane et al. 2000). Particularly, the standardized transects might be used for habitat intercomparisons, because their sample coverage was almost identical in both habitat types. Generally, transect walks need to be spread out across the entire season otherwise seasonal species would be missed. The main drawback of the transect methods was their strong collector bias. Because the variable transects were even less regulated than the standardized ones, we expected greater collector bias for this method. However, we did not observe marked differences in performance between transect methods except for the United Kingdom sites. This finding can be attributed to different levels of collector experience. Both transect methods require that the surveyors are able to spot large and conspicuous species as well as small and easily overlooked ones (Sutherland 1996, Cane et al. 2000). More specifically, the variable transects are open to the surveyors' bias (i.e., knowledge about food plant specializations,

microhabitats, and nesting sites). Hence, additional forage and nesting resources may be targeted by an observer, which may often be missed by standardized transect walks (Dafni et al. 2005). Variable transects can therefore be valuable in helping to provide full species lists for sites, provided sufficient time is allowed for the survey work and surveyor experience is high. In contrast to passive sampling methods (e.g., pan traps and trap nests), the transect walks allow the recording of associations between flowers and bees. This information is essential in studies focusing on pollination ecology (e.g., Moeller 2006) or plant–pollinator food webs (e.g., Memmott 1999). Furthermore, the standardized transect walks offer possibilities of combinations and synergisms with butterfly monitoring schemes (information *available online*),[14,15] as the setup of both transect methods is very similar (Pollard 1977, Kühn et al. 2005). Thus, it might be worth the effort to develop a unified bee and butterfly monitoring scheme.

Species–area curves (or species–accumulation curves) demonstrate strong, positive relationships between the numbers of detected species and the area that is surveyed (Rosenzweig 1995, Magurran 2004). The observation plots were surveyed for six minutes and covered only an area of 2 m^2, whereas one subunit of a standardized transect walk covered 100 m^2 during five minutes (while the surveyor was slowly moving). Hence, the poor performance of the observation plots can be attributed to the small area that was covered with this method in our study (see also Banaszak 1980, Williams et al. 2001). The discrepancy between the estimated species richness (Abundance-based Coverage Estimator, or ACE, values) and detected numbers of bee species (Figs. 4a and 5b; Appendix D: Fig. D2a; Appendix E: Fig. E1b) and the generally small numbers of detected individuals in the observation plots in both habitat types (Appendix D: Fig. D1a; Appendix E: Fig. E1a) also suggest that the sampling intensity of the observation plots was not sufficient (Colwell and Coddington 1994, Gotelli and Colwell 2001). The observation plots were not a good indicator method for bee species richness in our study, since they did not indicate the species richness in the agricultural habitats, and showed only a marginally significant correlation between the detected numbers of species and the total numbers of species in the seminatural habitats. Nevertheless, the species composition of the samples coming from the transect walks and observation plots were similar in both habitat types. These findings suggest that the observation plots detected bee species assemblages that are representative of the overall species composition of different habitat types. For this reason, observation plots (or focal plant populations) can be recommended for floral guild surveys investigating pollination services by linking pollinator abundance and richness to seed and fruit set of insect-pollinated plant species (e.g., Price et al. 2005).

Because only cavity-nesting bees inhabit trap nests, these nests had a naturally low sample coverage and detected fewer bee species than the other methods. However, the trap nests with reed internodes detected relatively high numbers of bee species, and tended to indicate the overall bee species richness of the study sites. The paper tube traps performed poorly relative to the reed internode traps. The contrasting efficiencies of the two trap nest methods might be explained by differences in the frequency distributions of the diameters of the cavities used. The trap nests with reed internodes offered a wider variety of internode sizes (Gathmann et al. 1994), whereas the paper tubes had only three distinct diameters. This suggests that the sampling efficiency of trap nests can be improved when a wide range of differently sized cavities is offered to trap-nesting bee communities. The natural reed cavities might also be more appealing to trap-nesting bees than the artificial paper tubes. However, the trap nests with reed internodes only weakly indicated the bee richness of a site. This might be due to the fact that we focused only on the trap-nesting bees, which may not reflect the overall bee species richness of the study sites so well. With a maximum of 10 trap-nesting bee species, the trap nests were naturally limited in their ability to indicate differences in the overall bee species richness between our study sites. Trap nests might, however, represent an appropriate indicator method for bee richness in other (tropical and Mediterranean) regions with more diverse cavity-nesting bee communities (e.g., Tylianakis et al. 2005). A benefit of the trap nests was that they were able to detect additional bee species that were not present in the samples of the other methods. For this reason, the trap nests represent a valuable tool to complement the findings of other methods, particularly in studies that aim at the comprehensive characterization of bee species richness (Appendix F: Fig. F1; Dafni et al. 2005). Like pan traps, trap nests are a passive sampling method, and thus are not biased by collector experience. Another advantage of the traps nests is their low maintenance once they are set up in the field. However, it should be noted that the reared individuals from the trap nests represent the next generation of pollinators, and that they are the outcome of a smaller number of nesting individuals. Thus, the absolute numbers of individuals recorded with the trap nests and other methods should be compared with caution, particularly as the trap nest data do not account for overwintering mortality in the natural environment or post emergence dispersal.

Effects of habitat characteristics on the method performance

Seminatural habitats tend to be more diverse in terms of the availability of food plants and microhabitats, such as nesting sites, than the agricultural habitats in this study that generally provide one homogeneously dis-

[14] ⟨http://www.ukbms.org/⟩
[15] ⟨http://www.tagfalter-monitoring.ufz.de/⟩

tributed food plant for a short period of bloom (e.g., Kremen et al. 2002, Steffan-Dewenter et al. 2002, Westphal et al. 2003). Indeed, the seminatural sites we studied supported more diverse bee communities than the agricultural sites.

The spatial and temporal heterogeneity of resources and higher proportion of rare species in the seminatural habitats might have resulted in the differences in sample coverage between habitat types. The sample coverage was lower in the more heterogeneous seminatural habitats, indicating that with increasing species richness and habitat heterogeneity the sampling effort, the size of the area that is surveyed, and also the spatial distribution of the sampling units (e.g., traps, plots, and transects) may need adjusting to optimize their effectiveness (see also Gotelli and Colwell 2001, Williams et al. 2001).

Not all three methods that were tested in both habitat types performed better in the agricultural habitats. The efficiency of the standardized transect walks was almost identical in both habitat types. The reduced efficiency of the standardized transect walks in the agricultural habitats might be explained by deficiencies in recording bee species in a mass-flowering crop, where it is very difficult to observe every flower and its visitors within a 4-m-wide transect.

Implementation of bee surveys

In addition to the selection of appropriate methods, some general aspects of methodology and analysis need to be considered for the successful development and implementation of bee surveys. Particularly for large-scale and long-term monitoring schemes, but also for other studies focusing on bee species richness, we recommend the following: (1) careful consideration of the method(s) that could be used to achieve the objectives of the survey most efficiently, (2) development of standardized sampling protocols, (3) estimation of the amount of time that is needed for preparation, field work, and processing, (4) standardization of collector experience in (international) training courses on bee taxonomy, species identification, and practical application of sampling methods, (5) sound evaluation of indicator methods prior to the survey, (6) validation of sampling effort based on species richness estimators (e.g., EsitmateS; Colwell 2005), and (7) application of rarefaction curves to identify potential collector biases.

Conclusions

Ongoing human activities, such as agricultural intensification and associated land use changes (Kremen et al. 2002, Steffan-Dewenter et al. 2002, Ricketts et al. 2004), habitat destruction and fragmentation (Rathcke and Jules 1993, Aizen and Feinsinger 1994, Hoekstra et al. 2005, Cane et al. 2006), and global warming (Walther et al. 2002, Parmesan and Yohe 2003, Thomas et al. 2004) might cause extensive shifts in pollinator populations. To quantify and potentially counteract declines in the diversity and abundance of pollinators, and to sustain the vital ecosystem service of pollination, long-term and large-scale pollinator monitoring schemes need to be conducted with standardized methods (São Paulo Declaration on Pollinators 1999, Williams et al. 2001, Ghazoul 2005, Committee on the Status of Pollinators in North America 2007).

We recommend UV-bright pan traps as the most suitable method for such pollinator monitoring schemes (see Plate 1), because this method proved to be highly efficient at sampling the overall bee fauna and was not biased by surveyor experience. Hence, pan traps are likely to provide reliable results when operated by many surveyors in different habitats, regions, and years. If the aim of monitoring schemes is to maximize the number of bee species recorded within a site, then trap nests with reed internodes represent a complementary, unbiased method to detect additional species (see Plate 1). In regions with species-rich cavity-nesting bee communities, trap nests with reed internodes, rather than paper tubes, can be used as an indicator method. Prior to the establishment of trap nest monitoring schemes, the indicator value of the method must be analyzed for the respective region. Because of their collector bias, the transect methods and observation plots can only be recommended for large-scale and long-term monitoring schemes after prior taxonomic training and standardization of surveyor experience. With experienced surveyors and a known fauna, the transect methods represent an efficient method for pollinator monitoring, which additionally avoids killing abundant and easy to identify bee species and other taxa that will be caught in the pan traps. Moreover, transect walks and observation plots represent principal methods in more detailed studies on plant–pollinator interactions, since they allow for the documentation of plant–pollinator interactions. The recommended methods operate efficiently in a wide range of entomophilous crops and extensively used European grassland habitats, and they may also be efficient in other habitat types and geographical regions. For all methods, the preparation and identification of the collected specimens are substantial parts of the work, which need expert knowledge, and thus should not be underestimated.

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APPENDIX A

Latitude and longitude of the study sites (*Ecological Archives* M078-026-A1).

APPENDIX B

List of the sampling dates for the tested methods (*Ecological Archives* M078-026-A2).

APPENDIX C

A complete list of the detected bee species along with the total numbers of individuals that were collected with the tested methods (*Ecological Archives* M078-026-A3).

APPENDIX D

Results of additional linear mixed-effects models testing for differences in the numbers of collected individuals and estimated species richness (ACE values) between the methods (observation plots, standardized transect walks, and pan trap) and habitat types (*Ecological Archives* M078-026-A4).

APPENDIX E

Results of additional linear mixed-effects models testing for differences in the numbers of collected individuals and estimated species richness (ACE values) between the methods that were tested in the seminatural habitats (*Ecological Archives* M078-026-A5).

APPENDIX F

The relative contribution of the tested methods in detecting additional species (*Ecological Archives* M078-026-A6).

APPENDIX G

A color version of Fig. 2 (*Ecological Archives* M078-026-A7).