Original Article

HABITAT-DEPENDENCY OF TRANSECT WALK AND PAN TRAP METHODS FOR BEE SAMPLING IN FARMLANDS

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Abstract

Bees are the most important group of flower visitors providing an essential ecosystem service, namely pollination. Due to the worldwide decline of bees, there should be standardized sampling methods in place to ensure consistent and comparable results between studies. We compared the two commonly used sampling methods of yellow pan traps and transect walk to determine (i) which habitat variables affect the species composition, abundance and species richness of sampled bee communities, (ii) which method potentially contains sampling bias towards some individuals or groups of bees and (iii) the efficiency of sampling in various habitats. We conducted fieldwork in different agricultural habitats distributed along landscape heterogeneity and topography gradients. Our results showed that the height of vegetation, the average number of flowers and the amount of woody vegetation had the greatest influence on the sampling efficiency. Our survey also demonstrated that sampling by transect walk captured less bees in general, especially in stubble, maize, and cereal fields. We found that *Apis mellifera* and *Bombus* spp. were well represented in samples collected by the transect walk method, while the abundance of other genera, especially *Dasypoda, Hylaeus* and *Panurgus* was higher in pan traps. Based on the results, we suggest (i) the transect walk method to compare samples of flower-visiting wild bee communities from various habitats of different vegetation and flower characteristics, (ii) application of the transect walk or pan traps to compare similar habitats and (iii) adoption of a comprehensive method which would incorporate both sampling techniques to gain a more complex insight into wild bee species composition.

Keywords: bee survey, insect pollinators, redundancy analysis, sampling bias, transformation-based, wild bee

INTRODUCTION

Honey bee (*Apis mellifera*, L.) and wild bee species (*Apoidea*) are the most important groups of flower visitors for a wide range of trees, bushes, herbaceous, and crop plant species in different climates all over the world (Michener, 2000; Potts et al., 2016). In the last fifty years, a notable decline in bee populations has been detected, leading to increased concerns on crop production and a decline in wild plant diversity (Kremen, Williams, & Thorp, 2002; Biesmeijer et al., 2006; Potts et al., 2010, 2016). Increasing agricultural intensification, including increased ap-

plication of pesticide at a local scale, as well as a homogenization of the vegetation at landscape scale, resulted in a loss of semi-natural habitats and decreased habitat diversity (Kremen, Williams, & Thorp, 2002; Kovács-Hostyánszki, Batáry, & Báldi, 2011; Kovács-Hostyánszki et al., 2017) and consequently to a loss of nesting places, pollen and nectar resources of bee populations (Holzschuh et al., 2016).

Well-established knowledge, long-term and large-scale monitoring systems and reliable sampling methods are necessary to support conservation measures (Ghazoul, 2005; Gilbert, 2014; Dicks et al., 2016; Senapathi et al., 2017). Bee communities are typically sampled through both active and passive methods, dependent on the scale of the study. Active sampling methods are fast and sample the actual community present at the moment and technically require a person sweeping, hand netting or vacuuming (Cane, Minckley, & Kervin, 2001; Roulston, Smith, & Brewster, 2007). Passive sampling methods including malaise, colored pan or vane traps are exposed to bees for several days. These are active during the whole day; therefore, they can potentially sample a wider community of species independently from their daily activity; but they are less selective for other insect groups (Stephen & Rao, 2005; Campbell & Hanula, 2007; Kimoto et al., 2012; Joshi et al., 2015; Hall, 2018). Former studies have applied a wide variety of sampling methods with different number of replicates and sampling frequency (Hopwood 2008; Matteson, Ascher, & Langellotto, 2008; Ptasznik, 2015; Moreira et al., 2016; Castro et al., 2017; Rhoades et al., 2017). However, there are potential limitations of each trapping method, as active sampling methods are associated with the unconscious influence of the sampling person (Rosenthal & Fode, 2007). Likewise, passive sampling using traps can bias toward certain species or groups depending on their color (Waser & Price, 1981), reflectance (Vrdoljak & Samways, 2012) or size (Wilson et al., 2016).

Methodological studies have already been conducted to compare different sampling methods and their efficiency in pollinator sampling. Westphal et al. (2008) compared observation plots, pan traps, standardized and variable transect walks, trap nests with reed internodes and paper tubes, and contrasted various biogeographical regions and habitats. They found that the pan trapping was the most efficient, unbiased and cost-effective method for sampling the widest range of species. Wilson, Griswold, & Messinger (2008) and Nielsen et al. (2011) also found that pan traps collected the largest number of bee species compared to other sampling methods and were similarly effective as the transect walk method by Grundel et al. (2011). However, Gibbs et al. (2017), McCravy & Ruholl (2017) revealed bias between the passive and active sampling techniques regarding the species richness, composition, and abundance of the sampled bee communities. For instance, Roulston, Smith, & Brewster (2007) determined that pan traps captured significantly less large bee species, while according to Westphal et al. (2008) cavity nesting bee species were underestimated with the transect walk method. However in some studies concerning the pan trap method, systematic bias was found between the number of captured bees and the amount of flowering plants at the sampling sites as blooming flowers were competing with colored pan traps for flower visitors resulting in an under- or oversampled abundance and number of bee species (Cane, Minckley, & Kervin, 2001; Kovács-Hostyánszki, Batáry, & Báldi, 2011; Mayer, 2005; Roulston, Smith, & Brewster, 2007; Wilson, Griswold, & Messinger, 2008; Popic, Davila, & Wardle, 2013). For transect walks, sampling procedure followed a stricter standard, independent from the amount of flower resources.

In this study, bee communities were sampled and compared with both the transect walk method and the colored pan trap method in different crop fields and grasslands, distributed along landscape heterogeneity and topography gradients. Our aim was to compare the efficiency of these two methods with different habitats of such local and landscape scale characteristics as vegetation structure and land-use type e.g. different crop fields versus grasslands and

habitat heterogeneity. We examined differences in bee species composition, abundance, richness and potential biases of sampling methods due to habitat characteristics.

MATERIAL AND METHODS

Study site

Our study was conducted in Romania, Central-East Europe in 2012. The study sites were located in the surrounding areas of nineteen village catchments (the area of one catchment was roughly 7x7 km), at a maximum distance of sixty kilometers from the city Sighisoara (for detailed map see Loos et al., 2014 and Kovács-Hostyánszki et al., 2016). The region was characterized by diverse landscape structure, a mosaic of traditionally managed agricultural fields and semi-natural habitats. In each village catchment, two croplands and two grasslands, in total thirty-eight from each land use type were surveyed.

Landscape characteristics were classified at wider and smaller spatial scales. At a wider scale, the sites differed along the topographical complexity of the extended area around the villages. Eight villages were in a low flat area dominated by open landscape elements, and eleven villages were in a high area with a high proportion of forest, steep hills, and small arable fields. At a small spatial scale of one hectare, all study sites were classified (Hanspach et al., 2014) by vegetation/land surface heterogeneity and woody vegetation cover in 56.42 m radius around the fields, which were described by three classes (1: low, 2: middle and 3: high) based on the measurements of the ARCGIS software (ESRI, 2011; Loos et al., 2014). Heterogeneity was defined as the standard deviation of a 2.5 m panchromatic Spot picture, stratified in quantiles where H1 belonged to lower third (low heterogeneity), H2 middle third and H3 upper third. Woody vegetation cover represented the proportion of shrubs and trees in the 1 ha circle, based on classified 10 m SPOT 5 data: W1: 0 to 5% woody vegetation cover; W2: 5 to 15% of woody vegetation; W3: 15 to 50% of woody vegetation (for more details see Hanspach et al., 2014; Loos et al., 2014). Furthermore, management and/or crop type (hereafter crop type) of the study sites were classified according to eight different categories (numbers correspond to the number of studied fields within the categories): alfalfa (n=15), cereal (wheat, barley, n=8), fallow (n=4), hayfield (n=10), maize (n=8), pasture (grazed by sheep or cattle, n=19), shrubby pasture (n=7) and stubble (mown or harvested, n=5). To compare the sampling methods caused by the flower density, the average number of blooming species was grouped.

Sampling methods

We used the transect walk method and yellow pan traps to sample bees in three time point in May (Period 1), in June (Period 2), and in July (Period 3) 2012. All samples were taken during two weeks per period.

When designing a study, a specific question or focus of inquiry that likely dictates the most suitable sampling method may be, "Do I want to sample a representative sample of the community (ecological target) or as many bee species as possible (faunistical target)?" or "Which sampling method should be used to compare habitats with great variability and to assess bee communities in a single habitat type?" There is an inevitable tradeoff, with respect to time and expense, which influences both the sampling efficiency and the sampling bias. Sampling efficiency was defined as a measure of the quality of the sampling, namely the absolute numbers of sampled species and individuals. Just as in statistics, we defined the sampling bias as a bias which impacts the trapped sample in such a way that some species of the intended (bee) community are less likely to be included than others. Sampling bias can lead to systematic over- or under-estimation of the bee community.

Transect walk method

In each study site, two 100 m long transects were assigned at least 50 m apart from each other and at least 30 m away from the edges of the sites due to the edge effects. Wild bees were actively netted parallel along the two

transects for 20 minutes by two collectors per transect. All flying, sitting and flower-visiting bees were recognized and collected frontwards by the collectors in a half-circle with a one-meter radius. Collectors were highly trained and experienced. There was no sampling in non-adequate weather conditions with rain, strong wind or temperatures below 20°C. Samples were frozen and species were identified by an expert, (Józan, 2011). Samples for cross-reference purposes were stored at the Institute of Ecology and Botany, Centre for Ecological Research in Hungary.

Yellow pan trap

In each of the study sites, two yellow pan traps (regular, unpainted plastic bowls filled with water) were placed in 50 m from each other and emptied one week after setting up/disposing. For comparative biodiversity assessments, the use of such high reflectance colors as white and yellow traps are most recommended although for full inventory surveys the application of other colors can be useful (Vrdoljak & Samways, 2012), and the effect of light reflectance of various trap paints on insect sampling is debated (Le Buhn et al., 2003; Diestelhorst et al., 2014). Our measurements in a former study (data not published) showed that our pan traps had relatively low reflectance in UV (<400 nm) but high reflectance in the visible spectrum (400-700 nm). Such differences in light reflectance of various pan traps in different studies should be acknowledged in direct comparison of the sampling data. The pan traps were set up above the vegetation since bees are most easily captured at 0.8-1 m high in the vertical zone, where they spend their active flying-time and foraging on flowers (Mayer, 2005). The pan traps were almost fully filled with water and with some added liquid detergent to reduce surface tension. The collected insects were stored in ethyl-alcohol, dried and pinned prior to species identification.

Botanical survey

Simultaneously with the bee samplings botanical survey was conducted in all sites along the sampling transects. All blooming flowers, insect-pollinated plant species and the number of blooming flowers per species were recorded in a 1x1 m quadrat area at every ten meters. The average number of blooming insect-pollinated flower species and the number of flowers were calculated per sites and sampling periods of one square meter. For the analyses, the eight crop type categories were classified according to the average number of blooming species and blooming flowers. When the average number of blooming species was under ten and the average number of flowers was under 100, the category was classified as a low flower density group (cereal, maize, stubble), otherwise crop types belonged to the high flower density group (alfalfa, fallow, hayfield, pasture, shrubby pasture). In addition to the number of blooming species and flowers being counted, the average vegetation height and cover (in percentages) of the vegetation were estimated at site level, after data from 1x1 m quadrats being averaged.

Statistical analysis

The analyses focused on two aspects. Our aim was to highlight that sampling methods affect the sampled species richness and the abundance of wild bees due to the influences of landscape and local vegetation parameters. To better fulfill the model assumptions regarding the normality of the residuals, we considered log-transformation for all metric variables: the average number of flowers, the vegetation height, the species richness and the abundance. For data analysis, classical linear regression (LM) with a model, the most suitable method, was used based on the literature and according to experimental design. After a full model was fitted to explain the logarithmic bee abundance, a stepwise back- and forward model selection was applied, terms were sequentially removed and added from the set of potential predictors until reaching the model with the lowest AIC value (Akaike's Information Criterion) (Hastie & Pregibon, 1991). When model fits are ranked, the one with the lowest AIC value was considered the 'best'. The model showed that some variables did not significantly affected the (log) bee abundance,

and therefore the following variables were removed: habitat heterogeneity, topography, method*topography, method*vegetation cover, method*flower density, method*wood and method*period (* indicates interaction between terms). The previous variable selection method

Table 1.

Abundance and species richness of bees collected in yellow water pan traps and transect walk samples in Romanian study sites in 2012

	Genus name		Abundance		Species richness	
	Scientific name	Common name	pan trap	transect	pan trap	transect
1	*Andrena* spp.	Mining bee	271	300	17	28
2	*Anthidium* spp.	Potter bee	1	1	1	1
3	*Anthophora* spp.	Flower bee	0	10	0	5
4	*Apis* spp.	Honey bee	96	528	1	1
5	*Bombus* spp.	Bumblebee	110	279	7	7
6	*Ceratina* spp.	Small carpenter bee	0	4	0	2
7	*Chelostoma* spp.	Scissor bee	3	2	3	1
8	*Coelioxys* spp.	Leaf cutter cuckoo bee	0	3	0	2
9	*Colletes* spp.	Plasterer bee	1	9	1	3
10	*Dasypoda* spp.	Pantaloon bee	99	0	1	0
11	*Diodontus* spp.		0	1	0	1
12	*Epeoloides* spp.		0	1	0	1
13	*Epeolus* spp.	Variegated cuckoo bee	0	1	0	1
14	*Eucera* spp.	Long-horned bee	6	85	3	5
15	*Halictus* spp.	Sweat bee	494	584	15	16
16	*Heriades* spp.	Resin bee	1	1	1	1
17	*Hylaeus* spp.	Yellow-face bee	94	21	11	9
18	*Lasioglossum* spp.	Base-banded furrow bee	1008	765	28	32
19	*Lithurgus* spp.		1	0	1	0
20	*Macropis* spp.	Oil-collecting bee	0	1	0	1
21	*Megachile* spp.	Leafcutter bee	13	33	4	5
22	*Melitta* spp.	Blunthorn bee	2	52	1	4
23	*Melitturga* spp.		0	7	0	1
24	*Micrandrena* spp.		0	1	0	1
25	*Nomada* spp.	Nomad bee	0	8	0	6
26	*Nomia* spp.		0	12	0	2
27	*Osmia* spp.	Mason bee	16	26	5	8
28	*Panurgus* spp.	Shaggy bee	96	2	1	1
29	*Pasites* spp.		0	3	0	1
30	*Sphecodes* spp.	Blood bee	0	31	0	7
31	*Systropha* spp.		0	15	0	2
32	*Tetralonia* spp.		2	14	2	3
33	*Triepeolus* spp.		0	2	0	1

For detailed naming of the species see Józan (2011). The complete list of species can be found in the Supplementary Table 1.

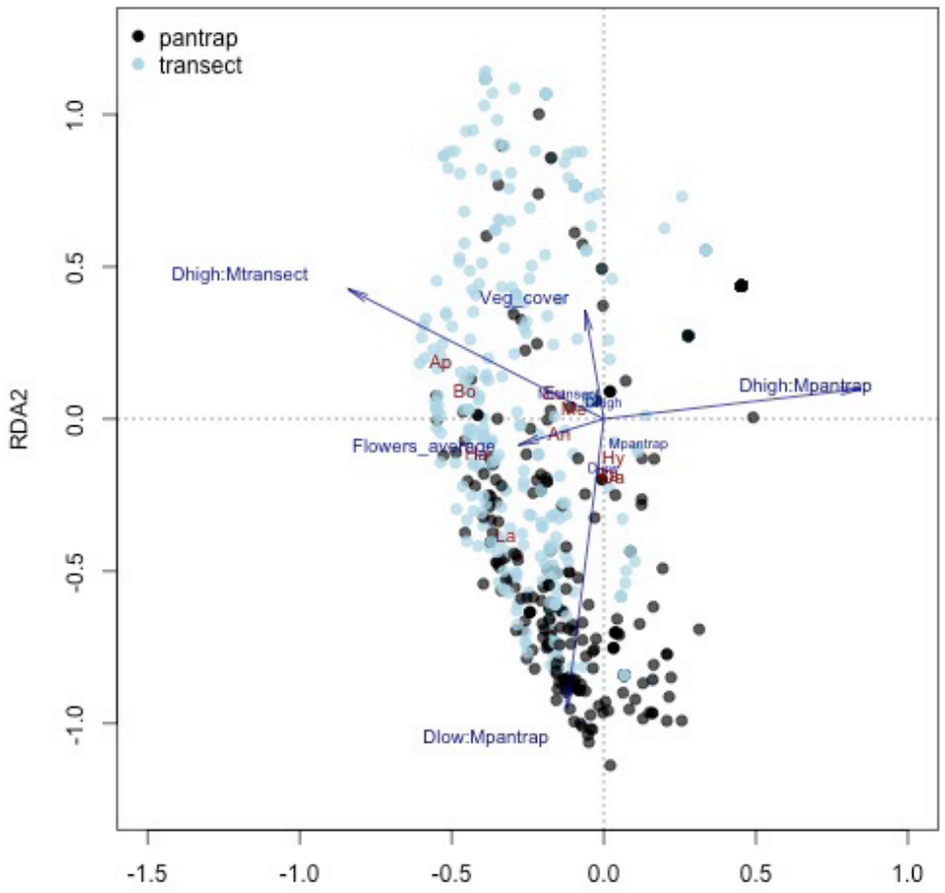

Fig. 1. Triplot, output of the tb-RDA, representing the variation in the response variables (abundance of wild bee species indicated by labels of the genera), explained by a set of explanatory variables: (Dhigh = high flower density, Dlow = low flower density, Mpantrap = pan trap sampling method, Mtransect = transect walk sampling method, the colon between two variables indicate interaction, Veg_cor = vegetation cover and Flowers_average = average number of flowers. The light color dots for the transect walk method and the dark color dots for the yellow pan-trap method represent the scores resulted from the tb-RDA. Distances between the scores and between labels approximate distances of the observations or the centroid of the nominal explanatory variable. The projection of a dot (score) onto the line of a response variable approximates the position of the corresponding object along the variable. Accordingly, the length of the arrows indicates the strength of the effect of continuous explanatory variables. If the angles between arrows is smaller, the degree of correlation between the individual variables increases. In addition, positively correlated variables are shown as arrows pointing in the same direction, while negatively correlated variables are pointing in opposite directions. Thus, the triplot representation is a multivariate view of the abundance of different genus types including the effects of explanatory variables. Abbreviations of the genera: Ap = Apis sp; Bo = Bombus sp; An = Andrena sp; Eu = Eucera sp; Da = Dasypoda sp; Ha = Halictus sp; Hy = Hylaeus sp; La = Lasioglossum sp; Me = Melitta sp; Pa = Panurgus sp.

was repeated and the best linear model specified for species richness included the following explanatory variables, transformations and interactions: the method (with the pan trap as the reference group), the period (the first period as the reference group), woody vegetation coverage (the smallest woody vegetation coverage group as the reference group), the logarithm of vegetation height, the logarithm of flower average, the interaction between method and crop field (pan trap and pasture as the reference groups) and the interaction between method and the logarithm of flower average. The model for abundance is very similar to the model used for species richness and contains various levels for crop types with pasture as the reference group but without the interaction of crop types and method.

The effects of model coefficients reported for each categorical variable (Tab. 2) are shown in

comparison to the reference category of the given variable. With more than two categories, each coefficient explains the difference between the mean of the given category and the reference category. In this study case, the first categories were chosen as pasture crop type due to the lack of significant difference between the abundance and/or species richness of wild bees sampled in such crop fields (see Fig. 2), pan trap method, period 1 and wood cover at level 1. The residual diagnostic plots i.e. Tukey-Anscombe plot, Q-Q plot, scale location plot and residual-leverage plot did not report any violations of the model assumptions, and neither leverage points nor observations with high Cook's Distance (Cook & Weisberg, 1982) were detected. Other transformations in the response and predictor variables did not improve the model nor lead to better model diagnostic results.

The second aim of the study was to gain insights into the community structure of wild bees trapped by two sampling methods. For the analysis of the genera composition of the sampled bee assemblages, the sampled species were grouped according to genera and then subjected transformation-based redundancy analysis (tb-RDA; see Legendre & Gallagher, 2001; Zuur, Leno, & Smith, 2007). The aim of redundancy analysis is to describe dependencies between groups of variables using two classical methods: linear regression analysis and principal component analysis (Ramette, 2007; Legendre, 2008; Reiter, 2014). In the first part of the tb-RDA, the functional relationship between the two groups of variables was modeled. In our case, the various bee genera (Tab. 1) were modeled by such environmental variables as average number of flowers or height of the vegetation and the sampling methods. In the second part of the tb-RDA, the fitted values for genus from the linear regression model were input for principal component analysis, whereby the variance-covariance matrix for both variable groups was used. Hellinger transformation was applied to standardize the counts on each genus, because otherwise, the influence of rare genera would have been too high (Legendre & Gallagher, 2001). The graphical output of tb-RDA is called triplot, which basically consists of two biplots that lay on top of each other, and an interpretation of it is given in the legend of Fig. 1. All analyses were conducted at species level except for the transformation-based redundancy analysis (which was genus-based, applied for community composition. The statistical analyses were done in R 3.5.0 (R Development Core Team, 2018). We used the *dplyr* and *reshape2* packages (Wickham, 2011; 2018) for data management, *ggplot2* (Wickham, 2009) for visualization and the *vegan* package (Oksanen et al., 2013) for transformation-based redundancy analysis.

RESULTS

During the sampling periods, 200 bee species were found - ninety only by transect walk, forty-one only by the pan traps and sixty-nine by both sampling methods. In total, we observed 2318 individuals presented in the transect walk samples and 2788 individuals in the pan traps. The complete list of species captured by the sampling methods is listed in Supplementary Tab. 1, while the abundance and species richness of genera is listed in Tab. 1.

The effects of habitat variables on the species composition of bee communities

The triplot (Fig. 1.) highlights our findings about the influence of the interactions among the flower density and the sampling method, the vegetation cover and the average number of flowers on the species composition of bee communities (R-squared: 0.92). The interaction between the flower density (Dlow, Dhigh) and method (Mpantrap, Mtransect) resulted in the longest loadings from the transformation-based redundancy analysis (Fig. 1), thus having the highest squared multiple correlation between the fitted values for the variable and the variable itself. Most of the varied abundance of wild bees collected by the transect walk method can be explained through vegetation cover (Veg_cov).

The triplot also shows (Fig 1.) which habitat character and/ sampling method potentially contains bias/higher sampling efficiency towards some species or group of bees. Such

bee genera as *Apis* (Ap), *Bombus* (Bo), *Eucera* (Eu) and *Melitta* (Me) highly correlated with high flowering density in the case of the transect walk method, because their loadings pointed towards these observations (Dhigh:Mtransect arrows in Fig. 1). Thus, more of these species were caught by the transect method in high flower density. The abundance of *Dasypoda* spp. (Da), *Hylaeus* spp. (Hy) and *Panurgus* spp. (Pa) correlated differently, as they were mostly sampled by pan traps in lower flower density (see Dlow:Mpantrap arrows in Fig. 1). The abundance of *Andrena* spp. (An) and *Halictus* spp. (Ha) could be best explained by the average number of flowers, but it is not clear for the *Lasioglossum* spp. (La) even though a higher average number of flowers seems to be relevant compared to the other species.

The effects of habitat variables on the abundance of bee communities

Our best model fit showed (Tab. 2.) a significantly positive coefficient for (log) vegetation height (coeff: +0.16; SE: 0.06) and the (log) average number of flowers (coeff: +0.21; SE: 0.04). It indicates that a higher height of the vegetation, as well as a higher number of flowers resulted in a higher sampled bee abundance. A greater abundance of wild bees was sampled within the second period (coeff: +0.49; SE: 0.07) and in woody vegetation cover level 2 (coeff: +0.26; SE: 0.08) compared to woody vegetation below 5% (Tab. 2).

We found transect walk generally sampled fewer bees pan traps especially in pastures within the first period and under woody vegetation cover level 1 (coeff: -0.70; SE: 0.19) (Tab. 2). Looking at the coefficients, this was also true for woody vegetation cover level 3 and period 3. When we focused on the efficiency of the transect walk method in various crop types, our model found that transect walk method sampled significant lower abundance of wild bees in cereal (coeff: -0.57; SE: 0.23), and maize fields (coeff: -0.67; SE: 0.27) and higher abundance in fallow (coeff: 0.59; SE: 0.29) relative to the captures of pan traps in pastures.

The effects of habitat variables on the species richness of bee communities

In Tab. 2, the coefficients with corresponding p-values are reported for each term included in the best model. The coefficients reported for categorical variables are related to the first category. Our best model fit found that the species richness increased (i) when the vegetation height increased (coeff: +0.11; SE: 0.04), (ii) in June (compared to May) (coeff: +0.33; SE: 0.05) and (ii) in the second woody vegetation coverage level (5 to 15%) compared to the first woody vegetation cover level (below 5%) (coeff: +0.16; SE: 0.06). Furthermore, we found that fewer bee species were collected by transect walk sampling (coeff: -0.47; SE: 0.14) than those by pan traps (Tab. 2). This was a slightly different (outcome compared to the abundance model when we focused on how crop types effected of the sampling method. In maize, cereal and stubble fields, the transect walk method sampled fewer bees than pan traps (Tab. 2).

All in all, we found no significant interaction between the sampling methods and topography, and landscape heterogeneity in the case of wild bee species richness and abundance. In general, the vegetation cover also had no significant effect on the abundance and species richness of wild bees. However, we showed that the efficiency of the sampling methods was influenced by the height of the vegetation which positively correlated with bee and species abundance. The average number of flowers did not significantly affect either bee abundance or species richness.

Bee species richness and abundance in various agricultural habitat types

Sampling efficiency was investigated within multiple habitats. We found differences in the abundance and species richness of wild bees sampled by the two methods in various crop types (Fig. 2) characterized by different flower density (Fig. 3). The pan traps sampled significantly more bee species than the transect walk in stubble, maize and cereal crop fields (Fig. 2). We also showed that the bee abundance and

Table 2.

The effect of local and landscape parameters (crop type, average number of flowers, height of the vegetation, wood cover), sampling period and their interaction (noted as ":") with the sampling method (yellow water pan trap method or transect walk) on the (log) abundance and (log) species richness of wild bees based on the best model fit. One feature to note in this table is whether the coefficient is significant or not. Furthermore, note that coefficients reported for categorical variables have meaning relative to the first category (explained in the statistical analysis section).

	Wild bee abundance		Wild bee species richness	
Term	Coefficients	Std Error	Coefficients	Std Error
(Intercept)	**1.19**	0.20	0.96	0.15
Crop-Alfalfa	-0.11	0.18		
Crop-Cereal	0.16	0.20		
Crop-Fallow	0.06	0.22		
Crop-Hayfield	-0.32	0.17		
Crop-Maize	0.19	0.26		
Crop-Shrubby pasture	-0.29	0.20		
Crop-Stubble	-0.05	0.21		
Log(Flowers_average)	0.00	0.03	0.00	0.02
Log(Vegetation height)	**0.16**	0.06	**0.11**	0.04
Methodpantrap:Crop-Alfalfa			-0.01	0.13
Methodpantrap:Crop-Cereal			0.20	0.15
Methodpantrap:Crop-Fallow			0.07	0.17
Methodpantrap:Crop-Hayfield			-0.12	0.13
Methodpantrap:Crop-Maize			0.13	0.19
Methodpantrap:Crop-Shrubby pasture			-0.16	0.15
Methodpantrap:Crop-Stubble			0.03	0.15
Methodtransect	**-0.70**	0.19	**-0.47**	0.14
Methodtransect:Crop-Alfalfa	0.12	0.20	-0.05	0.11
Methodtransect:Crop-Cereal	-0.57	0.23	**-0.34**	0.13
Methodtransect:Crop-Fallow	**0.59**	0.29	0.26	0.15
Methodtransect:Crop-Hayfield	0.26	0.21	0.02	0.10
Methodtransect:Crop-Maize	**-0.67**	0.27	**-0.40**	0.17
Methodtransect:Crop-Shrubby pasture	0.44	0.24	0.11	0.11
Methodtransect:Crop-Stubble	-0.35	0.26	**-0.36**	0.14
Methodtransect:Log(Flowers_average)	**0.21**	0.04	**0.15**	0.03
Period_2	**0.49**	0.07	**0.33**	0.05
Period_3	-0.02	0.05	**-0.08**	0.04
W2	**0.26**	0.08	**0.16**	0.06
W3	0.02	0.08	0.02	0.06

Relationships found to be significant ($p < 0.05$) are highlighted in bold. The following statistics were used in case of the wild bee abundance to evaluate the model fit: Adjusted R-squared: 0.3192 (se = 0.80), F statistic = 15.99, p-value: < 2.2e-16. Furthermore, in case of the species richness of wild bees, the following statistics were used to evaluate the model fit: Adjusted R-squared: 0.2992 (se = 0.59), F statistic = 14.65, p-value: < 2.2e-16. Abbreviations of the landscape parameters: crop types (alfalfa, cereal, fallow, hayfield, maize, shrubby pasture, stubble), flowers_average (average number of flowers), vegetation height (height of the vegetation within the crop field), sampling method type (pantrap or transect walk), sampling period (1: May, 2: June, 3: July), Woody vegetation cover (W1: low, W2: middle, W3: high).

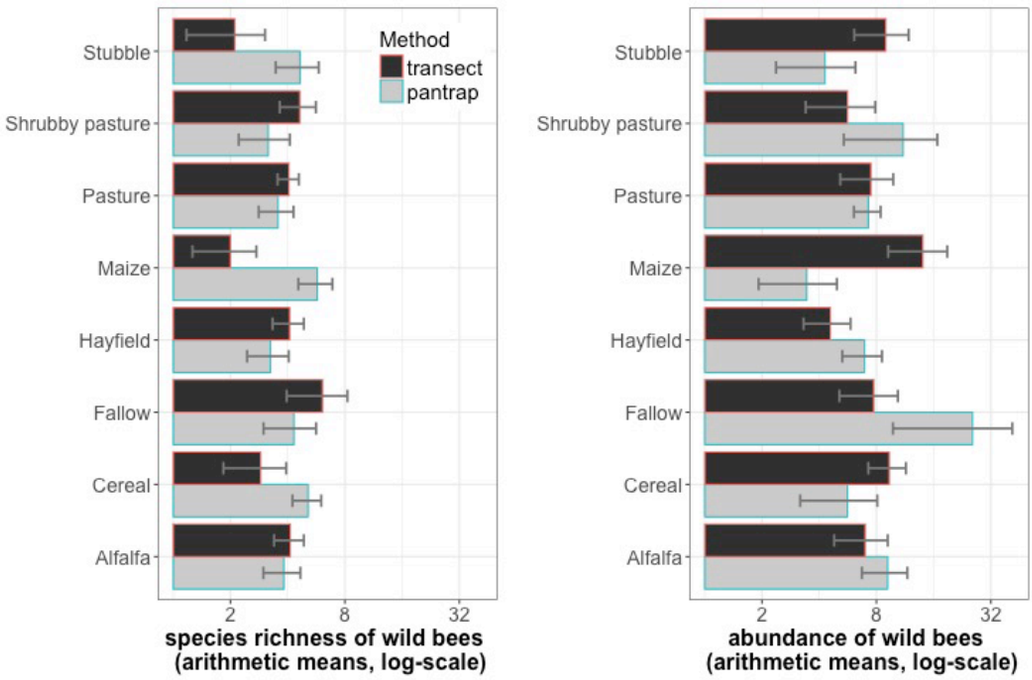

Fig. 2. Mean species richness (a) and mean abundance of wild bees (b) trapped within various crop types (cereal / fallow / hayfield / maize/ pasture / shrubby pasture / stubble) applying two bee sampling methods (yellow pan trap and transect walk). 95% confidence intervals are indicated for mean species richness and abundance of wild bees using a normal approximation.

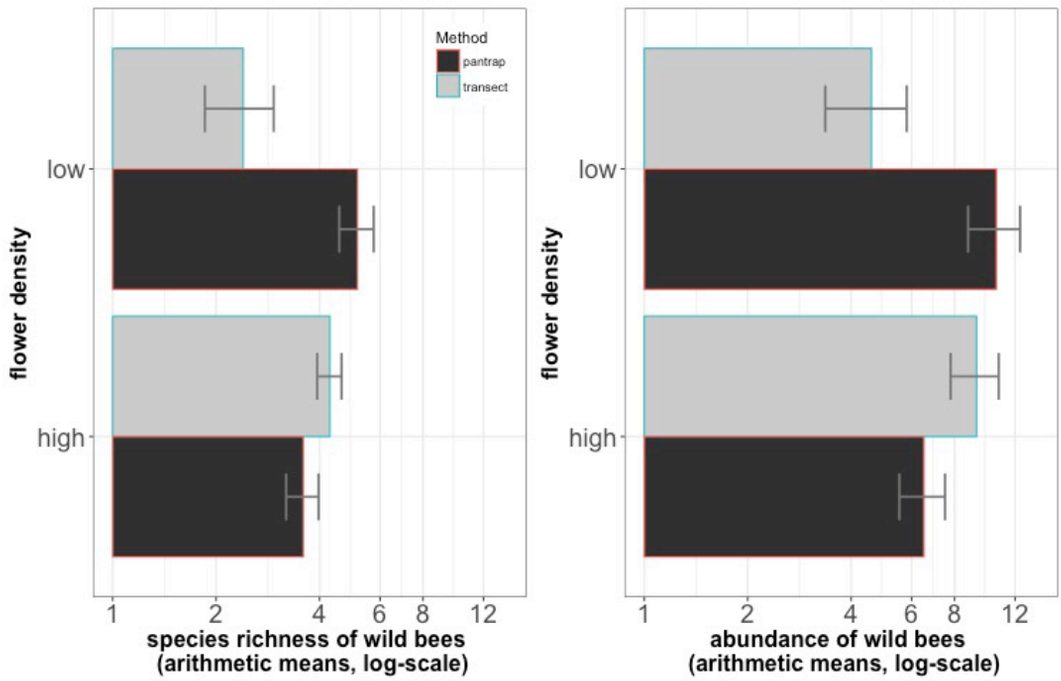

Fig. 3. Mean species richness (a) and mean abundance of wild bees (b) captured within different sampling sites characterized by low and high flower density applying two bee sampling methods (yellow pan trap and transect walk). 95% confidence intervals are indicated for the mean species richness and abundance of wild bees using a normal approximation.

species richness captured by the transect walk method were significantly higher in crop fields characterized by a higher average number of flowers. In line with this, we found that the flower density affected the efficiency of bee sampling (Fig. 3). More wild bee specimens were

collected by transect walk than pan traps when the study site was characterized by a high flower density. The opposite pattern was found in fields with low flower density, where the pan traps collected more bee species and individuals (Fig. 3).

DISCUSSION

In this study the transect walk method was compared to yellow pan traps in multiple crop fields and grasslands to observe the differences in their sampling efficiency on species richness, abundance and composition of wild bee communities. Westphal et al., (2008) had recommended the most effective bee sampling method in different agricultural fields and included in their findings our two chosen methods. However, to our knowledge, no such study had also considered the effects of local and landscape scale when comparing these two sampling methods. The application of the correct method is crucial for a representative sampling of wild bee communities, as sampling bias of different survey methods may result in under- or oversampling, under- or overrepresentation of certain bee species or groups (Aguiar & Sharkov, 1997; Bartholomew & Prowell, 2005; Kimoto et al., 2012). The performance of some sampling methods is debated based on different management, vegetation and landscape contexts (Munyuli, 2013; Nemesio & Vasconcelos, 2014).

Our study region in Transylvania harbored a highly diverse pollinator assemblage, thus providing a more sensitive area than the species-poor assemblages of intensive farmland regions characteristic in Western Europe (Kovács-Hostyánszki et al., 2016). Our results show that the average abundance and richness of bee species per site or per crop/land use types collected by the transect walk sampling method was smaller relative to that collected by the pan traps. In case of the transect walk sampling method, it provides only an actual state of the bee community, which could result in fewer bee species and individuals per sample than a longer time lapsed sampling captured by pan traps. On the other hand, a different sampling efficiency of the two methods was found in habitats of different vegetation and flower characteristics. Westphal et al. (2008) reported that pan traps had the highest sample coverage, collected the highest number of species and showed negligible collector bias, while the transect methods were also relatively efficient but had a collector bias.

In general, the density of flowers is considered as a marker for the quality and quantity of feeding resources, and the species richness and abundance of wild bees positively correlates with flower diversity (Kremen, Williams, & Thorp, 2002; Potts et al., 2003, 2004; Sárospataki et al., 2009; Nuttman et al., 2011). Our results support the "super flower" hypothesis (Kovács-Hostyánszki, Batáry, & Báldi, 2011), as the transect walk method was more efficient where floral resources were diverse and abundant in fields, whilst pan traps performed better where floral resources were sparse. However, in such agriculturally managed fields as like pastures and fallows with high flower density, pan traps are less attractive than flowering plants and underestimate the wild bee abundances. Baum & Wallen (2011) also found that pan traps undersample bee species richness and abundance when floral resources are abundant. Unfortunately, this can be a considerable disadvantage of the pan trap method and makes it more difficult to interpret the results (Cane, Minckley & Kervin, 2001; Roulston, Smith, & Brewster, 2007; Wilson, Griswold, & Messinger, 2008). On the contrary, pan traps can sample bee communities more efficiently in habitats of low flower resources, where transect sampling which is highly sensitive to flower-visiting individuals, cannot capture most of the wild bees in its short sampling time.

Flower species richness strongly associated with land use type and crop-management of the fields and showed higher values in grassland compared to arable fields (Kovács-Hostyánszki et al., 2016). Shrubby grasslands, hay meadows, fallows, pastures are considered as flower rich, alfalfa and cereal fields less so and maize fields are almost void of species (Kovács-Hostyánszki et al., 2016). Similarly, it was found that the abundance of bees and species captured by the

transect walk method were significantly higher in shrubby pastures, fallows and hayfields, crop fields characterized by a higher flower density, compared to that captured by pan traps. The pan traps collected significantly more bee species and individuals than the transect walk in stubble, maize and cereal crop fields, where flower density was lower.

An analysis of the community composition of wild bees may offer even a deeper insight into the mechanism behind the sampling methods and might reveal their biases. Composition of the samples may vary according to the bee body size which can often be used to predict their foraging behavior and thus the effect of landscape or habitat structure (Greenleaf et al., 2007; Sárospataki et al., 2009; Budrys, Budriene, & Orlovskyte, 2014; Hopfenmüller et al., 2014; Gonzalez et al., 2016). Our data shows that pan traps collected more individuals of genera characterized by smaller body size (*Dasypoda* spp., *Hylaeus* spp., *Panurgus* spp.), while larger wild bees were trapped more frequently in transect walk samples even in high flower density (*Apis* spp., *Bombus* spp., *Eucera* spp., *Melitta* spp.). This bias might be mediated either (i) by the sample collector, as one may detect smaller bees less often in the case of transect walking, or (ii) by the foraging behavior of the bees. In contrast to Westphal et al. (2008), we cannot prove that the *Andrena* species represented a lower sample size collected by the transect walk method. It was found that *Andrena* species were better sampled by the transect walk method, especially if the average number of flowers was high. Furthermore, our survey revealed that nest parasite wild bees (e.g. *Sphecodes* spp., *Pasites* spp., *Coelioxys* spp., *Epeolus* spp.) were more often in transect walk samples and less attracted by flower imitating pan traps. This is probably due to their smaller rate of flower visitation as they do not need to collect and transport pollen to their larvae (O'Toole & Raw, 1999). We have information about the body sizes of sampled bee genera from Sárospataki et al. (2009), but we did not measure them and therefore any consensus about their role in the changing habitat and landscapes can be taken only carefully.

Our study offers information about bee sampling methods that can improve planning and implementing research of wild bee communities to preserve their important role in the ecosystems. The results indicate that the transect walk method may detect more bee species and individuals in high-flower dense shrubby pastures, fallows and hayfields, whereas pan traps are more effective in low-flower dense stubble, maize and cereal crop fields. However, the positive impact of high flower density on bee species richness and abundance was mainly detected with the transect walk method, while the sampling data are biased when pan traps are applied. The authors therefore suggest (1) the application of the transect walk method for ecological studies which aim to compare flower-visiting wild bee communities in different habitats of different vegetation and flower characteristics; (2) application of both pan traps or transect walk method in ecological studies for sampling wild bee communities in habitats with similar habitat/flower resource characteristics; (3) adopting a more comprehensive sampling method that incorporates multiple sampling techniques (both transect walk and pan traps of even different colors) if the aim is to study species composition of wild bees and want to have a more complex species list on the present field. Whilst the two sampling methods yielded some good results, it would be wise to include them as part of the broader sampling protocol rather than putting our faith entirely in them. For example, the transect walk method could be appropriate to study plant-pollinator networks, if flower visitations are recorded on site (including the plant species data) and wild bee specimens are identified/stored separately.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. List of bee species collected in yellow pan traps and transect walk samples in Romanian study sites in 2012.

Genus	Species
Andrena spp.	*Andrena aeneiventris*
	Andrena bicolor
	Andrena bisulcata
	Andrena curvana
	Andrena dorsata
	Andrena falsifica
	Andrena flavipes
	Andrena fulvago
	Andrena fulvicornis
	Andrena gelriae
	Andrena gravida
	Andrena hattorfiana
	Andrena helvola
	Andrena humilis
	Andrena labialis
	Andrena labiata
	Andrena limata
	Andrena minutula
	Andrena minutuloides
	Andrena nigroaenea
	Andrena nitida
	Andrena nitidiuscula
	Andrena ovatula
	Andrena pallitarsis
	Andrena pandellei
	Andrena paucisquama
	Andrena polita
	Andrena proxima
	Andrena rosae
	Andrena schencki
	Andrena subopaca
	Andrena taraxaci
	Andrena thoracica
	Andrena varians
	Andrena ventralis

Bombus spp.	*Andrena ventricosa*
	Andrena viridescens
	Andrena wilkella
Anthidium spp.	*Anthidium byssinum*
	Anthidium laterale
	Anthidium punctatum
Anthophora spp.	*Anthophora crinipes*
	Anthophora furcata
	Anthophora plagiata
	Anthophora plumipes
	Anthophora pubescens
Apis spp.	*Apis mellifera*
	Bombus argillaceus
	Bombus hortorum
	Bombus humilis
	Bombus muscorum
	Bombus pascuorum
	Bombus pratorum
	Bombus ruderarius
	Bombus sylvarum
	Bombus terrestris
Ceratina spp.	*Ceratina cyanea*
	Ceratina nigrolabiata
Chelostoma spp.	*Chelostoma distinctum*
	Chelostoma florisomne
	Chelostoma rapunculi
Coelioxys spp.	*Coelioxys afra*
	Coelioxys mandibularis
Colletes spp.	*Colletes cunicularius*
	Colletes daviesanus
	Colletes hylaeiformis
	Colletes similis
Dasypoda spp.	*Dasypoda hirtipes*
Diodontus spp.	*Diodontus minutus*
Epeoloides spp.	*Epeoloides coecutiens*
Epeolus spp.	*Epeolus variegatus*
Eucera spp.	*Eucera clypeata*
	Eucera interrupta
	Eucera longicornis
	Eucera nigrescens

	Eucera pollinosa
	Eucera spectabilis
Halictus spp.	*Halictus brunnescens*
	Halictus calceatum
	Halictus confusus
	Halictus eurygnathus
	Halictus kessleri
	Halictus langobardicus
	Halictus leucaheneus
	Halictus maculatus
	Halictus malachurum
	Halictus morio
	Halictus patellatus
	Halictus quadricinctus
	Halictus rubicundus
	Halictus scabiosae
	Halictus semitectus
	Halictus sexcinctus
	Halictus simplex
	Halictus smaragdulus
	Halictus subauratus
	Halictus tataricus
	Halictus terrestris
	Halictus tumulorum
Heriades spp.	*Heriades crenulatus*
	Heriades truncorum
Hylaeus spp.	*Hylaeus angustatus*
	Hylaeus annularis
	Hylaeus brevicornis
	Hylaeus communis
	Hylaeus confusus
	Hylaeus cornutus
	Hylaeus duckei
	Hylaeus moricei
	Hylaeus nigritus
	Hylaeus pfankuchi
	Hylaeus sinuatus
	Hylaeus styriacus
	Hylaeus trinotatus
	Hylaeus variegatus

Lasioglossum spp.	*Lasioglossum albipes*
	Lasioglossum brevicorne
	Lasioglossum calcaratus
	Lasioglossum calceatum
	Lasioglossum corvinum
	Lasioglossum costulatum
	Lasioglossum discum
	Lasioglossum fulvicorne
	Lasioglossum glabriusculum
	Lasioglossum griseolum
	Lasioglossum interruptum
	Lasioglossum laevigatum
	Lasioglossum langobardicus
	Lasioglossum laticeps
	Lasioglossum lativentre
	Lasioglossum leucozonium
	Lasioglossum lineare
	Lasioglossum lucidulum
	Lasioglossum maculatus
	Lasioglossum majus
	Lasioglossum malachurum
	Lasioglossum malachurum/pauxillum
	Lasioglossum marginatum
	Lasioglossum morio
	Lasioglossum nigripes
	Lasioglossum pauxillum
	Lasioglossum politum
	Lasioglossum punctatissimum
	Lasioglossum puncticolle
	Lasioglossum sexcinctus
	Lasioglossum sexstrigatum
	Lasioglossum simplex
	Lasioglossum tataricus
	Lasioglossum truncaticolle
	Lasioglossum villosulum
	Lasioglossum xanthopus
	Lasioglossum zonulum
Lithurgus spp.	*Lithurgus chrysurus*
Macropis spp.	*Macropis europaea*
Megachile spp.	*Megachile centuncularis*

	Megachile ericetorum
	Megachile maritima
	Megachile melanopyga
	Megachile pilidens
	Megachile rotundata
	Megachile versicolor
Melitta spp.	*Melitta dimidiata*
	Melitta leporina
	Melitta nigricans
	Melitta tricincta
Melitturga spp.	*Melitturga clavicornis*
Micrandrena spp.	*Micrandrena sp.*
Nomada spp.	*Nomada alboguttata*
	Nomada basalis
	Nomada bluethgeni
	Nomada fucata
	Nomada pleurosticta
	Nomada trispinosa
Nomia spp.	*Nomia diversipes*
	Nomia unidentata
Osmia spp.	*Osmia adunca*
	Osmia aurulenta
	Osmia bicolor
	Osmia bidentata
	Osmia cerinthidis
	Osmia leaiana
	Osmia leucomelana
	Osmia rufa
	Osmia rufohirta
	Osmia spinulosa
	Osmia tergestensis
Panurgus spp.	*Panurgus calcaratus*
Pasites spp.	*Pasites maculatus*
Sphecodes spp.	*Sphecodes ephippius*
	Sphecodes gibbus
	Sphecodes majalis
	Sphecodes monilicornis
	Sphecodes reticulatus
	Sphecodes rufiventris
	Sphecodes scbricollis

Systropha spp.	*Systropha curvicornis*
	Systropha planidens
Tetralonia spp.	*Tetralonia alticincta*
	Tetralonia dentata
	Tetralonia salicariae
Triepeolus spp.	*Triepeolus tristis*

All analyses were based on species level except the transformation-based redundancy analysis (that was genus based) applied for community composition analysis.