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Assessing bee (Hymenoptera: Apoidea) diversity of an Illinois restored tallgrass prairie: methodology and conservation considerations

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Abstract Bee species diversity and the effectiveness of four sampling methods were investigated in a west-central Illinois restored tallgrass prairie. Bees were sampled using malaise traps, ground-level pan traps, elevated pan traps, and vane traps. A total of 4,622 bees representing 31 genera and 111 species were collected. Malaise traps collected the greatest number of bees and species, and groundlevel pan traps the least. Among the pan traps and vane traps, blue-colored traps collected the greatest abundance and species richness, and yellow traps the least. Chao1 estimator and rarefaction analyses showed that substantial increases in sample sizes would be necessary to achieve asymptotic species richness levels, particularly if groundlevel pan traps alone were used. Elevated pan traps and vane traps collected relatively similar species composition. Different colored pan traps at the same height collected more similar species composition than did those at different heights, but species composition of blue ground-level pan traps was relatively similar to elevated pan traps, regardless of color. Indicator species analysis revealed 22 species that were significantly associated with a specific trap type, and 11 species that were associated with a particular pan trap color/elevation. Results of this study show that elevated traps can increase the effectiveness of bee surveys in tallgrass prairie, and that a combination of trap types gives a more complete picture of the bee fauna than

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does a single survey method. These results should be considered along with cost, ease of use, and goals when planning and designing bee inventories.

Keywords Pan traps · Malaise traps · Vane traps · Bee inventory - Bee sampling methods - Grassland bees

Introduction

Pollination is an essential ecological function; it is estimated that over 90 % of angiosperms are animal-pollinated (Buchman and Nabhan [1996\)](#page-12-0), including a majority of major agricultural crops (Klein et al. [2007\)](#page-12-0). The complex nature of plant–pollinator relationships means that loss of important pollinators could lead to extinction cascades (Rathcke and Jules [1993](#page-13-0); Biesmeijer et al. [2006](#page-12-0).). Bees are among the most important pollinators. However, there is evidence that some bee populations are declining (Biesmeijer et al. [2006](#page-12-0); Byrne and Fitzpatrick [2009;](#page-12-0) Bartomeus et al. [2013](#page-12-0); Burkle et al. [2013](#page-12-0)), with habitat alteration playing an important role (Hines and Hendrix [2005;](#page-12-0) Byrne and Fitzpatrick [2009](#page-12-0); Grixti et al. [2009\)](#page-12-0). Habitat alteration and fragmentation are particularly acute in the Midwestern USA, where native forests and prairies have been reduced to small patches within large areas of cultivation and urbanization (Ramankutty and Foley [1999\)](#page-13-0). Prairies at one time covered more than 60 % of Illinois, but most have been replaced with agricultural fields and pastures (Bowles et al. [2003](#page-12-0); Guyette et al. [2003](#page-12-0)). Pastures and commonly planted crops such as corn and soybeans offer bees few resources in comparison with native prairies.

In such an environment, patches of restored prairie could serve as refugia for native bees (Hopwood [2008](#page-12-0)). Baseline data on bee species abundance and diversity is

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essential so that future monitoring can be used to evaluate trends in bee communities (Marlin and LaBerge [2001](#page-12-0); Biesmeijer et al. [2006;](#page-12-0) Cameron et al. [2011;](#page-12-0) Bartomeus et al. [2013;](#page-12-0) Burkle et al. [2013\)](#page-12-0). Furthermore, an understanding of the limits and biases inherent in bee sampling methodology is crucial in obtaining a complete picture of bee diversity and conservation status.

There are a variety of methods used to sample bees for inventory and monitoring purposes. The most commonly used method involves pan traps, colored plastic bowls placed on the ground and filled with soapy water to trap bees (Leong and Thorp [1999](#page-12-0); Cane et al. [2000;](#page-12-0) Roulston et al. [2007;](#page-13-0) Westphal et al. [2008;](#page-13-0) Droege et al. [2010](#page-12-0)). However, there is evidence of trap bias (some species are over or underrepresented) associated with pan trap sampling (Cane et al. [2000](#page-12-0); Bartholomew and Prowell [2005](#page-12-0); Roulston et al. [2007;](#page-13-0) Tuell and Isaacs [2009\)](#page-13-0), and trap color may influence bee catches as well (Cane et al. [2000](#page-12-0); Bartholomew and Prowell [2005](#page-12-0); Giles and Ascher [2006](#page-12-0); Campbell and Hanula [2007;](#page-12-0) Kwaiser and Hendrix [2008](#page-12-0)). In addition, trap height may affect species collections; it has been found that different bee species may forage at different heights in blueberry cropland (Tuell and Isaacs [2009\)](#page-13-0) and forests (Ulyshen et al. [2010](#page-13-0); Nuttman et al. [2011\)](#page-12-0). In addition to pan traps, at least two types of flight intercept traps have been used to sample native bees: vane traps and malaise traps. Vane traps have shown promise in sampling of large bees such as bumble bees that may be underrepresented in pan trap collections (Stephen and Rao [2005;](#page-13-0) Kimoto et al. [2012](#page-12-0)). Malaise traps are effective in collecting a variety of flying insects, including bees and other hymenopterans (Sugar et al. [1998](#page-13-0); Bartholomew and Prowell [2005;](#page-12-0) Ozanne [2005](#page-13-0); Ngo et al. [2013](#page-12-0)). These two methods have been less extensively tested for native bee sampling than have pan traps. In general, there have been few quantitative studies comparing bee survey methods, and none comparing all of these trapping methods in a tallgrass prairie. The objectives of this study were (1) to document the species composition of bees in an Illinois restored prairie, and (2) to compare the performance of different methods of sampling bee species richness and composition.

Methods

This study was conducted from early June to early October 2010 in a ca. 12 ha restored prairie $(40^{\circ}21'57''N,$ 91°24'24"W) at Western Illinois University's Alice L. Kibbe Life Science Station, located near Warsaw, in Hancock County, Illinois. This prairie is managed using prescribed fire and, to a lesser extent, $Tordon^{\circledR}$ and Garlon[®], herbicides used for the control of black locust and other woody invasives. The prairie is burned every 2–3 years, and had been burned most recently in Spring 2009.

Bees were sampled using four collection methods: pan traps (at ground level), pan traps (elevated 1 m), malaise traps, and vane traps. Pan traps were 354 ml colored plastic bowls (Hallmark, Kansas City, MO) which were blue, white, or yellow in color (Leong and Thorp [1999](#page-12-0)). Groundlevel pan traps were placed directly on the ground. Vegetation directly above the trap that might impede bee detection of the trap or entry into the trap was removed; otherwise, disturbance to vegetation was minimized as much as possible. Elevated pan traps were positioned 1 m above ground level via attachment to the top of a 3.8 cm by 3.8 cm wooden post placed in the ground. Two stacked bowls were attached to the post using a 2.5 cm course threaded black drywall screw. Once these bowls were secured a third removable bowl was stacked on for trapping and sample collection. Malaise traps (Townes [1972](#page-13-0)), purchased from Sante Traps, Lexington, KY, are large (ca. 2 m high) mesh fabric flight interception traps that are effective in collecting flying insects, which contact the central, vertical portion of the net, and move up the sloping roof to the collection container. Vane traps (SpringStar Inc., Woodinville, WA) are multidirectional traps consisting of two colored (blue or yellow) plastic cross vanes with a collection container beneath. Insects contact the vane and fall into the collection container. Vane traps were hung from shepherd hooks with the top of the collection jar 1 m above the ground.

A transect of twenty study plots (four trap types replicated five times) was established across the prairie. Each plot was composed of 3 elevated pan traps (1 blue, 1 white, and 1 yellow) or 3 ground-level pan traps (1 blue, 1 white, and 1 yellow) or 4 vane traps (2 blue and 2 yellow) or 1 malaise trap. Within each plot containing multiple traps each trap was separated by 5 m based on recommendations for pan trap sampling (Droege et al. [2010\)](#page-12-0), and plots were separated by 25 m. The order of the plots, as well as the order of traps within each plot, was randomly assigned. Traps contained soapy water (Method $^{\circledR}$ Natural and Biodegradable Dishwashing Soap, San Francisco, CA). Pan traps were filled with ca. 270 ml of soapy water, vane trap collection jars with ca. 800 ml and malaise trap collection bottles with ca. 450 ml. Trapping was done on 6 days within a 10-day period, based on weather conditions, from 0900 to 1800 hours. The first trapping period consisted of the dates 3 June, 6 June, 7 June, 9 June, 10 June and 11 June. The second trapping period consisted of the dates 16 July, 17 July, 19 July, 20 July, 21 July and 22 July. The third trapping period consisted of the dates 22 August through 27 August. The fourth trapping period consisted of the dates 27 September, 29 September, 30 September, 1

October, 2 October and 3 October. Collected bees were identified by Sam Droege (USGS Patuxent Wildlife Research Center, Beltsville, MD), Mike Arduser (Missouri Department of Conservation, St. Charles, MO), Rob Jean (Saint Mary-ofthe-Woods College, Saint Mary-of-the-Woods, IN), Jared Ruholl (Lewis and Clark Community College National Great Rivers Research and Education Center, Vandalia, IL), and by the authors, using the online Discover Life identification keys for the genera Andrena [\(http://www.discoverlife.org/mp/](http://www.discoverlife.org/mp/20q?guide=Andrena_female) [20q?guide=Andrena_female](http://www.discoverlife.org/mp/20q?guide=Andrena_female), and [http://www.discoverlife.](http://www.discoverlife.org/mp/20q?guide=Andrena_male) [org/mp/20q?guide=Andrena_male,](http://www.discoverlife.org/mp/20q?guide=Andrena_male) by Larkin L, Andrus R, Droege S), Ceratina ([http://www.discoverlife.org/mp/20q?](http://www.discoverlife.org/mp/20q?guide=Ceratina) [guide=Ceratina,](http://www.discoverlife.org/mp/20q?guide=Ceratina) by Droege S, Rehan S), and Melissodes [\(http://www.discoverlife.org/mp/20q?guide=Melissodes_](http://www.discoverlife.org/mp/20q?guide=Melissodes_female) [female](http://www.discoverlife.org/mp/20q?guide=Melissodes_female), and [http://www.discoverlife.org/mp/20q?guide=](http://www.discoverlife.org/mp/20q?guide=Melissodes_male) [Melissodes_male,](http://www.discoverlife.org/mp/20q?guide=Melissodes_male) by Andrus R, Droege S) and published references (Gibbs [2010](#page-12-0), [2011\)](#page-12-0).

Chi square tests were used to test for differences in abundance and species richness of bees collected by different colors of pan traps and vane traps. Bee species composition (species present and their relative abundances) was compared among collection methods using the multiresponse permutation procedure (MRPP). MRPP provides a measure (A value) of the extent to which species composition in different groups deviate from each other. The larger the A value, the more the groups deviate from one another. A values less than 0.1 are common in ecological studies, and values >0.3 are relatively high (McCune and Grace [2002\)](#page-12-0). MRPP was done including and excluding Lasioglossum versatum (Robertson). This was done because L. versatum comprised a large proportion (54 %) of the total collection (Table [1](#page-3-0)), and could potentially mask overall patterns. To examine differences between individual collection methods, series of paired MRPP comparisons were done. Holm's step-down procedure was used to correct for multiple comparisons (Holm [1979\)](#page-12-0). In this procedure, the nth smallest p value is compared with 0.05/(# of comparisons $+ 1 - n$). Hierarchical cluster analysis, with Euclidian distance measure and Ward's linkage method, was used to produce a dendrogram showing relative similarities in bee species assemblages among pan trap color and elevation combinations. Indicator species analysis (Dufrêne and Legendre [1997](#page-12-0)), or ISA, was used to identify particular bee species that were strongly associated with specific collection methods. ISA provides a measure of the extent to which individual species are exclusive (never occurring elsewhere) and faithful (always present) to a particular collection method (McCune and Grace [2002\)](#page-12-0). An indicator value can range from zero to 100, with the latter representing perfect indication, that is, perfect association with a particular collection method. ISA was also done including and excluding L. versatum. Cluster analysis, MRPP, and ISA were performed using PC-Ord Version 4.25 software.

The Chao1 estimator was used to estimate asymptotic species richness. This analysis estimates the total number of species present based on the frequency of rare species collected (Chao et al. [2009](#page-12-0)). Because species richness is affected by sample size, rarefaction analyses were done to allow comparisons of bee species richness among trap types. Rarefaction allows comparison of species richness associated with different sample sizes by standardizing all samples to a common sample size (Krebs [1989](#page-12-0)) which, in this study, was obtained with ground-level pan traps. The University of Alberta rarefaction calculator [\(http://www.biology.ualberta.](http://www.biology.ualberta.ca/jbrzusto/rarefact.php#Calculator) [ca/jbrzusto/rarefact.php#Calculator,](http://www.biology.ualberta.ca/jbrzusto/rarefact.php#Calculator) by Brzustowski J) and the Chao excel sheet calculator (Ecological Archives E090- 073-S1, Chao et al. [2009\)](#page-12-0) were used to calculate rarefaction and Chao1 estimates.

Specimens identified as Melissodes sp., Lasioglossum sp., Ceratina calcarata Robertson/Ceratina dupla Say, Ceratina calcarata/Ceratina mikmaqi Rehan and Sheffield, and Ceratina dupla/Ceratina mikmaqi were not included in the above analyses nor in species richness totals because these specimens were believed to belong to one or more species already present in the richness totals. However, specimens identified as Sphecodes sp. were included because they represented a unique morphospecies. The single individual identified as Nomada sayi Robertson/ Nomada illinoensis Robertson was also included in the above analyses. Hylaeus affinis (Smith) and Hylaeus modestus Say were treated as a single species in statistical analyses because most could not be determined as belonging to one or the other species due to outstanding difficulties in separating females in this species complex. However, in determining species richness, *H. affinis* and H. modestus were counted as separate species when identified males of each were present.

Results

A total of 4,622 bees representing 31 genera and 111 species were collected (Table [1](#page-3-0)). The June sampling period yielded 2,755 bees and 85 species, 1,581 bees and 60 species were collected during the July period, 204 bees and 33 species during the August period, and 82 bees and 26 species during the September–October period. Lasioglossum versatum was the most abundant species, accounting for 54 % of the total collection. The other six most abundant species [Augochlora pura (Say), Augochlorella aurata (Smith), Augochloropsis metallica (Fabricius), Ceratina calcarata, Ceratina dupla, and Lasioglossum smilacinae (Robertson)] comprised 22 % of the total collection. Elevated pan traps collected 763 bees and 69 species, ground-level pan traps collected 442 bees and 41 species, malaise traps collected 2,911 bees and 83 species, and vane traps collected 506 bees and 51 species. Elevated

Table 1 Numbers of bees collected using four trapping methods and dates of collection, with species richness, Chao1 estimates, additional sample size required to achieve a specified percentage of Chao1

estimate, and rarefaction estimates (±95 % confidence intervals) in Hancock County, Illinois, June–October 2010

Table 1 continued

Table 1 continued

Table 1 continued

EPT elevated pan traps, GPT ground-level pan traps, MT malaise traps, VT vane traps

For dates collected, $A = 3$ June–11 June, $B = 16$ July–22 July, $C = 22$ August–27 August, $D = 27$ September–3 October

pan traps collected significantly greater numbers of individuals $(X^2 = 85.511, df = 1, p < 0.0001)$ and species $(X^2 = 7.000, df = 1, p = 0.0082)$ than did ground-level pan traps. Among the elevated pan traps, blue traps collected 322 bees representing 51 species, white traps collected 238 bees representing 36 species, and yellow traps collected 203 bees representing 32 species. Distributions were significantly different from random for numbers of individuals ($X^2 = 29.413$, $df = 2, p < 0.0001$) but not for species richness ($X^2 = 5.059$, $df = 2, p = 0.0797$. Among the ground-level pan traps, blue traps collected 237 bees representing 29 species, white traps collected 125 bees representing 25 species, and yellow traps collected 80 bees representing 14 species. Distributions were again significantly different from random for numbers of individuals ($X^2 = 88.729$, $df = 2$, $p < 0.0001$) but not for species richness ($X^2 = 5.324$, $df = 2$, $p = 0.0698$). Among the vane traps, blue traps collected 465 bees representing 44 species and yellow traps collected 41 bees representing 19 species. Blue vane traps collected significantly more individuals $(X^2 = 355.289, df = 1, p < 0.0001)$ and species $(X^2 = 9.921, df = 1, p < 0.0016)$ than did yellow vane traps.

Chao1 analyses produced estimates of asymptotic species richness ranging from 65.22 to 108.00 for individual trap types and 132.53 overall (Table [1](#page-3-0)). Based on Chao1 estimates, an additional sample of 22,987.16, or 4.97 times the original sample, would be needed to reach the overall Chao1 estimate. For individual sampling methods, additional samples ranging from 4.61 (vane traps) to 11.04 (ground-level pan traps) times the original samples would have been required to obtain the Chao1 estimates of species richness (Fig. [1\)](#page-7-0). To reach 90 % of total richness, a 0.46 fold increase in overall captures would have been needed based on the Chao1 estimate, with sample increases ranging from 0.69-fold (vane traps) to 2.63-fold (ground-level pan traps) required to obtain 90 % of Chao1 estimates of species richness. To reach 80 % of species richness, sample increases ranging from 0.08-fold (vane traps) to 1.33-fold (ground-level pan traps) would have been needed.

Species richness for ground-level pan trap collections was less than the lower 95 % confidence limits of rarefaction estimates for elevated pan traps, vane traps, and for the overall total collection, but was within the 95 % confidence interval for malaise traps (Table [1](#page-3-0)). When L. versatum were excluded from analyses, rarefaction estimates for malaise traps (61.89 ± 6.12) and for the overall total (69.67 \pm 7.03) were significantly greater than the species richness obtained with ground-level pan traps and vane traps, but not elevated pan traps.

Significant differences in species composition (with L. versatum included in the analysis) were found among all trap types ($A = 0.1674$, $p < 0.0001$). Among paired comparisons (Table [2](#page-7-0)), species composition for elevated pan traps and ground-level pan traps were relatively dissimilar $(A = 0.1532)$, as were those for ground-level pan traps and malaise traps $(A = 0.1507)$. A values generally increased when L. versatum was excluded from analyses, with an overall A value of 0.2325 ($p < 0.0001$). For paired Fig. 1 Sample size increases required to achieve 80, 90, and 100 % of Chao1 estimates of actual bee species richness using four sampling methods in a restored prairie in Hancock County, Illinois USA, June– October 2010

Table 2 Multi-response permutation procedure A values (with p values in parentheses) for paired comparisons of bee species assemblages collected using four trapping methods in Hancock County, Illinois, June–October 2010

All differences between trapping methods were significant after adjustment for multiple comparisons using Holm's step-down procedure

EPT elevated pan traps, GPT ground-level pan traps, MT malaise traps, VT vane traps

comparisons excluding L. versatum (Table 2), the greatest similarity in species composition (lowest A value) was found for elevated pan traps versus vane traps (0.0977) and the least similarity for ground-level pan traps versus malaise traps (0.2345) and elevated pan traps versus ground-level pan traps (0.2029). In general, ground-level pan trap species composition was most dissimilar to that collected by other trap types, particularly elevated pan traps and malaise traps.

Results of ISA for trap types, including and excluding L. versatum, yielded the same indicator species associated with the same trap types. The values presented in Table [3](#page-8-0) are results of analyses that included L. versatum. There were a total of 22 species identified as significantly associated with a particular trap type. Fourteen of them were associated with malaise traps, four with elevated pan traps, three with vane traps and one with ground-level pan traps. Genus-level patterns were evident, with two Bombus species collected being associated with vane traps, two Heriades species associated with malaise traps, and all eight Lasioglossum indicator species associated with malaise traps or elevated pan traps.

Significant overall differences in species composition among pan trap position (ground-level vs. elevated) and color combinations were found ($A = 0.1659$, $p < 0.0001$). Results of paired comparison MRPP analyses of variation in species composition among pan trap position and color are shown in Table [4](#page-9-0), and the cluster analysis dendrogram of similarities in species composition is shown in Fig. [2.](#page-9-0) In general, species composition was more dissimilar in comparisons between pan traps at different elevations, but blue ground-level pan traps were relatively similar to elevated pan traps. A total of 11 species were significantly associated with a particular trap color/location, based on ISA (Table [5\)](#page-10-0). Four of these were associated with blue elevated pan traps, four with white elevated pan traps, two with yellow elevated pan traps, and two with blue ground-level pan traps [Hylaeus mesillae (Cockerell) was equally strongly associated with elevated white and yellow pan traps]. Both Ceratina indicator species were associated with blue ground-level pan traps, whereas *Lasioglossum* indicator species were associated with either white (three species) or yellow (one species) elevated pan traps. Both Melissodes indicator species were associated with blue elevated pan traps.

Table 3 Indicator values for species significantly associated with a particular trap type based on indicator species analysis of bees collected using four trapping methods in Hancock County, Illinois, June–October 2010

Species	p value	Indicator values				
		Elevated pan traps	Ground-level pan traps	Malaise traps	Vane traps	
Agapostemon sericeus	0.023			59	5	
Andrena robertsonii	0.003	4	0	71	0	
Augochlora pura	0.007	25	3	53	18	
Augochlorella aurata	0.024	25	21	43	9	
Augochloropsis metallica	0.001	6		86		
Bombus auricomus	0.001		0	Ω	96	
Bombus bimaculatus	0.003	0	0	2	91	
Heriades carinata	0.001	Ω	0	100	$\mathbf{0}$	
Heriades variolosa	0.017	5	0	69	0	
Hoplitis spoliata	0.022	2	65	2	0	
Hylaeus affinis/modestus	0.007	16	6	63	0	
Hylaeus mesillae	0.005	61	0	26	3	
Lasioglossum birkmanni	0.037	Ω	0	60	Ω	
Lasioglossum coriaceum	0.001	59	6		12	
Lasioglossum hitchensi	0.028	15	9	59	0	
Lasioglossum obscurum	0.001		0	90		
Lasioglossum oceanicum	0.022	59				
Lasioglossum smilacinae	0.002	22		74		
Lasioglossum subviridatum	0.001	2		76		
Lasioglossum truncatum	0.034	55	θ	11	16	
Megachile mendica	0.003		22	62	2	
Melissodes trinodis	0.004	29	$\boldsymbol{0}$	$\mathbf{0}$	64	

MRPP comparison of vane trap collections revealed a significant difference in species composition between blue and yellow vane traps $(A = 0.1453, p = 0.0010)$. ISA analysis of vane trap collections revealed eight species that were significantly associated with blue vane traps (with indicator values and p values): A. pura (IV = 98, $p = 0.0090$), Bombus auricomus (Robertson) (IV = 89, $p = 0.0090$), Bombus bimaculatus Cresson (IV = 100, $p = 0.0090$), Bombus griseocollis (DeGeer) (IV = 80, $p = 0.0470$), C. calcarata $(IV = 96, p = 0.0180), C. \text{ dupla } (IV = 100, p = 0.0090),$ *Eucera hamata* (Bradley) (IV = 90, $p = 0.0220$), and Melissodes trinodis Robertson (IV = 83, $p = 0.0090$). No indicator species were associated with yellow vane traps.

Discussion

Historical records of bee species composition have proven invaluable in assessing changes in the bee fauna of specific geographic locations over time (Marlin and LaBerge [2001](#page-12-0); Biesmeijer et al. [2006;](#page-12-0) Cameron et al. [2011;](#page-12-0) Bartomeus et al. [2013](#page-12-0); Burkle et al. [2013](#page-12-0)). Trapping represents a relatively time-efficient means of assessing bee species

composition, but our results suggest that different trapping methods can give different estimates of the bee fauna. While there have been other studies comparing bee sampling methods, these have focused primarily on pan traps or have been restricted to higher taxonomic levels or general abundance. Our study represents the most complete quantitative comparison of these four bee sampling methods at the species level.

The total of 111 species of bees collected in this study is generally similar to species richness values obtained in other recent bee studies done in North American grassland habitats. Reed [\(1995](#page-13-0)) collected 127 species in Minnesota tallgrass prairie using aerial netting. Davis et al. [\(2008](#page-12-0)), using pan traps, found 86 species of bees in northwest Iowa tallgrass prairie remnants, and Kwaiser and Hendrix ([2008\)](#page-12-0) collected 56 species of bees with pan traps and sweep netting in native tallgrass prairie fragments and ruderal areas, also in northwest Iowa. Hopwood [\(2008](#page-12-0)), using aerial netting and pan traps placed at ground level and three different heights, collected 95 species of bees in restored and weedy roadside habitats in northeastern Kansas. Kearns and Oliveras [\(2009](#page-12-0)) collected 104 bee species in Boulder, Colorado grasslands using hand-netting and pan

Table 4 Multi-response permutation procedure A values (with p values in parentheses) for paired comparisons of bee species assemblages collected using ground-level and elevated pan traps of three different colors in Hancock County, Illinois, June–October 2010

Comparison	MRPP $(p$ value)
EPT-B versus EPT-W	0.0815(0.0292)
EPT-B versus EPT-Y	0.0566(0.0439)
EPT-W versus EPT-Y	0.0367(0.1375)
GPT-B versus GPT-W	0.0712(0.0355)
GPT-B versus GPT-Y	$0.1469(0.0020)*$
GPT-W versus GPT-Y	0.0794(0.0308)
EPT-B versus GPT-B	0.0589(0.0497)
EPT-B versus GPT-W	$0.1742(0.0038)*$
EPT-B versus GPT-Y	0.2093 $(0.0018)*$
EPT-W versus GPT-B	0.0832(0.0298)
EPT-W versus GPT-W	0.1248(0.0054)
EPT-W versus GPT-Y	$0.1668(0.0012)$ *
EPT-Y versus GPT-B	0.0569(0.0748)
EPT-Y versus GPT-W	0.1546(0.0055)
EPT-Y versus GPT-Y	$0.1433(0.0032)$ *

EPT-B blue elevated pan traps, EPT-W white elevated pan traps, EPT-Y yellow elevated pan traps, GPT-B blue ground-level pan traps, GPT-W white ground-level pan traps, GPT-Y yellow ground-level pan traps

* Significant difference between trapping methods after adjustment for multiple comparisons using Holm's step-down procedure

trapping. Richards et al. [\(2011](#page-13-0)) collected 124 species in southern Ontario restored grasslands using a combination of pan traps and sweep and aerial netting. Kimoto et al. [\(2012](#page-12-0)), using blue vane traps, identified 94 species and 117 morphospecies in a study of bees inhabiting bunchgrass prairie in northeastern Oregon. A comprehensive review of the bees recorded in Canadian prairie ecozones documented 215, 218 and 299 bee species from prairies in Saskatchewan, Manitoba, and Alberta, respectively (Sheffield et al. [2014\)](#page-13-0).

In our study, the Chao1 estimate of 132.53 species (Table [1](#page-3-0)) suggests that a substantial proportion of bee species richness of Kibbe Life Science Station restored prairie remains undocumented. An estimated additional sample size of nearly 23,000 bees would have been required to collect all species. Use of additional methods such as trap-nests and insect nets might uncover additional species, though trap-nesting bees such as Hylaeus, Xylocopa virginica (L.), and several species of Megachilidae (Michener et al. [1994](#page-12-0)) were collected in our study. Active search-and-net collecting can complement trapping by yielding species that are absent or poorly represented in trap samples. For instance, relative to netting, ground-level pan traps generally yield low numbers of fast and highflying species such as *Megachile*, Colletes, and *Melissodes* (Giles and Ascher [2006\)](#page-12-0). In our study, Colletes were rare,

Fig. 2 Dendrogram showing relative similarities in bee species composition collected using three pan trap colors at ground level and elevated 1 m in a restored prairie in Hancock County, Illinois USA, June–October 2010. EPT-B blue elevated pan traps, EPT-W white elevated pan traps, EPT-Y yellow elevated pan traps, GPT-B blue ground-level pan traps, GPT-W white ground-level pan traps, GPT-Y yellow ground-level pan traps

but Melissodes were relatively abundant in elevated pan traps and vane traps (Table [1\)](#page-3-0), suggesting that these trap types may compensate for the ineffectiveness of groundlevel pan traps in collecting bees of this genus. Likewise, Megachile were relatively well represented in malaise traps in our study. Oligolectic bees are often more effectively collected by focused efforts on their host plants rather than by pan traps (Cane et al. [2000\)](#page-12-0). On the other hand, small, difficult-to-see bees can sometimes be less well represented in active search-and-net collections than in traps. Netting techniques such as less focused sweep netting across vegetation can sometimes yield higher numbers and richness of these smaller species than active searching alone. Earlier sampling in our study would have likely produced some additional early season species as well, such as springflying members of the genus *Andrena* and the cleptoparasitic genus Nomada. Some bee species have short phenologies often restricted to 6–8 weeks in the spring or summer.

Trap elevation and color had an important influence on abundance and species richness of bees collected in pan traps. Blue traps collected the greatest bee abundance and species richness at both trap elevations, and yellow traps the least. Cane et al. [\(2000](#page-12-0)) also found that blue pan traps outperformed yellow pan traps in a study of an Arizona desert shrub bee community, and Campbell and Hanula [\(2007](#page-12-0)) collected significantly more bees with blue than with white or yellow elevated pan traps in southeastern US forests. In contrast, blue was the least effective color in a northeastern US forest (Giles and Ascher [2006](#page-12-0)). Kwaiser and Hendrix [\(2008](#page-12-0)) collected greater numbers of bees with yellow than with blue or white pan traps in northwestern Iowa grasslands, and Bartholomew and Prowell ([2005\)](#page-12-0) found white pan traps collected significantly fewer bee

Table 5 Indicator values and randomization tests of significance for species significantly associated with a particular pan trap color/location based on indicator species analysis of bees collected using ground-level and elevated pan traps of three different colors in Hancock County, Illinois, June–October 2010

Species	p value	Indicator values					
		EPT-B	EPT-W	EPT-Y	GPT-B	GPT-W	GPT-Y
Agapostemon virescens	0.038	50	Ω	Ω	Ω		
Augochlora pura	0.001	45	18	24	10		
Ceratina dupla	0.010	16			39	19	
Ceratina strenua	0.012	3	0	Ω	57		
Hylaeus mesillae	0.043		40	40	Ω		
Lasioglossum coriaceum	0.014	21	43	10			
Lasioglossum timothyi	0.050	Ω	Ω	45	Ω		
Lasioglossum truncatum	0.002	10	75	0			
Lasioglossum smilacinae	0.038	21	43	11			
Melissodes bimaculata	0.011	53	Ω	$\mathbf 3$			
Melissodes trinodis	0.001	72	0				

EPT-B blue elevated pan traps, EPT-W white elevated pan traps, EPT-Y yellow elevated pan traps, GPT-B blue ground-level pan traps, GPT-W white ground-level pan traps, GPT-Y yellow ground-level pan traps

individuals and species than did yellow or blue pan traps in Louisiana longleaf pine savanna. It is clear that pan trap effectiveness varies with color, but the relative effectiveness of different colors of traps may be dependent on both spatial and temporal factors. The standard practice of using blue, yellow and white traps in combination is the most reliable method for using pan traps to capture a diverse sample of bees.

In our study, MRPP paired comparisons that included ground-level pan traps (the only trap type restricted to this lower elevation) produced relatively high A values, particularly when compared with malaise traps and elevated pan traps (Table [2\)](#page-7-0). Elevated pan trap comparisons with malaise traps and vane traps (all of which collect bees at higher elevations) produced relatively low A values, suggesting that these trapping methods are collecting higher foraging bee species and may be somewhat complementary to groundlevel pan traps in tallgrass prairie. MRPP comparisons of pan trap height and color (Table [4](#page-9-0)) supported this, with comparisons of pan traps at the same elevation generally producing lower A values and comparisons of pan traps at different elevations producing higher A values. However, blue ground-level pan traps collected relatively similar species composition to the elevated pan traps of each color, based on MRPP A values and cluster analysis (Table [4](#page-9-0); Fig. [2](#page-9-0)), which suggests that blue ground-level pan traps may be relatively detectable by and sample higher flying bees in tallgrass prairie. Ceratina dupla and Ceratina strenua Smith, which were associated with blue ground-level pan traps, were the only indicator species associated with a particular ground-level pan trap color (Table 5). ISA results of our study show that there are definite associations of particular bee species with particular trap types or colors (Tables [3,](#page-8-0) 5), and that some of these associations probably extend to the genus level. Information on species-sample method associations could be of great value in evaluating the conservation status of particular bee species.

Malaise traps collected by far the greatest species richness and abundance of bees among the four sampling methods in our study, whereas ground-level pan traps collected the least richness and abundance (Table [1](#page-3-0)). Malaise traps collected large numbers of Lasioglossum, particularly L. versatum. Similarly high abundances of a single Lasioglossum species have also been reported in malaise trap samples from a very different habitat, Costa Rican coffee agroecosystems (Ngo et al. [2013\)](#page-12-0). Lasioglossum in particular, and halictid bees generally are also known to be abundant in pan trap (Droege et al. [2010](#page-12-0); Richards et al. [2011\)](#page-13-0) and netting (MacKay and Knerer [1979](#page-12-0); Grixti and Packer [2006;](#page-12-0) Richards et al. [2011\)](#page-13-0) samples. In our study, malaise traps collected 75.9 % of the Chao1 estimated total of 108.0 species for this sampling method (Table [1\)](#page-3-0). This was a similar proportion to those collected by the other methods, except for ground-level pan traps, which only collected 59.3 % of the estimated total of 69 species for that method. This suggests that ground-level pan traps sampled an unusually low proportion of the bee fauna in this study. These results are supported by rarefaction analyses, which produced significantly greater species richness estimates for the three other sampling methods (with L. versatum excluded for malaise traps).

Elevated pan traps and vane traps were located at an elevation where greater numbers and diversity of foraging bees may have been present and the traps more obvious and

accessible to large, perhaps higher flying bees than the ground-level traps. MRPP analyses (Table [2\)](#page-7-0) suggest that elevated pan traps and vane traps collect relatively similar bee species composition. Bombus spp. and Melissodes spp. were particularly well represented in vane traps, which collected more individuals in these two genera than did all other trap types combined. Both of these genera were also much more abundant in elevated than in ground-level pan traps (Table [1](#page-3-0)). Bombus auricomus, B. bimaculatus, and M. trinodis were indicator species for vane traps (Table [3\)](#page-8-0), and, in particular, were most strongly associated with blue vane traps. Stephen and Rao ([2005](#page-13-0)) likewise found blue vane traps to be highly effective in collecting Bombus spp., and Bombus and Melissodes were also well represented in the Oregon bunchgrass prairie study of Kimoto et al. [\(2012](#page-12-0)). In the latter study, large proportions of Lasioglossum were also collected, as was the case in our study. However, in our study Lasioglossum were very abundant overall. In terms of overall bee abundance, blue vane traps were much more effective than yellow vane traps in our study, collecting over 10 times as many bees and about twice as many species. Stephen and Rao [\(2005](#page-13-0)) also collected many more (about three times as many) bees in blue versus yellow vane traps, and almost twice as many species.

Ground-level pan traps probably represent the most common method of surveying bee diversity in North America, and they have many advantages. These include low cost of traps (about \$0.15 each in our study) and easy transportation, which can be particularly advantageous when research is being done in remote locations. They are also quick and easy to deploy, meaning that it may be possible to compensate for low abundance and species yields by increasing trap numbers. However, this method has been criticized by Cane et al. [\(2000](#page-12-0)) as potentially producing results that are unrepresentative of the actual bee fauna present. In their survey of bees in Arizona desert, they found that many creosote bush-specialists were absent from pan trap samples. Based on their northern Virginia open field study, Roulston et al. [\(2007](#page-13-0)) also concluded that extrapolation from pan trap samples to entire bee communities should be done with caution, but pointed out that pan trapping can be an effective method in flower-poor habitats or when targeting easily trapped species. As these authors point out, this probably represents a dilution effect—fewer bees available for collection by pan traps in locations with abundant floral resources. Our results suggest that ground-level pan trap collections may not be representative of actual bee species composition (species present and their relative abundance) in the restored tallgrass prairie of Kibbe Field Station. Among the four trap types used in our study, it can be argued that malaise traps have the least bias, based on lack of color-based attraction. MRPP A values for ground-level pan trap/malaise trap comparisons were relatively high (Table [2](#page-7-0)). Ground-level pan trap species composition was also relatively dissimilar to that collected by other methods, based on MRPP A values (Table [2](#page-7-0)). However, this relative dissimilarity with other trap types also means that ground-level pan traps are to an extent complementary to the other trap types used in this study, as mentioned earlier.

Chao1 estimates suggest that, to collect species richness levels comparable to those of elevated pan traps and malaise traps, extremely large sample sizes would have been required for ground-level pan traps. For instance, elevated pan trap collections yielded 69 species from a sample size of 763 bees. To approach this species richness total, a ground level-pan trap sample size of roughly 5,000 bees would have been required (Table [1](#page-3-0)). Likewise, a much smaller malaise trap sample (2,911 bees) yielded substantially higher species richness (82 species) than would have been achieved with the greater ground-level pan trap sample size. These considerations could be important in assessments of bee species assemblages or the presence of rare species for conservation purposes. Using a combination of trap types, we collected 111 species with a sample size of 4,622 bees. Using ground-level pan traps alone, a much more destructive sample many times this size would have been needed to approach this level of species richness. The ease and low cost of ground-level pan traps must be balanced against the greater labor and expense of other trap types which may yield a more complete picture of bee species composition. Elevated pan traps collected 73 % greater abundance and 70 % greater species richness than did ground-level pan traps. But taken together, groundlevel and elevated pan traps (about \$2.20 each including posts and screws) collected 76 species, a number comparable to the malaise traps, a much more expensive (\$230 each) trap type. This suggests that, at least in tallgrass prairies, a combination of ground-level and elevated pan traps may be an effective and inexpensive approach for inventorying bee diversity. The addition of blue vane traps (about \$7 each, not including support post) could provide further improvement, particularly in collecting Bombus and Melissodes.

In conclusion, the restored tallgrass prairie of Kibbe Life Science Station harbors a diverse assemblage of well over 100 species of bees. The four trap types tested in this study varied greatly in numbers and species richness of bees collected. Malaise traps collected the most abundant and species rich bee samples, but the cost of these traps may be prohibitive for many bee monitoring programs. Elevation increased the effectiveness of pan traps substantially. Vane traps (particularly blue vane traps) were especially effective in collecting Bombus spp. and Melissodes spp. This study suggests that, when practical, bee monitoring programs that include multiple trap types are likely to obtain a

more complete assessment of bee species assemblages. Efficient collection efforts can document bee diversity without undue destructive sampling.

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