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The effect of repeated, lethal sampling on wild bee abundance and diversity

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Summary

1. Bee pollinators provide a critical ecosystem service to wild and agricultural plants but are reported to be declining world-wide due to anthropogenic change. Long-term data on bee abundance and diversity are scarce, and the need for additional quantitative sampling using repeatable methods has been emphasized. Recently, monitoring programmes have begun using a standardized method that employs a combination of pan traps and sweep netting, resulting in lethal sampling of bees. This standardized method can remove a large number of bees from sites during each sampling day, raising concern that the sampling itself could have a negative effect on bee populations.

2. We conducted an experiment to assess whether lethal sampling for bees using pan traps and netting affected bee abundance and diversity when done every two weeks throughout a season and over multiple years. We compared bee abundance, richness, evenness and functional group composition between sites that had been sampled every two weeks from 2009 to 2012 to similar sites not previously sampled.

3. We found that the standardized method for sampling bees, with specimens from 132 morphospecies, did not affect bee communities in terms of abundance, rarefied richness, evenness, or functional group composition. Thus, our results indicate that the bee communities we sampled are robust to such sampling efforts, despite removing an average of 2862 bees per season.

4. We discuss several explanations for why sampling did not affect bee abundance or community structure, including a density-dependent response to reduced competition for resources.

5. These results suggest that bee monitoring programmes sampling once every two weeks with pan traps and netting will not affect bee community structure. We urge researchers monitoring bees to utilize standardized protocols so that results can be compared across space and time.

Key-words: bee bowl, native bees, over-collecting, over-sampling, pan traps, Pollination, pollinator decline

Introduction

Environmental monitoring programmes are fundamental to ecological and environmental sciences, and play a crucial role in informing science-based environmental policy (Lovett *et al.* 2007). Environmental monitoring programmes are commonly used to assess environmental change, such as the effects of suburbanization on pollinator communities (Carper *et al.* 2014), the effects of habitat restoration on insect food webs (Albrecht *et al.* 2007), and the impact of dams on river benthic invertebrate communities (Statzner *et al.* 2001; Chaves-Ulloa, Umana-Villalobos & Springer 2014). Many environmental monitoring programmes require the collection and killing of organisms due to the difficulty of identifying specimens in the field (Reynoldson & Metcalfe-Smith 1992; Hawkins *et al.* 2000; Schonberg *et al.* 2004), creating concern that the monitoring itself may negatively impact the study taxa or system, and that changes documented by monitoring programmes may be caused by the monitoring efforts themselves. Monitoring of wild bees is of particular interest due to the pollination services they provide (Gallai *et al.* 2009) and the perception that bees are in decline (Potts *et al.* 2010), but most bees are difficult to identify in the field, necessitating collection to make or confirm species identification (Tepedino & Stanton 1981; Gibbs *et al.* 2013). The aim of this study was to determine whether a commonly employed bee monitoring protocol using lethal sampling techniques affected bee abundance and diversity when used repeatedly over several years.

Pollination by insects is an essential ecosystem service in both natural and agricultural systems. Approximately 90% of flowering species world-wide rely on pollination by insects and other animals for reproduction (Ollerton, Winfree & Tarrant

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2011), and pollinators provide approximately $202 billion per year globally in pollination services to agricultural systems (Gallai *et al.* 2009). Despite the ecological and economic importance of insect pollinators, and of bees in particular, little is known about their population trends beyond what can be learned from comparisons to historical records or museum specimens (Bartomeus *et al.* 2013; Burkle, Marlin & Knight 2013), prompting the development of standardized protocols for sampling bees (e.g. LeBuhn *et al.* 2003; Westphal *et al.* 2008). Concerns about global pollinator declines along with the development of standardized protocols have motivated the collection of long-term bee pollinator data using monitoring programmes (Committee on the Status of Pollinators in North America, & National Research Council 2007; Westphal *et al.* 2008) and the proposal of large-scale, long-term community efforts (Lebuhn *et al.* 2013).

One commonly used standardized bee catching protocol (outlined in Materials and Methods) (e.g. Wilson, Messinger & Griswold 2009; Neame, Griswold & Elle 2013; Winfree *et al.* 2014) employs a combination of sweep netting and pan traps (small plastic bowls painted fluorescent yellow, fluorescent blue, or white, filled with soapy water). Pan traps typically account for a large percentage of bees caught (Wilson, Griswold & Messinger 2008; Popic, Davila & Wardle 2013) and, by design, kill the sampled bees. Thus, a large number of bees can be collected each time the protocol is used, and bees are typically sampled repeatedly in the same site over the course of a season and sometimes for multiple years (e.g. Wilson, Griswold & Messinger 2008; Kearns & Oliveras 2009; Jha & Vandermeer 2010). A concern is that high bee capture rates of certain taxa, such as primitively eusocial *Lasioglossum* (*Dialictus*), obtained using pan traps and netting could affect bee abundance, diversity, and community composition. Unintentionally affecting wild bee populations would be problematic because the methods could (i) drive long-term patterns in measured bee abundance, diversity, and community composition, thus sampling would not accurately reflect how unsampled bee populations and communities would have responded to their natural environment, and (ii) negatively impact pollination in, and near, the study sites. Although concern has been voiced about the potential effects of over-collecting bees (Shepherd *et al.* 2003; Tepedino *et al.* 2015), little work to date has investigated the long-term impacts of pan trapping and netting.

The purpose of this study was to assess whether repeated use of pan trapping and netting had an impact on abundance and diversity of bee populations and communities. We used two approaches. First, we compared trends in bee abundance over 5 years of sampling from sites that were sampled every 2 weeks throughout the flight season for bees. We predicted that if repeated sampling negatively affected bee populations, bee abundance would decrease both over the course of a sampling season, and from one year to the next. Secondly, we used an experiment in which we compared bee abundance, species richness and evenness at sites that had been sampled twice per week for 4 years to sites that had never been sampled before. We predicted that if bee communities were affected by repeated pan trapping and netting, bee abundance and diversity (richness and evenness) would be lower in sites that had been repeatedly sampled than in newly sampled sites.

Materials and methods

STUDY AREA

We conducted this study in the West Elk Mountains of southwest Colorado, in Gunnison County near the Rocky Mountain Biological Laboratory (RMBL). The RMBL (2886 m elevation, 38°57′N, 106°59′W) is highly seasonal, with a growing season typically from late April or May until September (CaraDonna, Iler & Inouye 2014). Open meadow habitats around the RMBL are dominated by numerous perennial flowering plant species, many of which are visited by bee pollinators (Forrest, Inouye & Thomson 2010). Pollinator communities around the RMBL are dominated by native bees (Inouye 1977; Forrest & Thomson 2011); the European Honey Bee *Apis mellifera* (Hymenoptera: Apidae) is absent in this study system, and other non-native bee species nearly so.

STUDY SITES

We studied a total of 26 sites in three river valleys: East River, Slate River, and Washington Gulch (Appendix S1 and Fig. S1). To test whether bee sampling affected intra- and inter-annual variation in bee abundance, we used nine sites, all located in the East River valley (Appendix S1). For five consecutive years (2009–2013), these sites were sampled every two weeks throughout the flowering season. Each site was classified as either wet or dry (3 sites) meadow, and wet meadows were further divided into wet-*Veratrum* (3 sites) and wet-*Salix* (3 sites) based on dominant vegetation (*Veratrum tenuipetalum* and shrubby *Salix* spp., respectively). Wet and dry meadow are two dominant habitat types around the RMBL (Langenheim 1962). To test whether sites that were sampled repeatedly every two weeks for three years versus only sampled once differed in bee abundance and diversity in 2012, we used 23 sites. Seventeen were sampled only once (11 dry meadow and six wet-*Veratrum* sites), and were located in the East, Slate, and Washington Gulch river valleys (Appendix S1). We compared these 'single-sample' sites to sites of similar habitats that had been sampled every 2 weeks from 2009 to 2012 (the three wet-*Veratrum* and three dry meadow sites described above, Appendix S1). During the summer of 2012, each time we sampled a repeat-sample site, we would sample a comparable single-sample site within two days. We then assigned a numeric 'sample week' to the single-sample and repeat-sample sites that were blocked in time, for a total of seven sample weeks.

BEE SAMPLING

Each site consisted of two, fixed 45 m transects at approximately right angles. Pan traps were made from 9 cm diameter plastic bowls (Fisherbrand®; Hexagonal Polystyrene Weighing Dishes, Pittsburgh, PA, USA) painted fluorescent yellow or fluorescent blue using Krylon® Fluorescent Paint (colour numbers 3104 and 3109, respectively), or left unpainted (white). On each sample day, pan traps filled with soapy water (water with a small amount of Dawn® Ultra Dishwashing soap, original scent) were placed every 3 m along each transect with pan-trap colours assigned at random (as in LeBuhn *et al.* 2003). Pan traps were left in place from ~0800 to 1700, the peak hours of pollinator activity around the RMBL, after which the contents were strained and stored in 75% EtOH until processing. All bees were washed in water, dried with a hair drier, pinned and labelled as in LeBuhn *et al.* (2003).

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Pan traps typically catch many smaller bees but fail to consistently capture larger bodied, stronger flying bees including some Megachilidae and Apidae (Cane, Minckley & Kervin 2000). Thus, in conjunction with the pan traps, we systematically walked each transect, netting for bees for one hour each in the morning and afternoon when pan traps were in place (as outlined in LeBuhn *et al.* 2003). Netted bees that could not be identified to species in the field were collected, pinned, and labelled. We could identify most bumblebees (*Bombus*) to species in the field based on pile colours, with reference to the RMBL insect collection. Identified bumblebees were marked with a dot of indelible ink on their thorax to avoid recounting released individuals.

Pinned and labelled bees from the pan traps and netting were identified to species or morphospecies, although we were unable to identify some cryptic taxa beyond genus or subgenus, notably *Lasioglossum* (*Dialictus*) and *L.* (*Evylaeus* sensu lato).

To control for potential differences in biotic and abiotic conditions between sites sampled repeatedly versus once, we collected the following covariates each time we sampled for bees. First, along the transects at each site, we measured floral abundance using three, fixed, 4 m^2 flower census plots (six per site). On the same days that the sites were sampled for bees, flower censuses were conducted within all plots by recording all flowering plant species, number of flowers per plant for up to ten plants per species, and number of plants per plot. Secondly, we recorded daily maximum temperatures during each sampling period from a centrally located weather station near the RMBL (38° 57′ 47″N, 106° 59′ 36″W, 2945 m elevation). Temperature can vary dramatically at these subalpine sites, with strong effects on insect flight and activity (Corbet *et al.* 1993).

ANALYSES

We performed separate analyses for bees that were captured and killed (hereafter referred to as 'vouchered'), bees that were netted, identified in the field and released alive (hereafter referred to as 'released'), as well as with all bee data combined (hereafter referred to as 'combined'). Only vouchered specimens are included in the analyses described below except where otherwise indicated. Due to extreme drought conditions in 2012, abundance of *Bombus* workers was extremely low at all sites (both those that were sampled repeatedly as well as those only sampled once); thus, an analysis of only the released bees was not possible. For each sampling bout, the bee capture rate (a proxy for abundance) was calculated as bees caught per hour (bees/hour). For vouchered bees, we averaged the capture rates for bees caught in pan traps and bees caught by net. Floral density (flowers m^{-2}) was calculated by multiplying the average number of flowers per plant for each species by the number of plants per plot, summing across species, and dividing by four (each plot was 4 m^2). Plot means were then averaged at each site for each sample bout to calculate site mean floral density on each sampling day. Bee species richness was rarefied to the lowest common abundance across the combined data using the vegan package in R version 3.0.3 (R Development Core Team 2011; Oksanen *et al.* 2012), and evenness was calculated as E_{var} (Smith & Wilson 1996). E_{var} ranges from 0 to 1, with 0 representing minimum evenness and 1 maximum evenness (Smith & Wilson 1996).

We also wanted to determine whether community composition of particular bee functional groups (Winfree, Griswold & Kremen 2007; Williams *et al.* 2010) was affected by pan trapping. We categorized the nesting habits of each species (soil, wood, pithy stems, constructed cells, cavity or hive), diet (pollen generalists vs. pollen specialists), and sociality (eusocial vs. not eusocial) based on information compiled from published literature (Mitchell 1960; Hurd 1979; Cane, Griswold & Parker 2007; Michener 2007) and from extensive field experience by J.A. and E.W. We used Carper *et al.* (2014) as the basis for our nesting substrate designations, with the addition of the category 'constructed cells' for bees that construct cells on exposed surfaces using resins, masticated leaves, and other materials. We differentiated between bees that nest in existing cavities in wood ('cavity') and those that excavate nests in this substrate ('wood'), although the results were not qualitatively affected when 'cavity' and 'wood' were combined (analyses not shown). We classified all non-parasitic *Bombus* as 'hive' nesters due to the heterogeneity of nesting substrates used by *Bombus*, even within species. Parasitic bees were included in the nesting category of their host. The category 'diet' refers to pollen specialists versus pollen generalists, with 'specialist' referring to known oligolectic species, as well as taxa for which available evidence suggests oligolecty or mesolecty. 'Sociality' was broken into two categories: 'eusocial' and 'not eusocial', with 'eusocial' containing only non-parasitic *Bombus* spp. Species that could potentially be eusocial based on their phylogenetic placement, such as *Halictus* and *Lasioglossum* (*Dialictus*), were considered 'not eusocial' because eusociality is unlikely to be realized in high-elevation habitats with short growing seasons (Kocher *et al.* 2014), such as subalpine Colorado. Nonetheless, we also conducted a separate analysis of sociality, with *Bombus*, *Halictus* and *Lasioglossum* (*Dialictus*) treated as 'eusocial' given that in lower elevation areas not sampled in this study, *Halictus* and/or *Lasioglossum* (*Dialictus*) can be eusocial (Kocher *et al.* 2014). Parasitic bees were excluded from analyses of diet and sociality. We also categorized bee species by size using intertegular (IT) span to estimate dry body mass (Cane 1987; Greenleaf *et al.* 2007). We measured IT span using a stereoscope and calibrated ocular micrometer for up to five randomly selected female bees from each morphospecies, using only worker bees for social species. We classified species as small (<4 mg), medium (4–16 mg), or large (>16 mg) (Winfree, Griswold & Kremen 2007). We performed separate analyses for bee abundance (estimated as the bee catch rate), rarefied richness, evenness, and functional groups. We also calculated mean expected foraging distance for the bees using overall mean IT span and the statistical relationship between body size and foraging distance presented by Greenleaf *et al.* (2007).

To test whether bee abundance varied over the course of each sampling season and from year to year, we used generalized linear mixed models. We used AICc values to rank and compare multiple *a priori* hypotheses and pick the model best supported by the data. The candidate models included meadow type (dry, wet-*Veratrum*, or wet-*Salix*), mean floral density, and daily maximum temperature as factors. Site, year and sampling week were included as random effects. Sampling week was defined as the number of times a site was sampled within a season, which allowed for differences in timing of snowmelt (and thus access to the site for sampling) among sites and years. For example, sampling at higher elevation sites began slightly later than lower elevation sites due to later snowmelt and phenology, but the first time each site was sampled was lumped into a single phenologically similar sample week. Likewise, years with early snowmelt and phenology (e.g. 2012) had more sampling weeks than years with late snowmelt and phenology (e.g. 2011) due to a longer flight season. We log-transformed bee capture rates to fulfil the assumption of normality (here and below). We used the lmer function in the lme4 package to construct the models and the aictab function in the AICcmodavg package to rank and compare models using R version 3.0.3 (R Development Core Team 2011; Mazerolle 2013; Bates *et al.* 2014). We used type III sums of squares and Satterthwaite approximation of degrees of freedom to calculate F and *P* values.

To test for differences in bee abundance, rarefied richness, and evenness between single-sample sites and repeat-sample sites, we used nested

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ANCOVAS with treatment (single or repeated sampling), block (week), and river valley as factors, and mean floral density and daily maximum temperature as covariates. We included block as a random effect to control for the week the samples were collected, and we tested treatment over the block term (here and below). To test whether the effect of repeated sampling varied with bee functional group, we used general linear mixed models with functional group, treatment (single or repeated sampling), block (week), and river valley as factors, and mean floral density and daily maximum temperature as covariates. We included site as a random effect in the functional group analysis to control for variation among sites. Four separate analyses were performed for each functional group: nesting habits, diet, sociality, and body size. We used JMP 11.0 (SAS Institute 2013) to perform the nested ANCOVAS and functional group analyses. To visualize potential differences in community composition between single and repeat-sampled sites, we used non-metric multidimensional scaling (NMDS), using the Bray–Curtis dissimilarity index, using the vegan package (Oksanen *et al.* 2012) in R version 3.0.3 (R Development Core Team 2011). Stress values from models with two to six dimensions were compared to determine the most appropriate summary of the relationship between sampling and community composition.

Results

DATA OVERVIEW

Across five years and 26 sites, we vouchered 14 312 pan-trapped bees, netted-and-vouchered 995 bees, and netted-and-released 2489 bees. Collectively, these bees belonged to five families, 28 genera, and 132 species/morphospecies including 26 county records and three state records (Appendix S2), with a mean IT span of 1·39 mm and mean expected foraging range of 66 m (Greenleaf *et al.* 2007). Approximately 28% of the bees caught were male, and 40% of the bees caught were *Lasioglossum* (*Dialictus*) sweat bees. For bees identified to species, the two most common species were the sweat bees *Halictus virgatellus* and *Halictus rubicundus*, comprising 11·31% and 2·52% respectively of all bees vouchered. In addition, we captured 35 species represented by a single individual. In 2012, we collected 100% of the genera and 98% of the morphospecies that we would eventually catch after just 62 of the 95 total sampling events. A rarefaction curve of the 2012 sampling data (Fig. S2) revealed a saturating function, suggesting that our sampling effort adequately characterized common bee taxa but that we would likely continue to capture new, rarer species with additional sampling.

INTER- AND INTRA-ANNUAL VARIATION

Although we predicted that repeated sampling of sites would result in a decrease in bee catch rate both among years and within seasons, we found no evidence for either pattern. Among years, total bee catch rates (vouchered and released combined, divided by total sampling effort) ranged from 6·33 bees per hour in 2011 to 8·14 bees per hour in 2010 (Fig. 1). Catch rates for vouchered bees only were consistent across years, except for 2012, when the catch rate nearly doubled. Moreover, when we examined intra-annual variation in vouchered bee catch rates, we found no general pattern within years (assessed visually from Fig. 2, S3 and S4). The best-fit model for analysing inter- and intra-annual variation in bee abundance was supported by 88% of the Akaike weights and included significant interactions between flower density and maximum daily temperature, with site as a random effect. The model also allowed the intercepts (but not slopes) to vary among years and among sample weeks within years (nested random effects) (Appendix S3). In addition, we found significant main effects of both flower density and maximum daily temperature on vouchered bee catch rate ($F_{1,264·73} = 9·83$, $P < 0·002$ and $F_{1,\ 276·97} = 12·27$, $P < 0·001$, respectively), with bee catch rate increasing with increasing flower density and temperature ($\beta \pm 1$ SE: $0·48 \pm 0·15$ and $0·08 \pm 0·02$, respectively). We also found a

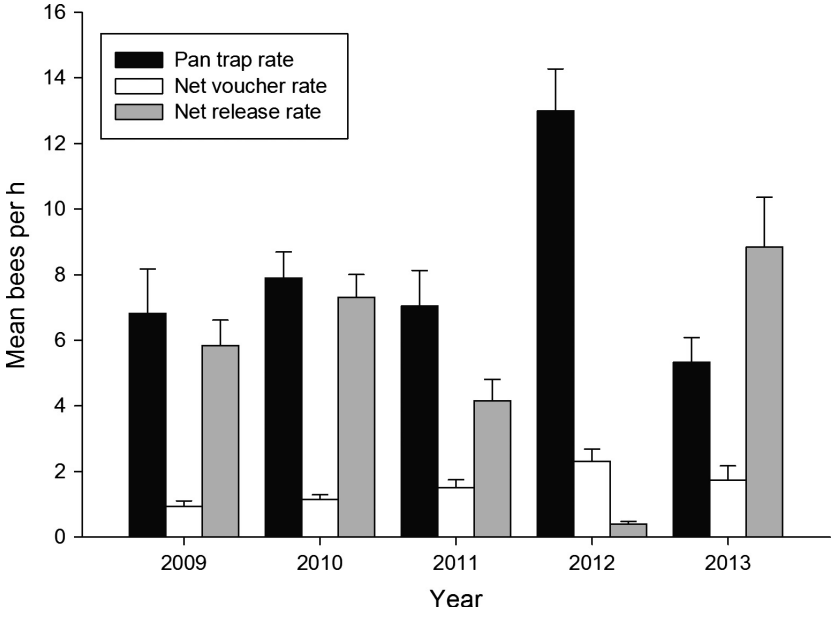

Fig. 1. Interannual variation in mean bee catch rates (± 1 SE) for bees that were caught in pan traps and vouchered (black bars), caught in nets and vouchered (white bars), and caught, identified in the field and released (gray bars). Only *Bombus* spp. were identified in the field and released. Bee catch rates varied among years with no consistent pattern over time.

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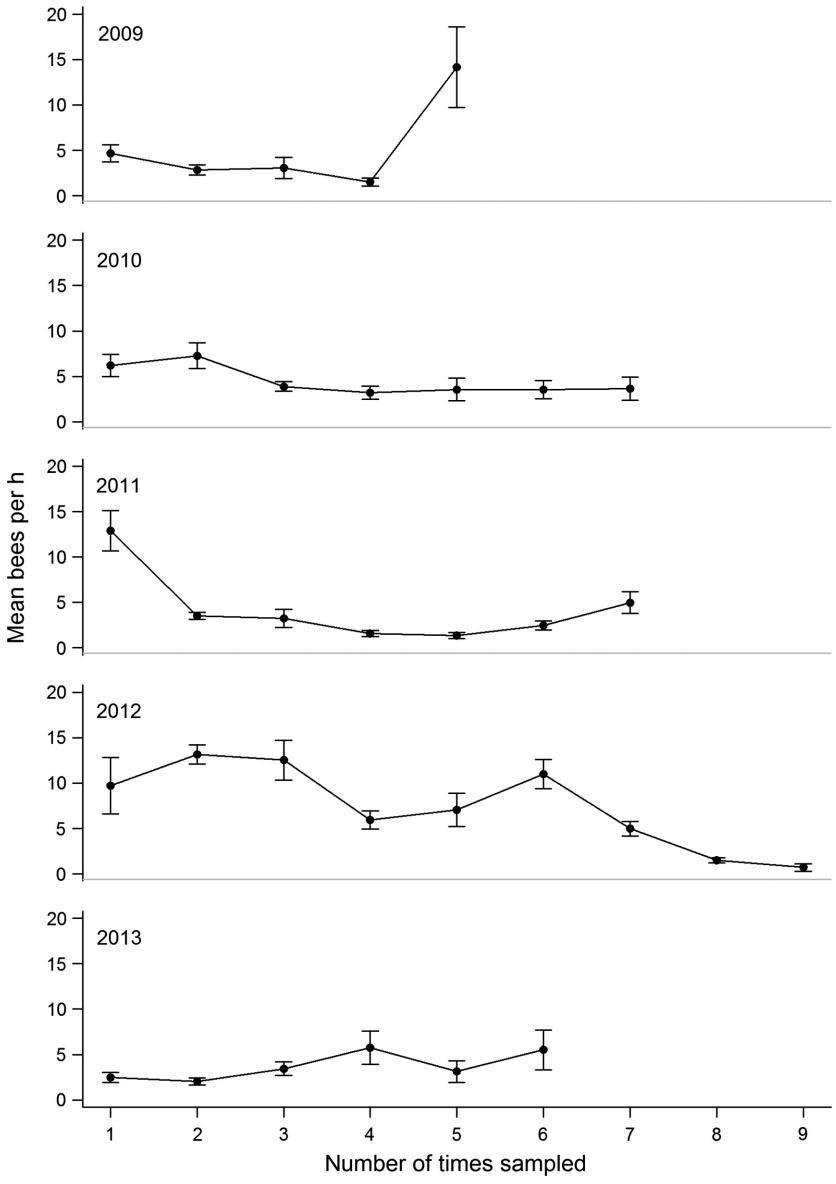

Fig. 2. Bee catch rates (mean bees caught per hour) for vouchered bees (collected using pan traps and netting) varied from year to year, with no consistent pattern related to the number of times the sites were sampled. Data represent mean (± 1 SE) catch rates across all sites after repeated sampling within the season. Years with earlier phenology (e.g., 2012) had more sampling bouts due to the longer flying season.

significant interaction between flower density and maximum daily temperature on vouchered bee catch rate ($\beta = -0{\cdot}02 \pm 0{\cdot}007$, $F_{1,\ 268{\cdot}85} = 9{\cdot}45$, $P = 0{\cdot}0023$).

REPEATED VERSUS SINGLE-SAMPLED SITES

The catch rate of vouchered bees was 31·28% higher on average in repeat-sampled sites versus single-sampled sites, although the difference was not statistically significant (Table 1, Fig. 3A). We also found no differences in rarefied richness or evenness (E_{var}) between repeat-sampled and single-sampled sites (Table 1, Fig. 3B and 3C, respectively). River valley had a significant effect on rarefied species richness, with the East River valley having higher rarefied richness (Least Squares Means ± SE: 5·68 ± 0·22 species) than Slate River valley (4·42 ± 0·58 species) and Washington Gulch (3·64 ± 0·56 species). Bee catch rate was 59·54% higher in wet-*Veratrum* sites than dry meadow sites (12·64 ± 5·03 and 7·92 ± 3·09, respectively). We also found a weak, positive relationship between rarefied richness and evenness and maximum daily temperature, and a weak, positive relationship between catch rate of vouchered bees and flower density (Table 1). Results were qualitatively similar for combined bee data (Fig. S5 and Appendix S4).

We also found no significant main effect of treatment (repeated versus single-sample) when we subdivided the bees by functional group (nesting habits, diet, sociality, and body size) (Table 2, Fig. 4). Likewise, we found no significant interaction between treatment and functional group for nesting habits and body size (Table 2, Fig. 4a,d). When only *Bombus* (and not *Halictus* and *Lasioglossum* (*Dialictus*)) were treated as eusocial, we found no significant interaction between treatment and sociality (Table 2, Fig 4c; see Appendix S5, Fig. S6 for analysis of these taxa categorized as eusocial). We found a significant interaction between treatment and diet (Table 2, Fig. 4b), with catch rates of pollen specialists being 43·88% higher, but catch rates of pollen generalists being 7·95% lower, in repeated- than single-sample sites. Bee catch rate varied by

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Table 1. Nested ANCOVAS testing the effect of treatment (repeated vs. single sampling) on (A) vouchered bee catch rate [log(bees/hour)], (B) rarefied richness and (C) evenness (measured as E_{var}). Type refers to the habitat type (dry or wet-*Veratrum*), valley is the river valley sites were located in (East River, Slate River, or Washington Gulch), and max temp is maximum temperature

	DF	*F* Ratio	*P*
(A) Catch rate			
Treatment	1, 8·93	0·27	0·619
Type	1, 21·41	6·31	0·020
Valley	2, 9·79	2·27	0·155
Flower density	1, 21·46	3·53	0·074
Max temp	1, 17·25	1·38	0·255
(B) Rarefied richness			
Treatment	1, 8·22	0·03	0·868
Type	1, 26·43	0·57	0·457
Valley	2, 14·34	5·34	0·019
Flower density	1, 26·51	0·39	0·538
Max temp	1, 15·76	4·11	0·060
(C) Evenness			
Treatment	1, 6·43	0·16	0·698
Type	1, 24·32	1·11	0·325
Valley	2, 10·94	3·60	0·063
Flower density	1, 24·34	0·14	0·713
Max temp	1, 12·60	3·80	0·074

functional group classification (Table 2). Soil-nesting bees comprised 66·63% of all vouchered bees, and were between 4·06 and 35·42-times more common than bees of any other nesting habits (Fig. 4a). The majority of vouchered specimens were pollen generalists (84·81%, Fig. 4b) and not eusocial (95·62%, assuming our montane *Halictus* and *Lasioglossum* (*Dialictus*) were not eusocial, Fig. 4c). Small- (48·91%) and medium-sized (38·75%) bees were both more common than large bees (12·33%, Fig. 4d).

NMDS indicated overlap in bee community composition between repeated versus single-sampled sites (Fig. 5a), among river valleys (Fig. 5b), and between wet versus dry habitats (Fig. 5c). Community composition changed dramatically over the course of the summer, as indicated by the distinct clusters of points when separated by sample week (Fig. 5d). The overall stress of the two-dimensional NMDS was 0·18, indicating fair confidence in the ordination distances. Models with higher dimensionality resulted in lower stress (e.g. three-dimensional solution, stress = 0·126), but the results were qualitatively similar (Fig. S7a–d) to the two-dimensional solution.

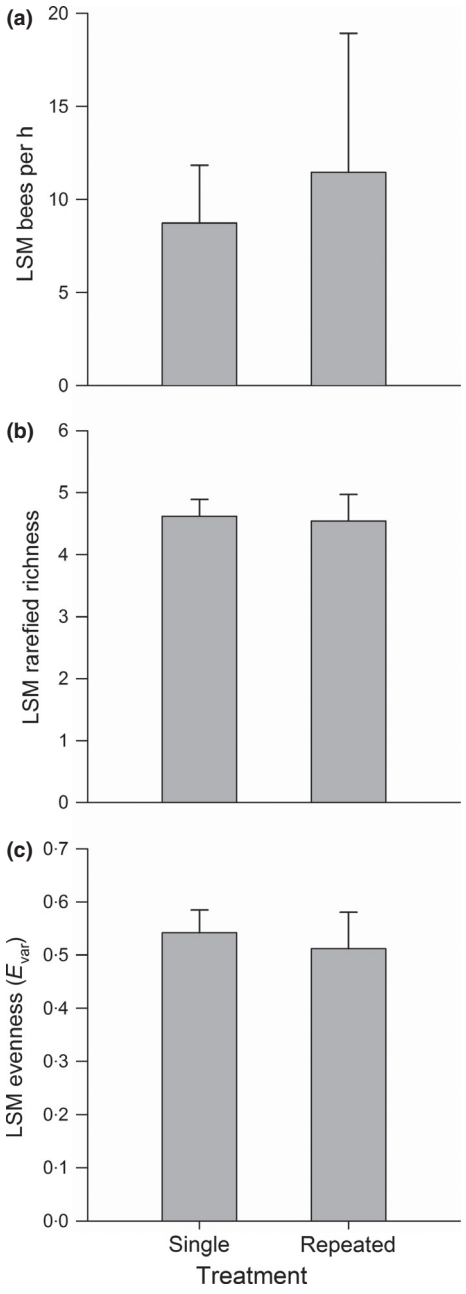

Fig. 3. Least Square Means (LSM) from ANCOVAS of vouchered bees between single sampled and repeated sampled sites. We found no significant difference in (a) bee catch rate (bees per hour), (b) rarefied richness, or (c) evenness between single and repeated sampling sites.

Discussion

Our results suggest that pan trapping for bees does not affect bee catch rate or diversity, even after sampling the same sites every other week during the flight season for three consecutive years. This lack of response was consistent across all bee functional groups with the exception of diet. Although we caught more pollen specialists in repeated-sample sites than in single-sample sites (and the opposite pattern for pollen generalists), the magnitude of effect associated with diet was relatively small and did not represent a large change in catch rate between treatments (Fig. 4b). Taken together, these results suggest that the bee populations sampled in this study are robust to repeated, lethal sampling twice monthly for multiple, consecutive years.

Several non-mutually exclusive mechanisms could explain a lack of sampling effect, four of which we explain here. The first two mechanisms reflect underlying ecological mechanisms related to population dynamics, and the second two are based on species life histories. First, compensatory mortality and natality could explain the lack of sampling effect. Bees often compete for limiting resources, including floral food resources (Minckley *et al.* 1994; Roulston & Goodell 2011) and nesting

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Table 2. Nested ANCOVAs testing the effect of treatment (repeated vs. single sampling) on vouchered bee catch rate [log(bees/hour+1)] by functional group. Type refers to habitat type (dry or wet-*Veratrum* meadow). Separate analyses were performed for each of four functional groups: (A) nesting substrate, (B) diet, (C) sociality and (D) body size. Body size was treated as a three-level categorical variable (small, medium and large)

	DF	*F* Ratio	*P*
(A) Nesting substrate			
Type	1, 12·9	6·18	0·027
Treatment	1, 8·44	0·15	0·711
Valley	2, 9·87	1·29	0·318
Flower density	1, 35·53	2·79	0·104
Max temp	1, 15·47	0·37	0·552
Nesting	5, 191·3	101·58	<0·0001
Nesting*Trmt	5, 191·3	0·47	0·801
(B) Diet			
Type	1, 13·32	5·19	0·040
Treatment	1, 8·77	0·31	0·593
Valley	2, 9·92	2·86	0·105
Flower density	1, 34·23	2·30	0·138
Max temp	1, 15·56	1·83	0·196
Diet	1, 49·94	189·17	<0·0001
Diet*Trmt	1, 49·94	6·11	0·017
(C) Sociality			
Type	1, 12·32	3·78	0·075
Treatment	1, 7·513	0·43	0·531
Valley	2, 9·88	2·75	0·113
Flower density	1, 31·84	1·17	0·287
Max temp	1, 13·01	1·46	0·248
Sociality	1, 51·86	326·25	<0·0001
Sociality*Trmt	1, 51·86	0·95	0·335
(D) Body size			
Type	1, 13·67	3·53	0·082
Treatment	1, 9·19	0·18	0·678
Valley	2, 9·89	2·38	0·143
Flower density	1, 37·60	3·05	0·089
Max temp	1, 18·18	0·77	0·393
Size	2, 83·57	53·72	<0·0001
Size*Trmt	2, 83·57	1·49	0·231

sites (e.g. Hubbell & Johnson 1977; Potts & Willmer 1997; Steffan-Dewenter & Schiele 2008). Reducing bee populations through lethal sampling may reduce competition for these resources and therefore increase the population growth rate via density-dependent processes. Compensation is a fundamental principle of wildlife management (Bartmann, White & Carpenter 1992), and although bee population dynamics are not well understood (Steffan-Dewenter & Schiele 2008), the same principles that allow for sustainable harvesting of fish (Worm *et al.* 2009) and hunting of wildlife (Boyce, Sinclair & White 1999) could apply to 'harvesting' of insects in environmental monitoring programmes. Secondly, lethal sampling may have no effect on subsequent bee population size if sampling simply removed individuals from the population that would not have had an opportunity to reproduce anyway. If bees are controlled by bottom-up rather than top-down forces (e.g. Steffan-Dewenter, Potts & Packer 2005), removing adult bees from the population via lethal sampling may not reduce population size as long as enough bees remain to utilize all limiting resources. Relative to the first mechanism proposed, here we would not expect to find a change in bee population growth rate following lethal sampling.

Thirdly, the reproductive habits of bees may lessen the effects of sampling. Solitary bees construct and mass provision brood cells as they forage (e.g. Eickwort *et al.* 1996); thus, many individual bees may be sampled after they have mated and carried out some reproduction, which could mitigate the effect of sampling among years. Unlike many vertebrate taxa, solitary bees do not require ongoing parental care after the female provisions and seals larval brood cells (sealing the entrance of a completed nest may or may not be essential for brood survival). Thus, the reproductive output of a vouchered specimen may be reduced but not eliminated. Moreover, lethally sampling some workers from eusocial species may have minimal effect on the colony level production of males and daughter queens (e.g. Harbo 1986). Finally, if males are removed from the population after mating, or females are not mate-limited, then sampling males may also have no effect on univoltine bee populations and communities in subsequent years.

The fourth mechanism we outline concerns the short flight season of most solitary bees, and thus high species turnover during a typical summer. In cases where bees have short flight seasons, sampling every two weeks may not result in within-season declines in bee abundance if new species or populations are being sampled during each sampling event. For example, *Halictus rubicundus* and other typically social halictine bees may have solitary populations in high-elevation regions of the Rocky Mountains due to short flight season, with individual bees only foraging for approximately two weeks (Eickwort *et al.* 1996). Our data support observations of short flight periods by Eickwort *et al.* (1996) in that detection of individual species within our sites spanned 11·88 ± 0·82 days on average (± SE). Thus, in systems with high species (and population) turnover, a biweekly sampling scheme may allow ample time for brood provisioning between sampling events.

Although our study specifically tested whether lethal sampling negatively affected bee populations, our results may be generalizable to other entomological systems. For example, Hunter *et al.* (2014) found that 90% of Finnish moth populations were stable or increasing during 32 years of repeatedly sampling 11 sites using light traps. Unfortunately, experimental data on the effects of sampling arthropods are extremely limited, and most accounts of negative effects of sampling only address the over-collection of rare and valuable specimens for the black market, such as alpine Lepidoptera in Asia (Hama, Ishii & Sibatani 1989). The dearth of information on the effects of sampling insects is unfortunate given the concerns about specimen collection in general (Wagner 1991; Minteer, Collins & Puschendorf 2014b; Minteer *et al.* 2014a) and of bees in particular (Tepedino *et al.* 2015), and the utility of insect collections for ecological research (e.g. Benton *et al.* 2002; Novotny *et al.* 2007).

Catch rates for both vouchered and released bees varied dramatically among years (Fig. 2 and S3, respectively), and there was no consistent decrease in vouchered bee catch rates from one year to the next as we would expect if lethal sampling

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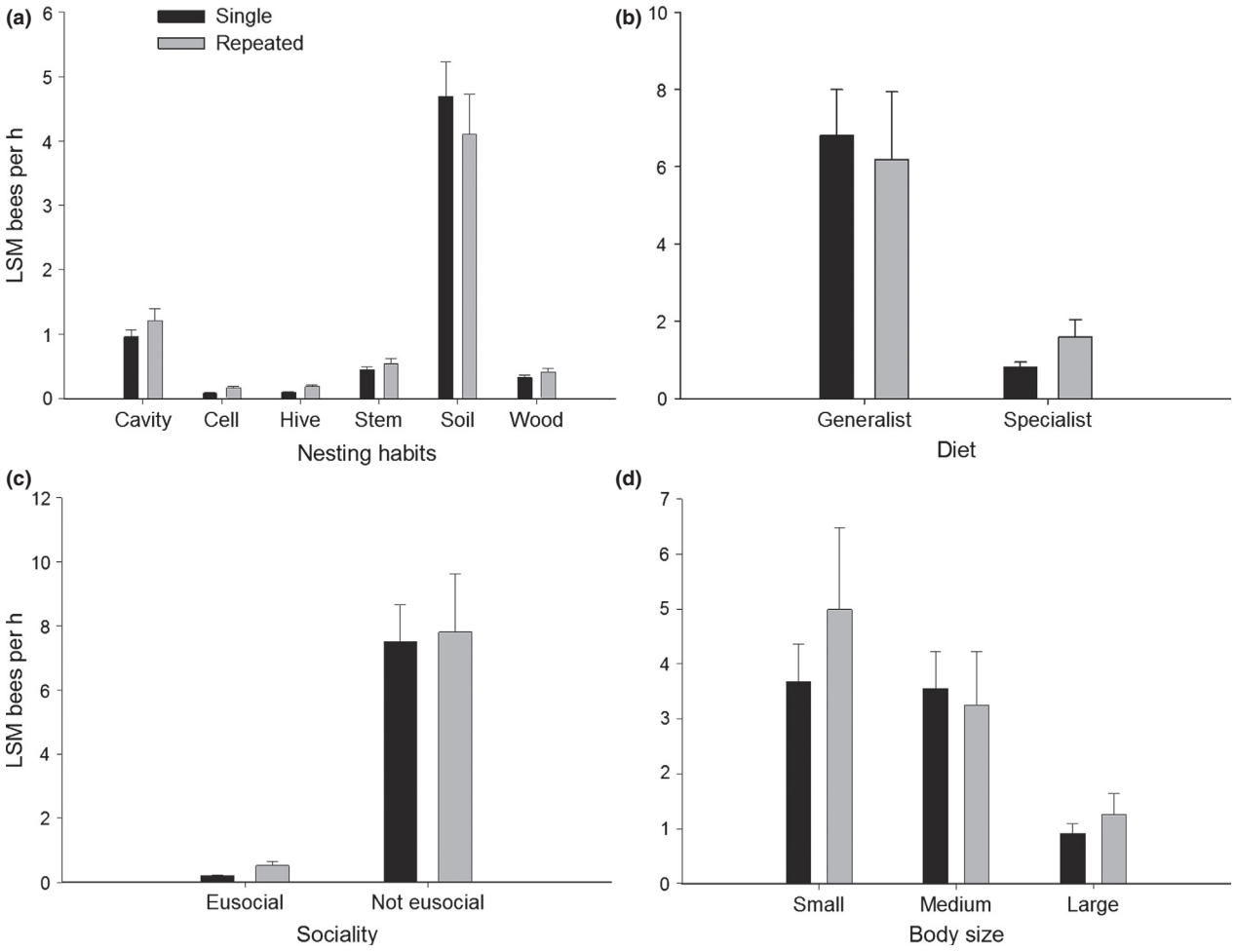

Fig. 4. Least Square Means (LSM) of vouchered bee catch rates for each of four functional groups, (a) nesting habits, (b) diet, (c) sociality, and (d) body size. In the sociality analysis, only *Bombus* were categorized as eusocial. The only functional group classification that interacted significantly with treatment (single versus repeated sampling) was diet. Note the different scale on the *y*-axes.

negatively impacted bee populations. Rather, the interannual variation in bee catch rates was likely related to seasonal differences among years. For example, high snowfall and late phenology in 2011 resulted in extremely high floral resources, and may have caused the solitary bee populations to increase, resulting in high vouchered bee capture rates in the subsequent year 2012. Likewise, extreme drought and freezing events (Inouye 2008) in 2012 resulted in extremely low floral resources during that summer, which may have caused the near absence of *Bombus* spp. workers. However, the capture rates of both vouchered and released bees returned to more average numbers in 2013, which was a more average year in terms of snowpack, phenology and floral abundance (Z. Gezon, personal observation). Our results reflect the extreme variability and unpredictability of floral resources observed in many systems, along with the corresponding effects on pollinator abundance and plant–pollinator interactions (Tepedino & Stanton 1980; Alarcón, Waser & Ollerton 2008; Olesen *et al.* 2008).

Five caveats are important to the interpretation of this study. First, our results apply to sampling bees every two weeks and cannot be used to assess whether more frequent sampling, for example weekly or even daily, may negatively affect bee populations or communities. Secondly, the distances between the repeated-sample sites (min = 125 m, max = 2400 m) were large compared to the mean expected foraging distance of the bees we sampled (66 m), and thus the maximum area that we could theoretically depopulate through sampling. Smaller distances between sampling sites in relation to bee foraging areas could affect the ability of bees to recolonize sampled areas and could therefore affect bee populations in some areas. Thirdly, the ecosystem investigated in this study (subalpine Rocky Mountain West) is highly seasonal with a short flight-season; thus, there is high species turnover when sampling once every two weeks. How sampling affects bee populations and communities in habitats with less seasonal turnover requires further investigation. Fourthly, we did not test for taxon-specific declines in abundance over sampling seasons. Looking at within-site patterns would be most appropriate for determining whether sampling affects individual species, but the probability of catching the same taxa within a site over multiple sampling periods was low even for the abundant taxa because of the infrequency of sampling compared to the short flight season of most species within our sites. Fifthly, we only explored whether repeated sampling affected bee catch

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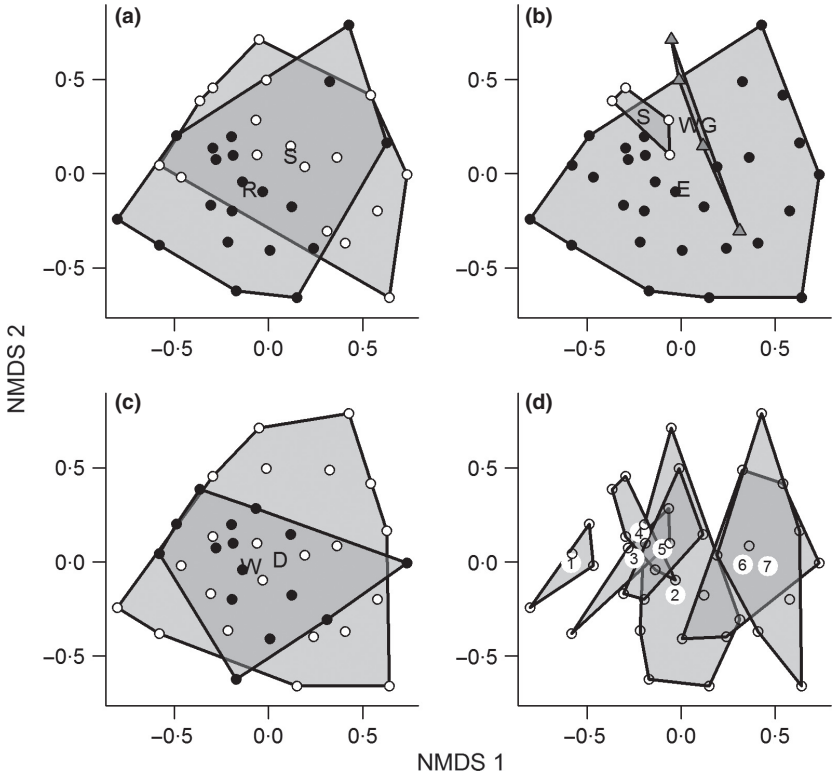

Fig. 5. Two-dimensional non-metric multidimensional scaling (NMDS) ordination plots indicating overlap in bee communities when separated by (a) treatment (S = single-sampled sites, R = repeat-sampled sites), (b) river valley (E = East River, S = Slate River, WG = Washington Gulch), (c) habitat type (D = dry meadow, W = wet-*Veratrum*), and (d) sample week. Abbreviations are located at the grand mean for each group, and the perimeter and area of each group is outlined and shaded. Note that only vouchered bees were included in this analysis.

rate and community structure and did not assess its impact on floral visitation and plant reproduction. Bees are critical in performing pollination services, and complete removal of even a single species of bee can have impacts on plant–pollinator interactions and plant reproduction (Brosi & Briggs 2013), so future studies should investigate whether intensive sampling results in pollen limitation of melittophilous plants.

Given the economic importance of bee pollinators and the mounting threat of anthropogenic disturbances such as climate and land-use change (Klein *et al.* 2007; Potts *et al.* 2010; Ollerton, Winfree & Tarrant 2011), bee inventorying and monitoring programmes are critically important for documenting the biodiversity of ecosystem service providers. Long-term and repeated sampling is needed to monitor bee populations across space and time (Committee on the Status of Pollinators in North America, & National Research Council 2007; Winfree 2010; Lebuhn *et al.* 2013), but implementing and funding such a programme remains a challenge due, in part, to divergent views on the relevance and statistical power of the proposed approaches, and the ability to overcome the taxonomic impediments (Lebuhn *et al.* 2013; Tepedino *et al.* 2015; and response by Lebuhn *et al.* 2015). The very effectiveness (lethality) of proposed trapping measures has also been cited as a concern, but our research provides compelling evidence that lethal sampling of bees using pan traps and netting every two weeks does not negatively affect bee populations in terms of abundance, species richness, evenness and functional group composition. While our study does not directly address the mechanisms that make bee populations robust to lethal sampling, we suggest several plausible explanations that draw from well-known ecological theories and species life histories. However, the generality of our results remains to be tested for bee populations and communities in other regions. Nonetheless, the lack of environmental impact of bee sampling we document is encouraging, suggesting that pan trapping and netting bi-monthly may be a sustainable method to inventory bee diversity and to document long-term trends in bee populations and communities.

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Data accessibility

All supplemental figures and appendices have been uploaded as supporting information. Specimen-related data are available at the RMBL website www.rmbl.org and will be archived at the American Museum of Natural History, which deposits their data in the Arthropod Easy Capture website and shares it with biodiversity portals including www.discoverlife.org, and, in the near future, with idigbio. All other data related to the experiment are available from the Dryad Digital Repository (Gezon *et al.* 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Map of the sampling sites. Treatment refers to sites that were sampled twice-monthly from 2009 to 2013 ('repeated') or only once in 2012 ('single').

Fig. S2. Rarefaction curve for the number of morphospecies caught over the course of the 2012 sampling season.

Fig. S3. Intra-annual variation in bee catch rates (mean bees caught per hour) for caught-and-released bees varied from year to year, with no consistent trend.

Fig. S4. Patterns of vouchered bee catch rates varied from year to year, with a trend toward higher catch rates early and late in the season, except in the year 2012.

Fig. S5. Least Square Means (LSM) from ANCOVAs of all bees (vouchered plus caught-and-released bees) between single-sampled and repeat-sampled sites.

Fig. S6. Least Square Means (LSM) from nested ANCOVA testing the effect of treatment (repeated vs. single sampling) on vouchered bee catch rate [log(bees/hour + 1)] by sociality.

Fig. S7. Three-dimensional non-metric multidimensional scaling (NMDS) ordination plots indicating overlap in bee communities when separated by (a) treatment, (b) river valley, (c) habitat type, and (d) sample week.

Appendix S1. Sampling site information and locations.

Appendix S2. Species, morphospecies, and functional group designations used in analyses.

Appendix S3. Table comparing *a priori* models of bee catch rate within and among years in the repeatedly sampled sites.

Appendix S4. Nested ANCOVAs testing the effect of treatment (repeated vs. single sampling) on (A) bee catch rate [log(bees/hour)], (B) rarefied richness, and (C) evenness (measured as E_{var}) for all bees combined (vouchered and catch-and-release).

Appendix S5. Nested ANCOVA testing the effect of treatment (repeated vs. single sampling) on vouchered bee catch rate [log(bees/hour + 1)] by sociality.

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