

Diverging landscape impacts on macronutrient status despite overlapping diets in managed (*Apis mellifera*) and native (*Melissodes desponsa*) bees

Christina L. Mogren^{1,*}, María-Soledad Benítez², Kevin McCarter³, Frédéric Boyer⁴ and Jonathan G. Lundgren⁵

¹Department of Plant and Environmental Protection Sciences, University of Hawai'i at Mānoa, 3050 Maile Way Gilmore 310, Honolulu, HI 96822, USA

²Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH 44691, USA

³Department of Experimental Statistics, Louisiana State University, Baton Rouge, LA 70802, USA

⁴Laboratoire d'Écologie Alpine, Centre National de la Recherche Scientifique, Université Grenoble Alpes, F-38000 Grenoble, France

⁵Ecdysis Foundation, Estelline, SD 57234, USA

***Corresponding author:** Department of Plant and Environmental Protection Sciences, University of Hawai'i at Mānoa, 3050 Maile Way Gilmore 310, Honolulu, HI 96822, USA Tel: 808 956 6745; Fax: 808 956 2428; Email: cmogren@hawaii.edu

Declining pollinator populations worldwide are attributed to multiple stressors, including the loss of quality forage. Habitat management in agricultural areas often targets honey bees (*Apis mellifera* L.) specifically, with the assumption that native bees will benefit from an 'umbrella species' strategy. We tested this theory using a conservation physiology approach to compare the effects of landscape composition and floral dietary composition on the physiological status of honey bees and *Melissodes desponsa* in eastern South Dakota, USA. The total glycogen, lipid and protein concentrations were quantified from field collected bees. Next-generation sequencing of the *trnL* chloroplast gene from bee guts was used to evaluate dietary composition. The effects of landscape and dietary composition on macronutrient concentrations were compared between bee species. As the mean land-use patch area increased, honey bee glycogen levels increased, though *M. desponsa* experienced a decrease in glycogen. Protein levels decreased in honey bees as the largest patch index, a measure of single patch dominance, increased versus *M. desponsa*. Lipids in both species were unaffected by the measured landscape variables. Dietary analysis revealed that honey bees foraged preferentially on weedy non-native plant species, while *M. desponsa* sought out native and rarer species, in addition to utilizing non-native plants. Both species foraged on Asteraceae, Oleaceae and Fabaceae, specifically *Melilotus* sp. and *Medicago* sp. Dietary composition was not predictive of the macronutrients measured for either species. Together, these data highlight the management importance of including patch area in conservation recommendations, as bee species may have divergent physiological responses to landscape characteristics. While solitary bees may forage on weedy introduced plants in agricultural areas, robust strategies should also reincorporate native plant species, though they may not be preferred by honey bees, to maximize overall health and diversity of pollinator communities.

Key words: Apidae, conservation physiology, dietary composition, honey bees, metabarcoding, nutrition

Editor: Steven Cooke

Received 3 September 2019; Revised 20 February 2020; Editorial Decision 3 November 2020; Accepted 4 November 2020

Cite as: Mogren CL, Benítez MS, McCarter K, Boyer F, Lundgren JG (2020) Diverging landscape impacts on macronutrient status despite overlapping diets in managed (*Apis mellifera*) and native (*Melissodes desponsa*) bees. *Conserv Physiol* 8(1): coaa109; doi:10.1093/conphys/coaa109.

Introduction

Habitat conversions at the landscape scale can negatively affect pollinator diversity by restricting available resources to those that promote agrobionts, or species thriving in areas heavily modified for agricultural production (Naug, 2009; Mogren *et al.*, 2016). In the Northern Great Plains of the Midwestern USA, prairie and grassland conversions to agricultural monocultures have greatly simplified the landscape of eastern South Dakota (Wright and Wimberly, 2013; Fausti, 2015; Wimberly *et al.*, 2017; Otto *et al.*, 2018). The additional removal of historic tree stands and flowering vegetation at field margins to maximize the field size and control weeds has further limited available floral resources upon which a large number of native bees rely (Mogren *et al.*, 2016). In these types of intensively managed agricultural areas, lands set aside for conservation have proven particularly important in maintaining pollination services of managed bees such as honey bees (*Apis mellifera* L.) (Gallant *et al.*, 2014; Otto *et al.*, 2016; Smart *et al.*, 2016) and native bees (Benjamin *et al.*, 2014), although the effects may vary among cropping systems (Campbell *et al.*, 2017; Quinn *et al.*, 2017). For this reason, the Pollinator Health Task Force (2015) recommended South Dakota along with Michigan, Minnesota, Montana, North Dakota and Wisconsin for a pollinator conservation initiative incentivizing habitat restoration. This focus on habitat restoration in agricultural areas is often intended to promote honey bees specifically, with an assumption that these conservation efforts will benefit native bees and other pollinators through an umbrella species paradigm (Kalinkat *et al.*, 2017).

However, significant differences between resource utilization exist between honey bees, which are social, and native bees, which are mostly solitary (e.g. Steffan-Dewenter and Tscharnatke, 2000). Honey bees have a high forage fidelity (Free, 1963) combined with the ability to mass recruit to plentiful resources and thus would be expected to forage mostly upon mass blooming resources. In contrast, solitary bees may visit numerous floral species in a single foraging visit. As such, floral management considerations for one type of pollinating bee may not completely satisfy the needs of the other.

One means for identifying differences between the diets of eusocial and solitary bees is through the use of next-generation sequencing analysis. DNA metabarcoding to identify dietary diversity in a number of animal species, including honey bees (Hawkins *et al.*, 2015; Smart *et al.*, 2017) is a technique that can be employed to compare the diets of coexisting species. DNA metabarcoding also results in greater taxonomic resolution than palynology alone (Keller *et al.*, 2015; Richardson *et al.*, 2015; Valentini *et al.*, 2010). In gut-content analysis applications, the chloroplast *trnL* (UAA) p6 intron region (Taberlet *et al.*, 1991) has been used to successfully categorize the diets of herbivorous mammals (Hibert *et al.*, 2013; Srivathsan *et al.*, 2015; Gebremedhin *et al.*, 2016; Espunyes *et al.*, 2019) and invertebrates (Valentini *et al.*,

2009; Wallinger *et al.*, 2015; Sint *et al.*, 2018). This gene region is highly conserved yet generally small enough that it may more easily be detected in degraded DNA samples as would be encountered in partially digested plant materials in herbivore guts (Taberlet *et al.*, 2007).

Discerning cause-and-effect relationships between the physiology of an organism and the environment in which they reside in order to refine resource management strategies to benefit that species is an emerging area of study, termed conservation physiology (Cooke *et al.*, 2013). This has important implications for management of pollinators (Alaux *et al.*, 2017). Assessment of health and nutrient parameters in field-collected bees adds an important, *in situ* perspective into the effects of habitat manipulations on their physiological status, as nutritional stress is associated with reductions in learning (Jaumann *et al.*, 2013), foraging ability (Schofield and Mattila, 2016) and immunity (Alaux *et al.*, 2010; Dolezal *et al.*, 2016). Floral resource availability (Di Pasquale *et al.*, 2016; Smith *et al.*, 2016) and landscape diversity (Dolezal *et al.*, 2016; Alaux *et al.*, 2017) have been previously correlated with bee physiology measures separately. However, they are not often considered simultaneously.

The purpose of this study was to define how landscape and plant diversity within a highly developed agricultural area impact the nutritional status of honey bees and a ubiquitous solitary native bee, *Melissodes desponsa* (Apidae, Eucerini). We tested the hypothesis that a more diverse agricultural landscape and access to more diverse forage resources would contribute to greater overall health in these bee species as measured by lipid, glycogen and protein macronutrients. By comparing these physiological measurements of a managed and native bee coexisting in the same region, we aim to translate these results into proactive management strategies for a region identified as important for pollinator conservation.

Materials and methods

Study region and bee collection

Bees were collected from 12 locations throughout Brookings County in east-central South Dakota in August of 2013 (Fig. 1), which reflected a gradient of landscape-level diversity conditions: within a 3-km radius of collection locations, row crops accounted for 26.3–77.6% of the landscape; grass and pasture, 8.1–65.1%; forage crops, 4.0–30.2%; small grains, 0–4.1%; and aquatic habitat, 0.2–25.4% (Mogren *et al.*, 2016). Precipitation totals fell below the historical average of 78 mm, while temperatures during bee collection ranged from 26.1°C to 33.3°C.

During the bee diversity assessment reported in Mogren *et al.* (2016), *M. desponsa* (Apidae: Eucerini) was the most abundant native bee species recovered from bee bowls and blue vane traps (SpringStar Inc., Woodinville, WA, USA) across collection sites and thus were chosen as a model soli-

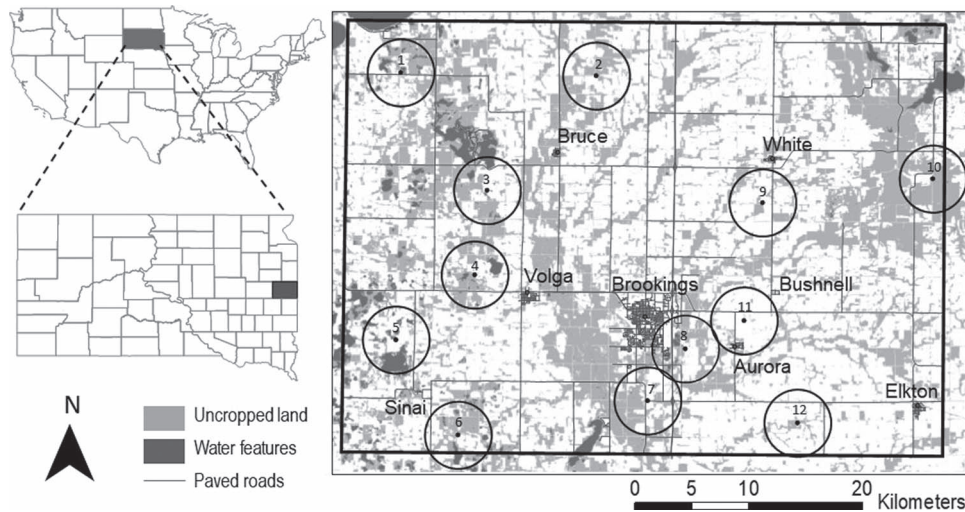


Figure 1: Map of the study region, showing 3 km sampling buffers for the landscape analysis. Figure originally published in *Environmental Entomology* (Mogren *et al.*, 2016).

tary bee species. These traps were deployed along crop field margins. Honey bee foragers were collected from apiaries located adjacent to each of the collection locations. Although the life histories for these bee species differ (e.g. honey bee foragers that are non-reproductive versus foraging *M. desponsa* females that are reproductive), comparing females actively engaged in foraging behaviours normalizes these differences. Samples were preserved in 70% ethanol and transported back to the laboratory on ice. Female *M. desponsa* were sorted and identified from the pollinator survey samples. All bees used in the present study were stored at -80°C until analysis. The number of bees of each species analysed per site is presented in Table 1.

Macronutrient assays

Bee nutrient analyses for lipids, glycogen and protein were conducted following Mogren and Lundgren (2016) and used as relative proxies for bee health. Briefly, bees were rinsed in 10% bleach and water. The entire alimentary canal was removed and stored in 70% ethanol at -20°C until DNA extractions. Glycogen and lipids from a homogenate of the remaining carcass were separated with a methanol-chloroform extraction. Lipids were quantified using a phospho-vanillin reaction of the supernatant compared with a standard curve of extra virgin olive oil (oleic acid) in chloroform (Vanhandel, 1985b) (54- μl olive oil in 50-ml chloroform; standard concentrations of 0, 1, 5, 10, 25, 50, 75, 100 μg), and the remaining pellet used in glycogen quantification with an anthrone assay compared with a standard curve of glycogen from oyster, type II (Sigma-Aldrich) (Vanhandel, 1985a) (25-mg oyster in 25-ml water; standard concentrations of 0, 1, 5, 10, 25, 50, 75, 100 μg). Protein assays were conducted following the Bio-Rad Bradford assay standard procedure for microtiter plates and calibration standards of bovine serum albumin (protein

standard II, Bio-Rad Laboratories) (standard concentrations of 0, 25, 50, 100, 250 and 500 μg).

Dietary breadth

DNA extraction and amplification

Total DNA from the alimentary canal was extracted using the DNeasy Blood and Tissue Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions and extracts recovered in a final volume of 400 μl . Because we were extracting DNA from pollen grains in the mid- and hindguts that had already undergone at least partial digestion by the bees, we reasoned that additional digests were not necessary. Dietary plant DNA from consumed nectar and pollen was amplified using a nested PCR procedure with primers targeting the *trnL* (UAA) p6 intron region of the chloroplast genome (Taberlet *et al.*, 1991, 2007). This approach amplified the small yet highly conserved gene region of interest, while simultaneously preparing individual samples for high-throughput sequencing by attaching sample-specific barcodes and sequencing oligonucleotides compatible with the Illumina MiSeq sequencing platform, as described by Hayden *et al.* (2008), Clarke *et al.* (2014) and Illumina (2013). This allowed for a cost-effective association of particular sequences to specific samples in a pooled DNA library (Pompanon *et al.*, 2012; Yu *et al.*, 2012).

The *trnL* primers were modified to include an overhanging adapter sequence (Table 2) such that sample-specific indexing primers would anneal to the first PCR product. In Phase 1 of the PCR, the amplification mixture included 8- μl PCR-grade water, 12.5 μl of KAPA HiFi HotStart ReadyMix 2x (KAPA Biosystems, Wilmington, MA), 1.0 μl each of 60 nmol modified forward and reverse *trnL* primers and 2.5 μl of template DNA, for a 25- μl total reaction volume. Samples were denatured at 95°C for 3 min, followed by 40 cycles of

Table 1: Summary of health metrics (mean \pm SE), total number of bees analysed (N) and number of bees testing positive for *trnL* by sampling site

| Site | Honey bees | | | | | <i>M. desponsa</i> | | | | |
|------|------------|---------------|-----------------------|-----------------|-----------------|--------------------|---------------|-----------------|-----------------|-----------------|
| | N | <i>trnL</i> + | Glycogen ^a | Lipids | Protein | N | <i>trnL</i> + | Glycogen | Lipids | Protein |
| 1 | 10 | 8 | 43.2 \pm 4.55 | 63.8 \pm 12.5 | 13.0 \pm 1.28 | 6 | 6 | 35.5 \pm 2.10 | 94.4 \pm 26.1 | 11.8 \pm 3.19 |
| 2 | 10 | 7 | 50.5 \pm 12.8 | 43.2 \pm 10.4 | 12.3 \pm 1.33 | 6 | 4 | 32.5 \pm 8.45 | 57.5 \pm 14.0 | 8.32 \pm 1.00 |
| 3 | 10 | 9 | 51.6 \pm 12.1 | 83.3 \pm 14.4 | 11.5 \pm 0.91 | 5 | 5 | 25.5 \pm 2.81 | 44.6 \pm 10.1 | 11.3 \pm 1.95 |
| 4 | 10 | 9 | 54.4 \pm 10.5 | 61.4 \pm 8.53 | 8.77 \pm 0.73 | 8 | 7 | 32.9 \pm 3.41 | 42.7 \pm 13.7 | 10.9 \pm 1.10 |
| 5 | 10 | 9 | 32.1 \pm 5.45 | 52.4 \pm 13.6 | 11.6 \pm 1.04 | 4 | 4 | 45.5 \pm 10.3 | 79.5 \pm 10.6 | 8.30 \pm 0.72 |
| 6 | 10 | 8 | 47.3 \pm 9.56 | 61.2 \pm 15.4 | 10.2 \pm 1.15 | 7 | 7 | 24.8 \pm 6.25 | 50.6 \pm 8.55 | 9.36 \pm 0.65 |
| 7 | 10 | 9 | 53.0 \pm 10.2 | 54.4 \pm 11.2 | 11.9 \pm 1.16 | 5 | 4 | 32.6 \pm 6.67 | 70.0 \pm 14.9 | 9.81 \pm 0.68 |
| 8 | 10 | 9 | 49.6 \pm 11.5 | 58.9 \pm 14.0 | 10.7 \pm 1.26 | 6 | 6 | 33.8 \pm 5.27 | 47.5 \pm 15.9 | 9.37 \pm 1.20 |
| 9 | 10 | 8 | 42.5 \pm 6.01 | 29.9 \pm 5.03 | 10.3 \pm 1.29 | 2 | 2 | 42.5 \pm 5.26 | 56.5 \pm 16.9 | 9.49 \pm 2.29 |
| 10 | 10 | 10 | 47.8 \pm 6.45 | 60.7 \pm 13.0 | 15.1 \pm 1.59 | 4 | 4 | 64.2 \pm 42.0 | 32.2 \pm 15.3 | 8.29 \pm 1.97 |
| 11 | 10 | 8 | 48.4 \pm 5.58 | 51.8 \pm 12.6 | 8.36 \pm 1.48 | 17 | 16 | 48.1 \pm 10.2 | 71.1 \pm 14.5 | 13.6 \pm 0.92 |
| 12 | 10 | 9 | 39.4 \pm 4.21 | 29.7 \pm 6.61 | 10.1 \pm 1.73 | 3 | 2 | 32.1 \pm 5.16 | 18.6 \pm 4.87 | 11.2 \pm 1.34 |

^a Units are μ g/bee for glycogen and lipids and mg/bee for protein.

Table 2: *trnL* primers used in this study

| Primer | Sequence (5'-3') |
|---------|---|
| Forward | <i>TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGGCAATCTGAGCCAA</i> |
| Reverse | <i>GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCATTGAGTCTCTGCACCTATC</i> |

Italics indicate the additional adapter sequence, with the amplicon specific sequence in bold (Taberlet *et al.*, 1991, 2007).

30 sec at 98°C, 30 sec at 50°C and 30 sec at 72°C, with a final 3-min elongation at 72°C.

To verify the presence of amplified DNA, samples were screened by electrophoresis on a 2% agarose gel. A total of 120 honey bees and 73 female *M. desponsa* specimens were evaluated for the presence of *trnL*, and only the positive samples underwent the second phase of amplification. This PCR mixture included 5.0 μ l of PCR-grade water, 12.5 μ l of KAPA HiFi HotStart ReadyMix 2x, 2.5 μ l each of i5 and i7 Nextera XT indexing primers (Nextera XT index kit v2, Illumina Inc., San Diego, CA) (Supplemental Table 1) and 2.5 μ l of *trnL* amplicon DNA from the first PCR. Samples were denatured at 95°C for 3 min, followed by 10 cycles of 30 sec at 98°C, 30 sec at 65°C and 30 sec at 72°C, with a final 3-min elongation at 72°C. Plant DNA from alfalfa, blue lupine, clover, dandelion, hairy vetch and a prairie seed mix were used as positive controls.

Library cleanup and sequencing

The library was cleaned using a 1.4:1.0 v:v ratio of AMPure XP beads (Agencourt Biosciences, Beverly, MA) to sample to remove fragments <150 bp (Lennon *et al.*, 2010). Bead-bound DNA was eluted in 1 \times TE buffer and then quantified

using the Quant-iT PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA). Sequencing was done at the University of Illinois Biotechnology Center (Urbana, IL) using an Illumina MiSeq v2 platform 2x250 paired-end run. There were on average 3385 \pm 23 raw data reads generated per sample. Because DNA from individual bee samples had unique indexing primers, 384 samples were combined and sequenced in a single library.

Sequence analysis

Sample de-multiplexing and quality filtering were performed using a modified OBITools pipeline (<http://metabarcoding.org/obitools/>) (Boyer *et al.*, 2016). This program was developed specifically for the analysis of next-generation sequencing data in a metabarcoding context, which is particularly relevant for dietary breadth analysis. This was installed and run within QIIME v.1.9.1 VirtualBox (http://qiime.org/install/virtual_box.html). Full-length amplicon sequences were first recovered by aligning the forward and reverse reads (`illumina-paired-end` command) and only well-recovered amplicon sequences were kept (`obigrep` command, minimum alignment score of 20). The primer sequences were identified and removed and the barcode sequence was reverse complemented if needed (`ngsfilter` command). Barcodes

were then dereplicated (`obiuniq` command). We selected sequences longer than 20 that occurred at least 10 times for the assignment of operational taxonomic units (OTUs) (`obigrep` command), as *trnL* (UAA) p6 intron region ranges from 10 to 143 bp (Taberlet *et al.*, 2007). A reference database of North American *trnL* sequences was downloaded from the NCBI database and sequences assigned to taxa with 90% sequence similarity (`obiconvert` and `ecotag` commands).

Raw sequence reads are deposited and publicly available in the Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra>) (BioProject ID: PRJNA558996; accession numbers: SAMN12510563 and SAMN12510732).

Statistical analyses

Landscape composition

The 2013 Cropland Data Layer (USDA, National Agricultural Statistics Service; <https://nassgeodata.gmu.edu/CropScape/>) land-use data were imported into ArcMap 10.3.1 (ESRI, 2015). The land cover was extracted by mask in 3-km buffers around each site, approximating the foraging range of honey bees and larger native pollinators (Cariveau *et al.*, 2013). A raster file of land use was exported in a GRID format and imported into FRAGSTATS v.4.2.1 (McGarigal *et al.*, 2012). A full landscape metrics analysis was run and included number of patches, largest patch index (LPI), total edges, landscape shape index, mean patch area (AREA_MN), radius of gyration distribution (GYRATE_AM), fractal dimension index, perimeter-area ratio, contiguity index, core area index, Euclidean nearest neighbour distance and relative patch richness. See Supplemental Table 2 for variable units and definitions.

Landscape and bee macronutrients

Multiple regression analysis was used in SAS (SAS Institute Inc, Cary, North Carolina, USA) to investigate relationships of the macronutrient variables (glycogen, lipids and total protein) between bee species. Because the landscape explanatory variables are site-level variables, the regression analysis was done at the site level ($n=12$). To accomplish this, the three macronutrient variables were averaged within each site per bee species, resulting in six response variables. Because the response within each site was multivariate in nature, in order to compare bee species, the modelled response variable for each health metric at a given site was calculated as the difference in the means of *M. desponsa* and honey bees within that site. Analysing each bee species in separate regressions would not have allowed for direct comparisons between their macronutrient responses to changing landscape conditions. Stepwise selection was used to select explanatory landscape variables for these models, with significance of 0.15 to enter and remain in the model.

Dietary composition and bee macronutrients

Linear mixed model regression analysis was used to model the macronutrients (glycogen, lipids and protein) as func-

tions of dietary composition. For each of the bee species, separate models were developed for the macronutrients, and when residual analysis indicated a violation of the normality assumptions, the natural log of the macronutrient was used as a response in the model. This occurred for glycogen for both bee species and lipids for honey bees. Dietary composition variables were identified for each taxon from the gut-content analysis and initially included 51 OTUs. In order to reduce the number of dietary variables, each bee species and macronutrient combination was subjected to an initial stepwise regression to identify the most significant plant resources, with a significance level of 0.15 used to enter and remain in the model.

Regression models were fit using fixed effects for the dietary composition variables and a random effect for site ($n=12$), resulting in linear mixed regression models. The random effect for site was included in order to account for potential variation from one site to another. Influence analysis was performed for each model to identify observations having unusual influence on the model, in conjunction with outlier identification.

Significant differences between bee species for dietary preferences, as measured by the number of reads from the family-level plant classification (OTU) ($n=9$), were tested within each of the 12 sites using a chi-square test of homogeneity, with post hoc pairwise comparisons for significant overall treatments using a Bonferroni correction. Family-level analyses were conducted as some plant taxa were only represented in a portion of the samples from some locations, and consolidating OTUs into families ensured adequate comparisons between bee species at each sampling location.

Results

Landscape and bee macronutrients

Stepwise selection revealed that the AREA_MN was the significant landscape predictor of the glycogen differences observed between *M. desponsa* and honey bees across sampling sites ($\hat{\beta}=10.1$; $F=9.17$, $df=1,10$, $P=0.013$). As the mean area of the patch size increased, so did the glycogen difference between *M. desponsa* and honey bees, indicating that uniformly larger habitat patches in the landscape were beneficial to glycogen levels in honey bees, while *M. desponsa* had relatively higher glycogen levels in areas with smaller habitat patches (Fig. 2). This could also imply that larger patches were dominated by honey bees, while *M. desponsa* were confined to relatively smaller habitat patches.

Protein differences were significantly influenced by the LPI, a measure of single patch dominance in the landscape ($\hat{\beta}=-0.50$; $F=8.26$, $df=2,9$, $P=0.018$) and GYRATE_AM, which measures how far an organism can move from a random starting point in a random direction without leaving a patch ($\hat{\beta}=0.024$; $F=4.51$, $df=2,9$, $P=0.063$), though

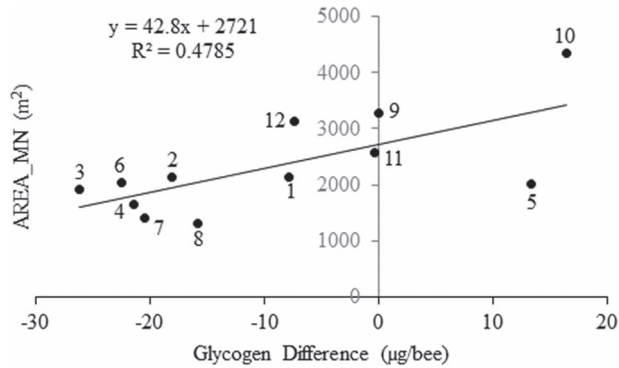


Figure 2: The relationship between mean habitat patch area and glycogen levels, expressed as the difference between *M. desponsa* and honey bees, per sampling site. Points are labelled with sampling locations.

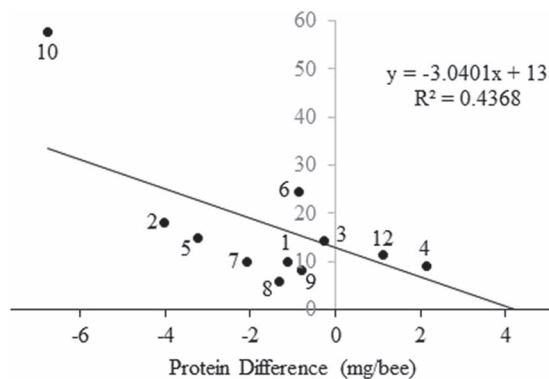


Figure 3: The relationship between largest patch index and protein levels, expressed as the difference between *M. desponsa* and honey bees, per sampling site. Points are labelled with sampling locations.

the parameter estimate for this second variable indicates the magnitude of this effect is small. The negative relationship between LPI and protein indicates that larger dominant patches reduce protein levels in honey bees relative to *M. desponsa* (Fig. 3).

None of the measured landscape variables explained differences in lipids between sampling locations. The glycogen, lipid and protein results for each of the bee species and collection site are shown in Table 2, as well as Supplemental Figs 1–3.

Dietary composition and bee macronutrients

The data for individual bee macronutrient and dietary results are presented in the supplemental spreadsheet. After quality filtering, there were a total of 221 842 sequence reads, from which 60.4% (134026) came from *trnL* positive honey bee samples ($n=103$) and 39.6% (87816) came from *M. desponsa* ($n=67$). Figure 4 shows the results of rarefaction analysis, indicating that the sampling depth was sufficient

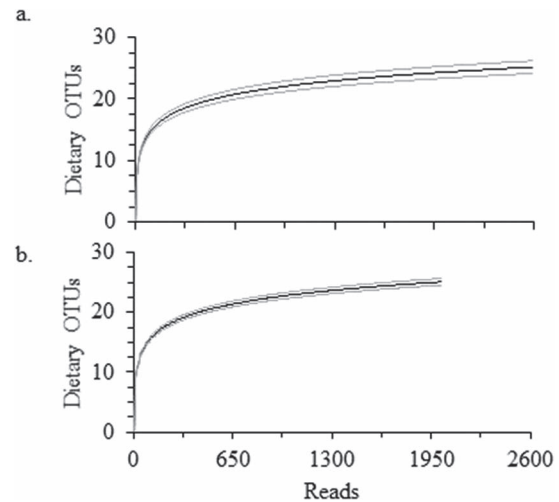


Figure 4: Alpha rarefaction curves of the filtered sequence reads showing the number of dietary plant OTUs, including Poaceae, recovered per sampling depth for honey bees (a) and *Melissodes desponsa* (b). Gray lines indicate the SE of the mean.

to capture the diversity of plant species in the bees' diets in the study region. A total of 51 OTUs were identified to species (10), genus (15) or family (22), with the remaining OTUs (4) determined to be unique but not matching known *trnL* sequences from the NCBI database. Recovered OTUs represent plant species from at least 18 different families. Individual honey bee samples ranged from 4–34 plant OTUs and *M. desponsa* ranged from 17–31 plant OTUs.

Poaceae accounted for 78% of sequence reads across all bee samples, which was attributed to forage contamination in the field. Though corn (*Zea mays*) is the dominant grass in the region and honey bees are frequently observed collecting corn pollen, this crop was not in anthesis during bee collection. Honey bees were not observed collecting pollen from other wild grasses, and as Poaceae was not present in controls, the abundance of this plant family in samples may have been due to possible preferential amplification of *trnL* during PCRs versus other plant species (Nichols *et al.*, 2018). Thus, this plant group was excluded from downstream analyses to prevent skewness in results.

Residual analysis of macronutrients and dietary composition regression analyses indicated non-normality of glycogen for both bee species and lipids for honey bees—the glycogen and lipid responses were therefore log transformed, which resulted in model residuals that conformed more closely to normality assumptions. The remaining models were run using untransformed data, and model results are presented in Supplemental Tables 3–8. While significant, the magnitude of the effects of individual plant OTUs on overall patterns of glycogen, lipid and protein levels were subtle regardless of

Table 3: Results of χ^2 test of homogeneity for bee dietary composition within sites

| Site | χ^2 | d.f. ¹ | P |
|------|----------|-------------------|--------|
| 1 | 450 | 8 | <0.001 |
| 2 | 30.0 | 7 | <0.001 |
| 3 | 81.9 | 8 | <0.001 |
| 4 | 78.0 | 7 | <0.001 |
| 5 | 250 | 7 | <0.001 |
| 6 | 1250 | 8 | <0.001 |
| 7 | 684 | 8 | <0.001 |
| 8 | 168 | 8 | <0.001 |
| 9 | 70.8 | 7 | <0.001 |
| 10 | 433 | 8 | <0.001 |
| 11 | 132 | 8 | <0.001 |
| 12 | 119 | 8 | <0.001 |

¹Bees from some sites did not contain DNA from Unk3 and thus that category was excluded for those analyses.

their overall significance in individual models, as evidenced by standardized coefficient values.

The proportion of sequence reads from each site for each bee species for the most common flowering families are shown in Fig. 5, which were significantly different between bee species within sites (Table 3). Between sites there was dietary overlap (Table 4); however, *M. desponsa* foraged more frequently on Ranunculaceae, Orobanchaceae and less common flowering species (Other) compared with honey bees (Table 5). Both bees foraged on flowers of Asteraceae, Fabaceae and Oleaceae. When honey bees foraged more heavily on Asteraceae than *M. desponsa* (sites 5, 6, 7 and 9; Table 5), this was explained by preferential foraging on species Asteraceae1 and Asteraceae7 specifically. When honey bees foraged more heavily on Fabaceae (sites 1, 7, 10; Table 5), this was explained by preferential foraging on *Medicago* sp., *Melilotus* sp. and Fabaceae1. *Melissodes desponsa* also foraged heavily on these species, particularly at sites 5, 6 and 12, in addition to *Trifolium* sp.

Discussion

This study adds to our understanding of how a co-occurring native and managed bees may be physiologically impacted in relation to each other by landscape and dietary variability across their environment. Our data show that while the macronutrient ratios of these bees are significantly affected by certain landscape patch characteristics, albeit divergently, the dietary composition recovered from *trnL* did not affect physiology as measured by glycogen, lipids and protein. Sig-

nificant differences in dietary preference within sampling sites, however, suggest that current management considerations favouring honey bee-oriented conservation plantings may be better served by augmenting vegetation in the environment preferred by native bees as well, which will benefit honey bees by proxy.

Physiological glycogen, lipid and protein levels in insects have been reported throughout the literature particularly as they relate to various stress responses. However, the value range that constitutes a healthy versus a deficient honey bee with regards to these cannot yet be defined due to variability in sampling methodology and the context of the experiments. Smart *et al.* (2019) reported glycogen, lipid and protein concentrations in honey bees as mg/g wet weight (we reported units as μg per individual bee) with the alimentary canal included in their analysis. They demonstrated a positive correlation between abdominal glycogen, lipids and proteins and frames of winter bees in the colonies, indicating that higher concentrations are associated with increased populations and survivorship within the colony. Mogren and Lundgren (2016) reported glycogen and lipid levels roughly two times greater in honey bees collected from pollinator strips adjacent to conventional and organic corn fields after the alimentary canal had been removed than in the present study. As more studies are published using these biomarkers in honey bees and other pollinators, comparisons will be possible that identify optimum macronutrient concentrations at various times during the year, corresponding with changes in colony physiology related to overwintering (Dolezal *et al.*, 2016), starvation stress (Wang *et al.*, 2016), pesticide exposures (Mogren and Lundgren, 2016; Cook, 2019) and pathogens (Lopienska-Biernat *et al.*, 2017). Presently, we are constrained to relative comparisons between locations and pollinator species.

From a landscape perspective, the solitary bee *M. desponsa* had higher glycogen and protein levels as compared with honey bees when there was patch dominance, as measured by LPI. In a previous study, Mogren *et al.* (2016) categorized land use types in eastern South Dakota, noting that 75% of the landscape comprised corn, soybeans and pasture, all of which represent highly simplified land-use designations. The same study found that these large patches of monocrops led to increased abundance of certain native bees, including *M. desponsa*, among others, contrary to what would be expected in a historic prairie region. These agrobiont pollinators appear to thrive in otherwise suboptimal habitat and increased glycogen and protein concentrations imply a physiological benefit resulting from some aspect of this otherwise degraded habitat that cannot be utilized by other bee species.

In contrast, honey bees experienced physiological benefits, relative to *M. desponsa*, in the form of increased glycogen and total protein concentrations when patch sizes were homogenous, which has also been shown to decrease pollen

Table 4: Dietary composition by pollinator species

| OTU | Honey bee (n = 103) | | | <i>M. desponsa</i> (n = 67) | | |
|--------------------------------------|---------------------|-----------------------------|--------------------|-----------------------------|----------------|--------------------|
| | Sequence reads | Proportion (%) ^a | Present in samples | Sequence reads | Proportion (%) | Present in samples |
| Apiaceae | 28 | 0.021 | 23 | 11 | 0.013 | 8 |
| Apocynaceae | 131 | 0.098 | 59 | 62 | 0.071 | 43 |
| Asteraceae1 | 17 669 | 13.2 | 102 | 5741 | 6.54 | 67 |
| Asteraceae2 | 714 | 0.533 | 94 | 461 | 0.525 | 60 |
| Asteraceae3 | 4 | 0.003 | 3 | 1055 | 1.20 | 17 |
| Asteraceae4 | 131 | 0.098 | 63 | 96 | 0.109 | 46 |
| Asteraceae5 | 332 | 0.248 | 74 | 105 | 0.120 | 45 |
| Asteraceae6 | 324 | 0.242 | 12 | 0 | 0 | 0 |
| Asteraceae7 | 531 | 0.396 | 96 | 175 | 0.199 | 57 |
| Asteraceae: | | | | | | |
| <i>Antennaria dioica</i> | 9 | 0.007 | 8 | 0 | 0 | 0 |
| <i>Eupatorium cannabinum</i> | 23 | 0.017 | 16 | 15 | 0.017 | 9 |
| <i>Pilosella officinarum</i> | 8 | 0.006 | 7 | 5 | 0.006 | 4 |
| <i>Solidago virgaurea</i> | 46 | 0.034 | 15 | 5 | 0.006 | 3 |
| Boraginaceae | 37 | 0.028 | 28 | 21 | 0.024 | 17 |
| Brassicaceae | 79 | 0.059 | 47 | 50 | 0.057 | 33 |
| Convolvulaceae | 20 | 0.015 | 16 | 35 | 0.040 | 27 |
| Curcubitaceae: <i>Curcubita pepo</i> | 1 | 0.001 | 1 | 187 | 0.213 | 7 |
| Cyperaceae: <i>Carex</i> | 13 | 0.010 | 13 | 9 | 0.010 | 9 |
| Fabaceae1 | 1247 | 0.931 | 27 | 21 | 0.024 | 6 |
| Fabaceae2 | 692 | 0.516 | 102 | 381 | 0.434 | 65 |
| Fabaceae3 | 44 | 0.033 | 32 | 117 | 0.133 | 26 |
| Fabaceae4 | 10 | 0.007 | 5 | 0 | 0 | 0 |
| Fabaceae: | | | | | | |
| <i>Amorpha fruticosa</i> | 101 | 0.075 | 54 | 55 | 0.063 | 33 |
| <i>Glycine max</i> | 479 | 0.357 | 62 | 318 | 0.362 | 49 |
| <i>Medicago</i> sp. | 2605 | 1.94 | 98 | 637 | 0.725 | 66 |
| <i>Melilotus</i> sp. | 751 | 0.560 | 78 | 714 | 0.813 | 44 |
| <i>Trifolium</i> sp. | 218 | 0.163 | 25 | 105 | 0.120 | 10 |
| Hypoxidaceae | 58 | 0.043 | 32 | 47 | 0.054 | 25 |
| Lamiaceae: | | | | | | |
| <i>Nepeta</i> sp. | 0 | 0 | 0 | 39 | 0.044 | 5 |
| <i>Salvia</i> sp. | 26 | 0.019 | 20 | 10 | 0.011 | 9 |
| Malvaceae1 | 0 | 0 | 0 | 133 | 0.151 | 2 |
| Malvaceae2 | 1 | 0.001 | 1 | 108 | 0.123 | 8 |

Table 4: Continued

| OTU | Honey bee (n = 103) | | | <i>M. desponsa</i> (n = 67) | | |
|--------------------------------------|---------------------|-----------------------------|--------------------|-----------------------------|----------------|--------------------|
| | Sequence reads | Proportion (%) ^a | Present in samples | Sequence reads | Proportion (%) | Present in samples |
| Oleaceae | 297 | 0.223 | 41 | 53 | 0.060 | 35 |
| Orobanchaceae: <i>Pedicularis</i> | 848 | 0.633 | 93 | 528 | 0.601 | 67 |
| Poaceae1 | 69 161 | 51.6 | 102 | 51 345 | 58.5 | 67 |
| Poaceae: | | | | | | |
| <i>Bouteloua</i> sp. | 439 | 0.328 | 92 | 242 | 0.276 | 59 |
| <i>Bromus</i> sp. | 22 110 | 16.5 | 97 | 15 145 | 17.2 | 67 |
| <i>Cenchrus</i> sp. | 381 | 0.284 | 84 | 268 | 0.305 | 65 |
| <i>Melinis repens</i> | 70 | 0.052 | 47 | 49 | 0.056 | 33 |
| <i>Muhlenbergia</i> sp. | 235 | 0.175 | 78 | 172 | 0.196 | 60 |
| <i>Panicum notatum</i> | 54 | 0.040 | 38 | 37 | 0.042 | 26 |
| <i>Paspalum</i> sp. | 66 | 0.049 | 42 | 39 | 0.044 | 33 |
| <i>Sorghastrum nutans</i> | 8757 | 6.53 | 97 | 5711 | 6.50 | 67 |
| Polygonaceae: <i>Persicaria</i> | 86 | 0.064 | 56 | 80 | 0.091 | 42 |
| Ranunculaceae: | | | | | | |
| <i>Thalictrum</i> sp. 1 | 2349 | 1.75 | 95 | 1600 | 1.82 | 66 |
| <i>Thalictrum</i> sp. 2 | 1062 | 0.792 | 92 | 615 | 0.700 | 64 |
| Solanaceae | 1 | 0.001 | 1 | 112 | 0.128 | 2 |
| Unknown1 | 865 | 0.645 | 87 | 613 | 0.698 | 66 |
| Unknown2 | 493 | 0.368 | 78 | 422 | 0.480 | 50 |
| Unknown3 | 319 | 0.238 | 15 | 18 | 0.020 | 8 |
| Unknown4 | 88 | 0.066 | 41 | 62 | 0.071 | 34 |

^aProportion refers to the percentage of total sequence reads from each pollen.

foraging distances (Steffan-Dewenter and Kuhn, 2003). This supports the management decisions of beekeepers in the region, who avoid corn and soybean fields when establishing apiaries (Otto *et al.*, 2016). Interestingly, we did not find an effect of landscape on lipid levels between bee species; elsewhere, increasing landscape diversity positively influenced bee lipid levels (Dolezal *et al.*, 2016; Smith *et al.*, 2016; Alaux *et al.*, 2017; Smart *et al.*, 2019). Lipids are synthesized and stored in the insect fat body as a source of energy; for honey bees, they are critically important to overwintering success and are higher in fall bees, with the temporal polyethism characterizing the species also contributing to lipid changes through time (Döke *et al.*, 2015). Solitary bees, such as *M. desponsa*, acquire all necessary nutrition themselves, as opposed to nest mates contributing to resource collection as in honey bees. In their case, lipids will be critical for egg production in females (Lawson *et al.*, 2017). We did not categorize the reproductive status of *M. desponsa* during

dissections, though developed eggs in the ovaries were not apparent. Thus, a lack of significance in our lipids data may be attributed to a pre- or post-reproductive biological status of *M. desponsa* that does not reflect potential impacts of landscape diversity.

Dietary composition, as indicated by DNA recovered in gut-content analyses, had no effect on glycogen, lipid or protein levels for either bee species. In the bumble bee *Bombus terrestris*, Kämper *et al.* (2016) found that forage quantity was more important than diversity in colony growth, which supports our finding, at least for the social honey bees. In contrast, Alaux *et al.* (2017) found that the presence of flowering catch crops increased pollen diet diversity, in turn leading to greater fat body mass and vitellogenin levels in honey bees. Honey bee hypopharyngeal gland development and vitellogenin expression were positively influenced by the nutritive quality of pollen as opposed to pollen diversity (Di Pasquale

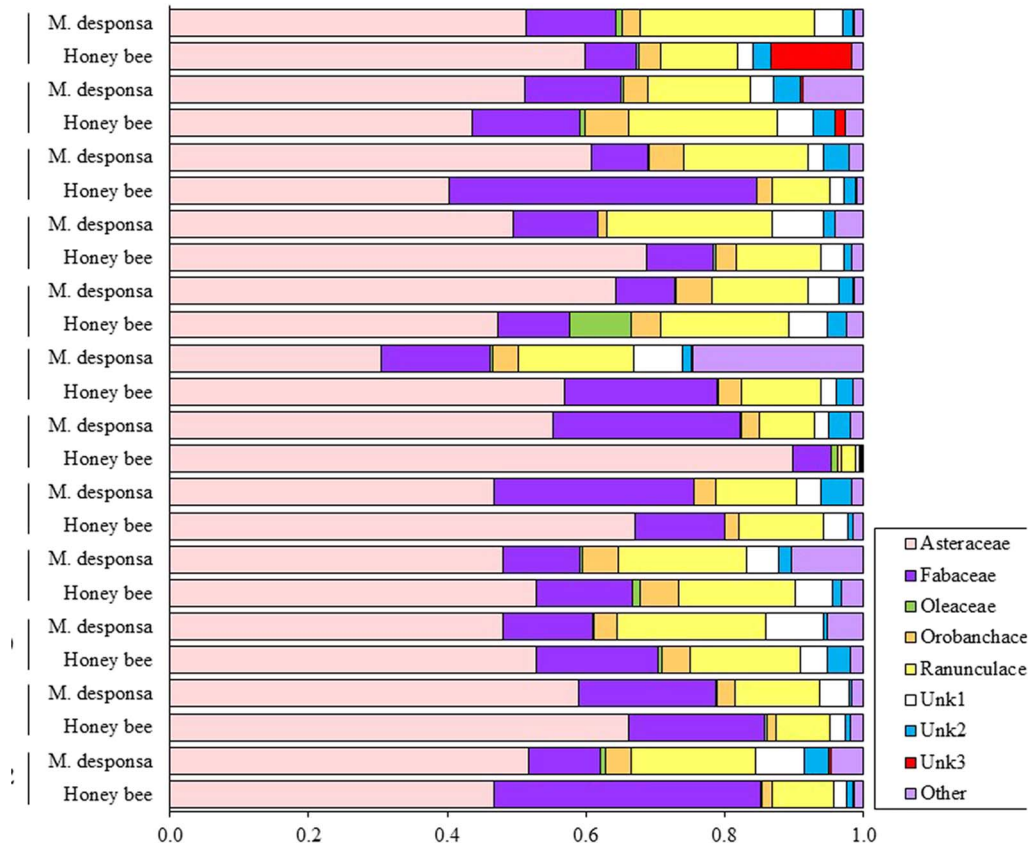


Figure 5: Average proportion of reads amplified for the most abundant dietary plant families, by bee species and site. Families representing <0.5% of reads were grouped as "other."

et al., 2016), though polyfloral diets may improve survival outcomes when honey bees are faced with a secondary stressor, such as the parasite *Nosema ceranae* (Di Pasquale et al., 2013). A lack of an observed physiological response to dietary composition in the present study could be attributed to both bee species obtaining sufficient nutrition from the quantity and diversity of forage available in this simplified landscape in eastern South Dakota. Alternatively, conditions may have been suboptimal for both species across the study region. More research is needed to determine exact macronutrient values that indicate healthy versus stressed bees.

Sequencing throughput per sample was lower for our samples than has been reported elsewhere in the literature for *trnL* dietary analyses (Valentini et al., 2009; Hibert et al., 2013; Srivathsan et al., 2015; Gebremedhin et al., 2016), though these studies examined faecal matter from herbivores as opposed to partially digested pollen. Low read counts may have been the result of using a DNA extraction protocol that was not plant-specific. However, despite low reads, rarefaction curves indicate sufficient coverage in our samples (Fig. 4).

While overall dietary composition may not have contributed to physiological differences in honey bees and *M.*

desponsa, there were some differences in foraging preferences between the species at different field sites. At sites where honey bees foraged heavily on Fabaceae, this was driven by a preference for non-native *Melilotus* sp. and *Medicago* sp. within the Fabaceae. These plant genera were identified as predominant floral resources in North Dakota as well (Smart et al., 2016; Otto et al., 2017). Similarly, honey bees in temperate regions have been found to utilize other agricultural weeds (Requier et al., 2015). While *M. desponsa* also foraged on *Melilotus* and *Medicago*, they preferred native, albeit less abundant floral species, such as Ranunculaceae and Orobanchaceae when they were present at study sites.

Studies on the floral preferences of many native bees are limited, though *Melissodes* as a genus are typically regarded as composite specialists, whose foraging is not thought to compete with that of honey bees (Dickinson and McKone, 1991). Our data showed dietary overlap between these two bee species particularly within Asteraceae and Fabaceae, though *M. desponsa* foraged on native flowers when they were apparently available (Table 5). Macronutrient ratios or other variables not measured in this study may also shape foraging strategies (Vaudo et al., 2016). Since we only evaluated the dietary compositions of two bee species in the region, in which 32 species of Apoidea were recently identified (Mogren et al.,

Table 5: Results of pairwise χ^2 tests of homogeneity for bee diet within sites

| Site | Asteraceae ^{a, b} | Fabaceae | Oleaceae | Orobanchaceae | Ranunculaceae | Unk1 | Unk2 | Unk3 | Other |
|------|----------------------------|-----------------------------|-----------------------------|-----------------------|-----------------------------|-----------------------------|-----------------------|-----------------------|----------------|
| 1 | 3.28 0.039 | 155 <0.001 | 8.08 0.001 | 15.1 <0.001 | 47.8 <0.001 | 57.2 <0.001 | 25.7 <0.001 | 0.443 0.449 | 34.7 <0.001 |
| 2 | 3.14 0.037 | 0.002 0.956 | 0.003 0.947 | 3.58 0.026 | 8.61 <0.001 | 5.26 0.007 | 1.13 0.212 | 0.015 0.885 | |
| 3 | 1.81 0.12 | 5.26 0.007 | 0.310 0.515 | 0.710 0.325 | 6.68 0.003 | 15.3 <0.001 | 11.9 <0.001 | 0.269 0.544 | 17.6 <0.001 |
| 4 | 1.91 0.066 | 2.55 0.034 | 2.19 0.049 | 0.205 0.547 | 0.590 0.307 | 0.473 0.360 | 1.25 0.138 | 34.9 <0.001 | |
| 5 | 40.3 <0.001 | 83.7 <0.001 | 0.239 0.557 | 2.77 0.045 | 0.166 0.624 | 0.002 0.955 | 45.3 <0.001 | 0.083 0.730 | |
| 6 | 163 <0.001 | 439 <0.001 | 6.25 0.003 | 44.0 <0.001 | 104 <0.001 | 26.8 <0.001 | 91.3 <0.001 | 1.63 0.135 | 37.2 <0.001 |
| 7 | 66.1 <0.001 | 8.95 0.001 | 1.74 0.140 | 0.158 0.656 | 10.5 <0.001 | 36.1 <0.001 | 2.78 0.062 | 3.13 0.047 | 416 <0.001 |
| 8 | 26.7 <0.001 | 1.78 0.094 | 63.6 <0.001 | 0.980 0.214 | 6.65 0.001 | 0.994 0.211 | 1.20 0.169 | 2.20 0.063 | 2.69 0.039 |
| 9 | 13.8 <0.001 | 1.63 0.178 | 0.733 0.365 | 2.79 0.078 | 25.5 <0.001 | 10.8 0.001 | 0.688 0.381 | 7.52 0.004 | |
| 10 | 55.4 <0.001 | 204 <0.001 | 6.58 0.004 | 13.8 <0.001 | 52.2 <0.001 | 0.202 0.616 | 9.95 <0.001 | 0.196 0.621 | 5.46 0.009 |
| 11 | 2.11 0.002 | 0.439 0.150 | 0.076 0.550 | 3.66 <0.001 | 4.68 <0.001 | 1.81 0.004 | 0.303 0.232 | 5.54 <0.001 | 9.31 <0.001 |
| 12 | 3.51 0.044 | 10.1 0.001 | 2.43 0.094 | 0.223 0.612 | 43.6 <0.001 | 4.25 0.027 | 1.74 0.157 | 36.6 <0.001 | 0.308 0.551 |

^a χ^2 value on top, *P*-value on bottom; *df* = 1 for each.

^bBonferroni correction applied; therefore, italic values indicate significance at *P* < 0.006. Bold values indicate that the significance was driven by higher than expected values in honey bees, while regular italicized indicates significance driven by higher values in *M. desponsa*.

2016), a full understanding of whether niche partitioning or competition occurs between the native solitary bees in the region and honey bees is not possible based on our data alone.

Although it is relatively simple to categorize the composition of honey bee collected pollen and nectar inside a managed colony, understanding the feeding biology of solitary native bees is more difficult. Thus, the application of metabarcoding techniques is an important tool for understanding their ecology in conservation applications. Unfortunately, being unable to assign a specific name to sequences in all cases can limit its utility when devising specific management plans. This emphasizes the need for bioinventories that simultaneously collect and deposit plant DNA for multiple genetic markers in public repositories, particularly in underrepresented regions of conservation importance. This is especially true for *trnL*, which has proven useful in dietary studies of herbivores using partially digested plant DNA (Valentini *et al.*, 2009; Hibert *et al.*, 2013; Srivathsan *et al.*, 2015; Wallinger *et al.*, 2015; Gebremedhin *et al.*, 2016; Espunyes *et al.*, 2019). While we were able to distinguish differences between the diets

of honey bees and *M. desponsa* using *trnL*, the taxonomic resolution to genus and family as opposed to species level identification may explain why we were unable to detect an effect on macronutrient levels across the study area. In some cases, we could distinguish OTUs despite not knowing species names (e.g. Asteraceae; Table 4), which still counted as dietary diversity, but in others, we were only able to resolve sequences at the family level. The limitations posed by our experimental design necessitated the use of a gene target that could identify DNA that had already undergone partial digestion, but future studies utilizing *trnL* for pollinator gut-content analysis may benefit from concurrent assembly of a DNA reference library for locally flowering species.

Our results imply that in highly developed agricultural landscapes such as eastern South Dakota, management practices focused on honey bee conservation that promote ubiquitous and potentially weedy flowering species may not completely capture the needs of native bees when honey bees are also present. Otto *et al.* (2017) similarly concluded that honey bees may not be appropriate umbrella species

for all pollinators within the landscape. However, we did find significant dietary overlap between these two bee species, specifically among weedy flowers. Dietary overlap may explain why we did not find an effect of dietary diversity on individual bee nutrient metrics. However, we did find a diverging physiological response to landscape characteristics, implying these bee species respond differently to agricultural development and have different ecological patch requirements despite dietary overlap. For honey bees, greater glycogen in response to increasing AREA_MN may be due to an enhanced success in floral patch discovery (Donaldson-Matasci and Dornhaus, 2014) versus smaller patches, which may be more difficult to discover and recruit to. Future pollinator conservation and management efforts in the region should incorporate minimum patch areas in planting recommendations to ensure these conservation plantings capture dietary and spatial needs for target species.

Supplementary material

Supplementary material is available at *Conservation Physiology* online.

Acknowledgements

We thank Janet Fergen, Ryan Bell, Jacob Pecenka, Nicole Berg and Marissa Layman for their help with field sampling and data collection. Karen Wright (University of New Mexico) assisted with the identification of *Melissodes* specimens. Emily Boothe (LSU AgCenter) assisted with ArcGIS and Fragstats analysis. The field portion of this work was conducted when C.L.M., M.-S.B. and J.G.L. were employees of the USDA-ARS in Brookings, SD, USA. Mention of any proprietary products does not constitute endorsement by the USDA-ARS.

Funding

This research was funded by the US Department of Agriculture Agricultural Research Service and a 2014 National Honey Board grant.

References

- Alaux CF *et al.* (2017) A 'Landscape physiology' approach for assessing bee health highlights the benefits of floral landscape enrichment and semi-natural habitats. *Sci Rep* 7: 40568.
- Alaux CF *et al.* (2010) Diet effects on honeybee immunocompetence. *Biol Lett* 6: 562–565.
- Benjamin FE *et al.* (2014) Pollinator body size mediates the scale at which land use drives crop pollination services. *J Appl Ecol* 51: 440–449.
- Boyer F *et al.* (2016) OBITOOLS: a UNIX-inspired software package for DNA metabarcoding. *Mol Ecol Resour* 16: 176–182.
- Campbell AJ *et al.* (2017) Do sown flower strips boost wild pollinator abundance and pollination services in a spring-flowering crop? A case study from UK cider apple orchards. *Agr Ecosyst Environ* 239: 20–29.
- Cariveau D *et al.* (2013) Response diversity to land use occurs but does not consistently stabilize ecosystem services provided by native pollinators. *Ecol Lett* 16: 903–911.
- Clarke LJ *et al.* (2014) Modular tagging of amplicons using a single PCR for high-throughput sequencing. *Mol Ecol Resour* 14: 117–121.
- Cook SC (2019) Compound and dose-dependent effects of two neonicotinoid pesticides on honey bee (*Apis mellifera*) metabolic physiology. *Insects* 10: 18.
- Cooke SJ *et al.* (2013) What is conservation physiology? Perspectives on an increasingly integrated and essential science. *Conserv Physiol* 1: <https://doi.org/10.1093/conphys/cot001>.
- Di Pasquale G *et al.* (2016) Variations in the availability of pollen resources affect honey bee health. *PLoS One* 11: e0162818.
- Di Pasquale G *et al.* (2013) Influence of pollen nutrition on honeybee health: do pollen quality and diversity matter? *PLoS One* 8: e72016.
- Dickinson JA, McKone MJ (1991) Insect floral visitors to four species of tall-grass prairie composite (Asteraceae: Heliantheae). *Prairie Naturalist* 24: 159–174.
- Döke MA *et al.* (2015) Overwintering honey bees: biology and management. *Curr Opin Insect Sci* 10: 185–193.
- Dolezal AG *et al.* (2016) Intensively cultivated landscape and *Varroa* mite infestation are associated with reduced honey bee nutritional state. *PLoS One* 11: e0153531.
- Donaldson-Matasci M, Dornhaus A (2014) Dance communication affects consistency, but not breadth, of resource use in pollen-foraging honey bees. *PLoS One* 9: e107527.
- Espunyes J *et al.* (2019) Comparing the accuracy of PCR-capillary electrophoresis and cuticle microhistological analysis for assessing diet composition in ungulates: a case study with Pyrenean chamois. *PLoS One* 14: e0216345.
- Fausti SW (2015) The causes and unintended consequences of a paradigm shift in corn production practices. *Environ Sci Pol* 52: 41–50.
- Free J (1963) The flower constancy of honeybees. *J Anim Ecol* 32: 119–131.
- Gallant AL *et al.* (2014) Mapping large area landscape suitability for honey bees to assess the influence of land-use change on sustainability of national pollination services. *PLoS One* 9: e99268.
- Gebremedhin B *et al.* (2016) DNA metabarcoding reveals diet overlap between the endangered Walia ibex and domestic goats—implications for conservation. *PLoS One* 11: e0159133.

- Hawkins J *et al.* (2015) Using DNA metabarcoding to identify the floral composition of honey: a new tool for investigating honey bee foraging preferences. *PLoS One* 10: e0134735.
- Hayden MJ *et al.* (2008) Multiplex-ready PCR: a new method for multiplexed SSR and SNP genotyping. *BMC Genomics* 9: 80.
- Hibert F *et al.* (2013) Unveiling the diet of elusive rainforest herbivores in next generation sequencing era? The tapir as a case study. *PLoS One* 8: e60799.
- Illumina. (2013) 16S metagenomic sequencing library preparation. Report #15044223 Rev B. 28.
- Jaumann S *et al.* (2013) Energetic cost of learning and memory can cause cognitive impairment in honeybees. *Biol Lett* 9: 20130149.
- Kalinkat G *et al.* (2017) Flagship umbrella species needed for the conservation of overlooked aquatic biodiversity. *Conserv Biol* 31: 481–485.
- Kämper W *et al.* (2016) How landscape, pollen intake and pollen quality affect colony growth in *Bombus terrestris*. *Landsc Ecol* 31: 2245–2258.
- Keller A *et al.* (2015) Evaluating multiplexed next-generation sequencing as a method in palynology for mixed pollen samples. *Plant Biol* 17: 558–566.
- Lawson SP *et al.* (2017) Effects of nutritional deprivation on development and behavior in the subsocial bee *Ceratina calcarata* (Hymenoptera: Xylocopinae). *J Exp Biol* 220: 4456–4462.
- Lennon NJ *et al.* (2010) A scalable, fully automated process for construction of sequence-ready barcoded libraries for 454. *Genome Biol* 11: R15.
- Lopienska-Biernat E *et al.* (2017) Biochemical status of feral honey bees (*Apis mellifera*) infested with various pathogens. *J Apic Res* 56: 606–615.
- McGarigal K *et al.* (2012) FRAGSTATS v4: spatial pattern analysis program for categorical and continuous maps. Computer software program produced by the authors at the University of Massachusetts, Amherst. Available at the following website: <http://www.umass.edu/landeco/research/fragstats/fragstats.html>.
- Mogren CL, Lundgren JG (2016) Neonicotinoid-contaminated pollinator strips adjacent to cropland reduce honey bee health. *Sci Rep* 6: 29608.
- Mogren CL *et al.* (2016) The effects of crop intensification on the diversity of native pollinator communities. *Environ Entomol* 45: 865–872.
- Naug D (2009) Nutritional stress due to habitat loss may explain recent honeybee colony collapses. *Biol Conserv* 142: 2369–2372.
- Nichols RV *et al.* (2018) Minimizing polymerase biases in metabarcoding. *Mol Ecol Resour* 18: 927–939.
- Otto C *et al.* (2018) Past role and future outlook of the conservation reserve program for supporting honey bees in the Great Plains. *Proc Natl Acad Sci USA* 115: 7629–7634.
- Otto CRV *et al.* (2017) Using publicly available data to quantify plant-pollinator interactions and evaluate conservation seeding mixes in the Northern Great Plains. *Environ Entomol* 46: 565–578.
- Otto CRV *et al.* (2016) Land-use change reduces habitat suitability for supporting managed honey bee colonies in the Northern Great Plains. *Proc Natl Acad Sci USA* 113: 10430–10435.
- Pollinator Health Task Force (2015) *National Strategy to Promote the Health of Honey Bees and Other Pollinators*, The White House, Washington, DC.
- Pompanon F *et al.* (2012) Who is eating what: diet assessment using next generation sequencing. *Mol Ecol* 21: 1931–1950.
- Quinn NF *et al.* (2017) Floral strips attract beneficial insects but do not enhance yield in cucumber fields. *J Econ Entomol* 110: 517–524.
- Requier F *et al.* (2015) Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. *Ecol Appl* 25: 881–890.
- Richardson RT *et al.* (2015) Application of ITS2 metabarcoding to determine the provenance of pollen collected by honey bees in an agroecosystem. *Appl Plant Sci* 3: 1400066.
- Scofield HN, Mattila HR (2016) Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. *PLoS One* 10: e0121731.
- Sint D *et al.* (2018) The effect of plant identify and mixed feeding on the detection of seed DNA regurgitates of carabid beetles. *Ecol Evol* 8: 10834–10846.
- Smart M *et al.* (2017) A comparison of honey bee-collected pollen from working agricultural lands using light microscopy and ITS metabarcoding. *Environ Entomol* 46: 38–49.
- Smart M *et al.* (2019) Nutritional status of honey bee (*Apis mellifera* L.) workers across an agricultural land-use gradient. *Sci Rep* 9: 16252.
- Smart M *et al.* (2016) Land use in the Northern Great Plains region of the US influences the survival and productivity of honey bee colonies. *Agric Ecosyst Environ* 230: 139–149.
- Smith GW *et al.* (2016) Bee abundance and nutritional status in relation to grassland management practices in an agricultural landscape. *Environ Entomol* 45: 338–347.
- Srivathsan A *et al.* (2015) Comparing the effectiveness of metagenomics and metabarcoding for diet analysis of a leaf-feeding monkey (*Pygathrix nemaeus*). *Mol Ecol Resour* 15: 250–261.
- Steffan-Dewenter I, Kuhn A (2003) Honeybee foraging in differentially structured landscapes. *Proc R Soc Lond B Biol Sci* 270: 569–575.
- Steffan-Dewenter I, Tscharntke T (2000) Resource overlap and possible competition between honey bees and wild bees in Central Europe. *Oecologia* 122: 288–296.
- Taberlet P *et al.* (2007) Power and limitations of the chloroplast *trnL* (UAA) intron for plant DNA barcoding. *Nucleic Acids Res* 35: e14.

- Taberlet P *et al.* (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol Biol* 17: 1105–1109.
- Valentini A *et al.* (2009) New perspectives in diet analysis based on DNA barcoding and parallel pyrosequencing: the *tmL* approach. *Mol Ecol Resour* 9: 51–60.
- Valentini A *et al.* (2010) DNA barcoding for honey biodiversity. *Diversity* 2: 610–617.
- Vanhandel E (1985a) Rapid determination of glycogen and sugars in mosquitos. *J Am Mosq Control Assoc* 1: 299–301.
- Vanhandel E (1985b) Rapid determination of total lipids in mosquitos. *J Am Mosq Control Assoc* 1: 302–304.
- Vaudo AD *et al.* (2016) Macronutrient ratios in pollen shape bumble bee (*Bombus impatiens*) foraging strategies and floral preferences. *Proc Natl Acad Sci USA* 113: E4035–E4042.
- Wallerger C *et al.* (2015) Detection of seed DNA in regurgitates of granivorous carabid beetles. *Bull Entomol Res* 105: 728–735.
- Wang Y *et al.* (2016) Starvation stress during larval development facilitates an adaptive response in adult worker honey bees (*Apis mellifera* L.). *J Exp Biol* 219: 949–959.
- Wimberly MC *et al.* (2017) Cropland expansion and grassland loss in the eastern Dakotas: new insights from a farm-level survey. *Land Use Policy* 63: 160–170.
- Wright CK, Wimberly MC (2013) Recent land use change in the Western Corn Belt threatens grasslands and wetlands. *Proc Natl Acad Sci U S A* 110: 4134–4139.
- Yu DW *et al.* (2012) Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. *Methods Ecol Evol* 3: 613–623.