- 1 Supplementary methods and analysis from "Dominant bee species and floral abundance
- 2 *drive parasite temporal dynamics in plant-pollinator communities*", Graystock *et al.*
- 3

## 4 Broad multiplex PCR panels to diagnose major bee parasites

5 We first screened samples for three groups of taxa known to contain common pollinator 6 parasites, trypanosomatids, Microsporidians and Apicystis, in multiplex 1. Samples 7 determined to be positive for either Trypanosome or Microsporidia were then used in further 8 multiplex assays to determine the presence of trypanosomes common in North American 9 bees (Crithidia bombi, C. expoeki) or Microsporidians (Nosema bombi, N. ceranae) respectively. Apicystis bombi is the only Apicystis species reported in bees, so further 10 11 multiplexes were not performed on these positives. In addition to this multiplex, we ran a 12 host control on all bee samples using Apidae general primers 2 which we found to amplify across the broad range of hosts used here. For all Primers, subsets of positives were 13 sequenced to confirm correct amplification. Primers, reagent concentrations, and thermal 14 15 cycling conditions are shown in Supplementary Table 1. 16 17 Trypanosomatid multiplex to diagnose two common Crithidia parasite species 18 Samples found to be positive for trypanosomatids in the broad multiplex were then further 19 screened in a multiplex designed to indicate single and dual infections of C. bombi and C. 20 expoeki, which was developed as part of previously published project 3. Primers, reagent

concentrations, and thermal cycling conditions are shown in Supplementary Table 1.

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23 Microsporidia multiplex to diagnose two common Nosema parasite species

24 Samples found to be positive for Microsporidia in the broad multiplex were then further

- screened in a duplex designed to indicate single and dual infections of *N. bombi* and *N.*
- 26 ceranae. Although most of the genetic data associated with the Microsporidia on GenBank is

27 based on portions of the small subunit region (16S), we were interested in using less variable regions that were highly discernable in each species. Using a modified version of primers 28 29 NoscRNAPol-F2 (5'-GGGTTCCCTAAACCTGGTGGTTT-3' and NoscRNAPol-R2 (5'-30 TCACATGACCTGGTGCTCCTTCT -3') that amplify a >600 bp region of largest subunit of the protein-coding DNA-dependent, RNA polymerase II (RPB1) 4, we obtained sequences of 31 N. bombi (MG494263) and N. ceranae (MN175395). These were aligned with available N. 32 33 ceranae (XM\_002995356) and N. apis (JX213670) sequences from GenBank in Geneious 34 v.6.1.8 (Biomatters, Auckland, NZ). With the aid of the Primer 3 plug-in 5, we selected 35 suitable priming regions unique to *N. bombi* or *N. ceranae*. 36 37 Potential primers were then examined to ensure that they would not produce amplicons for 38 non-target species and that amplicons for each species could be visually differentiated by 39 routine electrophoresis based on their size differential. Representative sequences of a variety of Microsporidia (N. apis: DQ996230, DQ996232, JX213663, JX213665, JX213751, 40 41 JX213670, HQ457438, HQ457435, HQ457436; N. bombycis: JX213751; N. disstriae: HQ457438; N. empoascae: DQ996232; N. fumiferanae: HQ457438; N. granulosis: 42 DQ996233; N. lymantriae: JX213749; N. sp.: HQ457436, HQ457437; N. trichoplusiae: 43 DQ996234; Varimorpha necatrix: DQ996236), were screened to ensure primer specificity. A 44 final set of differential primers was selected from those that had similar melting temperatures 45 46 and minimal interactions when combined in a single reaction. The novel primer set NB185F (5'- ACTAAGCCAATGTTCCACGTT -3') and NB185R (5'-47 CCAGTAAACCCACTTTCACAGAT -3') produces an amplicon 185 bp long if N. bombi 48 49 DNA is present, and NC288F (5'- TGAGGGAGAATTAACCGAGGC-3') plus NC288R (5'-AGCATCCCTTCCATAACAATAGATG-3') produces an amplicon 288 bp in the presence 50 51 of N. ceranae DNA.

53	Before being applied to the samples in this experiment, primer sets were tested in PCR both
54	singly and in combination with positives and negatives verified by microscopy to ensure
55	accurate diagnosis. Sequences of N. ceranae obtained with the NC288F-NC288R primer set
56	were deposited on Genbank (MG494264, MG494265; 242 bp with primer regions trimmed),
57	but because <i>N. bombi</i> sequences obtained with NB185F-NB185R were < 200 bp, they were
58	not eligible for deposition. A representative sequence (141 bp with the primer regions
59	trimmed) is 5'-
60	GGGTACATGAACAAGATCAAAAAGATTTTAGAATGTGTGTG
61	AAATAAAAATTGACAAGAAATCTTTGAAGAAAGACTTGAATTTTGTATGGAATG
62	CTTGTAAAGCTAAAGCAGTTTGTGAAGGAGAA-3' (Internal Sample Numbers AS107,
63	WC066). Extracted DNA and slide-mounted tissues are archived at the USDA-Pollinating
64	Insects Research Unit in Logan, Utah. Primers, reagent concentrations, and thermal cycling
65	conditions are shown in Supplementary Table 1.
66	

## Supplementary Table 1 | PCR mixes and conditions for the detection of the various parasites.

parasites.	Assay mix					Т	hermal cyclii	ng				
Primers & source	dNTP (mM)	MgCl2 (mM)	5xbuffer (μl)	Taq (U)	Primer F (μM)	Primer R (µM)	Template (µl)	Total volume (μl)	1 - Denaturing Min   Temp	2 - Replication Sec   Temp	3- Elongation Min   Temp	Amplicon size (bp)
Broad multiplex Trypanosomatidsta, Microsporidiama & Apicystisab 1 CB-SSUrRNA-F2 (5-3): CTTITGACGAACAGCGCCCATC CBISSR2 (5-3): TGCTCCTTTGTTATCCCATGCT MSporF2 (5-3): GGTGTGTCCATGGCCGTTTTC MSporDegR (5-3): GGTGTGTCCAAAGAACAGGG Apicyst357F (5-3): AGCGATGGATGTCTTGCGGCC Apicyst357F (5-3): CCTAGTTAGTTTCTTTCCTCCCGC	0.4	2.5	2.6	1	0.8 ta 0.8 ma 0.8 ab	0.8 та 0.8 ма 0.8 ав	1	10	2   94	10x 30   94 30   60 45   72 30x 30   94 30   57 45   72	5   72	584та 270ма 357ав
Crithidia Multiplex C. sp,c C. bombicb and C. expoekice 3 Crith18SF (5-3): TACCACTTCTACGGAGGGCA CBI8SR2 (5-3): TGCTCCTTTGTTATCCCATGCT CB279F (5-3): ATACTCATATTTTAGGTGTGGGCTGT CB279R (5-3): AACAAAACAAATGCATTACAATAACTA CE163F (5-3): TGTACTATGATGTCGTATTGAGGT CE163R (5-3): ATACAAATGGCAATAAAACATGTAAAA	0.4	2.5	2.6	1	0.8 с 0.8 св 0.8 се	0.8 с 0.8 св 0.8 се	1	10	2   94	40x 45   94 45   57 60   72	7   72	470с 279св 163се
Nosema Duplex N. bombin® and N. ceranaenc (This study) NB185F (5-3): ACTAAGCCAATGTTCCACGTT NB185R (5-3): CCAGTAAACCCACTTTCACAGAT NC288F (5-3): TGAGGGAGAATTAACCGAGGC NC288F (5-3): AGCATCCCTTCCATAACATGATG	0.4	2.5	2.6	1	0.8 NC 0.8 NB	0.8 NC 0.8 NB	1	10	2   94	40x 45   94 45   57 60   72	5   72	288nc 185nb
Host control Apidae 2 AGATGGGGGCATTCGTATTG AGATGGGGCATTCGTATTG ApidaeR(5-3): ATCTGATCGCCTTCGAACCT	0.2	1.5	2	1.25	0.2	0.2	1	10	2   94	35x 60   94 60   57 60   72	5   72	130

**Supplementary Table 2** | **Parasite prevalence among the 89 species of flowers that were screened.** (n = 2,631 individual flowers were screened in total.) 

Plant species	Samples screened	Percent positive with Nosema bombi	Percent positive with Nosema ceranae	Percent positive with Crithidia bombi	Percent positive with Crithidia expoeki	Percent positive with Neogreg arines
Achillea millefolium	3	0.0	0.0	0.0	0.0	0.0
Alliaria petiolata	1	0.0	0.0	0.0	0.0	0.0
Anaphalis margaritacea	2	0.0	0.0	0.0	0.0	0.0
Anemone nemorosa	2	0.0	50.0	0.0	0.0	0.0
Apocynum cannabinum	16	0.0	0.0	6.3	0.0	0.0
Asclepias incarnata	2	0.0	0.0	0.0	0.0	0.0
Asclepias syriaca	12	0.0	0.0	0.0	0.0	0.0
Brassica rapa	12	16.7	0.0	8.3	0.0	25.0
Calystegia sepium	3	0.0	0.0	0.0	0.0	0.0
Centaurea stoebe	265	2.3	0.8	6.0	2.3	6.0
Cerastium fontanum	2	0.0	0.0	0.0	0.0	0.0
Cichorium intybus	15	6.7	0.0	0.0	0.0	0.0
Cirsium arvense	35	0.0	0.0	5.7	0.0	0.0
Cirsium vulgare	8	0.0	0.0	0.0	0.0	0.0
Clematis virginiana	3	0.0	0.0	0.0	0.0	0.0
Clinopodium vulgare	18	0.0	0.0	0.0	0.0	0.0
Cornus racemosa	2	0.0	0.0	0.0	0.0	0.0
Daucus carota	79	5.1	1.3	1.3	2.5	5.1
Dianthus armeria	15	0.0	0.0	0.0	0.0	0.0
Dipsacus fullonum	53	0.0	0.0	5.7	1.9	0.0
Doellingeria umbellata	23	4.3	0.0	0.0	0.0	0.0
Epilobium ciliatum	2	0.0	0.0	0.0	0.0	0.0
Erigeron annuus	35	2.9	2.9	0.0	2.9	2.9
Erigeron sp.	2	0.0	0.0	0.0	0.0	0.0
Eupatorium perfoliatum	4	0.0	0.0	0.0	0.0	0.0
Eutrochium maculatum	13	0.0	0.0	0.0	0.0	0.0
Fragaria virginiana	56	5.4	1.8	5.4	0.0	0.0
Galium mollugo	5	0.0	0.0	0.0	0.0	0.0
Glechoma hederacea	3	0.0	0.0	0.0	0.0	0.0
Hesperis matronalis	3	0.0	0.0	0.0	0.0	0.0
Hieracium aurantiacum	3	33.3	33.3	0.0	0.0	0.0
Hieracium caespitosum	27	0.0	3.7	3.7	0.0	7.4
Hieracium pilosella	6	0.0	0.0	0.0	0.0	0.0
Hieracium scabrum	47	2.1	0.0	4.3	0.0	6.4
Hieracium sp.	29	6.9	0.0	3.4	6.9	3.4

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Hypericum perforatum	15	0.0	0.0	0.0	0.0	0.0
Impatiens capensis	8	0.0	0.0	0.0	0.0	0.0
Leucanthemum vulgare	100	5.0	2.0	1.0	5.0	2.0
Linaria vulgaris	3	0.0	0.0	0.0	0.0	0.0
Lobelia siphilitica	3	0.0	0.0	0.0	0.0	0.0
Lonicera morrowii	12	0.0	0.0	16.7	0.0	0.0
Lotus corniculatus	161	1.9	0.6	4.3	0.0	1.2
Lychnis flos-cuculi	50	8.0	2.0	10.0	0.0	6.0
Lycopus americanus	2	0.0	0.0	0.0	0.0	0.0
Lysimachia ciliata	2	0.0	0.0	0.0	0.0	0.0
Lythrum salicaria	107	0.0	0.0	0.9	0.0	0.0
Malva moschata	10	0.0	0.0	0.0	0.0	0.0
Melilotus albus	37	0.0	0.0	5.4	5.4	0.0
Melilotus officinalis	22	0.0	0.0	18.2	0.0	0.0
Mentha arvensis	28	3.6	3.6	0.0	0.0	7.1
Monarda fistulosa	53	0.0	0.0	0.0	0.0	1.9
Oxalis stricta	5	0.0	0.0	0.0	0.0	0.0
Pastinaca sativa	8	0.0	0.0	25.0	0.0	0.0
Penstemon digitalis	127	1.6	1.6	4.7	0.8	4.7
Plantago lanceolata	7	0.0	0.0	0.0	0.0	0.0
Potentilla canadensis	14	0.0	0.0	0.0	0.0	0.0
Potentilla recta	109	1.8	0.0	2.8	0.9	0.0
Potentilla simplex	5	0.0	0.0	0.0	0.0	0.0
Prunella vulgaris	36	0.0	0.0	0.0	0.0	0.0
Pycnanthemum						
tenuifolium	79	0.0	0.0	0.0	0.0	0.0
Ranunculus acris	184	1.6	0.0	1.6	1.6	4.9
Rosa multiflora	58	0.0	0.0	3.4	1.7	0.0
Rubus allegheniensis	78	1.3	0.0	1.3	1.3	1.3
Rubus hispidus	8	0.0	0.0	0.0	0.0	0.0
Rudbeckia hirta	18	0.0	0.0	5.6	0.0	0.0
Rumex crispus	1	0.0	0.0	0.0	0.0	0.0
Salix sp.	23	0.0	0.0	0.0	0.0	0.0
Silphium perfoliatum	16	0.0	0.0	0.0	0.0	0.0
Sisyrinchium	2	0.0	0.0	0.0	0.0	0.0
angustifolium	3	0.0	0.0	0.0	0.0	0.0
Sisyrinchium montanum	17	0.0	0.0	0.0	0.0	23.5
Solidago graminifolia	44	0.0	0.0	0.0	0.0	0.0
Solidago juncea	64	6.3	0.0	1.6	0.0	3.1
Solidago nemoralis	28	0.0	0.0	0.0	3.6	0.0
Solidago rugosa	37	2.7	0.0	0.0	2.7	5.4
Stellaria pubera	24	4.2	4.2	4.2	0.0	20.8
Stellaria sp.	8	0.0	0.0	0.0	0.0	37.5

Symphyotrichum						
ericoides	19	0.0	0.0	5.3	0.0	0.0
Symphyotrichum						
lateriflorum	24	4.2	0.0	4.2	0.0	0.0
Symphyotrichum novae-		0.0	0.0			0.0
angliae	3	0.0	0.0	0.0	0.0	0.0
Symphyotrichum novae-						
belgii	13	0.0	0.0	0.0	0.0	0.0
Taraxacum officinale	27	0.0	0.0	0.0	3.7	3.7
Trifolium aureum	3	0.0	0.0	0.0	33.3	0.0
Trifolium pratense	10	0.0	0.0	0.0	0.0	0.0
Trifolium repens	33	3.0	3.0	12.1	3.0	6.1
Veronica officinalis	4	0.0	0.0	25.0	0.0	0.0
Veronica persica	24	0.0	0.0	12.5	0.0	0.0
Veronica serpyllifolia	10	0.0	0.0	0.0	0.0	0.0
Vicia cracca	26	3.8	0.0	0.0	0.0	0.0
Vicia tetrasperma	8	0.0	12.5	0.0	0.0	25.0

76 Supplementary Table 3 | Parasite prevalence among the 110 species of bees that were 77 screened. (n = 2,685 individual bees were screened in total.)

Bee species	Samples screened	Percent positive with Nosema bombi	Percent positive with Nosema ceranae	Percent positive with Crithidia bombi	Percent positive with Crithidia expoeki	Percent positive with Neogreg arines
Agapostemon splendens	1	0.0	0.0	0.0	0.0	0.0
Agapostemon virescens	6	0.0	0.0	0.0	0.0	0.0
Andrena carlini	7	0.0	0.0	0.0	0.0	14.3
Andrena crataegi	2	0.0	0.0	0.0	0.0	0.0
Andrena cressonii	4	0.0	0.0	0.0	0.0	0.0
Andrena forbessi	3	0.0	0.0	0.0	0.0	0.0
Andrena frigida	1	0.0	0.0	0.0	0.0	0.0
Andrena hippotes	3	0.0	0.0	0.0	0.0	0.0
Andrena hirticincta	4	0.0	0.0	0.0	0.0	0.0
Andrena imitatrix	2	0.0	0.0	0.0	0.0	0.0
Andrena milwaukeensis	2	0.0	0.0	0.0	0.0	0.0
Andrena nasonii	44	0.0	0.0	0.0	0.0	13.6
Andrena nivalis	4	0.0	50.0	0.0	0.0	0.0
Andrena nuda	1	0.0	0.0	0.0	0.0	0.0
Andrena perplexa	10	0.0	0.0	0.0	0.0	0.0
Andrena platyparia	1	0.0	0.0	0.0	0.0	0.0
Andrena pruni	6	0.0	0.0	0.0	0.0	16.7
Andrena rugosa	1	0.0	0.0	0.0	0.0	0.0
Andrena simplex	2	0.0	0.0	0.0	0.0	50.0
Andrena spp.	8	0.0	0.0	0.0	0.0	0.0
Andrena wilkella	50	0.0	2.0	0.0	0.0	2.0
Anthidiellum notatum	1	0.0	0.0	0.0	0.0	0.0
Anthidium manicatum	1	0.0	0.0	0.0	0.0	0.0
Anthophora bomboides	1	0.0	0.0	0.0	0.0	0.0
Anthophora terminalis	17	0.0	0.0	0.0	0.0	5.9
Apis mellifera	632	0.9	18.8	0.3	0.5	4.0
Augochlora pura	20	0.0	0.0	10.0	0.0	15.0
Augochlorella aurata	79	0.0	0.0	1.3	0.0	1.3
Augochloropsis fulgida	8	0.0	0.0	0.0	0.0	0.0
Bombus bimaculatus	56	1.8	0.0	10.7	12.5	1.8
Bombus borealis	5	0.0	0.0	0.0	0.0	0.0
Bombus fervidus	9	0.0	11.1	0.0	0.0	22.2
Bombus griseocollis	45	0.0	0.0	22.2	2.2	0.0
Bombus impatiens	345	0.0	0.9	11.0	1.4	9.6
Bombus perplexus	2	50.0	50.0	0.0	0.0	0.0

Bombus rufocinctus	1	0.0	0.0	0.0	0.0	0.0
Bombus sandersoni	16	6.3	0.0	0.0	6.3	0.0
Bombus ternarius	4	0.0	0.0	0.0	0.0	0.0
Bombus vagans	22	0.0	0.0	4.5	27.3	18.2
Ceratina calcarata	142	0.0	0.0	0.7	0.0	2.8
Ceratina dupla	142	0.0	0.0	0.0	0.0	1.4
Ceratina mikmaqi	154	0.0	0.6	1.9	0.0	0.6
Ceratina spp.	28	0.0	3.6	0.0	0.0	0.0
Ceratina strenua	10	0.0	0.0	0.0	0.0	0.0
Coelioxys banksi	1	0.0	0.0	0.0	0.0	0.0
Coelioxys modesta	1	0.0	0.0	0.0	0.0	0.0
Coelioxys moesta	2	0.0	0.0	0.0	0.0	0.0
Coelioxys rufitarsis	1	0.0	0.0	0.0	0.0	100.0
Colletes inaequalis	1	0.0	0.0	0.0	0.0	0.0
Colletes simulans	3	0.0	0.0	33.3	0.0	0.0
Colletes solidaginis	1	0.0	0.0	0.0	0.0	0.0
Halictus confusus	12	0.0	0.0	0.0	0.0	0.0
Halictus ligatus	41	0.0	0.0	0.0	0.0	0.0
Halictus rubicundus	10	0.0	0.0	0.0	0.0	10.0
Heriades carinatus	14	0.0	0.0	0.0	0.0	0.0
Heriades spp.	3	0.0	0.0	0.0	0.0	0.0
Hoplitis pilosifrons	5	0.0	0.0	0.0	0.0	0.0
Hoplitis producta	8	0.0	0.0	0.0	0.0	37.5
Hoplitis simplex	1	0.0	0.0	0.0	0.0	0.0
Hoplitis spoliata	1	0.0	0.0	0.0	0.0	0.0
Hoplitis spp.	1	0.0	0.0	0.0	0.0	0.0
Hylaeus affinis	9	0.0	0.0	0.0	0.0	0.0
Hylaeus affinis/modestus	0	0.0	0.0	0.0	0.0	0.0
group	8	0.0	0.0	0.0	0.0	0.0
Hylaeus annulatus	11	0.0	0.0	0.0	0.0	0.0
Hylaeus illinoisensis	39	0.0	0.0	0.0	0.0	5.1
Hylaeus mesillae	21	0.0	0.0	0.0	0.0	0.0
Hylaeus sp. 1	3	0.0	0.0	0.0	0.0	0.0
Hylaeus spp.	33	0.0	0.0	0.0	0.0	3.0
Lasioglossum albipenne	20	0.0	0.0	0.0	0.0	0.0
Lasioglossum coeruleum	1	0.0	0.0	0.0	0.0	0.0
Lasioglossum coriaceum	16	0.0	0.0	0.0	0.0	0.0
Lasioglossum cressonii	28	0.0	0.0	0.0	0.0	0.0
Lasioglossum dreisbachi	1	0.0	0.0	0.0	0.0	0.0
Lasioglossum leucozonium	23	0.0	0.0	0.0	0.0	4.3
Lasioglossum lineatulum	23	0.0	0.0	0.0	0.0	0.0
Lasioglossum	2	0.0	0.0	0.0	0.0	0.0
macoupinense	1	0.0	0.0	0.0	0.0	0.0

Lasioglossum michiganense	1	0.0	0.0	0.0	0.0	100.0
Lasioglossum mitchelli	3	0.0	0.0	0.0	0.0	0.0
Lasioglossum nigroviride Lasioglossum	2	0.0	0.0	0.0	0.0	0.0
nymphaearum	4	0.0	0.0	0.0	0.0	0.0
Lasioglossum obscurum	3	0.0	0.0	0.0	0.0	0.0
Lasioglossum pectinatum Lasioglossum	1	0.0	0.0	0.0	0.0	0.0
quebecense	4	0.0	0.0	0.0	0.0	0.0
Lasioglossum spp.	126	0.0	0.0	0.0	0.0	0.8
Lasioglossum versans	3	0.0	0.0	0.0	0.0	0.0
Lasioglossum versatum	141	0.0	0.7	0.0	0.0	0.7
Lasioglossum weemsi	3	0.0	0.0	0.0	0.0	0.0
Lasioglossum zonulum	37	0.0	0.0	2.7	2.7	0.0
Megachile brevis	1	0.0	0.0	0.0	0.0	0.0
Megachile campanulae	7	0.0	0.0	0.0	0.0	0.0
Megachile centuncularis	3	0.0	0.0	0.0	0.0	0.0
Megachile gemula	2	0.0	0.0	0.0	0.0	50.0
Megachile latimanus	2	0.0	0.0	0.0	0.0	50.0
Megachile mendica	7	0.0	0.0	0.0	0.0	0.0
Megachile montivaga	9	0.0	0.0	11.1	0.0	0.0
Megachile pugnata	2	0.0	0.0	0.0	0.0	0.0
Megachile relativa	7	0.0	0.0	0.0	0.0	14.3
Megachile rotundata	1	0.0	0.0	0.0	0.0	0.0
Megachile sculpturalis	3	0.0	0.0	0.0	0.0	33.3
Melissodes desponsa	4	0.0	0.0	0.0	0.0	0.0
Melissodes druriella	4	0.0	0.0	0.0	0.0	0.0
Melissodes spp.	4	0.0	0.0	0.0	0.0	0.0
Melissodes subillata	3	0.0	0.0	0.0	0.0	0.0
Nomada luteoloides	1	0.0	0.0	0.0	0.0	0.0
Nomada pygmaea group	1	0.0	0.0	0.0	0.0	0.0
Nomada sp. 1	1	0.0	0.0	0.0	0.0	0.0
Nomada sp. 2	2	0.0	0.0	0.0	0.0	0.0
Nomada sp. 3	1	0.0	0.0	0.0	0.0	0.0
Nomada sp. 4	3	0.0	0.0	0.0	0.0	0.0
Nomada spp.	1	0.0	0.0	0.0	0.0	0.0
Osmia atriventris	1	0.0	0.0	0.0	0.0	0.0
Osmia bucephala	1	0.0	0.0	0.0	0.0	0.0
Osmia georgica	1	0.0	0.0	0.0	0.0	0.0
Osmia nigriventris	2	0.0	0.0	0.0	0.0	0.0
Osmia pumila Pseudopanurgus	1	0.0	0.0	0.0	0.0	0.0
andrenoides	2	0.0	0.0	0.0	0.0	0.0
Sphecodes aroniae	1	0.0	0.0	0.0	0.0	0.0

Sphecodes carolinas	1	0.0	0.0	0.0	0.0	0.0
Stelis lateralis	1	0.0	0.0	0.0	0.0	0.0
Xylocopa virginica	40	0.0	2.5	2.5	5.0	10.0

80 Supplementary Table 4 | Effects of week number on the prevalence of parasite groups/species in the pollinator community. GLMMs were fitted with parasite prevalence 81 as binomial response, week number as predictor, and site as random factor. Significance of 82 week number were evaluated using likelihood ratio tests. To account for multiple testing given 83 84 the number of parasite groups/species, we used a Bonferroni-corrected significance level of  $\alpha$ = 0.05/7 = 0.0071. Significant positive effects are shown in blue. Durbin-Watson tests using 85 scaled residuals were also conducted to check for temporal autocorrelation; a significant 86 87 temporal autocorrelation would have invalidated the use of a non-autoregressive model like 88 GLMM. The last parasite group refers to all four parasite species and neogregarines combined. Parasite groups/species with less than 20 positive samples were excluded from the analysis. 89 90 Sample size was n = 2,672.

91

Parasite	I	likelihood ratio	DW test for temporal autocorr.		
Parasite	Coef. est.	χ21	p-value	DW	p-value
Microsporidia	0.65	17	< 0.001	2.0	0.97
Trypanosomatids	0.11	45	< 0.001	2.4	0.39
N. bombi		< 20 positives			
N. ceranae	0.068	14	< 0.001	2.0	0.93
C. bombi	0.17	39	< 0.001	1.9	0.8
C. expoeki	0.071	2.9	0.087	1.5	0.24
Neogregarines	0.055	7.6	0.0059	1.8	0.58
Combined	0.092	56	< 0.001	1.6	0.36

93 Supplementary Table 5 | Effects of week number on the prevalence of parasite 94 groups/species in the floral community. GLMMs were fitted with parasite prevalence as binomial response, week number as predictor, and site as random factor. Significance of week 95 96 number were evaluated using likelihood ratio tests. To account for multiple testing given the number of parasite groups/species, we used a Bonferroni-corrected significance level of  $\alpha$  = 97 0.05/7 = 0.0071. Significant negative effects are shown in red. Durbin-Watson tests using 98 scaled residuals were also conducted to check for temporal autocorrelation. Parasite 99 100 groups/species with less than 20 positive samples were excluded from the analysis.

101

Parasite	I	ikelihood ratio t	DW test for temporal autocorr.		
rarashe	Coef. est.	X <sup>21</sup>		Coef. est.	χ21
Microsporidia	-0.028	2.9	0.090	1.5	0.28
Trypanosomatids	-0.017	1.6	0.21	2.7	0.11
N. bombi	-0.019	0.50	0.48	2.4	0.3
N. ceranae		< 20 positives			
C. bombi	-0.035	2.8	0.092	2.0	0.93
C. expoeki	-0.017	0.26	0.61	2.0	0.92
Neogregarines	-0.036	2.8	0.096	1.6	0.42
Combined	-0.038	8.3	0.0040	2.2	0.58

103 Supplementary Table 6 | Effects of bee genus and its interaction with week number on the prevalence of parasite groups/species in the pollinator community. GLMMs were fitted 104 with parasite prevalence in each genus as binomial response, week number (shifted), pollinator 105 genus and their interaction as predictors, and site as random factor. To account for multiple 106 107 testing, we used a Bonferroni-corrected significance level of  $\alpha = 0.05/7 = 0.0071$ . Significance of predictors were evaluated using likelihood ratio tests. Significant effects are shown in 108 fuschia. Note that none of the *Ceratina* samples tested positive for *C. expoeki* and were hence 109 110 excluded from the C. expoeki analysis, since they would have otherwise led to model degeneracy. Sample size was n = 2,196 for C. expoeki, and n = 2,672 for all other parasite 111 112 groups/species.

113

Parasite	Week	* genus	Genus		
I al asite	χ21	p-value	χ <sup>21</sup>	p-value	
Microsporidia	1.4	0.85	200	< 0.001	
Trypanosomatids	35	< 0.001	160	< 0.001	
N. ceranae	2.2	0.70	230	< 0.001	
C. bombi	15	0.0057	140	< 0.001	
C. expoeki	8.2	0.042	28	< 0.001	
Neogregarines	4.3	0.37	47	< 0.001	
Combined	31	< 0.001	230	< 0.001	

115 **Supplementary Table 7** | Post-hoc pairwise contrasts for the main effects of bee genus. 116 Results were based on the same models used in Supplementary Table 6. p-values were 117 calculated using the simultaneous inference procedure by Hothorn, Bretz and Westfall (2008), 118 which incorporated corrections for multiple testing given the number of contrasts. In addition, 119 we used a Bonferroni-corrected significance level of  $\alpha = 0.05/7 = 0.0071$  to account for the 120 number of parasite groups/species. Significant positive and negative contrasts are shown in 121 blue and red respectively.

Parasite	Pairwise contrast	Coef. est.	Z	p-value
	Apis - Bombus	1.5	6.4	< 0.001
	Apis - Ceratina	4.2	5.2	< 0.001
	Apis - Lasioglossum	2.4	6.6	< 0.001
	Apis - Others	2.4	8.2	< 0.001
	Bombus - Ceratina	2.7	3.2	0.0090
Microsporidia	Bombus - Lasioglossum	0.91	2.2	0.15
	Bombus - Others	0.88	2.6	0.066
	Ceratina -	-1.8	-2.0	0.23
	Lasioglossum	-1.8	-2.0	0.25
	Ceratina - Others	-1.8	-2.1	0.18
	Lasioglossum - Others	-0.035	-0.078	1.0
	Apis - Bombus	-1.7	-7.0	< 0.001
	Apis - Ceratina	1.0	2.3	0.15
	Apis - Lasioglossum	0.91	2.1	0.22
	Apis - Others	0.67	1.8	0.37
	Bombus - Ceratina	2.7	6.6	< 0.001
<b>Trypanosomatids</b>	Bombus - Lasioglossum	2.6	6.5	< 0.001
J 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Bombus - Others	2.4	7.1	< 0.001
	Ceratina -			
	Lasioglossum	-0.11	-0.20	1.0
	<i>Ceratina</i> - Others	-0.34	-0.68	0.96
	Lasioglossum - Others	-0.23	-0.47	0.99
	Apis - Bombus	3.0	6.4	< 0.001
	Apis - Ceratina	4.0	5.0	< 0.001
	Apis - Lasioglossum	4.6	4.0	< 0.001
	Apis - Others	3.5	6.7	< 0.001
	Bombus - Ceratina	1.1	1.2	0.74
N. ceranae	Bombus - Lasioglossum	1.6	1.2	0.64
IV. Ceranac	Bombus - Others	0.48	0.71	0.95
	Ceratina -	0.40	0.71	
	Lasioglossum	0.57	0.41	0.99
	<i>Ceratina</i> - Others	-0.59	-0.62	0.97
	Lasioglossum - Others	-1.2	-0.93	0.87
	Apis - Bombus	-1.2	-0.93	< 0.001
	Apis - Bombus Apis - Ceratina	-4.5	-4.2 -1.1	0.78
	-	-1.5 0.46	-1.1 0.20	1.0
	Apis - Lasioglossum			
	Apis - Others	-1.2	-0.93	0.87
C harrel:	Bombus - Ceratina	3.2	5.1	< 0.001
C. bombi	Bombus - Lasioglossum	5.0	2.3	0.11
	Bombus - Others	3.3	4.8	< 0.001
	Ceratina -	1.8	0.82	0.91
	Lasioglossum			
	Ceratina - Others	0.17	0.19	1.0
	Lasioglossum - Others	-1.6	-0.74	0.94
	Apis - Bombus	-2.1	-2.9	0.015
C. expoeki	Apis - Ceratina		No positive <i>Ceratina</i> sam	
<i>r</i> • • • • •	Apis - Lasioglossum	1.5	0.71	0.88
	Apis - Others	1.3	0.85	0.81

	Bombus - Ceratina	No positive Ceratina samples			
	Bombus - Lasioglossum	3.7	1.8	0.25	
	Bombus - Others	3.4	.5	0.048	
	Ceratina - Lasioglossum	No positive Ceratina samples			
	Ceratina - Others		No positive Ceratina samples		
	Lasioglossum - Others	-0.26	0.11	1.0	
	Apis - Bombus	-0.89	-3.2	0.010	
	Apis - Ceratina	1.1	2.4	0.12	
	Apis - Lasioglossum	1.4	2.4	0.093	
	Apis - Others	-0.33	-1.1	0.77	
	Bombus - Ceratina	2.0	4.5	< 0.001	
Neogregarines	Bombus - Lasioglossum	2.3	4.1	< 0.001	
	Bombus - Others	0.56	2.2	0.16	
	Ceratina - Lasioglossum	0.31	0.46	0.99	
	Ceratina - Others	-1.4	-3.1	0.013	
	Lasioglossum - Others	-1.7	-3.1	0.015	
	Apis - Bombus	-0.21	-1.4	0.60	
	Apis - Ceratina	2.3	7.7	< 0.001	
	Apis - Lasioglossum	2.7	6.9	< 0.001	
	Apis - Others	1.4	6.8	< 0.001	
	Bombus - Ceratina	2.5	8.4	< 0.001	
Combined	Bombus - Lasioglossum	2.9	7.4	< 0.001	
	Bombus - Others	1.6	7.8	< 0.001	
	Ceratina - Lasioglossum	0.41	0.85	0.90	
	Ceratina - Others	-0.98	-3.0	0.022	
	Lasioglossum - Others	-1.4	-3.3	0.0068	

124 Supplementary Table 8 | Effects of week number on parasite prevalence in different bee 125 genera. Results were based on the same models used in Supplementary Table 6. p-values were 126 calculated using the simultaneous inference procedure by Hothorn, Bretz and Westfall (2008), 127 which incorporated corrections for multiple testing given the number of genera. In addition, 128 we used a Bonferroni-corrected significance level of  $\alpha = 0.05/7 = 0.0071$  to account for the 129 number of parasite groups/species. Significant positive and negative effects are shown in blue 130 and red respectively.

131

Parasite	Genus	Coef. est.	Ζ	p-value	
	Apis	-0.079	-3.5	0.002	
	Bombus	-0.037	-0.73	0.96	
Microsporidia	Ceratina	-0.15	-0.81	0.93	
	Lasioglossum	-0.070	-0.80	0.94	
	Others	-0.022	-0.35	1.0	
	Apis	-0.093	-2.3	0.094	
	Bombus	0.065	2.0	0.21	
Trypanosomatids	Ceratina	-0.14	-1.5	0.49	
	Lasioglossum	0.034	0.32	1.0	
	Others	0.26	4.9	< 0.001	
	Apis	-0.098	-4.1	< 0.001	
	Bombus	0.049	0.38	1	
N. ceranae	Ceratina	-0.145	-0.80	0.94	
	Lasioglossum	-0.098	-0.36	1.0	
	Others	0.00041	0.004	1.0	
	Apis	-0.23	-1.7	0.37	
	Bombus	0.13	2.7	0.038	
C. bombi	Ceratina	-0.16	-1.3	0.69	
	Lasioglossum	0.51	1.1	0.80	
	Others	0.27	2.6	0.043	
	Apis	-0.099	-0.10	0.88	
	Bombus	-0.10	-0.11	0.25	
C. expoeki	Ceratina	No positive Ceratina samples			
	Lasioglossum	0.50	0.5	0.70	
	Others	0.32	0.32	0.33	
	Apis	-0.067	-1.5	0.50	
	Bombus	0.030	0.65	0.97	
Neogregarines	Ceratina	0.072	0.89	0.90	
	Lasioglossum	-0.043	-0.32	1.0	
	Others	0.039	0.94	0.88	
	Apis	-0.11	-4.8	< 0.001	
	Bombus	0.057	1.9	0.25	
Combined	Ceratina	-0.026	-0.40	1.00	
	Lasioglossum	0.064	0.60	0.98	
	Others	0.076	2.1	0.16	

133 Supplementary Table 9 | Effects of bee diversity (Shannon index) on parasite prevalence in the bee community. GLMMs were fitted with parasite prevalence as binomial response, 134 Shannon index as predictor, and site as random factor. We considered parasite prevalence 135 based on all bee genera, as well as without Apis and Bombus. Significance of pollinator 136 diversity were evaluated using likelihood ratio tests. To account for multiple testing, we used 137 a Bonferroni-corrected significance level of  $\alpha = 0.05/7 = 0.0071$  for prevalence based on all 138 genera, and  $\alpha = 0.05/4 = 0.13$  for prevalence without Apis and Bombus. Significant negative 139 140 effects are shown in red. Sample size was n = 2,446 for all bee genera, and 1,390 without Apis 141 and Bombus.

142

	All bee genera			Witho	ut Apis and B	ombus
Parasite	Coef. est. χ <sup>21</sup> p-value			Coef. est.	χ21	p-value
Microsporidia	-0.41	7.4	0.0065	0.52	0.54	0.46
Trypanosomatids	-0.74	21	< 0.001	-1.8 22 < 0.001		
N. bombi		< 20 positive	es	< 20 positives		
N. ceranae	-0.64	13	< 0.001	< 20 positives		
C. bombi	-1.1	21	< 0.001	< 20 positives		
C. expoeki	-0.0056	0.00016	0.99	< 20 positives		
Neogregarines	-0.37	3.6	0.060	0.020	0.0014	0.97
Combined	-0.69	34	< 0.001	-0.64 2.6 0.11		

143

144 Supplementary Table 10 | Effects of floral diversity (Shannon index) on parasite 145 prevalence in the floral community. GLMMs were fitted with parasite prevalence as 146 binomial response, Shannon index as predictor, and site as random factor. Significance of floral 147 diversity were evaluated using likelihood ratio tests. To account for multiple testing, we used 148 a Bonferroni-corrected significance level of  $\alpha = 0.05/7 = 0.0071$ . Sample size was n = 2,624.

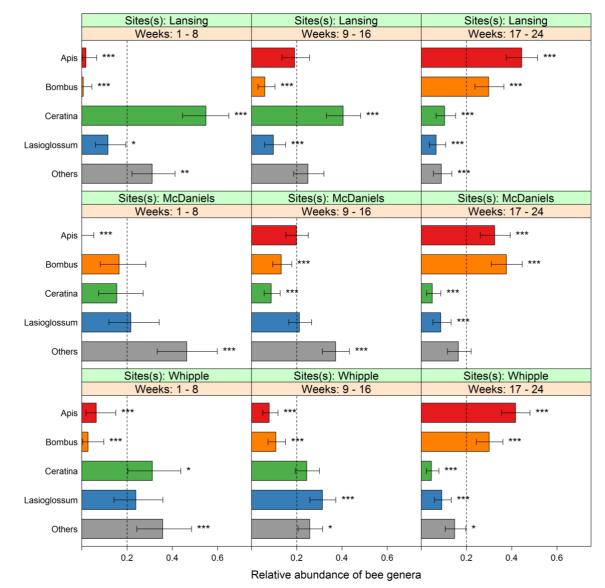
149

Parasite	Coef. est.	χ²1	p-value
Microsporidia	0.34	4.0	0.045
Trypanosomatids	-0.15	1.1	0.30
N. bombi	0.088	0.10	0.75
N. ceranae		< 20 positives	
C. bombi	-0.090	0.16	0.69
C. expoeki	0.063	0.031	0.86
Neogregarines	-0.56	6.3	0.012
Combined	-0.16	1.3	0.25

Supplementary Table 11 | Effects of total floral abundance on parasite prevalence in the floral community. GLMMs were fitted with parasite prevalence as binomial response, log10(mean total floral abundance per quadrat) as predictor, and site as random factor. Significance of floral abundance were evaluated using likelihood ratio tests. To account for multiple testing, we used a Bonferroni-corrected significance level of  $\alpha = 0.05/7 = 0.0071$ . Significant negative effects are shown in red. Sample size was n = 2,624.

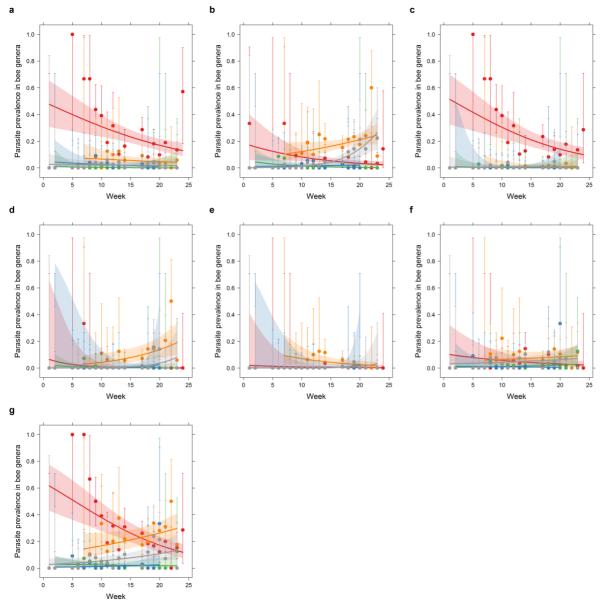
157

Parasite	Coef. est.	χ²1	p-value
Microsporidia	-0.26	2.2	0.14
Trypanosomatids	-0.17	1.2	0.27
N. bombi	-0.23	0.68	0.41
N. ceranae		< 20 positives	
C. bombi	-0.41	3.3	0.068
C. expoeki	-0.40	1.2	0.28
Neogregarines	-0.19	0.74	0.39
Combined	-0.39	8.0	0.0046



159

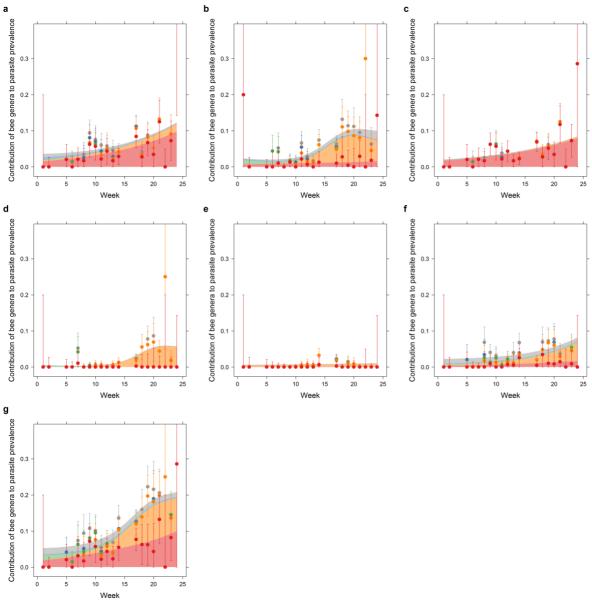
Supplementary Fig. 1 | Relative abundance of the four most common bee genera, in three 8-week periods of the field season. For each genus, post-hoc evaluations of the deviation from the null hypothesis (of equal multinomial proportions) were conducted using exact binomial tests with Bonferroni correction. 99% confidence intervals are presented here in accordance with this correction. Error bars are 95% Clopper-Pearson confidence intervals. Sample sizes are n = 164, 289, 349 for the three periods at Lansing, n = 97, 449, 348 at McDaniels, and n =109, 447, 420 at Whipple.





Apis 🚥 Bombus 🚥 Ceratina 🚥 Lasioglossum 🚥 Others

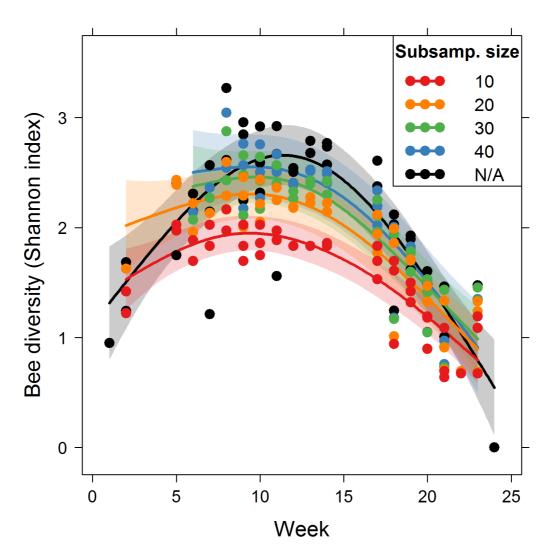
Supplementary Fig. 2 | Parasite prevalence in different bee genera across the season. Parasite species/groups shown here are (a) microsporidians, (b) trypanosomatids, (c) *N. ceranae*, (d) *C. bombi*, (e) *C. expoeki*, (f) neogregarines and (g) the four parasite species and neogregarines combined. Curves and confidence bands were fitted using binomial GLMMs with genus, week number and their interactions as predictors, and site as a random factor. To reduce plot clutter, points and error bars shown here are based on combining data from all three sites each week. Error bars are 95% Clopper-Pearson confidence intervals.



176

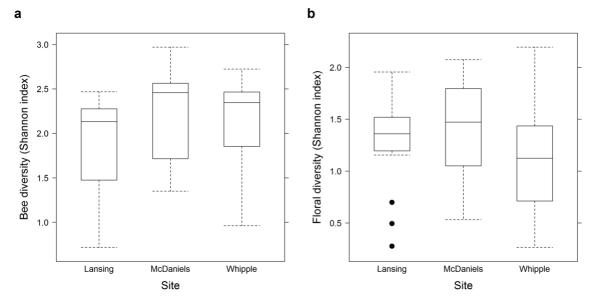
👐 Apis 👐 Bombus 👐 Ceratina 👐 Lasioglossum 👐 Others

Supplementary Fig. 3 | Contributions from each bee genus to overall prevalence of
parasites in the community across the season. Parasite species/groups shown here are (a)
microsporidians, (b) trypanosomatids, (c) *N. ceranae*, (d) *C. bombi*, (e) *C. expoeki*, (f)
neogregarines and (g) the four parasite species and neogregarines combined. Contributions
were defined as (relative abundance of genus) × (parasite prevalence in genus), and plotted as
stacked smoothing splines fitted using GAM with site as a random factor. Error bars are 95%
Clopper-Pearson confidence intervals.



185

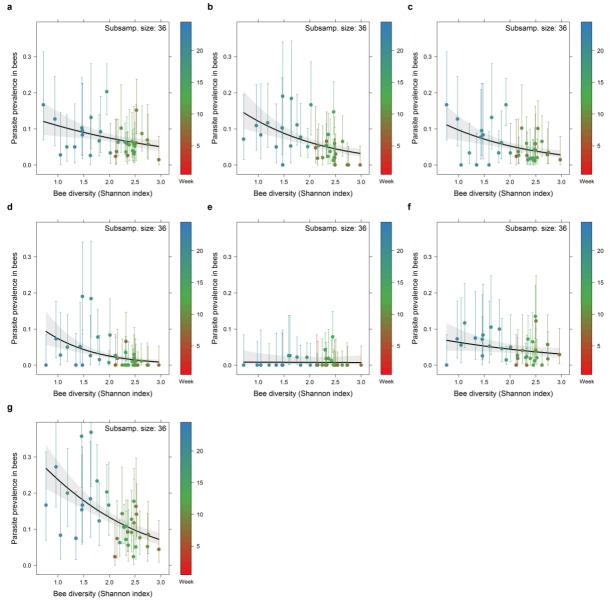
Supplementary Fig. 4 | Bee diversity at different site and weeks, with and without rarefaction. Smaller subsample sizes allowed more site/week samples to be included, especially those from earlier weeks, but at the expense of reducing the strength of any temporal trends. Curves shown here are smoothing splines fitted using GAM with site as a random factor.



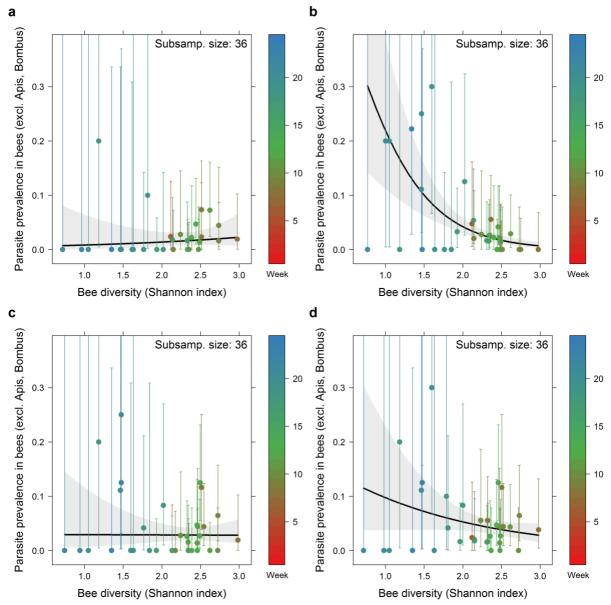
191
192 Supplementary Fig. 5 | Variation in bee and floral diversities across sites. Shannon indices

193 for the (a) bee and (b) flower communities were calculated using bee samples and site floral

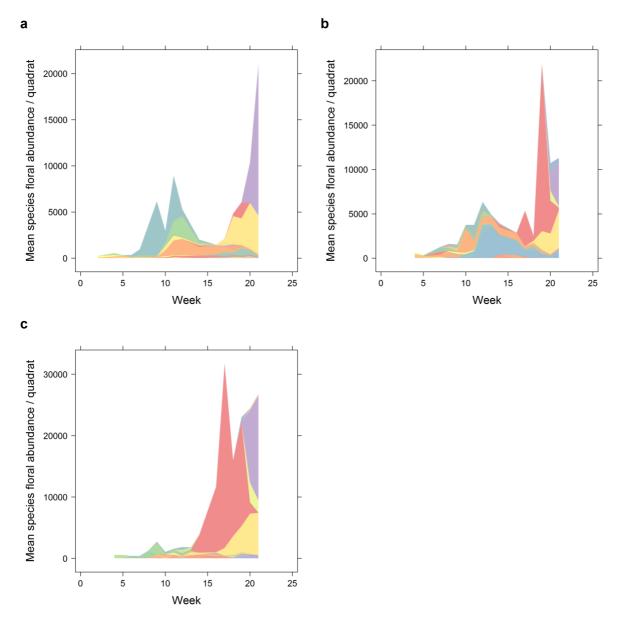
194 surveys respectively, with different weeks as replicates.



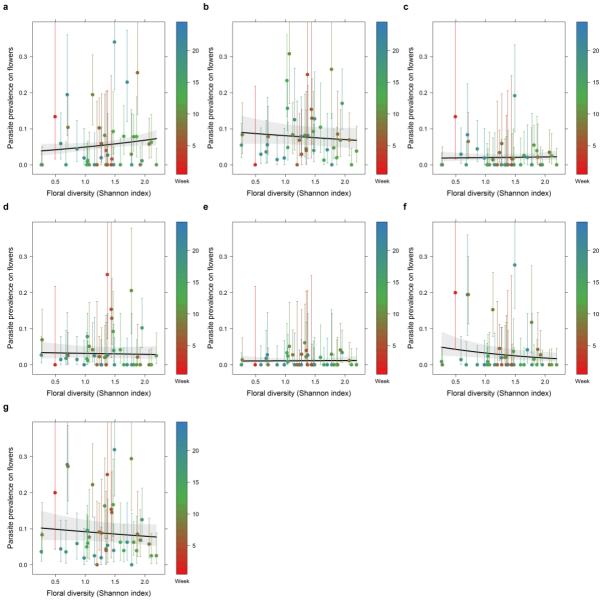
Bee diversity (Shannon index)
Supplementary Fig. 6 | Association between the prevalence of different parasite groups/species in bees and bee diversity. Parasite species/groups shown here are (a) microsporidians, (b) trypanosomatids, (c) *N. ceranae*, (d) *C. bombi*, (e) *C. expoeki*, (f) neogregarines and (g) the four parasite species and neogregarines combined. Colour indicates week number. Curves were fitted using binomial GLMMs with site as a random factor. Error bars are 95% Clopper-Pearson confidence intervals.



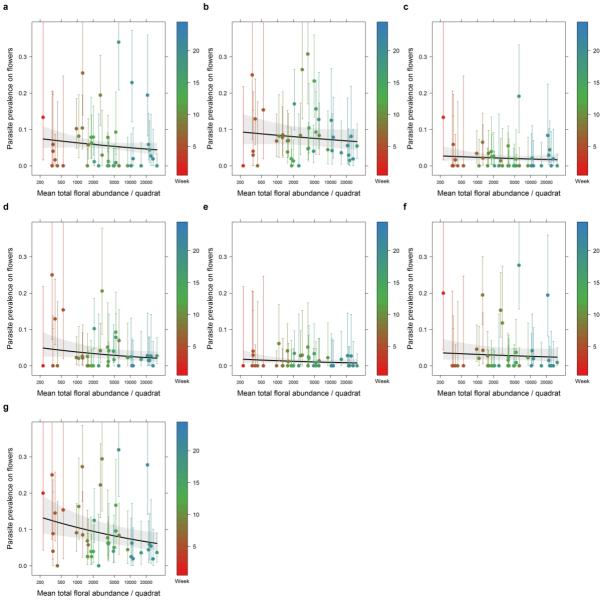
Supplementary Fig. 7 | Association between the prevalence of different parasite
 groups/species in bees (excluding *Apis* and *Bombus*) and pollinator diversity. Parasite
 species/groups shown here are (a) microsporidians, (b) trypanosomatids, (c) neogregarines and
 (d) the four parasite species and neogregarines combined. Colour indicates week number.
 Curves were fitted using binomial GLMMs with site as a random factor. Error bars are 95%
 Clopper-Pearson confidence intervals.



214 Supplementary Fig. 8 | Species floral abundances across the season. Mean floral abundances per quadrat were evaluated for each plant species at (a) Lansing, (b) McDaniels and (c) Whipple, and shown as stacked plots. 



Floral diversity (Shannon index)
Supplementary Fig. 9 | Association between the prevalence of different parasite groups/species on flowers and floral diversity. Parasite species/groups shown here are (a) microsporidians, (b) trypanosomatids, (c) *N. bombi*, (d) *C. bombi*, (e) *C. expoeki*, (f) neogregarines and (g) the four parasite species and neogregarines combined. Colour indicates week number. Curves were fitted using binomial GLMMs with site as a random factor. Error bars are 95% Clopper-Pearson confidence intervals.



Mean total floral abundance / quadrat
Supplementary Fig. 10 | Association between the prevalence of different parasite
groups/species on flowers and total floral abundance. Parasite species/groups shown here
are (a) microsporidians, (b) trypanosomatids, (c) *N. bombi*, (d) *C. bombi*, (e) *C. expoeki*, (f)
neogregarines and (g) the four parasite species and neogregarines combined. Colour indicates
week number. Curves were fitted using binomial GLMMs with site as a random factor. Error
bars are 95% Clopper-Pearson confidence intervals.

233	Supp	lementary References
234		
235	1.	Mullins, J. L., Strange, J. P. & Tripodi, A. D. Why Are Queens Broodless? Failed Nest
236		Initiation Not Linked to Parasites, Mating Status, or Ovary Development in Two
237		Bumble Bee Species of Pyrobombus (Hymenoptera: Apidae: Bombus). J. Econ.
238		Entomol. (2019). doi:10.1093/jee/toz330
239	2.	Meeus, I., de Graaf, D. C., Jans, K. & Smagghe, G. Multiplex PCR detection of
240		slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range
241		primers. J. Appl. Microbiol. 109, 107–115 (2010).
242	3.	Tripodi, A. D., Szalanski, A. L. & Strange, J. P. Novel multiplex PCR reveals multiple
243		trypanosomatid species infecting North American bumble bees (Hymenoptera:
244		Apidae: Bombus). J. Invertebr. Pathol. 153, 147–155 (2018).
245	4.	Gisder, S. & Genersch, E. Molecular differentiation of Nosema apis and Nosema
246		<i>ceranae</i> based on species–specific sequence differences in a protein coding gene. J.
247		<i>Invertebr. Pathol.</i> <b>113</b> , 1–6 (2013).
248	5.	Untergasser, A. et al. Primer3-new capabilities and interfaces. Nucleic Acids Res. 40,
249		1–12 (2012).
250		