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3   This manuscript is in preparation for submission to a peer reviewed journal. Please note that this

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5   may have slightly different content. If accepted, the final version of this manuscript will be

6   available. Please feel free to contact any of the authors; we welcome feedback.

7

8   Identifying drivers of sewage-associated pollutants in pollinators across urban landscapes
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10   Michael F. Meyer[1,4]*, Matthew R. Brousil[2,5,9], Benjamin W. Lee[3,6], Madison L. Armstrong[3,7],
11   Elias H. Bloom[3,8], David W. Crowder[3]
12

13   [1] School of the Environment, Washington State University, Pullman, Washington, United States
14   99164
15   [2] Center for Environmental Research, Education, & Outreach, Washington State University,
16   Pullman, Washington, United States 99164
17   [3] Department of Entomology, Washington State University, Pullman, Washington, United States
18   99164
19   [4] Current Address: U.S. Geological Survey, Observing Systems Division, Madison, Wisconsin,
20   United States 53726
21   [5] Department of Entomology, Washington State University, Pullman, Washington, United States
22   99164
23   [6] Current Address: Department of Entomology and Nematology, University of California, Davis,
24   California, United States 95616
25   [7] Current Address: Center for Population Biology, University of California, Davis, California,
26   United States 95616
27   [8] Current Address: Department of Plant Pathology and Plant-Microbe Biology, Cornell
28   University, Ithaca, New York, United States 14850
29   [9] Current Address: Department of Ecosystem Science and Sustainability, Colorado State
30   University, Fort Collins, CO, United States of America
31

32   *Corresponding Author: mfmeyer@usgs.gov
33

34   **ABSTRACT:** Human sewage can introduce pollutants into food webs and threaten ecosystem

35   integrity. Among the many sewage-associated pollutants, pharmaceuticals and personal care

36   products (PPCPs) are useful indicators of sewage in ecosystems and can also cause potent

37   ecological consequences even at minute concentrations (e.g., ng/L). Despite increased study over

38   the past three decades, PPCPs in terrestrial systems have been less studied than those in aquatic

39   ecosystems. To evaluate PPCP prevalence and drivers in a terrestrial ecosystem, we analyzed

40   managed and native bees collected from agroecosystems in Washington State (USA) for PPCPs.

41   Caffeine, paraxanthine, cotinine, and acetaminophen were detected in all three evaluated taxa

42   (*Bombus vosnesenskii*, *Agapostemon texanus*, and *Apis mellifera*), with *B. vosnesenskii* and *A.

43   texanus* having a higher probability of PPCP detection relative to *A. mellifera*. The probability

44   for PPCP presence in all three taxa increased in landscapes with more human development or

45   greater plant abundance, with significant but negative interactions among these factors. These

46   results suggest that human activity, availability of resources, and species-specific traits affect the

47   introduction and mobilization of PPCPs in terrestrial ecosystems. Consequently, monitoring

48   PPCPs and their ecological responses in terrestrial ecosystems creates opportunities to synthesize

49   consequences of sewage pollution across terrestrial and aquatic ecosystems and organism types.

50

51   **KEYWORDS:** Arthropoda, emerging contaminants, entomology, natural history, Persistent

52   Organic Pollutants

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54

INTRODUCTION

The introduction of wastewater and its byproducts into ecosystems can mobilize pollutants that reshape communities and food webs (Edmondson 1970). Historically, research on wastewater pollution has largely focused on changes in effluent nutrient concentrations as well as chemical and biological oxygen demand (Edmondson 1970; Brydon and Frodsham 2001; Tong et al. 2020). Recently, research emphasis has broadened to include micropollutants that are often found in sewage (Bernhardt et al. 2017). Pharmaceuticals and personal care products (PPCPs) in particular have garnered increased attention as an emerging organic micropollutant because they are consistently associated with human sewage and pose potent, yet often uncertain, ecological consequences (Richmond et al. 2017; Meyer et al. 2019).

As PPCPs are consistently associated with sewage, their presence, even at minute concentrations (e.g., ng/L), can indicate wastewater inputs into an ecosystem. Previous continental and worldwide surveys show that PPCPs such as antibiotics, non-prescription and prescription drugs, hormones, and fragrances are pervasive within subsurface and surface systems (Kolpin et al. 2002; Focazio et al. 2008; Wilkinson et al. 2022). Once introduced into ecosystems, PPCPs can propagate through food webs, where they can be metabolized (del Rey et al. 2011), accumulate within organisms (Meador et al. 2016), and be transferred across trophic levels (Richmond et al. 2018). However, patterns of PPCP prevalence across taxa can be related to individual behavior and tissue allocation (Meador et al. 2016), and biological responses to PPCPs are often uncertain and context-dependent. For example, activity and feeding rates of individual fish (*Perca fluviatilis*; European perch) increased when they were exposed to the anti-anxiolytic drug oxazepam, but sociality of fish populations decreased (Brodin et al. 2013). Certain algal taxa have been shown to have reduced photosynthesis and increased 16-carbon

78   unsaturated fatty acid production when exposed to fluoxetine, even though essential fatty acid

79   synthesis was not affected (Feijão et al. 2020). Bumble bees fed nectar primed with caffeine have

80   shown increased foraging behavior on nectars with similar aromatic compounds (Wright et al.

81   2013; Arnold et al. 2021). Overall, the literature suggests that biological responses to PPCPs are

82   common and often deleterious, although specific responses may also be uncertain (Richmond et

83   al. 2017).

84   Although considerable evidence from aquatic systems suggests that PPCPs are ubiquitous

85   and disrupt ecological processes, comparatively few studies on PPCPs have been conducted in

86   terrestrial systems (Meyer et al. 2019). This imbalance in the literature creates opportunity to

87   assess how PPCPs may propagate through terrestrial food webs in comparison to aquatic food

88   webs. For example, bees are exclusively terrestrial insect taxa that provide pollination services in

89   natural and managed ecosystems (Kleijn et al. 2015). As has been observed in earthworms

90   (Carter et al. 2021), bee pollinators may be commonly exposed to PPCPs through soil contact,

91   interactions with plants, applications of biosolids for fertilizer, or through contamination of water

92   in terrestrial ecosystems. Due to the dramatic, recent declines in bee pollinators within terrestrial

93   food webs worldwide (Potts et al. 2010; Kleijn et al. 2015), bee taxa may be promising,

94   societally important model organisms for expanding PPCP research from aquatic to terrestrial

95   ecosystems.

96   To assess PPCP prevalence and mobilization in pollinators within exclusively terrestrial

97   food webs, we evaluated the presence and drivers of PPCPs in three bee species: *Bombus*

98   *vosnesenskii*, *Agapostemon texanus*, and *Apis mellifera*. Two of these species are wild (*B.*

99   *vosnesenskii*, *A. texanus*), but *A. mellifera* (honey bee) is managed by humans. Our first goal was

100 to identify species-specific patterns of PPCP presence. We predicted that taxa interacting with

101 the soil (ground-nesting bees; i.e., *B. vosnesenskii*, *A. texanus*) would be more frequently

102 associated with PPCPs than managed, colony-forming species (i.e., *A. mellifera*), as ground-

103 nesting taxa would more likely encounter PPCPs within groundwater and biosolids, similar to

104 interactions observed in earthworms (Carter et al. 2021). Our second goal was to assess the

105 potential drivers of PPCP presence in each bee taxa. We predicted that PPCP presence would

106 increase at sites with greater human development and with a higher density of floral plant

107 resources at the sampling location, both of which can be potentially concentrated sources of

108 PPCPs (Carter et al. 2021; Meyer et al. 2022). Overall, our study provides some of the first

109 evidence for PPCP uptake in exclusively terrestrial bee species, while also demonstrating that

110 both landscape-context and organismal traits may affect exposure to and uptake of PPCPs.

111

112 **MATERIALS AND METHODS**

113 **Study System.** Our study of PPCPs in bees was nested within a study of bee ecology on 36

114 small (< 25 ha), diversified farms and community gardens (e.g., farms and gardens with more

115 than five unique flowering crops in simultaneous production) in western Washington (USA)

116 (Bloom et al. 2021). For the present study, bees were collected from a subset of 10 urban gardens

117 that were selected along an urbanization gradient (Fig. 1). Each garden was at least 1 km apart

118 for spatial independence (Bloom et al. 2021; Desaegher et al. 2022).

119

120 **Bee and plant surveys.** Bees were collected at each site using three blue vane traps

121 (SpringStar LLC, Woodinville, WA, USA) and 15 bee bowls placed along a 50 m transect. All

122 collected bees were preserved, pinned, and then identified to species (Bloom et al. 2021). Of the

123 6,539 specimens collected, we selected 101 specimens with approximately balanced specimens

124   from each of the three bee species (*B. vosnesenskii*, *A. texanus*, and *A. mellifera*); these taxa

125   reflect variation in social behavior (social vs. solitary taxa) and nesting strategies (i.e., cavity vs.

126   ground-nesting taxa). Because bee species were collected across three years (2014, 2015, 2016)

127   and three time points (spring, summer, and fall), we selected pinned specimens for this analysis

128   to have a balanced number of individuals across the sampling period. Specimen selection was

129   stratified by species, year, early/mid/late-summer timing, and sampling location, where each

130   species-timing-year-site combination included at least two individuals. As each species-timing-

131   year-site combination contained numerous individuals from the original sampling events,

132   personnel haphazardly selected individuals within a species-timing-site-year stratum.

133   To assess the role of plant communities in affecting PPCP presence in bees, plant richness

134   and abundance were measured at sites on the same date as bee sampling. Plants with flowers

135   serving as resources for bee visitation (i.e., having pollen and nectar) were recorded along the

136   same transect used for bee collection. A portable 1×1 m plot was placed over vegetation at 5 m

137   intervals and all plants with open flowers were identified to species. Each transect moved in a

138   serpentine fashion across each study site. Summarized field variables are detailed in Table S1.

139

140   **Landscape context.** As sites were located along an urbanization gradient, we determined

141   the amount of developed landscape within 1 km of each site using the United States Department

142   of Agriculture cropland data layer (CDL). Proportion development was defined as the count of

143   developed pixels within 1 km of each site divided by the total number of all pixels. Each pixel

144   within the CDL classifies a 30×30 m area as a landscape class, with 255 possible

145   classifications. To characterize all human development within a landscape, we summed across

146   low, moderate, and high intensity pixels (Table S1) (Han et al. 2014).

147

148   **PPCP extraction and quantification.** Each dried bee sample was massed and then ground

149   with mortar/pestle. Bee PPCPs were extracted using a three-phase sequential extraction, similar

150   to those described previously in Brodin et al. (2013) and Furlong et al. (2008). First, 1.5 mL of

151   methanol/water mix (7:3 ratio) with 0.1% formic acid was added into the mortar with the bee

152   parts and poured into a glass culture tube. An additional 1.5 mL methanol/water mix with 0.1%

153   formic acid was used to rinse the mortar and pestle, and then was poured into the same centrifuge

154   tube. Tubes with tissue and extract were centrifuged at 2,000 rpm for 5 minutes. Following

155   centrifugation, supernatant was then added to a clean centrifuge tube, and then immediately

156   wrapped in parafilm. A second 1.5 mL acetonitrile was next added to the first tube containing the

157   bee tissue. The tube containing bee tissue was then vortexed and centrifuged twice. After each of

158   the three extraction phases, the sample was placed under nitrogen flow in a 40°C bath. Once

159   samples were nearly evaporated completely, 1mL of formate buffer was added to each sample,

160   and the concentrated extract was transferred to a 1.5 mL amber glass autosampler vial. Samples

161   were preserved in the dark at -20°C until being analyzed with HPLC/MS.

162

163   **HPLC/MS Quantification.** PPCP identification and enumeration followed methods

164   similar to Furlong et al (2008) and Brodin et al. (2013). Standards for each PPCP are described

165   in table SM1. HPLC eluents included a 10-mM formate buffer and 100% acetonitrile solution

166   that varied in percent contribution over the quantification procedure (Table SM1). Minimal

167   detection limits were estimated to be 5 ng/g. A main difference between our methods and others

168   prior is that we split analyte signatures into separate channels, so as to avoid peak interference

169   (described in Table SM1). Mass spectrometer time-programmed operating conditions for

170   individual compounds are detailed in Table SM2-3.

171   Samples were also analyzed in a manner to account for potential cross-sample

172   contamination and peak drift. Following standard samples, 2 blanks of 100% methanol were

173   processed and assessed for contamination. Following the two blank samples, bee samples were

174   processed in batches of 10 followed by 1 blank, 1 standard (100 ng/L), and 1 blank. This routine

175   allowed us to purge the column from potential cross-sample contamination, to assess if samples

176   were contaminating downstream samples within a batch, and to control for peak drift.

177   Following peak quantification, we noticed that concentrations tended to be bimodal,

178   where PPCPs were either detected in higher concentrations or not detected (Figure 2). We

179   assumed that the bimodality of these detections was likely a product of low-to-intermediate

180   PPCP concentrations degrading since the time of specimen collection or diffusing from bee

181   tissues as samples were originally collected in an ethanol solution before pinning (Bloom et al.,

182   2021). In order to provide conservative estimates of bee PPCP presence, we reduced

183   concentrations into categorical presence/absence informatics, such that subsequent models and

184   model interpretations should be conservative.

185

186   **Quality Assurance and Quality Control Procedures.** During PPCP extraction and

187   quantification, an internal standard was unintentionally not included within each sample. To

188   account for potential biases and contamination that might arise during the PPCP extraction and

189   quantification process, we developed quality assurance and quality control protocols to ensure

190   high confidence of model results.

191   Throughout the entirety of the sampling process, laboratory personnel refrained from

192   consuming caffeinated beverages, nicotine products, and non-prescription medications.

193   Personnel wore an N95 mask and nitrile gloves during extraction to reduce chance of

194   contamination during extraction.

195   Samples were double-blind throughout the entire specimen selection, PPCP extraction,

196   peak quantification, and modeling procedures. Personnel extracting PPCPs from bees were only

197   aware of "Sample ID" for each specimen, which was written on the autosampler vial.

198   Experimental autosampler vials were haphazardly placed in the auto-sampler to reduce

199   likelihood of biases due to order in the auto-sampler. A separate laboratory personnel analyzed

200   ion spectra. Throughout the entire spectra analysis, the technician only knew the "Sample ID"

201   and not any metadata associated with that "Sample ID". Lastly, a third person modeled the data

202   from HPLC/MS quantification. Through each step, experimental personnel only knew samples

203   by a unique "Sample ID" and were not aware of any attributes associated with that sample.

204   Together, this scheme was intentionally designed to potentially eliminate personnel-specific

205   biases throughout the extraction and analysis process.

206

207   **Modeling PPCP presence.** We iterated through all combinations of logistic regression

208   models to predict PPCP presence based on six predictor variables: bee and plant abundance, bee

209   and plant diversity, percent development, bee taxon, and all two-way interactions between these

210   variables (Katz et al. 2015; Rheubert et al. 2020). To account for edge effects, all variables were

211   standardized by the perimeter:area ratio of their respective site. Once all models were generated,

212   we selected the best model based on AICc, R^2, AUC, accuracy (percent true results), and p-

213   value. When multiple models had similar performance (AICc within two points of lowest AICc

214   value and R^2, AUC, and accuracy above the median of all models), best performing models were

215   averaged to create a single statistical model. To validate our results, we repeated analyses 1,000

216   times with 80:20 train:test subsetted data, and compared the distribution of model parameters and

217   pseudo-R^2 values from subsetted data to those models generated with the entire dataset. This

218   subsetting routine allowed us to assess whether the distribution of possible model parameters

219   was multi-modal and to evaluate whether the model constructed with 100% of the training data

220   was overfit to those data (Rheubert et al. 2020).

221   All analyses were conducted within the R Statistical Environment (R Core Team 2022)

222   using the packages glmulti (Calcagno 2019), tidyverse (Wickham et al. 2019), janitor (Firke

223   2020), MuMIn (Barton 2020), ggpubr (Kassambara 2019), ggeffects (Lüdecke 2018), lubridate

224   (Grolemund and Wickham 2011), plotrix (Lemon 2006), Hmisc (Jr et al. 2020), corrplot (Wei

225   and Simko 2017), ggrepel (Slowikowski 2019), ggspatial (Dunnington 2021), tigris (Walker

226   2021), cowplot (Wilke 2019), sf (Pebesma 2018), and readxl (Wickham and Bryan 2019).

227   **RESULTS**

228   **Species-specific PPCP detections.** We detected four PPCPs across all three bee species:

229   caffeine, paraxanthine/1,7-dimethylxanthine, acetaminophen/paracetamol, and cotinine. We did

230   not detect evidence of seven other PPCPs: codeine, warfarin, trimethoprim, sulfamethoxazole,

231   diphenhydramine, thiabendazole, or albuterol. *Bombus vosnesenskii* (50% of samples; Table S2)

232   and *A. texanus* (44%; Table S2) tended to have a higher odds ratio of PPCP presence relative to

233   *A. mellifera* (26%; Table S2).

234

235   **Relating PPCP presence with human development and plant abundance.** Across all

236   bee taxa, the probability of PPCP presence increased in landscapes with greater human

237   development or higher plant abundance (Fig. 2). However, there was a significant negative

238   interaction between human development and plant abundance, suggesting the positive

239   association between development and PPCP presence decreased in landscapes with greater plant

240   abundance; and, the positive effects of plant abundance on PPCP presence decreased in

241   landscapes with greater development (Fig. 2, Fig. S1).

242   Our subsetting routine, which assessed the probability of observing the final model solely

243   by chance, suggested our final model coefficients were non-random (Fig. 3). Model parameter

244   estimates (Figure 3) as well as pseudo-R^2 values (Figure 4) were generally unimodal. Most

245   subsetted model runs included human development and plant abundance as positive predictors of

246   PPCP presence (Figure 3), and most coefficients for interactions between plant abundance and

247   development were negative (Figure 3). Coefficients from the subsetting routine also

248   corresponded with patterns of PPCP detections for each taxon, where *B. vosnesenskii* had higher

249   positive coefficients, and *A. mellifera* and *A. texanus* tended to have lower negative coefficients

250   (Figure 3).

251

252   **DISCUSSION**

253   Our study demonstrates the presence of PPCPs in a terrestrial ecosystem and highlights that

254   pinned insect specimens can be used along with environmental data to understand patterns of

255   PPCP prevalence. These results are notable, considering that the study of PPCPs in terrestrial

256   ecosystems is uncommon relative to aquatic environments (Meyer et al. 2019). Our results

257   corroborate those results from aquatic systems, showing that PPCP presence can be influenced

258   by human development and species-specific traits (Bendz et al. 2005; Meador et al. 2016) and

259   that PPCPs can be mobilized in terrestrial food webs (Lagesson et al. 2016; Richmond et al.

260   2018).

261   Our results suggest PPCPs are more prevalent in *B. vosnesenskii* and *A. texanus* than in *A.*

262   *mellifera* (Table S2), implying that differences in species' life history can affect PPCP

263   accumulation. Both *B. vosnenskii* and *A. texanus* create nests in the soil, which may increase

264   exposure to human contaminants in the soil matrix and groundwater relative to *A. mellifera*,

265   which is managed in artificial nests by humans (Gradish et al. 2019). Additionally, *A. mellifera*

266   may prefer different resources within the same habitat (Thompson and Hunt 1999; Leonhardt

267   and Blüthgen 2012), generating variation in exposure. Niche partitioning of floral resources due

268   to differences in traits, such as tongue length, could also mediate pollutant exposure (e.g.,

269   pesticides) (Brittain and Potts 2011). In addition to pollinator traits, heightened PPCP exposure

270   for *B. vosnenskii* and *A. texanus* may be due to variable production practices across sampling

271   locations, with famer's application of wastewater byproducts to soil contaminating certain

272   pollinator nesting sites (Karnjanapiboonwong et al. 2010; Shahriar et al. 2021). While we are not

273   aware of wastewater byproduct applications at our sampling locations, prevalence of external

274   pollutants in conventional and organic farms is common (Humann-Guilleminot et al. 2019).

275   Beyond species-specific patterns, our results suggest that human development and plant

276   abundance mediated PPCP presence across all three taxa. This result is consistent with previous

277   findings in aquatic systems, where the source concentration (e.g., amount of development) and

278   paths to trophic transfer (e.g., number of plants) are correlated with the presence of PPCPs at

279   higher trophic levels (Richmond et al. 2018). For example, PPCP concentrations in aquatic

280   systems have been shown to be directly proportional with human population size and inversely

281   proportional to distance from a human population center (Bendz et al. 2005; Meyer et al. 2022).

282   PPCPs also can transfer between trophic levels (Richmond et al. 2018), which may lead to non-

283   linear biological processes and consequences, such as our data showing a negative interaction

284   between plant abundance and human development. Consequences of PPCPs within food webs

285   are hard to predict, although they can cause biological responses at physiological (Feijão et al.

286   2020), behavioral (Brodin et al. 2013), population (Hoppe et al. 2012), community (Lee et al.

287   2016), and ecosystem (Richmond et al. 2019) levels. Aside from PPCPs eliciting direct

288   biological responses, PPCPs may co-occur with numerous human disturbances, such as nutrient

289   pollution, that may further obfuscate clear associations between biological consequences and

290   PPCP exposure.

291   To our knowledge, this is the first study to detect PPCPs in bee tissues that were preserved

292   and pinned in a manner similar to museums. While not originally intended for the present study,

293   detailed field notes and extensive covariate collection created the opportunity for us to evaluate

294   PPCP presence across taxa while testing potential mechanistic drivers of PPCP presence.

295   Considering the growing number of pharmaceuticals (Daughton and Ternes 1999) and changes

296   in pesticides used on national and global markets (Douglas et al. 2020), preserved collections,

297   such as in natural history museums, may offer ripe and previously untapped opportunities to

298   explore contaminant accumulation and mixtures within biota. For example, lake sediment cores

299   have been used to reconstruct interdecadal PPCP contamination loadings (Anger et al. 2013).

300   Similarly, museum collections may empower reconstructions of exposure, accumulation, and co-

301   contaminant histories, all of which may be useful for detailing how contaminant loading and

302   mixtures may change through time.

303

304   **Synthesizing PPCP patterns across ecosystem types.** Over the past three decades, the

305 study of PPCPs has expanded rapidly, and growing evidence suggests that PPCPs are pervasive

306 micropollutants across aquatic and terrestrial systems alike. Our results suggest that much like

307 aquatic systems, PPCPs tend to concentrate closer to their sources and have potential to enter

308 food webs when vectors for trophic transfer are present. Additionally, our results demonstrate

309 species-specific differences for PPCP uptake. Similar to patterns observed in PPCP accumulation

310 in aquatic and riparian systems (Meador et al. 2016; Richmond et al. 2018), *B. vosnesenskii* and

311 *A. texanus* had higher probabilities of PPCP detections relative to *A. mellifera*, implying that life

312 histories, behavior, or physiological differences between taxa may play a role in PPCP uptake.

313   Broadly, our results present novel opportunities for assessing PPCP presence throughout

314 food webs and suggest similarities to how terrestrial and aquatic systems accumulate PPCPs.

315 Where PPCP data are rarer, pairing human development, environmental, and ecological data may

316 aid managers in flagging systems that are more associated with PPCPs, and thus susceptible to

317 declines in ecosystem function and services (e.g., pollination). Regardless of the exact trajectory,

318 our study lays a foundation for future basic and applied PPCP research and creates a synoptic

319 view of how organic contaminants may mobilize within aquatic and terrestrial environments.

320

324

325 **AUTHOR CONTRIBUTIONS**

326   MFM, MRB, BWL, EHB, and DWC designed the experiment. EHB collected field samples. MFM,

327   BWL, and MRB developed scripts analyses and data visualization. MFM and MLA extracted PPCPs and

328   analyzed HPLC/MS outputs. All authors contributed text, edited, and approved the final manuscript.
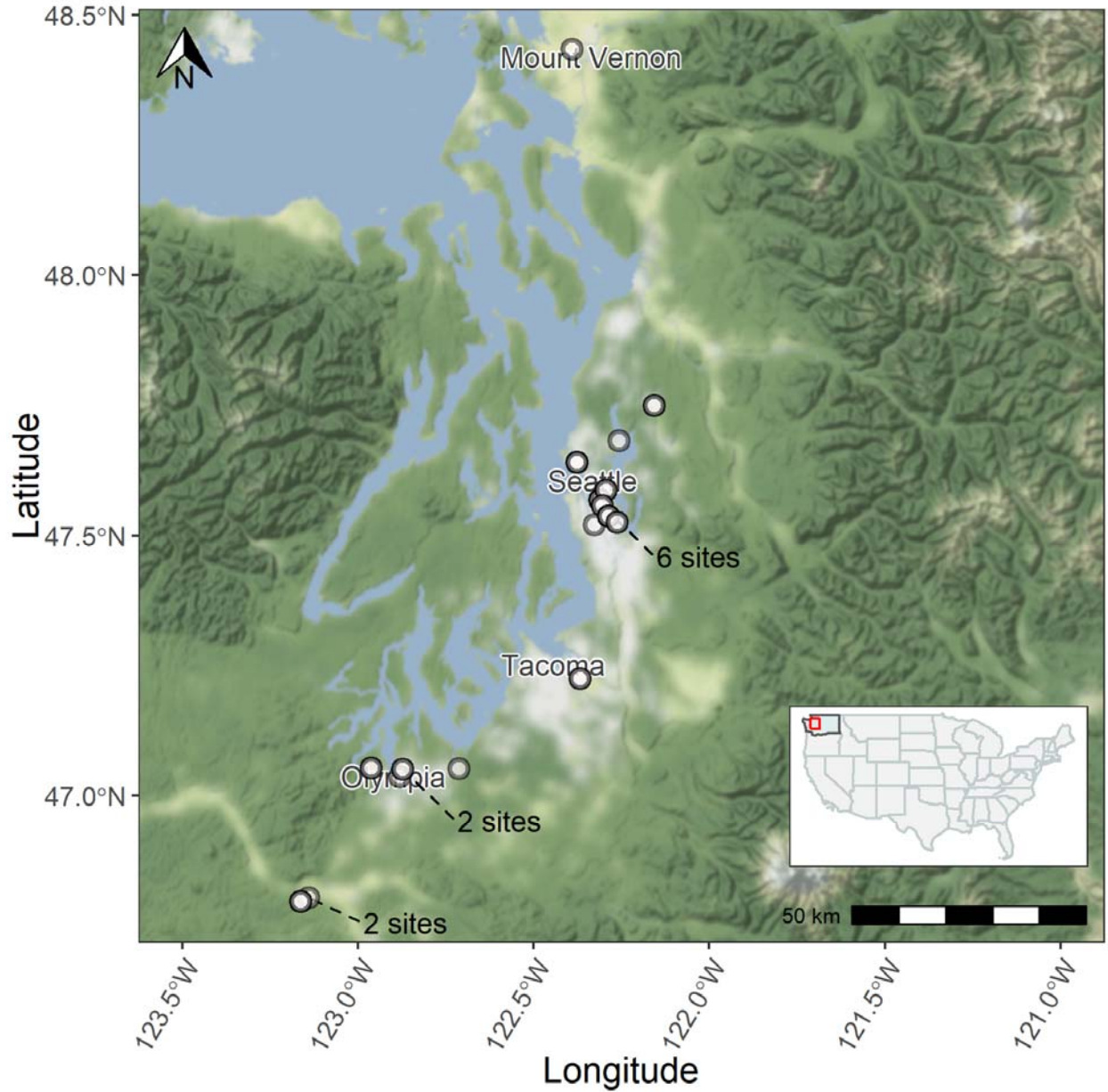
329

330   **FUNDING**

334

335   **DATA AVAILABILITY STATEMENT**

336   All data and scripts used to produce these analyses can be found in this project's companion

337   Open Science Framework repository (Brousil et al. 2022).

338

Figure 1: Map of bee sampling locations (white points). Map tiles by Stamen Design (stamen.com), under CC BY 3.0 (creativecommons.org/licenses/by/3.0). Data by OpenStreetMap (openstreetmap.org), under ODbL (openstreetmap.org/copyright). State data from US Census Bureau 2019.
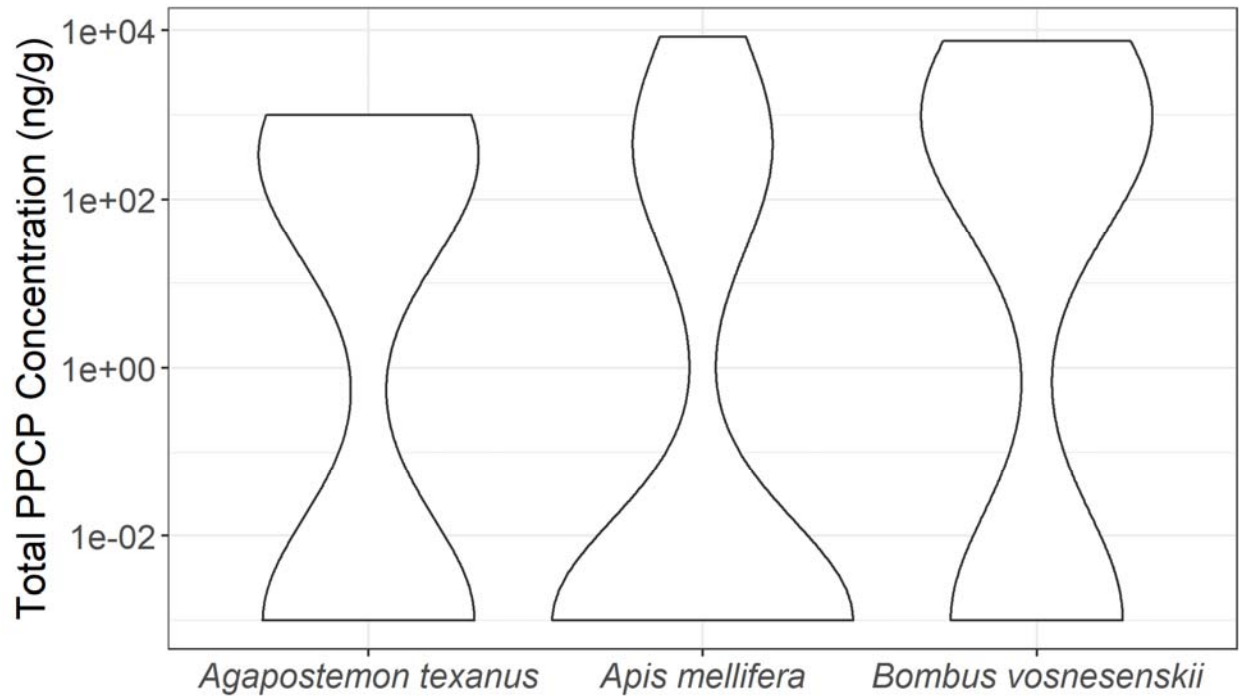
Figure 2: Violin plot of total PPCP concentrations (on log10 scale) in each of the three examined bee taxa. Although the figure shows continuous PPCP concentrations, few samples contained "intermediate" PPCP concentrations, meaning that concentrations were either high (i.e., greater than 500 ng/g) or not detectable. Given that this framework seemed unrealistic and could be a product of how bees were preserved, our main analysis only focused on presence/absence of PPCPs in samples. Therefore, our logistic regression approach should be more conservative than continuous, linear analyses. Nevertheless, trends in PPCP concentrations mirror patterns observed in PPCP presence/absence, where *B. vosnesenkii* and *A. texanus* have higher concentrations and probabilities of PPCP presence relative to *A. mellifera*.
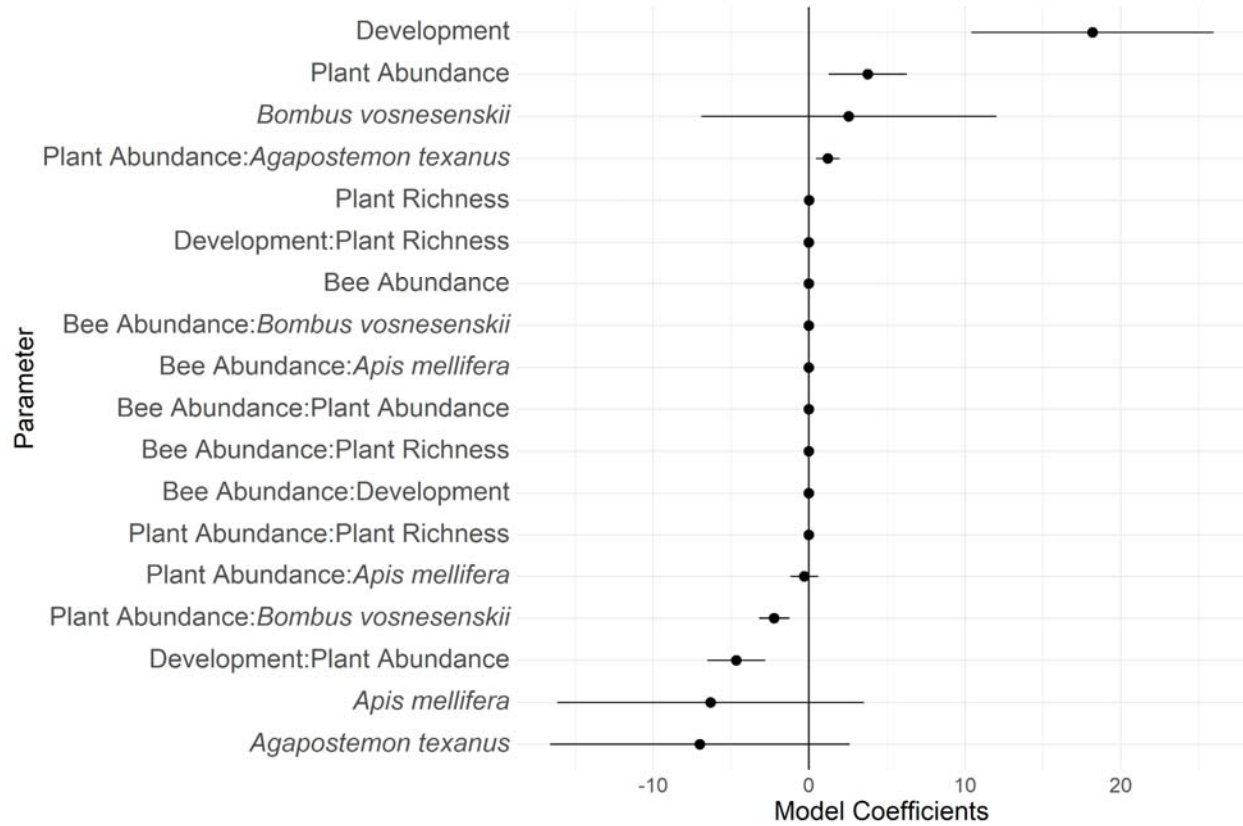
Figure 2: Coefficients for the final averaged model using all data for training. Points indicate the estimated model coefficient, and bars reflect the adjusted standard error. A value of 0 indicates a variable does not discriminate between PPCP presence or absence. Values greater or less than zero correspond to predictors that are more or less likely to be associated with PPCP presence, respectively. Parameters with a colon indicate two-way interaction terms. Parameters are arranged by decreasing coefficient estimate value.
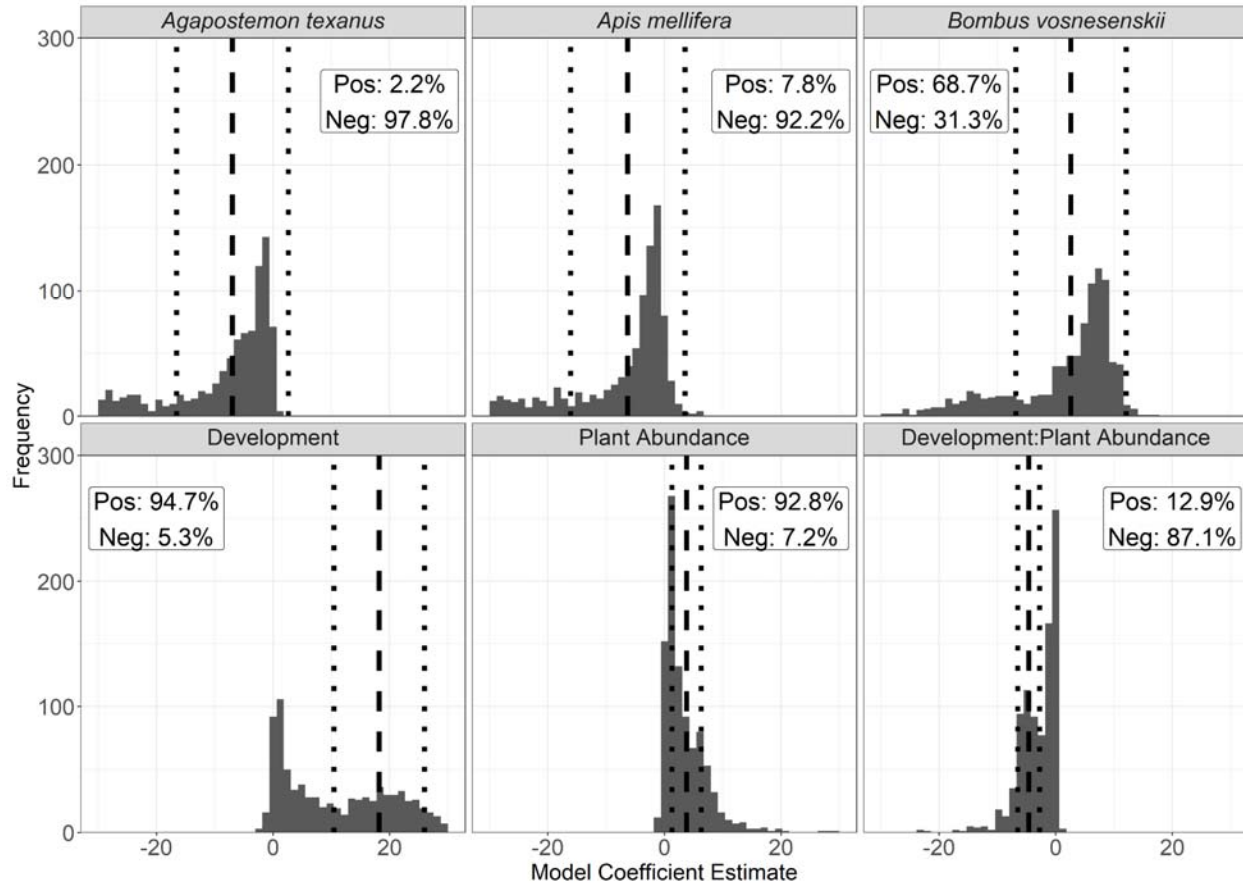
Figure 3: Distributions of most influential model coefficients for the final averaged model using 80% of original data as training data. Model averaging protocols were repeated 1,000 times, with each iteration starting with a species-stratified, random subsample. Vertical, dashed lines indicate the coefficient of the parameter that appeared in the final averaged model, when 100% of the data were used. Vertical, dotted lines indicate the adjusted standard error of the coefficient of the parameter that appeared in the final averaged model, when 100% of the data were used. Labels within each facet reflect the percent of coefficients that were positive (i.e., greater than zero) and negative (i.e., less than zero).
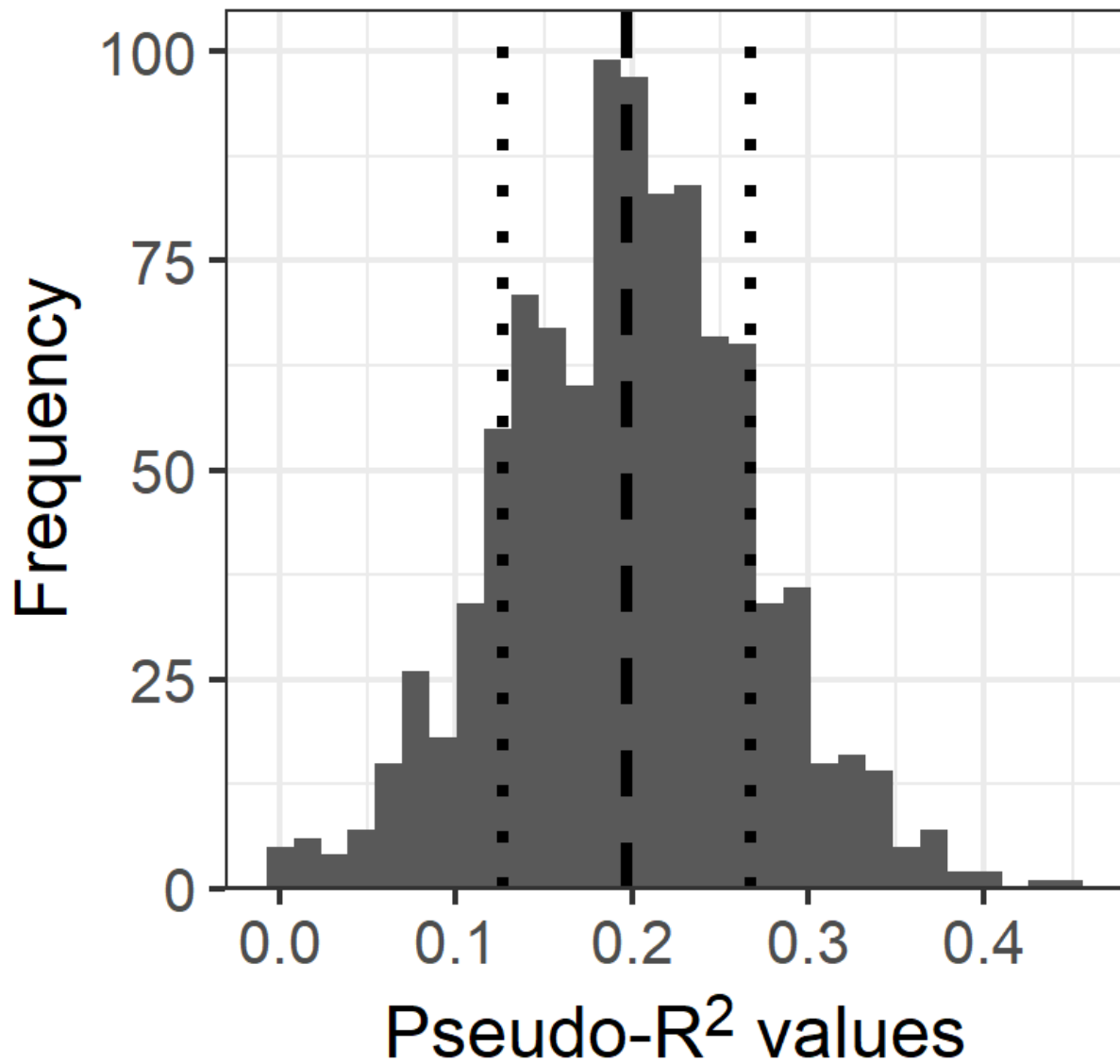
374
375   Figure 4: Distribution of pseudo R^2 values from all averaged models in the permuted analysis.
376   The vertical dashed line represents the mean of the distribution (Pseudo $R^2 = 0.197$), and
377   vertical, dotted lines represent one standard deviation from the mean (sd = 0.07).
378

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