

Soil properties and biochemical composition of ground-dwelling bee nests in agricultural settings

Rebecca A. Lybrand¹  | Jennifer Fedenko¹ | Malak Tfaily² | Sujaya Rao³

¹ Dep. of Crop and Soil Science, Oregon State Univ., Corvallis, OR 97331, USA

² Dep. Environmental Science, Univ. of Arizona, Tucson, AZ 85721-0038, USA

³ Dep. of Entomology, Univ. of Minnesota, Minneapolis, MN 55455, USA

Correspondence

Rebecca A. Lybrand, Dep. of Crop and Soil Science, Oregon State Univ., Corvallis, OR 97331.

Email: rebecca.lybrand@oregonstate.edu

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Abstract

Soils deliver under-recognized ecosystem services by supplying habitat for ground-dwelling pollinators, such as wild bees and other organisms, that pollinate 80% of insect-pollinated plants and play a critical role in securing resilient pollination provisions. Our objective is to identify soil properties of ground-nesting bee nests in agricultural settings of western Oregon. We confirmed ground-nesting bee and sand wasp activity in seven agricultural sites and one recreational park. Soils from 17 bee and sand wasp nests were analyzed for pH, particle size distribution, and carbon and nitrogen content. We visually confirmed that eight of the nesting bees were sweat bees from the *Halictidae* family and identified a captured bee specimen as *Lasioglossum (Dialictus)* (Hymenoptera: *Halictidae*). We located two sites with sand wasps where specimens were identified as *Cerceris* and *Bembix* (Hymenoptera: *Crabronidae*). The organic matter composition of three soil samples scraped from the linings of active nests was assessed using Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS). The FTICR-MS results identified unknown lipid compounds in the nest soil samples, which we hypothesize are waterproofing lipids secreted to line cell walls. Bee nests occurred in slightly acidic, bare-ground soils with low rock/vegetation coverage and low organic carbon content (<1%) and exhibited significantly higher silt-plus-clay fractions (>80%) vs. data published for bee nests in prior work. Our findings present important implications for textural controls on nest site selection in wet, cool environments and demonstrate the importance of integrating soil properties to improve our understanding of ground-dwelling organisms and associated soil habitats.

1 | INTRODUCTION

Soil provides various forms of ecosystem services to human populations, such as food production, carbon (C) seques-

tration, nutrient cycling, water purification, and climate regulation (Baveye et al., 2006). Soil also delivers a less well-known benefit to our society by supplying habitat to ground-dwelling, wild pollinators (Cane, 1991; Potts & Willmer, 1997), mammals (O'Brien, Rosenstock, Hervert, Bright, & Boe, 2005), amphibians (Dillard, Russell, &

Abbreviations: FTICR-MS, Fourier transform ion cyclotron resonance mass spectrometry; SOM, soil organic matter.

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Ford, 2008), and reptiles (Andersen et al., 2000; Bernheim, Shanas, & Bar-Massada, 2019; Heaton, Kiester, & Meyers, 2006), to name a few. However, the study of the soil habitat is often excluded entirely or poorly represented in conservation planning, microhabitat research, or habitat preference modeling activities (Bernheim et al., 2019).

Animals, including more than 20,000 species of bees, transfer the pollen for approximately 90% of the Earth's wild flowering plants that subsequently provide food, habitats, and resources for other organisms (IPBES, 2016). Bees contribute \$15 billion to crop value annually by pollinating approximately 75% of the fruits, vegetables, and nuts within the United States alone (Hamilton, 2013). Declines in honeybee colonies are a critical threat to agriculture, the economy, and our global food supply (Grossman, 2013). Growers who are interested in attracting alternative pollinators, such as wild bees, face a major challenge: The specific habitat requirements for many wild bee species remain understudied. The National Agroforestry Center estimates that 70% of wild bee species nest in the soil (Vaughan & Black, 2007), yet the soil properties that facilitate nesting activities require additional investigation despite an emerging emphasis on soil type as an important parameter to assess the impact of pesticides and pesticide residues on pollinating insects (Gierer et al., 2019; Uhl & Brühl, 2019).

The response of wild bee pollinators to anthropogenic land-use change varies within and by taxa (Tschardt, Klein, Krüss, Steffan-Dewenter, & Thies, 2005; Vazquez et al., 2002; Winfree, Griswold, & Kremen, 2007, 2011), making traits or attributes, such as an aboveground or belowground nesting preference, an important focus of study when monitoring the impacts of environmental change on bee communities (Potts & Willmer, 1998; Potts et al., 2005; Williams et al., 2010). An analysis of 19 studies from sites worldwide assessed the response of bee populations to human-induced disturbance and found that bees nesting aboveground were nine times more impacted by agricultural intensification than species nesting belowground (Williams et al., 2010). Several instances of ground nesting bee activity in agricultural landscapes have been documented in the Pacific Northwest of the United States. For example, one of the first studies to confirm ground-dwelling bee nesting activity in an agricultural environment identified bumble bee [*Bombus nevadensis* Cresson (Hymenoptera: Apoidea)] nests in a recently harvested annual rye grass seed field in the Willamette Valley, Oregon (Rao & Skyrn, 2013). In the 1950s, farmers had success with designing moist, salty soil areas, known as "bee beds," to attract ground-nesting alkali bees as pollinators to increase alfalfa yields in Walla Walla Valley, Washington (Stephen, 1959). Today, farmers still successfully cultivate both bees and alfalfa to increase yields (Moisset and Woj-

Core Ideas

- Our research assessed the soil properties of ground dwelling bee nests in Oregon, USA.
- Nests occurred in soils with high silt-plus-clay fractions compared with sandier soils in prior work.
- Unknown lipids in soil nest linings were identified and may be secreted waterproofing lipids.

cik, 2016). A knowledge deficit exists in understanding the soil physical and chemical properties that dictate nest-site selection despite the prevalence and resilience of ground nesting bees in agricultural landscapes.

Nesting resources shape the structure and organization of ground-nesting bee communities (Potts et al., 2005), with significant habitat characteristics spanning floral diversity, pollen availability, and abundance (Gathmann, Greiler, & Tschardt, 1994; Petanidou & Vokou, 1990; Potts et al., 2005); topographic factors, such as aspect and slope (Burkle & Alarcon, 2011; Potts & Willmer, 1997); and soil properties (Cane, 1991; Potts & Willmer, 1997; Sardinias & Kremen, 2014; Wuellner, 1999). Percentage of bare soil, sloped ground, and degree of soil compaction represented key microhabitat nesting proxies in a study of ground-nesting bee communities in northern California (Sardinias & Kremen, 2014), whereas percentage of litter cover and the density of potential nesting sites, in the form of cracks or holes in the soil surface, have also been identified as indicators of bee activity (Potts et al., 2005; Wuellner, 1999). An assessment of nest-site selection by the ground-nesting bee, *Halictus rubicundus*, across and within sites in the United Kingdom found less compact soils in sites with lower nesting densities versus the use of compacted soils in areas with high nest density and limited soil resources (Potts & Willmer, 1997). The U.K. study also confirmed that topographic factors enhanced solar insolation at the nesting sites (e.g., southerly aspects, sloped ground) and that the predominant soil textures occurred in the sand, loamy sand, or sandy loam textural categories, with silt-plus-clay fractions under 5% and sand percentages ranging from 35 to 100% (Potts & Willmer, 1997). Microhabitat nest selection and the potential for co-habitation of small habitat areas were also examined for five species of digger wasps (*Sphex funerarius*, *Ammophila heydeni*, *Ammophila pubescens*, *Ammophila sabulosa*, *Bembix tarsata*) in disturbed landscapes across central Europe (Srba & Heneberg, 2011). The authors found that nesting habits of the different species of digger wasps varied significantly by microhabitat preference, with specific emphasis on soil physical properties

including soil particle size, gravel content, soil compaction, and slope. The research summarized here indicates that soil–landscape relationships exhibit important control on nesting site preferences (e.g., Srba & Heneberg, 2011; Potts & Willmer, 1997; Sardinias & Kremen, 2014). However, the complex behaviors of individual bee species and communities coupled with the natural heterogeneity of the soil environment support the need to better examine the abiotic factors influencing nest site selection by ground-nesting bees, particularly in agricultural landscapes where growers are investigating avenues to enhance the activity of wild bees.

Our study examined the physical and chemical properties of soils collected from active bee and sand wasp nest sites in western Oregon to identify the soils conducive to ground-dwelling bee activity in mostly agricultural landscapes. The goal of our research was to assess soil properties associated with nest-site selection by ground nesting bees in the Willamette Valley, an agriculturally productive region of Oregon. We focused on ground-dwelling bees, predominantly native bees, with sand wasps included as indirect pollinators for one site comprised of imported sand and one site at an urban recreational park (referred to collectively herein as bees). We surveyed sites for bee nesting activity and then collected soils from active nests established mainly in agricultural landscapes. Agricultural sites were selected due to the expected benefits for crops dependent on pollination, yet the overall study design and presented analyses translate to ground-dwelling pollinators constructing nests in other soil environments. We compared soil properties among seven farm sites (and one urban recreational park) to identify similarities and differences in bee and sand wasp nesting sites based on soil pH, texture, rock fragment content, percent water stable aggregates, organic C and N percentage, gravimetric water content, and soil organic matter (SOM) molecular composition. Percent vegetation cover, soil temperature, and topographic factors (e.g., aspect, slope) were also examined. Nest sites were expected to occur on sloped, bare, exposed ground based on prior research (Potts & Willmer, 1997; Sardiñas & Kremen, 2014). We hypothesized that bee nesting sites in our study would present similarities to previous work with respect to soil properties, particularly soil texture, rock fragment percent, and aggregate stability, whereas we expected that sand wasp nests would present distinct soil properties (e.g., soil texture, aggregate stability) compared with soils from active bee nests. We also predicted that a high-resolution mass spectrometry approach, Fourier-transform ion cyclotron resonance mass spectrometry (FTICR-MS), would successfully detect lipid biomarkers potentially attributed to ground-dwelling bee activity compared to soil with no observed nests. Here, we advance understanding of ground-nesting bee activity as

a function of the associated soil environment by bridging disciplinary gaps among soil science, entomology, microbial ecology, and growers that can be translated to collaborative studies on soil and ground-dwelling organisms elsewhere.

2 | MATERIALS AND METHODS

2.1 | Study system

Our study was conducted in the Willamette Valley, given its reputation as Oregon's most productive agricultural land (Oregon Department of Fish and Wildlife, 2006). We established partnerships with several growers across the valley to survey agricultural soils for pollinator activity and to identify active nesting sites. The region is characterized by mild, wet winters and warm, hot summers with a mean annual temperature of approximately 11 °C and mean annual precipitation averaging 110 cm across sites (Table 1). Mean annual precipitation and temperature data were obtained from NOAA (Diamond et al., 2013) using Oregon State University's weather station at the Hyslop Field Laboratory for AH Farm, BDL Farm, BR Park, HY Farm, and TU Farm (Table 1). We also used climate data from the Dallas weather station for BO Farm and the Forest Grove weather station for BT Farm (Diamond et al., 2013). The distance between each farm and weather station is also provided (Supplemental Table S1). Environmental site properties were collected at each nesting location, including aspect, slope, vegetation cover, and percent rock cover. Soil temperature was monitored as point measurements in the surface and near-surface soils up to 65 cm in depth during the summer and winter seasons. All nesting sites occurred in soils classified as Aquultic Haploxeralfs (soils that experience aquic conditions for some time in normal years that contain a relatively thin subsurface horizon with clay accumulation), Aquultic Argixerolls (soils that experience aquic conditions for some time in normal years that contain a relatively thin subsurface horizon that has accumulated clay and shows an accumulation of organic matter in generally darker, C-containing surface horizons), Aquic Cumulic Haploxerolls (soils that experience aquic conditions for some time in normal years with minimal development in the subsurface horizons that show an accumulation of organic matter in generally darker, C-containing surface horizons), and Typic Haplohumults (soils with subsurface horizons showing clay accumulation that present a significant decrease in clay content within 150 cm of the soil surface) (Soil Survey Staff, 2019), except for BDL Farm, where nests were identified in surface soils comprised of imported sand.

TABLE 1 Site properties summarized for farms and urban park sites where active nesting sites were sampled

Location	Mean annual precipitation cm	Mean annual temperature °C	Aspect °	Slope	Vegetation cover %	Rock cover	Land use type (main crop)
AH Farm	108	11	90	12	5	2	agriculture (red clover)
BDL Farm	108	11	flat	1	1	3	agriculture (red clover; lavender)
BH Farm	108	11	flat	1	3	0	agriculture (red clover)
BO Farm	119	12	flat	5	20	0	agriculture (olive and fruit orchards)
BR Park	108	11	flat	3	5	0	recreation (urban park)
BT Farm	116	11	90	3	20	0	agriculture (red clover)
HY Farm	108	11	270	2	3	2	agriculture (wheat, barley, grass seed)
TU Farm	108	11	270	20	5	1	agriculture (livestock; blackberry, Oregon white oak)

Note. Climate data were collected from weather stations at Hyslop Field Laboratory and Dallas weather stations (Diamond et al., 2013). We determined topographic conditions, vegetation and rock cover percentages, and land use type, including the main crop(s) indicated in parentheses where applicable.

2.2 | Nest and pollinator identification

The first step in our study was to identify agricultural land with potential ground-dwelling bee activity where soil samples from active bee nests would be collected. We identified farms to survey for bee nesting activity by connecting with growers in the community by distributing an educational flyer summarizing potential evidence for ground-nesting bees, including the presence of emergence holes, tumuli (small mounds of soil around emergence holes indicative of active nesting activity), or bee activity observed on the soil surface. We surveyed for bee activity by monitoring transects established along the edges and rows of agricultural fields to ensure that we did not disrupt crops or farming activities as agreed upon when establishing partnerships with each grower. The size of the areas surveyed for bee activity on each farm, management practices, and overall farm size varied by site location (Supplemental Table S1).

Active nest sites were identified along the established transects by confirming wild bee foraging activity on nearby crops, surveying the surface soils for evidence of nest sites, including emergence holes and sometimes tumuli, and finally by monitoring emergence holes to visually confirm use by ground-nesting bees. Although we identified an abundance of emergence holes that were likely associated with bee nest-site activities, we only collected soil samples at sites where we observed a ground-nesting bee enter or exit an active nest site given that other ground-dwelling organisms also create emergence holes (Figure 1). In most situations, we visually confirmed ground-dwelling bee nesting activity and identified the



FIGURE 1 An example of the exposed bare ground surfaces where emergence holes were observed at AH Farm near Corvallis, OR. Soil samples were only collected from active nesting sites where we observed bees enter an emergence hole

specimen to the family level in the field (e.g., HY, TU; Supplemental Table S1). When possible, specimens were captured and preserved for identification purposes. The bees (or sand wasps for BDL and BR Park) were captured upon exit of nests using a small plastic vial or insect net. However, in several cases, we were unable to capture specimens because the bees either flew away or did not emerge from the nests within the duration of the survey at a farm site. The captured specimens were placed in a freezer for 24–48 h and then mounted on a #2 insect pin and maintained at room temperature to allow the specimens to dry for 14 d. The specimens were then identified to the genus/subgenus level by a bee taxonomist (L. Best, Oregon State University) and returned to insect specimen boxes for archiving in Oregon State University's Landscape Pedology Laboratory.



FIGURE 2 Three nesting sites located near Corvallis, OR, that were selected for FTICR-MS analysis based on differences in soil texture, vegetation cover, and land use type. (a) AH Farm: sloped, exposed edge of an agricultural field comprising soils with >80% silt+clay content. (b) BDL Farm: imported sand (>90% sand content) with minimal vegetation cover. (c) BR Park: a flat, grass-covered urban recreational park with soils that contain >70% sand. The image includes emergence holes (center) and includes snow cover as all nest sites were monitored year-round

2.3 | Soil chemical and physical analysis of nest soil samples

Soil samples from active bee nesting sites were air dried, sieved to the fine-earth fraction (<2 mm), and analyzed by Oregon State University's Landscape Pedology and Central Analytical Laboratory facilities. Soil pH was determined using 10 g of soil material in a 1:1 soil/water extract that was measured with a Horiba LAQUA F-74 benchtop pH meter (Soil Survey Staff, 2004). Total C and N contents were analyzed using an Elementar Vario Macro Cube that directly measures CO₂, N₂, and SO₂ following complete sample combustion at 1,150 °C. We assumed total C and N to equate to organic C and N given that no evidence of carbonate was observed in the samples. Organic C and N percentages are presented on an oven-dry basis.

Percentages of water-stable aggregates were assessed for each sample with a modified approach from the Cornell Soil Health Manual-Wet Aggregate Stability (Gugino et al., 2009), where dry soil (0.25–2 mm) is evenly spread on a 0.25-mm sieve and exposed to 1.25 cm of simulated rainfall delivered by an infiltrometer positioned 50 cm above the sample for 5 min. The aggregates remaining in the sieve following the rainfall event were dried at 105 °C and used to calculate the percentage of water-stable aggregates with respect to the total mass of the sample. The percentage of water-stable aggregates is an indicator of soil health (Guo, Zhang, Yang, Hua, & Chongfa, 2019) and was used as a soil variable to compare across bee nesting sites.

Samples were pretreated to remove organic matter using hydrogen peroxide prior to particle size analysis (Soil Survey Staff, 2004). The sieve-pipette analysis method was used to determine particle size classes by dispersing and shaking each pretreated sample with 5% sodium hexametaphosphate for 24 h, separating sand fractions using a wet sieve shaker, and differentiating silt and clay fractions by pipette according to Stokes' law (Soil Survey Staff, 2011).

2.4 | High-resolution mass spectrometry

We sampled soils from three active nest sites and one non-active site to test the prediction that FTICR-MS will detect potential lipid biomarkers to confirm bee nesting activity in field environments. Sites were selected to vary by dominant vegetation, soil, and land use type: AH Farm, BDL Farm, and BR Park (Table 1; Figure 2). Soil material was scraped from the upper ~5 mm of soil lining the walls of active nests and maintained at –80 °C until analysis by FTICR-MS. We also sampled an adjacent “non-activity” bulk surface soil at BDL Farm for comparison that was <50 cm from an active nest site and showed no emergence holes or other evidence of nesting activity.

Samples were prepared for FTICR-MS analysis using two solvents (water and chloroform) with different polarities to sequentially extract a representative fraction of organic matter from the soil (Tfaily et al., 2017). Briefly, extracts were prepared by adding 1 ml of solvent to 300 mg bulk soil and shaking in 2-ml capped glass vials for 2 h on an Eppendorf Thermomixer. Samples were removed from the shaker and left to stand before spinning down and pulling off the supernatant to stop the extraction. The soil residue was dried with N gas to remove residual solvent, and then the CHCl₃ was added. This protocol targets free or loosely bound organic matter compounds (e.g., organic matter that is accessible to microbes rather than large biopolymers or mineral-bound compounds) and extracts up to 7% of total organic matter in the sample (Tfaily, Hess, Koyama, & Evans, 2018b). Water extracts were desalted and concentrated using solid phase extraction (Dittmar et al., 2008), whereas the CHCl₃ samples were mixed with methanol (50:50) before analysis to increase ionization efficiency.

A 12 Tesla Bruker Solarix FTICR spectrometer was used to collect high-resolution mass spectra of the organic matter in the extracts. A standard Bruker electrospray

ionization source generated the negatively charged molecular ions. Samples were then introduced directly to the electrospray ionization source. The instrument is externally calibrated weekly to a mass accuracy of <0.1 ppm using a tuning solution from Agilent, which contains the following compounds: $C_2F_3O_2$, $C_6HF_9N_3O$, $C_{12}HF_{21}N_3O$, $C_{20}H_{18}F_{27}N_3O_8P_3$, and $C_{26}H_{18}F_{39}N_3O_8P_3$, with an m/z ranging between 112 and 1,333. The instrument settings were optimized by tuning on a Suwannee River Fulvic Acid standard. Blanks (high-performance liquid chromatography-grade MeOH) were run at the beginning and the end of the day to monitor potential carry over from one sample to another. The instrument was flushed between samples using a mixture of water and methanol. The ion accumulation time was varied to account for differences in C concentration. A total of 144 scans were averaged for each sample and internally calibrated using OM homologous series separated by 14 Da ($-CH_2$ groups). The mass measurement accuracy was <1 ppm for singly charged ions across a broad m/z range (i.e., $200 < m/z < 1,200$).

To reduce cumulative errors, sample peak lists for the dataset were aligned to each other prior to formula assignment to eliminate possible mass shifts that would affect formula assignment. Putative chemical formulas were assigned using Formularity software (Tolić et al., 2017). Chemical formulas were assigned using the following criteria: $S/N > 7$, and mass measurement error <1 ppm, taking into consideration the presence of C, hydrogen (H), oxygen (O), nitrogen (N), sulfur (S), and phosphorus (P) while excluding other elements. Formulae were classified as CHO, CHNO, CHOS, CHONS, CHONSP, and CHONP compounds such that CHO represents compounds that only contained C, H, and O elements; CHNO represents compounds that contain C, H, O, and N; and so on. Peaks with large mass ratios (m/z values >500 Da) often have multiple possible candidate formulas. These peaks were assigned formulas through propagation of CH_2 , O, and H_2 homologous series. Additionally, to ensure the consistent selection of a molecular formula when multiple formula candidates are found, the following rules were implemented. First, we selected the formula with the lowest error and the lowest number of heteroatoms. Second, the assignment of one phosphorus atom required the presence of at least four O atoms. The two datasets (water, chloroform) were combined for each sample to generate a composite. Peaks that were present in the blanks were subtracted from the sample data sets. All single peaks (i.e., peaks present in only one sample) were removed and not included in the downstream analysis.

We used van Krevelen diagrams to visualize compositional variations from the FTICR-MS data. A van Krevelen diagram places every assigned unique chemical formula

on a two-dimensional scatter plot of H/C ratio versus O/C ratio; thus, each dot in the diagram represents one or more molecular formulas with a specific O/C and H/C ratio. Regions of the van Krevelen plot can be tentatively associated with certain compound classes, such as lipid-like ($O/C < 0.4$, $1.4 < H/C < 2.5$), carbohydrate-like ($O/C > 0.8$ and $1.5 < H/C < 2.5$), or condensed hydrocarbon-like ($O/C < 0.2$, $H/C < 1$). In our study, labile/polar compounds refer to those that are readily utilized by microbial communities, such as sugars and proteins, whereas nonpolar compounds include lipid-like compounds that arise from microbial and/or plant origins. Here, lipid-like compounds are defined as a structurally diverse library of lipid-like formulated materials consisting of multiple lipid tails. Lipid-like compounds are characterized by O/C and H/C ratios similar to that of typical lipid molecules, such as fatty acids and their derivatives (including tri-, di-, monoglycerides and phospholipids) and other sterol-containing metabolites, such as cholesterol.

2.5 | Statistical analysis

All statistical tests were performed in JMP Pro (SAS Institute, 2018). A one-way ANOVA with the Tukey–Kramer HSD post hoc test was used to compare means of soil textural constituents (e.g., percentage of sand, silt, and clay) among soils from bee and sand wasp nests in our study and bee nest data published by Cane (1991).

3 | RESULTS AND DISCUSSION

3.1 | Nesting site features

We identified 17 active ground-dwelling bee and sand wasp nests among eight sites, including seven in agricultural settings and one site in an urban recreational park (Table 1; Supplemental Table S1). We sampled and monitored one active nest each at BO Farm, BR Park, BT Farm, HY Farm, and TU Farm; two active nests each at AH Farm and BH Farm; and eight active nests at the BDL Farm. The most common bees confirmed visually in the field were ground-nesting sweat bees (Hymenoptera: Halictidae), with one captured specimen identified as *Lasioglossium* (*Dialictus*) from AH Farm (Supplemental Table S1). Additionally, the BR Park and BDL Farm sites hosted ground-dwelling sand wasps, including captured specimens identified as *Cerceris* and *Bembix* (Hymenoptera: Crabranidae). The BR Park nesting site occurred in naturally sandy soil, whereas the active nests established at the BDL Farm were observed in imported sand placed on the soil surface by the landowners years earlier. Active nest sites for bees and sand wasps were included in our



FIGURE 3 Example of a sloped nesting site along the edge of an agricultural field at AH Farm in (a) warm summer months where (b) a tumulus was observed at an active bee nest. (c) The same bee nest was monitored throughout the winter months, where (d) the emergence hole remained open at the site surface for the wet, cool season

study to compare site and soil habitat requirements among these taxa in our region and to analyze with previously published bee nest site data (e.g., Cane, 1991).

Site features across all active nesting sites presented little to no rock cover (<3%) and low vegetation and organic matter coverage at <5%, except for two agricultural sites with 20% vegetation cover (Table 1). The sites presented a range in topographic features, such as slope (1–20°) and aspect, where four of the sites occurred on flat ground, two nesting sites were due north, and two were due south (Table 1). Microtopographic controls were also observed within sites, particularly at AH Farm where the sloped edge of an agricultural field showed the greatest number of emergence holes with confirmed active nesting activity (Figures 1 and 2). We monitored nest sites from the summer to winter months (July 2017–March 2019). Our observations confirmed that the active emergence holes remained open throughout the year and did not swell shut during the wetter, cooler seasons (Figure 3).

The site features observed at the active nesting sites in our study aligned with prior findings demonstrating that ground-dwelling bees prefer to nest in bare-ground soils

with minimal vegetation cover, likely due to the need for warm temperatures to enhance larval development (Potts et al., 2005; Sardinas & Kremen, 2014). Although we confirmed that active nest sites occurred on surfaces with low vegetation cover as predicted based on prior research, we did not observe strong topographic controls on nest site locations. For example, the active nests in our study occurred across a range of slopes and aspects (Table 1), whereas a preference for southerly aspects and slopes that maximize exposure to solar radiation represent important topographic requirements for ground-dwelling bees in other work (Burkle & Alarcon, 2011; Potts & Willmer, 1997). However, we emphasize that our study focused on identifying active bee nest sites and was limited in scope with respect to the number of bee species and nests analyzed (Table 1). Individual bee species present complex behaviors and contrasting preferences for nest site locations (Cane, 1991; Potts & Willmer, 1997; Sardinas & Kremen, 2014; Wuellner, 1999), as do digger wasps (Srba & Heneberg, 2011). Our study does not encompass enough nesting sites to fully understand how site features, such as aspect or slope, affect specific ground-nesting bee species overall but

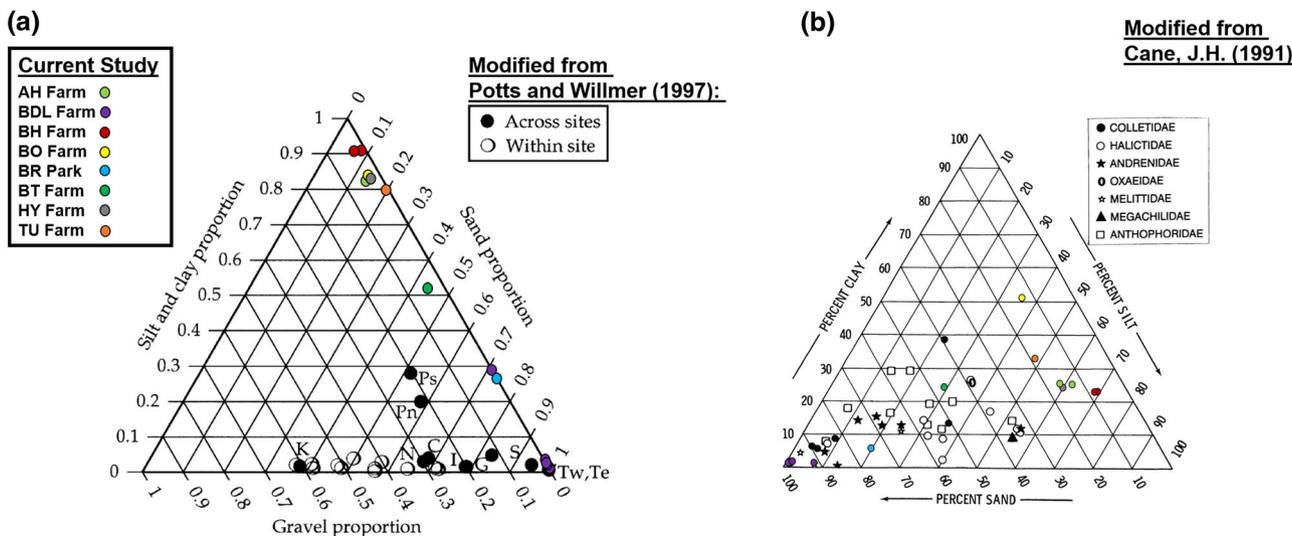


FIGURE 4 Soil texture triangles highlighting the particle size distribution of soils collected from active nest sites in our current study in comparison with (a) soil textural properties of nesting sites by *Halictus rubicundus* across and within sites in the United Kingdom (Potts & Willmer, 1997) and (b) a survey of soils associated with nests from contrasting ground-nesting bee species spanning different climates in the United States (Cane, 1991). Both figures were adapted from original publications with permission from respective journals

TABLE 2 Average values for bulk carbon (C), bulk nitrogen (N), wet aggregate stability, and particle size distribution for soils collected from active nesting sites

Location	pH (1:1 water)	Bulk C	Bulk N	Wet aggregate stability	%		
					Clay	Silt	Sand
AH Farm	5.77 ± 0.12	0.91 ± 0.00	0.08 ± 0.00	29 ± 0.00	24.05 ± 2.55	60.60 ± 4.61	15.36 ± 2.06
BDL Farm	5.23 ± 0.46	1.23 ± 2.36	0.04 ± 0.09	N/A	2.88 ± 4.37	2.61 ± 5.20	94.51 ± 9.54
BH Farm	4.99 ± 0.08	1.7 ± 0.06	0.15 ± 0.01	50 ± 4.24	22.53 ± 0.40	69.21 ± 1.29	8.27 ± 1.69
BO Farm	5.06 ± 0.00	1.21 ± 0.00	0.11 ± 0.00	34 ± 0.00	51.40 ± 0.00	33.98 ± 0.00	14.62 ± 0.00
BR Park	5.71 ± 0.00	1.40 ± 0.00	0.12 ± 0.00	32 ± 0.00	5.37 ± 0.00	19.54 ± 0.00	75.08 ± 0.00
BT Farm	5.08 ± 0.00	1.75 ± 0.00	0.18 ± 0.00	32 ± 0.00	24.20 ± 0.00	29.39 ± 0.00	46.40 ± 0.00
HY Farm	5.28 ± 0.00	2.80 ± 0.00	0.24 ± 0.00	62 ± 0.00	24.08 ± 0.00	60.20 ± 0.00	15.72 ± 0.00
TU Farm	5.30 ± 0.00	1.84 ± 0.00	0.17 ± 0.00	42 ± 0.00	33.01 ± 0.00	47.01 ± 0.00	19.91 ± 0.00

Note. Sites include BO Farm, BR Park, BT Farm, HY Farm, and TU Farm, where $n = 1$ active nest per site; AH Farm, where $n = 2$ active nests; BH Farm, where $n = 2$ active nests; and BDL Farm, with $n = 8$ active nests sampled. Average values were calculated and presented only when multiple nests were located within one farm site.

allow for comparisons in soil properties between active nest sites in our study with previously published work. Future research should also integrate methods that identify bees and/or wasps to the species level to enable interpretations of the results from an ecological point of view.

3.2 | Soil chemical and physical properties

We identified active nest sites in slightly acidic soils with unexpectedly high proportions of silt+clay (>75%) compared with earlier research in cool, wet climates where nesting sites were dominated by sandy, well-drained con-

ditions (Figure 4a,b) (Cane, 1991; Potts & Willmer, 1997). Sand percentages were <50% for six of the eight nesting sites; at BR Park and BDL Farm, sand wasps nested in coarser soil materials with sand contents of 75 and >90%, respectively. Soil pH, total C content, and total N content were well constrained across all nesting sites, with mean values ranging from 4.99 to 5.77, 0.91 to 2.80%, and 0.04 to 0.24%, respectively (Table 2). Wet aggregate stability presented more variability, with percentages spanning 0% at BDL, 32% at BR Park, 42% at TU Farm, 50% at BH Farm, and 62% at HY Farm. The lack of water-stable aggregates was observed in imported sands at BDL Farm compared with the highest degree of water-stable aggregates in the most fine-grained materials in our study (Table 2).

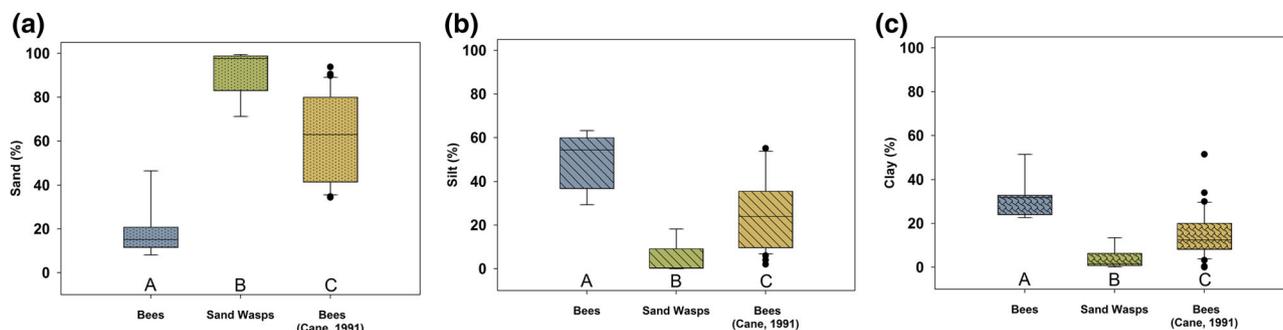


FIGURE 5 Soils from active bee and sand wasp nests from our study and bee nests from Cane (1991) as examined by percentage of (a) sand, (b) silt, and (c) clay. Different letters denote statistical significance ($P < .05$). Each box plot presents the median as a black horizontal line. The 25th and 75th percentile are represented as vertical boxes. The 10th and 90th percentile are indicated by error bars

Our study presents evidence for ground-nesting bees constructing nests in soils with high silt-plus-clay fractions, ranging from 54 to 92% (excluding sand wasp nests at BR Park and BDL Farm), with soil textural classes spanning silt loams, silty clay loams, loams, and clays (Figure 4; Table 2). Prior work found the greatest nesting densities in sandy, well-drained soils where silt-plus-clay fractions were less than 5% (Potts & Wilmer, 1997) or where nesting sites comprised at least 33–94% sand (Cane, 1991). Soils from active bee nest sites in our work exhibited significantly higher silt ($P = .0002$) and clay ($P < .0001$) percentages compared with soil textural data for bee nests published by Cane (1991) and for sand wasp nests at BDL Farm and BR Park ($P < .0001$) (Figure 5a,b). Conversely, sand percentages were significantly higher for sand wasp nests in our project versus those for soils from active bee nests herein ($P < .0001$) and for percentages published previously for bee nests ($P < .0001$; Figure 5c) (Cane, 1991). Our findings present important implications for soil textural controls on nest site selection and construction given that previous research attributed preferences for sandier soils to the need for ground-nesting solitary bees to avoid waterlogged soil conditions that would be detrimental to larval development (Potts & Willmer, 1997). We hypothesize that bees nesting in fine-grained soils use additional strategies for survival in relatively poorly drained environments, with one option being the secretion of hydrophobic compounds that line nest cell walls in the soil (Albans, Aplin, Brehcist, Moore, & O’Toole, 1980, Cane, 1991; Hefetz, Fales, & Batra, 1979).

3.3 | Soil organic matter molecular composition of active bee nest materials

We subsampled the upper 5 mm of soil lining active bee nesting sites from three sites (AH Farm, BDL Farm, and

BR Park) to assess the molecular composition of organic compounds putatively associated with nesting activity (Figure 6). Electrospray ionization FTICR-MS has proven to be a powerful technique for revealing the complexity and diversity of SOM molecules (Tfaily et al., 2015, 2017, 2018b). The compositional differences of SOM were clear between the active nest sites and appeared to be shaped in part by vegetation type and bee activity (Figure 6). In particular, distinct features were revealed among the active bee nest samples, such as a high abundance of specific molecular formulas with relatively low polarity at low O/C and medium-high H/C values (corresponding to lipid-like and unsaturated hydrocarbons) in the soil samples with known nesting activity compared with soil with no activity (Figures 6 and 7). Alternatively, the non-active soil was dominated by labile polar compounds (carbohydrates and amino-sugars). We visualized the FTICR-MS data using van Krevelen (Figures 7 and 8) and Venn diagrams (Figure 8), which also showed significant separation in SOM composition and quality between soils subsampled from the walls of active nests at the three sites and an adjacent non-active soil at BDL Farm (referred to as BDL-2; Figure 8). In particular, the percent composition of molecular species in active nest sites versus non-active soil showed distinct patterns (Figure 8a), capturing the heterogeneous molecular species shifts in composition that may be attributed to bee activity.

Soils with active nesting activity were relatively abundant in one or more of the following biogeochemical classes: lipid-like compounds, unsaturated and condensed hydrocarbons, lignin-like, and tannin-like compounds (Figures 6 and 7), possibly due to contributions from bees, sand wasps (BR Park, BLD Farm), and plants. Conversely, the soil sample with no observed nesting activity was dominated by labile compounds, such as carbohydrates and amino sugars, from plants and soil microbial communities (Tfaily et al., 2018b) (Figure 6; Supplemental

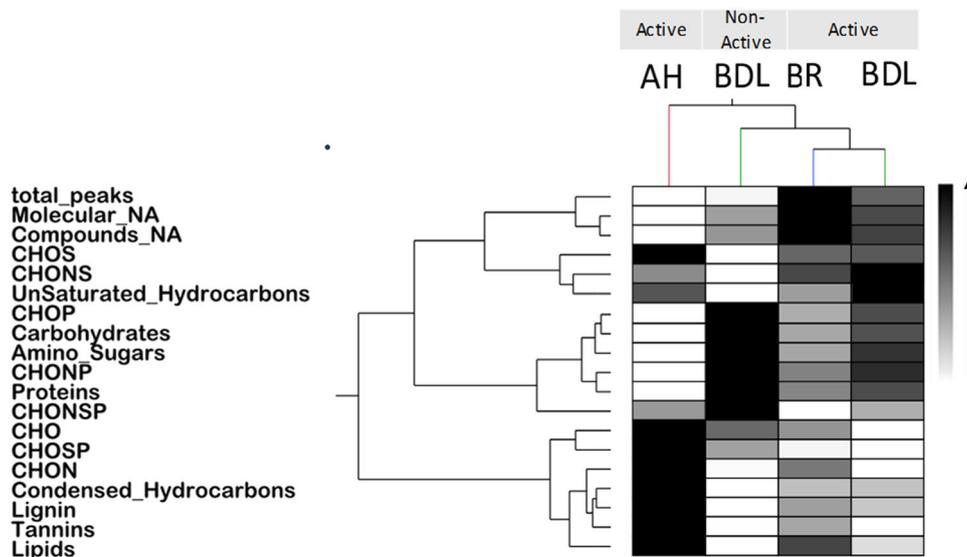


FIGURE 6 Two-way cluster analysis of the different characteristic parameters of soil organic matter (SOM) as inferred from FTICR-MS. Here, chemical formulae were assigned based on the presence of C, H, O, N, S, and P while excluding other elements. Chemical formulae were further assigned to biochemical compound classes (e.g., lipid-, protein-, lignin-, carbohydrate-, and condensed aromatic-like compounds) based on the H/C and O/C ranges. The relative abundance of different SOM compound classes and elemental composition (CHO, CHON, CHOPS, etc.) was then used for two-way cluster analysis

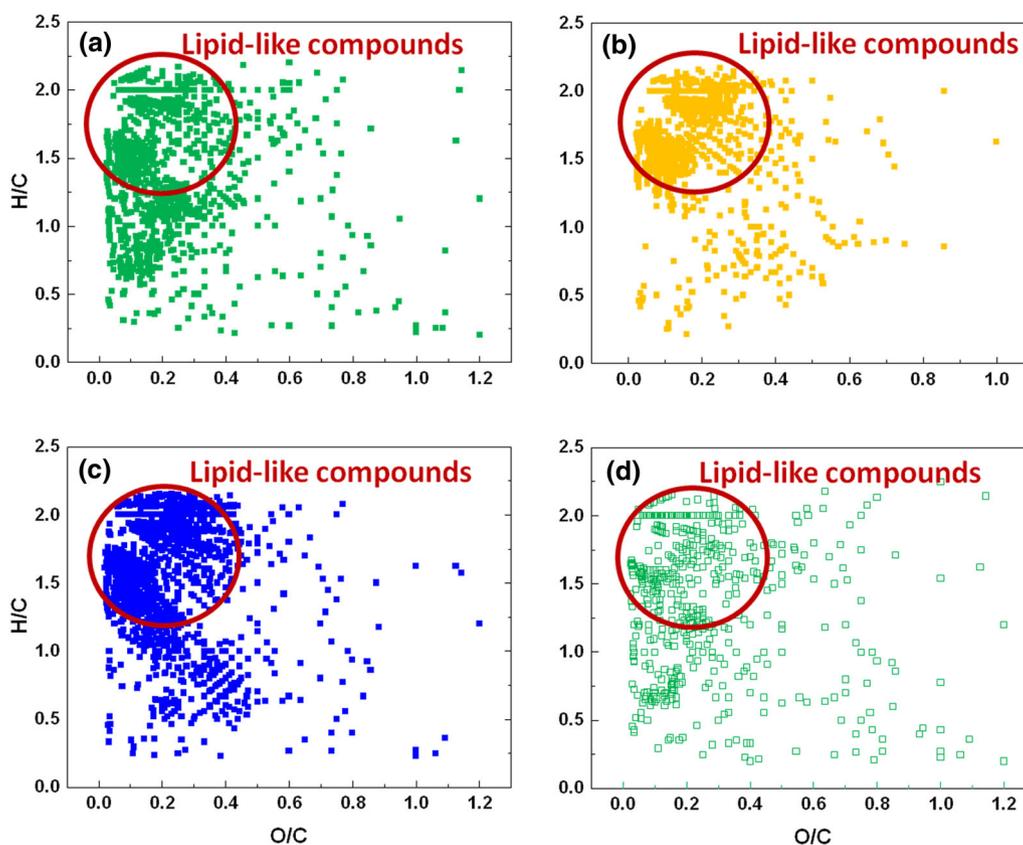


FIGURE 7 van Krevelen diagrams of all assigned formulas in soil organic matter from active nest sites at (a) BDL Farm, (b) AH Farm, and (c) BR Park compared with a non-activity soil sample at (d) BDL Farm. Compounds were plotted on the van Krevelen diagram on the basis of their molar H/C ratios (y-axis) and molar O/C ratios (x-axis), which provides a means to visualize and compare the average properties of organic compounds and to assign compounds to the major biochemical classes (e.g., lipid-, protein-, lignin-, carbohydrate-, and condensed aromatic-like). More data points within a specific region indicate a higher abundance of this class of compounds

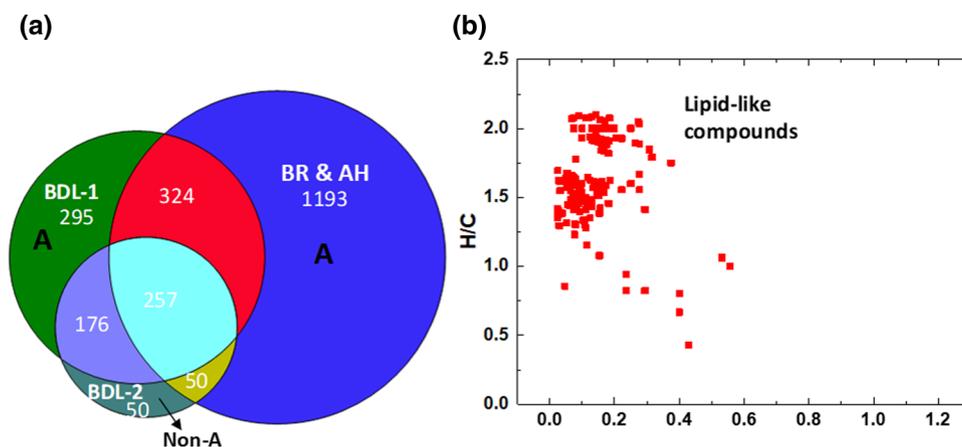


FIGURE 8 (a) Three-set Venn diagram showing overlap among the molecular compositions of soil nest linings from the different sites. (b) van Krevelen diagram of peaks that were unique to sites with active nest activity (i.e., not present in sample that lacked nesting activity). The percentage of overlap among all sites is approximately 11%. A, active nest soil samples; non-A, a non-active sample from BDL Farm

Table S1). In all samples, most formulas were CHO compounds (32–35%), followed closely by CHOP (15–23%), CHOS (5–9%), and CHON (3–9%), with other molecular series (CHONS, and CHOP [N, S]) accounting for only minor fractions (Figure 5; Supplemental Table S2). A high abundance of CHO and CHOS (including CHOSP and CHONS) were seen in active nest site soils compared with CHOP (including CHONP and CHNSOP) in non-activity soil (Figure 5; Supplemental Table S1). Venn diagrams also distinguished between shared and non-shared (unique) formulas observed among active nest sites (Figure 8a). Only 257 molecular formulas were common among all active nest sites. Sites with pollinator activity presented a more diverse range of molecular compounds compared with the sample with no nesting activity. Interestingly, all of the molecular formulas that were unique to soils with nesting activity and absent in the sample with no activity were lipid-like compounds, which further supported the notion that bees and sand wasps secrete and line nests with detectable lipid-like compounds (Supplemental Table S1; Figure 8b). Collectively, our data demonstrate that SOM composition contains integrative information on organic matter sources and that the application of FTICR-MS provides evidence that ground-dwelling bees and sand wasps may act as a contributing source to SOM pools by enhancing lipid-like compounds in soils.

Recent advances in ultrahigh-resolution analytical techniques, FTICR-MS in particular, have provided novel insights into the molecular composition of dissolved organic matter in fresh and marine water (Hertkorn, Harir, Koch, Michalke, & Schmitt-Kopplin, 2013; Lu et al., 2015; Sleighter et al., 2007; Tfaily et al., 2018a), SOM in different terrestrial systems (Bhattacharyya et al., 2018; Choi et al., 2017; Tfaily et al., 2018b), and the metabolic content of

plant root exudates (Pollier, Morreel, Geelen, & Goossens, 2011) and whisky (Roullier-Gall et al., 2018). Here, for the first time, we apply the capabilities of FTICR-MS to the analysis of soils lining active bee nesting sites to detect and identify molecular compounds possibly secreted by wild bees and sand wasps in field settings (Figures 2, 6–8). Our results align with prior research where patterns in the secretion of exudates were explored for Halictinae bees using gas chromatography and mass spectrometry (Johansson, Svensson, Tengo, & Bergstrom, 1982). The authors identified compounds extracted from Dufour's glands of individual bee specimens, including C₁₆, C₁₈, C₂₀, C₂₂, and C₂₄-lactones and straight chain hydrocarbons (Johansson et al., 1982), which were consistent with an abundance of lipid-like and unsaturated hydrocarbons found in the soil nest linings from our study. Lipids identified in secretions from the Dufour's glands of *Anthophora*, *Emphoropsis*, and *Megachile* bees comprised short-chain (C₂–C₂₀) fatty acid triglycerides, with shorter acids (C₂–C₁₆) and short-chain fatty acids (C₂–C₁₆), depending on the bee species analyzed (Cane & Carlson, 1984). Additional research examined the volatile components of Dufour's gland secretions from Halictine bees, which documented saturated and unsaturated macrocyclic lactones from C₁₈ to C₂₆ (including saturated C₂₆ and unsaturated C₂₀, C₂₂, and C₂₄ lactones identified as new products for Halictine bees). Here, the authors found that the major lactones in the Dufour's gland secretions also occurred in cell linings and pollen balls for *Augochlora pura pura* (Duffield, Fernandes, Lamb, Wheeler, & Eickwort, 1981). We also identified lipid-like compounds that were unique to the three active nest sites and contained several compounds with molecular formulas in the same C₉–C₂₆ range as described in the literature as well as other formulas up

to C49 that have not been identified previously (Supplemental Table S1). Although the exact function of the lipids secreted by ground-dwelling bees remains understudied, the hydrophobic nature of the lipid substances has been identified as a critical feature for maintaining moisture homeostasis for wild bees nesting across diverse climates and soil conditions (Cane, 1991; May, 1972; Shinn, 1967). We hypothesize that the lipids excreted by bees may function as an important waterproofing agent in nests constructed in exposed, fine-grained soils of the wet, cool climate found in the Pacific Northwest of the United States; however, future work should focus on confirming these predictions on function.

4 | CONCLUSION

Declines in wild and managed bee populations are a critical threat to agriculture, the economy, our global food supply, and the associated loss of biodiversity due to a decreasing diversity of pollinators. Wild bees represent a potential practical solution to these challenges given their widespread pollinating habits. However, growers interested in attracting alternative pollinators, such as wild bees, face a major challenge: The soil habitat preferences for many wild bees are understudied, yet 70% of wild bee species nest in soil. This obstacle presents an opportunity for soil scientists to partner with entomologists and growers to identify soil properties and nesting site features associated with ground-dwelling bee activity in agricultural settings. We identified eight active bee and sand wasp nesting sites in the Willamette Valley, a highly agriculturally productive region of western Oregon, and determined the biophysical soil properties of the nests. Our study was also the first to apply FTICR-MS to examine the composition of organic compounds putatively associated with ground nesting bee and sand wasp activity, specifically the biogeochemical nature of secretions produced to line active nests in the soil. Furthermore, we observed bee nesting activity in soils with unexpectedly high proportions of silt+clay percentages (>80%), which presents a distinct contrast to sandy, well-drained conditions typically associated with ground-nesting bee activity in previous work. We hypothesize that the lipid-like compounds associated with active nest sites serve as a waterproofing agent to enhance moisture homeostasis, although additional work examining the functional nature of these compounds is required to confirm. We also predict that ground-nesting bee activity is prevalent in agricultural settings and recommend that growers be trained on identifying wild bee activity in bare-ground soils exposed along the edges of agricultural fields that could intentionally be preserved to enhance wild bee activity, especially given the prevalence of active nests

in fine-grained agricultural soils. Our research provides a framework for ongoing collaborative endeavors among soil scientists, entomologists, and growers to identify soil habitats that support the activity of ground-nesting bees that reside in a given region. This, in turn, will improve understanding of connections between agriculture and the soil that bees, crops, and living organisms rely on to survive. Furthermore, we provide a demonstrative example of how the soil habitat itself can be assessed with respect to ground-dwelling organisms, a topic that remains under-represented in conservation planning and habitat preference research efforts.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Rebecca A. Lybrand  <https://orcid.org/0000-0001-7811-9093>

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SUPPORTING INFORMATION

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