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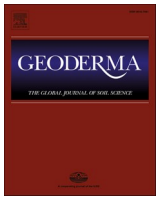

Morphology and temporal evolution of ground-nesting bee burrows created by solitary and social species quantified through X-ray imaging

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ABSTRACT

Most research on wild bees has focused on their role as pollinators, while their importance as soil ecosystem engineers has been largely overlooked, despite the fact that most species nest in the soil. There is limited quantitative knowledge regarding the architecture of nests created by wild bees and the temporal evolution of bee burrows. The aim of this study was to evaluate the feasibility of repeatedly scanning ground-nesting bee nests using X-ray computed tomography to quantify the morphology and temporal evolution of burrow systems created by both solitary and social species. We installed eleven large cylinders at locations with ongoing nesting activity of ground-nesting bees, and repeatedly scanned these soil columns with a medical X-ray scanner over a period of 16 months. From the X-ray images, we extracted bee burrows to visualize and quantitatively characterize their morphology and temporal evolution. The architecture and temporal evolution of burrows strongly differed between the studied social and solitary ground-nesting bee species. Burrows created by the solitary species were simple, linear and unbranched burrows, which were not reused and decayed with time. The burrow systems created by the social species were more complex, with highly branched networks of horizontal and vertical burrows, which increased in complexity and size over time during the bee activity period. The persistence of burrows created by ground-nesting bees varied greatly, with some decaying within a few weeks and others remaining mostly intact for the entire 16-month study period. This study demonstrates the potential of X-ray imaging to provide new insights into the underground life of ground-nesting bees, and highlights the locally important role of ground-nesting bees as soil ecosystem engineers.

1. Introduction

Bees (Hymenoptera: Apoidea: Anthophila), a group of insects with more than 20,000 described species, are considered the primary pollinators in most ecosystems (e.g., Garibaldi et al., 2013; IPBES, 2016; Michener, 2007; Neff and Simpson, 1993). The pollination services that they provide are critical to the functioning of both natural and managed ecosystems, with 87% of wild flowering plant species (Ollerton et al., 2011) and 75% of the world's most important crops dependent on or benefiting from pollinators (Klein et al., 2007). In most regions of the world, the wild bee fauna is dominated by species nesting below ground in the soil (e.g., Cane and Neff, 2011; Harmon-Threatt, 2020), many of which are considered key crop pollinators (Kleijn et al., 2015). While the pollination services provided by ground-nesting bees to natural ecosystems and crops are well recognized, their role as soil ecosystem engineers remains largely unexplored and underappreciated, despite their multitudinous direct and indirect impacts on soils (Christmann, 2022, 2019; Ruiz et al., 2023; Wilkinson et al., 2009).

Ground-nesting bees act as soil bioturbators by directly altering the pore network of the soil through their burrowing activity for nest construction (Danforth et al., 2019; Westrich, 2018). They dig burrows of large diameters (up to 10 mm; Sarzetti et al., 2013) with nest depths ranging from 1 to 530 cm (Cane and Neff, 2011). The nest structure comprises burrows and brood cells where the bees lay eggs on pollen moistened with nectar (Danforth et al., 2019; Westrich, 2018). Bee burrows are generally continuous, predominantly vertical, and connect the soil surface to the subsoil (Antoine and Forrest, 2021; Danforth et al., 2019), although some species form intricate networks of branching burrows and chambers (Stephen et al., 1969; Westrich, 2018). Some species nest gregariously and can form dense (up to 1,658 nests m^{-2};

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Visscher and Danforth, 1993) and enormously large nesting populations (up to 12 million individuals; Blagoveshchenskaya, 1963) that can persist for decades (e.g., Cane, 2003), and some species are capable of excavating significant amounts of soil (up to 27 Mg ha^{-1} yr^{-1}; Watanabe, 1998). Despite these impressive feats of ground-nesting bees that result in significantly altered soil pore network architecture and despite the well-established effects of such macropores on soil functions (e.g., enhanced water infiltration and soil aeration; Ruiz et al., 2023), the role of ground-nesting bees as soil ecosystem engineers – unlike earthworms, termites, and ants (Lavelle et al., 1997; Lee and Foster, 1991; Ruiz et al., 2023) – has been largely neglected (Christmann, 2022, 2019).

To properly assess the functional consequences on soils of burrowing activities by ground-nesting bees, it is important to quantify the morphology of the burrow systems that they create. Since soil porosity is dynamic (e.g., Rabot et al., 2018) and the shape or extent of burrow systems can be altered by physical processes, such as wetting–drying cycles (e.g., Le Mer et al., 2021), or biological activity of soil fauna (e.g., Meurer et al., 2020), it is important to also consider the temporal dimension, i.e., quantifying temporal changes in burrow morphology and their persistence. However, studies quantifying the structural changes and persistence of ground-nesting bee burrows over time are largely lacking, in part due to the difficulty of observing the cryptic subterranean life stage of bees.

Traditionally, nesting behavior and burrow morphology of ground-nesting bees have been studied in the field using excavation and casting techniques with various different materials (e.g., Linsley et al., 1952; Marinho et al., 2018; Michener et al., 1955) or in the laboratory using observation chambers (e.g., Batra, 1970; Leonard and Harmon-Threatt, 2019; Michener et al., 1955). Excavation and casting techniques are destructive and extremely tedious (Antoine and Forrest, 2021; Linsley et al., 1952; Marinho et al., 2018). Observation chambers allow the study of burrowing behavior and changes in burrow morphology over time, but alter bee behavior and restrict bee movement within a quasi two-dimensional container in an artificially created environment (Johnson et al., 2004). Furthermore, only a few ground-nesting bee species have been successfully reared in the laboratory, mostly social and communal halictid bees, but even with these, not all attempts are successful (Antoine and Forrest, 2021; Leonard and Harmon-Threatt, 2019; Michener et al., 1955). Therefore, none of these methods are suitable for studying the temporal evolution of ground-nesting bee burrows in their natural environment.

X-ray computed tomography (XRCT) has proven valuable for quantifying soil structure (see review Helliwell et al., 2013) and its temporal evolution (Koestel and Schlüter, 2019; Leuther et al., 2023), and has been used for studying burrowing of soil-dwelling fauna and nest systems of social insects (see review Gutiérrez et al., 2018). Greco et al. (2006) used X-ray imaging to examine the internal nest structure of the ground-nesting Australian blue-banded bee (*Amegilla holmesi*) in artificial mud-brick nests to count and measure brood cells, and to identify larval and pupal development stages. However, despite the potential of X-ray imaging to provide new insights into the cryptic and elusive subterranean life stage of ground-nesting bees, the technology remains largely unexplored in ground-nesting bee research. In particular, one of the greatest strengths of X-ray imaging – the ability to minimally invasively examine a ground-nesting bee nest over multiple time points – has remained unexploited; even X-ray imaging of a naturally occurring ground-nesting bee nest has not yet been performed to our knowledge.

The purpose of this study was to demonstrate the suitability (proof-of-concept) of repeated X-ray imaging of ground-nesting bee nests in their natural environment as a means to visualize and quantify bee nests and to study the temporal dimension in ground-nesting bee behavior and ecology and their consequences on soil structure and functions. In particular, we aimed to quantify the morphology of burrows created by different species of ground-nesting bees, and to monitor the temporal evolution of bee nest structures. We discuss the morphology and persistence of ground-nesting bee burrows and compare them with those of other soil-dwelling species, particularly earthworms.

2. Materials and methods

2.1. Study site description and soil column preparation

Five sites on the premises of the Swiss Federal Research Institute Agroscope in Zurich, Switzerland (47°25′43″N / 08°31′02″E) were selected based on the presence of ground-nesting bee nests and to cover a range of ground-nesting bee species, habitats, and soils (Table 1). Zurich has a temperate climate (Köppen: Cfb; Beck et al., 2018) with an average annual precipitation of 1022 mm and an average annual temperature of 9.8 °C (climate normal 1991–2020; MeteoSwiss, 2023). Freezing and thawing events can occur from October to April (Fig. 1). Soils at the sampling sites were classified as sandy loam, silt loam, and loam (USDA system; Soil Survey Staff, 2022). Soil organic carbon (SOC) content ranged from 1.37 to 2.45% (Table 1).

In early June 2021, eleven (hollow) PVC cylinders with an outer diameter of 20.0 cm and an inner diameter of 18.8 cm (wall thickness: 6 mm) and a height of 20 to 40 cm were carefully inserted into the ground at the selected sampling sites using a tractor front loader. Each cylinder was inserted into the soil so that the soil column contained at least one active bee nest, as evidenced by the presence of a characteristic tumulus (i.e., mount of excavated soil material) and visual observation of bee activity. The columns were then excavated and a strap system was installed to allow for rapid and minimally invasive removal and reinstallation of the columns for repeated X-ray imaging (Fig. 2). Air temperature at 2 m above the ground, soil temperature at 5 cm depth, and precipitation were recorded daily during the study period at a nearby MeteoSwiss weather station (47°25′39.6″N 08°31′04.4″E), located<300 m from all sampling sites (Fig. 1).

2.2. Sampling and identification of nesting bees

Ground-nesting bees were visually identified to species level where possible. Some species required capture for unambiguous identification, which we avoided during the study period to prevent disruption or cessation of their ongoing nesting activity. Instead, we captured nesting bees at the sampling locations at the end of the study period in March 2023 using mini-emergence traps, following the procedure detailed in Tschanz et al. (2023), and had them identified to species level by an expert bee taxonomist. Although this procedure does not allow to assign captured species with 100% certainty to the specific nest structures

Table 1
Description of soil column sampling locations. Nesting species are nesting females that were captured and/or visually identified at the sampling locations.

Site	Columns	Soil textural class	% Sand/ Silt/ Clay	% SOC	Nesting species	Habitat type
A	1–3	sandy loam	59.1/ 21.7/ 19.2	1.89	*Colletes cunicularius*	Extensively managed grassland
B	4–5	loam	49.5/ 30.7/ 19.8	2.32	*Colletes cunicularius*	Extensively managed grassland
C	6–8	silt loam	07.1/ 68.6/ 24.3	1.37	*Lasioglossum malachurum*	Bare soil SSO[a]
D	9–10	loam	42.4/ 37.2/ 20.5	2.45	*Lasioglossum malachurum*	Extensively managed grassland
E	11	loam	44.9/ 34.3/ 20.8	2.10	*Lasioglossum malachurum*	Extensively managed grassland

[a] See Keller et al. (2017) for a description of the bare soil plot from the soil structure observatory (SSO) experiment.

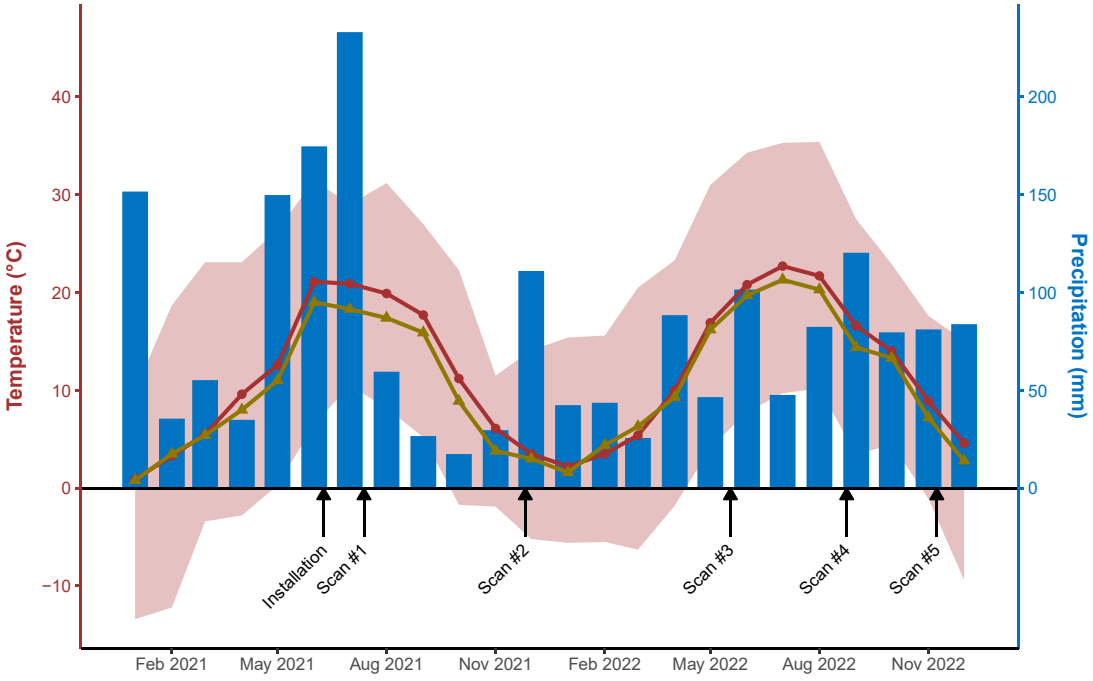

Fig. 1. Timeline of sampling and scanning with monthly weather data from a nearby weather station during the study period. The arrows indicate the dates when the cylinders were installed and the columns were taken to the X-ray device for imaging. The red line with circles indicates the mean air temperature at 2 m height, the brown line with triangles the mean soil temperature at 5 cm depth, and the blue bars the total precipitation. The red area indicates the minimum and maximum temperatures. Source: MeteoSwiss.

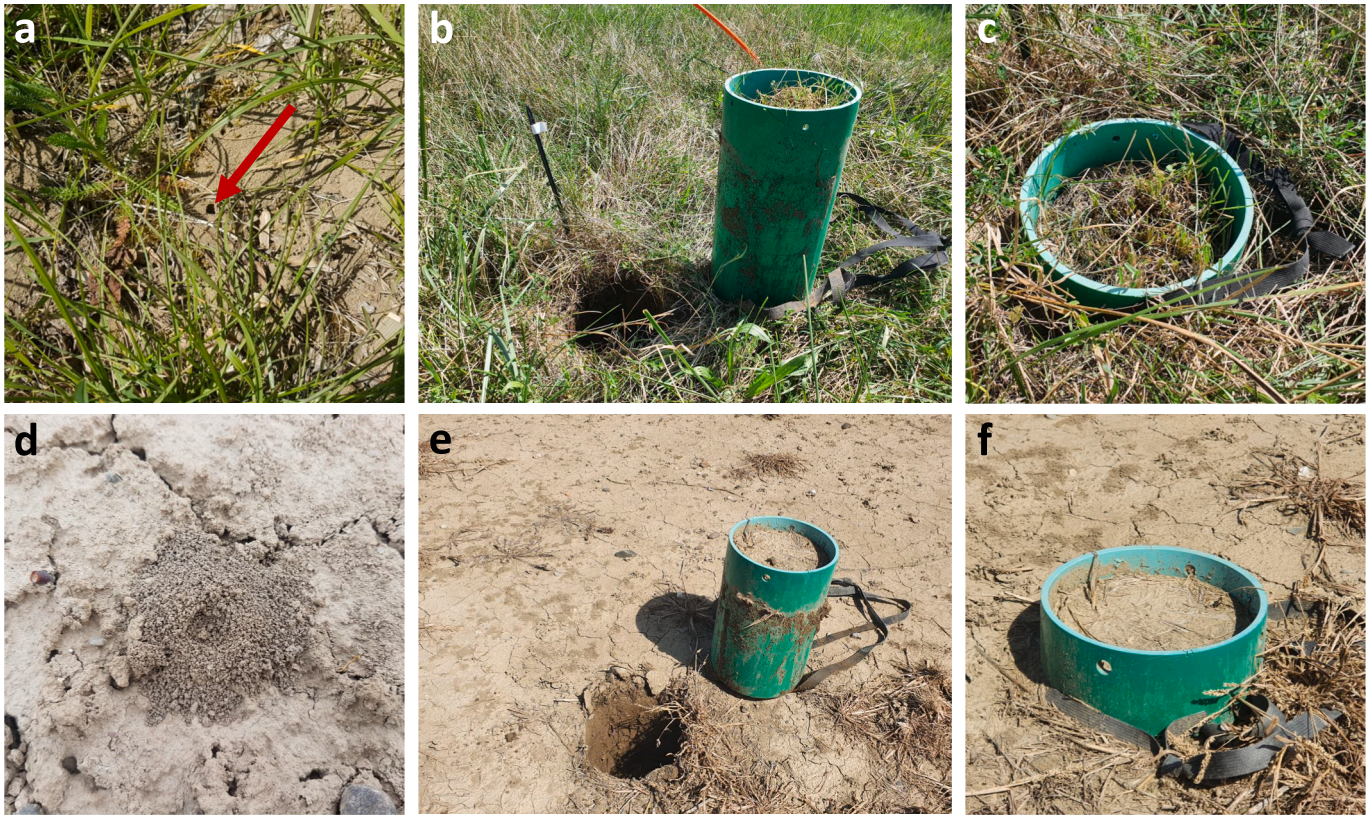

Fig. 2. Examples of two soil columns (b-c: column #1; e-f: column #7) used for repeated X-ray imaging. PVC tubes of 20 cm diameter and between 20 and 40 cm height were inserted using a tractor front loader at locations where at least one ground-nesting bee nest was found. (a, d) show examples of ground-nesting bee nests with visible nest entrances, indicated by the arrow in (a) and a typical tumulus (i.e., mount of excavated soil material) visible in (d) before the soil column was inserted. The black straps seen in the photos were used to pull out the soil columns for repeated scanning.

observed in the soil columns, it provides a good indication of the most likely nesting species.

Two nesting species of ground-nesting bees were captured: the spring mining bee *Colletes cunicularius* (Apoidea: Colletidae) and the sweat bee *Lasioglossum malachurum* (Apoidea: Halictidae; Table 1). *Colletes cunicularius* is a univoltine solitary (i.e., each female bee constructs her own nest and provisions her own offspring) species. Females are 13–14 mm long and are active from mid-March to mid-May (SwissBeeTeam, 2021). *Lasioglossum malachurum* is a multivoltine eusocial species. In eusocial species, adults of more than one generation co-exist, and cooperation among them in brood care and division of labor exists, with some queens reproducing and other female bees not reproducing but providing offspring with food resources (worker caste; Michener, 2007). Individuals are 8–9 mm long, and their activity period extends across several generations from early March to late October (SwissBeeTeam, 2021).

2.3. Soil column scanning and image acquisition

Soil columns were scanned every three to five months over a period of 16 months (scanning dates: 13 July 2021, 26 November 2021, 18 May 2022, 24 August 2022, 08 November 2022) (Fig. 1). Soil columns were only scanned at least three days after significant rainfall to ensure good drainage of the bee burrows. Due to hospital operational reasons, scanning was only possible in the late afternoon (around 5 pm) when bees may still be active. Columns were temporarily removed from the soil a few hours before scanning and immediately reinstalled in the same location after scanning.

Computed tomography images were acquired using a first-generation dual-source photon-counting X-ray CT scanner with quantum imaging (NAEOTOM Alpha, Siemens Healthineers) at the Zurich University Hospital in Switzerland. The scan parameters were based on those for the human lung with a fixed tube voltage of 120 kV and automatic tube current modulation. Multiple soil columns were scanned simultaneously and the smallest possible field of view was selected that covered all the columns on the bench. Images were reconstructed using a standard soft tissue convolution kernel (Br36) and Quantum iterative reconstruction at level 3 (Q3), with a slice thickness of 0.4 mm, an increment of 0.2 mm and a resolution of 512 × 512 pixels. The acquired images were a series of axial slices in the DICOM file format with 16-bit grayscale values calibrated to Hounsfields Units (HU).

2.4. Image processing

Image processing and analysis were conducted in Python version 3.8.15 (Python Software Foundation, 2022) using the libraries *PyImageJ* (Rueden et al., 2022) and *scikit-image* (Van der Walt et al., 2014). Manual image processing steps were conducted in Fiji/ImageJ (Schindelin et al., 2012). X-ray images were visualized using the Python library *Matplotlib* (Hunter, 2007) and the software Drishti (Limaye, 2012). Morphological and meteorological data were visualized using R version 4.2.2 (R Core Team, 2022). The detailed image processing workflow is visualized in Supplementary Fig. S1.

DICOM images were converted to 16-bit grayscale TIFF stacks and split by soil column. Soil columns were reoriented to ensure they were in a straight upright position and moved to the center of the canvas. All scans from the same cylinder were rotated in the horizontal plane so that they were aligned identically based on visual inspection of engravings in the PVC walls. Images were cropped along the vertical axis based on visual inspection of landmarks to ensure that all scans from the same cylinder contained an identical region of interest. Images were resampled to isotropic voxel sizes (0.3 × 0.3 × 0.3 mm^3). To eliminate edge effects caused by shrinkage and swelling of the soil from the column wall, a cylindrical region of interest with a diameter of 172 mm (8 mm smaller radius than the inner cylinder radius) was selected.

A joint histogram of the gray values from all 3D images was computed and smoothed to identify the histogram minimum between gray-values corresponding to air-filled pores and the soil matrix (Supplementary Fig. S2). This value (-760 HU) was then applied to all images for segmentation. As a result, we obtained binary images representing the air-filled pore space and the soil matrix. This approach was chosen because the focus was to study the effects of ground-nesting bees on the architecture of the soil pore network, i.e., the air-filled space created by ground-nesting bees. A 3D median filter (2 × 2 × 2 voxels) was applied to remove segmentation noise, and holes were filled using the ImageJ plugin *MorphoLibJ* (Legland et al., 2016). Connected pore-volumes smaller than 4630 voxels (0.125 cm^3) were considered noise.

We manually selected fourteen individual bee burrow networks (i.e., nests) from eight soil columns to quantify their morphology and evolution over time. Only nests that met three criteria were included: (*i*) they were unambiguously identifiable as bee nests, (*ii*) they were present in the first scan, and (*iii*) they were relatively intact and connected rather than fragmented into many small pieces. We identified individual nests in the first scan by first identifying all disjoint pore structures using the 26-connectivity connected component labeling function implemented in *MorphoLibJ* (Legland et al., 2016) and assigned a unique nest label to all components belonging to that nest. Then, all components belonging to the same nest were identified by visual inspection in all subsequent scans and reassigned to the corresponding nest label. Finally, each individual nest per image was extracted into a new TIFF stack for morphological analysis. Furthermore, each image was converted into a local thickness map using the largest inscribed sphere method (ImageJ *LocalThickness*) and a 3D skeleton was created (ImageJ *Skeletonize3D*) for morphological analysis. The 3D skeleton was pruned, i.e., all endpoint branches <10 mm were removed as they were considered artifacts created by the skeletonization procedure.

2.5. Morphological analysis

Soil columns and bee burrows for each scan date were morphologically characterized (see Supplementary Fig. S1 for an illustration of the workflow). Burrow volumes were computed using *MorphoLibJ*. Mean burrow diameter and macropore size distributions were computed based on the local thickness map in Python. The number of loops (1^{st} Betti number) was computed on basis of the binary images using the ImageJ plugin *BoneJ* (Doube et al., 2010). The burrow length, the number of branches, and the longest of the shortest path were computed on basis of the pruned skeletonized images using the ImageJ plugin *AnalyzeSkeleton* (Arganda-Carreras et al., 2010). Tortuosity was computed as the ratio of the total burrow length to the longest shortest path as a proxy for burrow complexity. Tortuosity values based on very short skeletons (<20 mm branch length) were strongly influenced by the skeletonization and pruning process and were not considered. Burrow persistence was visually assessed from the time series of rendered images of bee nest structures from the time of nest completion.

3. Results

Our method of sampling and X-ray imaging of large soil columns containing nests of ground-nesting bees successfully revealed the burrow systems made by different ground-nesting bee species in their natural environment (Fig. 3), and the evolution of the burrow systems over time (Figs. 4-6). Bee burrowing was the major contributor to the total imaged porosity in most soil columns, as reflected by peaks in pore size corresponding to the burrow diameter of the nesting species (Fig. 7).

3.1. Diversity of bee burrow architecture

The studied solitary and social ground-nesting bee species created burrow systems that differed fundamentally in their architecture. The burrow system made by the solitary *C. cunicularius* was simple, consisting of a single, predominantly vertical, linear, unbranched, large-

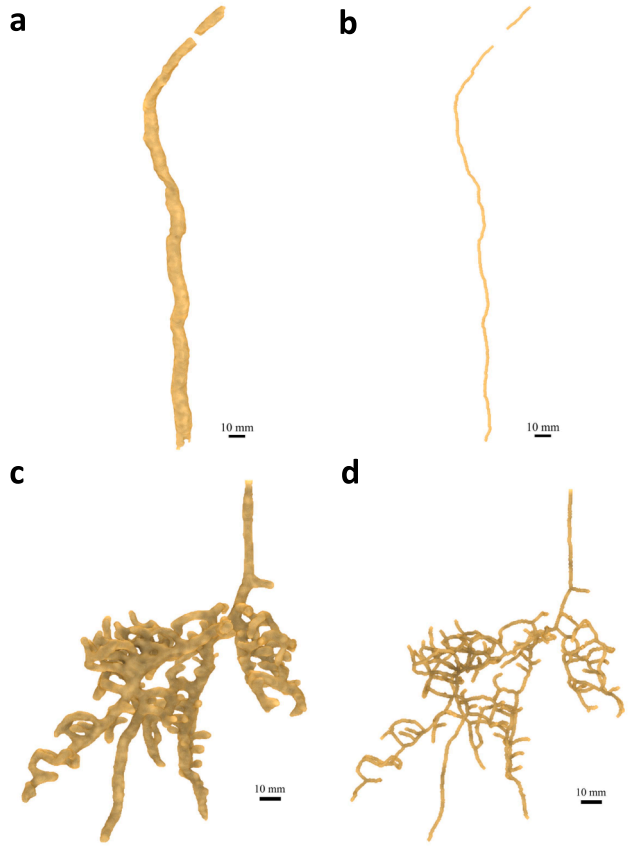

Fig. 3. Two representative examples illustrating the highly distinct architecture of burrows created by different species of ground-nesting bees. (a-b) An example of a relatively straight (low tortuosity), unbranched and predominantly vertically oriented burrow of large diameter created by the solitary ground-nesting bee species *Colletes cunicularius*. (c-d) An example of a complex, highly branched and curved (high tortuosity) burrow system of medium diameter created by the social bee species *Lasioglossum malachurum*. The same burrow systems are illustrated as: (a, c) volume; and (b, d) skeletonized volume (with a dilation step to improve visibility). Note the slightly different scales between (a-b) and (c-d).

diameter (~6 mm) main burrow (Fig. 3a-b). In contrast, the burrow system made by the social *L. malachurum* was a complex system of highly branched and tortuous burrows of much smaller diameter (~3 mm), running both horizontally and vertically, and containing numerous loops (Fig. 3c-d).

3.2. Temporal evolution of bee burrow systems

The temporal evolution of the bee burrow systems revealed two contrasting patterns associated with the nesting species. Burrow systems made by the social and multivoltine *L. malachurum* (columns #6–11 in Fig. 4 and Supplementary Fig. S3) initially increased in size (volume and length; Fig. 5a-b) and complexity (tortuosity, number of branches and loops; Fig. 5d-f) after they were created, and then decreased in size and complexity. Burrows made by the solitary and univoltine *C. cunicularius* (columns #1–5 in Fig. 4 and Supplementary Fig. S3) did not show any substantial increases in burrow size or complexity, but rather remained stable or decreased over time (Fig. 5). This different temporal pattern is illustrated in more detail for a selection of bee burrow systems of *C. cunicularius* and *L. malachurum* in Fig. 6.

3.3. Persistence of bee burrows

The persistence of biopores created by ground-nesting bees was highly variable between individual burrow systems (Figs. 4-5). In some soil columns, bee burrows were already no longer visible in the first scan (e.g., column #6 in Supplementary Fig. S3) or disappeared between consecutive scans (e.g., column #7 in Fig. 4), whereas some burrow structures remained largely intact over the entire 16-month study period (e.g., column #1 in Fig. 4; Fig. 6a). The differences in burrow persistence are also confirmed by the quantitative analysis of burrow morphology over time (Fig. 5), which show that some bee burrows remained stable in size (volume, mean diameter, length), whereas others decreased sharply or even disappeared. Differences in burrow persistence were not related to the nesting species (Fig. 5).

4. Discussion

4.1. Repeated X-ray imaging to study ground-nesting bee burrows and their temporal evolution

Our study demonstrates that X-ray imaging of large soil columns containing nests of ground-nesting wild bees is a suitable method to study nest architecture in their natural habitat. Traditionally, such studies have relied on methods that involve excavation and/or casting of nest structures (e.g., Linsley et al., 1952; Marinho et al., 2018; Michener et al., 1955). However, excavation and casting techniques are destructive, and tracking tunnels amid falling debris can be challenging (e.g., Linsley et al., 1952; Marinho et al., 2018; Michener et al., 1955). Moreover, these methods may only provide an incomplete picture of the nest architecture, especially in cases involving complex burrow networks or partially collapsed and discontinuous burrows. In such cases, X-ray imaging may be a more accurate and perhaps the only feasible method to capture the nest architecture.

Our study demonstrates the feasibility of repeatedly removing, scanning, and reinstalling the same soil columns containing active nests to quantify the temporal evolution of ground-nesting bee burrow systems. The fact that bees continued to develop their burrow system after the first X-ray scan (e.g., columns #10 and #11 in Fig. 4; Fig. 6b-c) indicates that our methodological approach did not majorly disrupt their nesting behavior and did not cause them to abandon their nest. The potential of repeated X-ray imaging as a minimally invasive method to study temporal aspects of the life of wild bee activity in the opaque medium of soil has been suggested nearly two decades ago (Greco et al., 2006), but we are not aware of any such previous studies.

Although X-ray imaging is considered to be largely non-destructive, it may affect the bees or their burrow structure. The procedure including installation of the cylinders, removal, transport and reinsertion of the soil columns for scanning, could result in the collapse of burrow structures. It is possible that the size of our soil columns has constrained nest development or may not have been large enough to capture the entire nest. For example, some of the vertical burrow structures in column #1 (Fig. 4) continued below 40 cm depth, which was the height of the cylinder. Therefore, a major drawback of X-ray imaging is the limited maximum sample size allowed by the scanner. However, since the burrow structure exceeding the cylinder height was created by a univoltine species and the nesting process was already complete at the time of scanning, it is unlikely to have affected the temporal evolution of this burrow structure. The sampling and scanning process may also cause disturbance to nesting bees, which could lead to nest abandonment. However, even though we scanned multivoltine species during their active period (columns #6–11), they continued to develop their nests (e.g., Fig. 6b-c). Finally, there is a possibility that radiation may cause biological harm to bees, but this seems unlikely in our study, since the radiation dose to bees from such scans has been shown to be much lower than the dose required to cause biological damage in *Drosophila melanogaster* (Greco et al., 2006, 2005; Kanao et al., 2003).

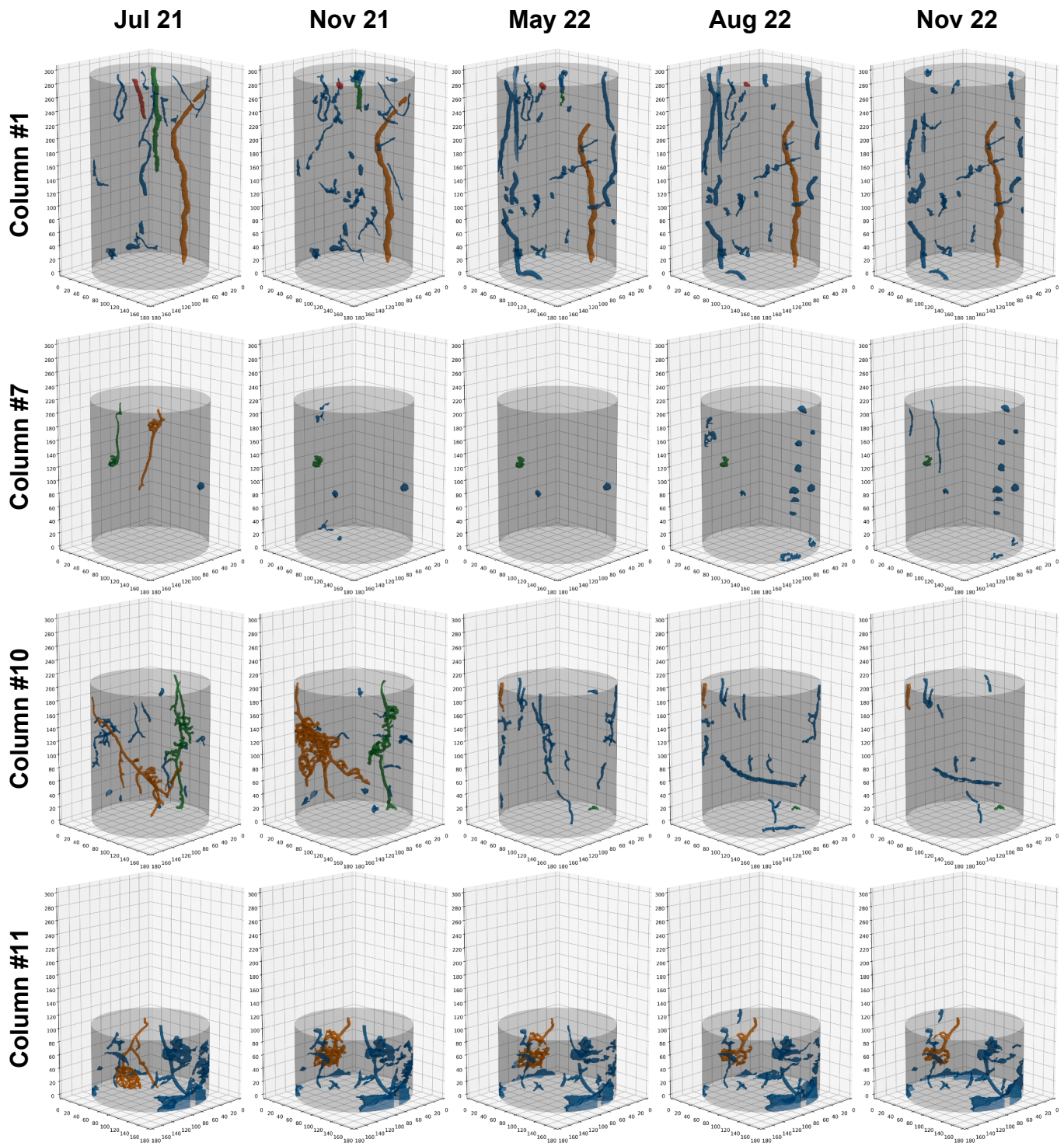

Fig. 4. Illustrative examples of the temporal evolution of macropores over the course of 16 months for selected soil columns. Macropores created by ground-nesting bees used for the quantitative analysis (based on three inclusion criteria; see Materials and Methods section) are shown in the colors red, green, and orange. Macropores not included in the analyses are shown in blue. Axis units are in mm, grid lines are 20 mm apart in all axis directions. Gray cylinders correspond to the selected cylindrical region of interest. See Supplementary Fig. S3 for an illustration of additional columns not shown here.

4.2. Morphology of ground-nesting bee burrow structures

Through their nesting activity, ground-nesting bees altered the physical structure of our soil columns in a variety of ways. They created pores of different diameter (from ~ 3 to 7 mm), orientation (from vertical to horizontal), and complexity (from linear and unbranched to highly branched and looped) (Figs. 3-6). With their extensive burrowing activity, they were the largest contributors to the total imaged porosity in many of our columns (Fig. 7).

Ground-nesting bees exhibit a remarkable diversity in nest architecture that varies among species. Typically, nests of solitary species, which comprise the majority of bee species (greater than 77%), consist of a main vertical burrow with lateral branches leading to brood cells (Antoine and Forrest, 2021; Danforth et al., 2019; Ullmann et al., 2020).

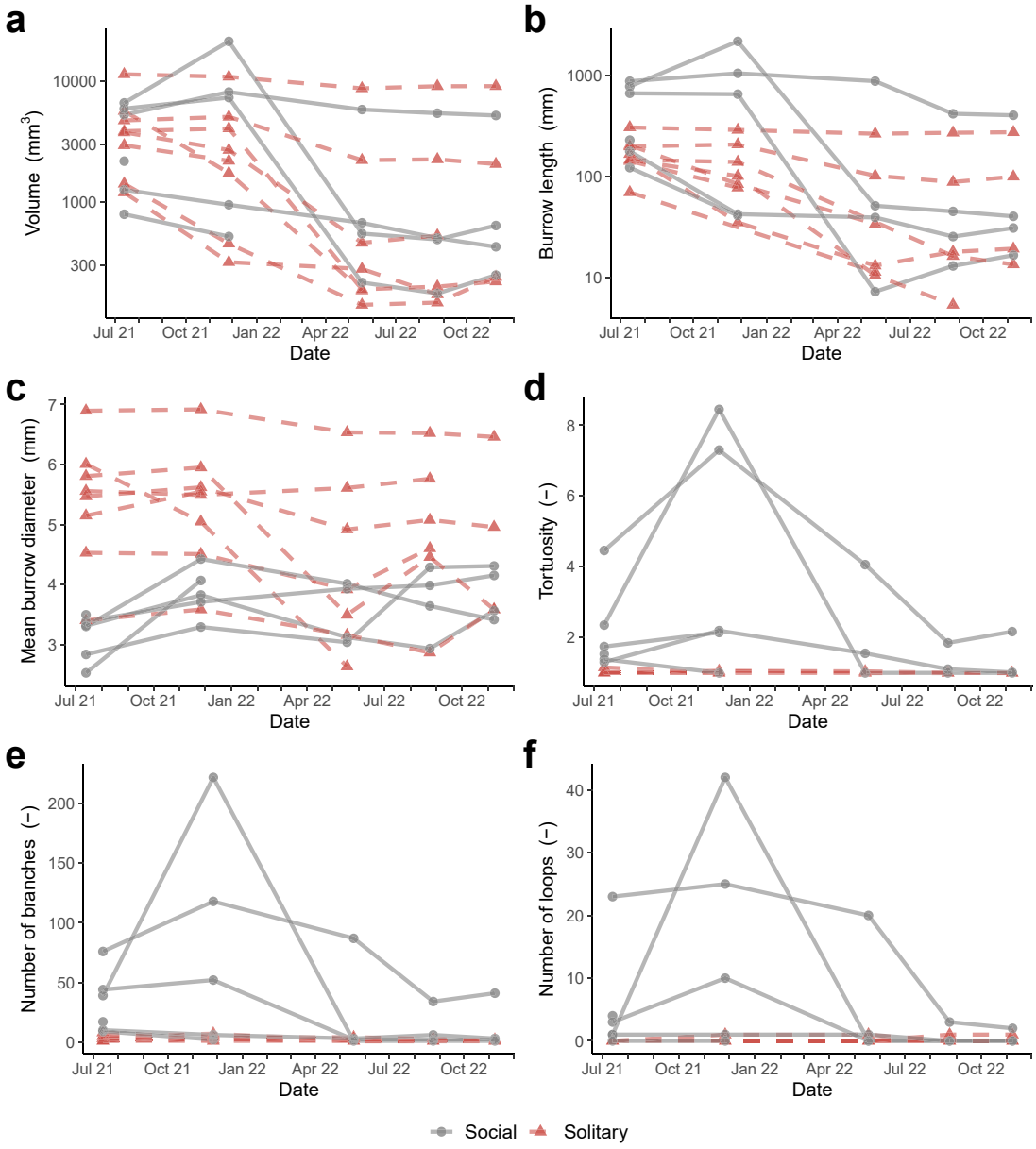

Fig. 5. Temporal evolution of morphological features of ground-nesting bee burrows created by solitary (red dashed lines with triangles) and social (gray solid lines with circles) ground-nesting bee species. Each line corresponds to an independent bee burrow system highlighted in color in Fig. 4.

However, nest complexity ranges from simple linear unbranched burrows directly terminating in a single brood cell to complex chamber-like structures with multiple brood cells clustered together (e.g., Antoine and Forrest, 2021; Sakagami and Michener, 1962; Stephen et al., 1969; Westrich, 2018). The nests of the solitary spring mining bee *Colletes cunicularius*, which produced the burrow structures visible in soil columns #1–5 (Fig. 3a-b, Fig. 4, Supplementary Fig. S3), are examples of a relatively simple nest architecture consisting of a main vertical burrow with several lateral branches each terminating in a single brood cell. Because *C. cunicularius* bees refill lateral branches with soil material, but not the main burrow (Malyshev, 1927), only the vertical main burrows were visible in the X-ray images (Fig. 4, Supplementary Fig. S3). The nest structures visible in columns #6–11 by social *Lasioglossum malachurum* species are examples of more complex chamber-like nest structures (Fig. 3c-d, Fig. 4, Supplementary Fig. S3) consisting of an intricate network of horizontal and vertical burrows, and with brood cells clustered in chambers (Sakagami and Michener, 1962).

Several attempts have been made to classify the diversity in nest architecture according to various criteria, such as branching patterns, and the number, orientation, and position of brood cells within the nest (e.g., Malyshev, 1936; Stephen et al., 1969). Ultimately, however, classification of nest architecture into discrete groups cannot adequately describe the nuances in continuous morphological quantities, and the subdivision of a continuum falls short for species with traits that lie between categorical boundaries. In particular, how such morphological quantities evolve over time, and how they may be affected by soil properties and conditions and by climate, cannot be adequately characterized by a discrete classification system. These inherent limitations of a classification system can be overcome by using a continuous measure as a proxy for the morphological characteristics of interest. For example, some of the quantitative characterization of bee nests performed in this study (i.e., tortuosity, number of branches and loops) could be used as proxies capturing different aspects of nest complexity instead of a classification system. This would allow us to detect even subtle changes in nest architecture over time and to compare variation in nest architecture between and within species.

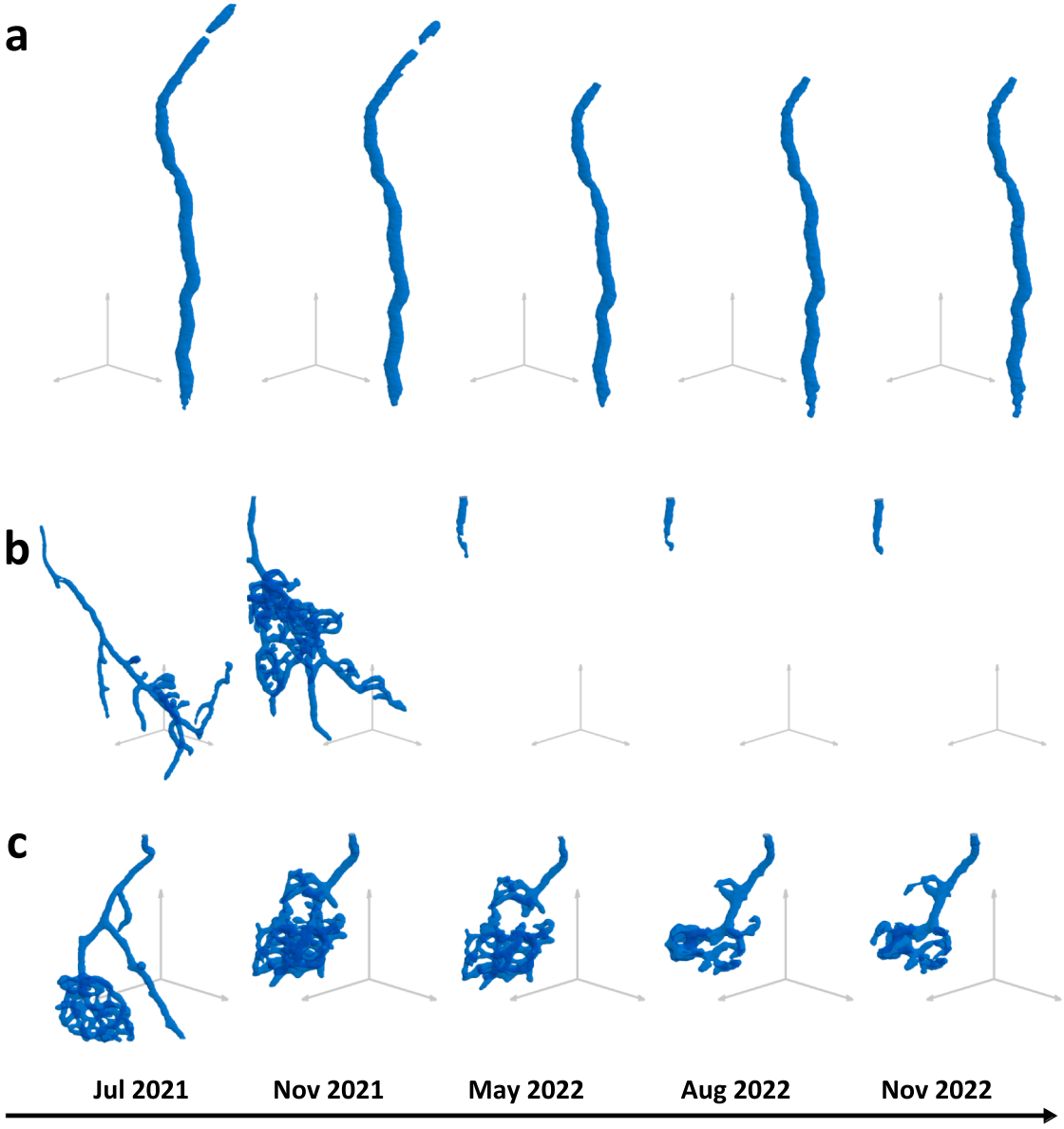

Fig. 6. Three examples illustrating the temporal evolution of burrow systems created by (a) ground-nesting solitary *Colletes cunicularius*, and (b-c) social *Lasioglossum malachurum* bees. These burrow systems correspond to the pores highlighted in orange in Fig. 4, which were isolated from soil columns #1 (a), #10 (b), and #11 (c), respectively. The axis arrows have a length of 50 mm each.

While more complex morphological measures beyond diameter and depth, such as volume, burrow length, and tortuosity, are well established in soil science to characterize the network architecture of pores created by abiotic processes, earthworms, and other macrofauna (e.g., Cheik et al., 2019; Koestel and Schlüter, 2019; Mele et al., 2021), we are not aware of such descriptions for ground-nesting bee burrows. This may be due to the scarcity of X-ray images of ground-nesting bee nests, as these metrics can be difficult, but also subjective, to compute without three-dimensional image processing techniques. The application of such standardized, continuous metrics to characterize different aspects of nest architecture opens up a number of interesting possibilities for advancing our still very limited understanding of various aspects of the nesting ecology of ground-nesting bees, with relevant implications for the conservation and management of these important pollinators and soil engineers. For example, it would allow to investigate whether and how bees adapt their nest architecture to specific local conditions, such as soil properties (e.g., density and texture) or soil and climatic conditions (e.g., moisture and temperature), to maximize their reproductive success. Such adaptations in nest architecture to local conditions may have implications for the functional role of the burrows (e.g., aeration and drainage), and how they resist disturbances, such as from wildfires or agricultural management practices.

The typical burrows of solitary ground-nesting bees, such as those in columns #1–5 (Fig. 3a-b, Fig. 4, Supplementary Fig. S3), are morphologically similar to those of anecic earthworm species. Typically, anecic (sensu Bouché, 1977) species cast above ground and create large continuous vertical burrows that are connected to the soil surface, whereas endogeic species typically create a network of branching and tortuous burrows that are often discontinuous (due to refilling by casts) and less connected to the soil surface (Capowiez et al., 2015, 2014, 2001; Edwards and Arancon, 2022; Jégou et al., 1999; Lee and Foster, 1991). Earthworm burrows range in diameter from 1 to 12 mm (Edwards and Arancon, 2022), with wider pores created by anecic species due to their larger body size (Capowiez et al., 2015; Pérès et al., 1998). While endogeic species typically burrow within the top 10–15 cm of soil, some anecic species burrow to depths of two meters and even

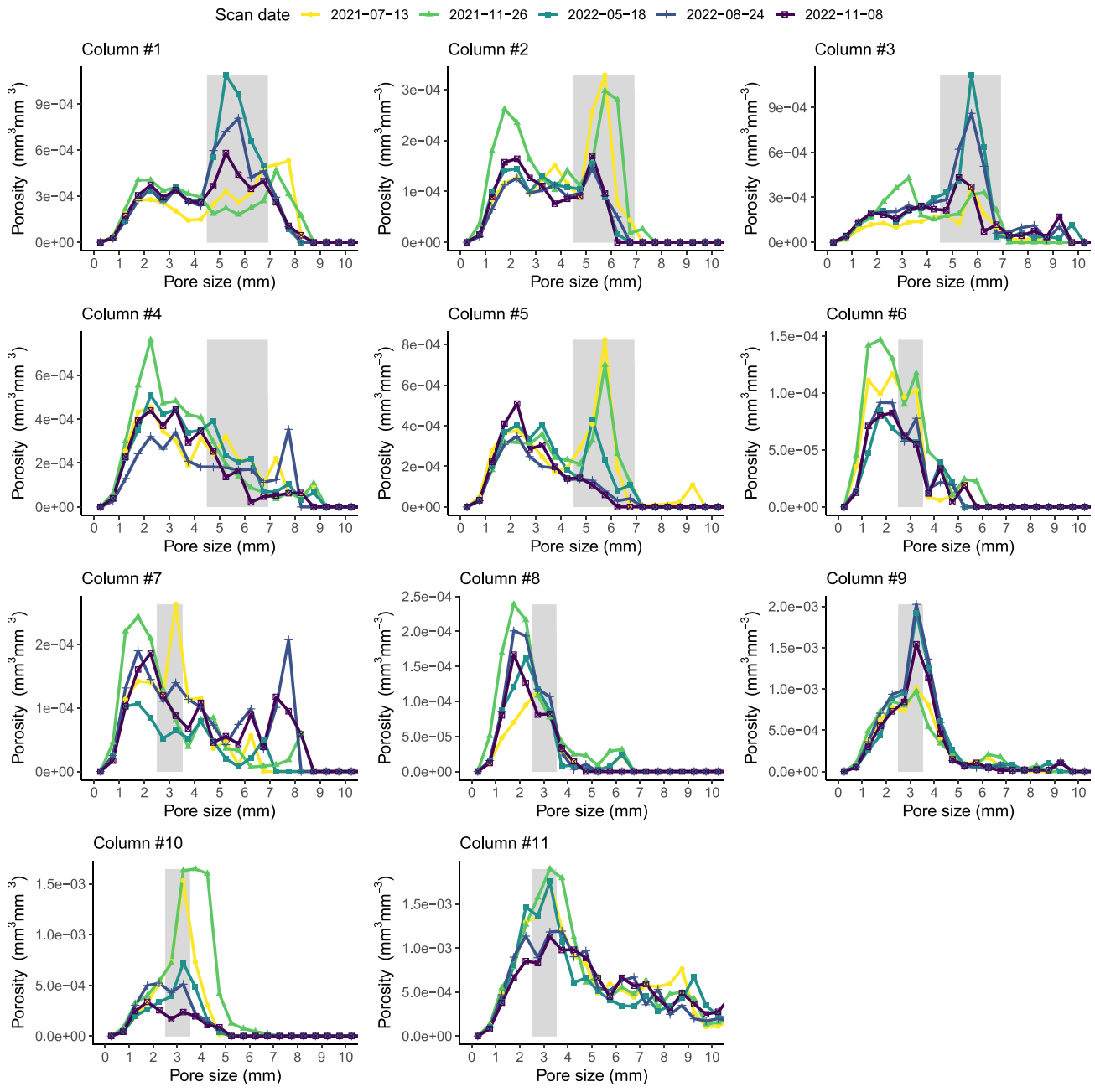

Fig. 7. Macropore size distribution per scan date and soil column. In contrast to Fig. 5, macropores not labeled as bee nests are also included. Porosity values are expressed as the volume of the macropore of the respective size class, relative to the volume of the region of interest. The gray areas indicate the range (min–max) of burrow diameters of the species captured at the respective column location (columns #1–5: *Colletes cunicularius*; columns #6–11: *Lasioglossum malachurum*). Ranges were obtained from the mean bee burrow diameter of the first scan of all extracted bee burrows per species.

more (up to 3 m; Bahl, 1950) (Edwards and Arancon, 2022). In comparison, ground-nesting bees create pores ranging from 2 to 10 mm in diameter (Rozen, 2016; Sarzetti et al., 2013; Westrich, 2018). The maximum median nesting depth for most bee species has been estimated at 20 cm (Cane and Neff, 2011) to 23 cm (Harmon-Threatt, 2020). However, several species have been reported to dig burrows several meters deep, even up to 530 cm (Cane and Neff, 2011; Danforth et al., 2019). In our study, bee burrow diameters were in the range of approximately 3–7 mm (Fig. 5), and some burrows extended below 40 cm (i.e., below the maximum cylinder height; column #1 in Fig. 4).

The similarity between burrow systems (burrow diameter, network architecture) created by anecic earthworms and certain ground-nesting bee species, and the fact that both anecic earthworms and ground-nesting bees transport soil to the surface (anecic earthworms: by soil ingestion and casting on the surface; bees: by carrying out material) suggests that these ground-nesting bees have similar effects on soil processes and functions as anecic earthworms, such as enhanced water infiltration and improved soil aeration (Edwards and Arancon, 2022; Le Mer et al., 2021; Lee and Foster, 1991).

4.3. *Temporal evolution of ground-nesting bee burrow morphology*

Burrows made by the social and multivoltine species *L. malachurum* increased in size and complexity after construction, whereas those made by the solitary and univoltine species *C. cunicularius* showed no substantial increase in burrow size or complexity after construction (Figs. 5-6). Burrow persistence was highly variable. Although nesting activity was visually confirmed in all soil columns during installation, some

columns (e.g., column #6 in Supplementary Fig. S3) had no visible bee burrow structures in the first scan, indicating that these burrows had already disappeared before the first scan. Other burrows disappeared within a few months (e.g., column #7 in Fig. 4), and some remained largely intact throughout the 16-month study period (e.g., column #1 in Fig. 4; Fig. 6a).

Species-specific differences in social behavior and voltinism, along with resulting variations in temporal activity patterns, are key considerations for comprehending the observed temporal evolution patterns in morphology of bee burrows. While solitary bees, such as *C. cunicularius*, typically construct their nest within a few days and do not maintain it thereafter, social bees, such as *L. malachurum*, engage in nest-building over several generations, lasting several months (Danforth et al., 2019; Malyshev, 1927; Sakagami and Michener, 1962; SwissBeeTeam, 2021; Westrich, 2018). For example, in *L. malachurum*, the queen initiates nest construction during the solitary phase in spring, followed by a social phase characterized by a division of labor between worker bees and the queen over several generations until autumn. Therefore, the nests are progressively expanded with new branches and brood cells organized in chambers during the bees' activity period (Sakagami and Michener, 1962; SwissBeeTeam, 2021; Westrich, 2018). These differences in nest construction between univoltine (e.g., *C. cunicularius*) and multivoltine (e.g., *L. malachurum*) bees are reflected in our X-ray images and the temporal evolution of morphological traits. Nests of the social and multivoltine species increased in complexity (reflected by the increase in tortuosity and number of branches and loops) and size (length and volume) after their initial appearance in spring until the end of their life cycle in fall, whereas those of the solitary and univoltine species started to disintegrate shortly after they were created in spring (Figs. 4-6; Supplementary Fig. S3).

In this study, ground-nesting bees did not reuse existing burrow structures from the previous year's nesting season (the study period included two spring nesting seasons), even when these structures were still present. Instead, they expended energy and time into the construction of new burrows as evidenced by column #1 (Fig. 4). This behavior seems perplexing given the time and energy required to identify ideal nesting locations and create burrows, and the wear and tear it causes on mandibles and wings (Antoine and Forrest, 2021; Leonard and Harmon-Threatt, 2019; Roulston and Goodell, 2011). However, reusing old burrows could lead to higher risk of pathogen and parasite infestation that can have fatal consequences for ground-nesting bees (Antoine and Forrest, 2021; Harmon-Threatt, 2020; Roulston and Goodell, 2011), and creating new burrow structures could be a way to minimize that risk (Harmon-Threatt, 2020), although reuse of burrows has been documented in other bee as well as digger wasp species (Polidori et al., 2006; Sakagami et al., 1984). The lack of burrow reuse behavior in our study may also be related to the difficulty for bees to relocate existing structures; even when burrows remained largely intact below the surface, entrance holes were no longer visible the following year's nesting season (pers. obs.).

4.4. Drivers of bee burrow persistence

While the temporal evolution of burrow morphology can be largely explained by the social behavior and voltinism, these factors do not appear to account for the observed variation in burrow persistence. Some of the burrows created by the solitary univoltine species disappeared within a few months of construction, while others remained largely intact throughout the study period of 16 months. These different trends were even observed within the same soil column (e.g., column #1, Fig. 4). Similarly, some burrow networks created by social species disappeared within a few months, while others remained largely intact over the study period (cf. columns #10–11 in Fig. 4; Fig. 6b-c). These findings demonstrate that even presumably abandoned and unmaintained burrow structures, as in solitary univoltine species, can persist for at least 16 months (and possibly longer), and that burrow persistence can vary widely even within the same species. Although unravelling underlying drivers of burrow persistence is beyond the scope of this study, it is likely that the burrow persistence is influenced by aspects of bee behavior, such as burrow architecture (e.g., depth, shape, orientation, diameter) and digging behavior (e.g., digging mechanisms, wall treatment, refilling behavior), as well as edaphic factors (e.g., texture and density), climate (e.g., rainfall frequency and intensity), and mechanical disturbance (e.g., by machinery or animals).

To our knowledge, the persistence of ground-nesting bee burrows has not yet been quantified, but there is limited knowledge on the persistence and stability of biopores created by other soil fauna. Biopores have been found to be more stable than pores or cracks created through abiotic processes. For example, earthworm burrows were more resistant to internal erosion than artificially created macropores in an infiltration experiment (Pelíšek, 2018), and galleries created by ants and termites were more stable than desiccation cracks when experimentally exposed to wetting–drying cycles (Cheik et al., 2021). Biopores can persist for years. For example, burrows created by the anecic earthworm *Amynthas khami* were reported to persist for 'at least several months' in clayey soil (Le Mer et al., 2021), and burrows created by the anecic earthworm *Lumbricus terrestris* persisted for more than seven years in sandy soils in a rhizotron facility (Potvin and Lilleskov, 2017). Since the improved stability of biopores created by earthworms and plants are associated with wall lining and compaction (see below), it is reasonable to assume that pores created by ground-nesting bees are also more stable than those created through abiotic processes, but this remains to be tested.

The stability of biopores is affected by organic substances secreted by different organisms, such as plant root mucilage (Czarnes et al., 2000) and earthworm mucus (Gray and Lissmann, 1938), which increase interparticle cohesion and water repellency in comparison to the surrounding soil (Czarnes et al., 2000; Leue et al., 2015). Most ground-nesting bee species also apply some type of lining, typically a hydrophobic film of glandular secretions (Cane, 1981; Lybrand et al., 2020) or plant-based materials (including leaves, flower petals, leaf trichomes, floral oils, and resins) to the walls of their brood cells, and sometimes even to their burrows (Danforth et al., 2019; Westrich, 2018). We expect that the hydrophobic nature of these substances leads to enhanced stability of bee biopores.

In addition to stabilization by secreted substances, wall compaction resulting from burrowing can also improve biopore stability (Cheik et al., 2021; Ruiz et al., 2017). Earthworms burrow through soil either by ingesting it or by pushing soil aside (Edwards and Arancon, 2022), which can result in increased soil bulk density in the vicinity of their burrows (Capowiez et al., 2021, 2011; Milleret et al., 2009; Rogasik et al., 2014). In contrast, the burrowing behavior of ground-nesting bees is not a cavity expansion mechanism, as they excavate nests using their mandibles and/or forelegs and generally push out loose soil using their legs and body parts (head, thorax, abdomen) (e.g., Batra, 1968; Martins and Antonini, 1994). As a result, the degree of compaction of biopore walls created by ground-nesting bees is likely lower compared to those created by earthworms. However, some ground-nesting bee species have been reported to tamp excavated soil into the walls of their burrows instead of disposing it at the surface (e.g., *Nomia melanderi*; Batra, 1970). This behavior could potentially lead to a significant increase in soil bulk density around the burrows, and therefore in biopore stability. Effects of wall compaction and lining behavior on biopore stability could be investigated in future studies.

Furthermore, the persistence of bee burrows is influenced by the species' specific behavior regarding burrow refilling. Some bee species have been documented to refill lateral branches (e.g., *Dasypoda visnaga*; Abdouni et al., 2021) and even main burrows (e.g., *Dasypoda hirtipes*; Westrich, 2018) with soil material after oviposition and provisioning of brood cells with pollen and nectar stores for developing offspring. Similarly, burrow refilling also occurs in earthworms. In general, anecic species produce more continuous burrows than endogeic species, as they

intensively reuse them and frequently cast on the soil surface, whereas endogeic species rarely reuse burrows and more frequently cast inside them (Capowiez et al., 2014; Edwards and Arancon, 2022; Jégou et al., 1997; Lee, 1985). The lack of lateral branches in the soil columns where *C. cunicularius* nested (columns #1–5, Fig. 4) could be explained by their documented refilling behavior. They start constructing brood cells at the bottom, and when digging out material for brood cells higher up, they use the excavated soil material to refill the previously dug lateral branches below (Malyshev, 1927).

Soil biotic factors can also influence biopore stability. Soil organic carbon is generally positively related to soil stability (Chaney and Swift, 1984; Chenu et al., 2000; Kay, 1997; Tisdall and Oades, 1982), and plant roots and fungal hyphae increase soil stability by enmeshing soil particles (Chaudhary et al., 2009; Tisdall and Oades, 1982). Thus, the short persistence of bee burrows in all columns (columns #6–8; Fig. 4, Supplementary Fig. S3) sampled in the bare soil plot (the plot has been kept vegetation-free since 2014; see Keller et al., 2017 and Fig. 2e-f) may be related to the lower organic carbon content compared to all other sampling sites within meadows (Table 1).

5. Conclusion

By providing a non-invasive glimpse into the elusive underground life of bees nesting in the opaque medium soil, X-ray imaging represents a valuable but hitherto largely neglected tool in ground-nesting bee research. As demonstrated in our study, it allows to investigate temporal aspects of the underground life of ground-nesting bees. Repeated X-ray imaging has the potential to provide new insights into their behavior and ecology that cannot be gained by traditional means, with implications relevant for the conservation and management of these crucial pollinators. Future studies could harness this potential, for example, by conducting pre- and post-disturbance scans or continuous nest monitoring to investigate the impact of disturbances (e.g., vehicular traffic, agricultural management practices) on bee nests, or to identify the effects of edaphic and environmental factors (e.g., soil texture, soil density, soil moisture) on bee behavior, ecology, and reproductive success.

We also showed that the nesting activity of ground-nesting bees significantly alters soil pore network architecture by creating a species-specific burrow system that varies in size and complexity. Our study demonstrates that bee burrows can persist for 16 months and possibly longer. Given the similarity of the biopores created by some ground-nesting bee species to those created by anecic earthworms, these bees may have similar effects on soil functions and associated ecosystem services as anecic earthworms, especially where they form locally dense nesting aggregations. Due to their specific biology and ecology, ground-nesting bees may contribute to bioturbation in a spatially and temporally complementary manner compared to other bioturbators (e.g., different diurnal/seasonal activity patterns, different habitat preferences). We believe that the role of ground-nesting bees as soil ecosystem engineers deserves more attention and should be further explored. Our study highlights the great potential of X-ray imaging as a novel and promising tool for this underexplored research field, and we encourage future studies to harness this potential to unravel our still limited knowledge of the elusive subterranean digging activity of ground-nesting bees.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2023.116655.

References

Abdouni, I.E., Lhomme, P., Hamroud, L., Wood, T., Christmann, S., Rasmont, P., Michez, D., 2021. Comparative ecology of two specialist bees: *Dasypoda visnaga* Rossi, 1790 and *Dasypoda maura* Pérez, 1895 (Hymenoptera, Melittidae). J. Hymenopt. Res. 81, 109–126. https://doi.org/10.3897/jhr.81.60528.

Antoine, C.M., Forrest, J.R.K., 2021. Nesting habitat of ground-nesting bees: a review. Ecol. Entomol. 46, 143–159. https://doi.org/10.1111/een.12986.

Arganda-Carreras, I., Fernández-González, R., Muñoz-Barrutia, A., Ortiz-De-Solorzano, C., 2010. 3D reconstruction of histological sections: Application to mammary gland tissue. Microsc. Res. Tech. 73, 1019–1029. https://doi.org/10.1002/jemt.20829.

Bahl, K.N., 1950. Pheretima (an Indian earthworm), 4th (rev. and enl.). ed, The Indian zoological memoirs on Indian animal types. Lucknow Publishing House, Lucknow.

Batra, S.W.T., 1968. Behavior of some social and solitary halictine bees within their nests. A comparative study (Hymenoptera: Halictidae). J. Kans. Entomol. Soc. 41, 120–133.

Batra, S.W.T., 1970. Behavior of the alkali bee, *Nomia melanderi*, within the nest (Hymenoptera: Halictidae). Ann. Entomol. Soc. Am. 63, 400–406. https://doi.org/10.1093/aesa/63.2.400.

Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., Wood, E.F., 2018. Present and future Köppen-Geiger climate classification maps at 1-km resolution. Sci. Data 5, 180214. https://doi.org/10.1038/sdata.2018.214.

Blagoveshchenskaya, N., 1963. Giant colony of the solitary bee *Dasypoda plumipes* PZ. (Hymenoptera, Melittidae). Entomol. Rev. 42, 60–61.

Bouché, M.B., 1977. Strategies lombriciennes. Ecol. Bull. 122–132.

Cane, J.H., 1981. Dufour's gland secretion in the cell linings of bees (Hymenoptera: Apoidea). J. Chem. Ecol. 7, 403–410. https://doi.org/10.1007/BF00995762.

Cane, J.H., 2003. Annual displacement of soil in nest tumuli of alkali bees (*Nomia melanderi*) (Hymenoptera: Apiformes: Halictidae) across an agricultural landscape. J. Kans. Entomol. Soc. 76, 172–176.

Cane, J.H., Neff, J.L., 2011. Predicted fates of ground-nesting bees in soil heated by wildfire: Thermal tolerances of life stages and a survey of nesting depths. Biol. Conserv. 144, 2631–2636. https://doi.org/10.1016/j.biocon.2011.07.019.

Capowiez, Y., Monestiez, P., Belzunces, L., 2001. Burrow systems made by *Aporrectodea nocturna* and *Allolobophora chlorotica* in artificial cores: morphological differences and effects of interspecific interactions. Appl. Soil Ecol. 16, 109–120. https://doi.org/10.1016/S0929-1393(00)00110-4.

Capowiez, Y., Sammartino, S., Michel, E., 2011. Using X-ray tomography to quantify earthworm bioturbation non-destructively in repacked soil cores. Geoderma 162, 124–131. https://doi.org/10.1016/j.geoderma.2011.01.011.

Capowiez, Y., Bottinelli, N., Jouquet, P., 2014. Quantitative estimates of burrow construction and destruction, by anecic and endogeic earthworms in repacked soil cores. Appl. Soil Ecol. 74, 46–50. https://doi.org/10.1016/j.apsoil.2013.09.009.

Capowiez, Y., Bottinelli, N., Sammartino, S., Michel, E., Jouquet, P., 2015. Morphological and functional characterisation of the burrow systems of six earthworm species (Lumbricidae). Biol. Fertil. Soils 51, 869–877. https://doi.org/10.1007/s00374-015-1036-x.

Capowiez, Y., Sammartino, S., Keller, T., Bottinelli, N., 2021. Decreased burrowing activity of endogeic earthworms and effects on water infiltration in response to an increase in soil bulk density. Pedobiologia 85–86, 150728. https://doi.org/10.1016/j.pedobi.2021.150728.

Chaney, K., Swift, R.S., 1984. The influence of organic matter on aggregate stability in some British soils. J. Soil Sci. 35, 223–230. https://doi.org/10.1111/j.1365-2389.1984.tb00278.x.

Chaudhary, V.B., Bowker, M.A., O'Dell, T.E., Grace, J.B., Redman, A.E., Rillig, M.C., Johnson, N.C., 2009. Untangling the biological contributions to soil stability in semiarid shrublands. Ecol. Appl. 19, 110–122. https://doi.org/10.1890/07-2076.1.

Cheik, S., Bottinelli, N., Minh, T.T., Doan, T.T., Jouquet, P., 2019. Quantification of three dimensional characteristics of macrofauna macropores and their effects on soil hydraulic conductivity in northern Vietnam. Front. Environ Sci. 7.
Cheik, S., Jouquet, P., Maeght, J., Capowiez, Y., Tran, T.M., Bottinelli, N., 2021. X-ray tomography analysis of soil biopores structure under wetting and drying cycles. Eur. J. Soil Sci. 72, 2128–2132. https://doi.org/10.1111/ejss.13119.
Chenu, C., Le Bissonnais, Y., Arrouays, D., 2000. Organic matter influence on clay wettability and soil aggregate stability. Soil Sci. Soc. Am. J. 64, 1479–1486. https://doi.org/10.2136/sssaj2000.6441479x.
Christmann, S., 2019. Do we realize the full impact of pollinator loss on other ecosystem services and the challenges for any restoration in terrestrial areas? Restor. Ecol. 27, 720–725. https://doi.org/10.1111/rec.12950.
Christmann, S., 2022. Regard and protect ground-nesting pollinators as part of soil biodiversity. Ecol. Appl. 32, e2564.
Czarnes, S., Hallett, P.D., Bengough, A.G., Young, I.M., 2000. Root- and microbial-derived mucilages affect soil structure and water transport. Eur. J. Soil Sci. 51, 435–443. https://doi.org/10.1046/j.1365-2389.2000.00327.x.
Danforth, B.N., Minckley, R.L., Neff, J.L., Fawcett, F., 2019. In: The solitary bees: biology, evolution, conservation. Princeton University Press. https://doi.org/10.1515/9780691189321.
Doube, M., Kłosowski, M.M., Arganda-Carreras, I., Cordelières, F.P., Dougherty, R.P., Jackson, J.S., Schmid, B., Hutchinson, J.R., Shefelbine, S.J., 2010. BoneJ: Free and extensible bone image analysis in ImageJ. Bone 47, 1076–1079. https://doi.org/10.1016/j.bone.2010.08.023.
Edwards, C.A., Arancon, N.Q., 2022. Biology and ecology of earthworms. Springer US, New York, NY. https://doi.org/10.1007/978-0-387-74943-3.
Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., Kremen, C., Carvalheiro, L.G., Harder, L.D., Afik, O., Bartomeus, I., Benjamin, F., Boreux, V., Cariveau, D., Chacoff, N.P., Dudenhöffer, J. H., Freitas, B.M., Ghazoul, J., Greenleaf, S., Hipólito, J., Holzschuh, A., Howlett, B., Isaacs, R., Javorek, S.K., Kennedy, C.M., Krewenka, K.M., Krishnan, S., Mandelik, Y., Mayfield, M.M., Motzke, I., Munyuli, T., Nault, B.A., Otieno, M., Petersen, J., Pisanty, G., Potts, S.G., Rader, R., Ricketts, T.H., Rundlöf, M., Seymour, C.L., Schüepp, C., Szentgyörgyi, H., Taki, H., Tscharntke, T., Vergara, C.H., Viana, B.F., Wanger, T.C., Westphal, C., Williams, N., Klein, A.M., 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science 339, 1608–1611. https://doi.org/10.1126/science.1230200.
Gray, J., Lissmann, H.W., 1938. An apparatus for measuring the propulsive forces of the locomotory muscles of the earthworm and other animals. J. Exp. Biol. 15, 518–521. https://doi.org/10.1242/jeb.15.4.518.
Greco, M., Spooner-Hart, R., Holford, P., 2005. A new technique for monitoring *Trigona carbonaria* nest contents, brood and activity using X-ray computerized tomography. J. Apic. Res. 44, 97–100. https://doi.org/10.1080/00218839.2005.11101156.
Greco, M., Bell, M., Spooner-Hart, R., Holford, P., 2006. X-ray computerized tomography as a new method for monitoring *Amegilla holmesi* nest structures, nesting behaviour, and adult female activity. Entomol. Exp. Appl. 120, 71–76. https://doi.org/10.1111/j.1570-7458.2006.00429.x.
Gutiérrez, Y., Ott, D., Töpperwien, M., Salditt, T., Scherber, C., 2018. X-ray computed tomography and its potential in ecological research: A review of studies and optimization of specimen preparation. Ecol. Evol. 8, 7717–7732. https://doi.org/10.1002/ece3.4149.
Harmon-Threatt, A., 2020. Influence of nesting characteristics on health of wild bee communities. Annu. Rev. Entomol. 65, 39–56. https://doi.org/10.1146/annurev-ento-011019-024955.
Helliwell, J.R., Sturrock, C.J., Grayling, K.M., Tracy, S.R., Flavel, R.J., Young, I.M., Whalley, W.R., Mooney, S.J., 2013. Applications of X-ray computed tomography for examining biophysical interactions and structural development in soil systems: a review. Eur. J. Soil Sci. 64, 279–297. https://doi.org/10.1111/ejss.12028.
Hunter, J.D., 2007. Matplotlib: A 2D graphics environment. Comput. Sci. Eng. 9, 90–95. https://doi.org/10.1109/MCSE.2007.55.
IPBES, 2016. The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn, Germany. https://doi.org/10.5281/zenodo.3402857.
Jégou, D., Cluzeau, D., Wolf, H.J., Gandon, Y., Tréhen, P., 1997. Assessment of the burrow system of *Lumbricus terrestris*, *Aporrectodea giardi*, and *Aporrectodea caliginosa* using X-ray computed tomography. Biol. Fertil. Soils 26, 116–121. https://doi.org/10.1007/s003740050353.
Jégou, D., Hallaire, V., Cluzeau, D., Tréhen, P., 1999. Characterization of the burrow system of the earthworms *Lumbricus terrestris* and *Aporrectodea giardi* using X-ray computed tomography and image analysis. Biol. Fertil. Soils 29, 314–318. https://doi.org/10.1007/s003740050558.
Johnson, S.N., Read, D.B., Gregory, P.J., 2004. Tracking larval insect movement within soil using high resolution X-ray microtomography. Ecol. Entomol. 29, 117–122. https://doi.org/10.1111/j.0307-6946.2004.00567.x.
Kanao, T., Okamoto, T., Miyachi, Y., Nohara, N., 2003. Parental exposure to low-dose X-rays in *Drosophila melanogaster* induces early emergence in offspring, which can be modulated by transplantation of polar cytoplasm. Mutat. Res. Mol. Mech. Mutagen. 527, 1–6. https://doi.org/10.1016/S0027-5107(03)00051-4.
Kay, B.D., 1997. Soil structure and organic carbon: a review, in: Soil Processes and the Carbon Cycle. CRC Press.
Keller, T., Colombi, T., Ruiz, S., Manalili, M.P., Rek, J., Stadelmann, V., Wunderli, H., Breitenstein, D., Reiser, R., Oberholzer, H., Schymanski, S., Romero-Ruiz, A., Linde, N., Weisskopf, P., Walter, A., Or, D., 2017. Long-term soil structure observatory for monitoring post-compaction evolution of soil structure. Vadose Zone J. 16, vzj2016.11.0118. https://doi.org/10.2136/vzj2016.11.0118.
Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L.G., Henry, M., Isaacs, R., Klein, A.-M., Kremen, C., M'Gonigle, L.K., Rader, R., Ricketts, T.H., Williams, N.M., Adamson, N.L., Ascher, J.S., Báldi, A., Batáry, P., Benjamin, F., Biesmeijer, J.C., Blitzer, E.J., Bommarco, R., Brand, M.R., Bretagnolle, V., Button, L., Cariveau, D.P., Chifflet, R., Colville, J.F., Danforth, B.N., Elle, E., Garratt, M.P.D., Herzog, F., Holzschuh, A., Howlett, B.G., Jauker, F., Jha, S., Knop, E., Krewenka, K.M., Féon, V. L., Mandelik, Y., May, E.A., Park, M.G., Pisanty, G., Reemer, M., Riedinger, V., Rollin, O., Rundlöf, M., Sardiñas, H.S., Scheper, J., Sciligo, A.R., Smith, H.G., Steffan-Dewenter, I., Thorp, R., Tscharntke, T., Verhulst, J., Viana, B.F., Vaissière, B.E., Veldtman, R., Ward, K.L., Westphal, C., Potts, S.G., 2015. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nat. Commun. 6, 1–9. https://doi.org/10.1038/ncomms8414.
Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B Biol. Sci. 274, 303–313. https://doi.org/10.1098/rspb.2006.3721.
Koestel, J., Schlüter, S., 2019. Quantification of the structure evolution in a garden soil over the course of two years. Geoderma 338, 597–609. https://doi.org/10.1016/j.geoderma.2018.12.030.
Lavelle, P., Bignell, D., Wolters, V., Roger, P., Ineson, P., Heal, O., Dhillion, S.P., 1997. Soil function in a changing world: The role of invertebrate ecosystem engineers. Eur. J. Soil Sci. 33, 159–193.
Le Mer, G., Jouquet, P., Capowiez, Y., Maeght, J., Tran, T.M., Doan, T.T., Bottinelli, N., 2021. Age matters: Dynamics of earthworm casts and burrows produced by the anecic *Amynthas khami* and their effects on soil water infiltration. Geoderma 382, 114709. https://doi.org/10.1016/j.geoderma.2020.114709.
Lee, K.E., 1985. Earthworms: their ecology and relationships with soils and land use. Academic Press, Sydney, Orlando.
Lee, K.E., Foster, R.C., 1991. Soil fauna and soil structure. Soil Res. 29, 745–775. https://doi.org/10.1071/sr9910745.
Legland, D., Arganda-Carreras, I., Andrey, P., 2016. MorphoLibJ: integrated library and plugins for mathematical morphology with ImageJ. Bioinformatics 32, 3532–3534. https://doi.org/10.1093/bioinformatics/btw413.
Leonard, R.J., Harmon-Threatt, A.N., 2019. Methods for rearing ground-nesting bees under laboratory conditions. Apidologie 50, 689–703. https://doi.org/10.1007/s13592-019-00679-8.
Leue, M., Gerke, H.H., Godow, S.C., 2015. Droplet infiltration and organic matter composition of intact crack and biopore surfaces from clay-illuvial horizons. J. Plant Nutr. Soil Sci. 178, 250–260. https://doi.org/10.1002/jpln.201400209.
Leuther, F., Mikutta, R., Wolff, M., Kaiser, K., Schlüter, S., 2023. Structure turnover times of grassland soils under different moisture regimes. Geoderma 433, 116464. https://doi.org/10.1016/j.geoderma.2023.116464.
Limaye, A., 2012. Drishti: a volume exploration and presentation tool, in: Developments in X-Ray Tomography VIII. SPIE, pp. 191–199. https://doi.org/10.1117/12.935640.
Linsley, E.G., MacSwain, J.W., Smith, R.F., 1952. Outline for ecological life histories of solitary and semi-social bees. Ecology 33, 558–567. https://doi.org/10.2307/1931531.
Lybrand, R.A., Fedenko, J., Tfaily, M., Rao, S., 2020. Soil properties and biochemical composition of ground-dwelling bee nests in agricultural settings. Soil Sci. Soc. Am. J. 84, 1139–1152. https://doi.org/10.1002/saj2.20085.
Malyshev, S.I., 1927. Lebensgeschichte des *Colletes cunicularius* L. Z. Für Morphol. Ökol. Tiere 9, 390–409. https://doi.org/10.1007/BF00408703.
Malyshev, S.I., 1936. The nesting habits of solitary bees: A comparative study.
Marinho, D., Andrade, J., Araujo, R.O., Vivallo, F., 2018. A new technique in the excavation of ground-nest bee burrows (Hymenoptera: Apoidea). Rev. Bras. Entomol. 62, 1–4. https://doi.org/10.1016/j.rbe.2017.11.003.
Martins, R.P., Antonini, Y., 1994. The biology of *Diadasina distincta* (Holmberg, 1903) (Hymenoptera: Anthophoridae). Proc. Entomol. Soc. Wash. 96, 553–560.
Mele, G., Buscemi, G., Gargiulo, L., Terribile, F., 2021. Soil burrow characterization by 3D image analysis: Prediction of macroinvertebrate groups from biopore size distribution parameters. Geoderma 404, 115292. https://doi.org/10.1016/j.geoderma.2021.115292.
MeteoSwiss, 2023. Climate norm values [WWW Document]. URL https://www.meteoswiss.admin.ch/climate/the-climate-of-switzerland/climate-normals/normal-values-per-measured-parameter.html (accessed 4.21.23).
Meurer, K., Barron, J., Chenu, C., Coucheney, E., Fielding, M., Hallett, P., Herrmann, A. M., Keller, T., Koestel, J., Larsbo, M., Lewan, E., Or, D., Parsons, D., Parvin, N., Taylor, A., Vereecken, H., Jarvis, N., 2020. A framework for modelling soil structure dynamics induced by biological activity. Glob. Chang. Biol. 26, 5382–5403. https://doi.org/10.1111/gcb.15289.
Michener, C.D., 2007. The bees of the world, second ed. Johns Hopkins University Press, Baltimore.
Michener, C.D., Cross, E.A., Daly, H.V., Rettenmeyer, C.W., Wille, A., 1955. Additional techniques for studying the behavior of wild bees (I). Insect. Soc. 2 (3), 237–246.
Milleret, R., Le Bayon, R.-C., Lamy, F., Gobat, J.-M., Boivin, P., 2009. Impact of roots, mycorrhizas and earthworms on soil physical properties as assessed by shrinkage analysis. J. Hydrol. 373, 499–507. https://doi.org/10.1016/j.jhydrol.2009.05.013.
Neff, J.L., Simpson, B.B., 1993. Bees, pollination systems and plant diversity, in: LaSalle, J., Gauld, I.D. (Eds.), Hymenoptera and Biodiversity. CAB International, Wallingford, UK, pp. 143–167.
Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? Oikos 120, 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x.
Pelíšek, I., 2018. Investigation of soil water infiltration at a scale of individual earthworm channels. Soil Water Res. 13, 1–10. https://doi.org/10.17221/283/2014-SWR.

Pérès, G., Cluzeau, D., Curmi, P., Hallaire, V., 1998. Earthworm activity and soil structure changes due to organic enrichments in vineyard systems. Biol. Fertil. Soils 27, 417–424. https://doi.org/10.1007/s003740050452.

Polidori, C., Casiraghi, M., Lorenzo, M.D., Valarani, B., Andrietti, F., 2006. Philopatry, nest choice, and aggregation temporal-spatial change in the digger wasp *Cerceris arenaria* (Hymenoptera: Crabronidae). J. Ethol. 24, 155–163. https://doi.org/10.1007/s10164-005-0176-0.

Potvin, L.R., Lilleskov, E.A., 2017. Introduced earthworm species exhibited unique patterns of seasonal activity and vertical distribution, and Lumbricus terrestris burrows remained usable for at least 7 years in hardwood and pine stands. Biol. Fertil. Soils 53, 187–198. https://doi.org/10.1007/s00374-016-1173-x.

Python Software Foundation, 2022. Python language reference, version 3.8.15.

R Core Team, 2022. R: A language and environment for statistical computing.

Rabot, E., Wiesmeier, M., Schlüter, S., Vogel, H.-J., 2018. Soil structure as an indicator of soil functions: A review. Geoderma 314, 122–137. https://doi.org/10.1016/j.geoderma.2017.11.009.

Rogasik, H., Schrader, S., Onasch, I., Kiesel, J., Gerke, H.H., 2014. Micro-scale dry bulk density variation around earthworm (*Lumbricus terrestris* L.) burrows based on X-ray computed tomography. Geoderma 213, 471–477. https://doi.org/10.1016/j.geoderma.2013.08.034.

Roulston, T.H., Goodell, K., 2011. The role of resources and risks in regulating wild bee populations. Annu. Rev. Entomol. 56, 293–312. https://doi.org/10.1146/annurev-ento-120709-144802.

Rozen, J.G., 2016. Nesting biology of the solitary bee *Epicharis albofasciata* (Apoidea: Apidae: Centridini). Am. Mus. Novit. 3869 (3869), 1–8.

Rueden, C.T., Hiner, M.C., Evans, E.L., Pinkert, M.A., Lucas, A.M., Carpenter, A.E., Cimini, B.A., Eliceiri, K.W., 2022. PyImageJ: A library for integrating ImageJ and Python. Nat. Methods 19 (11), 1326–1327.

Ruiz, S., Schymanski, S.J., Or, D., 2017. Mechanics and energetics of soil penetration by earthworms and plant roots: higher rates cost more. Vadose Zone J. 16, vzj2017.01.0021. https://doi.org/10.2136/vzj2017.01.0021.

Ruiz, S., Hallett, P., Or, D., 2023. Bioturbation—Physical processes, in: Reference Module in Earth Systems and Environmental Sciences. Elsevier, p. B9780128229743002000. https://doi.org/10.1016/B978-0-12-822974-3.00180-4.

Sakagami, S.F., Hoshikawa, K., Fukuda, H., 1984. Overwintering ecology of two social halictine bees, *Lasioglossum duplex* and *L. problematicum*. Res. Popul. Ecol. 26, 363–378. https://doi.org/10.1007/BF02515500.

Sakagami, S., Michener, C.D., 1962. The nest architecture of the sweat bees (Halictinae): a comparative study of behavior. University of Kansas Press.

Sarzetti, L., Genise, J., Sanchez, M.V., Farina, J., Molina, A., 2013. Nesting behavior and ecological preferences of five Diphaglossinae species (Hymenoptera, Apoidea, Colletidae) from Argentina and Chile. J. Hymenopt. Res. 33, 63–82. https://doi.org/10.3897/jhr.33.5061.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019.

Soil Survey Staff, 2022. Keys to soil taxonomy, 13th ed. USDA-Natural Resources Conservation Service.

Stephen, W.P., Bohart, G.E., Torchio, P.F., 1969. The biology and external morphology of bees with a synopsis of the genera of north-western America. Oregon State University, Corvallis.

SwissBeeTeam, 2021. Online Atlas der Schweizer Wildbienen.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141–163. https://doi.org/10.1111/j.1365-2389.1982.tb01755.x.

Tschanz, P., Vogel, S., Walter, A., Keller, T., Albrecht, M., 2023. Nesting of ground-nesting bees in arable fields is not associated with tillage system per se, but with distance to field edge, crop cover, soil and landscape context. J. Appl. Ecol. 60, 158–169. https://doi.org/10.1111/1365-2664.14317.

Ullmann, K.S., Cane, J.H., Thorp, R.W., Williams, N.M., 2020. Soil management for ground-nesting bees. In: FAO, pp. 23–44 https://doi.org/10.4060/ca8965en.

Van der Walt, S., Schönberger, J.L., Nunez-Iglesias, J., Boulogne, F., Warner, J.D., Yager, N., Gouillart, E., Yu, T., 2014. scikit-image: image processing in Python. PeerJ 2, e453.

Visscher, P.K., Danforth, B.N., 1993. Biology of *Calliopsis pugionis* (Hymenoptera: Andrenidae): nesting, foraging, and investment sex ratio. Ann. Entomol. Soc. Am. 86, 822–832. https://doi.org/10.1093/aesa/86.6.822.

Watanabe, H., 1998. Soil excavation by the deutzia andrenid bee (*Andrena prostimias*) in a temple garden in Hyogo Prefecture. Japan. Appl. Soil Ecol. 9, 283–287. https://doi.org/10.1016/S0929-1393(97)00054-1.

Westrich, P., 2018. Die Wildbienen Deutschlands, second ed. Eugen Ulmer KG, Stuttgart.

Wilkinson, M.T., Richards, P.J., Humphreys, G.S., 2009. Breaking ground: Pedological, geological, and ecological implications of soil bioturbation. Earth Sci. Rev. 97, 257–272. https://doi.org/10.1016/j.earscirev.2009.09.005.