Nesting preferences of ground-nesting bees in commercial fruit orchards

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Declaration of original authorship

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

There is increasing evidence in the literature of the importance of wild ground-nesting bees as pollinators of crops, especially for apple pollination. The survival of bees heavily depends on the availability of suitable food and nesting resources. Ways to provide floral resources are well established, however the nesting preferences of ground-nesting bees are poorly understood. This is a significant knowledge gap when considering that that three quarters of all wild bee species in the UK are ground nesters. This PhD used a combination of field surveys and manipulative experiments to address this gap with a focus on apple pollinators. In the UK the predominant apple pollinators are spring *Andrena* species.

The first study used artificially prepared plots of bare soil to attract ground-nesting bees to nest in orchards in the south east of England. Nine soil parameters were measured to determine the preference of ground-nesting bees: hydraulic conductivity, soil compaction, soil moisture, soil temperature, soil stoniness, soil organic matter, soil root biomass, soil texture and vegetation cover. Eighteen non-parasitic ground-nesting bee species (7 *Andrena*, 9 *Lasioglossum*, 1 *Halictus* and 1 *Colletes* spp.) were recorded founding nests in the study plots. Soil stoniness and soil temperature were positively correlated, and vegetation cover and hydraulic conductivity were negatively correlated, with the number of ground-nesting bees on the plots.

The second study used transect surveys to determine which ground-nesting bee species nest in apple orchards, and to identify their preferred orchard (alleyways, under trees, edges) and local (vegetation cover) nesting habitats. Fifteen non-parasitic ground-nesting bee species (12 *Andrena* spp., 2 *Lasioglossum* spp., 1 *Halictus* spp.) were recorded, nesting mainly under trees (36 %) and hedgerows (52 %) of apple orchards. Local habitat was a significant predictor of nest density, and bees mostly nested in areas of short grass (32 %), bare ground (31 %), and moss cover (18%). *Andrena haemorrhoa* Fabricius and *Andrena nitida* Müller nesting aggregations were also found under hedgerows of some apple orchards; these species have demonstrated a nesting preference for highly vegetated habitats. The nine soil parameters measured in the first study were also recorded for this study. Soil stoniness and soil temperature were positively correlated, and soil moisture was negatively correlated, with the nest numbers of nesting aggregations.

The third study used artificially constructed nest sites (90 litre tree tubs filled with soil) to establish the preferred soil compaction (determined from the other studies) and other abiotic factors for bee nesting. The study attracted three non-parasitic bee species (1 *Megachile*, and 2 *Lasioglossum* spp.) to nest, but the great majority of attracted ground-nesters were wasp species (95.3% of total specimens). Pebble treatments were added to half the area of the soil surface in in each tub during the fourth year of the study, which attracted a significantly higher number of bees/wasps to nest compared to the bare soil without pebbles. Soil temperature, compaction and pebbles were positively correlated, and soil moisture was negatively correlated, with the nest density of soil tubs.

Understanding wild bee nesting needs is an important component for the success of agri-environment schemes aiming to safeguard biodiversity and ecosystem services. Such knowledge would enable landowners and farm managers to create and maintain nesting habitats for wild bees and, with the provision of suitable food resources, contribute to the increase and diversification of bee populations. The findings of this PhD inform management practices on the nesting preferences of ground-nesting bees and provide recommendations on creating and maintaining nesting habitats for important crop pollinators in agricultural settings.

1 Chapter 1 – Introduction

Animal pollination supports more than 75% of the 115 leading crop species globally, (Klein et al. 2007). The annual market value of this service is estimated at £180-442 billion worldwide (Potts et al. 2016). Furthermore, both managed and wild pollinators are highly beneficial to society, contributing towards the maintenance of biodiversity, ecosystem stability and food security, playing a significant role towards farmer and beekeeper livelihoods and social and cultural values (Potts et al. 2016). Bees are vital for pollination services in agricultural (Klein et al. 2007) and natural ecosystems (Ollerton et al. 2011). However, bees are threatened by several disturbances, such as climate change, habitat loss, pesticide use, reduction in floral resources and spread of parasites and pathogens from managed bee species (Potts et al. 2016; Janousek et al. 2023).

Yields of some insect-pollinated crops in some regions of the world are managed by introducing honeybees (*Apis mellifera* Linnaeus) (Morse 1991; Goodwin et al. 2011; Rucker et al. 2012). However, there is evidence that honeybee hive numbers have been declining in some countries in Europe and North America (Potts et al. 2010; vanEngelsdorp & Meixner 2010) and might not be capable of supplying pollination service demands. A study by Breeze et al. (2011) shows that UK honeybee populations could supply just 34% of pollination demands in 2011, falling from 70% in 1984. Since insect-pollinated crop yields have increased by an average of 54% from 1984 to 2011, it is not likely that honeybees provide most pollination services (Breeze et al. 2011). Evidence in the literature indicates that the contribution of wild pollinators might have been underestimated. Garibaldi et al. (2013) investigated 41 crop systems and found significant positive associations between flower visitation by wild pollinators and fruit set, compared to flower visitation by honeybees which contributed significantly towards fruit set in only 14% of the studied systems. The study also found that visitation by honeybees and wild insects promoted fruit set independently, suggesting that honeybees should be considered a supplement rather than a substitute pollinator (Garibaldi et al. 2013). Kendall (1973) studied insect pollinators' visitation on apple blossom and found that wild solitary ground-nesting bee species *Andrena haemorrhoa* F. and *Andrena scotica* P. pollinated a significantly higher proportion of fruit ovules resulting in better fruit set (seed content) than honeybees. An explanation for this finding is given by Kendall & Solomon (1973), who indicated that solitary bee species such as *A. haemorrhoa* are capable of carrying large quantities of pollen grains (up to 15,000) to apple blossom, compared to honeybee workers who carry a much smaller amount (4000 on average). Also, Woodcock et al. (2013) provided evidence that honeybees tend to have lower rates of stigma contact than solitary bees, with the probability of pollen being transferred to the stigma to be estimated at 34.0% for the former and 71.3% for the latter.

Another study suggests that while the contribution of wild bees to crop yields is critical, delivery of pollination services is supplied by a small number of common bee species, with only 2% of bee species being responsible for almost 80% of crop pollination (Kleijn et al. 2015). Though not many studies have focused on the agricultural benefits of solitary bees, Garratt et al. (2016) estimated their value for UK apple pollination at £51 million p.a (Fig. 1.1). Hutchinson et al. (2021) reported the spring *Andrena* species *A. nigroaenea* K., *A. haemorrhoa* F., *A. cineraria* L., *A. nitida* M., *A. scotica* P. and *A. dorsata* K. as apple pollinating bee species in the UK. Holzschuh et al. (2012) found that the most common wild bee family visiting sweet cherry crops was Andrenidae, making up 92% of bees sampled. Another study that investigated the potential of small sweat bees as pollinators of melon (*Cucumis melo*) reported that *Lasioglossum* species accounted for more than 70% of species visiting the flowers, with *L. marginatum B.* and *L. malachurum* K. being the most abundant visitors (Rodrigo Gómez et al. 2016). Potts et al. (2005) suggest that a crucial element of wild bee presence in orchards and other agro- ecosystems is the availability of appropriate nesting resources. This statement is supported by Grundel et al. (2010), indicating that bee richness is positively associated with the availability of nesting and food resources. Ways to provide floral resources for pollinators are well developed (Sheffield et al. 2008; Rosa García & Miñarro 2014; Campbell et al. 2017);

however, methods for provisioning areas for ground-nesting bees are poorly understood. While the floral nectar and pollen diet is mostly shared between bee species, differing life-history traits and ecological characteristics, nesting sites can be specialised (Antoine & Forrest 2020). The increasing number of studies highlighting the effectiveness of several ground-nesting bee species as crop pollinators indicates that we should focus on understanding their nesting needs and providing the appropriate nesting resources to support conservation and delivery of ecosystem services like crop pollination.

Figure 1.1: Solitary bee on an apple flower

1.1 Bee ecology and life history characteristics

There are > 20,000 known bee species which differ significantly in their ecology, habits and size (IPBES 2016). More than 75% of existing bee species are not perennially social (e.g. honeybees) but solitary, where females work alone for the construction of their nest(s) and provide food for their offspring (Michener 2007). Solitary bee species are divided into six families: Megachilidae (long-tongued, leafcutter and mason bees) and Halictidae (short-tongued, sweat bees) with worldwide distribution; Andrenidae (short-tongued, digger bees) mainly present in the Northern Hemisphere; Colletidae (short-tongued, membrane bees), most diverse and numerous in the Southern Hemisphere; Melittiidae (short-tongued), a small, diverse group; and finally Apidae (long-tongued, carpenter bees, orchid bees, stingless bees, honeybees, bumblebees), the largest family within the superfamily Apoidea (Batra 1984; Else et al. 2016; Else & Edwards 2018). There are 277 bee species in the British Isles, where the great majority are ground-nesters, including species from *Andrena* Fabricius (68 species), *Lasioglossum* Curtis (33 species), *Halictus* Latreille (8 species), and *Colletes* Latreille (9 species) genera (Else & Edwards 2018).

There are bee species which are not solitary throughout their lifetime. Sweat bees (Halictidae) can be divided into three categories: solitary, obligately social and facultatively social (Richards 1994; Packer 1997). Obligatory social sweat bees cooperate with adult females in the nest to raise the next reproductive brood. Eusocial species have at least two phases in their colony cycle, with the females of the first brood (workers) helping raise reproductive or final brood (Richards 2000). Facultative social species comprise social and solitary populations and even social and solitary colonies within a single population. This eusocial habit has been reported in *Halictus rubicundus* C. (Eickwort et al. 1996) and *Lasioglossum calceatum* S. (Sakagami & Munakata 1972). An example of a kin-selected eusocial sweat bee could be one where a mother queen has complete control of the oviposition of eggs and her altruistic daughter workers, which neither mate nor lay eggs, are assisting with raising her brood (Eickwort 1985). *L. malachurum* K. is often seen as a classic example of a eusocial sweat bee (Richards 2000).

1.2 Parasitism

There is a minority of bee species which are parasites; a number of them specialise in using ground-nesting bee species as hosts (Sick et al. 1994). There are two recognised parasitic strategies: parasitoidism, which includes free-living mothers that lay eggs on or in the host, and kleptoparasitism, which encompasses stealing the host's food resources via the laying of eggs inside the nest of other species, avoiding the cost of brood care (O'Neill 2001). Approximately thirteen families of Aculeata contain parasitoids, with eight families containing obligate kleptoparasites (Bohart 1970; O'Neill 2001). Depending on the species, some parasitise one bee species whilst others have several bee hosts and various strategies for accessing the host's nest (Bogusch et al. 2006). The *Sphecodes* genus (Family: Halictidae), commonly known as cuckoo bees, are one of the main enemies of solitary bees and are mainly kleptoparasitic on halictid bees (*Halictus* and *Lasioglossum*), but also other bee families such as Melittidae, Andrenidae, Anthophoridae and Colletidae (reviewed by Michener 1978). *Lasioglossum malachurum* K. bees were observed blocking the nest's entrance with their head (K. Tsiolis, personal observation). A *Sphecodes monilicornis* K. arrived at a nest entrance determined to enter the nest. The *L. malachurum* guard was wrestling the kleptoparasite away, and suddenly the *S. monilicornis* K. grabbed the head of the *L. malachurum* K. guard and pulled it out of the nest. The guard bee died, and the kleptoparasite entered the nest. Several other *L. malachurum K.* bees were found dead by nest entrances with damaged wings or missing body parts (K. Tsiolis, personal observation).

1.3 Nesting behaviour

The great majority of bee species are ground-nesters. Cane & Neff (2011) reported that 64 % of bee species create their nests belowground (Fig. 1.2). Harmon-Threatt (2020) have reported that 84% of bee species in the USA and Canada are ground-nesters. This fossorial (burrowing) nesting behaviour is also typical of Crabronidae wasps which are thought to be the ancestors of ground-nesting bees (Debevec et al. 2012; Sann et al. 2018). Another group of bee species are considered cavity-nesters. Bee species of this group excavate their nest in wood or use pre-existing cavities in snail shells, stone walls, pithy stems, wood (Cane et al. 2007; Danforth et al. 2019), and belowground in the case of many bumblebee species (Liczner & Colla 2019). Bumblebees and honeybees are not considered ground-nesters as they do not dig burrows. Kleptoparasites also do not dig burrows.

Figure 1.2: Likely *A. haemorrhoa* F. ground-nesting bee exiting nest.

Despite ground-nesting behaviour being more widely observed across bee families, both social and solitary species, and being present in most habitats where bees exist, cavity nesters are proportionally far more studied than ground-nesters (Winfree 2010). Artificial nesting structures can be used to study cavity-nesters, but nests of ground-nesting bees are usually difficult to locate (Antoine & Forrest 2020). Laboratory and field studies are rare on living ground-nesting bees. Harmon-Threatt (2020) reported in a review that out of 527 bee species studied in the USA and Canada, there was nesting information for only 26% of them. This highlights the significant gap in knowledge on the ecology of this big group of bee species.

1.4 The life cycle of ground-nesting bees

The emergence of adult bees from belowground nests initiates the active period of ground-nesting bees. Males emerge first with female emergence following a week or two later, and mating occurring (Michener 2007). Males die shortly after mating, and female activity continues for a few more weeks. The active season of social bee species is much longer, lasting for months as the foundress produces successive cycles of adult workers. Both female social and solitary bee species have the spermathecae organ, where they store sperm. This organ enables bees to lay fertilised eggs, freeing them to spend the rest of their life constructing their nest, providing food to individual cells and laying their eggs. Egg hatching follows a few weeks after, and the progeny move into the larval stage, where they feed on stored food resource collected and stored by their mother or sisters (social species). Many solitary ground-nesting bee species in temperate regions overwinter in the prepupal stage before they complete metamorphosis in the following spring. Other bee species complete metamorphosis before overwintering as adults. Despite their developmental stage, solitary bees (including foundresses of some social species) overwinter in a dormant state inside their brood cells (Michener 2007) (Fig. 1.3). The nesting location chosen by the mother determines the overwintering location and often the survival of offspring bees. Immature bees can be below ground for several months (or sometimes over a year; Danforth 1999). During this period, they can be exposed to several environmental hazards, such as flooding (Fellendorf et al. 2004) and wildfires (Cane & Neff 2011).

The duration of life stages for ground-nesting bees varies and is highly dependent on species and the environment in which they are found, including temperature and the length of the flowering season. Some bee species are multivoltine, producing more than one generation per year (Michener 2007). Other bee species have just one generation or even take more than a year to complete a single generation (Forrest et al. 2019). Most *Andrena* species in the UK are univoltine (one brood of adults per year), such as *A. haemorrhoa* F. and *A. nitida* M. The flight period is species-dependent and can range from early spring to late summer. The small group of bivoltine (two adult broods per year) *Andrena* species, such as *A. dorsata* K. and *A. minutula* K., have a more extended flight period between April-July. The flight period of *Lasioglossum* and *Halictus* species is also season-dependent and can vary from early spring to the end of summer, and some species remain on the wing into mid-autumn. Mated females of eusocial species, such as *Lasioglossum albipes* F., *L. malachurum* K., *L. pauxillum* S. and *Halictus tumulorum* L. appear in March and can fly into October. By mid-summer, these species have produced a brood of workers, and the second brood of males and females flies at the end of summer (Else & Edwards 2018).

Figure 1.3: A general example of a ground-nesting bee nest. Foraging and mating take place above ground. The main life cycle stages are 1) Nest initiation, 2) Food resource provision and egg laying, 3) larval development, 4) Overwintering, and 5) Emergence (Harmon-Threatt 2020).

1.5 Belowground nest construction

Male ground-nesting bee species do not contribute to the construction of nests. However, it has been reported that males of some *Perdita* (Andrenidae) species occasionally dig shallow tunnels as shelter during unfavoured weather conditions or to spend the night. Female bees choose the nesting location and create their nest. Once the favoured nesting location is decided, bees begin excavating using their mandibles and/or hind legs to push the soil out (Martins & Antonini 1994). Dug soil can be used to strengthen the nest's structure, but it is mostly brought to the surface and forms a "tumulus" (soil mound) which can be used as a characteristic of freshly dug nests (Michener 2007). Female ground-nesting bees are equipped with specialised pygidial (on the sixth abdominal segment) and basitibial plates. These plates enable bees to dig and move easily inside their nest (Danforth et al. 2019). Ground-nesting bee nests typically comprise a main vertical tunnel and shorter lateral tunnels leading to elliptical-shaped brood cells. However, there is a variation in nest architecture between ground-nesting bee species (Fig. 1.4). Most bee species create several cells per nest, and Kamm (1974) reported a correlation between bee body size and cell size. Females of most ground-nesting bee species apply a coating of glandular secretions on the wall of their cells (Batra 1964; Danforth 1991). The Dufour's gland produces these secretions, forming a waxy layer around the brood cell (Cane 1981). Brood cell coating might help to protect the offspring from water infiltration and microbial infestation.

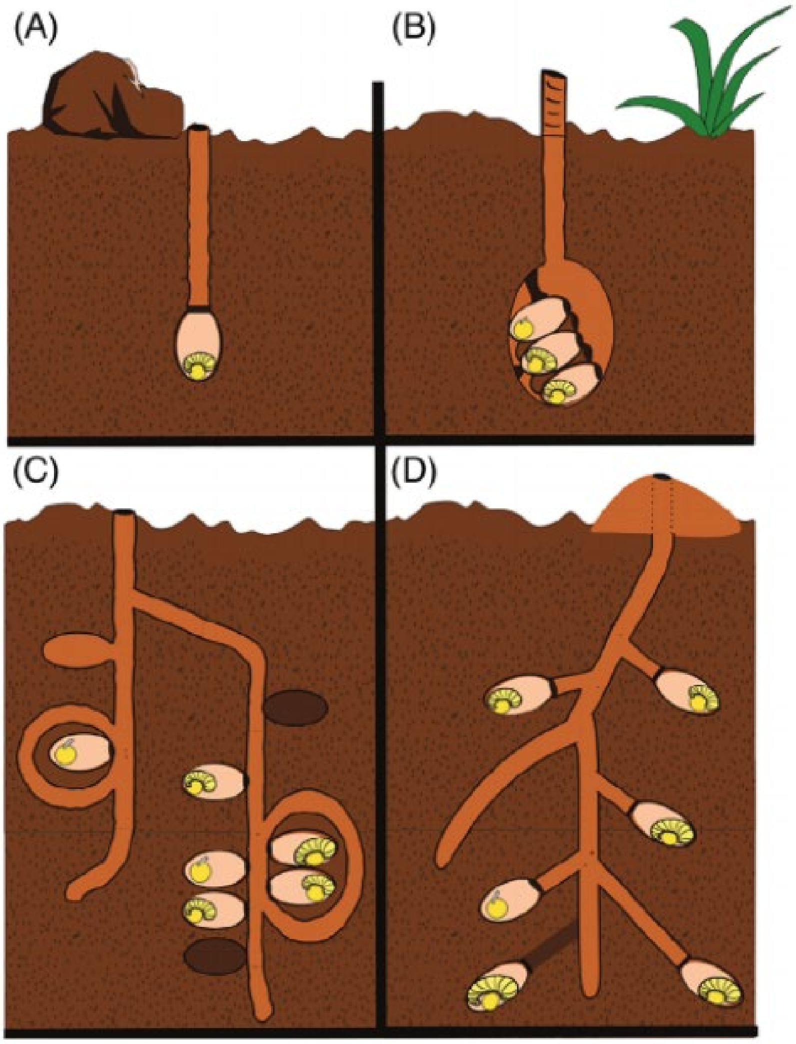


Figure 1.4: Ground-nesting bee nest architecture examples (Antoine & Forrest 2020). Nest design of A) *Epicharis zonata* S. (Roubik & Michener 1980), B) *Augochlorella striata* S. (Packer et al. 1989), C) *Halictus ligatus* S. (Packer & Knerer 1986), and D) *Peponapis pruinose* S. (Mathewson 1968).

1.6 Nest-site selection – Biotic and abiotic factors

Female ground-nesting bees do not care for their offspring other than constructing the nest and provisioning brood cells, therefore high-quality food and the best possible nesting location are the two factors that can maximise the chance of a successful brood. A site that protects from harmful weather conditions, parasites and predators could be considered a suitable nesting site (Roulston & Goodell 2010). Nevertheless, the more time the female bee spends looking for the ideal nesting location, the less time she will have to construct the nest, provision, and lay eggs. Therefore, time and energy spent on nest-site selection and construction of nests have fitness costs, the same as energy spent on long foraging trips can trade off against offspring production (Zurbuchen et al. 2010). This might suggest that nest-site selection has a critical role in the fitness of a female bee (Brockmann 1979).

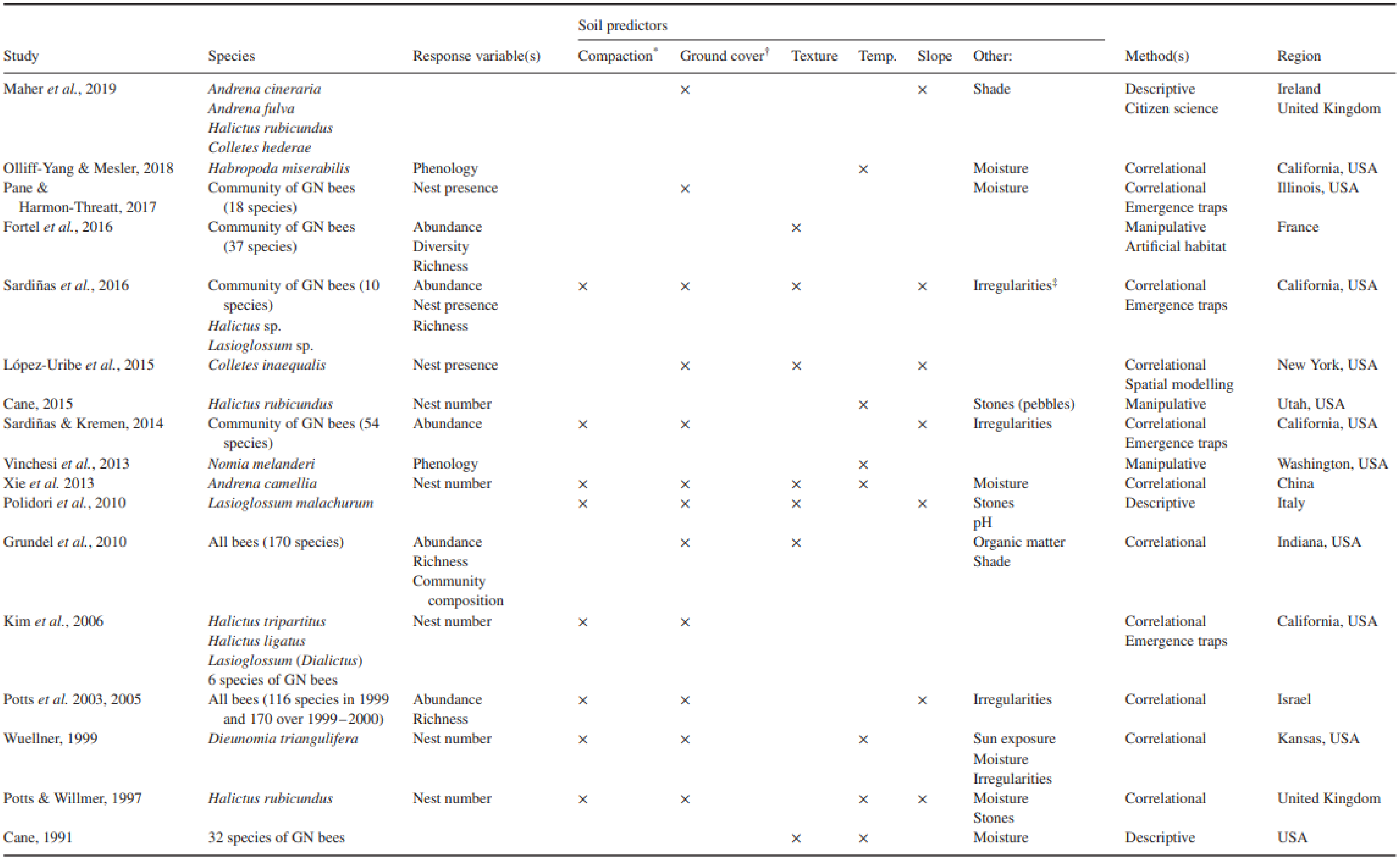
Ground-nesting bee nesting ecology and substrate preferences are relatively under-researched compared to their foraging ecology and floral preferences. Natural selection favours individuals capable of choosing the best nesting environment, which would contribute to the success of a given nest. Several abiotic and biotic factors might influence ground-nesting bees, including soil compaction, soil texture, soil temperature, soil moisture, organic matter, root biomass, stoniness and gradient of nesting habitat (Table 1.1).

1.6.1 Soil compaction

Studies have indicated that the compaction of soils is an important factor when ground-nesting bee females choose the best nesting ground (Brockmann 1979; Potts & Willmer 1997; Kim et al. 2006; Polidori et al. 2010; Tsiolis et al. 2022). The excavation process of a nest is a significant investment for most ground-nesting Hymenoptera having both energy and time costs (Michener 1956; McCorquodale 1989). Female *H. rubicundus* C. have been observed having nest-site selection behaviour, visually searching for the presence of other nest entrances in a landscape (Potts & Willmer 1997) and even biting the soil surface and carrying out digging tests to potentially determine the soil hardness of a specific soil landscape (S. G. Potts personal observation, cited in Potts & Willmer 1997. Highly compacted substrates might also have a negative impact on the fitness of digging female bees, such as in fossorial ant communities where the adult excavation specialist have higher mortality rates than the non-specialist, thought to be related to increased energy requirements and desiccation (Page Jr & Fewell 1999; Johnson 2000).

Nevertheless, harder soils have their benefits. Potts & Willmer (1997) noted that *H. rubicundus* C. had initially selected soft soils, but as the aggregation increased, this species favoured harder soils. Harder soils might better support the structure of bee nests, especially in dense nesting aggregations. Wuellner (1999) has reported that the Halictidae bee *Dieunomia* *triangulifera* V. shows nesting preference for compacted soils. The authors suggest that: 1) it might be easier for this species to start a nest on harder than loose powdery soils, which could cave in as the bees excavate, and 2) dense soils might retain higher temperatures contributing to the development of immatures in the ground. Conversely, Sardiñas & Kremen (2014) indicated that surface soil compaction had decreased the number of nesting bees. Srba & Heneberg (2012) recorded some sphecid wasp species showing a preference for low soil compactness and other sphecid species utilising highly compacted soils. A possible explanation for this variation is given by Ghazoul (2001), who found a positive correlation between soil hardness and the body size of solitary ground-nesting wasps. This suggests that the preferred soil compaction level might vary between bee species and the community composition of bees present at a given site.

Table 1.1: A list of studies (non-exhaustive) investigated the soil factors associated with the nests of ground-nesting (GN) bees (Antoine & Forrest 2020).



			Soil predictors							
Study	Species	Response variable(s)	Compaction*	Ground cover†	Texture	Temp.	Slope	Other:	Method(s)	Region
Maher *et al.*, 2019	*Andrena cineraria* *Andrena fulva* *Halictus rubicundus* *Colletes hederae*			×			×	Shade	Descriptive Citizen science	Ireland United Kingdom
Olliff-Yang & Mesler, 2018	*Habropoda miserabilis*	Phenology				×		Moisture	Correlational	California, USA
Pane & Harmon-Threatt, 2017	Community of GN bees (18 species)	Nest presence		×				Moisture	Correlational Emergence traps	Illinois, USA
Fortel *et al.*, 2016	Community of GN bees (37 species)	Abundance Diversity Richness			×				Manipulative Artificial habitat	France
Sardiñas *et al.*, 2016	Community of GN bees (10 species) *Halictus* sp. *Lasioglossum* sp.	Abundance Nest presence Richness	×	×	×		×	Irregularities‡	Correlational Emergence traps	California, USA
López-Uribe *et al.*, 2015	*Colletes inaequalis*	Nest presence		×	×		×		Correlational Spatial modelling	New York, USA
Cane, 2015	*Halictus rubicundus*	Nest number				×		Stones (pebbles)	Manipulative	Utah, USA
Sardiñas & Kremen, 2014	Community of GN bees (54 species)	Abundance	×	×			×	Irregularities	Correlational Emergence traps	California, USA
Vinchesi *et al.*, 2013	*Nomia melanderi*	Phenology				×			Manipulative	Washington, USA
Xie *et al.* 2013	*Andrena camellia*	Nest number	×	×	×	×		Moisture	Correlational	China
Polidori *et al.*, 2010	*Lasioglossum malachurum*		×	×	×		×	Stones pH	Descriptive	Italy
Grundel *et al.*, 2010	All bees (170 species)	Abundance Richness Community composition		×	×			Organic matter Shade	Correlational	Indiana, USA
Kim *et al.*, 2006	*Halictus tripartitus* *Halictus ligatus* *Lasioglossum* (*Dialictus*) 6 species of GN bees	Nest number	×	×					Correlational Emergence traps	California, USA
Potts *et al.* 2003, 2005	All bees (116 species in 1999 and 170 over 1999–2000)	Abundance Richness	×	×			×	Irregularities	Correlational	Israel
Wuellner, 1999	*Dieunomia triangulifera*	Nest number	×	×		×		Sun exposure Moisture Irregularities	Correlational	Kansas, USA
Potts & Willmer, 1997	*Halictus rubicundus*	Nest number	×	×		×	×	Moisture Stones	Correlational	United Kingdom
Cane, 1991	32 species of GN bees				×	×		Moisture	Descriptive	USA

∗Also called soil hardness / †Includes categories such as vegetation cover, bare ground, litter, etc. / ‡For example, cracks in the soil and small cavities.

1.6.2 Soil texture

Soil is composed of various proportions of sand (0.05 – 2.0 mm), silt (0.002 – 0.05 mm) and clay (> 0.002 mm) particles (US Department of Agriculture 1993; Fig. 1.5). Sand is a soil component that might be assumed as highly preferred by ground-nesting bees as it requires less energy and time to dig. However, highly sandy soils could be disadvantageous and unattractive for ground-nesting bees and Tsiolis et al. (2022) showed that a nesting plot with over 90% sand attracted no bees to nest, possibly due to its inability to support the structure of a nest compared to other highly utilised sandy loam plots. Osgood (1972) also found *Halictus*, *Colletes* and *Andrena* species nesting in gravelly sandy loam. Torchio (1966) reported that utilised nesting sites by the Alkali bee *Nomia melanderi* C. were generally of loam soil texture. Cane (1991) found ground-nesting bees, including *Halictus*, *Lassioglossum* and *Andrena* species, nesting in clay loam, sandy loam and silt loam but found no bees nesting in silt or clay soils. The author also suggests that bee species could be separated into two groups based on soil texture preferences; 1) species with nesting preference in loamy sands and sands, and 2) species with nesting preference in loams (sandy-, silt-, sandy clay- or clay-; Cane 1991). However, a study of human-made soil structures provided as bee nesting resources consisting of various soil textures, does not fully agree with this statement as it showed that bee species richness was not influenced by soil texture (Fortel et al. 2016). Potts & Willmer (1997) reported that soils where *H. rubicundus* C. nested were all in the sand/loamy sand/sandy loam category, but none of the soil texture variables was correlated with the number of bee nests. Another study focusing on the nesting requirements of the Halictidae bee *L. malachurum K.* shows a variation of preferred soil texture from high percentages of sand to high percentages of silt, but clay and gravel percentages were generally low (Polidori et al. 2010). Additionally, *N. melanderi* C. (Hymenoptera: Halictidae) is reported to prefer nesting in silty soil beds (Johansen et al. 1978). Soil texture might play a role in nest-site selection for ground-nesting bees, but currently it is not possible to identify the ideal texture for specific species as it might be highly dependent on other biotic and abiotic factors present at various nesting sites.

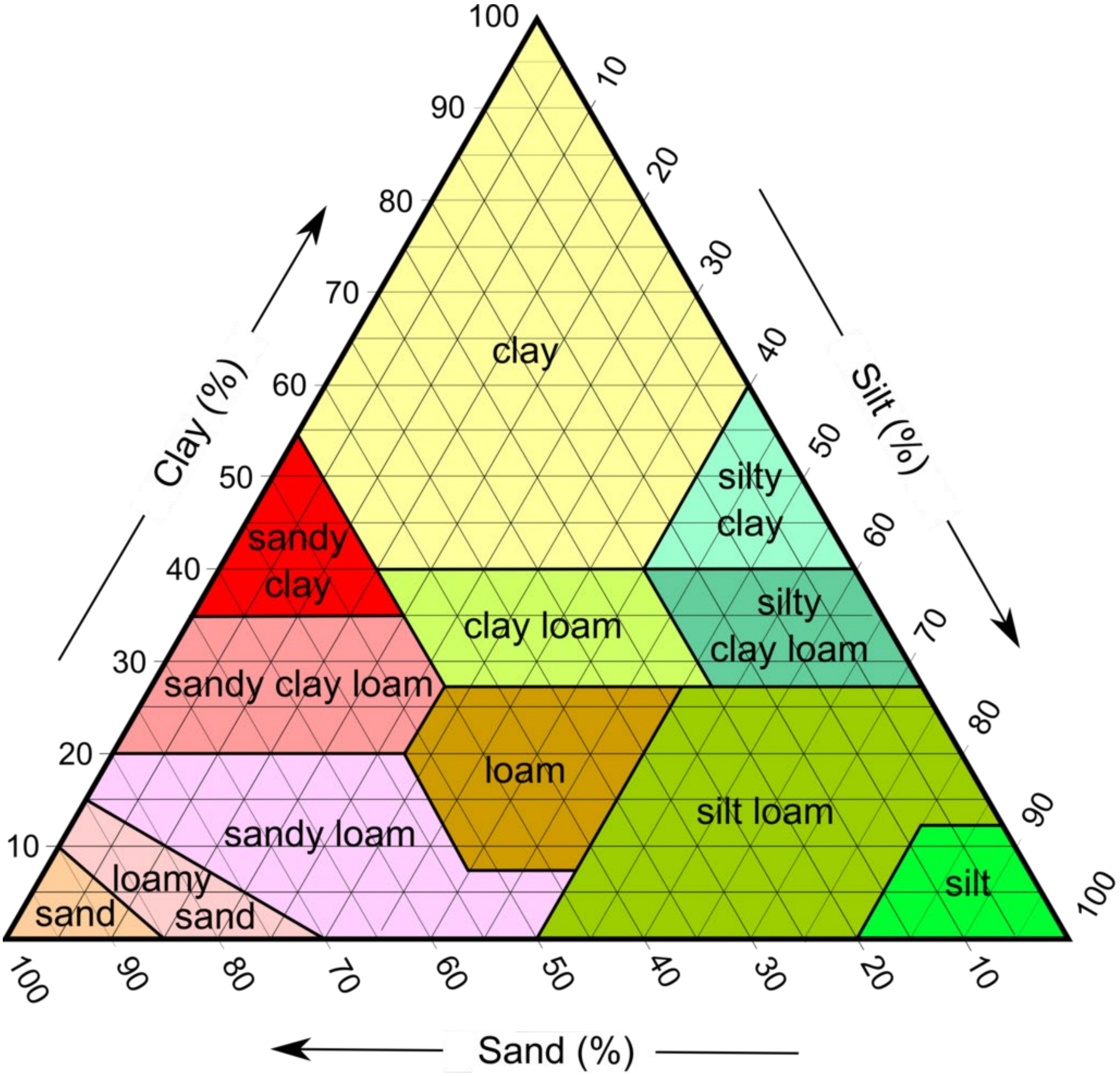


Figure 1.5: Soil Texture Triangle (Source: CEH Cosmos-UK).

1.6.3 Temperature

Bees are ectothermic and depend on temperature for activity (Woods et al. 2005) and mating behaviour (Larsson 1991). Nest temperature is also considered a significant component of nest productivity of Hymenoptera as it determines the development rates of eggs and larvae (Miyano 1981) and influences brood emergence timing, which can be closely related to survivorship (Jeanne & Morgan 1992; Forrest 2017). Ground-nesting bees cannot control the temperature of their nest, although they can be considered partially endothermic as they can increase their body temperature using their thoracic flight muscles (Stone & Willmer 1989). Additionally, the effort to raise body temperature when ambient temperatures are low have a metabolic cost (Willmer & Stone 2004). Subsequently, temperature can also influence voltinism in bee species (number of broods per year). For example, Forrest et al. (2019) reported that temperatures at early larval development determined whether the cavity-nesting bee *Osmia iridis* C. adopted a one- or two-year life cycle.

There is variability in soil surface temperature during a day (Xu et al. 2002), which could influence the nest-site selection of ground-nesting bees. For instance, the nest temperature of some ant and termite species can be influenced by nest orientation, which determines the amount of sunlight and heat absorbed by a nest (Jones & Oldroyd 2006). Indeed, *Lasioglossum duplex* Dalla Torre (*Halictus duplex*) preferentially nests in areas exposed to morning sunlight (Sakagami & Hayashida 1961). Potts & Willmer (1997) reported that nests of *Halictus rubicundus* were recorded at locations with significantly warmer soil temperatures than locations without bee nests. The authors also found that bees favour south-facing steep slopes, a factor that could be related to greater sunlight absorption. Soil surface temperature is considered a good indicator of the thermal properties of substrate area, and it is believed to be why females spend some time basking at different points on the substrate's surface during the nest founding period (Potts & Willmer 1997). A recent study reported that bee species richness and abundance was positively correlated with soil temperature at 5cm depth and south-facing 180° slope (Neumüller et al. 2022).

Bees are expected to nest in south- or south-east facing locations in the northern hemisphere with high sunlight exposure for maximum heat in the morning to benefit the female's activity and development of larvae (Antoine & Forrest 2020). However, bees could adopt the opposite strategy in very warm regions to prevent overheating of the brood. Indeed, ground-nesting bees were observed nesting in north-facing locations during summer months in south Europe and north Africa (personal communication, Stuart Roberts).

1.6.4 Soil moisture

Soil moisture could be considered a significant factor for bee nesting, as moist soils are usually easier to excavate. Cane (1991) reported that the soil moisture of 32 bee species' nests varied between 2.7 and 37.8 %. Potts & Willmer (1997) reported the mean soil moisture of *H. rubicundus* C. at 80 %. Bees have also been recorded nesting in soils submerged with water for a short period of time (Roubik & Michener 1980; Visscher et al. 1994) and even in constantly waterlogged soils (Pietsch et al. 2016). The ability of bee species to nest in sites with such high moisture content might be highly advantageous in areas with high competition for nesting resources.

Soil moisture is an important element for several ground-nesting species. For example, the ground-nesting bee *N. melanderi* C. prefers soils with high moisture content (Johansen et al. 1978), often maintained with irrigation (Cane 2008). *L. albipes* F., *A. haemorrhoa* F. and *Andrena marginata* F. create water-insoluble substances on pollen balls or interior walls that polymerise or solidify their subterranean nest cells, and it seems crucial for the maintenance of moisture homeostasis in the cell (Shinn 1967; May 1972; Cane 1981). Another function of these substances is possibly preventing excess water from entering the cells (May 1972; Roubik & Michener 1980). Excessive moisture inside brood cells could result in mould development or degradation of the food supply (Ordway 1984). In contrast, ground-nesting bees might choose soils with higher moisture content in dryer habitats. *D. triangulifera* V. has been recorded initiating the construction of nests only when the soil is moist enough (Wuellner 1999).

1.6.5 Stoniness

Aboveground and belowground stoniness is another factor that might determine nest-site selection. *H. rubicundus* C. was recorded as having a strong preference to nest near or under aboveground stones (Potts & Willmer 1997). Preference to nest near aboveground stones was also reported for *Lasioglossum* (*Evylaeus*) *comagenense*, *Lasioglossum* (*Evylaeus*) *cinctipes*, and *A. striata* S. (Packer et al. 1989). Additionally, Cane (2015) tested this nesting preference experimentally by adding pebbles on the surface of bare soil and found that the number of bee nests next to pebbles was significantly higher than in bare soil. A possible explanation could be the thermal role that stones might have by absorbing solar radiation and keeping the soil and nest entrance at a relatively warm temperature (Packer et al. 1989; Potts & Willmer 1997; Cane 2015). Ground-nesting bees can learn and evaluate the distance to visual landmarks; hence they could also use stones or pebbles as visual landmarks to locate their nests (Brünnert et al. 1994).

1.6.6 Organic matter

Bees could choose soils rich in organic matter as it makes soils softer, and bees require less effort to dig their nests. Alternatively, soils with a low percentage of organic matter could have high compaction (Hamza & Anderson 2005). Organic matter could also absorb and hold large amounts of water (Bescansa et al. 2006), resulting in waterlogged soils, which can be disadvantageous for the survival of bee nests. Osgood (1972) found species of Halictinae, *Colletes* and *Andrena* nesting frequently in thinner organic layers compared to soils present nearby.

1.6.7 Vegetation cover and root biomass

Studies in the literature reported that increased vegetation is associated with increased soil moisture and decreased soil temperature (Wuellner 1999; Anderson & Harmon-threatt 2016). Wuellner (1999) suggested that *D. triangulifera* V. might find it difficult to excavate their nests in highly vegetated habitats with high root biomass; hence recorded nesting in habitats with little or no vegetation. Roots may also grow into nests and damage cells, and vegetation could be used as perches by parasitic species such as the conopid fly (Wuellner 1999). Nevertheless, Packer & Knerer (1986) have reported *H. ligatus* S. nesting under vegetation. *Colletes succinctus* L. in grass-dominated landscapes with a vertical bare soil area, nested horizontally in the bare soil area, around 10-15 cm below the top soil layer (K. Tsiolis, personal observation) where there was a low density of roots.

1.6.8 Gradient

Ground-nesting bees have been found nesting on banks and other places with sloped surfaces. For example, *Neocorynura fumipennis* F. has been reported nesting in steep banks (Michener 1966), and *Anthophora abrupta* S. in a clay wall (Norden 1984). Two possible explanations could be that slope or vertical surfaces contribute to 1) decreasing the possibility of waterlogging and 2) increasing soil temperature compared to flat surfaces. Both factors can be highly influential to the success of ground-nesting bee nests (Potts & Willmer 1997; Michener 2007).

1.7 Thesis aims

Little is known about the nesting biology of important ground-nesting crop pollinators including what substrates, habitats and ground characteristics they prefer for nesting. In addition, only a few studies have analysed how farm landscape management can provide nesting resources for these valuable ground-nesting crop pollinators. This thesis aims to identify the key parameters influencing solitary bee nesting as a basis to develop practical ways to enhance nesting resources for ground-nesting bees in commercial orchards with a particular interest in the primary apple pollinators *A. nitida* M., *A. haemorrhoa* F., *A. nigroaenea* K. and *A. dorsata* K. (Garratt et al. 2016; Hutchinson et al. 2021).

This thesis focuses on answering three main research questions:

1. Where are ground-nesting bees found in apple orchards and which habitats do they prefer for nesting?
2. Which soil characteristics are preferred by ground-nesting bees in apple orchards?
3. How can nesting resources for ground-nesting apple pollinating bees be effectively provided and managed in commercial orchards?

2 Chapter 2 – The importance of soil and vegetation characteristics for establishing ground-nesting bee aggregations (Published)

Tsiolis K, Potts S, Garratt M, Tilston E, Burman J, Rintoul-Hynes N, Fountain M (2022) The importance of soil and vegetation characteristics for establishing ground-nesting bee aggregations. Journal of Pollination Ecology 31:186–200. [online] URL: https://pollinationecology.org/index.php/jpe/article/view/682

***The author of this thesis has contributed:**

- 50 % to conception and planning of study
- 100 % to literature review
- 100 % to data collection
- 100 % to data analysis
- 95 % to writing of the published paper

This study started in 2018 during my Master's by research and it was continued during my PhD.

2.1 Introduction

Bees (Apoidea: Anthophila) provide essential pollination services to natural (Ollerton et al. 2011) and agricultural (Klein et al. 2007) ecosystems. There are approximately 20,000 known species globally which differ in their ecology, habit preference, body size, and appearance, diverging according to the plants on which they forage (Michener 2007). There are 277 bee species in the British Isles, and the great majority are ground-nesters, including species from *Andrena* Fabricius (68 species), *Lasioglossum* Curtis (33 species), *Halictus* Latreille (8 species), and *Colletes* Latreille (9 species) genera (Else & Edwards 2018). Sixty-four per cent of global bee species are ground-nesters (Cane & Neff 2011).

Yields of many insect-pollinated crops are managed via the addition of honeybees (*Apis mellifera* L.) (Goodwin et al. 2011; Rucker et al. 2012). However, studies increasingly indicate that the contribution of wild pollinators has been underestimated (Kleijn et al. 2015). Garratt et al. (2016) estimated the value of solitary bees for UK apple pollination at £51 million p.a. and the value of honeybees at £21 million p.a. Kleijn et al. (2015) estimated that 81% of the top 100 pollinator bee species were wild ground-nesters.

Ground-nesting bees need floral resources for nutrition, shelter, and appropriate nest sites for the development of their offspring and hibernation until emergence. Ways to provide floral resources for pollinators are comparatively well developed (Sheffield et al. 2008; Rosa García & Miñarro 2014; Campbell et al. 2017); however, methods for provisioning areas for ground-nesting bees are poorly understood (Antoine & Forrest 2020). A recent review by Harmon-Threatt (2020) shows that information on nesting habits and preferences of ground-nesting bees was only available for 26% of the 527 species studied in the USA and Canada.

Since most of a ground-nesting bee's life cycle occurs underground, soil variables are highly likely to play a significant role in nesting bees' success and survival. Bare ground has been frequently linked with increases in fossorial bee nesting (Sardiñas & Kremen 2014). The nest locations of thirty-two bee species across the USA showed that ground-nesting bees did not nest in silt or clay soils but primarily in soils that were 33% - 94% sand (Cane 1991). Potts & Willmer (1997) studied a range of edaphic and microclimatic parameters involved in nesting of the ground-nesting bee *Halictus rubicundus* C. They observed that bees initially nested in soft soils, but gradually moved to hard soils as aggregations grew, probably because this provided a stronger nest structure, avoiding collapse. Soils with greater soil organic matter contents absorb and hold water and might make soils softer and easier for bees to dig (Bescansa et al. 2006). In addition, soil moisture is vital for the development of bee larvae (May 1972) but might also encourage the development of fungal pathogens inside nest cells affecting larval survival (Larsson 1991). The ground-nesting bee *Nomia melanderi* C. is reported to prefer nesting beds in silty textured soils with good hydraulic conductivity, moist subsoils, and without surface vegetation (Johansen et al. 1978). Furthermore, nest temperature is also considered a significant component of nesting success as it determines the rate of egg and larval development (Miyano 1981) and influences the brood's emergence timing, which can be closely related to survivorship (Jeanne & Morgan 1992). *H. rubicundus* C. prefers to nest in warm soils and favours south-facing steep slopes, probably due to high sunlight absorption and a decreased likelihood of waterlogging compared to flat substrates (Potts & Willmer 1997; Michener 2007).

A few studies have investigated entire communities of ground-nesting bees to understand nesting habitat characteristics (Potts et al. 2005; Sardiñas & Kremen 2014) and nest-site selection (e.g. Potts & Willmer 1997). Only a handful of species' preferences in relation to the soil characteristics of nesting habitats are documented (Harmon-Threatt 2020) and these abiotic nesting preferences cannot be reliably extrapolated to most unstudied ground-nesting bee species.

The aim of this study was to 1) determine whether the provision of bare ground plots can provide a nesting resource for ground-nesting bees, 2) identify which species are attracted to artificially constructed nest sites, and 3) identify the preferred biotic and abiotic nesting factors that drive nest site selection of ground-nesting bees.

2.2 Methods and Materials

2.2.1 Study site

The NIAB EMR commercial and experimental fruit farm in Southeast England (51°17′26″N 0°26′02″E) was used for the study. The farm has primarily level topography (< 2° slope) (DEFRA 2005), and the dominant soils are well-drained, non-calcareous Luvisols (IUSS Working Group WRB 2015) of the Malling and Fyfield series (Furneaux 1954). Eight south or southeast-facing bare soil plots were created in mid-February 2018 (Fig. 2.1), firstly by herbicide treatment to reduce vegetation growth, followed by the mechanical removal of surface vegetation and topsoil with a 0.91 m wide bucket digger. All sites were vegetated, primarily with a grass sward, before the construction of the bee plots. Each study plot was 10 x 2 m, and a slope of 10° (Fig. 2.1) was created in half of the plot by scraping off a 10 x 1 m section (0.88 m^3 soil), which was then heaped and pressed onto the adjoining 10 x 1 m section. Plots were created in field headlands, orchard margins, and other uncropped land (see Appendix 1).

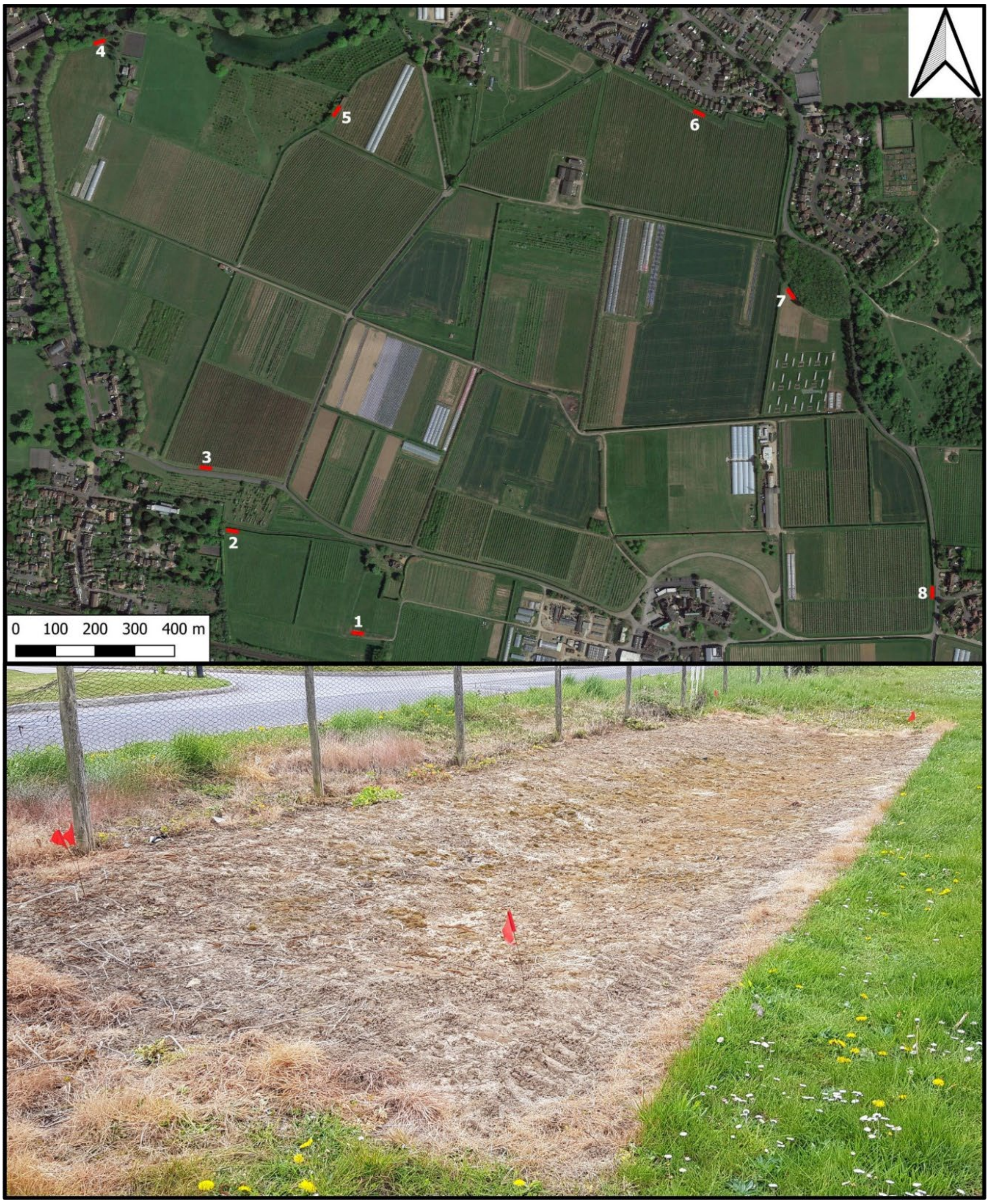


Figure 2.1: Map of bare ground bee nesting plots and a photograph of a plot at NIAB EMR. Above: Map of bare ground bee nesting plots at NIAB EMR (51°17′26″N 0°26′02″E; QGIS Development Team 2020). Below: Example of nesting areas (plot 8) at the beginning of the study in 2018 with a 10° slope (10 x 1 m) section. The entire plot was 10 x 2 m.

The growth of vegetation on plots was monitored visually as % estimates of flat and slope areas during the main solitary bee nesting period (April – July). When vegetation cover exceeded ≈10%, bare ground was reinstated by applying glyphosate at the recommended dose of 1,800 g/ha^{-1}. Eight glyphosate applications were made to all plots over the study period: 23 May 2018, 15 August 2018, 7 May 2019, 18 June 2019, 5 August 2019, 19 March 2020, 10 April 2020, and 24 June 2020.

2.2.2 Monitoring bee species on plots

No nest monitoring took place before the bare plots were established. Following plot establishment, bee activity observations were carried out during sunny and/or mild weather above 10°C if cloud cover did not exceed 4 oktas. On cloudier days, (5 – 8 oktas), surveys were conducted if the temperature was above 14°C. Also, wind speeds were below Beaufort scale of 5, or 29 km/h (Pywell et al. 2005). Surveys took place between mid-April and May in 2018 and between mid-April and mid-August in 2019 and 2020. These months are within the active flight period of most of *Andrena*, *Halictus* and *Lasioglossum* mining-bee species in the UK (Else & Edwards 2018). Plots were surveyed twice weekly in 2018 and every two weeks in 2019 and 2020 (weather permitting, 10 surveys per year) in a randomised order on each date, so over the study period, plots were sampled at different times of the day, including both the morning and afternoon. Each plot was observed for 30 minutes while walking slowly around the perimeter. Once a bee arrived on the plot, it was collected using a sweep net (Watkins & Doncaster, Leominster, UK) and placed into a 5 ml clear plastic tube in an ice bucket where bees were kept inactive until the end of the observation period (Grixti et al. 2009). Subsequently, bees were identified to species level in the field. If this was not possible, photographs including the key characteristics were taken for later identification and confirmation by Mr Mike Edwards, a professional entomological consultant (Else & Edwards 2018). Bees that were not identifiable by photographs were grouped by their body length (Small = 3 – 5 mm, Medium = 5 – 7 mm). Bees were subsequently released next to the plot.

Whenever notable numbers of kleptoparasites and wasps were observed, samples were collected for later identification in the laboratory.

2.2.3 Bee nesting observations

The number of bee nests on each plot's flat and slope sections prior to each 30-minute bee survey was recorded at each visit. Bee nests were distinguished from holes made by other invertebrates, including ants and earthworms, by the characteristic tumuli (volcano-shaped mounds of earth) that females make in the nest excavation phase. Ants also create similar soil structures, although these are usually formed of scattered soil particles compared to solitary bee tumuli, which have a more organised and firm structure (K. Tsiolis personal observation). Nests were not counted if there was any doubt about the nest occupant. On each plot's flat and sloped sections, fifteen nest entrance holes were measured using digital callipers to the nearest tenth of a millimetre (Preciva IP54) in the 2019 and 2020 surveys. Starting from the edge of each plot, three nests were measured from the middle or nearest to the middle every two meters. Once a nest was measured, a 12 cm long plastic plant label was placed on the north side of the nest to ensure that it would not be measured again. The total number of bee nests on each plot was also recorded. Wind or rain can destroy tumuli throughout the season, and so the number of bee nests recorded fluctuated between counts. Therefore, peak nest density (the highest number of nests recorded on a plot section each year) was used in all analyses.

2.2.4 Soil characteristics

From each plot, eight soil cores (D 10 cm x W 5 cm) were removed using a Buerkle soil sampler (Fisher Scientific International Inc., Hampton, US) in 2018 and 2020. Four samples were taken from each plot's sections (flat and slope). All samples were taken at least 2.4 m from each other and at least 30 cm away from the plot (Appendix 2). Soil water content was determined gravimetrically (80°C, five days; Martin-Vertedor & Dodd 2011). To measure root biomass, large aggregates were gently crushed with a mortar and pestle, and all visible root material was removed manually and weighed. The mass of root material was compared to the total weight per mass of each core to give root biomass in percentage. The stoniness of the samples was measured by hand sieving the sample through a 2 mm aperture test sieve for 30 seconds to separate the gravel fraction (Potts & Willmer 1997). The stones were weighed and calculated as a percentage of the total soil weight. Two grams of the remaining non-gravel fraction of each sample was then used to determine organic matter content by loss on ignition (550°C, 7 hrs; Goldin 1987). From two soil samples per slope and two per flat section collected in 2018, the percentage content by mass of gravel (> 2.0 mm), sand (0.05 – 2.0 mm), silt (0.002 – 0.05 mm) and clay (< 0.002 mm) was determined by sedimentation using the Bouyoucos hydrometer method (Lesikar et al. 2005). These measurements were used to determine soil texture according to the USDA texture classification system (Soil Survey Staff 2014). Hydraulic conductivity was measured from three samples per slope and three per flat section using mini disc infiltrometers (METER Group Inc., Washington, US). The devices were placed where soil cracks, bee nests and ant nests were absent. A suction rate of 2 cm s^{-1} was chosen except for plot 5, where a suction rate of 6 cm s^{-1} was used due to the high sandy soil texture of the plot. Water infiltration was recorded every 3 minutes (measurements were taken in 2018 and 2020).

Soil compaction (unconfined compression strength; kg/cm^2) was assessed in 2018 and 2020 by taking three probing readings per slope and flat section using an electronic recording cone penetrometer (Solutions for Research Ltd, Bedfordshire, UK), Readings were taken at 2.5 cm intervals and the mathematical mean calculated for those collected from the top 10 cm. the cone index was corrected for soil moisture content (measured using a MO750 model soil moisture meter; Extech Instruments Corp., New Hampshire, U.S.) according to Busscher et al. (1997).

Soil moisture and soil temperature were recorded twice in spring and twice in summer of 2020 as the mean of four positions per flat and slope sections. The HH2 moisture meter and a WET-2 sensor (Delta-T Devices Ltd., Cambridge, UK) were used. In addition, soil temperature was also measured in each plot every two hours from 28/03/20 to 12/08/20 by burying an iButton

Thermochron® data logger (model DS1921G-F5# (± 0.5°C), Premier Farnell Ltd., Leeds, UK) at 10 cm depth (following Potts & Willmer 1997) in the centre of each of the two plot sections. In situ soil measurements were made for the top 10 cm because this is the typical depth at which the brood cells of ground-nesting bees are found.

2.2.5 Vegetation cover

Percentage vegetation cover was visually monitored, using a 50 cm^2 quadrat, from the beginning of the plot creation. It was noted that there was an accumulation of moss and thatch on the soil surface from the spring of 2020. Hence this was recorded twice in the spring and twice in the summer of 2020, following the systematic method used for soil sampling (Appendix 2).

2.2.6 Statistical analyses

R studio (Version 1.2.5019; RStudio Team 2019) was used for all statistical analyses. Soil texture was plotted on soil texture triangles in the R package ‘plotrix’. Peak nest density was tested with the Shapiro–Wilk test, it was not normally distributed, and non-parametric statistical approaches were used.

Two Generalised Linear Mixed Effect Models (GLMMs, R package: lme4) were used to determine whether there was a significant relationship (positive or negative) between peak nest density (response) and soil variables measured (predictor). A negative binomial distribution was used due to excessive overdispersion with the Poisson distribution. Soil variables, year, and gradient (flat vs slope) were set as fixed effects, and plot was set as a random effect. The variance inflation factor (R package: car) was used to identify variables with high multicollinearity. Low multicollinearity was located (all variables VIF < 3). A few variables were only measured in 2020 (soil temperature and moisture (WET sensor) and vegetation cover), and as a result, a second GLMM was used, including all variables measured in 2020. After examination of model fit, a Poisson distribution was preferred, measured soil variables and gradient were set as fixed effects, and plot as a random effect. The variance inflation factor (R package: car) was used to identify variables with high multicollinearity, and as a result, soil water content and root biomass were removed due to high multi-collinearity (VIF > 10). As soil texture is determined by the percentage of sand, silt, and clay particles, there is high multi-collinearity between these measurements (VIF > 10). The effects of multi-collinearity were avoided using data for sand (highest percentage) for both models.

To explore the effects of slope, year and plot using data from all three years, a Kruskal-Wallis rank test was used to determine whether there was a significant change in peak nest density between years and between plots. The Dunn's Test of Multiple Comparisons (R package: rstatix) was used to identify which plots significantly differed from which. A Mann-Whitney U test was used to identify whether the slope section of plots showed significantly greater peak nest density than the flat section.

The data collected from the soil temperature data loggers were divided into four periods for analysis: early morning: 01:00 – 05:00, late morning: 07:00 – 11:00, afternoon: 13:00 – 17:00, and night: 19:00 – 23:00. A Kruskal-Wallis rank test was used to test whether there was a significant difference between the four periods, and a Dunn's Test of Multiple Comparisons (R package: rstatix) to identify significant differences between time periods.

To determine the range of each variable that bees can tolerate for nesting, the minimum and maximum values of each soil variable were calculated. Additionally, the value of soil variables at plots with the highest peak nest density was used to establish a potential optimum value promoting bee nesting.

2.3 Results

2.3.1 Bee activity

Over the three years of sampling, 870 bee specimens were identified (122 in 2018, 397 in 2019 and 351 in 2020), belonging to 26 species (14 in 2018, 14 in 2019 and 19 in 2020) (Appendix 3). Five hundred bee specimens were identified to species level and the remainder to genus level. The most abundant bee species (non-kleptoparasitic) was *Lasioglossum malachurum* Kirby (39.4%), second most abundant *Lasioglossum calceatum* S. /

Lasioglossum albipes F. (10.2%), and third most abundant *Andrena flavipes* P. (6.2%) (Fig. 2.2). Abundances of non-kleptoparasitic bee species vary across years, with *Lasioglossum calceatum* S. / *Lasioglossum albipes* F. being the most abundant species in 2018 and *Lasioglossum malachurum* being the most abundant species in 2019 and 2020. Eleven species, representing 49.7% of the total number of bee specimens, were kleptoparasites: *Sphecodes* spp. (79.2%) and *Nomada* spp. (14.8%) (Fig. 2.2). Furthermore, thirteen wasp species were recorded, representing 12% of the total number of specimens (Appendix 3).

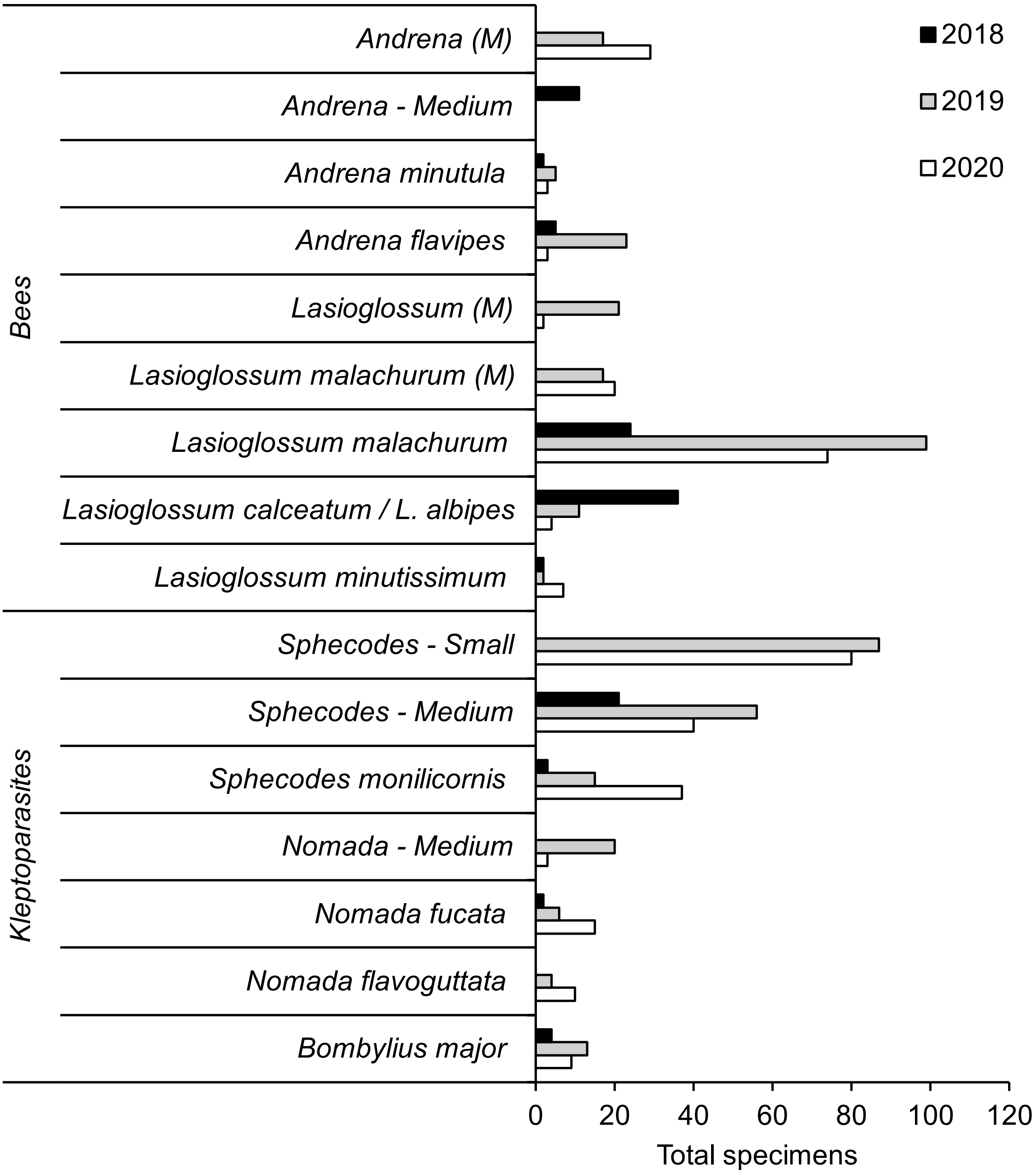


Figure 2.2: Total number of bees and kleptoparasites (> 10 individuals in total) sampled from all eight artificially created nesting plots in 2018, 2019 and 2020. Specimens that were only possible to identify to genus were grouped by their body length (small = 3 – 5 mm, medium = 5 – 7 mm, large = 7+ mm), and male specimens (M) were recorded separately. See full list of recorded species at Appendix 3.

2.3.2 Nest density

Peak nest density on plots did not significantly differ between years (Kruskal-Wallis rank test / *P* = 0.605; Fig. 2.3A). However, some plots attracted greater numbers of ground-nesting bees (Kruskal-Wallis rank test / *P* < 0.000; Fig. 2.3B) than others. The peak nest density on plot 5 was significantly less than on plot 2 (*P* = 0.010), 7 (*P* < 0.000) and 8 (*P* = 0.001), and the peak nest density on plot 7 was significantly greater than plot 1 (*P* = 0.013) (Dunn's Test of Multiple Comparisons). There was no significant difference in peak nest density between flat and slope sections (Mann-Whitney U test / *P* = 0.243; Fig. 2.3C). The mean diameter of nest entrances, measured in 2019 and 2020, was 3.08 ± 0.03 mm (*N* = 1,295, see Appendix 4), indicating that even though there were different species on the plots nest entrances were of similar size.

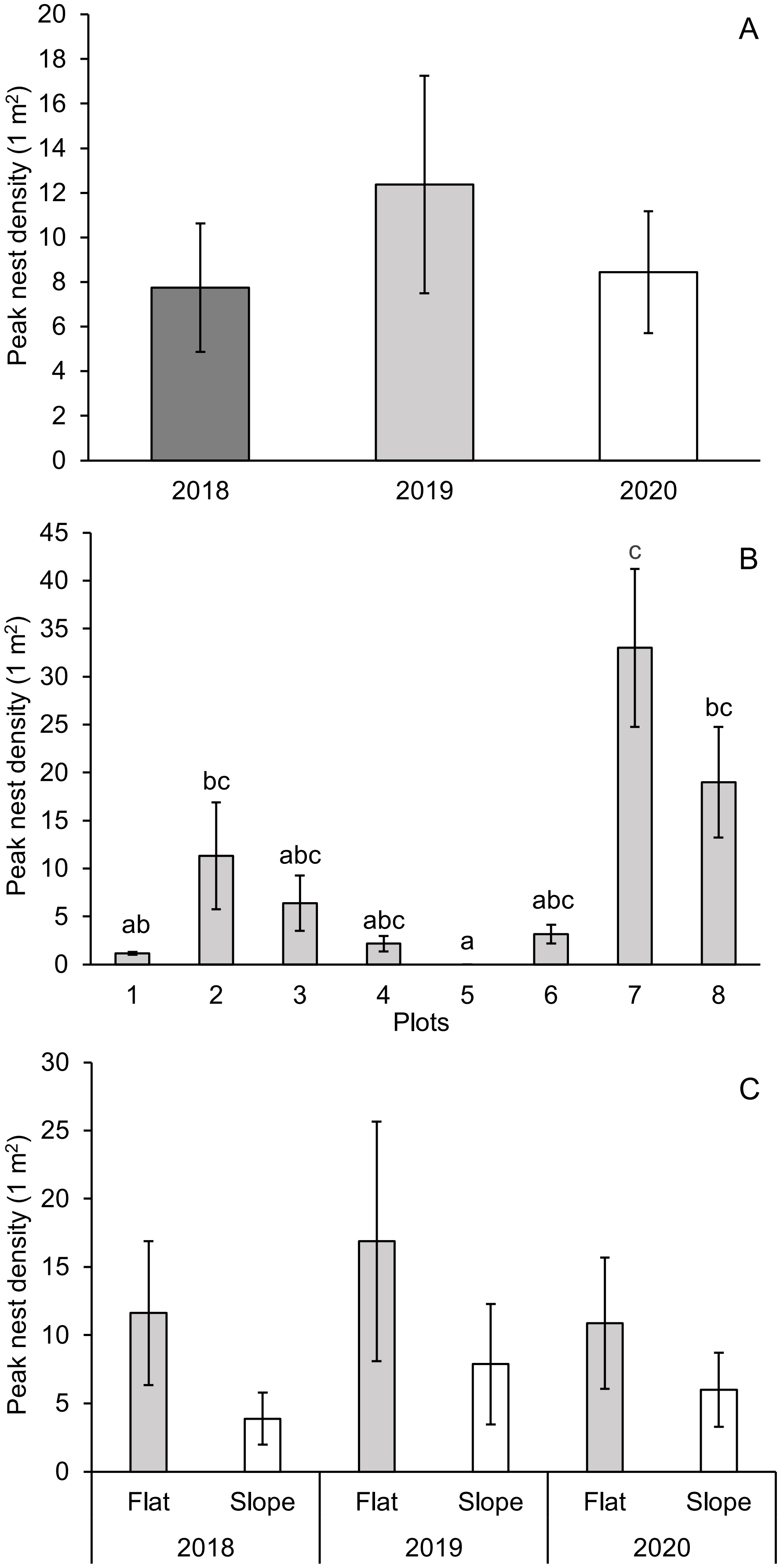

A
Peak nest density (1 m^2)
0
2
4
6
8
10
12
14
16
18
20
2018
2019
2020
B
Peak nest density (1 m^2)
0
5
10
15
20
25
30
35
40
45
ab
bc
abc
abc
a
abc
c
bc
1
2
3
4
5
6
7
8
Plots
C
Peak nest density (1 m^2)
0
5
10
15
20
25
30
Flat
Slope
Flat
Slope
Flat
Slope
2018
2019
2020

Figure 2.3: (A) Mean (± SE, *N* = 8) peak nest density for ground-nesting bees in artificially created nesting plots in 2018, 2019 and 2020. Peak nesting density was used because nest identification varies at each assessment depending on recent rain events, which can destroy the tumuli. There was no difference between years. (B) Mean (± SE, *N* = 8) peak nest density for ground-nesting bees in artificially created nesting plots over three years (2018 – 2020). Different letters indicate significant differences between plots ($P < 0.05$) according to Dunn's Test of Multiple Comparisons. (C) Mean (± SE, *N* = 8) peak nest density on the artificially created nesting plots over three years (2018 – 2020) on the flat and 10-degree sloped area. There was no difference between the flat and slope sections.

2.3.3 Soil characteristics

Measurements of abiotic soil variables from 2018 and 2020 are summarised in Table 2.1. The ranges (min-max) of soil variables where bees were observed to nest were; 4.1 – 14.6 kgf cm^{-2} for soil compaction, 5.1e-5 – 5.35e-4 (cm s^{-1}) for hydraulic conductivity, 1.0 – 34.6% for stoniness, 3.6 – 15.9% for organic matter, 25.5 – 29.9°C for soil temperature, 13.5 – 19.7% for soil moisture, 29.3 – 70.2% for vegetation cover and 0 – 0.1% for root biomass. Bees also tended to nest where vegetation cover on plots was 5 – 73% thatch, then bare ground 5 – 95%, moss 0 – 58% and finally green vegetation 0 – 27% (see Appendix 7).

The soil texture of study plots was mostly sandy-loam or sandy-clay-loam, except for plot 5, which was in the sandy category (see soil texture triangles in Appendix 5). The ranges of soil textures where bees mostly nested were sand 48.4 – 70.9%, silt 9.4 – 34.4% and clay 12.82 – 23.6%. The highest peak nest density was recorded at 8.6 kgf cm^{-2} for soil compaction, 2.4e-4 (cm s^{-1}) for hydraulic conductivity, 19.8% for stoniness, 3.8% for organic matter, 28.5°C for soil temperature, 13.6% for soil moisture, 40.6% for vegetation cover, 0.02% for root biomass, and for soil texture; sand 60.4%, silt 22.2%, and clay 17.4% (sandy-loam category). There was a significant difference in soil temperature between early morning (16.7 ± 0.06°C (*N* = 6,480)), late morning (17.0 ± 0.06°C (*N* = 6,480)), afternoon (21.8 ± 0.07°C (N=6,480)), and night (20.1 ± 0.07°C (*N* = 6,480)) (Kruskal-Wallis rank test / $P < 0.000$; Appendix 6).

Table 2.1: Mean ± SE, range and optimum values for soil and vegetation variables measured from eight study plots in 2018 and 2020.

	Variables	**Mean ± SE (*N*)**	**Range (Min – Max)**	**Optimum value**
Bee nests	Peak nest density (1 m^2)	9.3 ± 2.2 (28)	1 – 38	38
Plot characteristics	Hydraulic conductivity (cm s^{-1})	1.9e-4 ± 1.5e-5 (84)	5.1e-5 – 5.35e-4	2.4e-4
	Soil compaction (kgf cm^{-2})	9.0 ± 0.3 (84)	4.1 – 14.6	8.6
	Soil water content (%)	16.0 ± 0.1 (224)	13.5 – 19.7	13.6
	Soil temperature (°C)	27.5 ± 0.1 (224)	25.5 – 29.9	28.5
Vegetation cover	Green vegetation (%)	2.4 ± 0.4 (192)	0 – 27	0.9
	Thatch (%)	48.9 ± 1.5 (192)	5 – 73	34.6
	Moss (%)	9.5 ± 0.9 (192)	0 – 58	5.1
	Bare ground (%)	39.2 ± 1.5 (192)	5 - 95	59.4
Soil Composition	Organic matter (%)	5.7 ± 0.2 (112)	3.6 – 15.9	3.8
	Stoniness (%)	14.4 ± 1.0 (112)	1 – 34.6	19.8
	Root biomass (%)	1.9e-2 ± 3.0e-3 (32)	0 – 0.1	0.02
	Texture - Sand (%)	60.6 ± 1.2 (16)	48.4 – 70.9	60.4
	Texture - Silt (%)	21.7 ± 1.3 (16)	9.4 – 34.4	22.2
	Texture - Clay (%)	17.8 ± 0.6 (16)	12.82 – 23.6	17.4

2.3.4 Substrate parameters and nest density

The model which included variables measured in 2018 and 2020, showed no significant correlation with peak nest density (Appendix 8). The model which included variables measured only in 2020, indicated that hydraulic conductivity (GLMM, *P* = 0.028), soil temperature (*P* = 0.007), stoniness (*P* = 0.009) and vegetation cover (*P* = 0.005) were significantly correlated with peak nest density (Table 2.2).

Table 2.2: GLMM analyses of soil variables for 2020 only. The variables which had significant correlation with peak nest density were hydraulic conductivity, soil stoniness, soil temperature and vegetation cover. Significant variables: 0 '***' / 0.001 '**' / 0.01 '*' / 0.05 '

Variables	**Estimate**	**Std. Error**	**z value**	***P* value**	**VIF**
Slope	0.338	0.351	0.963	0.335	1.890
Hydraulic conductivity	-1.273	0.580	-2.194	0.028 *	1.355
Soil compaction	-0.060	0.332	-0.182	0.856	1.562
Soil organic matter	0.279	0.300	0.930	0.352	1.538
Soil stoniness	0.632	0.245	2.578	0.009 **	1.793
Soil texture (sand)	0.628	0.585	1.074	0.283	1.373
Soil temperature (WET)	0.968	0.359	2.699	0.007 **	1.367
Soil moisture (WET)	0.162	0.404	0.401	0.688	1.239
Vegetation cover	-0.669	0.238	-2.815	0.005 **	1.645

2.4 Discussion

Artificial and ecological experimental studies investigating the nesting preference of ground-nesting bee species are limited (Orr et al. 2022). This study demonstrates that the provision of bare ground plots can provide a nesting resource for ground-nesting bees. The key nesting factors influencing nest site selection and peak nest density were stoniness, soil temperature, hydraulic conductivity, and vegetation cover. Hydraulic conductivity and vegetation cover had a negative relationship, and soil temperature and stoniness had a significant positive relationship with peak bee nest numbers.

2.4.1 Species using the nesting sites

The 18 non-parasitic ground-nesting bee species identified were 7 *Andrena*, 9 *Lasioglossum*, 1 *Halictus* and 1 *Colletes* spp. (Appendix 3) representing 15% of mining bee species in Britain and Ireland (Else & Edwards 2018). Fortel et al. (2016) used human-made bare ground bee nesting structures and attracted 31 non-parasitic ground-nesting bee species within two years. This, and the present study, indicate that many ground-nesting bee species have similar nesting requirements. However, it is important to consider that in this study, 83.8% of recorded non-parasitic bee specimens (identified to species level) belonged to four bee species, *Lasioglossum malachurum* K. (62.1%), *L. calceatum* S. / *L. albipes* F. (13.5%), and *A. flavipes* P. (8.2%). These species may have dominated the plots as fast colonisers rather than because such plots and soil characteristics are more suitable for them than other bee species. The remaining recorded bee species might establish more slowly, so longer studies are necessary to capture their potential for colonisation.

In this study, 93% of identified bee specimens (species level) belonged to 9 species (non-kleptoparasites) which included bivoltine (spring and summer brood per year; *Andrena dorsata* K., *A. flavipes* P. and *A. minutula* K.), and eusocial (*Lasioglossum pauxillum* S., *L. malachurum* Kirby, *L. calceatum* S., *L. albipes* F., *Lasioglossum morio* F. and *Halictus tumulorum* L.) species (Else & Edwards 2018). Moreover, the mean nest entrance diameter (3.08 ± 0.03 mm, Appendix 4) indicated that small *Lasioglossum*, *Halictus* and *Andrena* species were utilising the plots rather than larger Andrenids, such as *Andrena cineraria* L., *Andrena haemorrhoa* F., and *Andrena nitida* M. (6+ mm, K. Tsiolis, personal observation). The preference for these species to nest in bare soil needs further investigation.

2.4.2 Parasitism

Parasitic species arrived at the same time as bees from the first year of plots' establishment. Kleptoparasitic species were mostly *Sphecodes* (79 %). The most abundant species was *S. monilicornis* K. (43.6 %), a well know kleptoparasite of *L. malachurum* K. (Legewie 1925; Knerer 1973). *S. monilicornis* K. is more active during peak foraging activity of their hosts and their activity decreases with the host foraging activity (Polidori et al. 2009). This behaviour is related to optimal foraging theory as during periods of high host's nesting activity, the kleptoparasite has an increased probability of finding fresh pollen in bee's nest (Polidori et al. 2009). *S. monilicornis* K. has also been observed as a kleptoparasite of *L. pauxillum* S. (Bogusch et al. 2006) and two medium-sized *Lasioglossum* species, *L. calceatum* S. (Stockhert 1933; Vegter 1993) and *L. albipes* F. (Alfken 1912; Blüthgen 1934). The presence of these ground-nesting bee species at the study plots (Appendix 3) explains the abundance of *S. monilicornis* K. However, it should also be considered that *S. monilicornis* K. is the most readily distinguished *Sphecodes* species in the field (K. Tsiolis, personal observation; M. Edwards, personal communication). The second most abundant species was *Bombylius major* L. (20.6 %) which attacks a variety of *Andrena* species by flicking its eggs near bees' nests entrances, and once hatched they enter a host cell (Falk, 2016). The third most abundant was *Nomada fucata* P. (18.2 %); a kleptoparasite of *A. flavipes* P. (6.2 %), and the fourth was *Nomada flavoguttata* K. (11.1 %) whose hosts are the *A. minutula* K. (2 %) (Else & Edwards 2018).

Parasitism can have a significant negative impact on host bee populations. A study by Strohm & Bordon-Hauser (2003) recorded parasitism of ~20% of *S. monilicornis* K. on a *L. malachurum* K. population, but parasitism of entire nests or colonies has also been recorded (Sick et al. 1994). During the surveys in this study, many bees were found dead next to the entrance of nests each year. Strohm & Bordon-Hauser (2003) have also observed this phenomenon; where *S. monilicornis* K. killed several *L. malachurum* K. workers (up to 19) inside the nest, extracting them from the nest one-by-one. More sampling seasons and detailed observations will be needed to determine whether density-dependent mortality exists at our study plots as demonstrated with *H. rubicundus* C. attacked by a *Leucophora* sp. (Diptera) (Eickwort et al. 1996). Potts & Willmer (1997) argue that the existence of gregarious nesting is an indication that specific factor(s) is/are present which outweigh(s) the cost of parasitism.

2.4.3 Abiotic factors and nest site-selection

Nest excavation is a significant investment for most ground-nesting Hymenoptera, encompassing energy and time costs (Michener 1956; McCorquodale 1989). Nevertheless, firmer soils can be beneficial for ground-nesting bees. Potts & Willmer (1997) noted that soft soils are initially selected, but as the nest aggregation grows, they favour harder soils which support larger nest architecture.

There was a significant negative correlation between hydraulic conductivity and peak nest density (Table 2.2). A faster hydraulic conductivity rate increases drainage, resulting in soils with limited water content and may be favourable to ground-nesting bees as waterlogging is reduced. Johansen et al. (1978) reported that increased water infiltration is favoured by alkali bees (*Nomia* spp.) for nesting. Faster hydraulic conductivity rates could also be linked to soils with a greater percentage of stoniness enabling nest construction as observed in earwig (*Forficula auricularia* L.) nests (Lamb 1976). Conversely, more rapid hydraulic conductivity in sandy or very soft soils could result in nest collapse. In this study, plot 5 was sandy, had the highest rate of hydraulic conductivity (0.0011 cm s^{-1}) and no bee nests were observed (Fig. 2.2B). This variable can also be influenced by soil compaction as the shape, size, and connectivity of microscale pores contribute to water flow in soils (Ebina et al. 2004).

Soil moisture ranged between 2.7 and 37.8% during the nesting activity of 32 ground-nesting bee species (Cane 1991), suggesting soil moisture content might be species-specific, but bees can tolerate a range of soil moisture. High fatality of *Andrena vaga* P. and local population decline (Fellendorf et al. 2004) and delayed emergence of male *Calliopsis pugionis* C. (Visscher et al. 1994) resulted from waterlogged soils. Soils considered suitable for bee nesting are neither waterlogged nor too dry, and moderating moisture levels will contribute to successful larva development and minimal risk of brood cell desiccation (Potts & Willmer 1997).

Soil texture influences soil temperature, moisture, and oxygen availability, which may impact bee survival inside a nest (Harmon-Threatt 2020). Ground-nesting bees, including *Halictus*, *Lasioglossum* and *Andrena* species, nest in clay-loam, sandy-loam, and silt-loam but not silt or clay soils (Cane 1991). Potts & Willmer (1997) reported that *H. rubicundus* C. nested in sand/loamy and sand/sandy-loam soils, although texture was not correlated to nest numbers. Harmon-Threatt (2020) reviewed the soil preferences of 527 species in the USA and Canada. Soil texture was only reported in 48 descriptions, but 75% of bee species were nesting in sand or sandy-loam soils. The review also highlighted the lack of qualitative data on soil texture preferences. Bees in our study were also primarily nested in sandy-loam and sandy-clay-loam soils.

Stoniness had a significant correlation with peak nest density. Potts & Willmer (1997) reported that 57% of nests of *H. rubicundus* C. were associated with stones which had higher temperatures compared to nests without stones. Soils of bee nests constructed under stones in halictid aggregations were 2 – 3°C warmer (at 5 cm) during the afternoon compared to bare soil (Packer et al. 1989). To test this, Cane (2015) introduced pebble mulch treatments to *H. rubicundus* C. nesting aggregations and found that 70% of bees built nests in plots with pebbles compared to bare soil control. Stones may also offer protection from parasitism (Potts & Willmer 1997) and/or could serve as visual landmarks (Brünnert et al. 1994).

Organic matter softens soils, reducing compaction (Hamza & Anderson 2005) and darkens soil (Jackson 2014), improving the absorbance of solar radiation. Conversely, thick layers of organic matter may create a waterlogged environment (Bescansa et al. 2006). Although organic matter did not impact peak nest density in this study, soil from nesting sites of *L. malachurum* K. contained 0.004% (± 0.0012) organic matter (Polidori et al. 2010), and Grundel et al. (2010) found bee nest density negatively correlated with organic matter.

Increased vegetation results in greater soil moisture and lower soil temperature (Wuellner 1999; Anderson & Harmon-threatt 2016). Wuellner (1999) reported that the ground-nesting bee, *Dieunomia triangulifera* V., preferred nesting in soil with little to no vegetation, attributed to roots which disturb digging and may grow into nests. The findings of this study suggested a significant negative impact of vegetation cover but no negative impact of root biomass on bee nesting (Table 2.2).

This study intended to treat vegetation with herbicides at early growth stages to keep soil bare. However, this was not always possible due to weather conditions, and at times considerable amounts of vegetation grew and became thatch (48.9 ± 1.5% (N=192)) after herbicide treatment. Additionally, the growth of moss was recorded (9.5 ± 0.9% (N=192)) in the third year of the study. Both habitat types could significantly affect bee nesting and should be carefully managed in future studies.

As solitary bees are ectothermic (Stone & Willmer 1989), they cannot reliably control nest temperature. Belowground, low temperatures can result in slower nest founding and construction (e.g. *L. malachurum* K. (Weissel et al. 2006)). Additionally, nest entrance temperature sometimes facilitated by “chimneys” may initiate foraging (Norden 1984), as observed in this study by small *Lasioglossum* species which positioned themselves inside their ‘chimneys’ (see Appendix 9) for 1-2 minutes in the morning before leaving their nest (K. Tsiolis, personal observation). This study has shown that the average soil temperature during late morning hours (07:00 – 11:00) was at 17.0°C and 21.8 °C during afternoon hours (13:00 – 17:00).

There was no significant difference in the number of bee nests between the 10° gradient slope and flat sections of our study plots. However, it should be acknowledged that the slope sections of plots were created artificially in 2018, and the soil was not as compacted as on the flat sections. Soil compaction of slopes had increased by 2020, but longer-term studies are needed to determine if any further soil compaction occurred and the impact on peak nest density. Soil compaction also influences other variables, such as soil moisture (Soane & van Ouwerkerk 1994) and hydraulic conductivity (Horton et al. 1994). The latter was shown to influence nesting in this study. Furthermore, it is possible that increasing slope gradient beyond >10° would be more beneficial for bee nesting; many bee nesting aggregations are often found in steep slopes such as riverbanks and drainage ditches (Michener 2007).

2.4.4 Management of ground-nesting bee populations

The availability of nesting and food resources are vital to sustaining bee populations; hence, both need to be considered as part of effective management for ground-nesting bees (Potts et al. 2003; Grundel et al. 2010; Murray et al. 2012). The provision of additional food resources in the form of nectar and pollen, such as flower strips (Haaland et al. 2011; Wratten et al. 2012), field margins (Rands & Whitney 2010), hedgerows (Garratt et al. 2017) and semi-natural land near farms (Martins et al. 2015) are highly beneficial for bees and improve bee abundance and diversity (Garibaldi et al. 2014; Venturini et al. 2017; Fountain 2022). Nevertheless, the nesting preferences of ground-nesting bees are under-researched (Antoine & Forrest 2020).

The parameters that need to be considered in construction are 1) soil hydraulic conductivity between 5.1e-5 – 5.35e-4 cm s^{-1} with the optimal value at 2.4e-4 cm s^{-1}, 2) soil temperature between 25.5 – 29.9°C with the optimal value at 28.5°C, 3) soil stoniness between 1 – 34.6% with optimal value at 19.8%, and 4) vegetation cover between 29.3 – 70.2% with optimal value at 40.6% as they are factors in nesting of recorded *Andrena*, *Lasioglossum*, *Halictus* and *Colletes* species. However, there is a strong relationship between soil variables, which should be considered in creating such habitats.

Our approach to nest site creation could be used to support the conservation of bees or even promote pollination in systems where ground-nesting bees are important pollinators, such as apples (Garratt et al. 2016). Providing both food and nesting resources would potentially increase bee abundance and diversity (Kline & Joshi 2020; Fountain 2022); which is essential for inter-annual stability in pollinator communities (Senapathi et al. 2021). Where both appropriate food resources and nesting habitats exist, ground-nesting bees could remain and multiply for many years. Examples of long-lived ground-nesting aggregations are *Panurginus polytrichus* C. for 20 years (Neff 2003), and *L. malachurum* K. for 37 years (Stöckhert cited in Michener (1974). Populations of the alkali bee (*N. melanderi* C.), the world's only intensively managed ground-nesting bee due to its value for pollinating alfalfa (*Medicago sativa* L.), grew nine-fold (16.7 million females) over eight years (Cane 2008). Nevertheless, it should be acknowledged that bees tend to nest near their parental nests (philopatry), and the nest density could be caused by both soil characteristics and the propensity to nest at natal sites (Michener et al. 1958).

Though not directly tested in this study, farming practices should be considered in the process of enhancing the population of ground-nesting bees in agricultural landscapes. For instance, minimum or no-till practices should be favoured, as 10-20 cm tillage may directly kill and have a negative impact on the following year's emergence of ground-nesting bees (Shuler et al. 2005). Pesticide use, including ground-applied herbicides, should be minimised to prevent exposure during foraging (Brown & Paxton 2009; Bloom et al. 2021) and nesting. A recent study has shown that contamination of nesting soils of the ground-nesting solitary squash bee (*Eucera pruinosa* S.) with a neonicotinoid (Imidacloprid, 18 mL/100 m row) insecticide; resulted in bees initiating 85% fewer nests, harvesting 5.3 times less pollen and producing 89% fewer offspring compared to untreated control soils (Willis Chan & Raine 2021).

Individuals interested in undertaking a similar study would be encouraged to be patient and spend in the field as much time as possible to familiarise themselves with the identification of bee nests and observe nesting behaviours that may play a significant role in understanding bee nesting preferences. There were several challenges during this study which should be considered for future experiments. The ability of the surveyor to identify bee nests was significantly reduced after heavy rain. As a result, it is suggested to pay close attention to the weather forecast and count nests after a minimum of 1-2 weeks of warm, dry weather during peak activity and before rainfall. Recording soil compaction was not a straightforward process due to the high percentage of stoniness at the site, which resulted in recording and discarding many false readings. Moreover, it was decided to mark assessed nests with plant labels, but it was soon realised that some labels were destroyed and removed by wildlife (possibly badgers). Vegetation growth is also a factor that requires close attention with the aim to remove this at early stages to prevent accumulation of thatch on the plots, which could significantly influence bee nesting. This study could have been improved by recording the availability of bare ground cover and food resources near study plots for the entire duration of the study, as they can both influence bee nesting. This is particularly significant when such experiments take place in agricultural landscapes, which often experience frequent local habitat changes. This additional data would have helped with interpretations of findings.

Conclusion

A critical component for sustaining ground-nesting bee populations is the availability of suitable nesting resources, which can be a limiting factor in both urban and agricultural environments. This study provides new insights into the nesting needs of ground-nesting bees. It shows that artificially created bare ground plots could help provide nesting resources for several ground-nesting bee species if key abiotic conditions are considered. Further research is needed to enrich the current limited knowledge on nesting preferences of these important ground-nesting bees and allow the improvement and sustainable enhancement of their populations in agricultural and urban environments. Nevertheless, the findings of this study can contribute to raising farmers’ awareness of the nesting needs of ground-nesting bee species, can inform agri-environment schemes rewarding good practices, and support environmental policy.

3 Chapter 3 – Nesting preferences of ground-nesting bees in apple orchards

3.1 Introduction

Apple (*Malus domestica*) is a globally significant crop. Its produce value was estimated at \$47,323 (US) per tonne in 2020 (FAO 2022), and its value in the UK is over £150 million per year from 23,000 hectares (Defra 2022). Many apple varieties depend on cross-pollination for higher fruit quality and yields (e.g., Garratt et al. 2014; Hünicken et al. 2020). The economic value of insect pollination (EVIP) was estimated at £12.8 billion per year in European Union, with apple being the most valuable insect-pollinated crop, accounting for 16% of the total EVIP (Leonhardt et al. 2013). Its value for two apple varieties (Gala and Cox) has been estimated at £36.7 million per year in the UK (Garratt et al. 2014). These studies highlight the high dependency of apple varieties on insect pollination. Researchers have also focused on understanding which pollinator guilds provide this valuable service. Large commercial orchards commonly use honeybees (*Apis mellifera* L.) to enhance productivity, but studies have shown that compared to wild bees, they are not always the most efficient apple pollinators (Delaphane & Mayer 2001; Stern et al. 2001; Pardo & Borges 2020). Blitzer et al. (2016) found that wild bees are essential for apple pollination even when large populations of honeybees are present. The economic value of UK apple-pollinating insect guilds was estimated at £51.4M for solitary bees, £21.4M for honeybees, £18.6M for bumblebees, and £0.7M for hoverflies (Garratt et al. 2016).

The sustainability of beneficial pollinators in apple orchards depends on both food and nesting resource availability. The duration of apple flowering is 2-3 weeks in the UK, but wild bee species are often active for 5-6 weeks or more (Else & Edwards 2018). Therefore, adding floral resources is necessary to sustain pollinators in apple orchards, and it has been relatively well studied (e.g. Carvell et al. 2022; Fountain 2022). However, the nesting preferences of wild bees are under-researched, especially for ground-nesting bees, which often have cryptic nesting behaviour, and it can be particularly challenging for ecologists to locate enough nests for robust replicated studies (Antoine & Forrest 2020). The dominant apple-visiting bee genera in Europe were identified as ground-nesting *Andrena* (10 spp.), eusocial *Bombus* (6 spp.), ground-nesting *Lasioglossum* (2 spp.) and aerial-nesting *Osmia* (1 sp.) (Hutchinson et al. 2021).

The nesting preferences of ground-nesting bees have been found to vary (Antoine & Forrest 2020). However, sandy soils (Cane 1991), soils with low organic matter (Grundel et al. 2010), sloped substrates (Sardiñas & Kremen 2014), soil temperature (Potts & Willmer 1997; Wuellner 1999; Xie et al. 2013), and availability of bare ground (Potts et al. 2005) are factors that have proven to generally be important in nest-site selection. The study in Chapter 2 has demonstrated that soil temperature and stoniness had a significant positive relationship, and hydraulic conductivity and vegetation significant negative relationship with bee nest density. Additionally, many bee species have been reported to prefer very moist and loose soils (McCorquodale 1989; Potts & Willmer 1998; Srba & Heneberg 2012), while others prefer compacted soils (Wuellner 1999; Ghazoul 2001; Polidori et al. 2010). A recent study identified fourteen ground-nesting bee species nesting in apple orchards, with higher nest densities in vegetation-free areas, such as under apple trees (Fountain et al. 2023). This study also demonstrated that the occupancy of ground-nesting bees doubled in three years by extending the vegetation-free areas at the end of tree rows. Since most key apple pollinators are ground-nesters, understanding their nesting preferences is vital in securing their presence in apple orchards.

The aims of our study were to 1) determine which species are nesting in apple orchards, 2) where they prefer to nest in apple orchards, and 3) which are the biotic and abiotic factors influence nest-site selection.

3.2 Methods

Twenty-three conventional apple (*Malus domestica*) orchards in Kent, UK (same as Carvell et al. 2022; Garratt et al. 2022), were rapidly surveyed (200 m^2 transect along randomly selected alleyways and under trees areas, and 200 m^2 transect along each South, East and west facing edges of apple orchards) for ground-nesting bee presence, and the eight orchards with the highest bee nest density were then intensively surveyed in 2019. It was impossible to continue surveying the same apple orchards in 2020 due to COVID-19-related travel restrictions, so surveys were confined to three conventional / research orchards at NIAB East Malling (Fig. 3.1). The surveys continued in 2021 in the five orchards with the highest nest density from the surveys in 2019 (Fig. 3.1). Four rounds of pollinator surveys took place each year in apple orchards, starting from the beginning of bee nesting and then continuing surveying weekly (weather permitting) (Table 3.1).

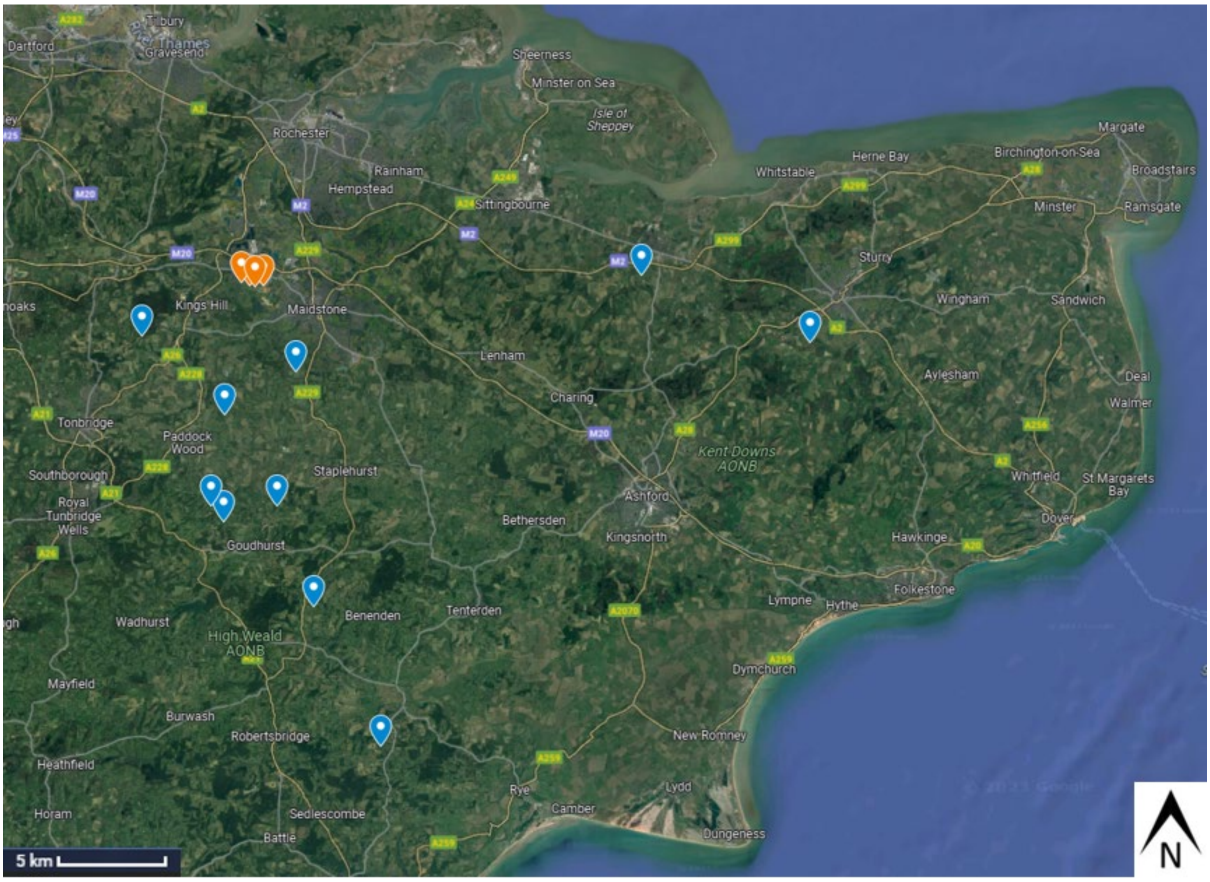


Figure 3.1: Map of sites where intensive surveys took place. Blue markers – Commercial apple orchards. Orange markers – NIAB East Malling research apple orchards.

Table 3.1: Dates of bee nest surveys.

Survey rounds	2019	2020	2021
1st	15 April – 21 April	08 April – 12 April	28 April – 05 May
2nd	22 April – 28 April	15 April – 20 April	08 May – 15 May
3rd	29 April – 04 May	23 April – 30 April	17 May – 23 May
4th	06 May – 13 May	04 May – 08 May	25 May – 03 June

Two thousand square meters transect took place for each of the orchard habitats; A) edges (South, East, and West-facing edges to tree rows), B) alleyways (area between tree rows), and C) under apple trees to determine nest density. A random number generator (Android mobile phone app, Random UX) randomly selected the alleyways and under-tree orchard habitats to survey. Bee and wasp nests were identified by the tumuli (volcano-shaped mounds of earth) females make in the nest excavation phase (Michener 2007).

Once a nest was found, a red-wired flag was placed 15 cm away and to the north of the nest's entrance. Then local habitat assessment took place, recording the percentage of long grass, short grass, thatch, moss and bare ground present 20 cm around the entrance of each nest. Additionally, a quadrat (50 cm x 50 cm) was used every 30 m along the transect to record the non-nesting local habitat. The nest entrance (hole) was measured using the external jaws of digital callipers to the nearest tenth of a mm (Preciva IP54) (Fig. 3.2). If the occupier of a nest was present, or could be seen leaving or entering the nest, it was captured using a 60 cm x 46 cm entomological hand net (Watkins & Doncaster, Leominster, UK) and placed into a 5 ml clear plastic tube. Identifiable bees were recorded and immediately released, and the non-identifiable individuals were placed into an ice bucket where they were kept inactive for approximately 30 minutes (Grixti et al. 2009). Then, photographs including the key characteristics were taken for later identification and confirmation by Mr Mike Edwards, a professional entomological consultant (Else & Edwards 2018). If the nest occupier could not be seen, a bee trap (supplied by NISBETS (https://www.nisbets.co.uk/) conical stainless-steel strainer, 7 cm wide, 4 cm tall)) with a clear plastic tube (30 mm x 45 mm) on the top of it was placed above the nest's entrance (Fig. 3.3). If the bee was inside the nest it was trapped on its emergence. Bees were seen flying around the traps in several occasions looking for the entrance of the nest after foraging (covered with pollen). The traps were checked every 20 minutes, and trapped bees were identified or photographed, as explained above.

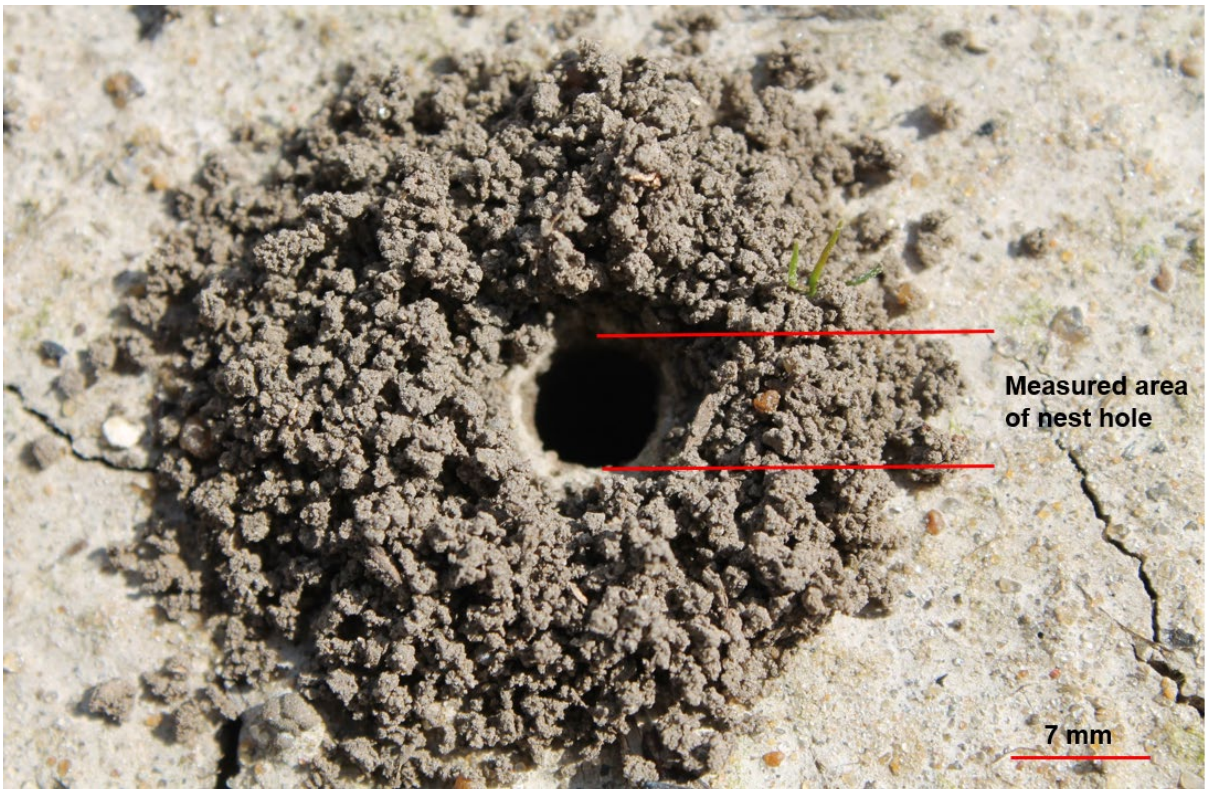


Figure 3.2: Method of measuring the diameter of ground-nesting bees nest's entrance.

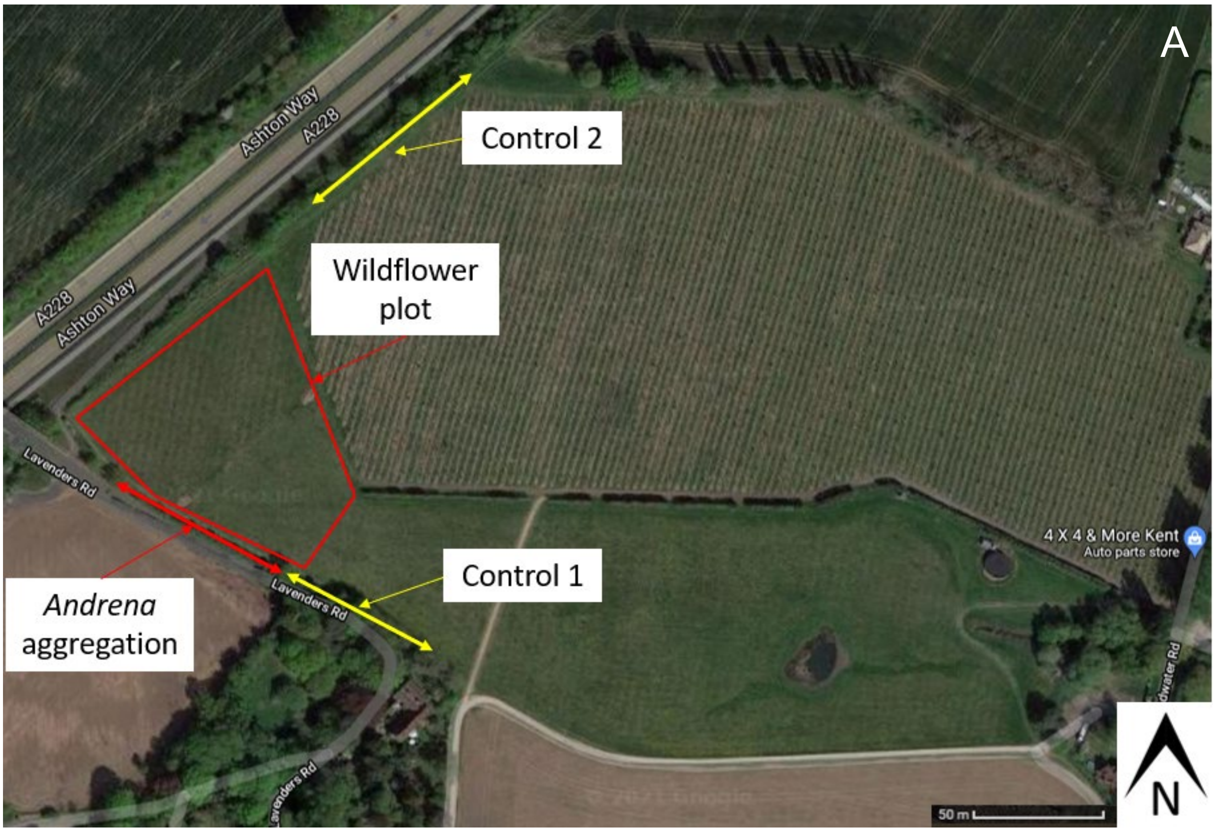
Figure 3.3: Conical trap of ground-nesting bees with a trapped *Andrena nitida* M*.*

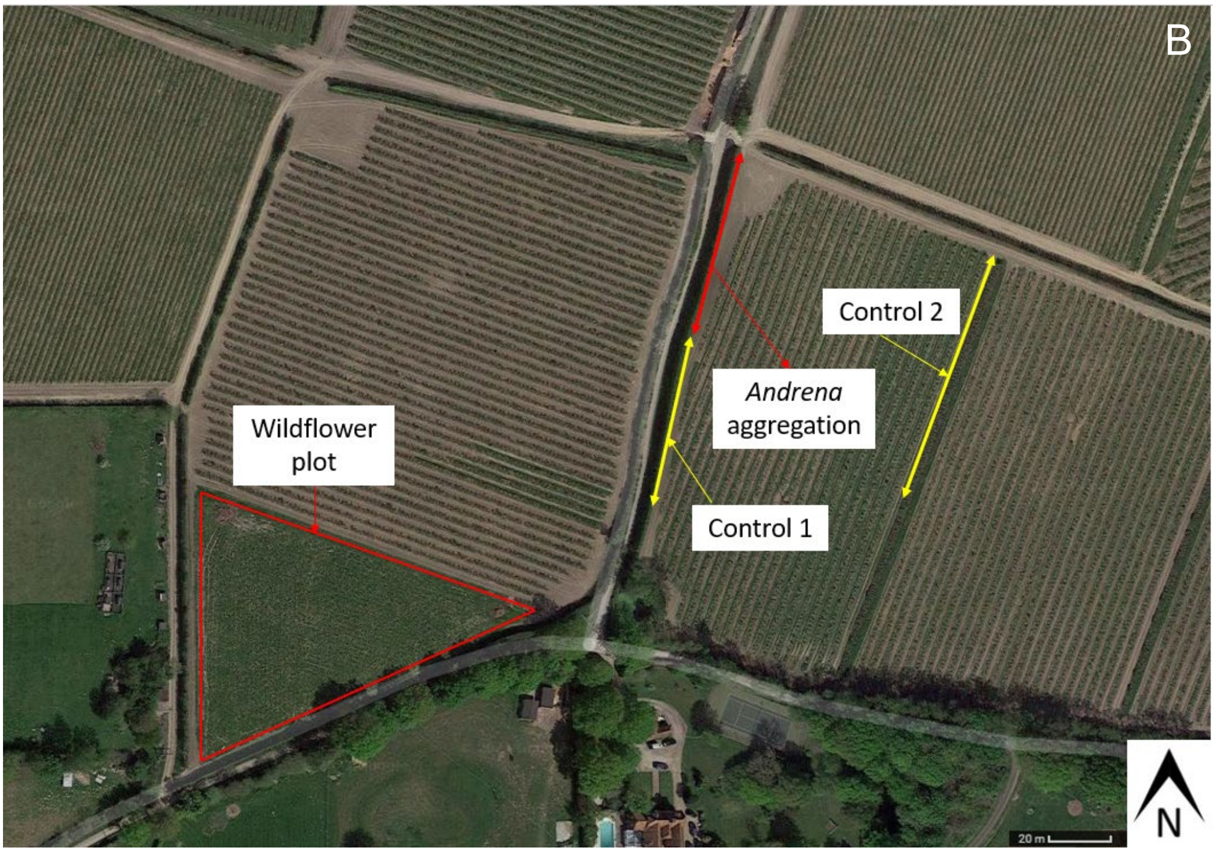
3.2.1 ***Andrena* nesting aggregations**

Three *Andrena* nesting aggregations were located along 1) North-facing, 2) East-facing, and 3) South East-facing hedgerows of apple orchards in June 2021 (Fig. 3.4). A nest was considered part of an aggregation if it was less than 3 meters apart from another bee nest. The distance between nests of a nesting aggregation ranged between 5 cm to 300 cm. Each nesting aggregation was divided into ten equal segments, and a 1 m^2 quadrat was placed on the highest nest density of each segment. Two control habitats were used for each nesting aggregation at locations where no bee nesting was recorded to determine which biotic and abiotic factors influence nest-site selection. The first control habitat was on the left of the aggregation along the same hedgerow where no bee nests were recorded, and the second control habitat was along the opposite hedgerow to the nesting aggregation (Fig. 3.5). The size of each control habitat was 50 m x 1.5 m, and they were divided into ten equal segments.

Each segment was marked and labelled to be easily recognisable for the following assessments.

Figure 3.4: *Andrena* nesting aggregation long a North-facing hedgerow of an apple orchard. Each red-wired flag indicates a bee nest or more.




B
Control 2
Andrena aggregation
Wildflower plot
Control 1
N
20 m

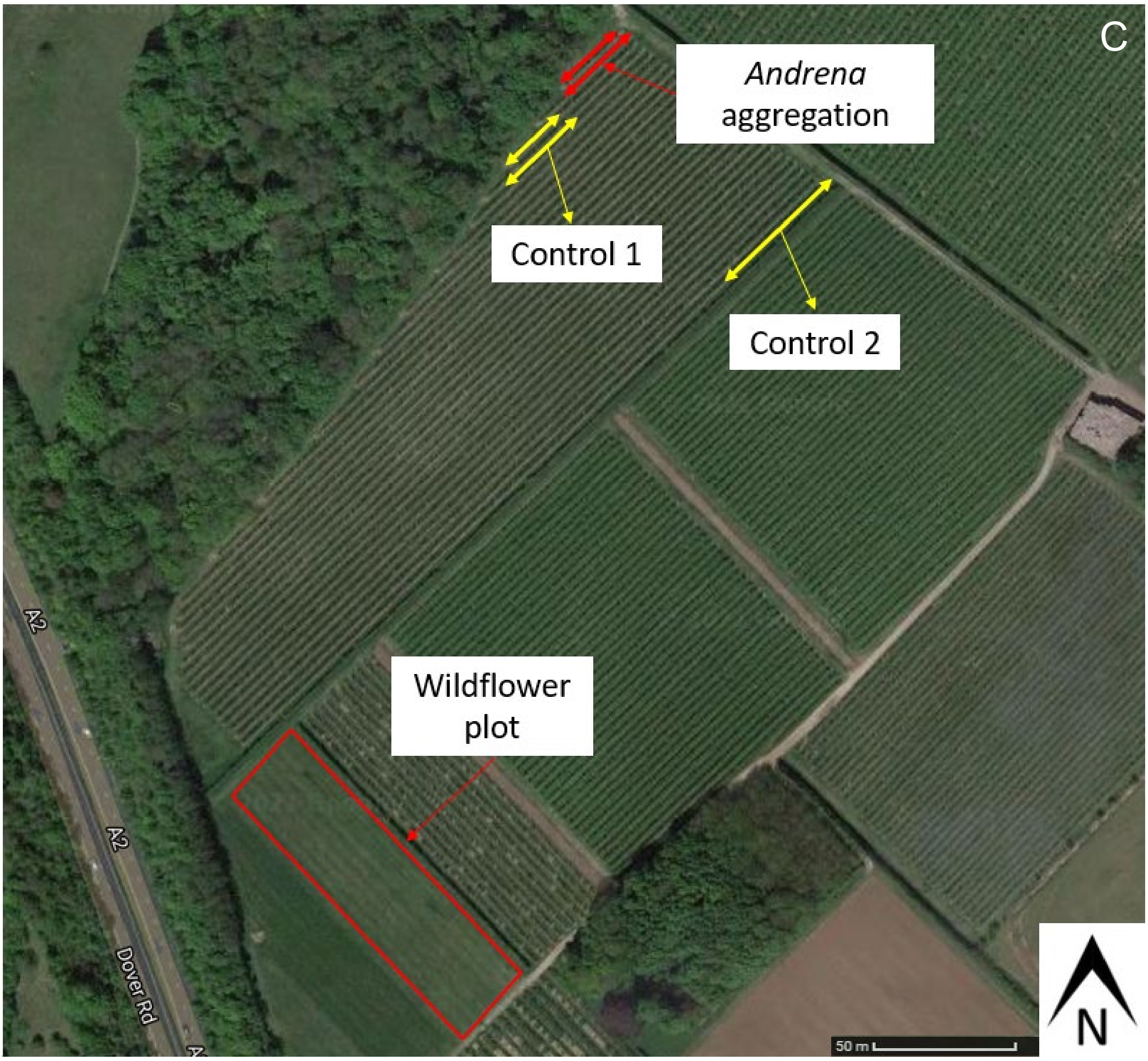


Figure 3.5: The three nesting aggregations (A, B, and C) with sampling design and controls along hedgerows of three apple orchards.

A soil core (depth 15 cm x width 5 cm) was collected from the centre of each segment at nesting aggregation and control habitats using a Buerkle soil sampler (Fisher Scientific International Inc., Hampton, US) in 2021. Large aggregates were gently crushed with a mortar and pestle for root biomass measurements, and all visible root material was removed manually and weighed. The mass of root material was compared to the total weight of each core to give root biomass in percentage. The stoniness of the samples was measured by hand sieving the sample through a 2 mm aperture test sieve for 30 seconds to separate the gravel fraction (Potts & Willmer 1997). The stones were weighed and calculated as a percentage of the total soil weight. Two grams of the remaining non-gravel fraction of each sample was then used to determine organic matter content by loss on ignition (550°C, 7 hrs; Goldin, 1987). Samples were analysed at the University of Reading for soil texture (particle size distribution, PSD) from the 23 orchards (Blott et al. 2004). According to the USDA texture classification system, these measurements were used to determine soil texture (Soil Survey Staff 2014). Hydraulic conductivity was measured from two samples per segment using mini disc infiltrometers (METER Group Inc., Washington, US). The devices were placed where soil cracks, bee nests and ant nests were absent. A suction rate of 2 cm s^{-1} was chosen. Water infiltration was recorded every 3 minutes (measurements were taken in 2018 and 2020).

Soil compaction (unconfined compression strength; kg/cm^{-2}) was assessed by taking three probe readings per nesting and control segments using an electronic recording cone penetrometer (Solutions for Research Ltd, Bedfordshire, UK). Readings were taken at 2.5 cm intervals, and the mathematical mean was calculated for those collected from the top 15 cm. The cone index was corrected for soil moisture content (measured using a MO750 model soil moisture meter; Extech Instruments Corp., New Hampshire, U.S.), according to Busscher et al. (1997).

Soil moisture and soil temperature were recorded once in June 2021 as the mean of three positions per nesting and control segments. The HH2 moisture meter and a WET-2 sensor (Delta-T Devices Ltd., Cambridge, UK) were used.

3.2.2 Statistical analysis

R (Version 4.1.2; R Core Team 2021) was used for all statistical analyses. A Generalised Linear Mixed Effect Model (GLMM, R package: lme4) with Poisson distribution was fitted to determine whether local and orchard habitats are significant predictors of bee nesting. Local habitat (vegetation cover) was estimated as percentage cover equalling 100%; hence there was collinearity between recorded habitats. A PCA was used for local habitats where Principal Component 1 (PC1) and Principal Component 2 (PC2), and orchard habitats were included in the GLMM as predictor factors. Nest density was calculated by having the total number of nests as the response factor and the size (m^2) of each surveyed orchard habitat as the offset. Surveyed sites were set as random effects.

A One-Way ANOVA and a Tukey test were used to determine whether nest density significantly differs between orchard habitats. A Welch Two sample t-test was used for each local habitat to determine whether there was a significant difference between habitat measures in non-nesting and nesting sections of the orchards.

A Generalised Linear Mixed Effect Model (GLMM, R package: lme4) with Poisson distribution was fitted to establish which soil variables measured from *Andrena* nesting aggregations were significant predictors of bee nesting. A negative binomial distribution was used due to excessive overdispersion with the Poisson distribution. Nest density was set as the response factor. A PCA was used for local habitat, and PC1 and PC2 (representing 88 % of local habitat) were included in the GLMM as predictor factors. Another PCA was used for soil textures (% sand, silt and clay), and PC1 (representing 83 % of soil textures) was included in the GLMM as a predictor factor. The rest of the predictor factors were soil moisture, temperature, compaction, root biomass, stoniness, organic matter, and hydraulic conductivity. Aggregation sites were set as random effects.

3.3 Results

3.3.1 Species

Over the three years of the study, 789 bee specimens were identified, belonging to 17 species (12 *Andrena* spp., 2 *Lasioglossum* spp., 1 *Halictus* spp., 1 *Nomada* spp., and 1 *Sphecodes* spp.). Specimens were identified to species level except for *Lasioglossum calceatum* S. and *Lasioglossum albipes* F., which were difficult to confidently separate as they have very similar identification features; hence, they were grouped. The most abundant bee species (non-kleptoparasitic) was *Andrena haemorrhoa* F. (36.5 %), the second most abundant *Andrena nitida* (34.2 %), and third most abundant *L. calceatum/albipes* (15.8 %) (Fig. 3.6). Two species, representing 0.7 % of the total number of bee specimens, were kleptoparasites: *Nomada goodeniana* K. (0.2 %) and *Sphecodes monilicornis* K. (0.5 %) (Fig. 3.6). The mean nest entrance diameter of *A. haemorrhoa* F. was 5.38 mm (SE ± 0.02, N = 288), *Andrena nitida* 6.90 mm (SE ± 0.03, N = 270), and *L. calceatum_albipes* 4.32 mm (SE ± 0.04, N = 125) (Table 3.2).

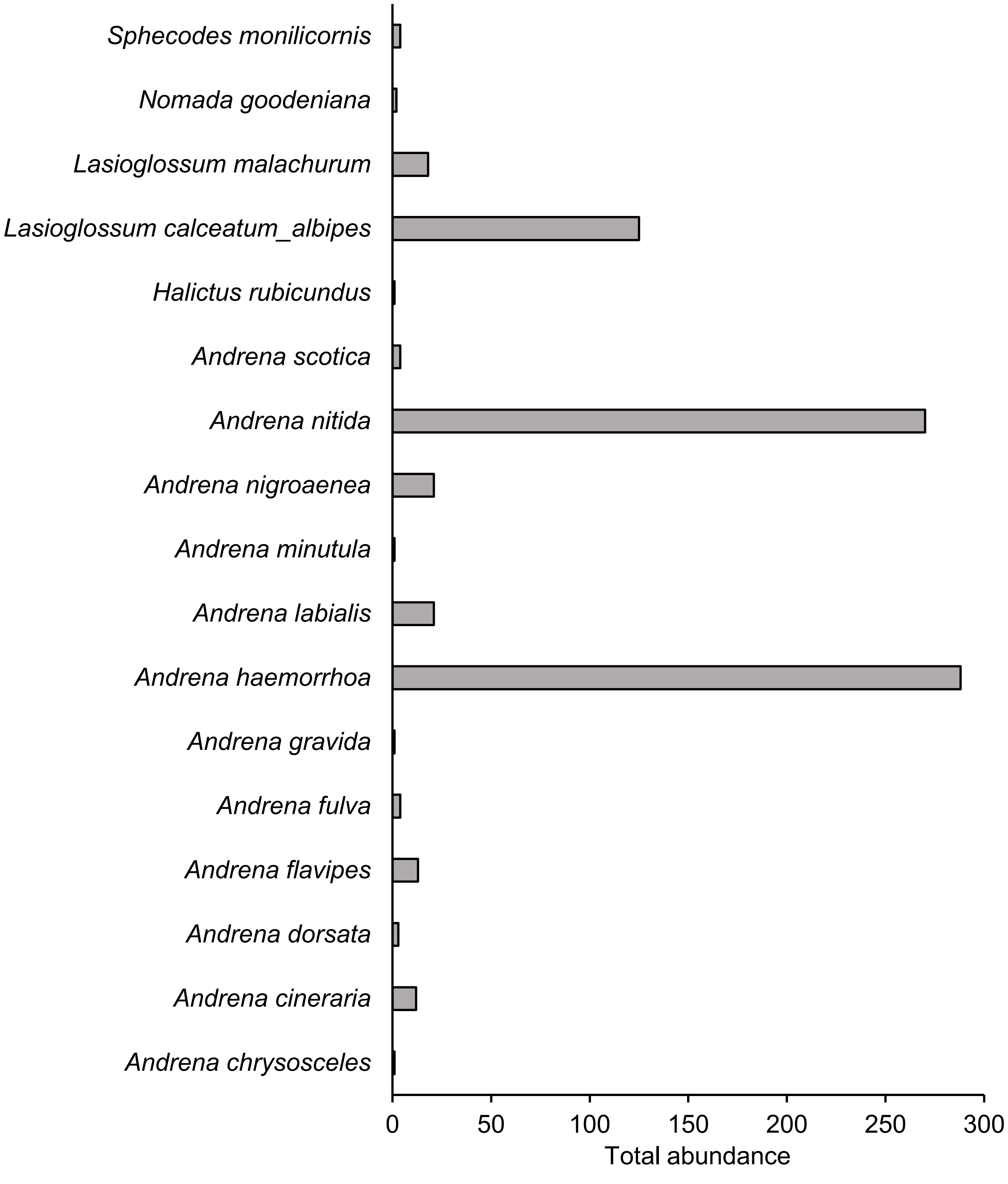


Figure 3.6: Bee species abundance of surveyed apple orchards during the three years of the study.

Table 3.2: Mean nest entrance's diameter and standard error of recorded bee species.

Species	Total	Mean diameter (mm)	Std. Error
Andrena haemorrhoa F	288	5.38	0.34
Andrena nitida M.	270	6.90	0.56
Lasioglossum calceatum_albipes F.	125	4.32	0.49
Andrena nigroaenea K.	21	6.07	0.45
Andrena labialis K.	21	6.10	0.22
Lasioglossum malachurum K.	18	3.57	0.58
Andrena flavipes P.	13	5.16	0.39
Andrena cineraria L.	12	6.51	0.82
Andrena fulva M.	4	6.63	0.69
Andrena scotica P.	4	6.68	0.60
Andrena monilicornis K.	4	4.31	0.63
Andrena dorsata K.	3	4.29	0.70
Nomada goodeniana K.	2	5.90	0.35
Andrena chrysosceles K.	1	3.35	NA
Andrena minutula K.	1	3.73	NA
Halictus rubicundus C.	1	6.28	NA
Andrena gravida I.	1	5.65	NA

3.3.2 Nesting – Orchard habitat

Over the three years of the study, 1,915 bee nests were recorded and assessed in apple orchards. Nest density significantly differed between orchard habitats (One-Way ANOVA, P = 0.012) (Fig. 3.7). The highest nest density was recorded in orchard edges and under trees, and there was no significant difference between the nest density of these two orchard habitats (Tukey HSD, P = 0.916) (Fig 3.7). Orchard habitats were significant predictors of nest density (GLMM, Alleyways (P < 0.001), under trees (P < 0.001) and edges (P < 0.001)). Ten of the recorded bee species were reported as dominant apple flower visitors (Hutchinson et al. 2021; Fig. 3.8). The most abundant dominant apple flower visitors were *A. haemorrhoa* F. and *A. nitida* M., nesting predominantly in orchard edges and under apple trees (Fig. 3.8).

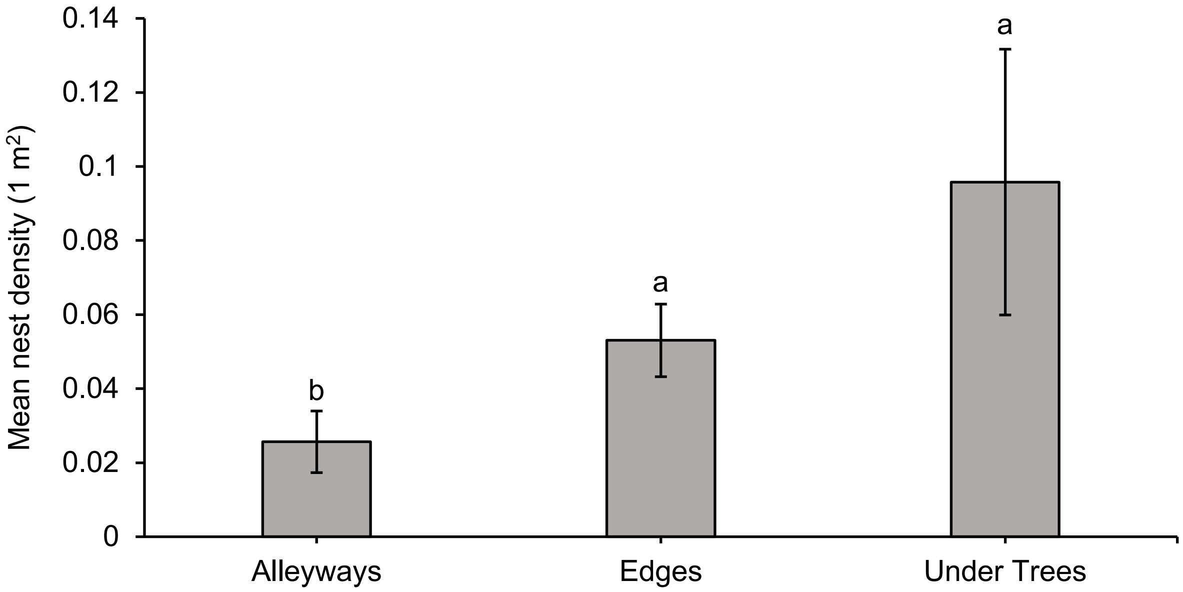


Figure 3.7: Mean nest density (1 m^2) of orchard habitats for the three years of the study. Different letters indicate significant differences between plots ($P < 0.05$), according to Tukey HSD. Nest density in alleyways differed significantly from nest density in edges ($P = 0.042$) and under trees ($P = 0.016$).

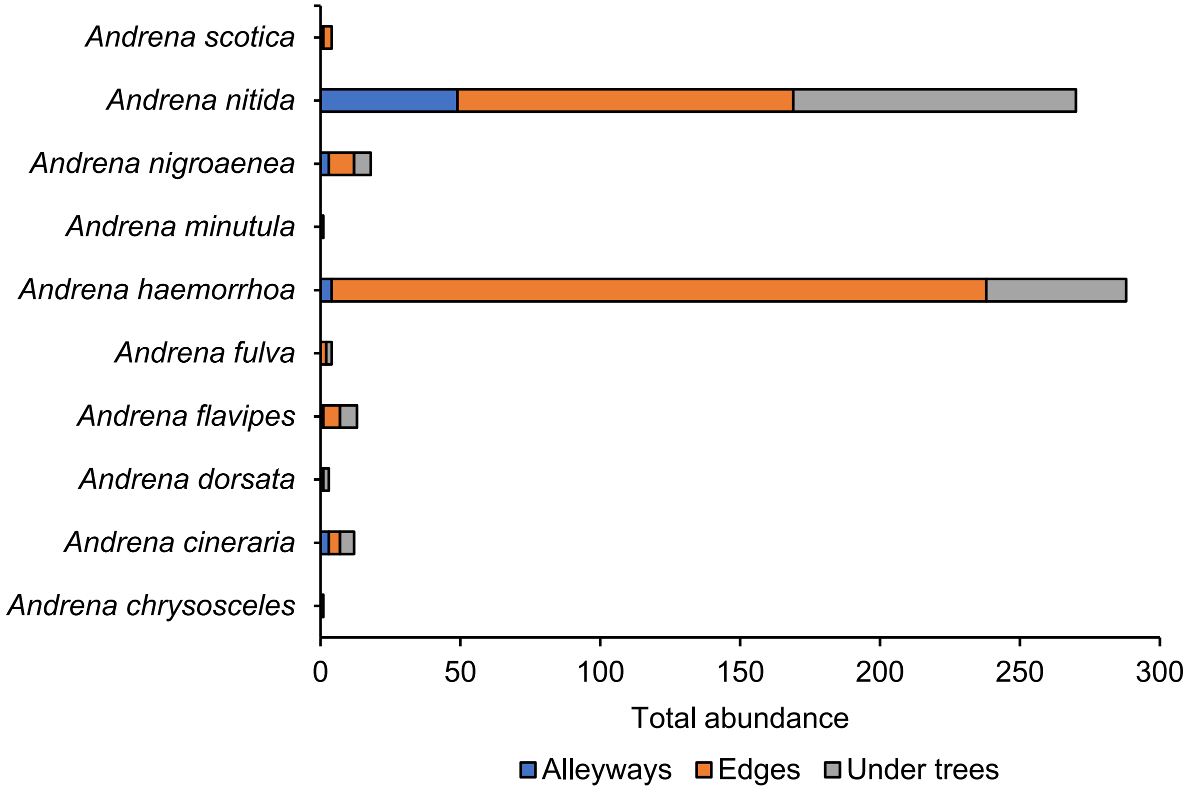


Figure 3.8: Bee species abundance per orchard habitat reported as dominant apple flower visitors (Hutchinson et al. 2021).

3.3.3 Nesting – Local habitat

The local habitat was a significant predictor of nest density (GLMM, PC1 (P = 0.014) and PC2 (P = 0.003)). In addition, the proportions of long (Two sample t-test, P < 0.001) and short vegetation (Two sample t-test, P < 0.001) were significantly higher in control than nesting locations, and the proportions of moss (Two sample t-test, P = 0.015) and bare ground (Two sample t-test, P < 0.001) were significantly lower in control than nesting locations (Fig. 3.9). The mean nesting local habitat for *A. haemorrhoa* F. was short vegetation 21.0 % (SE ± 1.50, N = 288), long vegetation 29.7 % (SE ± 1.39, N = 288), thatch 16.7 % (SE ± 0.85, N = 288), moss 22.1 % (SE ± 1.20, N = 288), and bare ground 10.5 % (SE ± 1.14, N = 288) (Appendix 10). The mean nesting local habitat for *A. nitida* M. was short vegetation 42.7 % (SE ± 2.04, N = 270), long vegetation 8.6 % (SE ± 1.16, N = 270), thatch 11.4 % (SE ± 1.04, N = 270), moss 13.2 % (SE ± 1.46, N = 270), and bare ground 24.1 % (SE ± 1.53, N = 270) (Appendix 10).

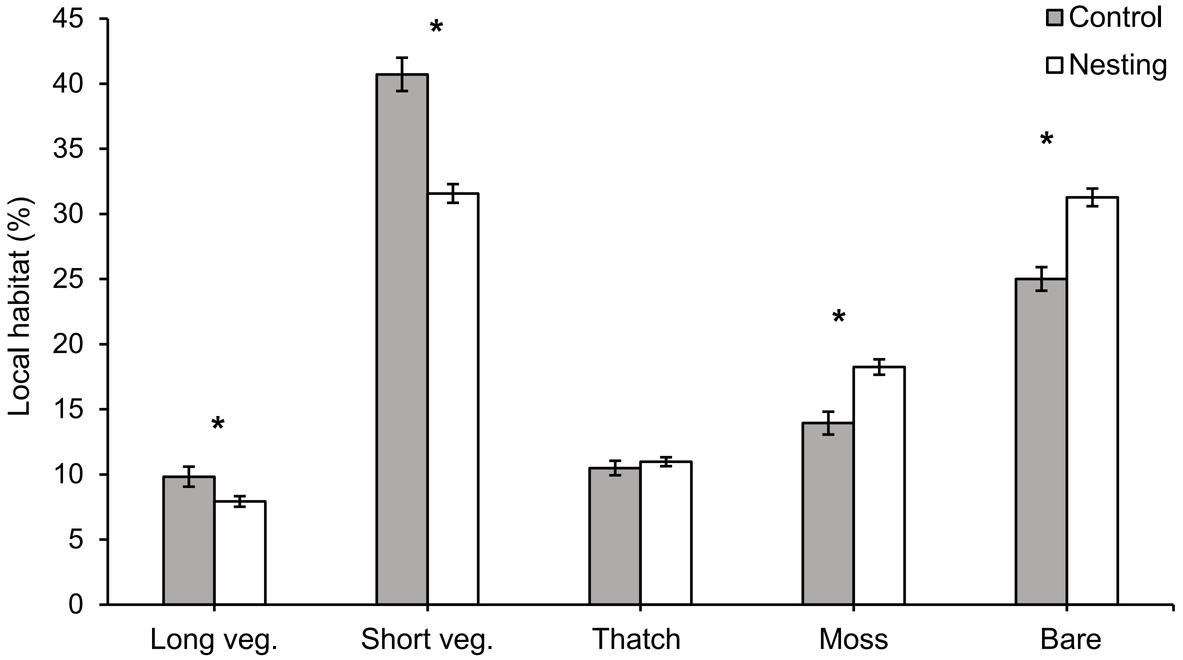


Figure 3.9: Nesting and control proportions of local habitats. The proportions of long (P < 0.001) and short vegetation (P < 0.001) were significantly lower in nesting than in control locations, and the ones of moss (P = 0.015) and bare ground (P < 0.001) were significantly

higher in nesting than in control locations. The star (*) on figure indicates significant differences (Welch Two sample t-test).

3.3.4 ***Andrena*** **nesting aggregations**

Over the three *Andrena* aggregations, 395 bee nests were recorded and assessed. The female occupiers were captured and recorded from 262 nests belonging to 214 *A. haemorrhoa* F., 47 *A. nitida*, and 1 *A. nigroaenea* K. Measurements of abiotic soil variables are summarised in Table 3.3. Soil temperature (GLMM, P = 0.039) and stoniness (GLMM, P < 0.001) were significant positive predictors, and soil moisture (GLMM, P = 0.048) was a significant negative predictor of nest density (Table 3.4). PC2 (vegetation cover) was also significant (GLMM, P < 0.001), but local habitat cannot be considered a significant factor as PC1 which represents most local habitat (62 %) is not significant (GLMM, P = 0.474), and PC2 represents only 18 % of local habitat. The ranges (min-max) of soil variables from the *Andrena* nesting aggregations were 1.3 – 22.3 % for stoniness, 18.3 – 29.9 °C for soil temperature, 3.0 – 14.5 % for soil moisture (Table 3.3). The highest nest density was recorded at 4.8 % stoniness, 18.3 °C soil temperature, 6.5 % soil moisture (Table 3.3).

Table 3.3: Mean ± SE, range and optimum values of variables recorded from *Andrena* nesting aggregations in apple orchards.

	Variables	Mean ± SE (*N*)	Range (Min-Max)	Optimum value
Bee nests	Nest density (1 m^2)	9.3 ± 2.2 (30)	2.0 – 27.0	27.0
Soil characteristics	Hydraulic conductivity (cm s^{-1})	7.3e-5 ± 1.5e-5 (60)	8.2e-6 – 3.9e-5	3.22e-4
	Soil compaction (kgf cm^{-2})	17.3 ± 0.7 (90)	12.7 – 25.8	15.9
	Soil moisture (%)	7.7 ± 0.5 (90)	3.0 – 14.5	6.5
	Soil temperature (°C)	22.1 ± 0.6 (90)	18.3 – 29.9	18.3
Vegetation cover	Short vegetation (%)	35.1 ± 4.1 (30)	5.0 – 73.0	10.0
	Short grass (%)	26.1 ± 3.5 (30)	5.0 – 67.0	10.0
	Short forbs (%)	9.0 ± 2.6 (30)	0.0 – 45.0	0.0
	Long vegetation (%)	20.2 ± 3.7 (30)	0.0 – 53.0	49.0
	Long grass (%)	13.8 ± 3.6 (30)	0.0 – 49.0	49.0
	Long fobs (%)	6.4 ± 2.6 (30)	0.0 – 53.0	0.0
	Thatch (%)	13.4 ± 1.9 (30)	0.0 – 35.0	8.0
	Moss (%)	11.5 ± 2.2 (30)	0.0 – 33.0	33.0
	Bare ground (%)	19.8 ± 3.9 (30)	0.0 – 80.0	0.0
Soil Composition	Organic matter (%)	9.5 ± 0.6 (30)	2.1 – 19.5	19.5
	Stoniness (%)	9.8 ± 1.0 (30)	1.3 – 22.3	4.8
	Root biomass (%)	0.9 ± 0.1 (30)	0.0 – 3.1	1.1
	Texture – Sand (%)	45.9 ± 1.7 (30)	28.2 – 72.7	40.0
	Texture – Silt (%)	52.6 ± 2.3 (30)	8.0 – 70	58.6
	Texture – Clay (%)	1.5 ± 0.1 (30)	0.0 – 2.5	1.4

Table 3.4: Results of the Generalised Linear Mixed Effect Model (GLMM) with nest density as the response variable and nine soil variables as predictors. Bold indicates significant effects (P < 0.05).

Predictor variables	Estimate	Std. Error	z value	*P* value	VIF
Soil stoniness	**1.042**	**0.269**	**3.879**	**< 0.001**	**1.077**
Hydraulic conductivity	-0.215	0.242	-0.888	0.374	1.585
Soil compaction	-0.445	0.232	-1.920	0.055	1.191
Soil organic matter	0.161	0.154	1.039	0.299	1.095
Soil root biomass	-0.004	0.231	-0.017	0.987	1.106
Soil moisture	**-0.720**	**0.363**	**-1.980**	**0.008**	**1.100**
Soil temperature	**1.005**	**0.488**	**2.059**	**0.039**	**1.201**
PC1 (Vegetation cover)	0.061	0.327	0.187	0.851	1.168
PC2 (Vegetation cover)	**-1.249**	**0.324**	**-3.859**	**< 0.001**	**1.304**
PC1 (Soil texture)	0.298	0.335	0.890	0.373	1.546

3.4 Discussion

This study contributes to our understanding of which ground-nesting bee species nest in apple orchards as well as their nesting preferences. Knowledge of ground-nesting bee nesting preferences in orchards and local habitats is vital in conserving existing, and creating additional, nesting substrates.

This study recorded 15 non-kleptoparasitic bee species nesting in apple orchards. Hutchinson et al. (2021) reported 10 of the species recorded in this study as dominant apple visitors, including the two most abundant bee species of this study, *A. haemorrhoa* F. and *A. nitida* M. (Fig. 3.7). It has often been shown that bees prefer bare open ground than vegetation cover for nesting (Wuellner 1999; Sardiñas & Kremen 2014; Tsiolis et al. 2022). However, this study shows that the preferred nesting habitat of bees in apple orchards was mainly under trees and at orchard edges, and their preferred local habitat was predominantly short vegetation (31 %), moss (18 %) and bare ground (31 %). This finding suggests that the recorded bee species prefer to nest in semi-shaded locations compared to fully exposed bare soil (e,g. Tsiolis et al. 2022).

Notably, nine of the recorded bee species, accounting for 79 % of the total specimens in this study, were univoltine (one brood per year) (Else & Edwards 2018). In contrast, the study in Chapter 2 (Tsiolis et al. 2022) reported nine bee species, accounting for 93% of total specimens, as either bivoltine (spring and summer brood per year) or eusocial bee species (Else & Edwards 2018), were nesting in bare ground plots. Bare soils are warmer than semi-shaded or vegetated soils and soil temperature has a significant role in many aspects of bees' lives, such as the development of larvae and emergence (Forrest 2017), activity rates (Borrell & Medeiros 2004), and the number of broods bees can have per year (Forrest et al. 2019). Bivoltine bee species might require faster larval development times than univoltine species, and their nesting location/habitat may reflect a need for substrates that provide warmer conditions to accelerate brood growth. This hypothesis (Michelle Fountain and Mike Edwards, personal communication) is supported by this study and the study in Chapter 2. However, further research is needed to confirm or reject this hypothesis and could involve direct manipulation of substrate temperatures for univoltine and bivoltine species, and recording development rates.

Identification of bee nests is important for understanding nesting behaviours but can be a challenging process for untrained eyes. Ants and worms also create structures similar to solitary bee tumuli, but usually, ant tumuli are made out of scattered soil particles compared to solitary bee tumuli, which have a more organised and firm structure (K. Tsiolis, personal observation). The size of nest holes can be a valuable tool in identifying nesting bee species if used in conjunction with other nest/species characteristics. The diameter of nest holes ranged from 3.35 mm (*Andrena chrysosceles* K.) to 6.90 mm (*A. nitida* M.) in this study (Table 3.2). Nest hole diameter can be very similar between ground-nesting bee species (see Table 3.2); hence, it cannot be a reliable predictor of nesting bee species alone. However, once bee nests are identified, the nest hole diameter can be used with the time of the year (active ground-nesting bee species) and body size of active ground-nesting bee species to determine possible nesting bee species. It was also observed that the tumuli of smaller bee species (e.g.

Lasioglossum malachurum K.) were made out of smaller soil particles than the tumuli of bigger bee species (e.g. *A. nitida* M.), and this can use as an additional characteristic for identification of bee species (Fig. 3.10).

Figure 3.10: A nest of A) *L. malachurum* K., and B) *A. nitida* M., which demonstrate that the tumulus of the former is made of smaller soil particles than the latter.

The three *Andrena* aggregations were a valuable find, enabling the further study of the nesting preferences of two prominent apple-pollinating species, *A. haemorrhoa* F. and *A. nitida* M. The first aggregation was located by observing bees' "diving" into long vegetation. It was then noted that semi-shaded hedgerows might be an important nesting habitat for some bee species, which led to locating another two aggregations in a similar habitat type. Subsequently, soil temperature and stoniness at *Andrena* nesting aggregations were identified as significant positive predictors of nest density (Table 3.4). Stones can be beneficial in bee-nesting soils due to their thermal properties. Potts & Willmer (1997) found that nests of *Halictus rubicundus* C. associated with stones were warmer than those without stones. In addition, studies have shown that pebbles on the soil surface can attract more bees to nest than bare soil (Cane 2015; Chapter 4). Soil moisture was a significant negative predictor of nest density. Soil moisture is important for larval development (May 1972), but excessive soil moisture can contribute to mould growth inside cells, degrade food resources, and risk bee survival (Ordway 1984). The optimum, or preferred, values for the significant factors in this study were: soil temperature 18.3 °C, stoniness 4.8 %, and moisture 6.5 % (Table 3.3). Another factor that might play a significant role in bee nest site selection is sunlight exposure. It was observed in early June 2021 that one of the *Andrena* nesting aggregations was exposed to the sun from around 08:00 to 12:30, and it was shaded for the rest of the day (K. Tsiolis, personal observation). Future studies should consider measuring sunlight exposure at the beginning of bee nesting activity when female ground-nesting bees are looking for a suitable nesting site.

Furthermore, distance from food recourse could be another factor determining nest-site selection (Westrich 1996). Studies have shown that solitary bees have relatively short flight distances relative to eusocial bee species. The maximum foraging range of *Andrena* species is estimated to be between 100-300 m (Gathmann & Tscharntke 2002). The nesting aggregations were typically between 5 and 300 m from wildflower plots (established for the study by Carvell et al. (2022)). The aggregation with the highest nest density (27 nests per m^2) was closest to a wildflower plot (5 m). However, this study did not include distance to food resources as a predictor factor.

This study aimed to locate additional *Andrena* nesting aggregations in 2022, to investigate further the factors which might determine nest-site selection by spring *Andrena* species. However, despite intensive efforts, no such nesting aggregations were located. It was also noticed that there was very low nesting activity under trees which was the orchard habitat with the highest nest density during the previous three years of the study. A few other PhD students and Mike Edwards (Entomological consultant/bee expert) also observed very low activity of spring *Andrena* species in 2022 (K. Tsiolis, personal communication). It may therefore be that the weather conditions during the spring of 2021 could have negatively impacted *Andrena* populations. There was a warm and dry two-week period between late March and early April of 2021, and male *Andrena* emergence was recorded. However, weather conditions suddenly changed to atypically wet and low temperatures for three weeks (below 0 °C for 4-5 days). Consequently, the question was whether emerged males would be able to survive the unfavourable weather conditions and remain present at female emergence for mating to occur? If not, how would this impact the following year's spring *Andrena* populations? Unmated females can lay unfertilised eggs, producing male bees (Michener 1974). Anecdotally, there was high male activity in the spring of 2022, but very low female activity following that. Weather data from a Kent-based weather station (Met office – East Malling) shows that in April and May (*Andrena* active period; Else & Edwards 2018), ambient and soil temperatures were lower in 2021 than 2019 and 2020 (Appendix 11). It also shows that rainfall was higher and solar irradiation lower in May (peak *Andrena* activity) 2021 than the previous two years (Appendix 11). Climate change can significantly impact the phenology of wild bees (Bartomeus et al. 2011), but this study indicates that rapid weather changes during specific stages of bees' life cycle may potentially negatively affect bee populations. However, further research is needed to test these observations.

3.4.1 Creating and managing nesting habitats for ground-nesting bees

Creating and effectively managing nesting habitats for beneficial ground-nesting bee species is essential for the sustainability of pollinator communities in apple orchards. Farmers should aim to create and maintain nesting habitats, taking into account the preferred nesting conditions and forage distances of solitary ground-nesting bees. This study shows that sparsely vegetated habitat, with approximately 30% bare ground, was generally the most preferred local habitat for bee nesting in apple orchards (Fig. 3.9). Such local habitat can be created by mechanical (e.g. scrapes) or chemical (spraying off vegetation with herbicides) means, and maintained under apple trees and at north, north-east and east facing hedges, which were the predominantly bee nesting orchard habitats in this study.

Planting of hedges should be less than 300 m away from tree rows to be within the foraging range of *Andrena* species (Gathmann & Tscharntke 2002). Providing nesting habitat both under trees and hedges would help ensure that all apple trees within an orchard would potentially increase the chance to be visited and pollinated by *Andrena* species. Farmers can manage vegetation cover under trees and hedges via herbicide treatments. The aim should be to have approximately 70% vegetation growth from April to the middle of June, which is the nesting period of univoltine *Andrena* species of interest for apple pollination (Hutchinson et al. 2021; Else & Edwards 2018; K. Tsiolis, personal observation). Hence, a suggested approach is to monitor vegetation growth and apply a herbicide treatment under trees, if necessary, a month before bee activity (early March) and then allow vegetation growth until the end of the bee nesting phase.

This study also demonstrates that the two most abundant apple-pollinating bee species, *A. haemorrhoa* F. and *A. nitida* M.*,* favour heavily vegetated hedgerows as the highest nest density (27 nests per m^2) at nesting aggregations was recorded at 0% bare ground. Consequently, it is suggested to create 100 % grass-vegetated plots, with dimensions 20 m x 2 m (approximate size of dense highest nest density in this study) at north, north-east and east facing hedges of apple orchards to serve the nesting needs of these species. The nesting habitats should receive sunlight exposure in the morning and be shaded in the afternoon (K. Tsiolis, personal observation). Additional factors that need to be considered are soil moisture at 6.5 %, soil temperature at 18.3 °C, and stoniness at 4.8 %. Farmers could pre-survey various potential locations for establishing nesting substrates and then select those which are closest to the known soil moisture temperature preferences. In addition, potential nesting sites could be partially covered with a variety of stones of different sizes, which may create more thermally suitable conditions.

Disturbance of potential nesting habitat (e.g. by cultivation, scarification, vehicle traffic) should be minimised wherever possible, especially during the bees' nesting period and beyond. The nesting locations at edges or hedgerows could be marked and have signs to highlight to farm workers the importance of avoiding disturbance. Wuellner (1999) reported that although vehicular traffic did not impact nest initiation, but it did negatively impact nests' success.

Overall, the best approach to enhancing nesting opportunities for apple pollinators and other ground-nesting bees is to provide a wide variety of nesting conditions that meet the known requirements of these species. Providing local scale heterogeneity in both nesting and floral resources can help ensure that the right pollinator communities can persist year on year, even under changing weather, farm management, and other conditions.

Conclusion

In summary, this study found that orchard productive areas and uncultivated local habitats are important for the nesting of apple pollinators. Bees mostly nested in orchard edges and under orchards trees, and their nest entrance was mainly surrounded by short vegetation, moss and bare ground. Moreover, high soil temperature, low moisture and high stoniness were significant predictors of *A. haemorrhoa* F. and *A. nitida* M. nesting aggregations, and these two bee species have shown nesting preference in heavily vegetated habitats. Future studies on orchard landscapes should consider these findings to maintain and increase the nesting habitats of beneficial apple pollinators.

4 Chapter 4 – Soil compaction preferences of ground-nesting bees

4.1 Introduction

The nesting ecology and soil properties preferred by ground-nesting bee species are relatively under-researched compared to foraging behaviours. Previous research has highlighted that soil compaction is a significant nesting factor for ground-nesting bees (Brockmann 1979; Potts & Willmer 1997; Kim et al. 2006; Polidori et al. 2010; Sardiñas & Kremen 2014). The excavation process of a nest is a substantial investment for most ground-nesting Hymenoptera incurring energy and time costs (McCorquodale 1989). Females of *Halictus rubicundus* C. in the UK have been observed visually searching an area to nest (Potts & Willmer 1997) and even biting the soil surface and carrying out digging tests to potentially determine the soil hardness of a specific soil landscape (S. G. Potts personal observation, cited in Potts & Willmer 1997). Nevertheless, harder soils have their benefits as they can support the structure of bee nests, especially in nesting aggregations. The ease of excavation outweighs the risk of nest collapse as the distance between bee nests decreases. Potts & Willmer (1997) found that soft soils are initially selected, but as nesting aggregations increase in size, *H. rubicundus* C. favour harder soils, which can support the architecture of their nests.

Soil temperature, moisture, and the presence of pebbles on the soil surface can also be considered important nesting factors for ground-nesting bees. Below-ground temperatures may influence the developmental rate or survival of larvae (Forrest 2017) and, at the soil surface level, the time that a bee can start foraging activities in a day (Stone 1994). The ability of heat to penetrate the soil decreases with rising soil bulk density and increases with soil water content (Marshall et al. 1996). Soil moisture also has a beneficial role in the development of bee larvae (May 1972), but excessive soil moisture could encourage the development of fungi and kill overwintering bees (Stephen 1965) or delay the emergence of ground-nesting bees (Visscher et al. 1994). The general advice to conservationist and land managers is to provide bare soil as nesting habitat for ground-nesting bees (Frankie et al. 2014; Tsiolis et al.

2022). Nevertheless, Cane (2015) has demonstrated that stream pebbles on the soil surface were a preferred nesting factor for the ground-nesting bee species *H. rubicundus* C. in the US. The aim of this study was to 1) test different soil compaction levels and determine the preferred range of soil compaction of ground-nesting bees, 2) identify which bee species are attracted to artificially constructed nest sites, and 3) identify the preferred abiotic nesting factors that drive nest site selection of ground-nesting bees.

4.2 Methods and Materials

4.2.1 Experimental design

The study took place in Kent, UK (Fig. 4.1), from March 2019 to August 2022. Previous studies have shown sandy loam to be the preferred soil texture for nesting by several ground-nesting bee species (Cane 1991; Harmon-Threatt 2020; Tsiolis et al. 2022) as it is soft enough to dig their burrows and firm enough to support the structure of their nests. Consequently, sandy loam was the chosen soil texture for this experiment, provided by a Kent (UK) based soil company (Bourne Amenity Ltd). Five samples from stock soil were taken to the University of Reading for particle size distribution analysis (Blott et al. 2004) in 2022, and the soil texture used was determined to be in the sandy silt loam category, which is very similar to sandy loam though with a slightly higher silt and lower sand content.

Sixteen heavy-duty tree planting tubs (Diameter 60 cm x Height 48 cm, Volume 90 Ltr.) were used for each block of soil compaction treatments and untreated controls (Fig. 4.2). Five centimetres of gravel (Diameter 20 mm) were placed at the bottom of each tub to facilitate drainage, and then 45 cm of the sandy silt loam subsoil was added. Subsoil was chosen as it is suspected to have less plant seeds than topsoil. A row of 10 mm drainage holes, 20 cm apart, were drilled at the soil surface height to allow any standing water to drain, another at 20 cm soil depth, and a third at 40 cm soil depth. A year after the establishment when the soil had settled, another two rows of 10 mm holes, 10 cm apart, were drilled at 10 cm and 30 cm soil depth on the downward slope of each tub to allow rainwater to drain away.

Figure 4.1: Map of three sites (blue markers) where the study was undertaken.

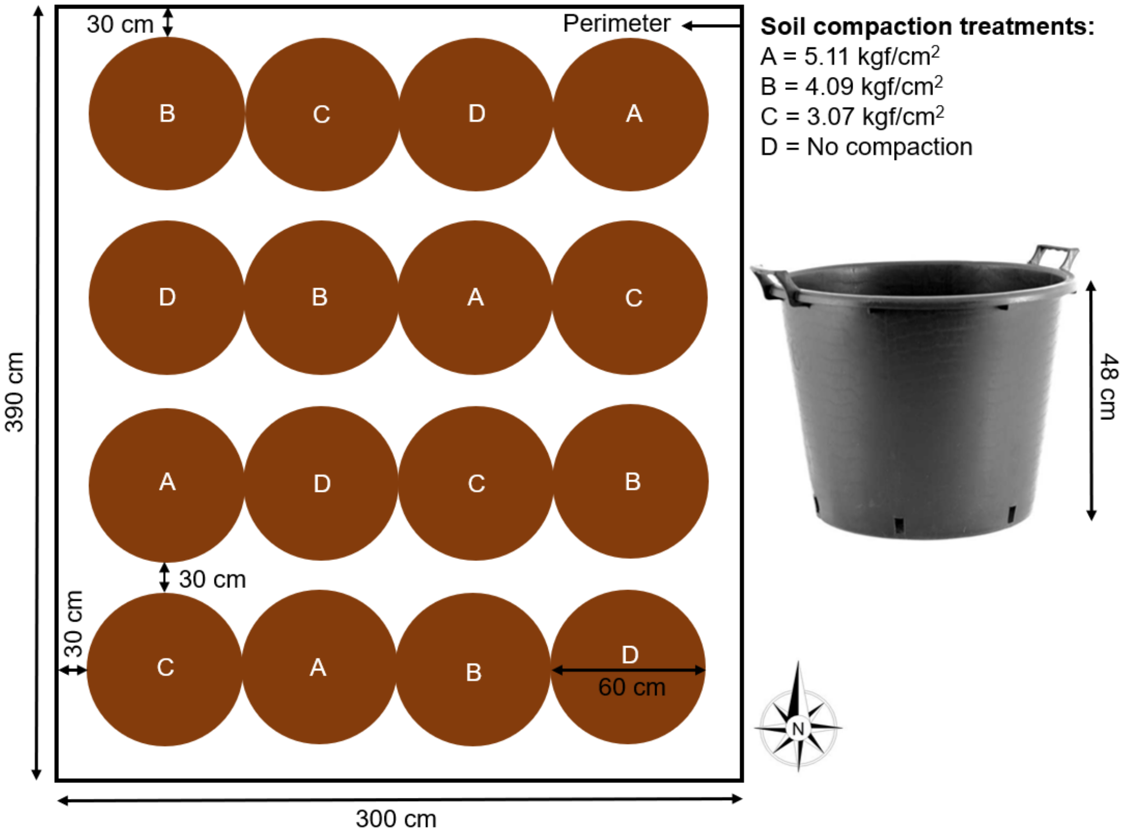


Figure 4.2: Arrangement of soil compaction treatments and untreated controls in each block.

The experiment had four compaction treatments A) 5.11 kgf/cm^{-2}, B) 4.09 kgf/cm^{-2}, C) 3.07 kgf/cm^{-2}, and D) an uncompacted control. Treatment B was chosen as the mean soil compaction of studies that reported the preferred soil compaction of bee nesting sites (Potts & Willmer 1997; Kim et al. 2006; Polidori et al. 2010; Sardiñas & Kremen 2014; Tsiolis et al. 2022). This level of compaction was increased by 25% for treatment A and reduced by 25% for treatment C. The desired soil compaction levels were achieved by placing a circular piece of plywood (diameter 55 cm x height 1.2 cm) on the surface of the soil and applying pressure cruciformly (see Fig. 4.3) using a tamper tool. The cruciform method was repeated six times for treatment A, four times for treatment B, and twice for treatment C. Treatment D was left untreated. Several preliminary trials took place to determine the pressure required to achieve the chosen soil compaction levels.

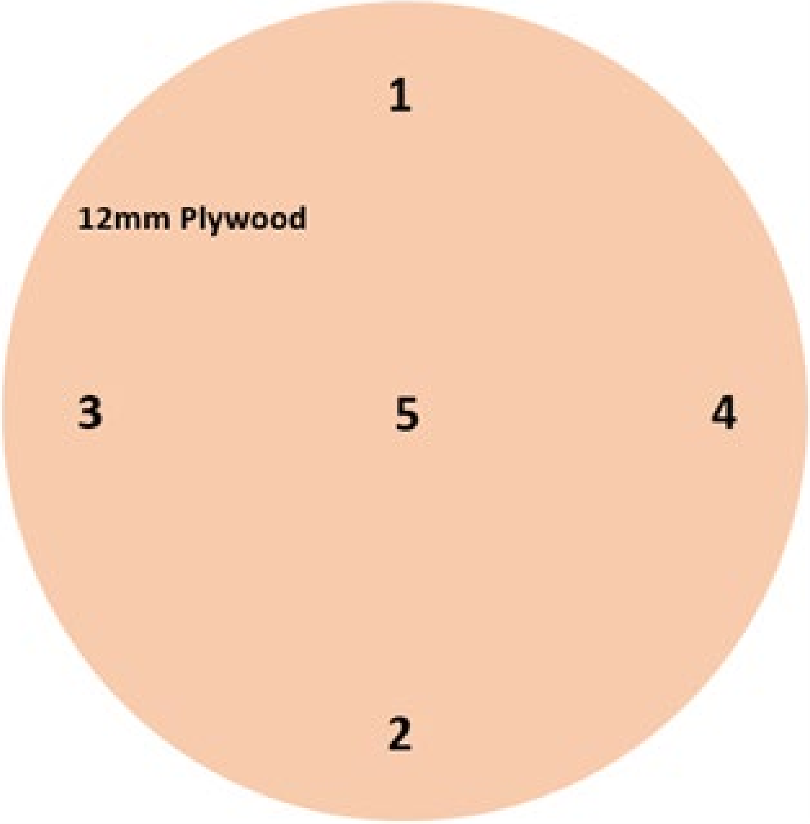


Figure 4.3: The "cruciform method" used to form the three soil compaction treatments. The pressure was applied on circular plywood in the following order, 1) North, 2) South, 3) West, 4) East, and 5) middle using a tamper tool.

A block design (Fowler et al. 1998) of 16 tubs (4 tubs x 4 tubs) of soil compaction treatments and untreated controls was created. Each row and column of the block had each of the three soil compaction treatments and a control (Fig. 4.2). Previous studies reported ground-nesting bees often nest in more or less vertical landscape profiles (i.e., cliffs), which is thought to enhance the thermal gain of soils (c.f flat surfaces) and prevent soil waterlogging (Potts & Willmer 1997; Potts et al. 2005; Sardiñas & Kremen 2014). Therefore, the soil compaction treatment tubs were placed on wooden Euro pallets (length 120 cm x width 80 cm, two tubs per pallet). The north side of each pallet was lifted to a 14.4 degrees (25%) slope by placing a wooden railway sleeper (height 20 cm x width 10 cm x length 60 cm) underneath. In addition, a wooden batten (height 2.5 cm x width 3.8 cm x length 100 cm) was placed in front of the tubs to prevent them from sliding off the pallet.

Cane (2015) showed that the addition of stream pebbles on the soil surface was positively associated with the nesting of *H. rubicundus* C. Consequently, pebbles were added to the surface of the soil compaction treatments in February 2022. Each treatment's surface area was divided into two equal-sized sections using a measuring tape and natural twine string. Half the tubs had a vertical split and half a horizontal split. A monolayer of rounded, flat stream pebbles (30-50 mm) was randomly allocated to one of the two halves, with each row of tubs having a top, bottom, left, and right pebble position and a bare section (Fig. 4.4). Pebbles had approximately 10 mm gaps between them, and were lightly pressed into place using a tamper tool.

Three replicate blocks of soil compaction treatments were used for the study; a block was placed at the south-facing headland of two Kent apple orchards and the third between apple and pear orchards at NIAB East Malling. The study blocks were located approximately 10 m from known ground-nesting bee nesting sites to increase the opportunity for bees to use them as nesting resources. The tubs faced south, and the chosen location for each block was in full sun during morning and afternoon hours. High-visibility tape was placed on each tub (side facing away from the trial) to increase its visibility to farm staff, and a sign at the front and rear of each block of soil compaction treatments to emphasise the importance of not being disturbed.

Figure 4.4: Soil compaction trial with pebble and bare soil treatments in each block.

4.2.2 Species activity

Three surveys took place in the spring and three in the summer of 2019, 2020, 2021, and 2022 to record nesting bee species. The duration of surveys was an hour and undertaken during sunny and/or mild weather with temperatures above 10°C, if cloud cover did not exceed 4 oktas. On cloudier days (5-8 oktas), surveys took place when temperatures were at least 14°C. Wind speeds were below Beaufort scale 5, or 29 km/h (Pywell et al. 2005). Insect specimens were collected if they exhibited positive nesting behaviour (such as antennal tapping, inspecting digging, biting of soil, and digging a nest) associated with the trial blocks. Insects were collected using a hand net (Watkins & Doncaster, Leominster, UK) and placed into a 5 ml clear plastic tube in an ice bucket. They were kept inactive until the end of the observation period (Grixti et al. 2009). Subsequently, they were identified to species level in the field. If this was not possible, photographs including the key characteristics were taken for later identification and confirmation by Mr Mike Edwards, a professional entomological consultant (Else & Edwards 2018). Specimens were subsequently released at the site. In addition, a few samples were collected for later identification in the laboratory whenever significant numbers of kleptoparasites and wasps were observed. The blocks were sampled randomly, ensuring that each block of soil compaction treatments was visited in the morning and afternoon.

4.2.3 Nest density

The nest density in each tub was recorded and marked on an annotated map to record the continuity of nesting. Bee and wasp nests were identified by the tumuli (volcano-shaped mounds of earth) females make in the nest excavation phase (Michener 2007; K. Tsiolis, personal observation). Ants can create structures similar to solitary bee tumuli, but usually, ant's tumuli are made out of scattered soil particles compared to solitary bee tumuli, which have a more organised and firm structure (K. Tsiolis, personal observation). It was impossible to confidently separate bee and wasp's nests as their tumuli were very similar in many cases. The nest density in the pebbles and bare soil treatments was also recorded in 2022. It was noted that bees and wasps were utilising the vertical drainage holes of tubs for nesting; hence, nesting was recorded as “Top” for surface area and “Side” for drainage holes. These surveys took place immediately after the species surveys.

4.2.4 Soil conditions

Soil compaction (defined as unconfined compression strength; kg/cm^{-2}) was measured in 2019 using a pocket penetrometer. Subsequently, an electronic recording cone penetrometer from Solutions for Research Ltd (Bedfordshire, UK) providing measurements with higher accuracy was used for assessments in February 2020, 2021 and 2022 by taking two probing readings per soil tub. Readings were taken at 2.5 cm depth intervals, and the mathematical mean was calculated for those collected from the top 20 cm (typical ground nesting depth (Michener 2007). The cone index was corrected for soil moisture content (measured using a MO750 model soil moisture meter; Extech Instruments Corp., New Hampshire, U.S.), according to Busscher et al. (1997) . A piece of plywood (depth 65 cm x height 1.2 cm) was adapted and used as a platform for the penetrometer as it is heavy (9 kg), and it would have caused significant disturbance if it had been put directly on the soil surface. A hole (depth 5 cm) was created on plywood, and only the shaft of the penetrometer's probe had contact with the soil.

Soil moisture and soil temperature were recorded twice in the spring and the summer of 2022 by measuring pebble and bare soil areas in each tub. The HH2 moisture meter and a WET-2 sensor (Delta-T Devices Ltd., Cambridge, UK) were used.

4.2.5 Maintenance

Some ground-nesting bee species prefer bare ground as their nesting habitat (Sardiñas & Kremen 2014). Consequently, every 2-3 weeks, weeds were removed by hand. When vegetation cover exceeded ≈10%, bare ground was reinstated by applying glyphosate at the recommended dose of 1,800 g/ha^{-1}. Four glyphosate applications were made (July 2019, 2020, 2021 and 2022) on the surface of each treatment, around the blocks (1 meter), and within each row of treatments to prevent vegetation from getting above tubs' height and influencing the experiment.

Wildlife disturbance on soil tubs during the autumn months of 2020 and 2021 was minimal at one block and severe at another. Firstly, a small number of holes in the shape of a bird's beak were noted on the surface area of soil tubs in the autumn of 2020, but no action was taken as the disturbance was not considered significant. Soon after, disturbance was noted on the surface area of soil tubs at a different block, which was believed to be from badgers (footprints on soil). An electric fence was set up on the block's perimeter of soil tubs to prevent further disturbance and was successful. Then, severe disturbance was noted in the autumn of 2021 in all soil tubs (soil was dug at approximately 5 cm in depth) of the block that previously experienced minimal disturbance. It was by unknown wildlife (there is a badger population on the farm). Omnivores such as birds, badgers and other mammals might have been attracted to feed on nests of bees and wasps.

4.2.6 Statistical analysis

R studio (Version 4.1.2; R Core Team 2021) was used for all statistical analyses. Two Kruskal-Wallis rank tests were used: 1) to determine whether nest density significantly differed between compaction treatments on the top, and 2) on the side of tubs. Two Kruskal-Wallis rank tests were used: 1) to determine whether nest density significantly differed between years on the top, and 2) on the side of tubs. A Welch Two sample t-test was used to explore the effects of pebbles and compaction treatment on nest density in 2022. A Kruskal-Wallis rank test was used to explore the difference in soil compaction between treatments in 2020, 2021, and 2022. The Dunn's Test of Multiple Comparisons (R package: rstatix) was used to identify which treatments significantly differed from which. Two Welch two sample t-tests were used; to determine if there was a significant difference between pebble and bare soil treatments with respect to 1) soil temperature, and 2) soil moisture. Then, two one-way ANOVA tests were used to determine if there was a significant difference between soil compaction treatments with respect to: 1) soil temperature, and 2) soil moisture. A Generalised Linear Mixed Effect Model (GLMMs, R package: lme4) was used to determine whether there is a significant correlation between bee/wasp nests on the top of soil tubs (response), and soil temperature, moisture, compaction and pebbles/bare soil (predictors) irrespective of treatments. The variance inflation factor (R package: car) was used to identify variables with high multicollinearity. Low multicollinearity was apparent (all variables VIF < 3).

4.3 Results

4.3.1 Species activity

Over the three years of sampling, 870 insect specimens were recorded, 605 were collected from the top, and 265 from the side of soil compaction treatments. The specimens belong to 5 bee species (4.7% of total specimens) and 10 wasp species (95.3% of total specimens). The most abundant bee species was *Megachile willughbiella* K. (2.8% of total specimens), the second most abundant *Coelioxys elongate* L. (1.3% of total specimens), and the third most abundant *Lasioglossum smeathmanellum* K. (0.3% of total specimens). The most abundant wasp species was *Oxybelus uniglumis* L. (70.9% of total specimens) (Table 4.1).

Table 4.1: Total records of bee and wasp species (K = Kleptoparasites) sampled from the top and side of soil compaction treatments in 2020, 2021, and 2022, and the percentage of species present based on the total records.

Species	Bee/wasp	Side	Top	Total	%
Oxybelus uniglumis Linnaeus	Wasp	22	595	617	70.9
Anoplius nigerrimus Scopoli	Wasp	171		171	19.6
Crossocerus distinguendus Morawitz	Wasp	25		25	2.9
Megachile willughbiella Kirby	Bee	24		24	2.8
Coelioxys elongata Lepeletier	Bee (K)	11		11	1.3
Gorytes laticinctus Lepeletier	Wasp	4	3	7	0.8
Lasioglossum smeathmanellum Kirby	Bee		3	3	0.3
Mimumesa dahlbomi Wesmael	Wasp	3		3	0.3
Lasioglossum minutissimum Kirby	Bee		2	2	0.2
Nysson trimaculatus Rossi	Wasp (K)	1	1	2	0.2
Crossocerus annulipes Brullé	Wasp	1		1	0.1
Ectemnius continuus Fabricius	Wasp	1		1	0.1
Lasioglossum fulvicorne Kirby	Bee		1	1	0.1
Oxybelus mandibularis Dahlbom	Wasp	1		1	0.1
Pemphredon inornata Say	Wasp	1		1	0.1
Total		**265**	**605**	**870**	

4.3.2 Nest density

Bees and wasps were observed and recorded nesting both on top and on the side of soil compaction treatments (Fig. 4.5). The highest number of bee nests (68) belonged to *M. willughbiella* K., which nested through the side (drainage holes) of the soil compaction treatments and untreated controls at Ockford Farm. Nest density did not significantly differ between soil compaction treatments either on top (Kruskal-Wallis rank test / $P = 0.785$) or side (Kruskal-Wallis rank test / $P = 0.591$) of soil tubs (Fig. 4.6). Nest density differed significantly at the top (Kruskal-Wallis rank test / $P < 0.000$) and side (Kruskal-Wallis rank test / $P < 0.000$) of soil compaction treatments between years. Nest density significantly increased between

2020 – 2021 (P < 0.000) and 2020 – 2022 (P < 0.000) on top; and between 2020 – 2022 (P < 0.000) and between 2021 – 2022 on the side of soil compaction treatments (Dunn's Test of Multiple Comparisons) (Fig. 4.7). There was also a significant difference in nest density between bare soil and pebble treatments with pebbles treatments attracting a significantly higher number of nests than bare soil treatments (Welch Two sample t-test / P = 0.006) (Fig. 4.8). Soil temperature (P < 0.000), compaction (P = 0.024) and pebbles (P < 0.001) were significant positive predictors, and soil moisture (P = 0.001) a significant negative predictor of nest density (Generalised Linear Mixed Effect Model (Table 4.2)).

Table 4.2: GLMM analyses of soil variables. All variables tested had a significant correlation with nest density. Significant variables: 0 '***' / 0.001 '**' / 0.01 '*' / 0.05 '.'

Variables	Estimate	Std. Error	z value	P value	VIF
Soil temperature	0.185	0.014	13.111	< 0.001	1.019
Soil moisture	-0.014	0.004	-3.293	0.001	1.032
Soil compaction	0.029	0.013	2.256	0.024	1.004
Pebbles	0.530	0.081	6.572	< 0.001	1.021

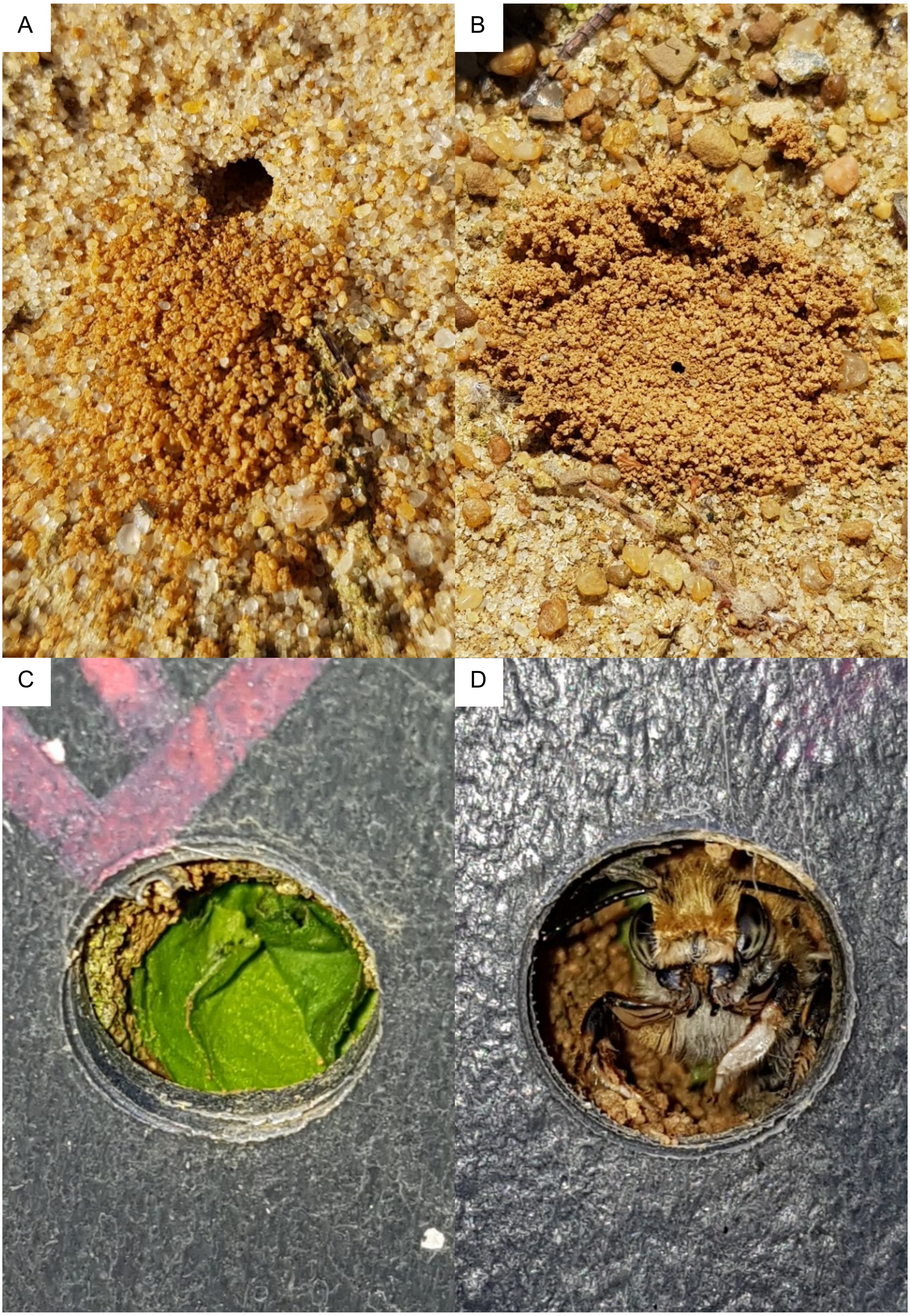


Figure 4.5: Nest entrances of bee and wasp species nesting in soil compaction treatment tubs. A) Nest of *O. uniglumis* L. (top), B) Nest of *Lasioglossum minutissimum* K. (top), C) Nest of *M. willughbiella* Kirby (side), and D) *M. willughbiella* K. (side) at the entrance of its nest.

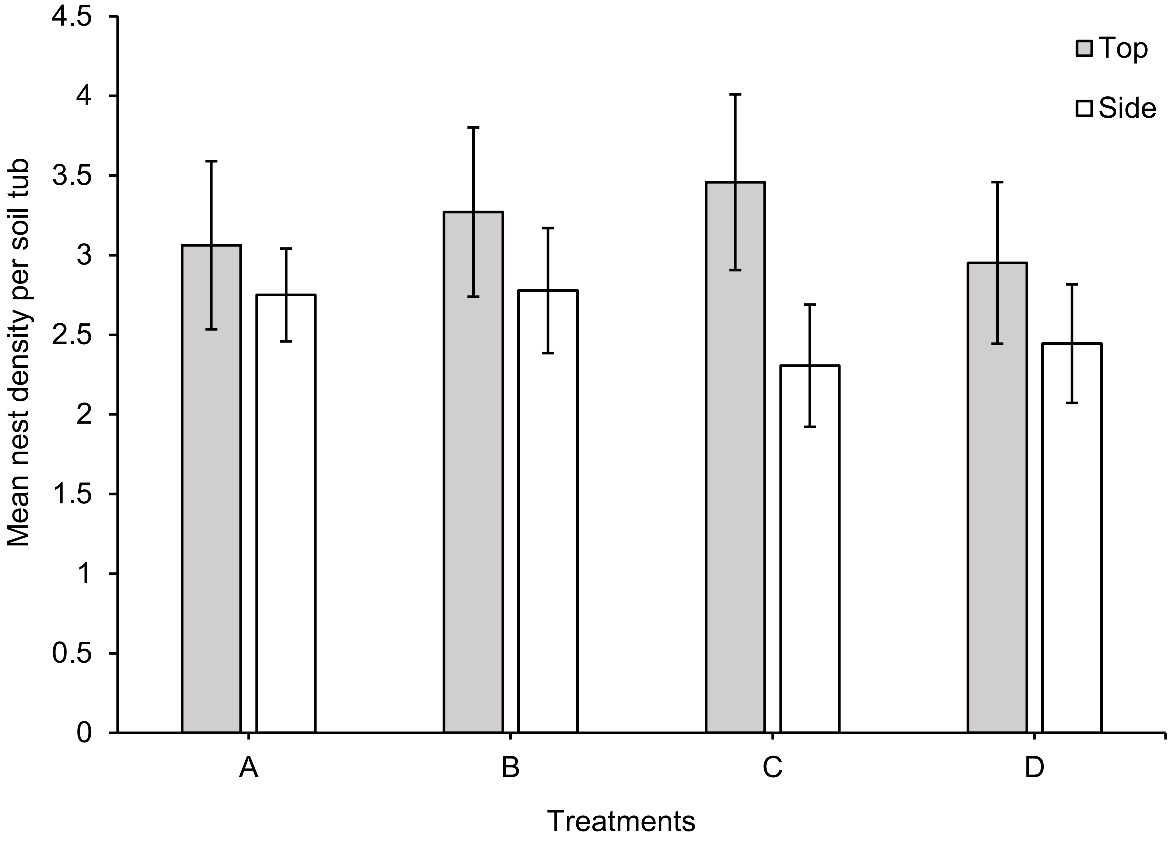


Figure 4.6: Mean (± SE, N = 288) nest density at the top and side of soil compaction treatments. There was no significant difference in either nest densities between soil compaction treatments (A-C, highest to lowest respectively) and untreated control (D).

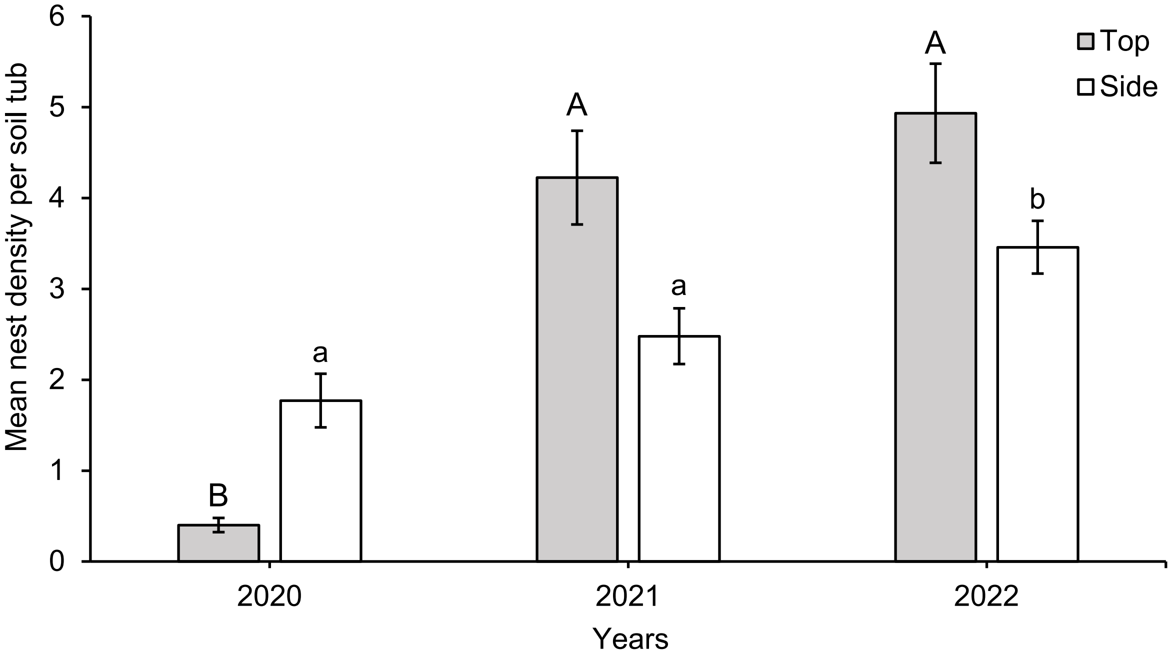


Figure 4.7: Mean (± SE, N = 288) nest density at the top and side of soil compaction treatments between years. Nest density differed significantly at both top (P < 0.001) and side (P < 0.001) sections of soil compaction treatments between years. According to Dunn's Test of Multiple Comparisons, different letters indicate significant differences between years (P < 0.05) (Capital letters – Top, Lower case – Side).

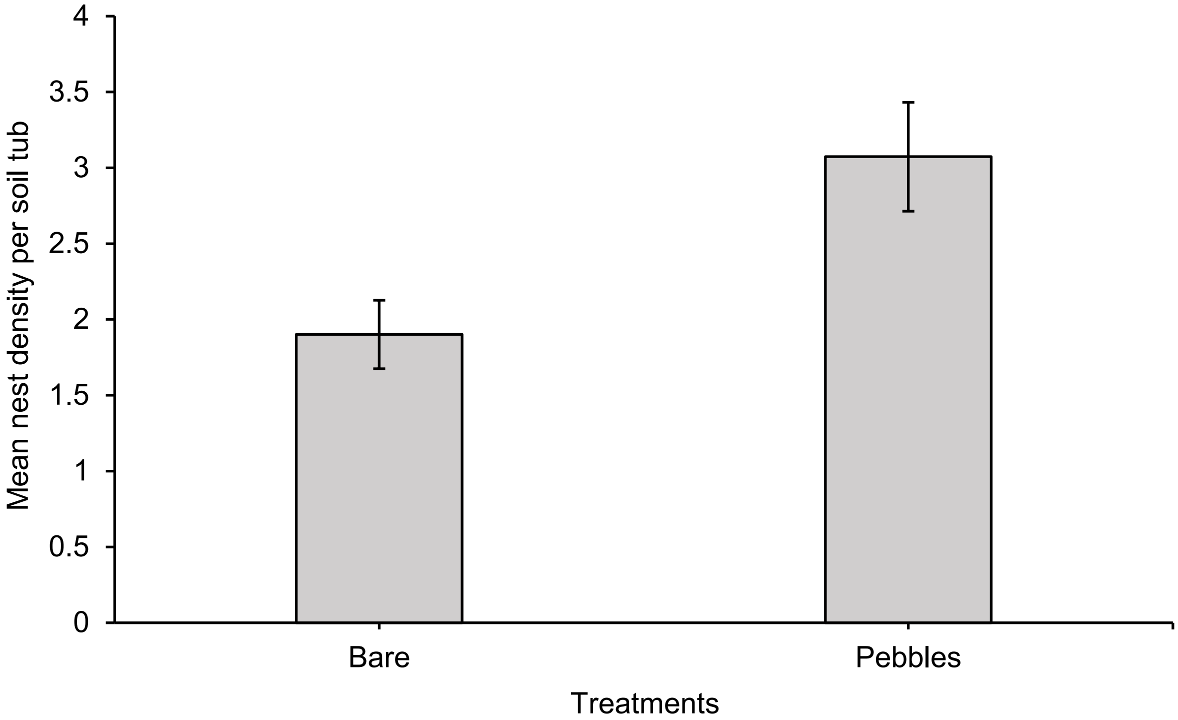


Figure 4.8: Mean (± SE, N = 288) nest density of bare soil and pebble treatments in 2022. Nest density was significantly higher in pebble than bare soil treatments (P = 0.006).

4.3.3 Soil compaction

Soil compaction differed significantly in 2020 (Kruskal-Wallis rank test / P < 0.001), 2021 (Kruskal-Wallis rank test / P < 0.001), and 2022 (Kruskal-Wallis rank test / P < 0.001). There was a significant difference between treatments A – C (P = 0.019), A – D (P < 0.001), B – D (P < 0.001), and C – D (P = 0.008) in 2020; A – C (P = 0.025), A – D (P < 0.001), B – D (P < 0.001), and C – D (P = 0.002) in 2021; and A – D (P < 0.001), B – D (P = 0.001), and C – D (P = 0.039) in 2022 (Dunn's Test of Multiple Comparisons) (Fig. 4.9).

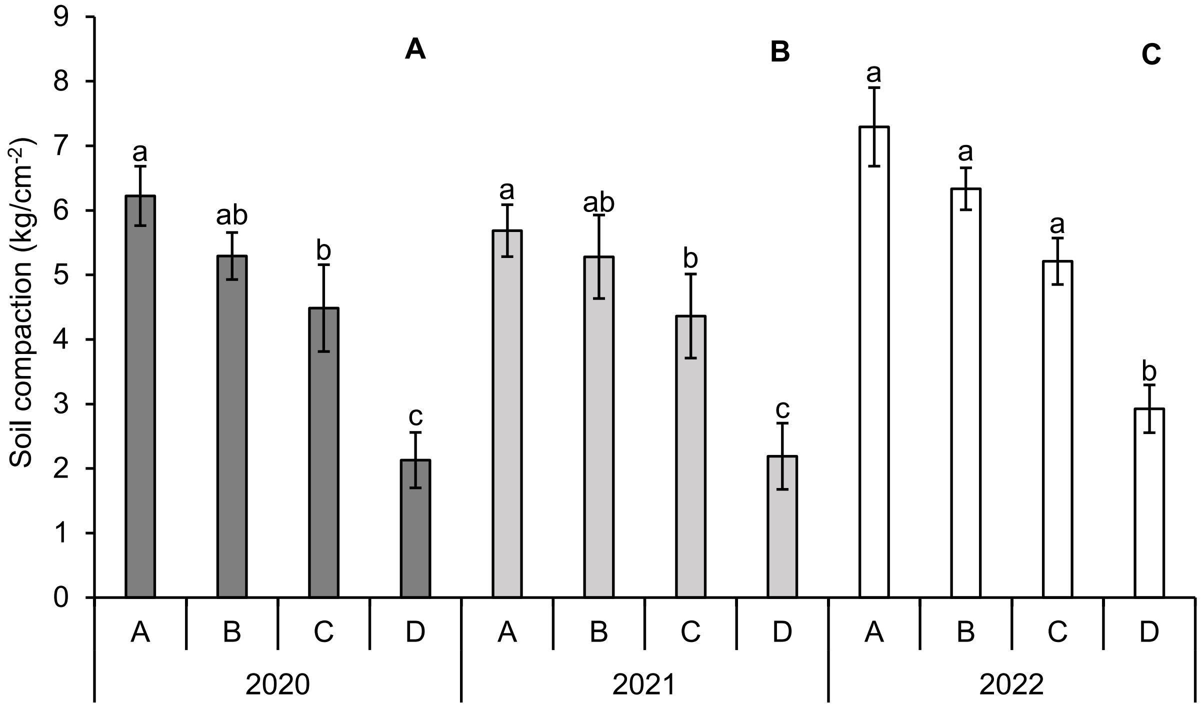


Figure 4.9: Mean (± SE, N = 24) soil compaction of soil compaction treatments and untreated controls in 2020, 2021, and 2022. There was a significant difference between soil compaction treatments in (A) 2020 ($P < 0.000$), (B) 2021 ($P < 0.000$), and (C) 2022 ($P < 0.000$). According to Dunn's Test of Multiple Comparisons, different letters indicate significant differences between treatments ($P < 0.05$).

4.3.4 Soil moisture and temperature

The mean temperature was 33.9 ± 0.4 °C (N = 192) for bare ground and 34.3 ± 0.4 °C (N = 192) for pebble treatments. There was no significant difference in soil temperature (Welch Two sample t-test / $P = 0.568$), but there was a significant difference in soil moisture (Welch Two sample t-test / $P = 0.008$) between bare soil and pebble treatments. The mean soil moisture was 18.9 ± 0.9 % (N = 192) for bare ground and 22.4 ± 1.0 % (N = 192) for pebble treatments (Fig. 4.10). There was no significant difference in soil temperature (one-way ANOVA / $P = 0.862$), and soil moisture (one-way ANOVA / $P = 0.491$) between soil compaction treatments and untreated controls.

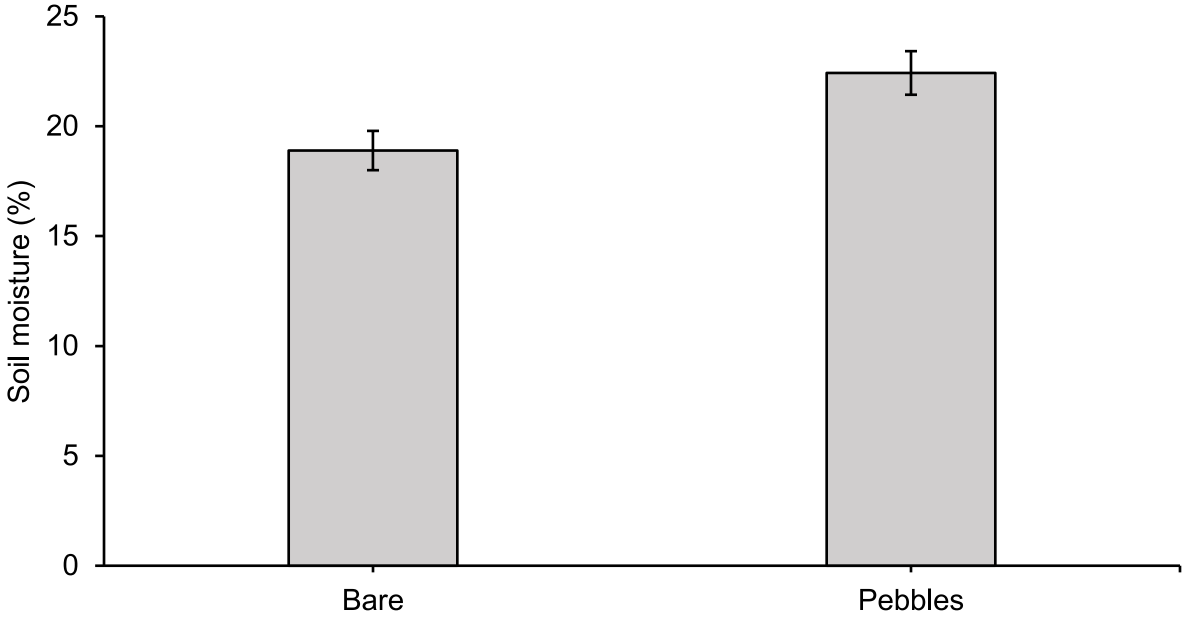


Figure 4.10: Mean (± SE, N = 192) percentage of soil moisture of bare soil and pebble treatments in 2022. Soil moisture was significantly higher in pebble than bare soil treatments (P = 0.009).

4.4 Discussion

The great majority of species that used the blocks of soil compaction treatments and untreated controls as nesting resources were the predatory wasp species *O. uniglumis* L. (70.9 %) nesting mainly on the top of the soil tubs and *Anoplius nigerrimus* S. (19.7 %) nesting entirely on the site of the soil tubs. Additionally, the leafcutter bee *M. willughbiella* K. was the bee species with the highest abundance (2.8 %), and it was unexpectedly nesting through the draining holes of soil tubs. Female *M. willughbiella* K. were observed cutting circular pieces of young apple leaves and taking them to their nests to construct their cells (Else & Edwards 2018). *C. elongate* L. was the second most abundant bee species, a kleptoparasite of *M. willughbiella* K. (Else & Edwards 2018)*.* Fighting between the host and kleptoparasite was observed in the effort of the latter to enter a nest.

There was no visitation or nesting activity in the first year (2019) of this study. However, the study attracted ground-nesting wasp species to nest in soil tubs after being exposed to various weather conditions for a year. Such weathering might be necessary for the soil to settle and become suitable for bee/wasp nesting. Untreated soils remained significantly less compacted than treated soils through the whole experiment. Nesting on the top surface of soil tubs significantly increased between 2020 and 2021.

The disturbance of soil in 2020 and 2021 could have negatively impacted the bee and wasp populations nesting in soil tubs, despite the increase in nest density in 2022. Such disturbance could have been avoided if the top surface area of the soil tubs had been covered with a metal mesh.

There was a significant nesting preference in pebble treatments over bare soil in 2022. Bees can use pebbles as visual landmarks to locate their nest (Brünnert et al. 1994), which can be particularly important when nest tumuli are washed away after rain or strong winds. A nest entrance next to the pebble could also be advantageous as it could reduce the chances of blocking entrance by vehicle or foot traffic (Cane 2015). Soil temperature had a significant positive relationship with nest density in this study (Table 4.2). Despite the mean soil temperature of pebble treatments being only 0.3 °C higher than bare soil treatments, pebbles absorb higher levels of solar radiation than bare soil, and could act as thermoregulators during the night (Cane 2015). Preference for nesting under stones was observed in the ground-nesting *Forficula auricularia* L. (Beall 1932). Lamb (1976) suggested this could be due to heat regulation of its nests as soil temperature near the surface is less stable than at deeper levels; *F. auricularia* L. is possibly able to regulate egg temperature and speed of hatching by moving her eggs against the stone or near the soil surface. Ground-nesting bee species do not move their eggs near the surface, but they may use stones in the upper level of their nests for the same biological reason as *F. auricularia* L. There was a significant difference in soil moisture between pebble and bare soil treatments, with mean soil moisture being 3.5% higher in pebble treatments than in bare soil in this study. Soil moisture also had a significant negative relationship with nest density in this study (Table 4.2). Soil moisture is essential for larva development (May 1972) and particularly important during dry and warm summer months. However, waterlogged soils can negatively affect nesting populations of ground-nesting bees

(Visscher et al. 1994; Fellendorf et al. 2004). Optimal moisture can result in more successful larva development (Potts & Willmer 1997). Soil moisture ranged between 1.1 – 43.2 % for bare soil and 2.7 – 43.9 % for pebble treatments in this study.

Ground-nesting bees could be assumed to prefer uncompacted soils for nesting as they require less effort and time to dig however, soils with low soil compaction might not be able to support the structure of a bee nest (Potts & Willmer 1997). The preferred soil compaction for nesting might be highly related to bee species and their nesting behaviour. For instance, compacted soils can be advantageous for ground-nesting bees, which tend to form nesting aggregations (Bennett & Breed 1985; Torchio et al. 1988; Orr et al. 2016). Tsiolis et al. (2022) have reported the preference of such species to nest in bare ground plots (i.e. *Lasioglossum malachurum* K.). The authors also reported that areas with higher levels of soil compaction attracted more nesting activity than less compacted areas. The study reported the optimal soil compaction for attracted bee species were 9.92 ± 0.53 kg/cm^{-2}. As nest density increases, structural integrity may decrease unless the soil is sufficiently compacted. Soil compaction was a significant positive predictor of nest density in this study (Table 4.2). There was a significant difference between soil compaction treatments A and C in 2020 and 2021 and between soil compaction treatments and untreated controls (D) in all three years of this study (Fig. 4.9). It should be noted that the disturbance by wildlife in autumn of 2021 may have possibly contributed to the increase of soil compaction by approximately 1 kg/cm^{-2} in 2022.

Conclusion

In conclusion, this study has demonstrated which soil factors are influential for bee nesting and require more consideration in future studies. Vertical holes on the side of sandy silt loam soil tubs provided a nesting resource for *M. willughbiella* K. (Cane 2015) and this study demonstrated that areas of bare soil with pebbles are a preferred nesting habitat by ground-nesting bees and wasps compared to adjacent bare soil. Soil texture, temperature, moisture and pebbles should be considered when creating nesting habitats for ground-nesting bees and wasps. Future studies should also take measures to avoid soil disturbance by wildlife.

5 Chapter 5 – Novel method for locating nests of ground-nesting bees in orchards

5.1 Introduction

Wild solitary ground-nesting bees make an important contribution to apple pollination (Garratt et al. 2016; Hutchinson et al. 2021; Weekers et al. 2022). However, their nesting preferences are understudied, which could be a limiting factor in providing sustainable pollination services (Antoine & Forrest 2020). In addition, nests of solitary ground-nesting bees can be difficult to locate which make them difficult to study (Sardiñas & Kremen 2014). Even when a possible nest site has been located, confident identification of the bee nesting can be a challenge when "tumuli" (mounds of earth created during the excavation stage) are not present or are highly disturbed due to heavy rain or strong winds (K. Tsiolis, personal observation). Furthermore, some ground-nesting bee species, such as *Andrena haemorrhoa* Fabricius and *Andrena nitida* Müller, nest in well-hidden locations such as under vegetation (see Chapter 3). It is often impossible to locate nests if the tumuli are absent or not visible.

Tracking of bees can provide valuable information to ecologists, such as enhancing knowledge of foraging behaviours (Saville et al. 1997). Studies have previously used the harmonic radar system to investigate bee search strategies (Reynolds et al. 2007) and orientation flights (Capaldi et al. 2000), and radio telemetry to locate nests of Asian hornets (*Vespa velutina* L.) (Kennedy et al. 2018). However, the size and weight of tags that need to be attached to individual insects limit their use to large bee species only (e.g. bumblebees), and they are costly to deploy (Smith et al. 2021). For instance, the approximate cost of radio telemetry equipment is £2,000, plus £140 per radio tag (Kennedy et al. 2018). Therefore, such technologies are not generally suitable for tracking movements and locating nests of solitary ground-nesting bees considering the small size of insects and the fact that they are not cost-effective due to the high number of bees need to be tagged to locate a considerable number of nests.

Fluorescent powder is a relatively cheap method previously used to track the movements of bees and other insects. Mayer & Lunden (1991) marked honeybees with fluorescent powder to determine whether they forage on apple flowers once dandelion flowers close in the afternoon and Frankie (1973) and Sardiñas et al. (2016) tracked wild bee movements in sunflower (*Helianthus annuus*) fields. Other studies have used fluorescent powder to trace the movements of beetles (Kareiva 1985), ladybeetles (Egerer et al. 2018), and parasitoids (Corbett & Rosenheim 1996). Boyle et al. (2018) investigated the persistence of protein and fluorescent powders on adult *Osmia lignaria* S. bees throughout the 18-day study as they emerged from cocoons. The study found that powdered egg albumin and orange fluorescent powder were very persistent for the entire study duration. Nevertheless, fluorescent powders have never been reported in the literature as a way to locate bees' nests.

Developing an effective method to locate nests of ground-nesting bees could be highly advantageous in understanding bees' nesting preferences. Such a method would allow researchers to identify nesting preferences of particular ground-nesting bee species, assess population densities and inform habitat management. Nesting ecology is relatively understudied compared to foraging ecology, but could be the missing link in managing crop pollinating bees (Potts et al. 2005). This study aimed to test a low cost method to locate nests of four *Andrena* species known to be important apple pollinators, *A. haemorrhoa* F.*, A. nitida* M., *A. nigroaenea* K. and *A. dorsata* K. (Hutchinson et al. 2021) with the use of fluorescent powders in UK apple orchards.

5.2 Methods

5.2.1 Collecting specimens

The method, based on mark-release-recapture, involved capturing ground-nesting bees visiting apple orchard blossoms, marking individuals with a fluorescent dye and then releasing them. Three orchard visits took place in two farms in the apple growing region of Kent (Fig. 5.1). These apple orchards were chosen because they had the highest numbers of *Andrena* species according to previous surveys by Konstantinos Tsiolis and Mike Edwards (professional entomological consultant) in 2018 and 2019. Visits were undertaken during sunny and/or mild weather with temperatures above 10°C if cloud cover did not exceed 4 oktas. Windspeed was below Beaufort scale 5, or 29 km/h (Pywell et al. 2005). Surveyors walked along apple tree rows, collecting *A. haemorrhoa* F., *A. nitida* M., *A. nigroaenea* K. and *A. dorsata* K. from apple flowers with a 60 cm x 46 cm entomological hand net (Watkins & Doncaster, Leominster, UK) and carefully placed individuals into separate 5 ml clear plastic tube. Some bees were also collected by catching them in a tube while they were foraging on an apple blossom. A red-wired flag was placed every 30 m in shaded locations along tree rows, and surveyors were instructed to leave the potted specimens next to the red flag closest to where the bee was collected.

Figure 5.1: Map of two apple orchards (blue markers) where surveys were undertaken.

5.2.2 Mark and release

A portable marking station was set up in the flagged area. At the station were four fluorescent powders (FTX series, Swada London); yellow for *A. nitida* M., orange for *A. haemorrhoa* F., purple for *A. nigroaenea* K. and pink for *A. dorsata* K., four powder insufflators with a glass jar (Fig. 5.2; BARBER PRO - C/o BeautyPro Ltd) each containing one of the fluorescent colours, three paper bags (L 12 × W 7 × D 21.5 cm), and a nylon sheet (50 x 50 cm). Paper bags and powder insufflators were colour-labelled. Konstantinos Tsiolis was responsible for the mark and release process. Collected specimens were brought to the marking station and, if correctly identified, were subsequently marked via the following method and released. Firstly, the nylon sheet was placed on the ground to prevent the contamination of the ground with the fluorescent powder. Then, a specimen was placed in the allocated colour-labelled paper bag, and the nozzle of each equivalent colour-labelled powder insufflator was inserted 4-5 cm into the paper bag. Next, the top of the paper bag was squeezed, and the rubber bulb pump of the powder insufflator was gently compressed and released twice. This process collects the fine fluorescent dust from the attached glass jar and forces it out of the nozzle and onto the specimen (Stern & Mueller 1968). Then, the nozzle was removed from the paper bag; the bag was softly shaken for 3 seconds, and the bee released. Finally, the number of each bee species marked and released was recorded.

Figure 5.2: Powder insufflator used to mark the *Andrena* species of interest (BARBER PRO - C/o BeautyPro Ltd).

5.2.3 Locating nests of marked bees

To locate possible nest sites, surveyors walked along the alleyways and edges of study orchards during the night (dark) hours following the day of marking and releasing, using a UV torch (uvBEAST V2 (UV wavelength: 385-395 nm / UV Radiant Intensity: 1400 mW / UV Irradiance: 4500 uW/cm^2) uvBEAST TM) and searching for luminous powder on the ground. The search radius was approximately 200 m from marking and releasing locations, surveying 3 m wide the South, East and West facing edges, and every other alleyway and under tree areas. If fluorescent powder was found, the intention was to write the species' initials (according to colour) on a red wire flag mark and place this 15 cm away and to the north of detected fluorescent traces. The plan was for Konstantinos to revisit the orchard the following day, measure each nest's entrance, record its location in the apple orchard, and perform a habitat assessment for each nest following the method used in Chapter 3.

5.3 Results

Overall, 47 *A. nitida*, 62 *A. haemorrhoa* F.*,* 21 *A. nigroaenea* K. and 9 *A. dorsata* K. were marked and released over three days of collections. No luminous powder was detected on the ground, and consequently, no nests were located using this survey methodology, but powder was detected on apple flowers and wildflowers (Fig. 5.3).

Figure 5.3: Marked *Andrena* bees and fluorescent powder traces on flowers under UV-light. A) Marked *A. nitida* M. and B) corresponding powder on apple flowers. C) Marked *A. haemorrhoa* F. and D) corresponding powder on a dandelion flower.

5.4 Discussion

Using this method, we were able to identify areas where ground-nesting bees had foraged, but it was not possible to locate specific bee nest locations. Several factors might have contributed to the failure of the methodology to detect nests of ground-nesting bees in apple orchards. Despite paying close attention to the weather forecast, unexpected heavy rainfall in the evening of the first day of marking at one apple orchard may have washed fluorescent powder away, and no fluorescent powder could be detected on flowers or the ground at night hours. Bees can lose fluorescent powder during normal bee behaviour, such as grooming (Thorp 1979), flight, and landing on flowers (Rademaker et al. 1997). Another consequence could be that fluorescent powder had behavioural or physiological effects that disoriented or harmed the bees so that they did not return to their nests. Several bees were observed removing fluorescent powder from their eyes and antennae before flying away, and no marked bees were seen again during the mark and release process (K. Tsiolis, personal observation). Sardiñas et al. (2016) reported that marked ground-nesting bees returned to locations where they were initially caught and marked and have even been observed entering their nests. However, the authors did not report whether they could detect fluorescent powder at the nest entrance. Sardiñas et al. (2016) used a different marking technique than this study, possibly dispensing less fluorescent powder on bees. This study used a similar marking technique as Frankie (1973), who also reported no significant effect of fluorescent powder on the mortality of *Centris* spp., but no behavioural or physiological effects were recorded.

Furthermore, bee nests might not have been detected as surveys with UV torches at night hours did not occur at bees' preferred nesting locations. For instance, surveys in May 2021 took place mainly in alleyways (area between tree rows), under apple trees, and south-facing and East-facing hedgerows at one apple orchard, and traces of fluorescent powder were found on the ground. Then, a nesting aggregation predominantly of *A. haemorrhoa* F. and *A. nitida* M. was discovered along the north-facing hedgerow of the apple orchard in June 2021 by simply observing bees "diving" into tall vegetation (see Chapter 3). The distance between the nesting aggregation and marking locations was between 100 and 350 meters, which is within the maximum foraging ranges of similar size *Andrena* species (Gathmann & Tscharntke 2002). Bees might not have deposited enough fluorescent powder next to or at the nest's entrance while entering and exiting during foraging journeys. The nests of those two *Andrena* species found at hedgerows were under vegetation, and in such cases, enough fluorescent powder must be deposited on the vegetation surrounding the nest for the surveyor to detect it with the hand-held torch.

This experiment aimed to be repeated in 2022 but was not possible due to the low number of apple blossom foraging *Andrena* species of interest in that year (see Chapter 3). A critical component for the methodology's success is marking and releasing as many bees as possible. This method should be tested during ideal weather conditions (i.e. warm, dry, little wind) and the peak activity of bee species of interest. However, it is worth noting that despite choosing the best-known sites for bee species of interest, the mark and release of an average of 48 bees per day required 20 person-hours. Hence, it is a labour-intensive procedure, and the higher the number of person-hours would reflect a higher number of marked and released bees. The small number of marked bees in this study also reduced the likelihood of locating nests of *Andrena* species of interest.

The proposed method could be used to locate nests of other beneficial pollinators, such as wild bumblebees (Button & Elle 2014). Bumblebee nests are usually well-hidden and difficult to locate in sufficient numbers for research perposes (Liczner & Colla 2019). O'Connor et al. (2012) tested the efficacy of dogs in finding bumblebee nests in agricultural habitats, and they were able to locate nests at a rate of one nest for 19 h 24 min of searching time. Considering that bumblebees are social and their colonies have between 40 and 150 active workers (Edwards & Jenner 2018), there is a higher possibility of marking several members of individual colonies, which increases the chance of leaving more traces at the nest entrance than a single solitary female bee in the case of *Andrena* species of interest marked in this study (Else & Edwards 2018). Consequently, marking bumblebees with fluorescent powder could be more effective in locating their nests than any previously intended methodology.

The proposed method can also be used for other research purposes in ecology. For example, it could help researchers understand what plant species bees are foraging on. Commercial *Bombus terrestris* L. colonies were used in polytunnels at East Malling Research to enhance strawberry pollination services (data unpublished). Wildflower plots were created outside strawberry polytunnels, and they were in flower at the same time as strawberry plants. The question was raised whether *B. terrestris* L. prefer visiting wildflowers over strawberry plants

(Figure 5.3). The fluorescent powder was applied inside the bumblebee colonies early in the morning and before the bumblebees were active. Then, surveys with UV torches for traces of fluorescent powder on strawberry flowers and wildflowers took place during the evening, when it was dark. The trial outcome showed that the commercial *B. terrestris* L. preferred visiting strawberry flowers over wildflowers in this small pilot study (Fountain et al. 2021, unpublished work).

Figure 5.3: Left) Marked commercial *B. terrestris* L. at hive entrance and Right) fluorescent powder traces on strawberry flowers under UV-light.

Furthermore, this mark and trace technique could help to increase our understanding of floral competition and inform the management of floral resources in apple orchards for the *Andrena* species of interest. Additionally, bees foraging behaviour on apple trees could be mapped to inform orchard design. The proposed methodology could help answer research questions: 1) Are *Andrena* bees visiting polliniser trees or just the crop trees? 2) Do they visit adjacent trees and move down a tree row or just visit one tree and fly off? 3) Which bee species is the most frequent visitor of apple flowers?

Conclusion

This experiment should be repeated with increased surveying efforts to determine whether the proposed methodology is effective for locating ground-nesting bees' nests. An additional study should also take place alongside the field study to monitor dusted bees in flight cages and determine the possible adverse effect of the proposed marking technique and fluorescent powders on bees' health and behaviour. Finally, the proposed methodology could provide valuable insights related to the nesting, movements, and foraging behaviour of *Andrena* bees in apple orchards.

6 Chapter 6 – General Discussion

6.1 Overview

Insect pollination is an essential ecosystem function (Ollerton et al. 2011) and contributes significantly to agricultural productivity (Klein et al. 2007). Solitary bees need flowers for food and a place to live, such as bare ground in which to nest. However, there is little known about the nesting habitat requirements of ground-nesting solitary bees. Agri-environment schemes aim to safeguard biodiversity and ecosystem services (Batáry et al. 2015), and knowledge of wild bee nesting needs is an important component for the success of such schemes (Image et al. 2022). Improving our knowledge of the nesting preferences of ground-nesting bees would potentially enable landowners and managers to increase bee populations by providing and maintaining their preferred habitat, which might also result in more sustainable pollination services. The research described in this thesis has contributed to increasing the knowledge on the nesting preference of ground-nesting bees, including those species known to be beneficial crop pollinators.

6.2 Summary of Chapter findings

In Chapter 2, it was determined that soil stoniness and soil temperature had a positive relationship, whereas vegetation cover and hydraulic conductivity had a negative relationship, with nest density. The study also demonstrated that bare ground plots were a valuable nesting resource for eighteen non-parasitic ground-nesting bee species (7 *Andrena* spp., 9 *Lasioglossum* spp., 1 *Halictus* spp. and 1 *Colletes* spp.), and in particular for *L. malachurum* Kirby, *L. calceatum* S. / *L. albipes* F., and *A. flavipes* P. (83 % of recorded specimens). Nevertheless, only a few of the *Andrena* species known to be important for apple pollination (Hutchinson et al. 2021) were recorded utilising the bare ground plots, and these were mostly found in low numbers (<10 specimens in 3 years). This observation informed the study in Chapter 3, which aimed to determine where wild solitary ground-nesting apple pollinators nest in greatest abundance in apple orchards. This Chapter explored the preferred orchard (alleyways, under trees, edges) and local (vegetation cover) habitats for nesting, as well as biotic and abiotic factors influence nest-site selection.

Fifteen non-parasitic ground-nesting bee species (12 *Andrena* spp., 2 *Lasioglossum* spp., 1 *Halictus* spp.) were recorded nesting in apple orchards, from which ten are regarded as dominant apple flower visitors (Hutchinson et al. 2021). The most abundant species were *A. haemorrhoa* F. (37 %) and *A. nitida* M. (34 %). The study also identified which orchard and local habitats were most favoured for bee nesting. Bees mainly nested under trees (36 %), and at the hedgerows (52 %) of apple orchards. Local habitat was also a significant predictor of nest density, and bees mostly nested in areas of short grass (32 %), bare ground (31 %), and moss cover (18%). Three *Andrena* nesting aggregations were also located along hedgerows of apple orchards with predominant nesters being *A. haemorrhoa* F. and *A. nitida* M. This finding enabled further investigation of the nesting preferences of these two species. It was determined that soil temperature and underground stoniness had a significant positive, and soil moisture had a significant negative relationship, with nest density. These species were also found to favour nesting in highly vegetated habitats.

Chapter 3 also highlighted the potential negative impact of unsuitable weather conditions on the population of key apple pollinators. A recent study supports this observation, as it reported responses of apple-pollinating bees to spring temperatures resulting in climate change-driven ecological mismatches between apple pollinators' flight dates and apple flowering (Wyver et al. 2023). Senapathi et al. (2021) highlighted the importance of pollinator diversity in agricultural landscapes for the sustainability of crop pollination services.

The Chapter 4 study aimed to identify the species of ground-nesting bee attracted to artificially constructed nest sites (tree tubs) and determined the preferred soil compaction and other abiotic factors for nesting. Most ground-nesters utilising the artificial nest sites were wasp species (95.3% of total specimens), but four non-parasitic bee species were also attracted to nest (*M. willughbiella* K.*, L. smeathmanellum* K.*, L. minutissimum* K. and *L. fulvicorne* K.). The soil compaction level of uncompacted treatments (controls) remained significantly lower than the rest of soil compaction treatments through the duration of the study. Significantly higher nest density was recorded in pebble treatments (set up in the final year of the study) than bare soil. Soil temperature, compaction and pebbles were all significant positive predictors, and soil moisture was a significant negative predictor, of nest density.

The experiment in Chapter 5 tested a novel method aiming to locate nests of important apple pollinators, *A. haemorrhoa* F.*,* *A. nitida* M., *A nigroaenea* K., and *A. dorsata* K. (Hutchinson et al. 2021) in apple orchards with the use of fluorescent powders. Unfortunately, no bee nests were located using this methodology, but additional work is needed to confirm whether it has potential for studying nesting behaviour. However, there proposed method can be use for other studies such as to understand on what plants bees are foraging on and determine the flight range of bee species.

6.3 Future research

Until the early 1990s, most studies were field-based and provided descriptions of bees' nest architecture, immature stages, parasites, and taxonomy. Such work was extremely valuable in building knowledge of ground-nesting bees' life history and nest structure (Antoine & Forrest 2020). Later, studies focused more on nesting habitat characteristics of ground-nesting bee communities (Potts et al. 2005; Sardiñas & Kremen 2014). Nevertheless, only a few studies (e.g. Potts & Willmer 1997) recorded soil characteristics in nesting aggregations to determine nesting preferences. Furthermore, Harmon-Threatt (2020) argues that many existing studies have focused on a single species; hence, generalisation on abiotic preferences across bee species is not always appropriate.

The lack of quantitative studies on ground-nesting bee nest site characteristics implies that there might be factors determining nest-site selection that we are no aware of. Such factors include soil chemistry, including the presence of pollutants, salinity, organic matter, below-ground oxygen levels, hours of sunlight exposure and soil temperatures at different times of the day . Similarly, the influence of natural enemies on nest-site selection has been explored only in a handful of studies (e.g. Sick et al. 1994; Rozen et al. 2019). For instance, does the presence and quantity of parasites or predators influence nest-site selection? Do specific soil types create conditions susceptible to pathogen infection inside brood cells and affect the nest-site selection?

Another factor that deserves further investigation are olfactory cues. Studies suggested that chemical signals might influence solitary bees' mating and nesting behaviour (Michener et al. 1958; Guédot et al. 2006). Observations suggest that aggregating bees might deposit chemical signals at the nest entrance, to help recognise their nesting location (Raw 1992). Nest site odour played a significant role in attracting *A. flavipes* P. nesting in aggregations towards conspecifics (Butler 1965). This may eventually lead to nest site fidelity and philopatry, where offspring return to their natal site. During the surveys of Chapters 2 and 3, *Sphecodes* and *Nomada* kleptoparasites (cuckoo bees) were observed flying approximately 15-20 cm above the soil surface, reducing flight speed whenever approaching a nest hole, and often hovering above nest holes for a few seconds. Many nests of the *Andrena* aggregations studied in Chapter 3 were found by following flight routes of *Nomada* species, which are often kleptoparasites of *Andrena* species (Else & Edwards 2018). Indeed, Tengö & Bergstrom (1977) found that identical chemical compounds are present in the cephalic secretion of male *Nomada* bees and in the Dufour's gland secretion of female *Andrena* bees. As discussed in this thesis, several variables are associated with nest-site selection, but the scent and chemical signalling should also be further studied, especially when studying aggregated nesting bees (Cane 1981; Cane & Tengö 1981).

Ground-nesting bees contribute significantly to the yields of some crops (Kleijn et al. 2015). However, there are many unknowns regarding the effect of agricultural machinery, and other practices, on ground-nesting bee populations. Chapter 3 has demonstrated that the edge and adjacent hedgerows of apple orchards are attractive nesting habitats for spring bee species. However, such habitats might act as ecological traps if nests are destroyed by machinery or exposure to harmful pesticides. For instance, heavy agricultural machinery can create uneven landscapes when the soil is very wet on the edges of apple orchards. Consequently, farmers often use tractors to flatten the landscape, potentially destroying bee broods in soils. A such disturbance event was observed in a Kent apple orchard in 2020, where trenches (roughly 20 cm deep and 30 cm wide) were created by the tyres of agricultural machinery after heavy rain in the middle of a recorded *Lasioglossum* spp. nesting aggregation along a south-facing edge of an apple orchard. Then, efforts were made to flatten the landscape, resulting to soil being noticeably disturbed. The undisturbed edges of the nesting aggregation continued to be active, but the disturbed area has not recovered (since 2019) (K. Tsiolis, personal observation). Tilling of 10-20 cm can also directly kill shallow ground-nesting bee species, but could also negatively impact the following year's emergence due to interference with nest structure (Shuler et al. 2005). Williams et al. (2010) also showed that tilling negatively affected ground-nesting bees, but whether this was due to direct mortality or changes in soil characteristics is unclear. Additionally, little is known regarding the impact on nesting of plastic covers, mulches, cover crops or straw beneath crop canopy. Increasing our knowledge of common agricultural practices is vital for the sustainability of ground-nesting bee populations. Further research is needed to understand such practices' physiological, behavioural, reproductive and survival effects, and how nesting areas can be managed to reduce risks.

Furthermore, pesticide use can have damaging effects on bees (Brown & Paxton 2009; Bloom et al. 2021), but most studies have considered impacts via adult feeding and application trials which do not explore their effect on bees while nesting (Harmon-Threatt 2020). A recent study has shown that contamination of nesting soils of the ground-nesting solitary squash bee (*Eucera pruinosa* S.) with a neonicotinoid insecticide, resulted in bees initiating 85% fewer nests, harvesting 5.3 times less pollen and producing 89% fewer offspring compared to untreated control treatments (Willis Chan & Raine 2021). More research is needed to determine the impact of soils contaminated with commonly used insecticides on different ground-nesting bee species. Removing herbicides from approval will inevitably result in more reliance on mechanical weeding, which may potentially harm bee nests in bare soil. Some weed species provide food and refuge to many beneficial insects in agricultural landscapes, and should be maintained wherever possible if they do not interfere significantly with crops (Nicholls & Altieri 2013; Rollin et al. 2016). Alterations in habitat quality and reduced availability of natural habitats are two of the most significant causes of bee declines (Potts, Biesmeijer, et al. 2010).

A study that examined 41 European bee species showed bees have high quantitative pollen requirements, with 85% of species needing more than 30 flowers to rear a single larva (Müller et al. 2006). This finding highlights the importance of ample pollen and nectar sources near nesting sites. Methods to provide floral resources in agricultural settings are already established (Carvell et al. 2022; Fountain 2022). However, the estimated flight range of bee species needs further investigation. Gathmann & Tscharntke (2002) reported the flight range of solitary bees to be between 150-600 m and positively correlated with bee size. Nevertheless, this estimate was based on a small number of species only, and may not be relevant to all ground-nesting species. For example, *Andrena* or other species of interest could be captured on emergence early in the morning, marked, released and then searched for traces of fluorescent powder on apples and wildflowers at night hours following the methodology of Chapter 5, to estimate maximum flight range. Determining the flight range of ground-nesting bees from nest to forage area is essential for the provision of nesting resources for such species.

Locating enough nests of ground-nesting bees for replicated studies is often considered a challenging procedure (Antoine & Forrest 2020). This PhD project has proved that it is possible, and proposed methodology for locating and recording ground-nesting bee nests which should be adopted by future studies. Surveyors need to spend considerable time in the field and observe patterns and nesting behaviours of ground-nesting bees to familiarise themselves with species behaviour and nest site characteristics; an essential component in understanding the nesting preferences of ground-nesting bees.

6.4 Implications for orchard management practices

We are in a biodiversity crisis (Rull 2022). Wild bees provide vital pollination services in agricultural ecosystems but are threatened by changing climate and loss of habitat due to agricultural intensification and expansion (Potts, Biesmeijer, et al. 2010; de Palma et al. 2015; Potts et al. 2016; Settele et al. 2016). Management practises to provide/enhance food and nesting resources and avoid harm to pollinating bee species are essential to sustain pollination services (Potts et al. 2016; Martin et al. 2019). Apples are a crop where the benefits of wild bees and particularly ground-nesting bees have been quantified (Garratt et al. 2016; Pardo & Borges 2020; Hutchinson et al. 2021). In order to support these species it is important to provide not only floral resources but also suitable nesting habitat.

This PhD has determined that provision of both bare ground and vegetated habitats tailored to ground-nesting bee requirements could increase pollinator diversity and abundance in and around orchards. It has also identified important nesting habitat characteristics to inform the management of nesting resources for ground-nesting bees in agricultural settings. The following is recommended for the creation and maintenance of suitable bee nesting habitats:

- Bare ground habitats – The ground-nesting bee species *L. malachurum* Kirby, *L. calceatum* S. / *L. albipes* F., and *A. flavipes* P. have recorded predominantly nesting in bare ground areas. It is suggested to create bare ground plots by scraping off 5 cm of topsoil to remove vegetation and dormant seeds in the soil. The suggested soil characteristics to be considered are 1) hydraulic conductivity at 1.9e-4 ± 1.5e-5 s^{-1}, 2) soil temperature at 27.5 ± 0.1 °C, 3) stoniness at 14.4 ± 1.0 %, and 4) vegetation cover at 48.9 ± 1.5 %. It should be considered that there is a strong relationship between soil characteristics. The vegetation cover should be monitored and maintained at recommended levels via herbicide treatments. Chapter 4 has demonstrated that pebbles on the soil surface increased nest density and could be added to the nest site using a variety of stones of different sizes, which may create more thermally suitable conditions.

- Vegetated habitats – Such habitats were utilised mainly by *A. haemorrhoa* F., *Andrena nitida* M. (key apple pollinators), and *Lasioglossum calceatum* S. / *albipes* F. Therefore, protecting or creating semi-shaded, semi-vegetated (grass) nesting habitats is also recommended. Such plots should be created at north, north-east, and east-facing locations, ensure that receive sunlight exposure in the morning and be shaded in the afternoon. The suggested soil characteristics to be considered are 1) soil temperature at 22.1 ± 0.6 °C, 2) soil moisture at 7.7 ± 0.5 %, 3) stoniness at 9.8 ± 1.0 %, and 4) vegetation cover at 80.2 ± 4.1 %. These were the conditions in which key apple pollinators were found nesting. The vegetation cover should be monitored and controlled via herbicide treatments before the active period of bee species. Herbicide treatment can be applied if necessary, in early March for the spring species.
- Farmers should pre-survey various potential locations for establishing new bee nesting habitats and select those closest to identified soil moisture and temperature preferences. It is important to ensure that the chosen location would be able to accommodate the bee nesting habitat for several years.
- Farmers should pre-survey hedgerows of orchards to identify existing nesting bee aggregations.
- Disturbance of nesting habitats should be avoided. Markers and signs should be put along the plots to inform farm workers and the public of such habitats' existence and the importance of not being disturbed.
- Flight ranges positively correlated with a bee's body length (Gathmann & Tscharntke 2002). Orchard pollinating female bee species are typically around 6 – 12 mm in length (Falk 2016; Hutchinson et al. 2021). Therefore, nesting habitats should be created no further than approximately 150 – 200 meters from orchards so they remain in foraging range for small and larger solitary bee species.
- Additional floral resources such as flower margins should be provided for solitary bees within foraging range of the nest sites. This is particularly important in orchards and

landscapes where additional forage resources are scarce (Martin et al. 2019). Ideally these will include a diversity of flowering plants which provide nectar and pollen for the duration of important bee foraging and when apples are not in flower (Carvell et al. 2022).

6.5 Concluding remarks

In conclusion, this PhD thesis has identified important variables determining the nest-site selection of UK orchard ground-nesting bees. It demonstrated that some bee species prefer nesting on warm bare ground and others in vegetated and semi-shaded locations of apple orchards. It also identified gaps in knowledge on the nesting preferences of ground-nesting bees and highlighted where future research need to focus to enhance this knowledge. Increasing the understanding of the variables influencing ground-nesting bees will help policy-makers and farmers provide suitable nesting habitats, and potentially promote the conservation of this forgotten group of bee species, which is critical for the sustainability of crop pollination services.

Even though it is challenging to locate and study nesting sites of ground-nesting bees, it is an essential element in the effort to understand their nesting preferences. This PhD has demonstrated that it is possible to locate enough nests in agricultural settings when a considerable amount of time and efforts are invested. A vital component of such success was the interest in observing and recording patterns and nesting behaviours of ground-nesting bees and the ones of their kleptoparasites. Future studies on the nesting preferences of ground-nesting bees should adopt the proposed methodologies. There are currently many unknowns, and research efforts should be increased, including encouraging early career researchers to investigate the fascinating world of ground-nesting bees.

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8 Appendices

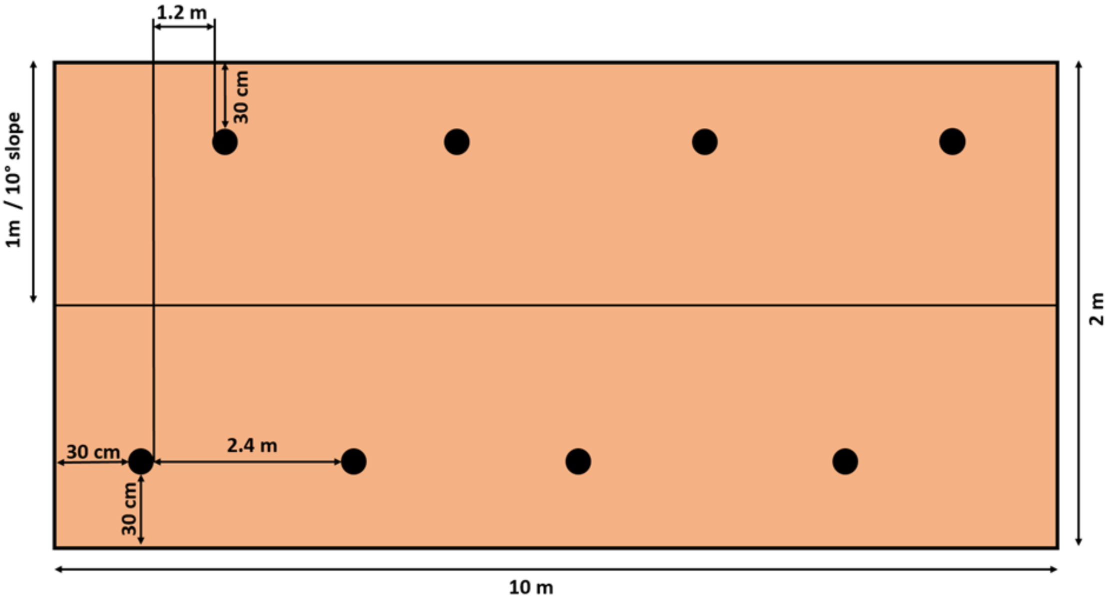
8.1 Appendix 1: Surrounding habitat type and dominant local vegetation type of plots.

Surrounding habitat type and dominant local vegetation type of plots. Habitats with perennial grasses contained approximately 10% *Taraxacum officinale*.

Plot	General habitat type	Dominant local vegetation type
1	Field headland adjacent to soil-grown strawberry crops	Perennial grasses, strawberry plants
2	Field headland adjacent to soil-grown strawberry crops	Perennial grasses, strawberry plants
3	Field headland adjacent to apple orchards	Perennial grasses, apple trees
4	Field headland adjacent to a deciduous woodland	Perennial grasses, deciduous trees
5	Field headland adjacent to cherry orchards	Perennial grasses, cherry trees
6	Field headland adjacent to pear orchard	Perennial grasses, pear trees
7	Adjacent to perennial wildflower meadow and a deciduous woodland	Perennial wildflowers, deciduous trees
8	Field headland adjacent to apple orchards	Perennial grasses, apple trees

8.2 Appendix 2: Soil sampling method.

Soil sampling method showing the arrangement of soil sampling points (black dots) from the 10 x 2 m bee nesting plots.



8.3 Appendix 3: Bee, wasp and bee fly species sampled from eight study plots in each year of the study.

NIAB EMR, Kent, England (51°17′26″N 0°26′02″E) and percentage of species present based on the total records.

Taxon	Family	Year 2018	Year 2019	Year 2020	Years Total	Species %
Andrena haemorrhoa Fabricius	Andrenidae	2	0	0	2	0.2
Andrena nitida Müller	Andrenidae	2	0	0	2	0.2
Andrena scotica Perkins	Andrenidae	5	0	0	5	0.49
Andrena dorsata Kirby	Andrenidae	1	4	1	6	0.59
Andrena florea Fabricius	Andrenidae	0	0	1	1	0.1
Andrena flavipes Panzer	Andrenidae	5	23	3	31	3.04
Andrena minutula Kirby	Andrenidae	2	5	3	10	0.98
Andrena (M)	Andrenidae	0	17	29	46	4.51
Andrena - Medium	Andrenidae	11	0	0	11	1.08
Lasioglossum malachurum Kirby	Halictidae	24	99	74	197	19.3
Lasioglossum malachurum Kirby (M)	Halictidae	0	17	20	37	3.62
Lasioglossum minutissimum Kirby	Halictidae	2	2	7	11	1.08
Lasioglossum pauxillum Schenck	Halictidae	3	0	2	5	0.49
Lasioglossum villosulum Kirby	Halictidae	2	0	0	2	0.2
Lasioglossum morio Fabricius	Halictidae	0	5	3	8	0.78
Lasioglossum leucozonium Schrank	Halictidae	0	1	0	1	0.1
Lasioglossum calceatum Scopoli / Lasioglossum albipes Fabricius	Halictidae	36	11	4	51	5
Lasioglossum - Small	Halictidae	0	0	6	6	0.59
Lasioglossum (M)	Halictidae	0	21	2	23	2.25

Appendix 3 continued

Taxon	**Family**	**Year 2018**	**Year 2019**	**Year 2020**	**Years Total**	**Species %**
Halictus tumulorum Linnaeus	Halictidae	1	1	4	6	0.59
Colletes similis Schenck	Colletidae	0	2	0	2	0.2
Sphecodes - Medium (K)	Halictidae	21	56	40	117	11.5
Sphecodes - Small (K)	Halictidae	0	87	80	167	16.4
Sphecodes monilicornis Kirby (K)	Halictidae	3	15	37	55	5.39
Sphecodes geoffrellus Kirby (K)	Halictidae	0	0	1	1	0.1
Sphecodes longulus von Hagens (K)	Halictidae	0	0	1	1	0.1
Sphecodes puncticeps Thomson (K)	Halictidae	0	0	2	2	0.2
Nomada fabriciana Linnaeus (K)	Apidae	0	0	1	1	0.1
Nomada fucata Panzer (K)	Apidae	2	6	15	23	2.25
Nomada flavoguttata Kirby (K)	Apidae	0	4	10	14	1.37
Nomada zonata Panzer (K)	Apidae	0	1	0	1	0.1
Nomada marshamella Kirby (K)	Apidae	0	0	1	1	0.1
Nomada ruficornis Linnaeus (K)	Apidae	0	0	1	1	0.1
Nomada - Medium (K)	Apidae	0	20	3	23	2.25
Bombylius major Linnaeus (CF)	Bombyliidae	4	13	9	26	2.55
Lindenius albilabris Fabricius (W)	Crabronidae	0	9	4	13	1.27
Lindenius panzeri Vander Linden (W)	Crabronidae	0	0	2	2	0.2
Cerceris rybyensis Linnaeus (W)	Crabronidae	0	7	31	38	3.72
Cerceris quinquefasciata Rossi (W)	Crabronidae	0	0	2	2	0.2

Appendix 3 continued

Taxon	**Family**	**Year 2018**	**Year 2019**	**Year 2020**	**Years Total**	**Species %**
Mimumesa unicolor Vander Linden (W)	Crabronidae	0	1	0	1	0.1
Hedychrum niemelai Linsenmaier (W)	Chrysididae	0	10	23	33	3.23
Vespula germanica Fabricius (W)	Vespidae	0	0	23	23	2.25
Ancistrocerus parietum Linnaeus (W)	Vespidae	0	0	1	1	0.1
Microdynerus exilis Herrich-Schäffer (W)	Vespidae	0	0	2	2	0.2
Tiphia minuta Vander Linden (W)	Tiphiidae	0	1	0	1	0.1
Priocnemis parvula Dahlbom (W)	Pompilidae	0	0	7	7	0.69
Total		**126**	**438**	**457**	**1,021**	

(K) Kleptoparasitic bee species

(CF) Kleptoparasitic fly species

(W) Wasp species

(M) Males

Sizes (length):

Small = 3-5 mm

Medium = 5-7 mm

Large = 7+ mm

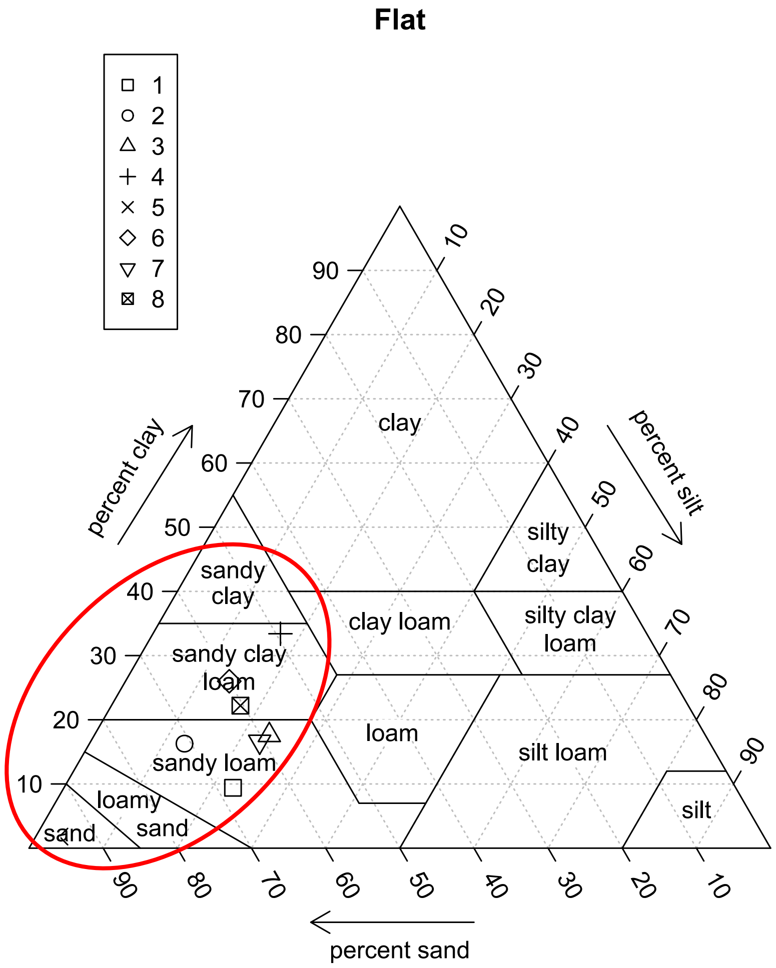
8.4 Appendix 4: Mean diameter of nests’ entrance on flat and slope sections of plots.

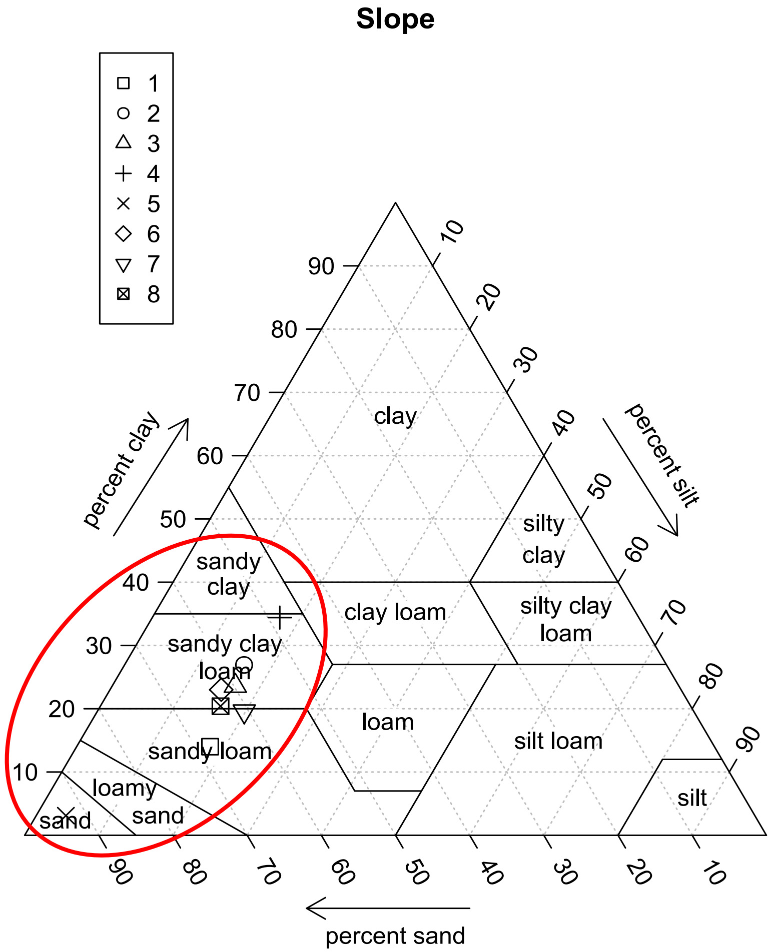
Mean diameter of nests’ entrance of each plots’ gradient (±SE, *N* = 1,295) measured in 2019 and 2020 surveys.

	Mean ± SE (n)	
Plot	**Flat (mm)**	**Slope (mm)**
1	2.731 ± 0.220 (21)	2.771 ± 0.212 (19)
2	3.398 ± 0.122 (103)	3.335 ± 0.139 (64)
3	3.073 ± 0.082 (138)	2.726 ± 0.231 (14)
4	3.312 ± 0.185 (59)	3.682 ± 0.149 (105)
6	2.598 ± 0.122 (84)	2.955 ± 0.139 (103)
7	2.861 ± 0.062 (171)	2.910 ± 0.066 (152)
8	3.078 ± 0.069 (138)	3.201 ± 0.074 (124)

8.5 Appendix 5: Soil texture of flat and slope areas of plots.

Each symbol in legends indicates the soil texture of the numbered plots determined from % of sand, silt, and clay.





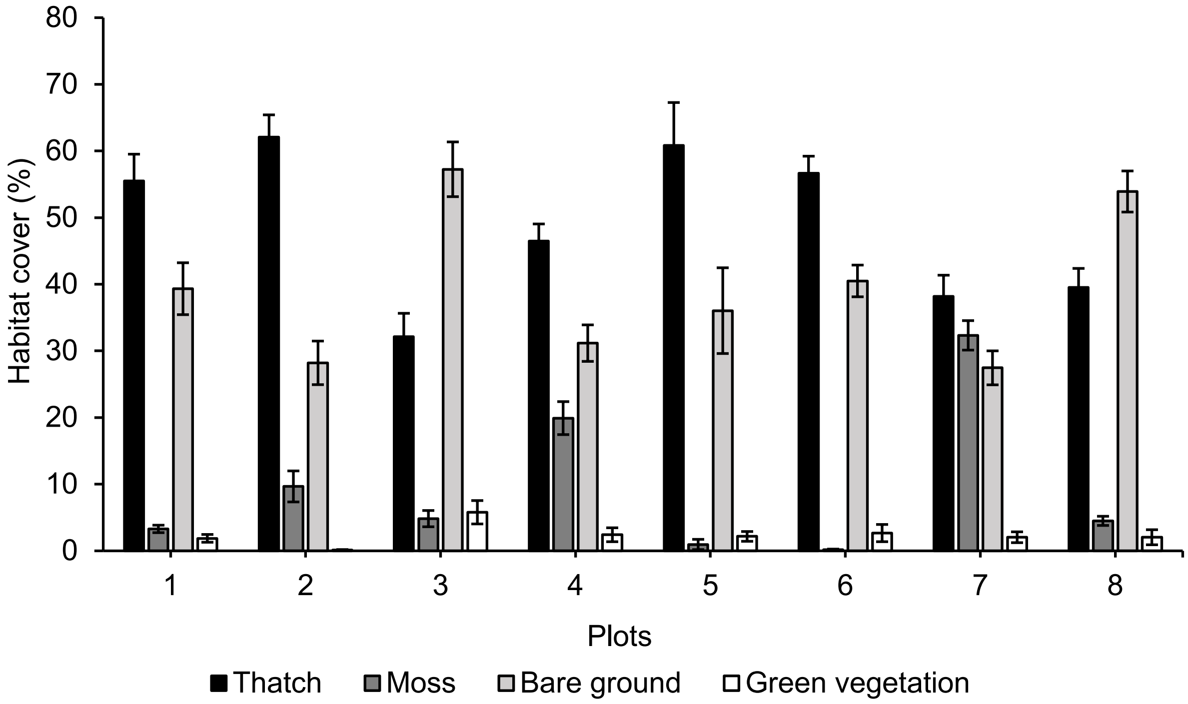
8.6 Appendix 6: Mean soil temperature (°C) of plots in the morning, afternoon, and night hours.

Mean soil temperature (°C) for the flat and sloping areas of each plot (± SE, *N* = 405) for early morning (01:00 – 05:00), late morning (07:00 – 11:00), afternoon (13:00 – 17:00), and night (19:00 – 23:00) from 30/03/2020 to 11/08/2020.

		Early morning	**Late morning**	**Afternoon**	**Night**
Plot	**Gradient**	**Mean ± SE (n)**	**Mean ± SE (n)**	**Mean ± SE (n)**	**Mean ± SE (n)**
1	Flat	17.58 ± 0.22 (405)	17.06 ± 0.22 (405)	21.30 ± 0.24 (405)	20.60 ± 0.25 (405)
1	Slope	16.61 ± 0.21 (405)	15.88 ± 0.20 (405)	21.83 ± 0.26 (405)	20.73 ± 0.26 (405)
2	Flat	16.73 ± 0.24 (405)	16.69 ± 0.24 (405)	20.59 ± 0.26 (405)	19.67 ± 0.26 (405)
2	Slope	16.87 ± 0.25 (405)	16.93 ± 0.24 (405)	20.73 ± 0.27 (405)	19.83 ± 0.27 (405)
3	Flat	16.91 ± 0.25 (405)	17.31 ± 0.25 (405)	22.35 ± 0.27 (405)	20.60 ± 0.27 (405)
3	Slope	16.02 ± 0.25 (405)	16.44 ± 0.25 (405)	20.77 ± 0.27 (405)	19.32 ± 0.27 (405)
4	Flat	17.31 ± 0.27 (405)	17.24 ± 0.22 (405)	22.82 ± 0.27 (405)	20.77 ± 0.24 (405)
4	Slope	16.52 ± 0.21 (405)	17.47 ± 0.23 (405)	23.64 ± 0.26 (405)	20.03 ± 0.23 (405)
5	Flat	15.07 ± 0.21 (405)	16.01 ± 0.22 (405)	19.21 ± 0.21 (405)	17.31 ± 0.21 (405)
5	Slope	15.12 ± 0.22 (405)	16.25 ± 0.23 (405)	19.63 ± 0.27 (405)	17.25 ± 0.20 (405)
6	Flat	17.11 ± 0.25 (405)	17.33 ± 0.25 (405)	21.68 ± 0.27 (405)	20.42 ± 0.27 (405)
6	Slope	16.86 ± 0.24 (405)	17.99 ± 0.26 (405)	23.84 ± 0.29 (405)	20.87 ± 0.27 (405)
7	Flat	17.23 ± 0.23 (405)	17.46 ± 0.23 (405)	22.80 ± 0.27 (405)	20.74 ± 0.26 (405)
7	Slope	17.56 ± 0.24 (405)	17.31 ± 0.23 (405)	23.07 ± 0.27 (405)	21.38 ± 0.27 (405)
8	Flat	17.24 ± 0.24 (405)	17.96 ± 0.25 (405)	23.44 ± 0.28 (405)	21.12 ± 0.27 (405)
8	Slope	17.01 ± 0.24 (405)	17.04 ± 0.24 (405)	22.03 ± 0.27 (405)	20.54 ± 0.27 (405)

8.7 Appendix 7: Mean percentage of plot's vegetation cover.

Mean (± SE, *N* = 24) percentage of vegetation cover on plots in spring and summer of 2020.



8.8 Appendix 8: Summary of GLMM analyses of soil variables for both 2018 and 2020.

No variables predict peak nest density.

Predictor variables	Estimate	Std. Error	z value	*P* value	VIF
Year	-0.270	0.805	-0.336	0.737	2.989
Slope	-0.315	0.387	-0.815	0.415	1.463
Hydraulic conductivity	-1.078	0.697	-1.546	0.122	1.422
Soil compaction	0.184	0.169	1.087	0.277	1.300
Soil organic matter	0.096	0.170	0.567	0.571	1.108
Soil root biomass	-0.620	0.883	-0.702	0.482	1.737
Soil water content	0.299	0.292	1.026	0.305	1.343
Soil stoniness	0.325	0.323	1.007	0.314	2.253
Soil texture (Sand)	0.008	0.557	0.014	0.989	1.400

8.9 Appendix 9: Nests of solitary ground-nesting bees with “chimneys” extending from nest entrance.

8.10 Appendix 10: Mean nesting local habitats of reported non-kleptoparasitic bee species.

Species	No. of Samples	Short veg. (%)	SE	Long veg. (%)	SE	Thatch (%)	SE	Moss (%)	SE	Bare ground (%)	SE
Andrena haemorrhoa	288	21.0	1.5	29.7	1.4	16.7	0.9	22.1	1.2	10.5	1.1
Andrena nitida	270	42.7	2.0	8.6	1.2	11.4	1.0	13.2	1.5	24.1	1.5
Lasioglossum calceatum_albipes	125	31.3	2.8	1.4	0.6	12.0	1.3	27.3	2.4	28.1	2.1
Andrena nigroaenea	21	48.8	6.1	5.2	4.0	9.0	2.8	7.1	4.6	29.8	5.5
Andrena labialis	21	61.9	5.6	0.0	0.0	2.4	2.4	0.0	0.0	35.7	5.7
Lasioglossum malachurum	18	12.8	2.8	0.0	0.0	1.1	0.9	10.6	6.5	75.6	5.8
Andrena flavipes	13	30.0	6.0	0.0	0.0	9.2	3.2	6.2	3.5	54.6	6.0
Andrena cineraria	12	49.6	10.0	0.0	0.0	5.8	2.7	7.1	2.8	37.5	8.0
Andrena fulva	4	17.5	6.6	0.0	0.0	40.0	14.0	21.3	12.3	21.3	6.6
Andrena scotica	4	33.8	17.7	17.5	17.5	2.5	2.5	12.5	12.5	33.8	19.7
Andrena dorsata	3	1.7	1.7	0.0	0.0	35.0	25.7	6.7	6.7	56.7	23.2
Andrena chrysosceles	1	70.0	NA	0.0	NA	0.0	NA	0.0	NA	30	NA
Andrena minutula	1	20.0	NA	0.0	NA	5.0	NA	0.0	NA	75.0	NA
Andrena gravida	1	65.0	NA	0.0	NA	0.0	NA	0.0	NA	35.0	NA

SE – Standard Error

8.11 Appendix 11: Weather data for March, April and May of 2019, 2020, and 2021 from Met office - East Malling weather station.

This data explains the low presence of spring *Andrena* species in 2022. A) Ambient temperature, B) solar irradiation, C) Soil temperature at 10 cm depth, and D) Rainfall.