Basic and Applied Ecology 91 (2026) 1–9

Contents lists available at ScienceDirect

Basic and Applied Ecology

journal homepage: www.elsevier.com/locate/baae

Research Article

Cichorioideae abundance and local land use drive *Osmia leaiana* pollen use in semi-natural grasslands

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ARTICLE INFO

Keywords:
Calcareous grassland
Habitat fragmentation
Pollen metabarcoding
Semi-natural grasslands
Solitary bees

ABSTRACT

Land-use change is a major driver of bee decline, with solitary and specialist bees being particularly vulnerable due to their nesting and foraging behavior. Understanding how local land use and management practices influence solitary bee resource use is therefore critical for informing conservation in fragmented ecosystems. In this study, we investigated brood cell number and pollen resource use of *Osmia leaiana*, a solitary bee specializing on Asteraceae, across a network of Estonian calcareous alvar grasslands. We hypothesized that due to the short foraging range of *O. leaiana* and its dependence on Asteraceae, local management variables such as grazing intensity, shrub cover, and tree cover, affect the taxonomic composition of nest-tube pollen provisions through their effects on local Asteraceae community composition. In addition, we predicted that higher Asteraceae abundance corresponds to greater brood cell production by increasing the availability of preferred resources. Nest blocks were deployed to sites that differed in the composition of the surrounding landscape, management, and plant community composition. Pollen metabarcoding of nest contents was used to assess floral resource use. While Asteraceae abundance did not affect brood cell number, higher proportions of cropland and forest had significant negative effects. Of all assessed variables, proportion of semi-natural grassland within 500 m, tree cover, and the abundance of the subfamily Cichorioideae within grassland patches significantly influenced the composition of pollen provisions. These results indicate that *O. leaiana* prefers a narrow subset of Asteraceae and primarily forages on plants locally available within semi-natural grasslands, while brood cell production decreased with increasing amounts of alternative land-use types in the surrounding landscape.

Introduction

Pollinators play a vital role in plant reproduction, with animal-mediated pollination estimated to support about 90 % of angiosperm species (Herbertsson et al., 2021; Tong et al., 2023). In turn, plants provide essential floral resources that sustain pollinator fitness (Parreño et al., 2022). However, substantial declines have been observed across pollinator populations (Powney et al., 2019; Zattara & Aizen, 2021), with bees experiencing particularly sharp decreases in both abundance (Turley et al., 2022) and species richness (Goulson et al., 2015). Many factors contribute to this current decline, but land use change has emerged as a primary driver (Halsch et al., 2025; Tsang et al., 2025) as it often greatly reduces floral resource availability and diversity. These changes can significantly alter pollinator foraging patterns and diets (Fijen et al., 2025; Peters et al., 2022) in ways that can adversely affect pollinator health, reproduction, and population growth (Hass et al., 2019; Lau et al., 2023; Pluta et al., 2024). Solitary bees may be particularly vulnerable to these pressures, as their typically shorter foraging ranges make them more susceptible to reduced floral availability and isolation in fragmented landscapes (Hofmann et al., 2020; Zurbuchen

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https://doi.org/10.1016/j.baae.2025.12.011
Received 18 September 2025; Accepted 23 December 2025

et al., 2010c). One habitat where these risks are especially pronounced are semi-natural grasslands, which are among the most important yet most threatened environments for pollinators in Europe (Öckinger & Smith, 2007).

The decline of semi-natural grasslands has been primarily driven by abandonment and agricultural intensification (Poschlod & WallisDeVries, 2002). As a result, many pollinators have lost access to diverse and continuous floral resources (Goulson et al., 2015), making the conservation and restoration of these habitats a priority. Restoration typically involves removal of excessive trees and shrubs and subsequent reintroduction of traditional land use practices such as grazing. Grazing with moderate intensity has been shown to increase plant species richness and subsequently floral resource diversity, and is therefore vital for pollinator conservation (Johansen et al., 2019; Wehn et al., 2017). However, despite these ecological benefits, maintaining or reintroducing extensive instead of intensive farming practices remains challenging for farmers under current political and economic conditions (Gorris et al., 2025).

Surveys have shown that European solitary bees generally require pollen from dozens or even hundreds of flowers to successfully provision a single larva (Cane et al., 2011; Müller et al., 2006). Such high pollen demands likely make these and similar species more vulnerable to floral resource scarcity, particularly given the strong link between pollen provisioning and reproductive success. Provisioned pollen mass has been shown to strongly correlate to larval mass in *Osmia cornuta* (Bosch & Vicens, 2002) and *Osmia bicornis* (Radmacher & Strohm, 2010), and *O. cornuta* females that provisioned their nests at a higher rate had higher fecundity (Bosch & Vicens, 2006).

Most prior research, however, has focused on polylectic *Osmia* species. Research on oligolectic species is more scant, despite their greater vulnerability to changes in habitat quality and land use as they rely on a narrower range of pollen resources (Peters et al., 2022; Winfree et al., 2011). Abundances of individual specialist bee species often increase with landscape heterogeneity as this increases the likelihood that their preferred dietary resources are present within their foraging range (Parreño et al., 2024). However, this pattern may vary depending on the specific taxonomic specialization of a species. For example, Torné-Noguera et al. (2014) found that of 21 oligolectic species in their community, the only highly abundant species was a specialist on Asteraceae, which is consistent with the widespread distribution and ecological versatility of these plant species (Funk et al., 2009). This suggests that local abundance of preferred floral resources is a major driver of bee fitness and abundance. Nonetheless, more research is required to elucidate how land use change and habitat quality affect the foraging and reproductive success of solitary specialist bees.

To address these gaps, we assessed the pollen resource use of the solitary, cavity-nesting bee *Osmia leaiana* in Estonian alvar grasslands and examined how local land use and site characteristics affect nest pollen composition and brood cell number. *Osmia leaiana* is oligolectic on Asteraceae, with females extensively foraging on these flowers during their May-August flight period to provision their larvae with pollen (Falk, 2019; Westrich, 2018). Given the likely low foraging distance of *O. leaiana* and its dependence on Asteraceae, we hypothesized that grazing intensity, shrub cover, and tree cover in each site affect the species composition of nest tube pollen provisions through their effects on local Asteraceae community composition. Differences in land use type were not anticipated to affect pollen species composition as we expect that Asteraceae resources are sufficiently abundant within semi-natural grasslands, reducing the need for foraging in lower-value land use types. We additionally predicted that Asteraceae abundance drives brood cell number given the reliance of *O. leaiana* on this floral resource. To test these hypotheses, nest blocks were installed across 32 alvar grasslands, nests were checked for occupancy, and metabarcoding was used to determine the composition of pollen provisions.

Methods

Study area

The study was conducted in western Estonia (22.2 - 24.0 E; 58.2 - 58.8 N) and encompasses a large portion of the national distribution range of dry calcareous grasslands (EU Habitat Directive codes 6210 and 6280; Fig. 1a). Situated in the hemi-boreal climate zone, the region contains relatively large patches of semi-natural grasslands, with individual pastures often comprising a mosaic of multiple grassland types embedded within a matrix of forests and agricultural land. Each study site consisted of a single grassland ranging in size from 0.92 to 9.76 ha. Sites were separated by a mean of 39.2 km (range: 1.4 – 108.6 km). Half of the 32 study sites were managed by cattle, sheep or horse grazing, while the other half was abandoned and partly overgrown by shrubs and trees (mostly *Juniperus communis* and *Pinus sylvestris*). Increasing coverage of shrubs and trees have shown to decrease flower abundance and pollination services in these grasslands (Prangel et al., 2023).

Nest blocks and pollen collection

Two nest blocks constructed from medium-density fiberboard were placed out to each of the 32 study sites between 17th and 21st of May 2022. Each nest block contained 10 × 5 holes, ca 8 mm in diameter (Fig. 1b). The blocks were attached to the trees or juniper shrubs at a height of about 1.2 m (Fig. 1c). In grassland patches with high shrub cover, blocks were positioned to remain unobstructed by thick shrubbery. In more open areas, placement depended on the availability of suitable trees or shrubs. The distance between the nest blocks in each site ranged from 39 to 290 m. From June 23rd to August 16th 2022, the nest blocks were checked for occupancy by *O. leaiana*, and pollen was collected from occupied nest tubes for metabarcoding analysis to assess floral resource use. Each sample consisted of pollen provisions from a single nest tube sampled transversely along the length of the most recently stocked consecutive brood cells that contained unhatched larvae. The samples were preserved in 95 % ethanol until DNA extraction.

Nest blocks from three sites were excluded due to collapse or damage, leaving 29 sites to be used in the analysis. Of the 58 nest blocks from these remaining 29 sites, only 27 contained nest tubes occupied by *O. leaiana*. Furthermore, each occupied block contained only a single such nest tube due to extremely high rates of nest usurpation by eumenine wasps.

Land use variables

Data on agricultural fields in the matrix surrounding the study sites was extracted from Estonian Agricultural Registers and Information Board WFS maps (https://avaandmed.eesti.ee/). Data on semi-natural grasslands in the surroundings of the study sites was extracted from EELIS (Estonian Nature Information System, data extracted on 1st of May 2022). All land use variables were derived from a base map generated during a recent Estonian Mapping and Assessment of Ecosystems and their Services (Helm et al., 2023) and calculated using the '*st*' package (Pebesma, 2018) as proportions within a 500 m radius around a centroid between the two nest blocks at each site. The land use types included agricultural pasture, semi-natural grasslands, annual crops, and forest, which are described in detail in Table A1. Although no prior studies have directly measured foraging distances of *O. leaiana*, this radius was chosen based on estimates for the similarly sized *O. bicornis*, which suggest maximum foraging distances of 250 m (Hofmann et al., 2020), 550 m (Rathjen, 1994), and 600 m (Gathmann & Tscharntke, 2002).

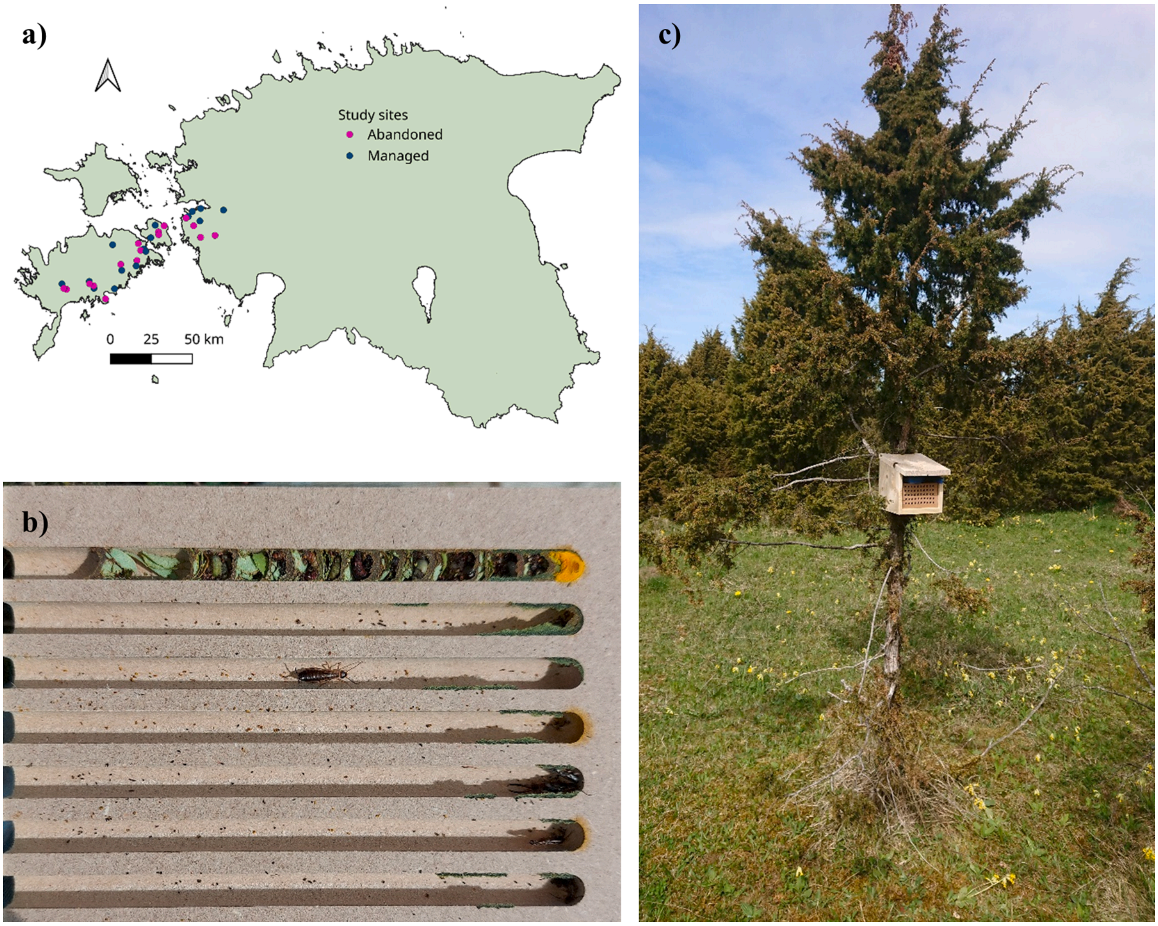

Fig. 1. a) Map of managed and abandoned study sites within Estonia. b) Cross-section of a trap nest block with chewed leaf material and pollen provisions. c) Nest block mounted on a juniper tree at one of the study sites.

Local plant community and management variables

To assess plant community composition, four transects 50 m in length and 2 m in width were placed at locations in each site to best represent the local floral community. All insect-pollinated flowering plants were counted and cover area was measured in cm^2 for each species to calculate flower cover per transect. These values were then summed across transects within each site to represent floral abundance by species and overall. Plant species richness per grassland was determined according to the species recorded in the transects as well as any additional species observed at each site. Asteraceae species richness and abundance were then calculated as subsets of overall species richness and abundance, with these values further split into the three Asteraceae subfamilies of Asteroideae, Carduoideae, and Cichorioideae. Given that *O. leaiana* exhibits oligolectic foraging on Asteraceae (Falk, 2019; Westrich, 2018), only the richness and abundance values of Asteraceae and it's subfamilies were used in the analyses to represent the floral resources used by the study species. As *O. leaiana* females can provision one to two brood cells per day and the transects were conducted on the same day as pollen sampling, the transect data provide an accurate snapshot of the available floral resources. Due to the large number of geographical dispersed sites and variation in nest establishment timing, transect walks were conducted in late June, late July, and mid-August.

To calculate tree and shrub cover, five circular plots with a 5-meter radius were randomly selected to capture the range of vegetation heterogeneity present at each site. Cover was then estimated for each plot with the mean value of the five plots used as the estimate for the site. Shrub removal is often done in semi-natural grassland management such that woody cover is an important indicator of management or abandonment. A grazing index was used as a measure of grazing intensity and defined as:

$$G_j = \frac{\frac{\sum_{i=1}^{n} N_{ij} * M_{ij} * L_i}{12}}{A_j},$$

where N_{ij} is the number of individuals of animal species i in site j, M_{ij} is the number of months animal species i grazed in site j in 2022, L_i is the livestock unit of the animal species i and A_j is the grazed site area of site j. Livestock units for each animal species were 0.1 for goats, 0.1 for sheep, 1 for cattle, and 1.1 for horses (see Fischer et al., 2010). Lastly, we counted the number of eumenine wasp nests in each nest block as high rates of nest usurpation were observed during data collection.

Pollen metabarcoding

Pollen DNA was extracted using the Plant/Fungi DNA Isolation 96 Well Kit (Norgen Biotek). Pellets were first resuspended in 200 μL of Lysis Buffer L and transferred to a 96-well plate pre-loaded with ceramic beads for mechanical homogenization using a bead mill. After homogenization, an additional 200 μL of Lysis Buffer L was added to each well, and DNA extraction was conducted following the manufacturer's protocol. PCR amplification targeted the ITS2 region using the ITS2-S2F/ITS4R primer pair (Chen et al., 2010; White et al., 1990) optimized for dual indexing (Sickel et al., 2015) on an Illumina MiSeq system (Illumina, San Diego, CA, USA). PCR was performed using an ALLin Mega HiFi Red Mastermix (highQu, Kraichtal, Germany), beginning with an initial denaturation at 95 °C for 1 min followed by 40 cycles of denaturation at 99 °C, annealing at 52 °C, and extension at 72 °C, each lasting 15 s. The amplified products were purified using AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) with a 1.8:1 bead-to-sample ratio. Sequencing was conducted at the Genomics Core Leuven in Leuven, Belgium.

Bioinformatics

Bioinformatic analysis was conducted using the pipeline available at https://github.com/chiras/metabarcoding_pipeline (version 8c8536b; Leonhardt et al., 2022). This workflow employs VSEARCH (Rognes et al., 2016) for quality filtering, merging, dereplication and defining amplicon sequence variants (ASVs) through denoising. Taxonomic assignment was completed using an iterative approach. Initially, direct global alignments were performed using a 97 % similarity threshold against a localized reference database. This database was assembled with BCdatabaser (Keller et al., 2020) and based on a list of plant species occurring in Estonia. ASVs that could not be assigned in this first step were subsequently compared to a comprehensive global vascular plant database (Quaresma et al., 2024; Ratnasingham & Hebert, 2007) using VSEARCH with the same 97 % threshold. For any remaining unclassified ASVs, hierarchical classification was conducted using SINTAX (Edgar, 2016) against the global plant database, with a confidence threshold set at 0.8 to reach the deepest reliable taxonomic level. Finally, ASVs were aggregated to the species level and converted into relative read abundances (i.e. the proportion of reads assigned to each taxon within the sample. Taxa contributing less than 0.1 % of the total reads in a sample were removed using the sequencing strategy found in Sickel et al. (2015), while samples with fewer than 1000 quality-filtered reads were excluded entirely.

Statistical analyses

To assess whether the abundances of the three subfamilies of Asteraceae differed across the study sites, a generalized linear mixed model with a gamma distribution and log link function was constructed using the '*glmmTMB*' package (Brooks et al., 2017). Pairwise comparisons of estimated marginal means among subfamilies were then assessed, with Tukey's adjustment for multiple comparisons. The prevalence of each plant species within the pollen mix of each sample was described using relative read counts in each sample. Shannon diversity (H') was calculated for each sample and converted to the effective species richness (Hill number of order 1, $q = 1$) by taking the exponent of H'. Relative abundances across samples were summed by family to calculate the percentage of the total relative abundance of reads attributable to each family in overall pollen use. They were further summed by subfamily for Asteraceae species.

To test our hypothesis that local management would drive pollen resource use, redundancy analyses (RDA) were performed using the R package '*vegan*' (Oksanen et al., 2024). The influences of land use proportions, shrub and tree cover, grazing intensity, and overall Asteraceae subfamily abundance on the relative species composition of Asteraceae within pollen provisions were evaluated. Multicollinearity was assessed using the vif.cca function from '*vegan*'. Analyses were conducted using Hellinger-transformed read abundances per sample, and the significance of the relationships was evaluated using the anova.cca function from '*vegan*' (n = 10,000 permutations). The varpart function from '*vegan*' was then used to partition the variation in pollen provision composition into three predictor sets, which were selected using the vif.cca function from '*vegan*': (i) land use composition (% semi-natural grassland, % forest, % annual crops, and % agricultural pasture), (ii) management (% shrub cover, % tree cover, and grazing intensity), and (iii) floral resources (Asteraceae subfamily abundances).

To test our hypothesis that Asteraceae abundance drives reproductive success, a generalized linear model with a negative binomial distribution and log link function was then constructed using the '*glmmTMB*' package. The same variables described above, in addition to the number of eumenine wasp nests, were tested for significant effects on the number of brood cells per *O. leaiana* nest tube. Brood cell count is a typically used measure of reproductive success in cavity-nesting bees (Rosanigo et al., 2020; Spendal & Cane, 2022). Forward and backward selection was used to determine the best model according to AICc values as calculated using the AICc() function from the '*MuMIn*' package (Bartoń, 2025). The '*DHARMa*' package was used for model diagnostics, including testing residuals for overdispersion, uniformity, and outliers (Hartig, 2024).

Results

Floral resource use

Pollen metabarcoding revealed that Asteraceae comprised 94.3 % of the total relative abundance of sequences among all samples (Fig. 2a). The next most abundant family was Rosaceae, which comprised 3.0 % of total relative abundance. Examining these data at the species level shows that the pollen provisions in each nest tube contained a mean plant species richness of 9.9 and a mean Shannon diversity of 1.01, which corresponds to an effective species richness of 3.03 (Hill number, $q = 1$). Within Asteraceae, *Crepis, Leontodon*, and *Pilosella* were the most abundant genera, respectively comprising 21.1 %, 19.5 %, and 14.7 % of total relative abundance (Fig. 2b). Many of the nests were dominated by pollen from few species, which included *Crepis biennis, Leontodon hispidus*, and *Tragopogon pratensis* (Fig. 3). The great majority of species are Asteraceae from the subfamily Cichorioideae, which also dominated total relative abundance of sequences (84.1 %) compared to Asteroideae

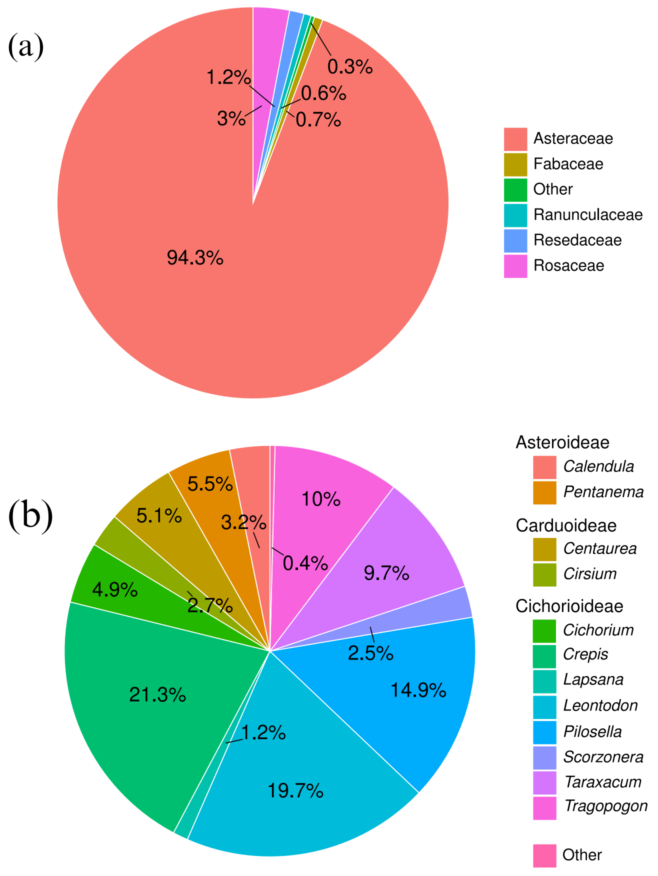

Fig. 2. Pie charts illustrating the percent of total relative abundance of sequences across all samples represented by a) each plant family and b) each Asteraceae genus. Those with percentages ≥ 0.5 % of total relative abundance among all samples are shown individually, while all others are grouped into the "Other" category.

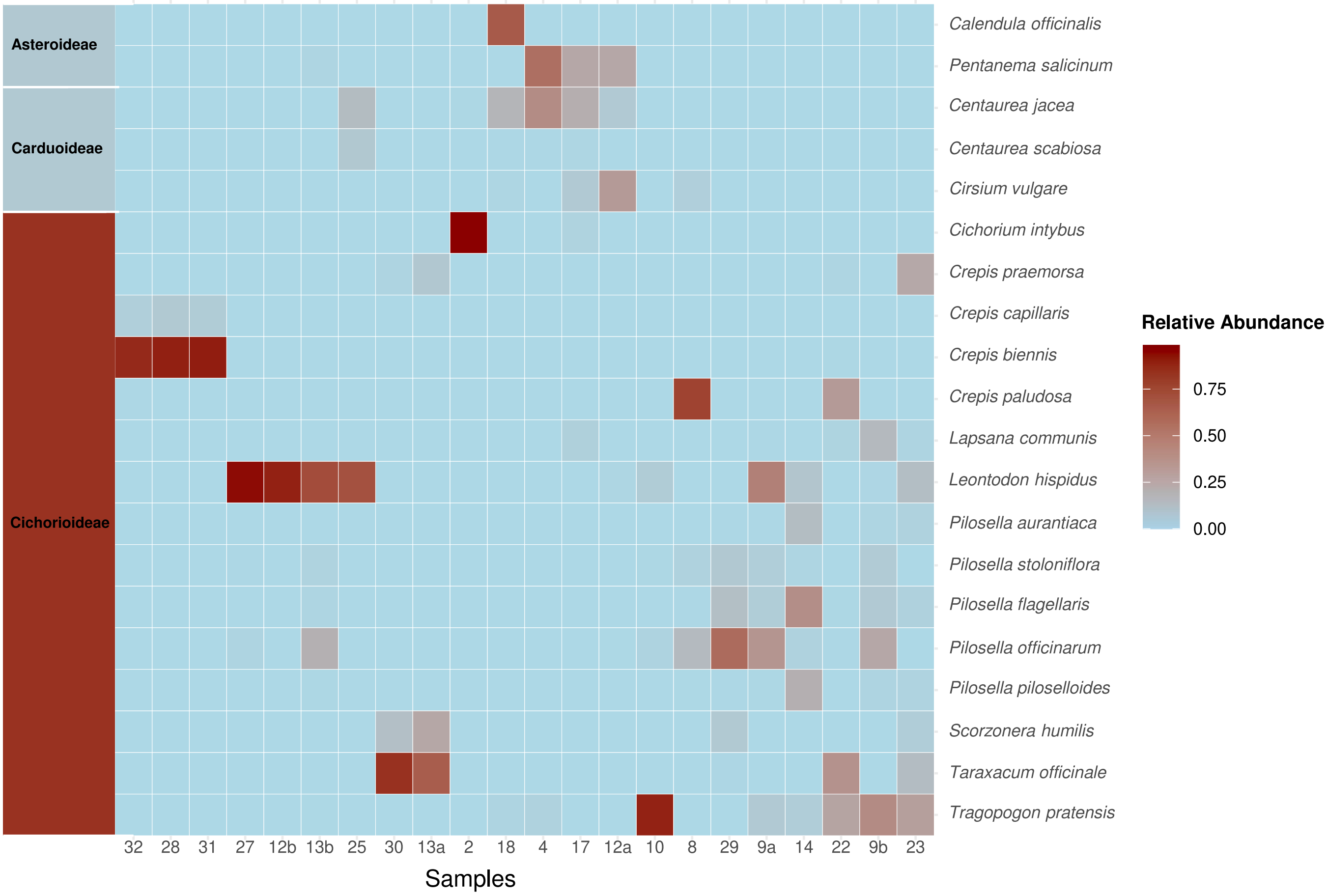

Fig. 3. Heatmap of Asteraceae species relative abundances within each sample. Rows are organized by subfamily and color-coded by relative abundance. Columns are nest block IDs. Note that only plant species with percentages ≥ 0.5 % of total relative abundance among all samples are shown.

(8.3 %) and Carduoideae (7.6 %). A GLMM assessing differences in abundance among the three subfamilies across the 29 sites supported that this represents a foraging preference and not a mere artifact of community composition. The post-hoc comparison of estimated marginal means showed that plants of the subfamily Cichorioideae had significantly lower abundance than those of Carduoideae (SE = 0.548, $p = 0.001$) and Asteroideae (SE = 0.581, $p = 0.001$), with raw means of 237.3 cm^2, 4175.1 cm^2, and 2535.5 cm^2, respectively.

Impact of land use, management, and floral resource variables on pollen resource use

Variance partitioning revealed that floral resources, land use, and management uniquely explained 11 %, 9 %, and 6 % of the variance in pollen assemblages, respectively, with an additional 4 % explained jointly by land use and management (Fig. A1). The best RDA model (adj-$R^2 = 0.213$, $F = 1.57$, $p = 0.005$; Fig. 4) found that the proportion of semi-natural grassland ($F = 2.56$, $p = 0.006$), tree cover ($F = 2.07$, $p = 0.025$), and Cichorioideae abundance ($F = 2.24$, $p = 0.009$) significantly explained variation in the composition of pollen provisions. In study areas with higher proportions of semi-natural grassland, which correlated with higher tree cover, pollen provisions had substantially higher relative abundances of L. *hispidus*, which is a species characteristic of calcareous grasslands. In contrast, sites with lower proportions of semi-natural grassland had higher relative abundances of *Calendula officinalis*. In sites with higher Cichorioideae abundance, several species of the subfamily Cichorioideae (*Taraxacum officinale, Scorzonera humilis,* and *Crepis paludosa*) had higher relative abundances in pollen provisions. Lastly, *Pentanema salicinum* (Asteroideae) and *Centaurea jacea* (Carduoideae) had somewhat higher relative abundances in pollen provisions from sites with lower Cichorioideae abundance.

Reproductive success

Of the 58 nest blocks from 29 sites, 27 (46.6 %) from 23 sites were occupied by *O. leaiana*. Generalized linear models (GLMs) were used to test for significant effects of land use, management, and ecological variables on the number of brood cells in occupied nest tubes. The best model found that brood cell number was significantly lower in sites with higher proportions of cropland (estimate = −2.79, SE = 1.24, $p = 0.024$; Fig. 5a) and forest (estimate = −2.61, SE = 1.09, $p = 0.017$; Fig. 5b).

Discussion

Pollen resource use

Our results showed that 94.3 % of the total relative abundance of sequences across all samples were from Asteraceae, which corroborates prior research that *O. leaiana* females are oligolectic on this family for pollen collection (Falk, 2019; Westrich, 2018). This included 36 species of Asteraceae across all samples. However, the effective species richness in individual nest tubes was broadly low (mean = 3.03), indicating that pollen provisions were dominated by a limited number of species (Fig. 2). These results suggest that despite a high diversity of available resources in the land use matrix, individual bees provision their larvae with pollen from few plant species. This supports prior research on *O. cornuta* and *O. bicornis* showing that larval mass was dependent on provision amount (Bosch & Vicens, 2002; Radmacher & Strohm, 2010)

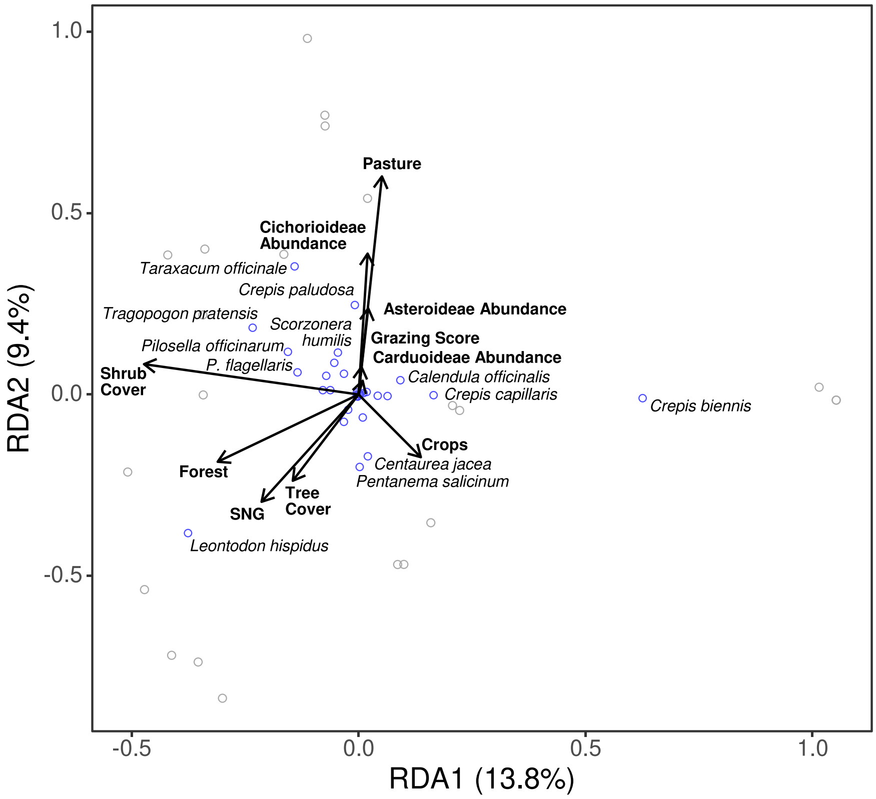

Fig. 4. RDA plot showing the effects of land use, management, and floral resource variables on the community composition of pollen samples taken from Osmia leaiana nest tubes. Samples and species are respectively displayed in gray and blue. Labels were only added to species that were most influenced by the RDA axis.

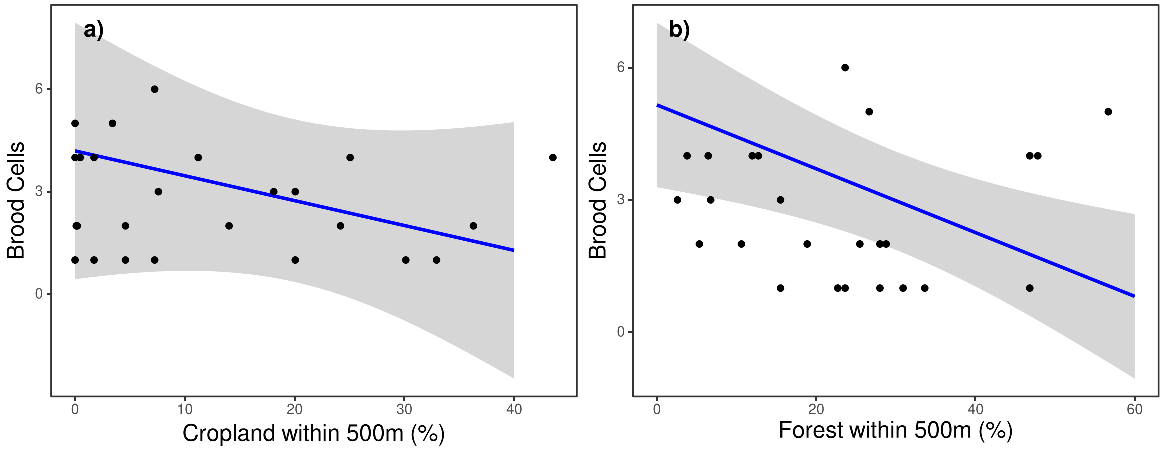

Fig. 5. Effect plots of (a) the proportion of cropland and (b) forest within 500 m from the best GLMs assessing the influence of land use, management, floral resource variables on numbers of brood cells in Osmia leaiana nest tubes.

rather than diversity (Radmacher & Strohm, 2010).

Although *O. leaiana* has long been known to specialize on Asteraceae (Raw, 1974), few prior studies have investigated preferences below the family level in this diverse plant lineage. By assessing pollen resource use at the subfamily level, we found that pollen provisions were mainly dominated by species from the subfamily Cichorioideae, which represented 84.1 % of the total relative abundance of sequences among all samples. This corresponds to previously noted preferences for the tribes Cichorieae and Cardueae (Westrich, 2018), the former of which falls within Cichorioideae and includes all such species with ≥ 0.5 % of total relative abundance among all samples (Fig. 3). Our study did not find a preference for Cardueae, despite a significantly greater abundance of Carduoideae than Cichorioideae at the study sites ($p = 0.001$).

The only other plant families with species representing greater than 0.5 % of total relative abundance of reads across samples were Rosaceae and Resedaceae. The former comprised 3 % of total relative abundance, the largest amount after Asteraceae, and included the species *Potentilla reptans* and *Rosa canina.* Although the exact source of this material is not certain, *O. leaiana* has been observed gathering and chewing leaf material from various Rosaceae species for nest construction (Jelle Devalez pers. obs.). This has also been observed in Central Europe in *O. leaiana* and seven other species of osmiine bees to build nest partitions and nest plugs (Else & Edwards, 2018; Müller & Richter, 2018). In 3 subgenera, specific use of *Fragaria* and *Potentilla* leaves for nest construction has also been recorded (Müller & Richter, 2018; Prosi et al., 2016). It is therefore reasonable to assume that the DNA from these Rosaceae species comes from leaf tissue rather than pollen.

Impact of land use, management, and floral resource variables on pollen resource use

The RDA revealed that floral resource use of *O. leaiana* is driven by the proportion of semi-natural grassland, tree cover, and local abundance of species of the subfamily Cichorioideae. The higher relative abundances of calcareous grassland plants, such as L. *hispidus*, in areas with more semi-natural grassland suggest that *O. leaiana* preferentially foraged in these habitats. However, one sample from a site with little semi-natural grassland contained a very high relative abundance of *Calendula officinalis* (sample 18, see Fig. 3), which is an ornamental plant and thus likely came from a nearby garden. However, three other sites with even lower proportions of semi-natural grassland showed no evidence of foraging in other land use types, so it remains unclear whether this single example is an exception or representative of more general foraging behavior. Our assertion that differences in land use would not affect pollen species composition due to sufficient abundance of Asteraceae resources within semi-natural grasslands thus remains ambiguous. In particular, the lack of significant effects of other land use types is in contrast with findings obtained for bumblebees, which showed that *Bombus lapidarius* and *B. pascuorum* regularly foraged in the broader landscape and collected pollen from plant species found in alternative land use types such as non-productive agri-environmental schemes (AES) (Sloan et al., 2025). Differences in foraging range may therefore have a large impact on how bee species respond to changing land use, with reduced effects for most *Osmia* species and other small, solitary bees due to lower foraging ranges.

Higher abundance of Cichorioideae was associated with higher relative abundances of *Taraxacum officinale, Scorzonera humilis,* and *Crepis paludosa* (all Cichorioids) in pollen provisions, suggesting that these floral resources were favored when available. Along with the descriptive pollen use data (Fig. 3), this indicates a strong preference for floral resources from this subfamily. Despite substantial variation within taxonomic groups, pollen nutrient content or ratios are often conserved even up to the family level (Stephen et al., 2024; Vaudo et al., 2020, 2024). Within Asteraceae, prior research has shown that pollen from Cichorioideae species consistently contained higher sterol content than that of species from Asteroideae and Carduoideae (Baker et al., 2025). Given that diets richer in sterols increased larval growth in *O. bicornis* (Martel et al., 2025), this and other subfamily-level nutritional differences may drive the observed preference for Cichorioideae. In particular, the higher total relative abundance of *T. officinale* within pollen provisions from sites with higher Cichorioideae abundance is supported by the prior finding that of 275 sampled wildflower species in England, *Taraxacum sect. Taraxacum* contained the highest pollen sterol concentrations (Baker et al., 2025).

In pollen provisions from sites with lower Cichorioideae abundance, *Pentanema salicinum* (Asteroideae) and *Centaurea jacea* (Carduoideae) had somewhat higher relative abundances, providing evidence that these species were more frequently selected as floral resources in the absence of more preferred Cichorioideae species. While *C. jacea* was plentiful at almost every site, its sequences were often abundant in nest tubes alongside those of *P. salicinum*, raising the possibility that pollen from these two species may nutritionally complement one another. Prior studies have shown that *Osmia cornifrons* likely forages pollen to achieve ideal protein to lipids ratios for larval nutrition (Cane & Sipes, 2006; Vaudo et al., 2020).

Impact of land use, management, and floral resource variables on reproductive success

Although we predicted a positive relationship between local Asteraceae abundance and brood cell number, this effect was not observed. One possible reason is that these floral resources were only directly measured within the semi-natural grasslands and not across the full landscape. Due to this sampling limitation, the observed negative effects of cropland and forest proportions on reproductive success may reflect lower floral resource availability in these land-use types compared to semi-natural grasslands and pastures. If properly managed, the latter can also provide preferred floral resources, such as *Taraxacum* spp. (Orford et al., 2016). Particularly in smaller semi-natural grasslands, higher proportions of forests or cropland in the surrounding landscape most likely reduce overall Asteraceae abundance in the matrix. Although Asteraceae contains many common species that thrive in disturbed habitats (Funk et al., 2009), solitary bees may still experience reduced reproductive success if required to travel longer distances to access these resources (Zurbuchen et al., 2010b). This may also explain the negative impact of increased forest cover on the number of brood cells. Although small solitary bees are capable of traversing relatively large forest fragments (Zurbuchen et al., 2010a), this may still impose costs on their foraging efficiency. Importantly, these results illustrate that not only anthropogenic factors such as land use intensification but also differences in natural habitat cover (i.e., forest vs. semi-natural grassland) must be considered when evaluating the conservation needs of pollinators.

Despite high rates of nest usurpation by eumenine wasps, we found no negative effects on reproductive success. Prior research also found that sites with higher rates of wasp kleptoparasitism did not significantly reduce mean brood cell numbers in *O. caerulescens* nests, particularly because wasps tended to target nests with fewer brood cells (Tobajas et al., 2021). Nonetheless, biotic interactions with natural enemies can have substantial impacts on reproductive success. Brood parasitism is a main cause of offspring mortality among solitary cavity-nesting bees, with other parasites, parasitoids, and predators also reducing reproductive success (Minckley & Danforth, 2019). However, other than the aforementioned extensive nest usurpation, no such interactions were observed in our study, and we therefore do not believe they had a major effect on our analysis.

Conclusions

Metabarcoding of nest tube pollen provisions supports previous observations that *O. leaiana* exhibits oligolectic foraging on Asteraceae, with an observed preference for Cichorioideae in the study populations. The proportion of semi-natural grasslands and tree cover also affected the composition of pollen provisions, demonstrating that differences in land use and management affect pollen resource use. Brood cell number was negatively related to the proportion of cropland and forest, suggesting that land use may also impact reproductive success. Finally, these findings illustrate the potential of pollen metabarcoding to evaluate floral resource use of cavity-nesting solitary bee species at fine taxonomic scales in relation to local factors and to inform conservation measures that account for species-specific requirements.

Funding

This research was funded through the 2020–2021 Biodiversa+ and Water JPI joint call for research projects, under the BiodivRestore ERA-NET Cofund (GA N° 101,003,777), with the EU and the funding organizations Belgian Science Policy (BelSPO) through research grant B2/22E/InterRest, Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) through research grant 491,739,988, and the Estonian Research Council. JD, TR, AH and EP acknowledge the support by Estonian Ministry of Education and Research, Centre of Excellence for Sustainable Land Use (TK232) and Estonian Research Council grant PRG874. CW is grateful for funding from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—Project number: 493,487,387. ES is additionally grateful to FWO for funding through research grant S009023N.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any generative AI and AI-assisted tools during the writing process.

CRediT authorship contribution statement

Evan Taylor Sloan: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jelle Devalez:** Writing – review & editing, Investigation, Conceptualization. **Alexander Keller:** Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Elisabeth Prangel:** Writing – review & editing. **Aveliina Helm:** Writing – review & editing, Funding acquisition. **Annika L. Hass:** Writing – review & editing, Funding acquisition, Conceptualization. **Catrin Westphal:** Writing – review & editing, Funding acquisition, Conceptualization. **Triin Reitalu:** Writing – review & editing, Project administration, Funding acquisition, Data curation, Conceptualization. **Hans Jacquemyn:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for this journal and was not involved in the editorial review or the decision to publish this article.

Acknowledgements

We are grateful to all the landowners who have allowed us to use their pastures for this project.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.baae.2025.12.011.

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