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Floral resources in the surrounding landscape matrix augment plant species richness of bumblebee pollen loads in small, fragmented calcareous grasslands☆

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A B S T R A C T

Pollinator populations have shown substantial declines worldwide due to habitat loss and fragmentation, with bees experiencing particularly sharp reductions in both species richness and abundance. While local management measures in semi-natural grasslands and agri-environmental schemes (AES) intend to improve farmland biodiversity, the extent to which these measures affect foraging behavior and pollen resource use of bees in fragmented landscapes remains poorly understood. In this study, we tested the hypothesis that landscape and grassland management affected floral resource use of two bumblebee species (*Bombus lapidarius* and *B. pascuorum*) in fragmented calcareous grasslands in Central Germany. Pollen metabarcoding was used to assess the richness and community composition of pollen loads from both bumblebee species. Moreover, we compared the interactions detected by pollen analysis to those observed in transect walks. The results showed that both species, despite their ecological differences, regularly foraged in the broader landscape and collected pollen from plant species found in AES. The percentage of land within 1 km allocated to non-productive AES (e.g., flower strips) was associated with higher pollen species richness in both *B. lapidarius* and *B. pascuorum*. In the latter species, this relationship was particularly strong in smaller sites. Landscape and site management variables affected the species composition of pollen assemblages collected from both bumblebee species. Our study showed that bumblebees utilize floral resources from the broader landscape, especially from non-productive AES, illustrating that these schemes can support local pollinator communities of semi-natural grasslands in fragmented agricultural landscapes.

1. Introduction

Pollinators are crucial for ecosystem functioning as they are estimated to provide pollination services to over 90 % of angiosperm species (Herbertsson et al., 2021; Tong et al., 2023). However, recent trends have shown substantial declines in pollinator populations (Powney et al., 2019; Zattara and Aizen, 2021), with bees in particular experiencing sharp reductions in abundance (Koh et al., 2015; Turley et al.,

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2022) and species richness (Goulson et al., 2015). One of the main reasons for the current decline of pollinators is land use change (Tsang et al., 2025; Shipley et al., 2024), which often leads to a strong decline of floral resource availability and diversity that consequently alters pollinator diets (Carvell et al., 2006; Roulston and Goodell, 2011; Parreño et al., 2024; Fijen et al., 2025). These changes in food resource utilization can have severe impacts on pollinator health, reproduction, and population growth (Hass et al., 2019; Lau et al., 2023), particularly as dietary imbalances or deficiencies among key elemental nutrients can adversely affect both adults (Filipiak et al., 2023) and larvae (Filipiak, 2019). Moreover, a monotonous diet may increase the susceptibility of pollinators to diseases and the harmful effects of pesticides (Goulson et al., 2015). Therefore, it is important to understand how land use changes affect the floral resource use of pollinators and whether conservation measures at the local and landscape scale can counteract the impoverishment of their diets.

Calcareous grasslands are an important habitat for pollinators that have been severely reduced by land use change. These semi-natural grasslands are one of the most species-rich habitat types in Europe (Eriksson and Eriksson, 1997), but have suffered dramatic declines through the 19th and 20th century due to agricultural intensification and abandonment (Poschlod and WallisDeVries, 2002). Restoration measures have been slowly but increasingly adopted since the 1970s, mostly by reintroducing traditional land use practices such as rotational grazing, mowing, or shrub removal, to conserve their exceptional biodiversity (Poschlod and WallisDeVries, 2002; Willems, 2001). These measures have been shown to increase plant species richness and therefore also the diversity of food resources available to pollinators (Johansen et al., 2019; Wehn et al., 2017). Moreover, most present-day calcareous grasslands are fragmented and isolated within predominantly agricultural landscapes (Adriaens et al., 2006; Helm et al., 2006; WallisDeVries et al., 2002). Small and isolated habitat patches can only support a limited number of plant and insect species (Steffan-Dewenter et al., 2006) and the remaining populations are often small and prone to extinction.

To mitigate the effects of fragmentation and improve pollinator biodiversity, attempts have been made to improve the ecological quality of agricultural landscapes through an array of European Union or nationally-funded agri-environmental schemes (AES). These schemes are programs designed to encourage environmentally-friendly agricultural practices that enhance biodiversity, conserve natural resources, and mitigate negative environmental impacts caused by intensive farming (Batáry et al., 2015; Kleijn and Sutherland, 2003). AES can be categorized into productive schemes, which are implemented on cultivated areas such as arable cropland or grasslands (e.g., organic agriculture), and non-productive schemes, which are implemented on areas out of production such as field margins (e.g., flower strips). Regardless of type, AES can have positive effects on pollinators in adjacent habitats. For example, field margins of organically managed crop fields have been shown to support more diverse interactions between bumblebees and floral species (Marja et al., 2021), while flower strips contributed to more diverse pollen diets (Piko et al., 2021). Flower strips sown with species chosen to augment nectar and pollen availability effectively provided forage that increased bumblebee abundance and richness (Pywell et al., 2006). Similarly, sowing with native wildflowers increased bumblebee richness and provided early-season foraging resources (Carvell et al., 2007). However, knowledge regarding how productive and non-productive AES supplement pollen diets of bee populations within semi-natural areas of conservation concern, such as calcareous grasslands, remains largely unknown.

Prior research has shown that the quality and diversity of resource patches greatly influence bumblebee foraging behavior with respect to both patch selection and floral choice within a patch (Jha and Kremen, 2013; Takagi and Ohashi, 2025). The major aim of this study was therefore to assess how landscape and local management affect foraging behavior and floral resource use of two bumblebee species (*Bombus lapidarius* and *Bombus pascuorum*) in fragmented calcareous grasslands. We used visual observations of plant-pollinator interactions and pollen metabarcoding of pollen carried by both bumblebee species to achieve this aim. While visual observations of plant-pollinator interactions can only capture interactions that occur within study sites, pollen found on insects is collected from a wider area, thus offering clear advantages when addressing ecological questions at the landscape scale (Bänsch et al., 2020; Danner et al., 2017; Schweiger et al., 2022; Richardson et al., 2021; Verbeke et al., 2023). Furthermore, high-throughput sequencing-based pollen identification provides superior taxonomic resolution than morphological identification and reduces the need for specialized taxonomic expertise (Bänsch et al., 2020; Bell et al., 2017; Keller et al., 2015).

Specifically, we hypothesized that (i) bumblebees frequently forage outside local patches and use landscape-scale resources and that (ii) local and landscape management will be important drivers of pollen species richness and community composition collected by bumblebees. In particular, we expect that (i) at the local scale, species richness of pollen will be higher in sites with more conservation management and lower indications of abandonment; (ii) at the landscape scale, the availability of calcareous grasslands and AES will increase pollen species richness and lead to higher use of resources typical for these habitats; (iii) the percentage of conventional agriculture will have a negative effect on pollen species richness; and (iv) landscape-scale effects will be stronger when calcareous grassland sites are small.

2. Methods

2.1. Study species

Bombus pascuorum and *B. lapidarius* are two common bumblebee species found throughout Europe and western Asia where they often inhabit gardens, grasslands, farmland, and woodland edges (Goulson, 2003). *Bombus pascuorum* tends to nest on or directly beneath the soil surface, while *B. lapidarius* typically nests under the ground, but has also been observed to opportunistically use structures such as bird nests and anthropogenic artifacts (Fussell and Corbet, 1992; Svensson et al., 2000). Colonies of *B. pascuorum* typically have 60 to 150 workers, while the larger colonies of *B. lapidarius* consist of up to 300 (Falk, 2019). The flight period of these species typically extends from spring to midsummer or early autumn in *B. lapidarius* and *B. pascuorum*, respectively. Prior studies have estimated foraging distances in agriculturally dominated landscapes from 272 m to 775 m for *B. pascuorum* and 536 m to 755 m for *B. lapidarius* (Carvell et al., 2012; Knight et al., 2005; Wood et al., 2015; Redhead et al., 2016). Although both species show a preference for species of Fabaceae, they forage on a diverse range of plant species and adjust their resource use according to local floral availability (Marja et al., 2021).

2.2. Study areas

Sampling was conducted across 31 calcareous grassland fragments in the German federal state of Lower Saxony in the districts of Göttingen and Northeim (Fig. 1). These study sites are interspersed throughout open agricultural landscapes dominated by intensive cultivation of primarily cereals, oilseed rape, and sugar beet, but also include *Fagus sylvatica*-dominated forests, intensively managed grasslands, and settlements. Most calcareous grassland patches in the region are widely dispersed and small, with the selected study sites ranging in size from 0.63 to 8.1 ha. Two decades ago, calcareous grasslands covered approximately 0.26 % of the region (Krauss et al., 2003). Over the past few decades, nearly all study sites were overgrown by shrubs though some were later restored. Management, when present, consisted of regular shrub removal and/or grazing with various livestock.

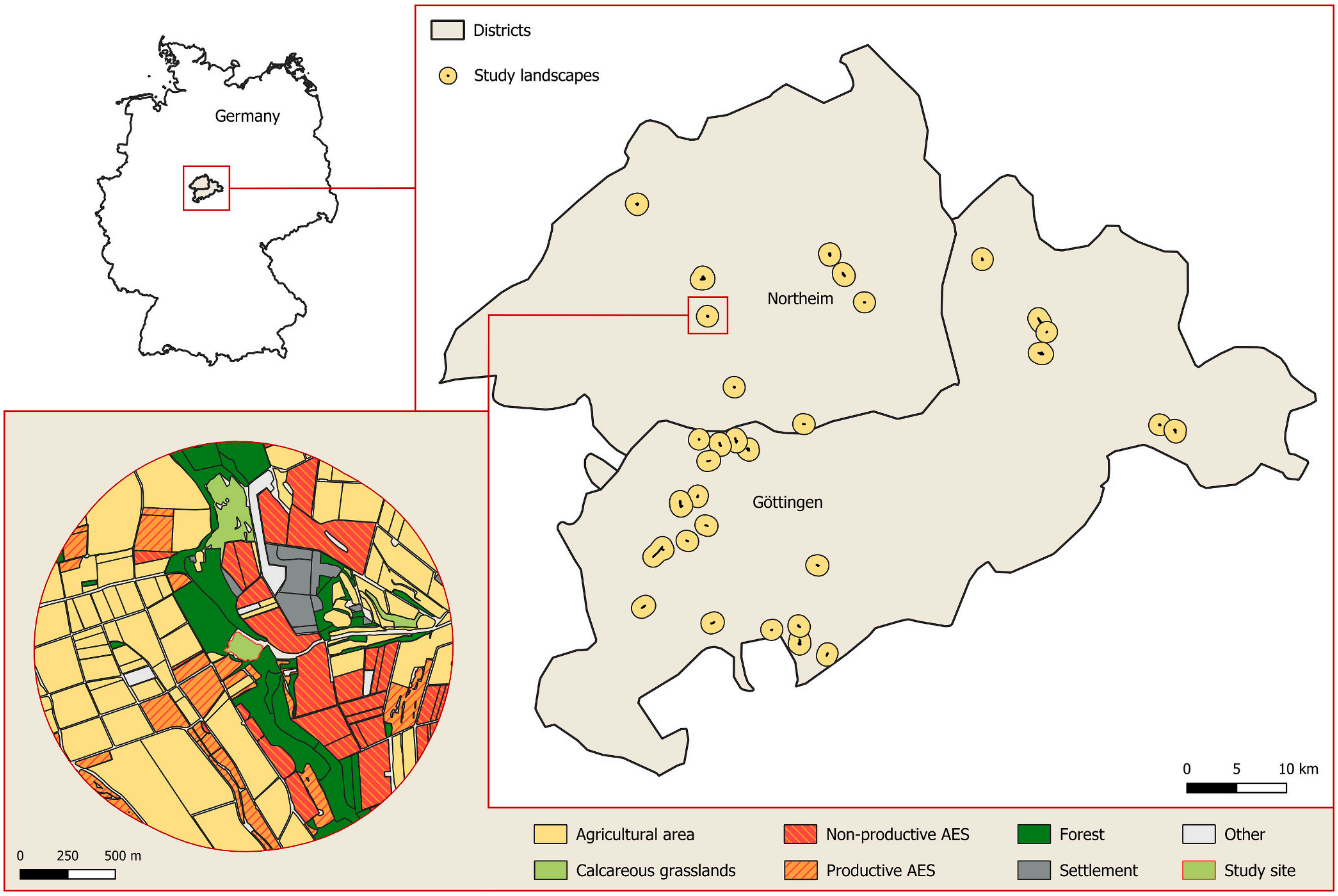

Fig. 1. Overview of the study area. The main map shows the two administrative districts and all of the grassland sites therein. The lower inset map provides an example of the land use surrounding one of the sites.

2.3. Bumblebee surveys and pollen collection

Each site was visited three times during the bumblebee flight season (mid-May to mid-August 2022). Sampling was conducted only during sunny weather with temperatures above 15 °C, no strong wind, and between 9:00 AM and 5:00 PM. Bumblebees were collected in each grassland along 200 m of transect walks divided among four 50 m sub-transects with a width of 2 m. Sub-transects were placed in variable locations and aimed to include most flower-rich patches while remaining representative of the entire site area (Westphal et al., 2008). Each sub-transect was walked for 5 min in each of the three sampling periods, totaling 60 min of observation per site. Sub-transect walks occurred throughout each day with only a single site sampled per day. During the walks, the observer captured every flower-visiting individual of the two study species within the transect area using an insect net and assigned a unique ID on the data sheet and corresponding vial. The time was paused during bumblebee handling and identification to ensure a standardized observation period of 5 min per sub-transect. The visited flowering plant species was then identified and recorded. For pollen collection, captured individuals of *B. lapidarius* and *B. pascuorum* were gently placed in a plastic vial filled with distilled water to remove pollen from their bodies, after which they were released. In total, pollen from up to 15 individuals was collected per species and site. Other observed interactions involving the focal bumblebee species were also recorded even if specimens could not be collected for pollen. After sampling was completed, the collected pollen samples were sent to the Plant Population Biology & Conservation Lab at KU Leuven in Belgium and frozen at −20 °C until further processing.

2.4. Landscape variables

To assess how the surrounding landscape affected pollen resource use, the percentage cover of conventional agriculture, various AES, and calcareous grasslands within a 1 km buffer surrounding each study site was calculated. All land use variables were determined using QGIS (QGIS.org, 2024) and R (R Core Team, 2024) based on land use data from the LEA portal of Lower Saxony (https://sla.niedersachsen.de/landentwicklung/LEA/), the IACS (Integrated Administration and Control System) of Germany and ground truthing in the field. The agri-environmental schemes were organized into two categories of productive and non-productive AES given that productive schemes such as organic agriculture have different ecological effects compared to non-productive schemes such as flower strips (Batáry et al., 2015; Mader et al., 2017). Productive AES included organic grasslands, organic crops, extensive grasslands, and organic plantations, while non-productive AES included annual flower strips, perennial flower strips, green strips, conservation fields, conservation grasslands, and fallows (Table A1). Lists of plant species potentially included in flower strips were obtained from the Lower Saxony Ministry of Food, Agriculture, and Consumer Protection and lists of flowering crops were obtained from the LEA portal of Lower Saxony. A 1 km radius was chosen for these land use variables based on estimated foraging distances for *B. lapidarius* and *B. pascuorum* (Carvell et al., 2012; Knight et al., 2005; Wood et al., 2015; Redhead et al., 2016).

2.5. Local variables

To survey the plant community, five subsites with a 5 m radius were chosen in each grassland to best represent the full vegetation community of each site. These did not necessarily overlap with the sub-transects. Abundance of vascular plant species per site was scored from 0 to 5 based on its occurrence in the 5 subsites. For each grassland site, total plant species richness was calculated based on these surveys with the addition of any plant species encountered in the transect walks that were not present in any of the subsites. Site area was calculated using QGIS (QGIS.org, 2024) and R (R Core Team, 2024) as above.

In addition, a management-abandonment index was developed to combine indicators of conservation management with that of abandonment and thus facilitate comparison of sites with heterogeneous management. The index uses the following variables: (1) continuous management over the last 25 years (yes = 1, no = 0), (2) regular shrub removal (yes = 1, no = 0), (3) grazing index, and (4) woody cover. Low to moderate intensity grazing is broadly accepted as necessary to both restore and maintain the species richness and unique community composition of calcareous grasslands (Škornik et al., 2010; van Wieren and Bakker, 2008) and to support higher pollinator richness and abundance (Lazaro et al., 2016; Weiner et al., 2011). 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). Woody cover was assessed by calculating the average proportion of ground covered by shrubs or trees within the five vegetation survey plots. All of the above variables other than woody cover were determined through interviews with land managers.

To create the index, sites were ordinally ranked according to the management measures (continuous management over the last 25 years, shrub removal, and grazing), as well as their state of abandonment as indicated by woody cover. First, a PCA was conducted on the management measures using the '*vegan*' package (Oksanen et al., 2024). The first PCA axis (PC1) represented 41.76 % of the explained variance and was positively correlated with all of the constituent variables. Sites were then ranked by their PC1 values with the highest score set as the highest management value. Second, the sites were ranked by their proportion of woody cover with the lowest shrub cover receiving the highest rank. Lastly, the mean of both ranks was assigned to each site to create the final management-abandonment index.

2.6. Pollen metabarcoding

Pollen DNA was extracted using the Plant/Fungi DNA Isolation 96 Well Kit (Norgen Biotek). First, the pellets were resuspended in 200 μL of Lysis Buffer L and placed into a 96-well plate containing ceramic beads. The samples were then mechanically homogenized using a bead mill. Following this step, an additional 200 μL of Lysis Buffer L was added to each well and DNA extraction proceeded according to the manufacturer's instructions. PCR amplification was then conducted with the ITS2-S2F/ITS4R primer pair (White et al., 1990; Chen et al., 2010), which was optimized for dual indexing (Sickel et al., 2015) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). Using an ALLin Mega HiFi Red Mastermix (highQu, Kraichtal, Germany), the reaction began with 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 for 15 s. The PCR products were purified using AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) at a bead-to-sample ratio of 1.8:1. Lastly, Illumina sequencing was conducted at Genomics Core Leuven in Leuven, Belgium.

2.7. Bioinformatics

Bioinformatics followed the pipeline available at https://github.com/chiras/metabarcoding_pipeline (version 8c8536b, Leonhardt et al., 2022). The pipeline uses VSEARCH (Rognes et al., 2016) for quality filtering, merging, dereplication and definition of amplicon sequence variants (ASVs) through denoising. Taxonomy was assigned with an iterative approach, with first direct global alignments and a threshold of 97 % against a localized database. This database was created using BCdatabaser (Keller et al., 2020) and a plant species list of Germany. ASVs that remained unclassified were classified against a global vascular plant database (Ratnasingham and Hebert, 2007; Quaresma et al., 2024) as well using VSEARCH global alignments and a threshold of 97 %. Remaining unclassified reads were hierarchically classified using SINTAX (Edgar, 2016) against the global plant database to get taxonomic levels as deep as possible with a threshold of 0.8. ASVs were aggregated at species level, transformed to relative read abundances, and low abundance taxa below 0.1 % sample contribution were removed from such samples according to positive control sample results. Samples with <1000 total quality-filtered sequences were also removed.

2.8. Statistical analyses

For both sampling methods (visual observations and pollen metabarcoding), we calculated the number of plant species uniquely identified by each method as well as the number of plant species detected by both methods. To characterize foraging behavior according to each method, we compared the frequencies with which plant species were recorded in the visual observation and metabarcoding data. Analyses were performed for the most commonly visited plant species as ranked by total relative abundance across the metabarcoding samples. Additionally, to assess whether plant species detected exclusively through metabarcoding were likely foraged in the surrounding landscape, we reviewed whether these species were known to occur in study sites based on their detection via manual plant surveys and transect walks. Finally, to see whether the collected pollen assemblages differed between the bumblebee species, we used a permutational multivariate analysis of variance (perMANOVA) with the adonis2 function in the '*vegan*' package, with bumblebee species included as the explanatory variable. Differences in pollen composition were visualized with a non-metric multidimensional scaling (NMDS) and a Venn diagram was produced to show the overlap in resource use between the two species.

Generalized linear mixed models (GLMMs) with a negative binomial distribution and log link function were constructed using the R package '*glmmTMB*' to assess how the local and landscape variables influenced the species richness of pollen loads on individual bumblebees (Brooks et al., 2017). Site was included as a random factor and interaction effects were evaluated between site area and each variable other than plant species richness per site. Both forward and backward selection were used to determine the best model for each bumblebee species, which was selected according to AICc values. The '*DHARMa*' package was used for model diagnostics, including testing residuals for overdispersion, uniformity, and outliers (Hartig, 2024). Because the 1 km buffers around each site overlapped in a few instances, the testSpatialAutocorrelation() function from this package was also used to test for spatial autocorrelation by applying Moran's I to the simulated residuals of the models.

Redundancy analyses (RDA) were conducted using the R package '*vegan*' to elucidate the effect of the landscape and management variables on both the species composition of pollen samples and the relative abundance of individual plant species within samples. These analyses were based on Hellinger-transformed relative abundances of read counts in each sample. Forward and backward selection was executed with the ordi2step function in '*vegan*' to identify the local and landscape variables that explained most of the variation in the data. The significance of the observed relationships was assessed using the anova.cca function in '*vegan*' (n = 999 permutations).

3. Results

3.1. Floral resource use

The visual observations and pollen metabarcoding provide different pictures of pollen resource use and flower visitation. Metabarcoding detected 97.7 % and 94.1 % of all plant species recorded by both methods for *B. lapidarius* and *B. pascuorum*, respectively (Table 1). Conversely, visual observations detected only 19.4 % and 20.4 % of all plant species recorded for each respective bumblebee species. Although the observation data of both *B. lapidarius* and *B. pascuorum* contained a small number of plant species that were not found in the metabarcoding (2.3 % and 5.9 %, respectively), the majority of the detected plant species were only found in the metabarcoding (80.6 % for *B. lapidarius* and 79.6 % for *B. pascuorum*). Some of the most important floral resources according to relative abundance in the metabarcoding data were observed at either much lower frequencies or not at all in the visual observations (Fig. 2). For example, *Ononis spinosa* was found in 37.5 % and 44.1 % of *B. lapidarius* and *B. pascuorum* samples, respectively, while interactions were never observed with *B. lapidarius* and comprised only 1.9 % of those observed for *B. pascuorum*. Similarly, *Hypericum perforatum* occurred in 19.9 % and 32.4 % of samples from *B. lapidarius* and *B. pascuorum*, respectively, while it comprised 0 % and 3.6 % of observed interactions, respectively.

The composition of pollen assemblages differed between the two bumblebee species ($p = 0.032$, $F = 2.56$, $R^2 = 0.009$). However, the effect size was small and there was substantial overlap in pollen communities collected by both bumblebee species (Fig. A1a). In total, 126 and 142 plant species were observed in *B. lapidarius* and *B. pascuorum* samples, respectively, of which 99 were found in those of both species (Fig. A1b). Pollen samples from *B. lapidarius* contained the highest relative abundances of sequences from *Lotus corniculatus*, *Centaurea jacea*, *C. scabiosa*, and *O. spinosa*, which together comprised 50.3 % of the total relative abundance among all samples. *B. pascuorum* samples contained the highest relative abundances of sequences from *O. spinosa*, *Trifolium pratense*, *L. corniculatus*, and *C. scabiosa*, which together comprised 51.0 % of the total relative abundance. The bumblebees also each showed distinct preferences for certain plant species. For example, *B. lapidarius* had higher total relative abundances of sequences from *Hippocrepis comosa* and *Onobrychis viciifolia*, while *B. pascuorum* samples contained higher relative abundances of *H. perforatum* and *Pilosella officinarum*. Overall, both *B. lapidarius* and *B. pascuorum* primarily relied on a small number of abundant plant species for pollen resources, with the top seven species comprising nearly two-thirds of total relative abundance. Species of the Fabaceae were particularly prominent within the sampled pollen assemblages. In *B. lapidarius* samples, five of the seven most abundant plant species belonged to Fabaceae, while all three of the most abundant species in *B. pascuorum* samples belonged to this family.

3.2. Impact of local and landscape variables

Of the 126 plant species found on *B. lapidarius* and 142 found on *B. pascuorum*, 49 (38.9 %) and 67 (47.1 %) respectively were not found in any of the sites according to plant surveys and transect walks, suggesting that they were foraged throughout the surrounding landscape. Examples include *Helianthus annuus* and *Lythrum salicaria* in *B. lapidarius* and *Lathyrus heterophyllus* and *Digitalis purpurea* in *B. pascuorum*. Many such species were known to occur in agri-environmental schemes (e.g., flower strips) and agricultural fields in the surrounding landscape (Table 2).

Table 1
Number of plant species interacted with by each bee species. Total number of visited plant species is separated into those that were exclusively detected by either or both methods.

	Total	Metabarcoding only	Transects only	Both
Bombus lapidarius	129	104	3	22
	100 %	80.6 %	2.3 %	17.1 %
Bombus pascuorum	151	121	9	21
	100 %	79.6 %	5.9 %	14.5 %

The final GLMM of *B. lapidarius* after variable selection included only the percentage of land within 1 km dedicated to non-productive AES, which was associated with higher pollen species richness (estimate = 3.34, SE = 1.64, $p = 0.042$; Fig. 3a; Table A2). That of *B. pascuorum* included the percentage of land dedicated to non-productive AES, site area, and their interaction effect (Table A3). Percentage of land dedicated to non-productive AES was associated with higher pollen species richness (estimate = 7.95, SE = 2.33, $p < 0.001$; Fig. 3b). The negative interaction between non-productive AES within 1 km and site area illustrated that bumblebees foraged more in the surrounding landscape when calcareous grasslands were small (estimate = −1.89, SE = 0.729, $p = 0.009$; Fig. 3c).

For *B. lapidarius*, the best RDA model identified that the percentage of conventional agriculture and non-productive AES within 1 km, site area, and plant species richness per site had significant effects on pollen load species composition (adj-$R^2 = 0.069$, $F = 3.52$, $p < 0.001$; Fig. 4a). All terms had large effect sizes, though those of conventional agriculture and plant species richness were most pronounced (Table A4). *L. corniculatus* had higher relative abundance in smaller sites, while the specialist *C. scabiosa* had higher relative abundance in samples from sites with higher plant species richness. A greater proportion of conventional agriculture in the landscape was associated with greater relative abundance of L. *corniculatus* and *O. spinosa* pollen, while *H. comosa* and *C. jacea* (both calcareous grassland specialists) pollen had greater relative abundance in bumblebee samples from sites with less surrounding conventional agriculture.

For *B. pascuorum*, the best RDA model identified the percentage of conventional agriculture, productive AES, non-productive AES, and calcareous grasslands within 1 km as well as the abandonment-management index, site area, and plant species richness of each site were explanatory and significant (adj-$R^2 = 0.095$, $F = 3.16$, $p < 0.001$; Fig. 4b). All of the terms other than the percentage of land as non-productive AES and calcareous grassland were of comparably high effect size (Table A5).

4. Discussion

4.1. Pollen load composition

Our results showed that 38.9 % and 47.1 % of plant species detected in *B. lapidarius* and *B. pascuorum*, respectively, were not known to occur in any of the plant surveys or transect walks of the study sites (Table 2), indicating that bumblebees forage regularly outside calcareous grasslands. These interactions cannot be captured by visual observations in the study sites alone, indicating that pollen metabarcoding provides a more comprehensive view of floral resource use, both within sites and in the surrounding landscape (Bänsch et al., 2020; Bell et al., 2016; Bell et al., 2023; Verbeke et al., 2023). This is in line with previous studies that have also noted these spatial limitations of observational interaction data relative to the higher spatial coverage provided by pollen-based methods (Arstingstall et al., 2021; Souza et al., 2021). Moreover, these statistics are likely conservative as they exclude plant species with uncertain taxonomy and those known to occur both in study sites and in the surrounding landscape.

The phenomenon of detecting plant species with a high frequency in pollen samples that were never or hardly observed in visual interactions was widespread, even among important floral resources. Although the reasons for these discrepancies are likely multifaced, the differences in time duration and interaction strength captured by the two methods likely play a substantial role. Visual observations capture only the

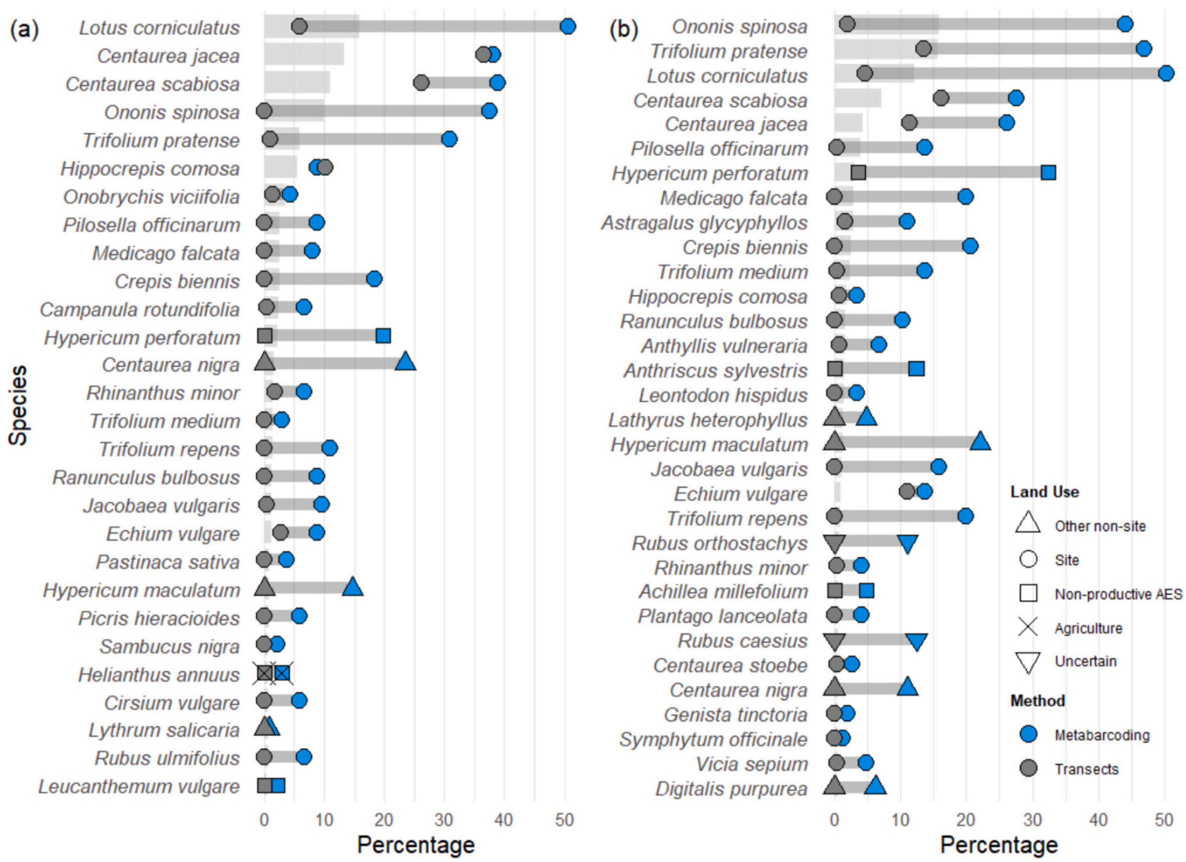

Fig. 2. Plant species that comprise >0.5 % of total relative abundance in all metabarcoding samples from (a) *Bombus lapidarius* and (b) *Bombus pascuorum*. Species are ordered from highest to lowest relative abundance shown in the light gray bars. Connected dots compare the frequency of each plant species in percent of metabarcoding samples and percent of interactions. Symbols indicate the land use type in which each species occurred. Species designated "Other non-site" were not found in any of the sites nor were they among the flowering crop species or those found in flower strips (non-productive AES). *H. perforatum*, *A. sylvestris*, *A. millefolium*, and *L. vulgare* were found in sites in addition to non-productive AES, but are given only the symbol for the latter. Unidentified *Rubus* species were also present in the sites, so these species were designated as "uncertain". The only other species present in multiple land use types was *Helianthus annuus*, which has symbols for both "Agriculture" and "Non-productive AES".

Table 2
Number of plant species from metabarcoding found only in the landscape. These are then separated into those present in flower strips and crop fields.

	All metabarcoding	Only landscape	Flower strips	Crops
B. lapidarius	126	49	7	2
	100 %	38.9 %	5.6 %	1.6 %
B. pascuorum	142	67	12	3
	100 %	47.1 %	8.5 %	2.1 %

Note: Estimates include only species with certain taxonomy and exclude species found in the landscape as well as the sites. This ensures that these estimates are highly conservative. Lists of plant species found in flower strips and flowering crop species were obtained from the Lower Saxony Ministry of Food, Agriculture, and Consumer Protection and the LEA portal of Lower Saxony, respectively.

moment when interactions occur, while metabarcoding reflects all floral visitations over a longer preceding period. This may explain cases such as *Hypericum perforatum*, which appeared frequently in the metabarcoding data, but very rarely in the visual observations (Fig. 2). Generally, *H. perforatum* is most attractive to *Bombus* spp. in the early morning when nectar production is likely highest (Devalez, personal communication). Although nectar and pollen resources may thus already be depleted before transect walks have begun, this does not preclude detection of pollen via metabarcoding.

Nonetheless, pollen metabarcoding did not capture all foraging activity, as three (2.3 %) plant species in *B. lapidarius* and nine (5.9 %) in *B. pascuorum* were only recorded in the visual observation data. Morphological mismatches may explain why pollen was not transferred during interactions, but the large and hairy bodies of bumblebees tend to retain pollen (Roquer-Beni et al., 2020; Velthuis and Van Doorn, 2006) such that pollen transport data more closely aligns with visually observed interactions (Librán-Embid et al., 2024). Most likely, the failure to detect these species via metabarcoding resulted from sample processing and PCR amplification. Pollen morphology can influence the effectiveness of mechanical and/or chemical methods to extract DNA, while primer choice can cause amplification to greatly vary across taxa (Bell et al., 2017; Swenson and Gemeinholzer, 2021).

Despite these methodological imperfections, previous studies have shown that both pollen metabarcoding (Baksay et al., 2022; Baksay et al., 2020; Polling et al., 2022) and metabarcoding in general (Lamb et al., 2019) can be considered semiquantitative. Even when treated as approximations, the selection and ranking of plant species by relative abundance in Fig. 2 remain informative. Furthermore, although rare pollen may not be amplified and thus fail to appear in metabarcoding results (Devriese et al., 2024), plant species with very low relative abundances should be viewed with caution as they may be due to incidental contact rather than foraging choice.

4.2. Impact of the local and landscape management on pollen resource use of bumblebees

Our results provide strong evidence that non-productive AES, such as

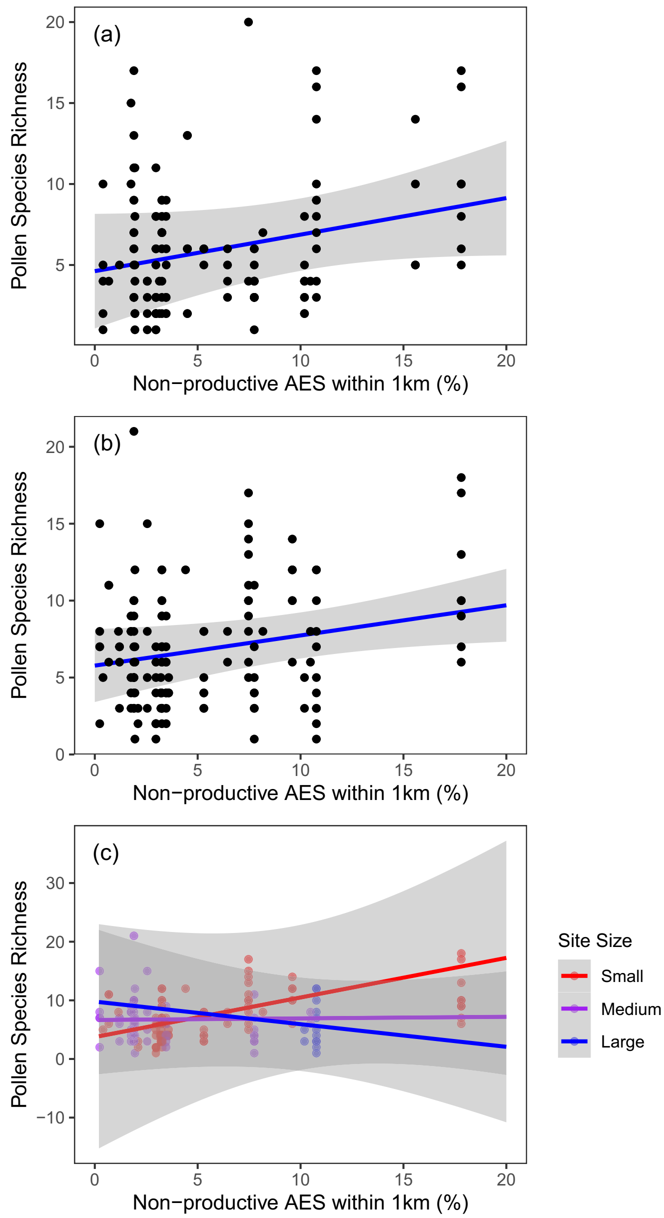

Fig. 3. Effect plots of significant variables from the best GLMMs assessing the influence of landscape characteristics on pollen species richness found on individual (a) *Bombus lapidarius* and (b, c) *Bombus pascuorum* samples. 3c illustrates the interaction effect between non-productive AES and site area.

flower strips, increased the species richness of floral resources upon which the bumblebees forage. This aligns with prior findings that higher land cover diversity decreases network specialization and increases dietary breadth by offering diverse floral resources in the broader landscape (Librán-Embid et al., 2024). These results thus illustrate that the presence of non-productive AES provides supplemental floral resources to these two bumblebee species. The fact that several of the species detected in pollen samples were known to occur in non-productive AES or be cultivated as mass flowering crops (e.g., *Helianthus annuus*) further validates the importance of these features as food sources in the landscape. Other landscape features may also contribute to the diversity of available floral resources. For example, *Sambucus nigra*, *Rubus orthostachys*, and *Digitalis purpurea* are typically found near forest edges (Fig. 2). The model for *B. pascuorum* also included an interaction effect of non-productive AES with site area (Fig. 3c), indicating that bumblebees foraging in small calcareous grasslands more frequently use pollen resources from neighboring areas than those foraging in large grasslands. Smaller sites likely contain fewer resources, prompting bumblebees to forage in the surrounding landscape, while larger sites may offer an abundance of highly desirable and common plant species (e.g., *L. corniculatus*, *Centaurea* spp.) that encourage bumblebees to remain within the site.

In contrast to non-productive AES, the percentage of productive AES, calcareous grasslands and conventional agriculture did not affect the plant species richness of pollen collected by the two bumblebee species. Resources in productive AES such as organic fields seem much less frequently visited by bumblebees than those in non-productive AES such as flower fields (Geppert et al., 2020). Moreover, productive AES may offer a much lower number of flowering plant species due to their focus on crop production. While positive effects of organic farming on bumblebees in directly adjacent semi-natural habitats are evident (Marja et al., 2021), this effect was not detected at the landscape level in our study. That being said, species richness alone may not capture the potential contributions of floral resources from productive AES to bumblebee diets in semi-natural calcareous grasslands. Non-productive AES not only drove pollen species richness, but also pollen species composition collected by both bumblebee species, further highlighting their importance as a food resource. Given the supplemental resources that non-productive AES provide, great consideration should therefore be given to sown seed mixes. Current seed mix protocols are often poorly designed with respect to both bumblebees (Marja et al., 2021) and the broader pollinator community (Nichols et al., 2019). Use of local seeds may additionally prevent changes in the gene pool of nearby plant populations in semi-natural habitats through gene flow resulting from pollination.

Surprisingly, the percentage of calcareous grasslands did not affect pollen load species richness. We had expected that a high percentage of calcareous grasslands surrounding focal grasslands would increase local and landscape plant species richness (Johansen et al., 2019; Wehn et al., 2017) and therefore also the pollen richness collected by bumblebees. However, the bumblebees may have foraged primarily on the most abundant and nutritious plants in the study sites as well as in the connected calcareous grasslands. This mechanism could also explain why local management did not influence collected pollen species richness. Although local conservation management had no effect on plant species richness collected by bumblebees, it has been found to be important for specialized and endangered pollinator species (Biegerl et al., 2025).

The percentage of conventional agriculture also did not affect the species richness of pollen loads. We expected a negative effect, as intensive agriculture provides few floral resources (Geppert et al., 2020, Fijen et al., 2025). However, given its dominance in the landscape, the land use types that replace conventional agriculture (i.e., AES) may be more influential than conventional agricultural cover itself. Lastly, while our choice of a 1 km radius for land use variables is justified based on known bumblebee behavior (Carvell et al., 2012; Knight et al., 2005; Wood et al., 2015; Redhead et al., 2016), this does not preclude the possibility that landscape-scale effects may emerge at other spatial scales (Librán-Embid et al., 2024; Westphal et al., 2006).

Nonetheless, it is important to note that most local and landscape variables had significant effects on the plant species composition of pollen samples. Changes in land use in the surrounding landscape inevitably alter the available floral resources relative to those found within the calcareous grasslands themselves. Variation in intrinsic site characteristics (e.g., management) can also result in changes in the plant community, which may in turn affect foraging opportunities. These results indicate that with local conservation management as well as changes in the landscape percentages of productive AES, conventional agriculture, and calcareous grasslands, bumblebees do not collect pollen from more plant species, but rather change their dietary choices according to availability.

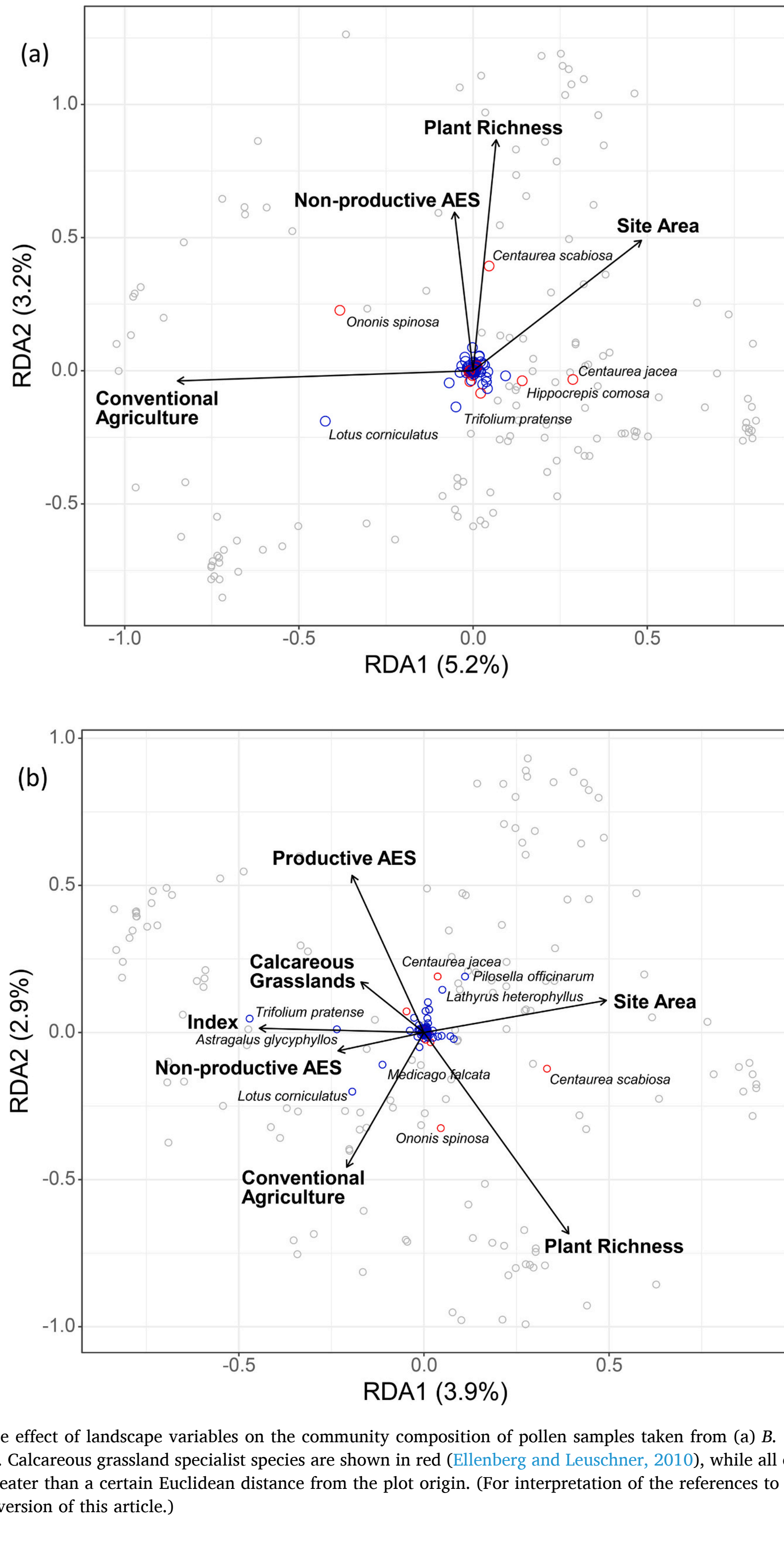

Fig. 4. RDA plots showing the effect of landscape variables on the community composition of pollen samples taken from (a) *B. lapidarius* and (b) *B. pascuorum*. Samples are displayed in gray. Calcareous grassland specialist species are shown in red (Ellenberg and Leuschner, 2010), while all others are shown in blue. Labels were only added to species greater than a certain Euclidean distance from the plot origin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.3. Implications for conservation

Our results demonstrate that, despite their ecological differences, *B. lapidarius* and *B. pascuorum* regularly forage outside calcareous grasslands patches and that higher proportions of non-productive agri-environmental schemes (such as sown flower strips) surrounding grasslands were associated with higher species richness of pollen loads. This effect was especially pronounced for *B. pascuorum* in smaller grassland fragments. These findings may have direct implications for bumblebee colony fitness, as several studies have demonstrated that more diverse pollen diets can positively affect colony growth and reproduction. For example, colony growth (Hass et al., 2019) and reproductive success (Schweiger et al., 2022) of *B. terrestris* were clearly positively affected by diverse pollen diets. Bumblebees in intensively cultivated landscapes with sown flower patches were also shown to produce a greater biomass of sexuals and this increased with flower patch area (Carvell et al., 2015). In addition, more abundant high-value foraging habitat within 250 to 1000 m from *Bombus terrestris*, *B. pascuorum*, and *B. lapidarius* colonies corresponded with higher interannual colony lineage survival in an intensive agricultural landscape (Carvell et al., 2017). Overall, these findings suggest that non-productive AES can provide valuable floral resources for pollinators in adjacent calcareous grasslands and corroborate previous results that non-productive schemes (e.g., flower strips) effectively increase the availability of resources for many grassland species. However, to fully understand how AES affect the relationship between individual foraging behavior and colony fitness, further research is needed. Our results further demonstrate that pollen metabarcoding can be used to inform the design of non-productive AES, for example by identifying the preferred taxa that bees forage upon. With that said, this must be done with consideration of whole pollinator communities rather than only specific taxonomic groups such as bumblebees. With sufficient information, however, well-designed non-productive AES may be crucial conservation tools to support pollinators and should be promoted in the areas surrounding semi-natural habitats.

Declaration of Generative AI and AI-assisted technologies in the writing process:

The corresponding author confirms on behalf of all of the authors that no generative AI tools were used in the writing of the manuscript.

CRediT authorship contribution statement

Evan Taylor Sloan: Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – original draft. **Ira Hannappel:** Investigation, Formal analysis, Data curation, Conceptualization, Writing – review & editing. **Annika L. Hass:** Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization, Writing – review & editing. **Alexander Keller:** Software, Methodology, Formal analysis, Data curation, Writing – review & editing. **Felipe Librán-Embid:** Conceptualization, Writing – review & editing. **Elena Velado-Alonso:** Methodology, Writing – review & editing. **Triin Reitalu:** Conceptualization, Writing – review & editing. **Catrin Westphal:** Project administration, Funding acquisition, Conceptualization, Writing – review & editing. **Hans Jacquemyn:** Supervision, Project administration, Methodology, Conceptualization, Writing – review & editing. **Jelle Devalez:** Writing – review & editing.

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Declaration of competing interest

The corresponding author confirms on behalf of all of the authors that there are no competing interests that may have caused bias or otherwise influenced the outcomes and conclusions in this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2025.111379.

Data availability

Data and R code used in this study are available upon request from the corresponding author.

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