

# Environmental variables and species traits as drivers of wild bee pollination in intensive agroecosystems—A metabarcoding approach

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## Abstract

Wild bees are known to be efficient pollinators of wild plants and cultivated crops and they are essential ecosystem service providers. However, wild bee populations have been suffering from significant declines in the last decades mainly due to the use of agrochemicals. Within this framework, we aimed to characterize wild bees' pollination spectrum (i.e., the community of pollinated flowering plants) in intensive agroecosystems, and describe the environmental variables and wild bee species traits influencing the pollination. To do this, we conducted metabarcoding analyses of pollen loads from wild bees collected in sunflower crops in the French region of Nouvelle-Aquitaine. Our study revealed that wild bees visited flowering plants corresponding to 231 different operational taxonomic units, classified into 38 families of which Asteraceae, Brassicaceae, and Apiaceae were the most visited and more than 90% of the visited taxa turned out to be wildflowers. We also analyzed the potential effect of environmental variables and wild bee species traits in governing their choice of pollinated plants. The community composition of pollinated plants varied depending on the flowering stages of the sunflower and the farming system. Our results also show that pollination niche breadth (alpha diversity) varied depending on the flowering stages of the sunflower but was not different between organic and conventional farming systems. Regarding wild bee species traits, the community composition of pollinated plants varied in relation to wild bees' body sizes and sociality levels. Our results are consistent with previous studies, suggesting that solitary bees are more specialist when it comes to flower selection than social bees, which are more generalist. The metabarcoding of pollen loads enabled us to draw a global picture of plant–wild bee interactions in an intensive agroecosystem. Our findings support the hypothesis that a higher diversity of weeds may increase wild bee diversity in intensive agroecosystems.

## KEYWORDS

biodiversity, pollen DNA metabarcoding, pollination, sunflower, wild bees

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## 1 | INTRODUCTION

Wild bees (monophyletic group Apiformes, clade Antophila, non-*Apis* bees) are a diverse group from a species-richness, phylogenetical, and behavioral perspectives, and comprise around 18,000 species worldwide divided into seven different families (Michener, 2000; Winfree, 2010). This diversity has enabled wild bees to colonize a wide variety of ecosystems, being present in every continent of the planet, with the exception of Antarctica (Danforth, 2007). Communities of wild bees are among the most efficient pollinators (Garibaldi et al., 2013) and they are responsible for 20% of the global pollination services (Losey & Vaughan, 2006; Sánchez-Bayo & Wyckhuys, 2019). They are, thus, providers of an essential ecosystem service given that, at least, one-third of our food, including animal-origin food, depends on animal-pollinated (usually bee-pollinated) crops (Aizen et al., 2009; Klein et al., 2007; McGregor, 1976). Be that as it may, several species are food specialists with narrow-foraging ranges and nesting habitat resources (Roulston & Goodell, 2011; Sánchez-Bayo & Wyckhuys, 2019), which makes them vulnerable to environmental changes. Indeed, in the course of the last century, wild bee populations have suffered widespread declines (Zattara & Aizen, 2021) alongside the rest of insect taxa (Basset et al., 2012; Basset & Lamarre, 2019; Cardoso et al., 2020; Didham et al., 2020; Leather, 2017). One of the main causes of the decline in wild bee populations is the loss of suitable nesting sites and foraging habitats (Brown & Paxton, 2009; Foley et al., 2005; Steffan-Dewenter et al., 2005), which has been reported to be linked to intensive agriculture, and the use of agrochemicals within agricultural landscapes (Basset et al., 2012; Basset & Lamarre, 2019; Brühl & Zaller, 2019; Cardoso et al., 2020; Didham et al., 2020; Leather, 2017).

Agrochemicals have not only direct toxic effects on insects but also indirect effects such as habitat alteration (Hayes & Hansen, 2017), which may modify the abundance and diversity of weeds (wildflowers) within and around crops, thereby altering the availability of floral resources for pollinators (Potts et al., 2010). These threats can cause the disappearance of rare pollinator species and lead to the homogenization of natural plant and pollinator communities (Hallmann et al., 2017; Seibold et al., 2019), which in turn affects the functioning of agroecosystems, rendering them more vulnerable to perturbations such as droughts or pest invasions (Roy et al., 2016; Silverman & Brightwell, 2008). There is therefore an urge to find solutions to halt the extinctions that insect populations are currently facing (Basset & Lamarre, 2019; Sánchez-Bayo & Wyckhuys, 2019). In the scenario of extinction of pollinators, estimations of high economical losses have been made (Gallai et al., 2009; Sandhu et al., 2016). Also, potential failures in pollination would decrease long-term survival of plants (Biesmeijer et al., 2006).

Conventionally, pollination is studied through observational approaches (Arizaga et al., 2000; Cordeiro et al., 2017; dos Santos & Wittmann, 2000; Valido et al., 2019), which require significant efforts to obtain only limited information (Nicholson & Egan, 2020), as the focus is mainly on the plant being pollinated. In the last decade, the boom of high-throughput DNA-sequencing technologies and,

especially, the use of environmental DNA (eDNA) metabarcoding has enabled scientists to acquire more information about community composition in an efficient and cost-effective manner for large-scale surveys (Deiner et al., 2021). eDNA has become a valuable tool to monitor biodiversity and interactions (Bohmann et al., 2014; Deiner et al., 2017; Evans et al., 2016) in a wide variety of ecosystems, including plant-pollinator interactions within agroecosystems (Gous et al., 2019; Lowe et al., 2022; Lucek et al., 2019; Smart et al., 2017). One of the strengths of eDNA is the fact that it provides information about composition of ecological communities. Exploring pollination and acquiring knowledge regarding the quantity of pollen carried by pollinators is key to providing an accurate view of the global pollination picture than those estimations obtained through observational approaches (Baksay et al., 2020; Deagle et al., 2019; Lamb et al., 2019). In fact, molecular-based identification techniques of pollen have been shown to identify a higher number of plant taxa with greater taxonomic resolution than observational approaches and it does not rely on taxonomy experts (Evans & Kitson, 2020; Pornon et al., 2017). Within this framework and with the final objective of integrating the knowledge acquired into conservation programs, we developed an ITS metabarcoding approach on pollen loads to describe the pollination spectrum of wild bees in sunflower crops, as a model for intensive agroecosystems. We also aim to describe which environmental variables govern their choices of pollinated plants. Lastly, we tested the impact of two wild bee species traits, body size, and sociality level (social vs. solitary), not only on pollinated flowering plant community composition but also on pollination niche breadth (diversity of pollinated plants). Acquiring knowledge of flowering plants pollinated by wild bees' communities (hereafter called pollination spectrum) and the drivers of this pollination process might help in understanding pollinator-plant interactions in agroecosystems and potentially inform effective ecological intensification approaches to improve the conservation status of wild bees.

## 2 | MATERIALS AND METHODS

### 2.1 | Wild bee collection and characterization

We captured wild bees (non-*Apis* species) in the LTZER Zone Atelier Plaine & Val de Sèvre (SW France, Nouvelle-Aquitaine Region) (Bretagnolle et al., 2018) in 25 sunflower fields with a maximum of 10 individuals per field. A total of 212 specimens were collected, exclusively during the pre-flowering (7<sup>th</sup>–9<sup>th</sup> July 2020) and flowering (17<sup>th</sup>–22<sup>nd</sup> July 2020) periods (Table S1a,b). Wild bees were collected on any flowering plant within the field and its edges using either a sweep net or directly with a 25-mL sampling tube. The specimens were then placed in a cooled ice box for transportation to the laboratory, where they were stored at –20°C until subsequent processing.

Together with wild bee specimen collection, we took records of the following environmental variables in each crop: percentage of ground covered by weeds in the field, sunflower crop flowering

stage (pre-flowering or flowering), height of the sunflower (cm), percentage of sunflower flowering, and farming system (organic or conventional). Percentage of weeds and height of sunflowers were categorized into four quartiles.

Wild bee specimens were identified morphologically to species level. Subsequently, they were classified by two species traits: size (categorized as T1 to T4) and sociality level (social or solitary). Moreover, we also calculated abundance and species richness of wild bees for each environmental variable explained above: sunflower flowering period, farming system, percentage of weeds, and height of sunflower. Finally, we measured the differences in wild bee community composition (Whittaker index) or the beta diversity between the levels of the four environmental variables, which ranged from 0 (no species turnover) to 1 (every sample holds a unique set of species), using the *vegan* R package (Oksanen et al., 2013).

## 2.2 | Wild bee sample processing, pollen DNA library preparation, and flowering plant identification

Prior to DNA extraction, all wild bee specimens were individually washed in their original collection tube to recover the pollen from their cuticle and wings following a method developed by Batuecas et al. (2022). Following DNA extraction, an ITS2 metabarcoding library was prepared. To that purpose, we used the primer pair (5'-ATGCGATACTTGGTGTGAAT-3') and ITS4R (5'-TCCTCCGCTTATTGATATGC-3') (Chen et al., 2010; White et al., 1990) modified for high-throughput sequencing (HTS), and we attached Nextera XT adaptors through a second amplification before performing a final equimolar pooling (see detailed protocol in [Supplementary Information](#)). This library was sequenced in Illumina MiSeq using V2 chemistry (250×300bp, 500cycles) in the Sequencing Center within the Biozentrum of the Ludwig-Maximilian University in Munich (Germany).

## 2.3 | ITS metabarcoding library filtering and taxonomic assignment

The raw library was filtered using a standard toolbox of software. The expected sequencing depth was around 78,000 reads per sample. The quality of the library was checked using *FastQC* (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>), primers were trimmed using *cutadapt* (Martin, 2011), and reads were merged with *PEAR* (Zhang et al., 2014). The remaining filtering and Operational Taxonomic Unit (OTU) clustering (97% of cut-off threshold) was performed using *vsearch* v2.8.2 (Rognes et al., 2016). At this point, we produced an OTU table. When possible, OTUs were classified until species level using *rjson* (Couture-Beil & Couture-Beil, 2018), *taxize* R packages (Chamberlain & Szöcs, 2013), and a phylogenetic confirmation through *IQ-TREE* (Nguyen et al., 2015)

(see detailed bioinformatic protocol in [Supplementary Information](#)). All samples with <1000 reads were discarded for subsequent analyses. Flowering plant species (OTUs) for which the DNA was detected in our samples were considered to have been visited by the bees and therefore potentially pollinated.

## 2.4 | Flowering plant biodiversity data analysis

At this point, only the wild bee samples with a minimum of 1000 reads were kept for subsequent analyses. Thus, we estimated the completeness of the sampling by computing a rarefaction curve and individual rarefaction curves for each of the environmental variables and species traits studied using R package *vegan* (Oksanen et al., 2013). As another measure of quality, we calculated the number of OTUs detected in each sample and averaged this number at the population level to determine the number of pollinated plants detected per wild bee specimen. We used the multivariate BIO-ENV procedure (Clarke & Ainsworth, 1993), which is a dissimilarity-based method to identify the set of environmental variables that are most correlated with the OTU table, and thus best explain the bee pollination spectrum. The four environmental variables for which we had records were included in the BIO-ENV procedure: flowering period, farming system, percentage of weeds, and height of sunflower.

The flowering plants pollinated by wild bees on sunflower crops were described using two different biodiversity metrics, relative read abundance (RRA) and frequency of occurrence (FOO). RRA refers to the proportion of reads obtained per OTU in each sample, each variable, or for the full dataset; and FOO informs about the number of samples (counts) in which an OTU is present (Deagle et al., 2019). The calculation of both metrics, RRA and FOO, for the whole sampling and each of the selected variables' levels was calculated using customized scripts based on R package *dplyr* (Wickham et al., 2021).

The importance of environmental variables and wild bee species traits in the pollination spectrum was inferred by fitting them onto an unconstrained ordination and applying *envfit* analysis (Oksanen et al., 2013). Alpha diversity (exponential of Shannon index,  $q=1$ ) of pollinated plants was calculated using *hilldiv* R package (Alberdi & Gilbert, 2019). To test the statistical significance of the differences in alpha diversity, we computed a Kruskal–Wallis (K-W) rank-sum test and the post hoc Dunn test in the case of variables with more than two levels, using *stats* (Team et al., 2018) and *dunn.test* (Dinno & Dinno, 2017) R packages, respectively. Beta diversity (community composition) was calculated using an unconstrained ordination through a principal coordinate analysis (PCoA) and a constrained ordination through canonical analysis of principal coordinates (CAP). Both these analyses were based on Bray–Curtis distance matrix to account for differences in abundance, applying the *phyloseq* R package (McMurdie & Holmes, 2012). Statistical significance of both ordinations was tested through a non-parametric multivariate analysis or PERMANOVA (999 iterations) and constrained CAP permutation

test, respectively (Oksanen et al., 2013). For the case of these two permutational analyses, aside from the main effects, the interaction between the environmental variables and the species traits was also tested.

We explored the effects of environmental variables and species traits selected, together with the interaction between them through the computation of a negative binomial generalized linear model (GLM), using a nested model, and applying a log-link function (999 iterations) using *mvabund* R package (Wang et al., 2012).

## 2.5 | Differentially abundant flowering plant

For each of the selected environmental variables and species traits, we identified the differentially abundant flowering plant OTUs by applying a *DESeq* negative binomial Wald test using *DESeq2* R package (Love et al., 2014), that is to say, the OTUs, which show a significantly higher abundance in a particular factor within the environmental variables or species traits. *DESeq2* analysis is a procedure originally designed for differential expression analyses of genes, however, it is widely used in count/abundance data. The identified plant species (OTUs) were considered indicator species of each factor of the environmental variable or species trait and were further analyzed. The significance of differential abundance among levels of variables was tested through Kruskal–Wallis test, and the RRA of significantly abundant species was visualized through a hierarchically clustering (complete linkage method) using the *heatmap* R package (Kolde & Kolde, 2015).

## 2.6 | Plant–wild bee ecological networks

For each species trait (body size and sociality level), we constructed bipartite networks based on the RRA of flowering plant OTUs using the *bipartite* R package (Dormann, 2020). OTUs representing <1% of the reads were discarded from the network to obtain a better visualization. All the biodiversity analyses, *DESeq2* analysis, and bipartite networks were done using R version 3.6.1 (R Core Team, 2019).

# 3 | RESULTS

## 3.1 | Characterization of wild bee communities

We collected 212 wild bees from sunflower crops, which were identified at species level and corresponded to 33 different species (Table S2; Figure S1). The most abundant wild bee species was the social sharp-collared furrow bee (*Lasioglossum malachurum*), while the yellow-legged mining bee (*Andrena flavipes*) was the most abundant among the solitary bees (Figure S1a). The wild bees included in this study belonged to four families (of seven described): Andrenidae, Colletidae, Halictidae, and Megachilidae (Figure S1a),

with the majority belonging to Halictidae. The overall distribution of wild bee families was similar among both flowering periods and farming systems (Figures S1b,c).

Wild bee species richness did not show important differences among the selected environmental variables, flowering period, and farming system. Between the two flowering periods, the Whittaker index (beta diversity) was 0.387 and between the two farming systems 0.36 (values ranged from 0 to 1) (Figures S1b,c).

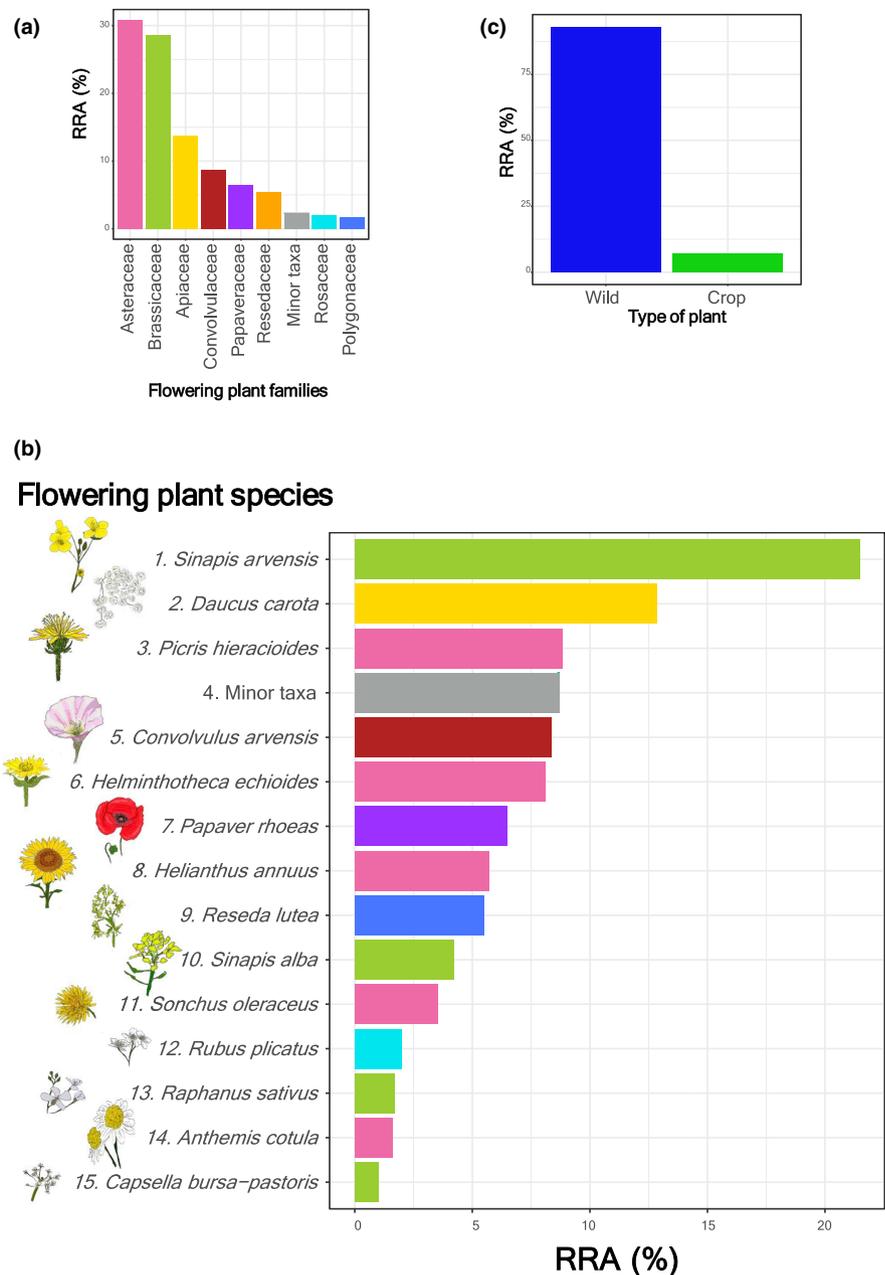
## 3.2 | Description of library quality and wild bee's pollination spectrum

Of the 212 pollen samples, 185 passed the sequencing depth filter (>1000 reads) and produced a total of 15,153,994 reads (i.e., an average of 71,484.1 reads per sample). Reads assigned to sunflower *Helianthus annuus* (10,707 reads, 1.18%) were found in the extraction control and subtracted from all the samples.

According to the rarefaction curves (Figure S2a), the sequencing quality was adequate to study the plants pollinated by wild bees in sunflower crops. All rarefaction curves reached a plateau (Figure S2b) and we detected an estimated 90.53% of the OTUs of plant taxa pollinated by wild bees. The mean number of plant OTUs detected per wild bee sample was 12.12. A total of 269 OTUs were detected, of which 231 were kept for subsequent analyses as they corresponded to flowering plants (class Magnoliopsida) and 150 were identified at species level (Table S2). Of the 38 OTUs discarded, 1 was assigned to a fungus (class Ascomycota), 13 to uncultured eukaryotes, and the 24 OTUs remaining did not reach our quality thresholds (percentage of identity >75% and query coverage >60%).

The flowering plant community was taxonomically classified into 21 orders, 38 families, 108 genera, and 153 different species within the 231 OTUs (Figure 1a,b). Of the 38 families detected, 8 represented more than 1% of the filtered reads and 29 were present in more than 1% of the samples (Figure 1a). Asteraceae (RRA = 30.85%) and Brassicaceae (RRA = 28.64%) were the most abundant families followed by Apiaceae (RRA = 13.78%) and Convolvulaceae (RRA = 8.68%) (Figure 1a). With regards to prevalence, the most common families (highest FOO) were Asteraceae (FOO = 72.29%), Brassicaceae (FOO = 57.58%), Apiaceae (FOO = 53.25%), Plantaginaceae (FOO = 34.63%), and Rosaceae (FOO = 29%) (Table S2). At species level (Figure 1b, Table S2), white mustard *Sinapis arvensis* (RRA = 21.47%) was the most abundant, followed by wild carrot *Daucus carota* (RRA = 12.85%), hawkweed oxtongue *Picris hieracioides* (RRA = 8.84%), and field bindweed *Convolvulus arvensis* (RRA = 8.36%) (Figure S3). In terms of occurrence in samples (Table S2), *D. carota* (FOO = 48.92%) and *S. arvensis* (FOO = 44.16%) were the most common species followed by the stinking chamomile *Anthemis cotula* (FOO = 37.66%) and the common sowthistle *Sonchus oleraceus* (FOO = 28.57%). Globally, 184 OTUs (RRA = 93.1%) corresponded to wild plants while 47 OTUs (RRA = 6.9%) were crop or ornamental plants (Figure 1c).

**FIGURE 1** Relative read abundance (RRA) of the flowering plant community pollinated by wild bees in sunflower crops in the study area of Plaine et Val de Sèvre. (a) assigned until family level (families comprising <1% of reads were grouped in the category “Minor taxa”), (b) assigned until species level (species comprising <1% of reads were grouped in the category “Minor taxa”) and colored by family, and (c) categorized by type of plant (wild or crop plant).



### 3.3 | Environmental variables and species traits driving change in pollination spectrum

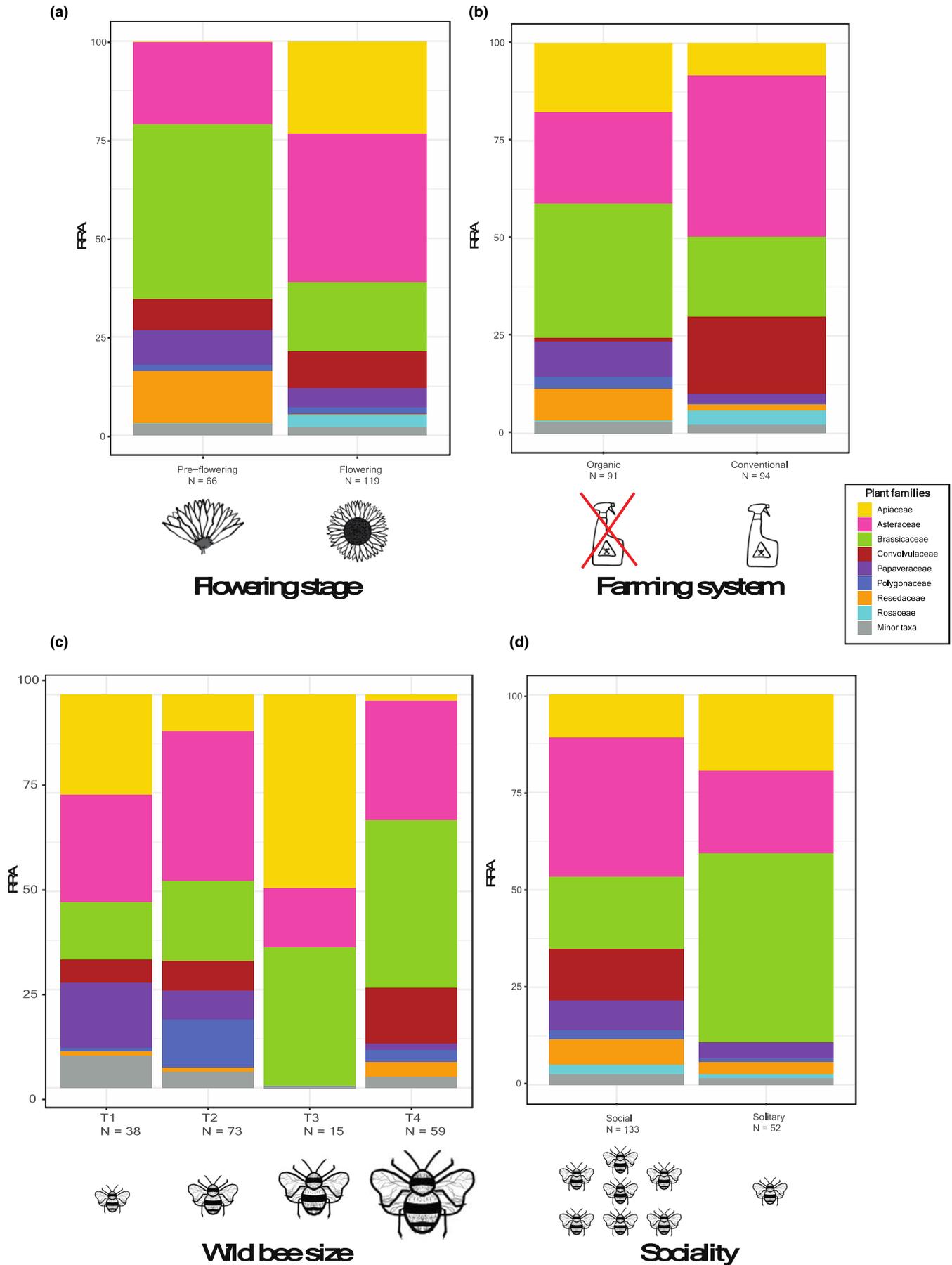
According to the BIO-ENV procedure, the flowering period (pre-flowering vs. flowering) and the farming system (organic vs. conventional) ( $r=0.14$ ) were the environmental variables best correlated with the OTU table. Deeper investigation through *envfit* showed that these two environmental variables as well as the two species traits, body size and sociality level, influenced the pollination spectrum the most ( $\text{prand} < 0.001$ ). According to *envfit* outcome, the most important variable explaining the variation in the pollination spectrum was the size of wild bees ( $R^2=0.218$ ), followed by farming system ( $R^2=0.1469$ ), social behavior ( $R^2=0.0941$ ), and flowering period ( $R^2=0.0744$ ).

In terms of pollinated plant community composition (based on RRA), there were a number of differences between the two

flowering periods and the two farming systems (Figure 2a,b; detailed analysis in Supplementary Information).

With regards to environmental variables, we found a significant difference in alpha diversity between both flowering periods with higher diversity of plants pollinated by bees before flowering of the crop compared to the flowering period (K-W  $\chi^2=4.7447$ ,  $p\text{-value}=0.02939$ ) (Table S4; Figure S4a). In contrast, we did not find significant differences in pollinated plant alpha diversity when comparing the two farming systems (K-W  $\chi^2=0.3358$ ,  $p\text{-value}=0.5623$ ) (Table S4; Figure S4b).

Beta diversity between the two different flowering periods and the farming systems showed not only a separation between the communities of pollinated plants but also a certain degree of overlap in both cases on the PCoA (Figures S4a,b). PERMANOVAs indicated that both the flowering period and the farming system



**FIGURE 2** Stacked bar plots of the taxonomic composition of the flowering plants pollinated by wild bees (RRA) at family level in relation to (a) stages of the crop (pre-flowering vs. flowering), (b) type of agriculture (organic vs. intensive), (c) wild bee size categories (T1–T4), and (d) sociality level (social vs. solitary bees).

had a significant effect on the pollination spectrum of wild bees ( $R^2_{\text{period}}=0.03159$ ,  $\text{prand}<0.001$ ;  $R^2_{\text{farming}}=0.03234$ ,  $\text{prand}<0.001$ ) (Table S4). Deeper constrained investigation of beta diversity among both levels of each environmental variable was visualized through CAP (Figures S6a,b). Permutation test for CAP confirmed that the differences visualized were significant ( $\chi^2_{\text{period}}=0.379$ ,  $\text{prand}<0.001$ ;  $\chi^2_{\text{farming}}=0.3562$ ,  $\text{prand}<0.001$ ) (Table S4).

As for species traits (body size and sociality level), differences in alpha diversity between the four wild bee size categories were not significant according to Kruskal–Wallis test (K-W  $\chi^2=6.58$ ,  $p\text{-value}=0.09$ ). Regarding sociality level, we found significant differences in alpha diversity between social levels (K-W  $\chi^2=3.9292$ ,  $p\text{-value}=0.04745$ ) (Table S4; Figure S4d and Figure S7), and social wild bees showed a more diverse pollination spectrum than the solitary community.

Regarding community composition (beta diversity) of the species traits, body size and sociality level were visualized through the unconstrained PCoA, showing a certain degree of dissimilarity among communities (Figures S5c,d). PERMANOVAs showed a significant effect of, both, the four wild bee body size categories and the two sociality levels on the pollination spectrum of wild bees ( $R^2_{\text{size}}=0.04865$ ,  $\text{prand}<0.001$ ;  $R^2_{\text{social}}=0.01764$ ,  $\text{prand}<0.001$ ) (Table S4).

Further constrained investigation of beta diversity was visualized through CAP (Figure 4c,d). Permutation test for CAP permutation test confirmed the statistical significance of the visualized differences ( $\chi^2_{\text{size}}=0.7709$ ,  $\text{prand}<0.001$ ;  $\chi^2_{\text{social}}=0.2243$ ,  $\text{prand}<0.001$ ) (Table S4). In the case of the wild bee size, CAP shows an overlap in community composition with the biggest separation between T1 and T3 (Figure S6d). In the case of bee sociality level (Figure S6c), we detected not only a clear separation between social and solitary bees but also a certain degree of overlap between both sociality levels. The interaction between the two environmental variables and the two species traits was significant in the case of PERMANOVA ( $R^2_{\text{interaction}}=0.2717$ ,  $\text{prand}<0.001$ ) and CAP permutational analysis ( $\chi^2_{\text{interaction}}=4.6465$ ,  $\text{prand}<0.001$ ) (Table S4).

Effects of each environmental variable and species trait (main effects) and interaction on the pollination spectrum of wild bees showed up to be significant according to GLM (Dev<sub>period</sub>=797.1,  $\text{prand}<0.001$ ; Dev<sub>farming</sub>=746.3,  $\text{prand}<0.001$ ; Dev<sub>size</sub>=1235,  $\text{prand}<0.001$ ; Dev<sub>social</sub>=436.8,  $\text{prand}<0.002$ ; Dev<sub>interaction</sub>=4924,  $\text{prand}<0.001$ ) (Table S4).

### 3.4 | Flowering plant species as indicators of environmental conditions and wild bee traits

DESeq2 and K-W detected 15 plant species (25 OTUs), which were differentially abundant in relation to environmental conditions or wild bee species traits (Table S3). Among these 15 plant species, those showing the highest RRA for a specific environmental condition or wild bee species trait are considered indicator species. *Reseda lutea*

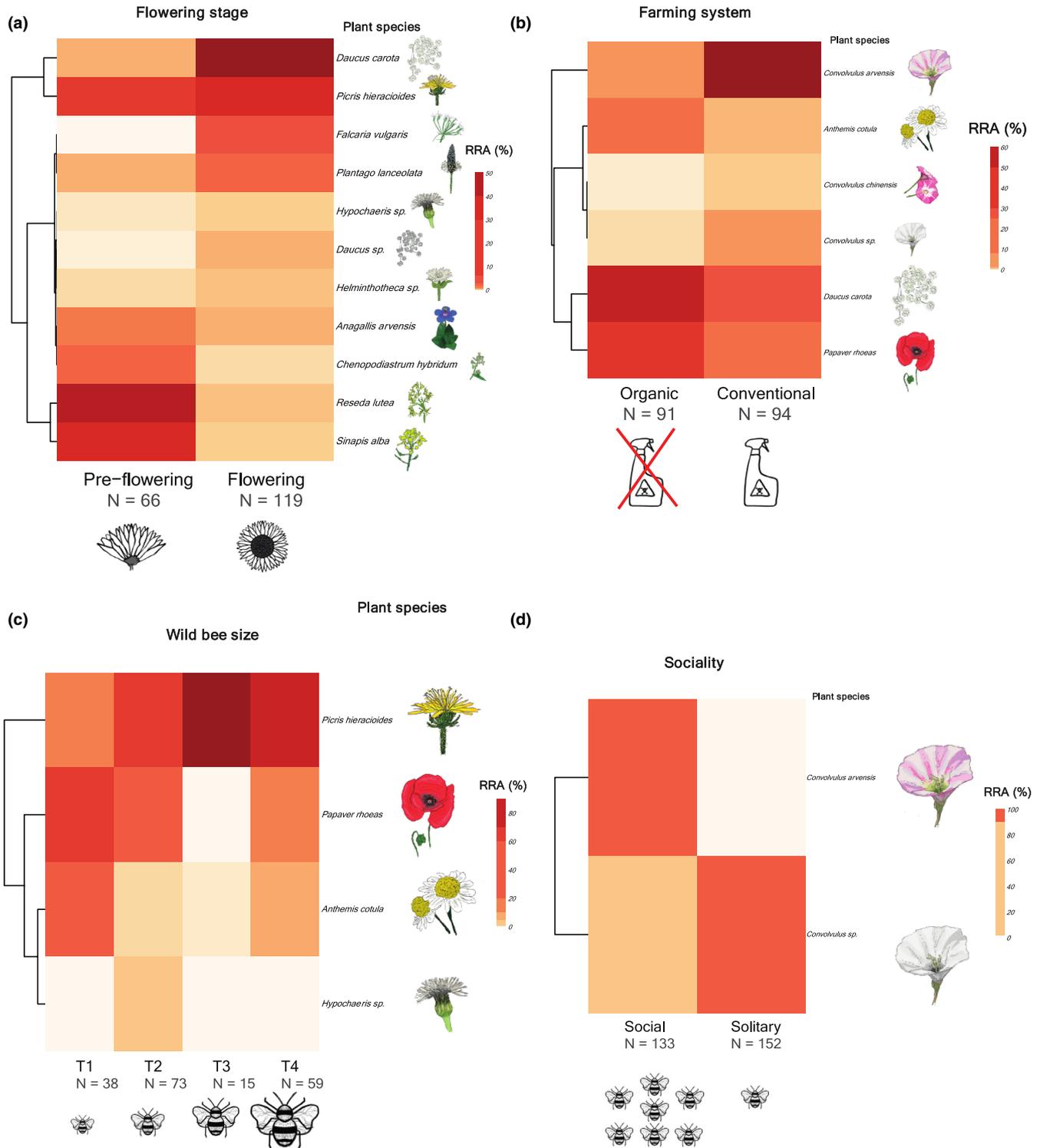
was an indicator species during pre-flowering while *D. carota* was an indicator during flowering of sunflowers (Figure 3a). Concerning the farming system (Figure 3b), *D. carota* was also an indicator of organic crops while *C. arvensis* turned out to be an indicator of conventional crops. Among the wild bee size categories (Figure 3c), the common poppy *Papaver rhoeas* was preferred by small bees (T1 category), while T2 bees preferred *Hypochoeris sp.* and T3 *P. hieracioides*. No plant indicator species was detected for the large wild bee category T4. As for social levels (Figure 3d), social bees preferred *C. arvensis* while *Convolvulus sp.* was preferred by solitary bees.

### 3.5 | Plant–pollinator ecological networks

Bipartite ecological networks showed wide overlap in the pollination spectrum of solitary and social bees (Figure 4a). Five plant species, however, were exclusively pollinated by social bees (*Cirsium arvense*, *Crepis capillaris*, *Polygonum boreale*, *C. arvensis*, and *A. cotula*), while only one plant species (*Capsella bursa-pastoris*) was exclusively pollinated by solitary bees. With regards to bee body size (Figure 4b), eight plant species were exclusively pollinated by one bee size category (*A. cotula*, *Lactuca sativa*, *C. capillaris*, and *P. boreale* were pollinated only by T1 bees; *P. lanceolata*, *F. vulgaris*, and *C. arvense* were pollinated only by T2 bees; and *C. bursa-pastoris* was pollinated only by T3 bees). In contrast, six species of plants provided resources to all four different size categories of bees, these plants included the main crop plant (*H. annuus*) as well as common weeds (*P. hieracioides*, *S. oleraceus*, *D. carota*, *S. alba*, and *S. arvensis*).

## 4 | DISCUSSION

We analyzed plant species composition from pollen collected by wild bees that were captured in sunflower crops. Aside from characterizing the pollination spectrum of wild bee communities, this study revealed that wild plants are key for the survival of wild bees even within an intensive agroecosystem and even during sunflower blooming period, as they show an overall preference for wild floral resources. Moreover, despite the overlap in floral resources used by different species of wild bees, we found clear differences in the choice or availability of plant taxa in relation to several environmental variables, such as flowering period and farming system. In addition, the size of wild bees and their sociality appears to have an influence on their pollination spectrum in sunflower crops, together with the interaction of the mixed effect between environmental variables and species traits. However, because we worked at community level, there are different sampling sizes between the factors of environmental variables and species traits. Therefore, these results should be taken with caution as these sampling biases inherent to the type of study could have an impact, especially in the case of alpha diversity.



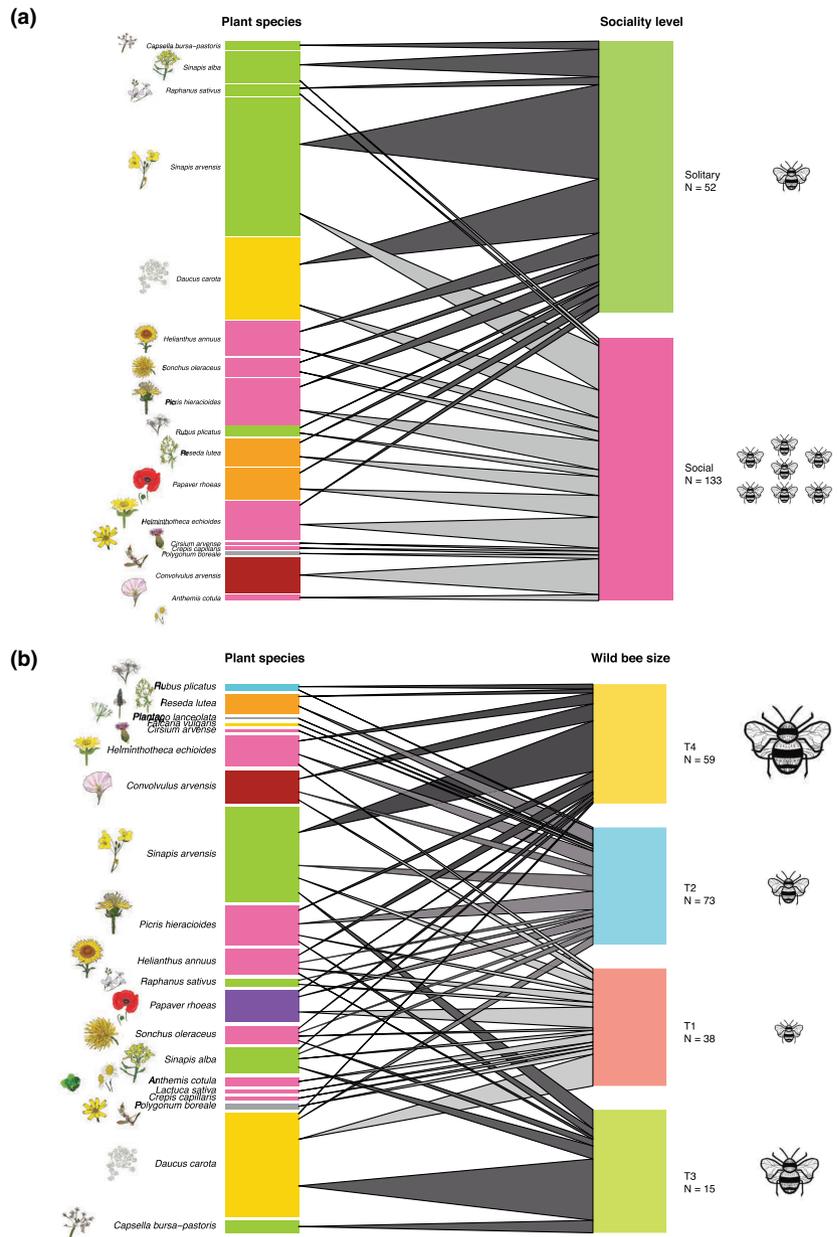
**FIGURE 3** Clustered heatmap of RRA from differentially abundant OTUs clustered by species according to *DESeq2* analysis and Kruskal-Wallis test in relation to (a) stages of the crop (pre-flowering vs. flowering), (b) type of agriculture (organic vs. intensive), (c) wild bee size categories (T1–T4), and (d) sociality level (social vs. solitary bees).

#### 4.1 | Pollen DNA metabarcoding as an efficient tool to describe pollinated plant communities

ITS2 DNA metabarcoding has been used with success to understand trophic interactions in agricultural landscapes (Batuecas et al., 2022;

Bell et al., 2017). Here, pollen DNA metabarcoding has allowed us to identify plant species visited, and therefore potentially pollinated, by wild bees. This method has enabled us to not only directly detect and identify to species level a high percentage (69%) of the pollination spectrum of the collected wild bees but also relate this pollination

**FIGURE 4** Ecological bipartite network showing the interactions between species of pollinated plants (>1% of reads) and wild bee communities clustered and classified by (a) sociality level and (b) wild bee body size.



spectrum to environmental variables and wild bee species traits. Such accuracy would be challenging to reach using pollination observational studies (Arizaga et al., 2000; Cordeiro et al., 2017; dos Santos & Wittmann, 2000; Valido et al., 2019), as these studies are plant based and produce information only from one plant at each observation. In contrast, our metabarcoding analysis led to the identification of an average of more than 10 plant species per wild bee, which means that, as suggested by previous studies (Evans & Kitson, 2020; Pornon et al., 2017), we are discovering additional hidden information compared to conventional methods. Even though, metabarcoding approaches are not free of biases. The confirmation that our pollen load DNA metabarcoding approach is able to recover such a high-quantity and high-quality information about plant–pollination interactions opens a promising avenue of research as this method requires less effort and less botanical expertise than traditional observation methodologies.

## 4.2 | Weeds as essential floral resources for wild bees

Our study illustrates that, even if flowering crops within intensive agricultural landscapes can provide floral resources to pollinators (Garibaldi et al., 2014), these are usually transient resources, which contribute to an increase in pollination rates only during reduced periods of time (Diekötter et al., 2010). Thus, crop plants do not always overlap with pollination season and many wild pollinators not only need enough number of flowering plants to pollinate but also a continuity in the availability of floral resources across a prolonged period (Ganser et al., 2018). For instance, sunflower blooming stage lasts around 4 weeks per year (Minckley et al., 1994), which is a short period to fulfill the requirements of wild bee communities. In contrast, the presence of weeds inside or on the edges of the crops, which are more diverse in terms of flowering periods,

sizes, and shapes, increases pollination rates of wild bees (Biesmeijer et al., 2006; Forup & Memmott, 2005; Nichols et al., 2019; Rollin et al., 2016). This goes in line with our study showing that flowering plants pollinated by wild bees are in their majority weeds (93.1%). In our study system, *S. arvensis* and *D. carota* were the most abundantly pollinated floral resource. In line with our results, previous studies have already indicated that patches of wild vegetation in between the crops provide essential pollen resources when crops are not in flower (Carvalho et al., 2012; Ganser et al., 2018; Kells et al., 2001; Korpela et al., 2013; Morandin & Kremen, 2013; Robson, 2014). In fact, declines in pollinators are known to be linked to local extinctions of wild plants (Biesmeijer et al., 2006). It is worth remarking that sunflower *H. annuus* turned out to be neither very abundant (5.7% reads) nor very common (9.1% FOO) floral resource within our study area. Sunflowers produce abundant pollen (Neff & Simpson, 1981), which is easily available to certain groups of pollinators due to the morphology of its flowers. However, this pollen is lower quality (low protein content) than that of many wild flowering plants (Nicolson & Human, 2013), such as *P. rhoeas*, which is among the 10 most pollinated plants by our wild bee communities (Hanley et al., 2008). Therefore, a diet relying heavily on sunflower pollen could have detrimental effects on wild bee fitness if it is not complemented with other pollen types (Nicolson & Human, 2013). Our ITS2 metabarcoding approach has successfully enabled us to precisely identify the highly diverse floral requirements of wild bees, supporting the idea that an abundant and diverse community of weeds makes sunflower crops a higher-quality habitat for wild bees. Hence, wild bees may be able to enhance agricultural production if the non-crop flowering plants around the agroecosystem are diverse enough to cover their feeding requirements (Morandin & Winston, 2005).

### 4.3 | Effects of flowering stage and farming system on the composition and diversity of the pollination spectrum of wild bees

Our metabarcoding data illustrate how environmental variables may have a strong influence on the selection of floral resources by wild bees. However, it also points out that the biodiversity metrics used, RRA and FOO, were not always perfectly congruent. For instance, the common sowthistle *Sonchus oleraceus* was the most visited plant during pre-flowering but not the most abundant species in terms of sequences. This is consistent with the fact that *S. oleraceus* produces low quantity of pollen during a short period (Percival, 1955; St John-Sweeting, 2011). Therefore, even if the flower is available in sunflower fields, wild bees may only collect small quantities of its pollen. This brings to light the need to consider not only the strong points of each metric but also their potential biases and assumptions (Deagle et al., 2019). While RRA provides a semi-quantitative measure of relative biomass (Elbrecht & Leese, 2015; McClatchie & Dunford, 2003), which could be a useful proxy of pollen biomass if treated with caution, FOO provides information about the low or high presence of the floral resource (Alberdi et al., 2018; Deagle

et al., 2019). On one hand, RRA has shown a higher variance among laboratory and bioinformatic protocols used (Alberdi et al., 2018; Deagle et al., 2019) but it is more accurate and descriptive than FOO. On the other hand, FOO is more consistent, but the information it provides is less precise and this metric gives importance to rare taxa (Deagle et al., 2019). Combining RRA and FOO, after a robust laboratory and bioinformatic protocol (Alberdi et al., 2018), is potentially the best approach to accurately describe and monitor ecological communities (Deagle et al., 2019). In our study, both biodiversity metrics help in providing a global picture of the pollination spectrum of wild bees in sunflower crops.

Seasonal climatic variability is known to drive changes in pollination spectrum (Andersen, 1980; Hirao et al., 2006). Our results showed seasonal differences in the taxa of floral resources pollinated by wild bees when comparing pre-flowering and flowering seasons, which may be partly due to the phenology of flowering plants in our study area. In fact, we detected 11 plant species whose abundance in pollen loads varies significantly between both flowering periods. These plant species could be considered as indicators of a certain environmental condition, like, for example, *D. carota* and *Daucus sp.* during sunflower flowering stage. Pollination niche breadth was significantly higher during sunflower pre-flowering season compared to the flowering season. Despite the imbalance in sample size ( $N_{\text{pre-flowering}} = 66$ ,  $N_{\text{flowering}} = 119$ ), a potential explanation could be the higher percentage of weeds flowering during the pre-flowering season. Indeed, more than half of the fields (54.6%) showed high density of weeds (>75% ground cover), while during flowering 63% of the fields had low density of weeds (<25% ground cover). Thus, we observed a congruence between the percentage of weeds covering the crop ground and the number of reads (RRA) detected in the pollen loads. Consistently, it has been reported that in more diverse environments, bees, especially social bees, not only increase the number of visitations of flowering plants (Ebeling et al., 2008) but also their intake of pollen (Kaluza et al., 2017, 2018). This goes in line with our results, which support the need of making weeds available for wild bees. Enhancing the biodiversity of weeds would have a positive effect not only on wild bee fitness and social bee colony survival (Kaluza et al., 2017, 2018) but also on the productivity of pollinator-dependent crops, such as the sunflower (Morandin & Winston, 2005).

Although the negative effects of agrochemicals on plant biodiversity have been widely reported (Aktar et al., 2009; Brühl & Zaller, 2019), our comparison of plant species diversity between organic and conventional crops shows no statistical effect of the farming system. Even if the lack of agrochemicals is known to increase wild bee diversity (Winfree, 2010), there may other factors influencing the number of plants available for the pollinators, such as, landscape complexity, which has been known to have a positive effect on pollinator-plant interactions (Power et al., 2012). Therefore, the landscape homogeneity occurring in intensive agricultural areas, such as LTSEZ ZA Plaine & Val de Sèvre, would be affecting the pollination spectrum in both organic and conventional crops. In contrast, there is a compositional shift in terms of floral resources, as wild

bees prefer different flowering plants depending on the farming system within the crop. For instance, *C. arvensis* is the most pollinated species in terms of abundance and an indicator plant species within conventional crops. Interestingly, this weed has been reported to be naturally tolerant to glyphosate (Huang et al., 2019), which is a widely used and non-selective herbicide (Bradshaw et al., 1997). Hence, weeds more pollinated within intensive crops may be resistant species to certain agrochemicals or the ones present only in organic fields could be more vulnerable to the use of agrochemicals. The differences in pollination spectrum could also be explained by the differences in wild bee communities inhabiting both types of crops. For instance, social bees were more often collected in conventional crops (79.8%) compared to organic crops (63.3%).

#### 4.4 | Body size and sociality as modulators of pollination spectrum in wild bees

Our findings highlighted a clear relation between wild bee body size on the pollination spectrum, which is consistent with previous studies. However, according to our ecological network, wild bees of different sizes could pollinate similar taxa albeit with different intensities. This suggests that, even if wild bee body size had an effect, it would be in combination with other factors, such as social level as indicated the significant interactions of PERMANOVA, CAP permutational test, and GLM. Consistently, we found that in T1, most wild bee species are social. In contrast, T3 shows the lowest diversity, almost the same number of bee species (6), and more than 75% of wild bee species are solitary (Table S1). This could be an effect of the low sample size of T3 ( $N=15$ ) compared to the other categories. However, this low diversity level makes sense as solitary bees tend to be more specialists (Ebeling et al., 2008; Kaluza et al., 2017, 2018). As shown in Figure S7, when comparing the most pollinated floral resources of the most abundant social wild bee *L. malachurum* with the most abundant solitary bee *A. flavipes*, we confirm the higher specialization of solitary bees compared to social bees as 67% of the pollination spectrum of *A. flavipes* was a single species, *S. arvensis*, while *L. malachurum* visited a higher number of plant species as shown by a more balanced percentage of read abundance. Moreover, this sheds light on the fact that social bees are capable of foraging in different types of floral landscapes and thus may be more resistant to changes in flowering community composition. In contrast, certain solitary bees may depend on one or few specific plant species whose absence could lead to local extinctions of these bee species. There is, thus, an urge to manage agroecosystems in a way that they provide resources not only for social bees but also for solitary bees (Ebeling et al., 2008; Kaluza et al., 2017, 2018). This pollen metabarcoding study has enabled us to draw a broad picture of plant-wild bee interactions in an agroecosystem. Although our findings suggest some level of redundancy among the community of wild bees, several specific interactions illustrate the need for increased weed diversity in agricultural landscapes to conserve a diverse community of wild bees.

## 5 | CONCLUSION

Using an pollen eDNA approach, we characterized the pollination spectrum and shed light on the interactions among wild bees, pollinated plants, environmental variables, and wild bee species traits in sunflower crops. Despite a somewhat imbalanced sampling, our study confirms that metabarcoding is an efficient tool to describe ecological interactions at the community level. Moreover, our study has brought to light hidden information about pollinated plants, which may not be available by conventional observational studies of visitation rates (Evans & Kitson, 2020; Pornon et al., 2017). In line with previous studies (Biesmeijer et al., 2006; Forup & Memmott, 2005; Nichols et al., 2019; Rollin et al., 2016), our work confirms the importance of weeds diversity for the conservation of wild bees in agricultural landscapes. The crop flowering stage and the farming system were shown to be strong drivers of change in the composition of plants pollinated by wild bees. The effect of crop flowering stage might be related to the seasonal climatic variability and the differences in the percentage of weeds available (Andersen, 1980; Hirao et al., 2006). In fact, the diversity of weeds available has been suggested to affect fitness and survival of wild bees (Kaluza et al., 2017, 2018). Although the composition of the community of pollinated plants varies according to the farming system, the diversity of plants pollinated is similar in organic and conventional fields. This may be due to the landscape homogenization within intensive agricultural areas (Power et al., 2012), such as in this case in the LTSER Zone Atelier Plaine & Val de Sèvre. Wild bee species traits, specifically body size and sociality, also impact the pollination spectrum, confirming results reported in previous studies (Ebeling et al., 2008; Kaluza et al., 2017, 2018). This work emphasizes the potential of eDNA to decipher plant-insect interactions and stresses the need to maintain weeds in intensive agroecosystems to conserve diverse communities of social and solitary wild bees in the context of global insect declines.

### AUTHOR CONTRIBUTIONS

Stéphane Boyer obtained the funding; Stéphane Boyer and Marina Querejeta designed the research; Vincent Bretagnolle and Sabrina Gaba provided the geographical and environmental data as well as logistic support for the fieldwork; Marilyn Roncoroni identified the wild bee specimens; Stéphane Boyer, Paul Pfeiffer, Lorène Marchal, and Marina Querejeta performed the fieldwork; Paul Pfeiffer and Marina Querejeta carried out the laboratory work; Marina Querejeta performed the statistical analysis; Stéphane Boyer and Marina Querejeta analyzed the data and wrote the manuscript; and all authors contributed to revision of drafts and approved the final manuscript.

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### CONFLICT OF INTEREST STATEMENT

The authors of this study have no conflicts of interest to disclose.

### DATA AVAILABILITY STATEMENT

The raw dataset generated and analyzed during the current study has been submitted to the NCBI Sequence Read Archive (SRA) under the BioProject PRJNA874523.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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