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The neonicotinoid Acetamiprid alters the chemical profile of the primitive eusocial bee *Lasioglossum malachurum*

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ABSTRACT

The widespread use of agrochemicals, particularly neonicotinoids, poses a significant threat to the health of (pollinating) insects. Various health traits are affected, but the impact on the chemical communication of wild bees remains a poorly studied aspect. Here, we assessed how field-realistic exposure to the 'honeybee-safe' neonicotinoid Acetamiprid affects the behaviour, cuticular lipids and microbiome of *Lasioglossum malachurum*, a small ground-nesting sweat bee. *L. malachurum* is an important, abundant pollinator of several crop plants with primitive social behaviour which relies on cuticular lipids for communication. We collected bees in the field for a controlled pesticide treatment in the lab. Pesticide-treated individuals increased their sugar-water consumption rate compared to the control group. After 7 days of experiment, the treatment group showed a trend towards less developed ovaries and an increased amount of odour with significantly altered queen pheromones. While the microbiome was not affected by the treatment, a comparison with field individuals showed an erosion of their gut microbiome with a reduction in *Apilactobacillus* during laboratory keeping. Our findings indicate that neonicotinoids may disturb chemical communication in *L. malachurum* and thus might impair social behaviour. This raises concerns about the threats of currently approved pesticides to wild pollinators.

1. Background

Solitary and social bees are the most important pollinators worldwide of cultivated (Klein et al., 2007) and wild plants (Ollerton et al., 2011). However, wild bees are severely affected by species and population decline, with more than 50 % of species critically endangered in Germany (Westrich et al., 2011). Similar or even higher threats have been reported from the Alps and Eastern Europe (Nieto et al., 2014) and are expected as declines worldwide. Although the reasons for pollinator decline are manifold, the intensification of agricultural land-use is one of the major factors, causing a general loss of valuable habitat and food resources (Wagner, 2020; Goulson et al., 2015; Millard et al., 2021; LeBuhn and Vargas Luna, 2021; Brunet and Fragoso, 2024). Within this context, the use of pesticides can directly influence foraging behaviour or learning abilities and lastly bee health (Blacquière et al., 2012), and has been linked to pollinator decline since the turn of the millennium (Sponsler et al., 2019).

The influence of pesticides on pollinators can be very diverse; from loss of food resources in case of herbicides (Goulson et al., 2008); to direct effects of fungicides and insecticides on non-target organisms

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(Degrandi-Hoffman et al., 2015; Pisa et al., 2015). Among these, the effects of insecticides are particular problematic because their impacts are manifold: These are physiological problems and direct damages to health (Dworzańska et al., 2020; Straub et al., 2023, 2021), but also influence on reproduction (Camp et al., 2020a; Bernauer et al., 2015; Birkenbach et al., 2024) and changes in learning ability and behaviour (Iwasa et al., 2004; El Hassani et al., 2008; Lambin et al., 2001). Even the exposure to sub-lethal amounts of pesticides influenced wild bee behaviour or odour profiles (Straub et al., 2023; Boff and Ayasse, 2024). Neonicotinoids pose a particular high risk to various insects (Mamy et al., 2025; Godfray et al., 2015) because they persist in nectar and pollen (Godfray et al., 2015; Lundin et al., 2015) and target the nervous system as neurotoxins (Dworzańska et al., 2020), binding highly effectively to the acetylcholine receptors of insects (Camp et al., 2020a; Wang et al., 2018). Besides this, neonicotinoids have multiple cytotoxic side effects that can even be a risk to vertebrates (Wang et al., 2018; Xu et al., 2022). This makes neonicotinoids a major threat to insect diversity (Mamy et al., 2025) and even to non-target animals, such as amphibians (Wan et al., 2025).

So far, effects of neonicotinoid exposure have been mainly studied in a few, large species, such as honeybees, bumblebees and mason bees (Straub et al., 2023; Alkassab and Kirchner, 2017). Transferring these results to other species is difficult, as wild bees differ in size and lifestyle, and therefore react very differently to pesticides (Iwasa et al., 2004; El Hassani et al., 2008; Godfray et al., 2015; Whitehorn et al., 2012; Botías et al., 2017). In particular, the effects on ground-nesting wild bees have hardly been investigated to date, although 75 % of wild bee species are ground-nesting (Antoine and Forrest, 2021). Moreover, wild bees show differences in their social behaviour. For example, communication as a basic requirement for social behaviour varies greatly from species to species (Leonhardt et al., 2016). Nevertheless, little is known about the effects of neonicotinoids on the chemical communication of wild bees (Tappert et al., 2017; Hopwood et al., 2016). Pheromones play an important role in the communication of solitary and social bees (Ayasse, 1991; Wittwer et al., 2017; Ayasse and Jarau, 2014; Ayasse et al., 2001). Among these, cuticular hydrocarbons enable communication on the one hand and provide desiccation protection on the other (Chung and Carroll, 2015). Pesticides have been reported to affect the cuticular hydrocarbons of wild bees (Straub et al., 2023; Boff et al., 2022) and leaf beetles (Müller et al., 2017). Neonicotinoids in particular can cause behavioural changes upon contact among conspecifics, as has been shown in parasitoid wasps (Tappert et al., 2017), honeybees (Schuehly et al., 2021) or stink bugs (Sessa et al., 2021). Although the use of neonicotinoids has been severely restricted in the EU since 2018 (Blake, 2018), the first generation neonicotinoid Acetamiprid is the only neonicotinoid that is still authorised in the EU until 2033, as it is considered safe for bees (Lewis et al., 2016). But when its approval as a pesticide was renewed in the EU in 2018, risk assessment based mainly on the impact on human health, with no further consideration of insect pollinators (Hernandez-Jerez et al., 2024). However, even sublethal dosages of Acetamiprid can have negative effects on the behaviour or colony development of honeybees and bumblebees (Straub et al., 2023; El Hassani et al., 2008; Shi et al., 2019, 2020; Camp et al., 2020b).

Several studies have also investigated the influence of pesticide exposure on the microbiome of honeybees and bumblebees, since it is an important factor for bee health (Wang et al., 2022; Daisley et al., 2022; Zhang et al., 2022; Cuesta-Maté et al., 2021; Hotchkiss et al., 2022). While social maintenance allows the honeybee to compensate for changes in the gut microbiota to a certain degree, the microbiome of wild bees is more vulnerable to environmental changes (Voulgari-Kokota et al., 2019a; Nguyen and Rehan, 2023). The majority of wild bee species are ground nesting (Antoine and Forrest, 2021), yet their gut microbiota have been only little investigated.

Here, we test the effect of Acetamiprid on a small ground-nesting sweat bee, *Lasioglossum malachurum* of the family Halictidae, which is common and an important pollinator species (Westrich, 2019). *L. malachurum* is considered an obligate primitive eusocial species with effective division of labour by communication via queen pheromones where the ovarian activity signal of the queen becomes an honest queen signal (Leonhardt et al., 2016; Soro et al., 2009; Garibaldi et al., 2014). In sweat bees, macrocyclic lactones function as queen pheromones by suppressing the ovary development (primer function) and by inducing submissive behaviour of workers (releaser function) (Steitz and Ayasse, 2020). This makes *L. malachurum* a good model for ground-nesting bees to investigate the effects of pesticides on the chemical communication. Furthermore, Acetamiprid is widely used in oil-seed rape which is also used as pollen resource by *L. malachurum* (Polidori et al., 2010; Rollin et al., 2015). We therefore test the non-exclusive hypotheses:

Hypothesis 1. Does Acetamiprid exposure change the composition of cuticular lipids in *L. malachurum*?

Hypothesis 2. Does Acetamiprid exposure influence the development of ovaries of *L. malachurum*?

Hypothesis 3. Does Acetamiprid exposure change the composition of the gut microbiome of *L. malachurum*?

2. Methods

2.1. Field collection

Lasioglossum malachurum workers were collected at a nest aggregation between mid-July 2021, timed with the emergence of the workers, near Ulm in Reichenbach im Täle, district of Göppingen, Germany. All nests were numbered before collection to determine their colony affiliation. When emerging in the morning, the workers were collected in plastic vials that were placed over the entrances of the nests. In addition, workers were collected with a vacuum exhauster out of their nests, as described in (Soro et al., 2009). In total, 60 bees were captured from 16 colonies. Individual bees were put into Eppendorf cups, cooled during transport in a cooling bag and transferred alive to the laboratory setup within the same day. Additionally, six workers were directly frozen to compare the gut microbiome under field conditions. These samples were supplemented with 14 additional individuals from the field collected in 2023 from the same location.

2.2. Laboratory setup and treatments

The colonies were divided into two test groups of ten microcolonies each, with three workers per microcolony. One group was fed with a sugar solution containing the neonicotinoid Acetamiprid (IUPAC: (*E*)-N^1-[(6-Chlor-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidin, Formula: $C_{10}H_{11}ClN_4$). The control group was fed with sugar solution only (760 ml API-Invert®, Südzucker AG, Ochsenfurt, Germany; 240 ml water; 3 g potassium sorbate, VWR Chemicals; 1 g citric acid, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) via a glass capillary (length 125 mm, capacity 100 µl, Brand GmbH & Co. KG, Wertheim, Germany), which was replaced daily. A field-realistic Acetamiprid concentration of 5 ng/g was used (Pohorecka et al., 2012) with a standard Acetamiprid solution in water of 100 µg/ml, PESTANAL®, CAS-Number: 160430–64-8, Sigma-Aldrich, Taufkirchen, Germany), diluted with the sugar solution. The bees were allowed to drink *ad libitum* and the capillaries were weighed before and after 24 h to measure the consumption rate/day. The laboratory colonies were kept in artificial nests, which consisted of a 20 cm long acrylic tube with an internal diameter of 6 mm and were modelled on natural *L. malachurum* nests, following (Steitz and Ayasse, 2020). The nests were kept in the dark at all times, or for approximately one hour daily for handling in red light. The temperature was kept constantly at 25°C and the relative humidity at 55 %. After 7 days, the experiments were finished, and the bees were freeze-killed at −20°C. Before the end of the experiment, eight individuals from the control group and eleven from the Acetamiprid group

have died and were removed from further analysis.

2.3. Sample processing and dissections

Before dissections, the bees were washed in 200 µl pentane for 15 s to extract cuticular lipids for chemical analyses. After washing, the bees were dissected to obtain guts and ovaries. The guts were pulled out with the stinger and stored in DNA/RNA Shield (Zymo Research) at −20°C for subsequent microbiome analyses. Furthermore, one wing was cut off with microscissors and cubital cells measured as proxy for body size to account for a size effect on the amount of surface extracts. However, body size had no effect on the amount of cuticular lipids and was therefore not accounted for in further analyses. Lastly, the tergites were removed and the development of the ovaries was categorised into three categories according to (Duchateau and Velthuis, 1989). Category one describes ovaries whose ovarioles are empty and undeveloped. In category two, ovaries were categorised in which ovarian development had already begun but no mature oocytes were present. Ovaries with fully developed eggs were categorised in category three (Fig. 1 B).

2.4. Chemical analysis of cuticular lipids

The cuticular lipids were concentrated to a volume of 50 µl using a gentle stream of nitrogen. For quantitative analysis of the extracts, 10 µl n-octadecane (standard solution 99.9 %; 100 µg/ml) was added to the samples as an internal standard. At 50°C oven temperature, 1 µl of each sample was injected splitless into an Agilent 7890B Gas Chromatograph (Agilent Technologies, Waldbronn, Germany) with a DB-5 capillary column (30 m × 0.25 mm ID) and a flame ionisation detector (FID). The carrier gas was hydrogen with a constant flow of 2 ml × min^{-1}. After one minute, the splitter was opened and the temperature was increased by 10°C × min^{-1} until the final temperature of 310°C was reached, which was maintained for 23 min. The peaks in the chromatograms were identified with reference substances and comparisons of previous work (Ayasse, 1991; Steitz et al., 2019). Therefore, we performed GC runs with synthetic mixtures of already identified compounds and compared them with the GCs of the cuticular lipid samples. Since the same compounds elute on the same chemical column (DB-5) always in the same order they can be identified by superimposing gas chromatograms. The absolute amounts and relative proportions of all identified substances were determined by using Agilent ChemStation Software (Agilent Technologies, Germany) and the internal standard as a reference.

2.5. PCR and library preparation for gut microbiome analysis

DNA of the gut samples was isolated using ZymoBIOMICS 96 DNA Kits (Zymo Research) with a bead beating step at 3200 rpm for 15 min. As positive control the ZymoBIOMICS Microbial Community Standard (Zymo Research) was included. PCR amplification of the V4 region of the 16S rRNA gene was performed using the Phusion Plus PCR Master Mix (Thermo Scientific). Cycling conditions were as follows: 30 sec at 98°C, 30 cycles with 10 sec at 98°C, 10 sec at 55°C, 30 sec at 72°C and a final extension step for 5 min at 72°C. We followed a dual-indexing approach as done by (Kozich et al., 2013) with PCR amplification in triplicates (3 × 10 µl) as outlined by (Sickel et al., 2015). Barcoded primers contained Illumina adapters, indexing sequence, pad sequence and a linker, as well as specific primer based on 515 f (GTGCCAGCMGCCGCGGTAA) and 806r (GGACTACHVGGGTWTCTAAT) (Caporaso et al., 2011). PCR amplification was checked on a 1 % Agarose Gel with SYBR Safe using the E-Gel Power Snap Plus Electrophoresis System (Thermo Fisher Scientific). PCR amplicons were normalized with SequalPrep Normalisation Plates (Invitrogen) before pooling. Correct fragment sizes of the libraries were checked on a 2100 Bioanalyzer instrument using a High Sensitivity DNA Chip (Agilent Technologies). Concentration of each plate pool was quantified on a Qubit 4 Fluorometer using the 1 ×dsDNA HS Assay (Thermo Fisher Scientific) and pooled equimolarly to 2 nM final concentration. Sequencing was performed on an Illumina MiSeq platform at the Genomics Service Unit of the LMU Biocenter using Illumina Reagent Kits v2 (2 × 250 paired-end sequencing).

2.6. Sequence processing and bioinformatics

Sequencing data was processed using the metabarcoding pipeline available at https://github.com/chiras/metabarcoding_pipeline (Leonhardt et al., 2022). Paired ends of forward and reverse reads were joined using VSEARCH v2.14.2 (Rognes et al., 2016). A quality filtering step (EE < 1) was included as described by (Edgar and Flyvbjerg, 2015) and all reads shorter than 170 bp as well as singletons removed. Amplicon sequencing variants (ASVs) were defined using VSEARCH (Rognes et al., 2016). *Denovo* chimera filtering of ASVs was done with UCHIME3 (Edgar, 2016a). Final ASVs were mapped against the RDP (v18), Greengenes (v13.5) and SILVA (v123) reference databases using a global alignment identity threshold of 97 %. All remaining reads without taxonomic allocation were hierarchical classified using SINTAX (Edgar, 2016b) using a cut-off threshold of 0.9 against the RDP (v18)

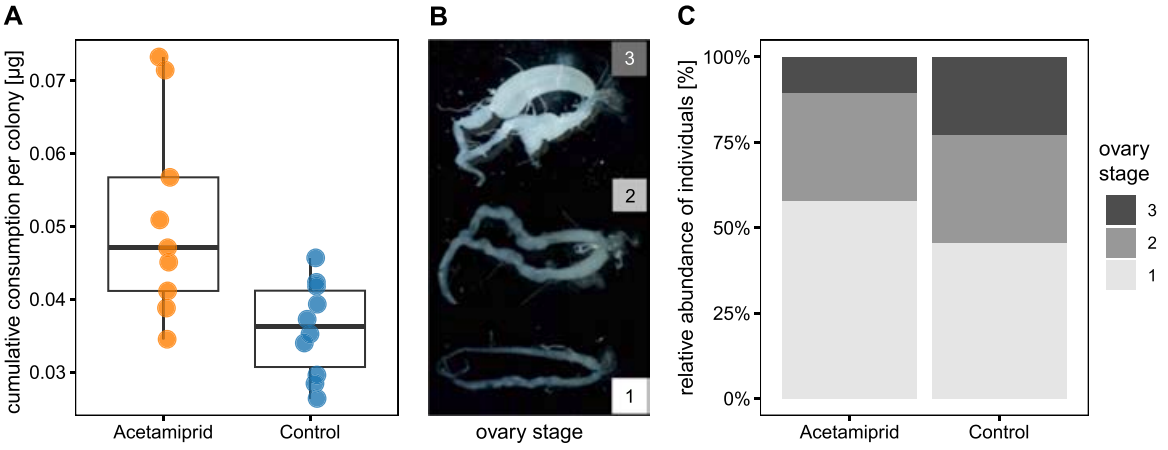

Fig. 1. Sugar water consumption and ovary development due to Acetamiprid treatment in the halictid bee *Lasioglossum malachurum*. A: Cumulative consumption of sugar water was higher when containing Acetamiprid compared to the control solution. n = 10 colonies per treatment group ($p < 0.01$). B: Ovary development was grouped into three different stages: Stage 1 (undeveloped), stage 2 (ovarian development in progress) and stage 3 (fully developed ovaries with fully developed eggs). C: Distribution of the three ovarian development stages among the acetamiprid and control group was not significant ($p = 0.55$), Acetamiprid group n = 19, control group n = 22.

database.

All non-bacterial reads (e.g. ASVs assigned to plant chloroplasts or Oomycetes) were removed. Additionally, ASVs below 0.005 permille relative abundance (less than 50 reads within the entire dataset of 9.4×10^6 reads) were filtered from the dataset to remove spurious phyla. ASVs from the positive controls as well as those accounted to other samples processed on the same extraction plate were removed from the dataset. Final dataset contained 311 quality ASVs from 174 different genera. Two low throughput samples (<2500 reads) were removed resulting in 59 samples with a median throughput of 42,000 reads per sample (Acetamiprid group n = 18, control group n = 22, field group n = 19).

2.7. Statistics

Statistical analysis was performed in R version 4.4.2 (R Development Core Team, 2025). The consumption rate of sugar solution with and without Acetamiprid was measured per nest and was divided by the number of individuals living in the colony to account for the mortality of single individuals throughout the experiment. For the analysis of the cuticular lipids and development of ovary stages only those individuals were selected which survived the full seven days of experiment so that we could be sure that the number of days the bees spend in the experiment did not blur the results (Acetamiprid n = 19, control n = 22). The treatment and time effect on the consumption rate was tested by an ANOVA, as well as the treatment effect on the absolute amount of cuticular lipids. The treatment effect on the ovary development was tested by a Chi-squared test. In order to compare the whole cuticular-lipid profile, we calculated Bray-Curtis distances, using the metaMDS function in the 'vegan' package and performed a non-metric multidimensional scaling (NMDS), based on a community matrix with relative abundance data of chemical compounds. To test the interaction between ovary development and treatment, we did a PERMANOVA using the adonis2 function as implemented in the 'vegan' package (Oksanen et al., 2001) with a random factor for the nest as collected in the field to account for nest-specific smell. A similarity percentage analysis (SIMPER) was used to determine the cuticular lipids which were most different in relative abundance between the two treatments and between the three different ovarian development stages. Microbiome data was handled using the packages 'phyloseq' (McMurdie and Holmes, 2013). Shannon diversity was tested by a linear model ('lm') and the 'anova' function applied to the fitted model. Taxa abundance was tested by a generalized linear model ('glm') using quasipoisson distribution. To test for differences in microbial community composition a PERMANOVA based on bray-curtis distance matrix was performed as outlined above with 9999 permutations. Homogeneity of variances was evaluated using a Bartlett Test and normality of residuals was tested by a Shapiro Test. In case of violation, data was square root transformed. Data visualization was performed using 'ggplot2' (Wickham, 2016) and the 'microViz' package (Barnett et al., 2021).

3. Results

3.1. Influence of Acetamiprid on feeding behaviour and ovary development

To control for an equal uptake of the Acetamiprid, we investigated the consumption rates among the treatment groups over a span of 7 days. The cumulative amount of sugar water consumed per nest varied significantly between the acetamiprid-treated bees and the control group, with the acetamiprid-treated bees consuming more than the control group (ANOVA $F_{1,17} = 9.57$, $p < 0.01$) (Fig. 1 A). Consumption rates also varied greatly in the course of the experiment. Amounts decreased in the first half of the experiment and then slightly rose and dropped again in both treatments (ANOVA treatment: $F_{1,18} = 11.47$, $p < 0.01$, date: $F_{1,19} = 11.70$, $p < 0.01$) (Supplementary, Figure S1).

To investigate if the Acetamiprid treatment interferes with ovary development, bees were dissected after the 7-day feeding experiment and ovaries grouped into three different developmental stages (Fig. 1 B). The Acetamiprid treated bees showed the trend for slightly more undeveloped and less fully developed ovaries, but this pattern was not significant ($\chi^2(2) = 1.20$, $p = 0.55$) (Fig. 1 C).

3.2. Acetamiprid treatment changed the chemical profile of cuticular lipids

Since the developmental stages of ovaries are important for chemical communication of bees, we investigated if the Acetamiprid treated group differs in the cuticular lipid composition in comparison to the control. The GC analysis of cuticular extracts revealed a total of 81 different substances, from which 52 could be identified and 29 remained unknown (Supplementary, Figure S2.1, Table S2.2). The identified substances belonged to n-alkanes, n-alkenes, saturated and unsaturated macrocyclic lactones, isopentenyl esters, ethyl esters, unsaturated fatty acids and wax esters. In a quantitative comparison, the absolute amount of surface extract was significantly greater in the acetamiprid treatment group than in the control group (ANOVA $F_{1,39} = 7.45$, Adjusted R-squared = 0.13, $p < 0.01$) (Fig. 2 A).

Regarding the composition of cuticular compounds, we found an effect of the acetamiprid treatment, ovary stage and an interaction of both, when corrected for the colony-specific odour (PERMANOVA treatment: $F_{1,37} = 2.696$, $p < 0.05$, ovaries: $F_{1,37} = 4.407$, $p < 0.001$, treatment x ovaries: $F_{1,37} = 2.974$, $p < 0.05$) (Fig. 2 B). These differences were mainly based on lower amounts of the n-alkanes tricosane, pentacosane, heptacosane and nonacosane and the n-alkene (*Z*)-9-nonacosene and higher amounts of the macrocyclic lactones 20-eicosanolide and 22-docosanolide, the n-alkenes (*Z*)-9-pentacosene and (*Z*)-9-heptacosene and two unknown substances in the Acetamiprid treatment compared to the control (Supplementary, Table S2.3, Table S2.4).

3.3. Change of microbiota composition during lab experiment

For the gut-microbiota analysis we did not only investigate the bees from the treatment groups, but included also untreated field samples, which were directly frozen after field collection and had not been reared in the laboratory. The microbiota of *L. malachurum* showed overall a very simple composition and low diversity. All samples were dominated by *Wolbachia* as major endosymbiont, followed by *Apilactobacillus* as the major gut-bacterium (Supplementary, Figure S3). These two genera made together more than 97.1 % of the microbial community composition in all samples, with up to 99.2 % relative abundance in the field samples. Here, *Wolbachia* showed a median of 51 % (SD ± 24 %) and *Apilactobacillus* 48.2 % (SD ± 24 %) relative abundance (Fig. 3 A, B). Following the seven days of treatment experiment in the laboratory microbial community composition changed and *Wolbachia* dominated the microbial community with 93.6 % (SD ± 19 %) in the control group and 94.6 % (SD ± 9 %) in the Acetamiprid group (Fig. 3 A, B). At the same time the relative abundance of *Apilactobacillus* decreased to 2.1 % (SD ± 15 %) in the control group and 1.7 % (SD ± 9 %) in the Acetamiprid group (Fig. 3 A, B). *Apilactobacillus* sp. relative abundance differed significantly between field vs lab samples (GLM t = -6.18, $p < 0.001$), but showed no significant difference between the control and Acetamiprid treatment groups (GLM t = -0.595, $p = 0.554$). Field samples collected in different years showed a similar composition (Supplementary, Figure S3).

As a consequence, the overall low alpha diversity of the samples showed a further decrease when brought into the lab (Fig. 4 A). Shannon diversity differed in the field samples ($F_{1,56} = 35.14$, $p < 0.001$) but not between the treatment groups ($F_{1,56} = 0.005$, $p = 0.947$).

The overall community composition based on Bray Curtis distance matrix showed in the NMDS a clear separation of the field and lab samples (PERMANOVA $F_{1,55} = 70.91$, $R^2 = 0.562$, $p < 0.001$), but no differences between the control and Acetamiprid treatment groups

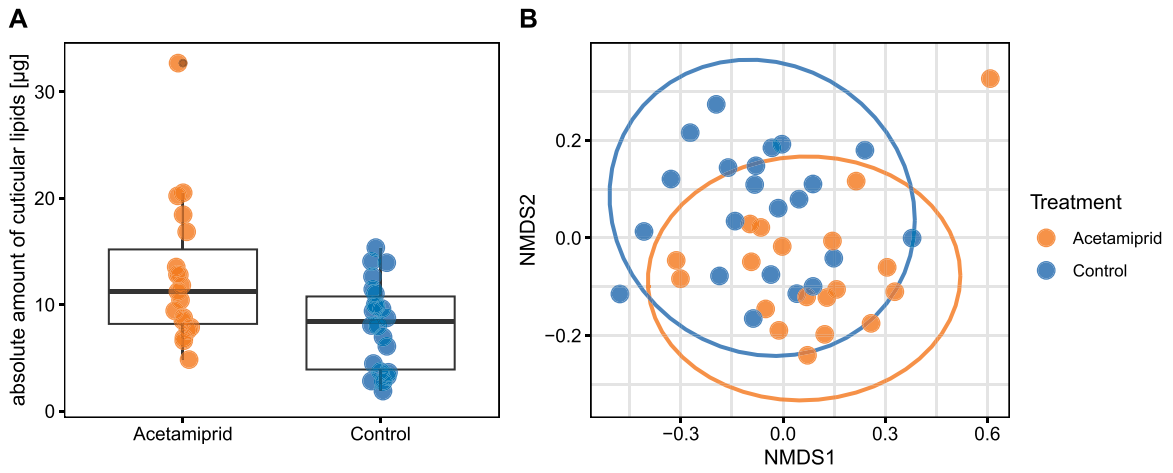

Fig. 2. Change in chemical profile of *L. malachurum* due to Acetamiprid treatment. A: The absolute amount of cuticular lipids from surface extracts differed significantly ($p < 0.01$) between the acetamiprid and the control group. B: The composition of the cuticular compounds in the surface extracts differed significantly ($p < 0.05$) among acetamiprid-treated and control bees as illustrated by non-metric multidimensional scaling (NMDS) based on Bray-Curtis similarity, 2D stress-value = 0.16, Acetamiprid group n = 19, control group n = 22.

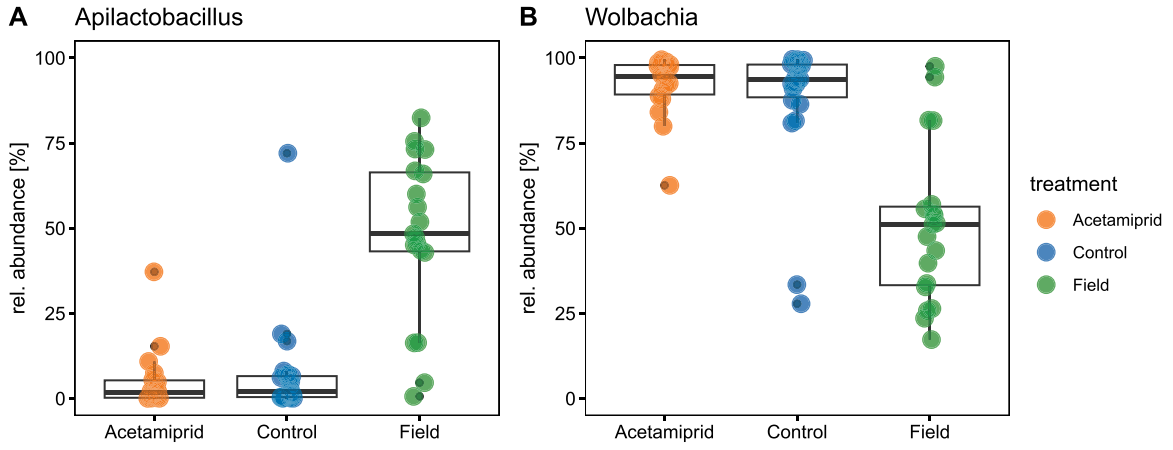

Fig. 3. Relative abundance of the two most important microbial taxa from the microbiome analysis of the *L. malachurum* from the field, compared to the treatment groups from the laboratory experiment (Acetamiprid and control). The abundances of (A) *Apilactobacillus* and (B) *Wolbachia* did not differ between treatment groups (Acetamiprid vs control). But the 7-day experiment in the laboratory altered the microbiota of the treatment groups compared to the field collected samples showing a loss of *Apilactobacillus*. Acetamiprid group n = 18, control group n = 21, Field group n = 19.

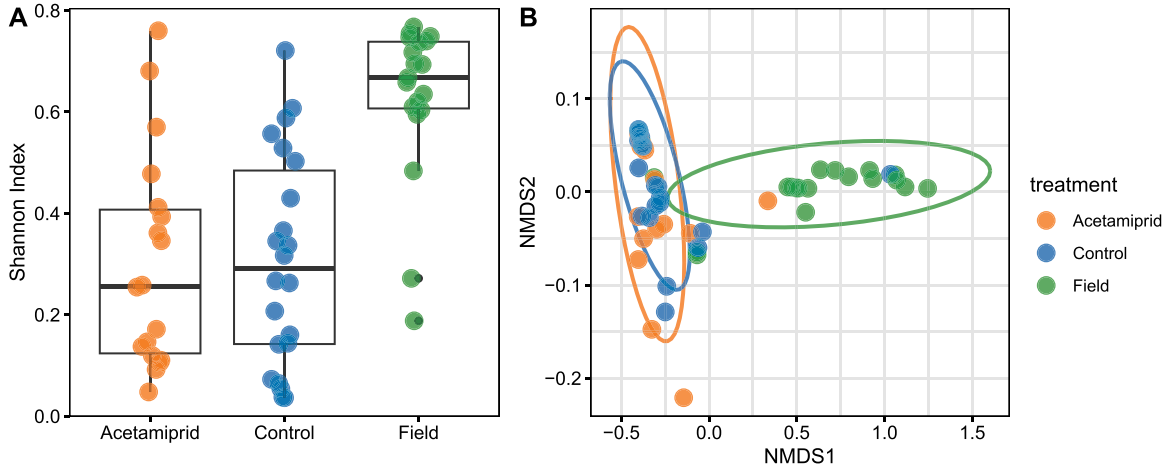

Fig. 4. Microbiome diversity of *L. malachurum* did not differ between treatment groups (Acetamiprid vs control), but between laboratory and field samples. (A) Shannon diversity as well as (B) Bray-Curtis dissimilarity was markedly different in the field samples compared to the treatment groups reared for 7 days in the laboratory. For the NMDS plot one control sample (dominated by *Spiroplasma*) was removed (Supplementary, Fig. S3). Stress value = 0.0073. Acetamiprid group n = 18, control group n = 21, Field group n = 19.

(PERMANOVA $F_{1,55} = 0.209$, $R^2 = 0.0017$, $p = 0.666$) (Fig. 4 B). Test results were similar when performed only with laboratory samples (PERMANOVA $F_{1,37} = 0.35$, $R^2 = 0.0094$, $p = 0.683$).

4. Discussion

In this study, we revealed significant effects of the neonicotinoid Acetamiprid on the overall quantity and relative composition of cuticular lipids, as well as the consumption rate of the ground-nesting bee *Lasioglossum malachurum,* even though it is considered to be bee safe and has a lower toxicity than other neonicotinoids (Lewis et al., 2016). Our data further indicate that Acetamiprid could potentially alter the development of ovaries, which could be harmful for colony development—something which already has been found for bumblebees (Straub et al., 2023). Interestingly, we found that the microbiome of *L. malachurum* changed drastically during the seven days of lab experiment, independent from Acetamiprid exposure. A finding that highlights the limitations of lab-based microbiome studies, that test pesticide exposure only under laboratory conditions. Thus, our study provides novel insights into possible effects of insecticides on primitively eusocial social ground-nesting wild bees.

4.1. Changes in the cuticular lipid profile of Acetamiprid-treated bees

Cuticular lipids play a key role in the communication of social insects (Leonhardt et al., 2016; Ayasse and Jarau, 2014; Steitz et al., 2018). Any anthropogenic disturbance of such fragile communication system could have unforeseen consequences for colony development, mating behaviour and thus populations in the wild. We found that field-realistic Acetamiprid exposure alters the amount and composition of cuticular lipids. Acetamiprid-treated bees had overall a higher amount of cuticular lipids and the abundance of some substances changed compared to the control. Such influences of neonicotinoids, and more specific of Acetamiprid, on the chemical composition are also known for *Bombus terrestris (*Straub et al., 2023*)*. For the solitary bee *Heriades truncorum* changes in the chemical composition of cuticular hydrocarbons have been found after flupyradifurone exposure, impacting the mating behaviour in this oligolectic bee (Boff and Ayasse, 2024). These observed changes in the cuticular lipids highlight the susceptibility of the chemical communication towards pesticides (Straub et al., 2023). The n-alkanes and macrocyclic lactones that contributed most to the separation of the Acetamiprid treatment and control are known to act as queen pheromones (Steitz and Ayasse, 2020). While the n-alkanes tricosane, pentacosane, heptacosane and nonacosane and the n-alkene (*Z*)-9-nonacosane occurred in lower abundances in Acetamiprid-treated bees, the two macrocyclic lactones 20-eicosanolide and 22-docosanolide and the two n-alkenes (*Z*)-9-pentacosane and (*Z*)-9-heptacosane occurred in higher abundances. These substances are electrophysiological active compounds (Steitz et al., 2019) and the above-mentioned n-alkanes and macrocyclic lactones occur in higher abundances, while the n-alkenes occur in lower abundances in queens of *Lasioglossum malachurum (*Steitz and Ayasse, 2020*)*. Interestingly, the chemical profile of the workers treated with Acetamiprid changed specifically in lactones and hydrocarbons, the substances known as queen pheromones. These queen pheromones of *Lasioglossum malachurum* play a key role in intra-colonial communication, ensuring effective division of labour. Released by the queen they suppress ovary development in workers, which leads to subordinate behaviour in workers as a so called releaser function (Steitz and Ayasse, 2020; Steitz et al., 2019). Thus, compositional changes in the chemical profile could have unpredictable effects on the communication within colonies at native nesting sites. Such effects on the social behaviour remain to be tested in further experiments. Changes in social behaviour due to neonicotinoid exposure have been reported for stingless bees (Straub et al., 2023; Boff et al., 2018) and in bumblebees (Straub et al., 2023), which could have negative consequences on the performance and pollination services of these groups.

4.2. Compounds affected by the Acetamiprid-treatment correlate with ovarian developmental stages

Patterns of cuticular hydrocarbons of many solitary and social insect species have a function as a fertility signal and change with the development of the ovaries (Steitz et al., 2018; Oi et al., 2015; Sramkova et al., 2008). More specifically, in social insects fertility signals of the queen (queen pheromones) regulate reproduction and inhibit the ovarian development of workers (Ayasse and Jarau, 2014; Steitz and Ayasse, 2020). In our study, we found a positive correlation between the developmental stages of the ovaries and the overall amount of cuticular lipids, as well as clear compositional changes in the cuticular compounds. Interestingly, the compounds which differ in quantity in the Acetamiprid-treated bees compared to the control bees are components of the fertility signal and are characteristic for differentiating the chemical profiles of bees with different stages of ovary development. However, ovarian developmental stage was not significantly affected when testing only by treatment. The relative abundances of these n-alkanes, n-alkenes and macrocyclic lactones, are usually separating queens and workers (Steitz and Ayasse, 2020). The circumstance that those compounds are affected by the Acetamiprid-treatment, which serve as important queen pheromones, regulating the development of the ovaries in workers, might have detrimental effects on the colony performance of *Lasioglossum malachurum*. In a study with bumblebees, acetamiprid changed as well the odour profile, which had an effect on the colony and population development (Straub et al., 2023). In mammals, Acetamiprid had direct negative effects on the reproductive function of male mice related to oxidative stress and mitochondria degeneration (Wang et al., 2018). Other studies which investigated the effects of Neonicotinoids on bumblebee population dynamics found fewer offspring in the treatment group (Elston et al., 2013; Laycock et al., 2014). Hence, the influence of neonicotinoid exposure on reproduction might affect population dynamics in primitively eusocial wild bees, contributing to their decline. This would have major impact on ecosystem functions, as primitively eusocial wild bees are common and important pollinators in agricultural landscapes.

4.3. Behavioural changes induced by neonicotinoid exposure

We monitored the consumption of sugar water, to examine whether the Acetamiprid group takes up as much sugar solution as the control group. Other experiments demonstrated that honeybees showed an altered responsiveness towards sugar solution when exposed to either one of the three neonicotinoids Thiamethoxan, Imidacloprid or Clothianidin (Démares et al., 2018). In our experiment, bees consumed even more sugar water including Acetamiprid than the control sugar water solution. These higher consumption rates of a pesticide containing solution are concerning and need to be observed over a longer experimental time period. A study with *Bombus impatiens* showed that the consumption increased for a high Acetamiprid treatment only in the first week compared to the control and then decreased again (Camp et al., 2020a). While other studies of *Bombus impatiens* and *B. terrestris* did not observe differences in the consumption rates of the Acetamiprid and the control group in bumblebee colonies (Straub et al., 2023; Camp et al., 2020a, 2020b). However, bumblebees seem to get adapted to the taste of neonicotinoids and increase their visits and consumption rates of Thiamethoxam-laced feeding solution after a period of ten days (Arce et al., 2018). Still, we observed a preference and higher consumption of the Acetamiprid sugar solution already from day 1 on.

Neonicotinoids are neurotoxins that bind with high affinity to the acetylcholine receptors of insects. They have according to a meta-analysis more negative effects on learning ability and memory of honeybees than on bumblebees, while data on other wild bees is largely lacking (Siviter et al., 2018). Neonicotinoids showed various influences

on cognitive perception and odour recognition of bees (Straub et al., 2021; Paoli and Giurfa, 2024). This might be an explanation why *L. malachurum* from the treatment group showed even higher amounts of surface extracts. If self-perception of odour emission is inhibited, they might produce more as compensation due to a disturbed auto-regulation. This shows that even subtle effects of pesticide exposure could have larger ecological impact on wild populations. Ground-nesting bees, with primitive social structures, could suffer to a larger extend from neurological or behavioural alterations, compared to bees with already highly developed social structures (like *Apis* and *Bombus*). It becomes clear that more research on solitary and sub-social wild pollinators is sorely needed, to better understand the environmental impact of neonicotinoid application.

4.4. Microbiota erosion during lab experiment highlights limitation of lab-based studies

Our local population collected near Ulm in Germany had a very simple microbiota structure with *Apilactobacillus* as major gut-bacterium and *Wolbachia* as major endosymbiont, a similar pattern as previously reported from *Megalopta* spp. (Halictidae) (McFrederick et al., 2014) as well as *Crawfordapis* spp. (Colletidae) (Hammer et al., 2023). *Wolbachia* are in general commonly reported among Halictidae (Saeed and White, 2015; Gerth et al., 2015). *L. malachurum* sampled in the Tuscany in Italy showed likewise mainly *Apilactobacillus* in their gut-microbiota data, but *Spiroplasma* as major symbiont (Ronchetti et al., 2022). Interestingly, we observed a single individuum in the control group that showed *Spiroplasma* instead of *Wolbachia*. Other studies report mainly about the presence of *Sodalis* in *L. malachurum* sampled in France and Italy (Rubin et al., 2018).

However, during the course of the laboratory experiment *L. malachurum* seem to lose its association with *Apilactobacillus,* when only fed with sugar solution. After 7 days in the experimental setup, most individuals show mainly *Wolbachia* as remaining endosymbiont. This emphasised that microbiota experiments with bees conducted only under laboratory conditions should be taken with caution. Similar observations have been made in a study with the ground nesting alkaline bee *Nomia melanderi* (Halictidae), which lose their association with *Apilactobacillus* when reared in the lab for 10 days (Kapheim et al., 2021). In nature, such a microbiome turnover has been observed at an elevational gradient at Mt. Kilimanjaro, where *Lasioglossum* spp. lose their association with lactic acid bacteria at higher elevations (Mayr et al., 2021). On the other hand, bumblebees can show a reverse process and a recovery of lactic acid bacteria when placed outdoors following artificial rearing (Weinhold et al., 2024).

The microbiome of primitive or facultatively social bees, like *L. malachurum*, is shaped through a combination of environmental as well as social influences (Nguyen and Rehan, 2023). While *Wolbachia* can be vertically inherited, *Apilactobacillus* needs to be acquired from the environment or food provision (Leonhardt et al., 2022; Voulgari-Kokota et al., 2019b; Argueta-Guzmán et al., 2025). Hence, their microbiota were suspected to be predominantly vulnerable to the effect of sublethal pesticide exposure (Nguyen and Rehan, 2023). While some studies claim that neonicotinoids can influence the microbiome of bees, our experiment clearly showed that this was a result of the rearing condition and not due to the treatment. Interestingly, *Apilactobacillus* has been reported to be able to mitigate the toxicity effect of Acetamiprid exposure in honeybees (Liu et al., 2022).

The influence of pesticides on the microbiome of bees is still debated controversially (Daisley et al., 2022; Hotchkiss et al., 2022). Though several neonicotinoid pesticides have been tested, in particular with the western honeybee *A. mellifera*, their influence on the gut microbiome of bees is not always clear (Cuesta-Maté et al., 2021). The exposure with Imidacloprid decreases honeybee survival, but did not show an effect on the gut-microbiome (Raymann et al., 2018). Although some studies have applied unrealistic high dosage of pesticides, they report no effect on the microbiome of honeybees (Wang et al., 2022). While experiments with low dosage of pesticide exposure are more field realistic, they showed likewise no effect (Zhang et al., 2022; Almasri et al., 2022).

5. Conclusions

Though Acetamiprid is considered “bee safe”, we found changes in behaviour and culticular lipid composition in the primitive-social sweat bee *L. malachurum*. To our knowledge, this study investigates the effects of Acetamiprid for the first time on a small ground-nesting bee, in contrast to previous studies on honeybees, bumblebees and mason bees. In our study, the composition of the cuticular lipids of bees treated with Acetamiprid was significantly different to the control bees, while there was only a trend observable on the ovary development. However, the chemical substances separating the Acetamiprid and control group, correlated significantly with different ovary stages. Thus, treatment with the neonicotinoid Acetamiprid could have a more subtle, but barely investigated effect on wild-bee communication. This might even result in changes in the social structure, especially because lactones which act as queen pheromones were affected by the treatment. Therefore, research on pesticides should also include small, ground-nesting species before pesticides are authorised and permissions renewed, worsening the situation of pollinator decline. Additionally, investigations of the effects of pesticides on the microbiome of wild bees should consider microbiome alterations under laboratory conditions. In contrast to the microbiomes of honeybees are wild bees strongly influenced by environmental factors and conclusions from lab-based studies should be taken with caution.

CRediT authorship contribution statement

Antonia Veronika Mayr: Conceptualization, Supervision, Project administration, Investigation, Data curation, Formal analysis, Validation, Visualization, Writing - original draft. **Arne Weinhold:** Investigation, Data Curation, Formal analysis, Validation, Visualization, Writing - original draft. **Manfred Ayasse:** Conceptualization, Supervision, Project administration, Investigation, Data curation, Resources, Writing - review & editing. **Amelie Nolzen:** Investigation, Data curation. **Alexander Keller:** Resources, Writing - review & editing.

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Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.119311.

Data availability

data availability statement is included in title page

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