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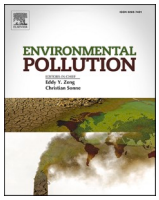

Field-realistic doses of the neonicotinoid acetamiprid impact natural soil arthropod community diversity and structure☆

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ABSTRACT

The neonicotinoid acetamiprid is used as a foliar insecticide spray, which results in direct exposure of a wide variety of soil organisms. Laboratory testing indicated that acetamiprid is toxic to the Collembola (springtails) species *Folsomia candida*, while Acari (mites) seem relatively insensitive to neonicotinoids. Since such opposing effects on different soil arthropods might imbalance natural arthropod communities, this study determined: (i) if field-realistic doses of acetamiprid affect the abundance and diversity in soil arthropod communities, and (ii) whether these potential effects are short-term or persist after degradation of acetamiprid. Intact soil cores collected from an untreated grassland field were placed in the mesocosm set up 'CLIMECS', and the naturally sourced communities were exposed to a control and increasing field-realistic doses of acetamiprid (i.e. 0, 0.05, 0.2, 0.8 mg a.s./kg dry soil). Before and 7 and 54 days after spraying the insecticide, the abundance of mites and springtails and springtail diversity were assessed. Springtail and mite abundances were similar at the start of the experiment, but springtail abundance was significantly lowered while mite abundance increased shortly after exposure to increasing doses of acetamiprid. At the highest dose, springtail numbers decreased by 53% on average while the number of mites increased by 26%. This effect was no longer visible after 54 days, suggesting recovery of the community as a whole reflected by observed changes in community dissimilarity: shortly after application springtail communities clearly diverged from the control in terms of species composition, while communities converged again in the long-term. With our results, we are the first to show that field-realistic applications of N-nitroguanidine neonicotinoids can significantly impact natural soil fauna communities, which might have implications for soil ecosystem functioning.

1. Introduction

To minimize the damaging effects on crops inflicted by insects, insecticides are used (Gupta and Gajbhiye, 2007). Over time, different insecticide classes followed each other (McAfee, 2017; Renaud et al., 2018). Currently, neonicotinoids dominate the insecticide market as they are the most used insecticide worldwide (Jeschke et al., 2011). However, in Europe, all neonicotionoids, expect for acetamiprid, were discontinued for particular uses since 2013. Acetamiprid is still allowed to be used and yearly emergency authorizations are granted to individual member states for the discontinued compounds (European Commission, 2018 a,b,c; European Commission, 2024).

Neonicotinoids have several benefits over most of their predecessors as they can be used as both foliar spray and seed coating, and are considered relatively less toxic to mammals and birds since they specifically target insects (Godfray et al., 2014; McAfee, 2017). The latter can be explained by their mode of action as an agonist of insect neuronal nicotinic acetylcholine receptors (nAChRs) (Cartereau et al., 2018) by binding to the AChRs which induces neuronal hyperexcitation, possibly leading to the death of the insects within minutes (Van der Sluijs et al.,

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2013).

Neonicotinoids are divided in two main classes: N-cyanoamidines and N-nitroguanidines. The latter share a high water solubility and relatively high environmental persistence, making them effective to be used as seed coating (Jeschke et al., 2011). However, these properties also enhance the possibility of exposure of non-target species as they are prone to environmental dispersal via runoff and leaching (Hladik et al., 2014). Studies indicate that field-realistic doses of these neonicotinoids are considered toxic to a wide range of non-target insects, including pollinators (Van der Sluijs et al., 2013; Klatt et al., 2016) and aquatic invertebrates (Morrissey et al., 2015).

Acetamiprid is a neonicotinoid belonging to the other group, the N-cyanoamidines, which are relatively less well-studied for their ecotoxicity (Morrissey et al., 2015). Similar for the N-nitroguanidines, there is also scientific support that N-cyanoamidines are toxic at field-realistic doses to aquatic insects (Barmentlo et al., 2021), but relatively less so to terrestrial pollinators such as bees as their detoxification potential is higher (Bass and Field, 2018; Manjon et al., 2018). However, due to its foliar spray application, fauna are directly exposed to the compound with possible negative consequences. As shown by De Lima e Sillapawattana and Schäffer (2017), acetamiprid is toxic to both the earthworm (Opisthopora) *Eisenia andrei* (LC_{50} of 0.80 mg a.s./kg dry soil) and the springtail (Collembola) *Folsomia candida* (LC_{50} = 0.12 mg a.s./kg dry soil). Acetamiprid may already pose a risk to natural springtail populations after a single application. This risk increases when applied frequently during a growing season, such as in potato cultivation (De Lima e Silva et al., 2020). On the other hand, non-insect soil fauna like mites (Acari) appear insensitive to neonicotinoids as effect concentrations are a factor of 450 times higher than those for Collembola (based on the EC_{50} of imidacloprid for effects on reproduction; De Lima e Silva et al., 2017). Hence, it is likely that acetamiprid affects natural soil fauna community composition.

Given the high environmental toxicity of the closely resembling N-nitroguanidine group and the observed laboratory toxicity at field application rates, it is crucial to investigate if N-cyanoamidines affect natural communities of soil fauna. However, up till now research mainly focused on short-term single species toxicity tests (e.g. Pisa et al., 2021), while exposure of natural communities is rarely addressed. Yet it is recognized that single species tests inadequately predict neonicotinoid effects on natural communities (Barmentlo et al., 2019, 2021). As mesocosms can act as a bridge between the laboratory and actual field conditions (Moser et al., 2004), a substantial body of literature has investigated the effects of several neonicotinoids to aquatic life (e.g. Barmentlo et al., 2021; Merga and Van den Brink, 2021; Duchet et al., 2023). However, mesocosm experiments that investigate the relationships between neonicotinoids and soil fauna communities are scarce (Pisa et al., 2021), except for short-term data on imidacloprid exposure (Sjursen Konestabo et al., 2022).

To reduce the knowledge gap, this study investigated: i) if field-realistic doses of the neonicotinoid acetamiprid affect the abundance and diversity of soil arthropod communities, and (ii) whether potential effects are short-term or persist after degradation of acetamiprid. We focus on natural springtail diversity as the first apparent risk for single species has already been indicated by the available literature (e.g. De Lima e Silva et al., 2020), but we also considered potential effects on mites because of differential ecotoxicological thresholds and because both groups are major parts of the terrestrial food web that occupy similar ecological niche space (Culliney, 2013). We hypothesize that mites will not be affected by direct toxicity (De Lima e Silva et al., 2017), while springtails, on the other hand, are expected to decrease in abundance with increasing concentrations of acetamiprid. We also expected that the relative abundance of different ecomorphological life forms would change due to changes in acetamiprid exposure. For example, hemiedaphic species (i.e. species that reside in topsoil and leaf litter) potentially receive both direct spray application as well as by ingestion as they live in and on top of the soil.

To test these hypotheses, a mesocosm experiment was conducted with naturally sourced soil cores harboring fully naturally formed soil communities. We used a unique terrestrial mesocosm setup: the CLIMECS (CLImatic Manipulation of ECosystem Samples). This setup has a size in the microcosm-mesocosm range with standardized and controlled environmental conditions, thus creating unique opportunities to study the impact of environmental pollutants on whole soil communities (Franken, 2019). We exposed soil communities from the naturally sourced soil cores to a control and three different field-realistic doses of acetamiprid and sampled the communities before, shortly after (7 days) and long after application (54 days) to study the effects on the soil arthropod community.

2. Methods

2.1. Acetamiprid application, mobility, and degradation

The doses used were based on the application rate in potato cultivation in the Netherlands, which is 250 g/ha per cycle of a formulation containing 20% of the active substance acetamiprid (=50 g a.s./ha). In a season there are three application moments, hence 750 g/ha in total every year (CTGB, 2023). In this experiment, the formulation Mospilan (also 20% acetamiprid as the active substance) was used. Mospilan (with 20% acetamiprid (CAS160430-64-8) as the active substance) was purchased in Brazil. The dose of 150 g a.s./ha (corresponding with a concentration of approx. 0.2 mg a.s./kg in the top 5 cm soil layer) was used for the 'medium' treatment. The treatment 'low' was four times lower (37 g a.s./ha ~0.05 mg a.s./kg) and the treatment 'high' four times higher (600 g a.s./ha ~0.8 mg a.s./kg). Stock solutions of the different dose rates were prepared in demineralized water. Each mesocosm received 50 mL of the stock solution, applied using a pipette (simulating foliar application), followed by 50 mL artificial rainwater (see calculation S1 in Supporting Information) using the water basin of the CLIMECS system (Fig. S1 in the Supporting Information).

For both the intended and measured doses of acetamiprid, we calculated how far the compound would enter the soil over time (see Calculation S1). These calculations showed that during the whole experiment, acetamiprid would be present only in the top centimeter of the soil and any deviations from this are likely the result of bioturbation.

2.2. Soil core collection and inoculation

Soil samples were taken on July 6th, 2020 from a fenced-off field at Unifarm, Wageningen University & Research, Wageningen, The Netherlands. The field had sandy soil and was dominated by grass (*Poa trivialis* L.), with creeping buttercup (*Ranunculus repens* L.), common dandelion (*Taraxacum officinale* L.), and plantain species (*Plantago* sp. L.). Soil cores were collected following Franken (2019), using a 16.6 cm diameter soil corer. The metal soil corer contained a polyethylene ring (12 cm height), in which the sample remained after soil extraction. The bottom of the core was cut off at the end of the polyethylene ring and placed on an LDPE plastic dish to prevent the soil from moving in the ring. This combination was placed in a plastic bag to contain soil fauna and stored in a climate room. The next day all but five soil cores were transferred into the CLIMECS mesocosms. The remaining five cores were placed in a similar setup (only difference was that the top was open) and analyzed later to determine the initial soil conditions and soil fauna. All soil cores were left untouched (except for adding springtails and earthworms, see 'Experimental design') in the mesocosms or climate room for 7 days to allow the soil communities to acclimatize to the laboratory conditions.

2.3. Experimental design

After the 7-day acclimatization period, exposures were started (referred to as time 0). We used three acetamiprid doses and a control

(no added acetamiprid). Based on the amount of rainfall that would be applied, the bulk density of the soil, and the half-life of acetamiprid (5–7 days in laboratory tests, De Lima e Silva et al., 2020), we estimated that 7 days would be the most suitable moment for the first sampling to identify direct exposure effects. At this time point, acetamiprid should not have leached through the soil core and approximately 38% of the initial dose should still be present in the soil. Considering the aim to investigate potential long-term (and indirect) effects, sampling also took place 54 days after acetamiprid application, when most acetamiprid was degraded. Each treatment (including the control) was replicated five times, for both sampling points. In addition, five soil cores were sampled at the start of the experiment (time 0) to determine the base abiotic conditions and natural fauna presence (see 'sampling'). Hence, in total 45 soil cores were collected from the field and incubated in the CLIMECS setup (see 'incubation conditions', Fig. S1).

Before acetamiprid application, each mesocosm received 100 individuals of *Folsomia candida* as a sentinel species. These springtails were taken from a culture at the Vrije Universiteit Amsterdam, which was kept at 15 °C and 75% relative humidity. In addition, we added five sentinel earthworms of the species *Dendrobaena veneta* to each mesocosm to further stimulate bioturbation and thus more closely approximate natural conditions. As the size of the earthworms was variable, we added three larger and two smaller individuals to each mesocosm, with an average (±SD; n = 40) biomass of 1.42 ± 0.208 g/mesocosm. The earthworms were obtained from a commercial breeder (LASEBO, Nijkerkerveen, The Netherlands).

2.4. Incubation conditions

Two times a week, 50 mL of artificial rainwater, prepared according to Moser et al. (2004), was applied to each mesocosm, corresponding with an amount of approx. 240 mm/year. This approximates the average net rainfall in the Netherlands (approximately 300 mm/year), but also takes lower evapotranspiration in the closed mesocosms into account. The mesocosms were kept in a climate room at 15 °C and a 16:8 h light: dark cycle. In the individual mesocosms only LED lights (for specifications see Franken, 2019) were used to make sure (almost) no heat was produced. The exact temperature in each individual mesocosm was monitored by a central computer.

2.5. Sampling

At the start (time 0), after 7 days and after 54 days mesocosms were sampled on floral and faunal communities and abiotic conditions.

Upon sampling, we first identified the vegetation composition with a local plant expert and identification guide by Van der Meijden (2005). The composition was recorded as the estimated percentage cover of the total mesocosm surface area at the soil level. Next, the whole vegetation was cut at ground level. To collect root material, dried soil samples of the Tullgren extractions (see below) were sieved using a hand strainer with mesh size 2 mm and weighed. The aboveground plant material was dried at 70 °C for 48 h. In case vegetation was also cut in between samplings (because it outgrew mesocosm height) this was also collected and added to the total biomass. For plant mass, all vegetation, both above and belowground was taken together as the total mass.

Thereafter, a soil sample was taken (diameter 10 cm, 5 cm deep) to extract soil arthropods using a Tullgren apparatus. Extraction lasted for 21 days following Van Straalen and Rijninks (1982). For soil fauna identification and counting, a Leica MZ 12.5 stereomicroscope with 0.8-10x magnification was used. A springtail reference collection was made based on morphospecies after which we identified the specimens to the lowest possible taxonomic level by using the identification guides by Krediet (2018) and Hopkin (2007) and cross-referencing with a local expert. For all identified taxa, the ecomorphological life form (euedaphic, hemiedaphic, and epedaphic) was identified to investigate if Collembola were affected differently upon where they reside in the soil. To compare the abundance data of mites and springtails with literature, abundances were expressed on a per m^2 basis.

The Tullgren extraction sample was also used to determine soil moisture content: soil samples were weighed before Tullgren extraction, dried at 70 °C for 48 h after extraction and reweighed. To measure soil pH, soil samples were shaken for 2 h with distilled water at a soil:solution ratio 1:5. After settling of the soil particles, the pH of the supernatant was determined with a manual pH meter (WTW INOLAB Level 2).

From the soil core we also collected four smaller soil samples (diameter 1.5 cm and 5 cm height), which were mixed and divided into subsamples for the following measurements: soil pH, soil C/N ratio, and acetamiprid concentration. These smaller soil samples were also dried for 48 h at 70 °C. Large plant material and visible animals were removed. To determine C/N ratio, the soil was ground using a ball mill and subsequently the carbon and nitrogen contents of the samples were measured by dry combustion using a FLASH Elemental Analyzer 1112 (Thermo Scientific). To assess acetamiprid concentrations, soil samples were analyzed by the commercial certified analytical laboratory Normec Groen Agro Control in Delfgauw, The Netherlands. Acetamiprid analysis was done using the QuEChERS extraction method, followed by reversed-phase LC-MS analysis. The detection limit was 0.01 mg a.s./kg dry soil.

2.6. Statistical analyses

Before investigating if environmental variables (soil moisture content, plant mass, soil pH, and soil C/N ratio) affected the soil fauna responses to acetamiprid, collinearity between these variables was tested (package 'corrplot'). No collinearity between environmental variables was observed. We used linear regressions (function 'lm') to investigate whether individual environmental parameters were affected by either the concentration acetamiprid, time or their potential interaction. We tested for normality of the data and model residuals using QQ-plots.

In a similar fashion we tested for potential effects of acetamiprid, time and their interaction on mite or springtail abundance). Next, we used dose-response modeling (function 'drm', package 'drc') to investigate if the total springtail abundance and that of the three ecomorphological forms were affected by acetamiprid application per time point. We also used dose-response modeling to test for potential changes in springtail richness and Shannon diversity ('*H*'). Effects on community dissimilarity were investigated via PERMANOVA using the function 'adonis' (package 'Vegan') with nominal acetamiprid concentration as a numerical explanatory variable. Abundance data was log10(x + 1) transformed and we used Bray-Curtis as the measure for dissimilarity with 999 permutations. As each sampled mesocosm was an individual specimen, there was no repeated measure design. We included time (in days) and its respective potential interaction with acetamiprid as an explanatory variable. In order to test whether or not the data showed homogeneous dispersion, we used distance-based dispersion tests using the function 'betadisper'. Finally, in case significant effects of acetamiprid concentration were detected per time point, we used the function SIMPER ('similarity percentage', package 'Vegan') to calculate the average contribution of individual taxa to the overall observed dissimilarity. All data analyses were performed using RStudio version 4.3.2 (R Core Team, 2023).

3. Results

3.1. Environmental impacts

Seven days after acetamiprid application, soil pH_{H20} significantly differed between the control and low dose, which was mainly due to the small variation at the low dose and the relatively low values of the controls. The range of soil pH_{H2O} values was, however, small, ranging between 5.65 and 5.86 (see Table S1), nor did it show collinearity, hence it was not explored in further data analyses.

3.2. Test conditions

The vegetation composition was highly similar between individual mesocosms, irrespective of sampling time or concentration of acetamiprid that was applied. In all columns the vegetation composition consisted of a minimum of 95% *P. trivialis*. Some columns contained some *T. officinale*, *R. repens*, and *Plantago* sp. in very low abundances. Like the vegetation, soil C/N ratio was unaffected by either time or treatment (Table S1).

However, soil moisture contents were highest at the start of the test (Table S1), but also showed relatively high variation at this point (SEM = 7.45) due to mesocosms without a closed top. After 54 days, soil moisture contents were on average 9.44% and 7.44% lower than at time 0 and after 7 days, although this change was not statistically significant ($p > 0.05$). We also tested this separately for 7 and 54 days after application. A significant difference ($p = 0.038$) between the treatments was found for soil moisture content after 54 days.

In all analyzed soil samples, both after 7 and 54 days and for the different acetamiprid concentration applications, we did not detect acetamiprid (detection limit of 0.01 mg/kg dry soil; Table S1). The acetamiprid stock solutions were measured twice, resulting in low, medium, and high acetamiprid concentrations of on average 1.4 μg/L (only one measurement, the other was inexplicably low and therefore considered an outlier), 5.4 μg/L, and 1.5 mg/L, respectively, confirming that the application of acetamiprid was indeed effective.

3.3. Faunal abundance and springtail richness

Before acetamiprid application, mesocosms contained on average 67,940 mites per m^2 (SEM = 3585). Seven days after acetamiprid application, control mesocosms contained on average 68,908 mites per m^2 (SEM = 4716). No significant difference in mite abundance was found between time 0 and the control samples after 7 days ($p > 0.05$). However, mite abundance increased significantly by acetamiprid application and by time, but not in interaction with each other ($F_{1,41} = 6.58$, $p = 0.014$ and $F_{1,41} = 6.32$, $p = 0.020$, respectively). After 7 days, mite abundance increased by 22.7 and 26.1% relative to the control at 0.2 and 0.8 mg a.s./kg, respectively (Fig. 1A). After 56 days this increase in abundance with increasing test concentration persisted with averages of 28.0 and 42.2% at concentrations 0.2 and 0.8 mg a.s./kg, respectively (Fig. 1B).

At time 0, on average 70,818 springtails were found per m^2 (SEM 20,196) and after 7 days this was 53,476 per m^2 (SEM 11,639) in the control mesocosms. In total, only 100 *F. candida* individuals were found in the soil cores extracted from all mesocosms together, corresponding with a total of 275 individuals when corrected for the surface area sampled compared with the total mesocosm area (~7 % of the added total of 4000 individuals). As for the mites, the springtail abundance did not significantly differ between the start of the experiment and the control samples 7 days after acetamiprid application ($p > 0.05$). After 7 days the total springtail abundance showed a dose-related decline; dose-response curve fitting of the abundance data of springtails per m^2 showed an EC_{50} of 0.577 mg acetamiprid/kg dry soil, 95% CI:0–1.26 mg/kg dry soil (~EC_{50} of 427 (95% CI:0–935) g acetamiprid/ha = 2135 g Mospilan/ha). This corresponded to a 52.9% decrease in springtail abundance at the highest test concentration (0.8 mg/kg). In the period between 7 and 54 days of exposure, springtail abundance increased in all test concentrations by 380 and 709% on average, but significant differences between individual treatments did not persist over time (Fig. 1B). The ratio between springtails and mites changed over time but also between acetamiprid doses as a result of the described increases and decreases of both groups. With an almost 1:1 ratio (springtails:mites = 1.06) at the start, springtails and mites were equally abundant. With an increasing concentration of acetamiprid, the mites took the overhand

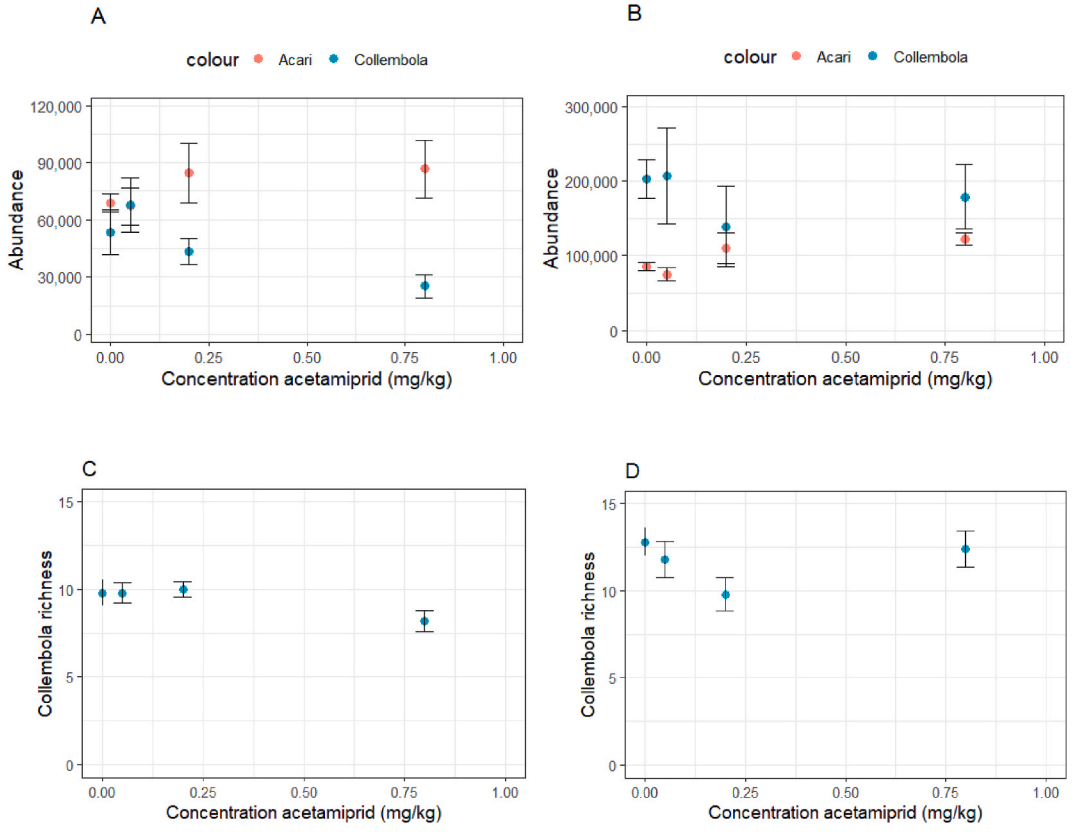

Fig. 1. Effects on average Collembola and Acari abundances (top; per m^2, ±SEM) and Collembola taxon richness (bottom; ±SEM) of increasing acetamiprid concentrations (0.05, 0.2, 0.8 mg a.s./kg dry soil, N = 5) 7 (left) and 54 (right) days after application to field soil cores incubated in mesocosms in the laboratory.

after 7 days with an average springtail:mite ratio of 0.79, 1.12, 0.62 and 0.30 in the control, 0.05 mg/kg, 0.2 mg/kg and 0.8 mg/kg treatments, respectively (Fig. S2). After 54 days this pattern reversed with springtails highly increasing in abundance (Fig. S2), but relatively less so at the two higher test concentrations: average springtail:mite ratios were 2.44, 2.95, 1.24 and 1.51 for control 0.05 mg/kg, 0.2 mg/kg and 0.8 mg/kg treatments, respectively.

At t = 0, in total 12 unique Collembola taxa were identified and each mesocosm replicate contained on average 9.2 taxa (SEM 0.86) belonging to three different taxonomic orders (Entomobryomorpha, Poduromorpha and Symphypleona). In the control, the number of taxa did not differ significantly after the first 7 days of the experiment (9.8 taxa, SEM 0.73, $p > 0.05$). Acetamiprid application had a slightly negative effect on Collembola taxon richness ($F_{-1,41} = 6.27$, $p = 0.016$), while throughout the whole experiment taxon richness increased (12.8 taxa in the control 54 days after acetamiprid application, SEM 0.80, $F_{-1,41} = 5.08$, $p = 0.030$) (Fig. 1C and D). After 7 days, taxon richness decreased slightly in the highest test concentration (0.8 mg acetamiprid/kg dry soil) to 8.2 (SEM 0.58) (Fig. 1C). After 56 days, however, the average number of taxa at this test concentration was comparable to the control (12.4 and 12.8, respectively), while a decrease was observed at 0.2 mg acetamiprid/kg dry soil (9.8 taxa) (Fig. 1D). The observed effects on taxon richness did not coincide with effects on alpha diversity as no statistically significant effects of acetamiprid were observed on either Shannon diversity (*H*) or Shannon evenness (*J*) ($p > 0.05$ for all comparisons).

3.4. Springtail community structure

There were significant differences in ecomorphological life form abundance ($F_{2,125} = 148.68$, $p < 0.001$) and all ecomorphological life forms of the springtails increased in abundance over time ($F_{1,125} = 29.22$, $p < 0.001$). At the start of the experiment, the number of hemiedaphic springtails was highest (372 on average) followed by euedaphic (172 on average) and epedaphic springtails (6 on average). Between the start and end of the experiment, the average abundance in the controls of euedaphic springtails remained stable ($p > 0.05$), hemiedaphic springtails significantly increased with a factor 3.81 ($F_{1,41} = 9.32$, $p = 0.004$), and epedaphic springtails with a factor 8.73 ($F_{1,41} = 6.82$, $p = 0.0126$). Throughout the experiment, we only found a significant interaction of acetamiprid and time for hemiedaphic springtails ($F_{1,41} = 8.62$, $p = 0.005$), meaning that the abundance of this ecomorphological life form was differently affected by acetamiprid over time. After 7 days of exposure to acetamiprid, we did find significant dose-related declines of both euedaphic ($EC_{50} = 0.184$ mg/kg acetamiprid/kg dry soil, 95% CI 0.002–0.366; 680 g Mospilan/ha) and hemiedaphic ($EC_{50} = 0.791$ mg acetamiprid/kg dry soil, 95% CI 0.267–1.355; 2927 g Mospilan/ha), but not epedaphic springtails, which is possibly related to the fact that counts of epedaphic springtails were already low in general (Fig. S2B). After 54 days, dose-related effects on abundances were not observed for any of the three ecomorphological life forms.

Acetamiprid application also significantly impacted Collembola community structure in interaction with time: a significant dissimilarity compared to the control was observed ($R^2 = 0.041$, $F_{1,44} = 2.31$, $p = 0.040$) (Fig. 2). When investigating per time point, significant effects on dissimilarity were only observed 7 days after acetamiprid application ($R^2 = 0.107$, $F_{1,19} = 2.16$, $p = 0.036$), meaning that effects of acetamiprid on springtail community structure were likely not as persistent over the measured points in time, although considerable dissimilarity compared to the control was present at 0.2 mg acetamiprid/kg dry soil (Fig. 2). In contrast, the observed dissimilarity after 7 days of exposure was most strong in the 0.8 mg acetamiprid/kg treatment (Fig. 2). We found no significant effects of acetamiprid on community beta dispersion ($p > 0.05$ for all timepoints).

Corresponding with the changes in total springtail abundances, the changes in dissimilarity could be explained from changes in the abundance of individual springtail taxa (Table 1). With increasing concentration of acetamiprid, the SIMPER analysis indicated that effects on individual taxa abundance were increasingly more negatively per individual taxa that explained most of the observed dissimilarity between

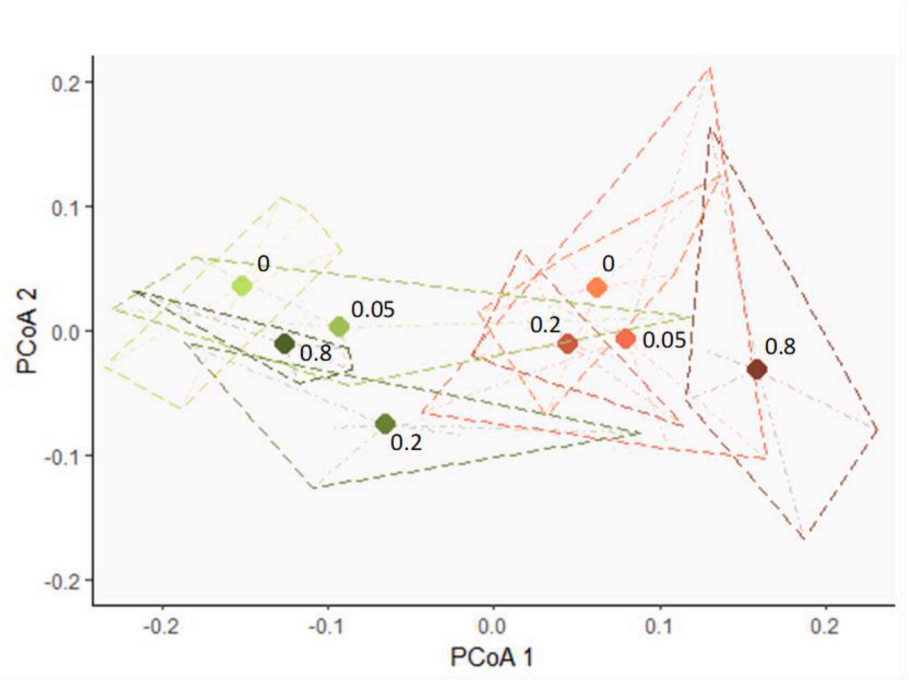

Fig. 2. Principal coordinate analysis (PCoA) of springtail communities (N = 5 per treatment) with Bray-Curtis dissimilarity, in soil mesocosms sprayed with acetamiprid. Note that t = 0 is not shown for clarification purposes. Red colors show springtail communities 7 days and green color 54 days after acetamiprid application. Darker shades indicate increasing acetamiprid concentrations (in mg a.s./kg dry soil). Dots show the community centroids per acetamiprid concentration and time point. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Average contribution (in percentage and sorted from highest to lowest) to the observed dissimilarity per taxon (log10[x+1] transformed) of springtail communities in soil mesocosms sprayed with acetamiprid. Doses (0, 0.05, 0.2 and 0.8) are shown in mg a.s./kg dry soil. Only data is shown for 7 days after the initial exposure since after 54 days no significant differences in community dissimilarity were observed ($p > 0.05$). Shown are the top five taxa that together contributed most to the observed dissimilarity between groups (about 90% or more). 'Abundance control' indicates the average control abundance and 'abundance perturbed' indicates the respective acetamiprid dose. Blue colors indicate higher average abundances in the acetamiprid treatments compared to the control while yellow indicates lower average abundances.

Comparison	Taxon	Abundance control (per m^2)	Abundance perturbed (per m^2)	Contribution to observed dissimilarity (%)
0 - 0.05	Onychiuridae sp.	17367	31780	43.3
	Isotominae spp.	11790	17520	22.6
	Paraisotoma notabilis	8556	6926	13.0
	Lepidocyrtus M2	7589	3743	8.1
	Hypogastruridae M1	3081	662	4.3
0 - 0.2	Onychiuridae sp.	17368	7436	26.0
	Parisotoma notabilis	8556	15381	24.7
	Isotominae spp.	11790	7003	21.2
	Lepidocyrtus M2	7589	5933	11.4
	Hypogastruridae M1	3081	2190	6.1
0 - 0.8	Onychiuridae sp.	17367	7945	28.1
	Isotominae spp.	11790	6748	25.3
	Parisotoma notabilis	8556	3973	16.1
	Lepidocyrtus M2	7589	3998	13.2
	Hypogastruridae M1	3081	738	6.7

'M' indicates morphospecies.

treatments. For all comparisons between control and treatment, the taxonomic family of Onychiuridae explained most of the observed dissimilarity (26% or more). The taxonomic subfamily of Isotominae and the species *Parisotoma notabilis* explained the second or third most depending on the control-treatment comparison. All three taxa showed on average the highest abundance in the control out of all taxa identified.

4. Discussion

We investigated if and how actual field application levels of the neonicotinoid acetamiprid affect soil arthropod communities using naturally sourced soils incubated in mesocosms. Increasing doses of acetamiprid significantly reduced the total number of springtails while mites simultaneously increased in abundance. Collembola taxa richness also decreased slightly with increased acetamiprid application and overall springtail communities were dissimilar compared to the control state.

4.1. Acetamiprid fate

In all soils, after 7 and 54 days measured acetamiprid concentrations were below the detection limit (0.01 mg a.s./kg dry soil). Assuming an average DT_{50} of 6 days (De Lima e Silva et al., 2020), after 7 days the compound should still have been detectable at concentrations around 0.02, 0.09, and 0.36 mg/kg for nominal treatments of 0.05, 0.2, and 0.8 mg a.s./kg, respectively, while after 54 days all concentrations were expected to be below the detection limit. It should be noted that De Lima e Sillapawattana and Schäffer (2017) spiked acetamiprid into LUFA 2.2 soil that was dried and stored in the lab till use. Consequently, microbial

activity probably was lower than in the field-collected fresh soil used in our mesocosm test, possibly explaining the faster degradation (Potts et al., 2022). In addition, part of the applied acetamiprid was likely intercepted by the vegetation during application. Although it could still end up in the soil after simulated rainfall, due to photolysis it might have been partly degraded before reaching the soil (Liang et al., 2019). Finally, it should be noted that soil by definition is not homogenous, and chemical analysis usually is performed on only a small fraction of the total soil sample. These factors probably explain why acetamiprid concentrations in the soil were below the detection limit already after 7 days. However, our stock solutions did contain the intended acetamiprid concentrations, confirming the applied doses. Furthermore, significant biological effects were observed after 7 days of application, also showing that acetamiprid dosing of the mesocosms was successful, but the dynamics of the natural system likely have affected its fate in several ways.

4.2. Mites and springtails

In control mesocosms at the first two timepoints (0 and 7 days after application of acetamiprid), we observed on average 68,000 and 69,000 mites per m^2 and 71,000 and 53,000 springtails per m^2, respectively. Comparable grassland ecosystems in the UK showed slightly lower mite and springtail abundances of 48,000 and 42,000 per m^2, respectively (Cole et al., 2005), although the authors used a shorter and therefore possibly not optimal Tullgren extraction time (96 h compared to the optimal 21 days suggested by Van Straalen and Rijninks, 1982). Chauvat et al. (2007) found similar springtail abundances depending on the age of grasslands (around 35,000 and higher) in their land-use change and grassland succession experiment. Hence, we conclude that the abundances of mites and springtails in our mesocosms accurately reflected densities of natural communities throughout the experiment.

Acetamiprid clearly affected both mites and springtails. For the mites, a dose-related increase was observed shortly after application (7 days), which was even more pronounced after 54 days (Fig. 1). The opposite was found for springtails, with after 7 days an EC_{50} of 427 mg a.s./ha (i.e. $EC_{50} = 0.577$ mg acetamiprid/kg dry soil). These results are in close agreement with the study of Sjursjen Konestabo et al. (2022), who also observed a dose-related decline of springtail numbers in *in situ* mesocosms dosed with the N-cyanoamidine imidacloprid with an EC_{50} of 0.30 mg/kg, which is very comparable to observed EC_{50} for acetamiprid.

After 54 days the decrease in springtail abundances was no longer visible. The increase in abundance of mites is likely explained by natural interactions occurring in soils. One of these interactions is the competition for food, and both groups contain many scavenging species with similar food preferences (De Oliveira et al., 2016). The depletion of many springtails by acetamiprid thus probably opened up niche space for mites. This specific explanation is focused only on biotic-biotic interactions. However, the impact of any given disturbance (in this case the insecticide acetamiprid) is not only dependent on the fauna, but also on soil characteristics, weather, and other abiotic factors (Coyle et al., 2017). Even though the exact consequences of each factor are unknown, the loss of biodiversity likely will alter the productivity and regeneration capacity of ecosystems (often negatively) (Tresch et al., 2019).

Both the hemiedaphic and euedaphic ecomorphological life forms of springtails were significantly impacted by acetamiprid shortly after application (after 7 days). We found no indication of different direct toxic effects of acetamiprid on individual ecomorphological lifeforms (i.e. there was no significant difference between dose-response curves). However, the epedaphic springtails that live at the soil surface and in the vegetation were found in low numbers at first and no direct toxicity was observed (likely due to the low initial abundances). This can be explained by their ability to jump away at the moment of soil collection. Therefore, it was harder to detect potential effects of acetamiprid on this ecomorphological life form in the present setup, although it may be more susceptible to surface-sprayed neonicotinoid applications as found by Sjursen Konestabo et al. (2022) in field-exposed mesocosms dosed with imidacloprid.

The springtail community as a whole (in terms of taxon composition) became increasingly more dissimilar with increasing acetamiprid concentration compared to the control. Such community altering effects of field realistic doses of a neonicotinoid insecticide have been observed earlier in aquatic mesocosms (Barmentlo et al., 2019; Beketov et al., 2008; Rico et al., 2018), but to our knowledge not in a terrestrial mesocosm. Moreover, studies that experimentally show the effects of neonicotinoids on naturally established communities are even rarer (Barmentlo et al., 2019). Thus with our results, we show that the effects of neonicotinoids on the natural environment are ubiquitous and transgress hemispheres.

The springtail community composition changed in response to increasing acetamiprid concentration compared to the control. With increasing concentration of acetamiprid, the SIMPER analysis showed increasingly negative effects on individual taxa abundance, which contributed significantly to dissimilarity between treatments. For all comparisons between control and treatment, the taxonomic family of Onychiuridae explained most of the observed dissimilarity (26% or higher), followed by the taxonomic subfamily of Isotominae and species *P. notabilis* depending on the control-treatment comparison. All three taxa showed on average the highest abundance in the control out of all taxa identified. Onychiuridae, characterized by a euedaphic ecomorphological life form, primarily inhabit deeper soil layers. This may explain its initial resistance to the treatment and potential for thriving as other groups were more affected. The Isotominae, including *P. notabilis*, have a hemiedaphic ecomorphological life form, live in the upper soil layer, which makes them initially more susceptible to acetamiprid exposure.

The applied acetamiprid doses were close to the EC_{50} of 0.10 mg a.s./kg dry soil found in a laboratory toxicity test with *F. candida* (De Lima e Silva et al., 2020). Thus, the results of this experiment, which incorporated 15 taxa, simulating a more natural situation, are in line with the results of a single species laboratory toxicity test. It should, however, be mentioned that it is difficult to compare single species and multispecies mesocosm tests as in laboratory tests the compound is homogeneously mixed in with the soil, while in the mesocosm experiment it was dosed by spraying to simulate pesticide application. Furthermore, the standardized OECD toxicity test guideline relies on only one species: *F. candida*, which was also added to all mesocosms as a reference species, but only a few individuals were retrieved.

Since there was a clear dose-related decrease in the abundance of springtails after 7 days, we investigated if abundances were increasing between 7 and 54 days compared to the control. This was indeed the case and can be interpreted as regular population dynamics or an indication of recovery. This suggested recovery might be explained by a springtail's ability to detoxify neonicotinoids (seen in *Folsomia candida*) (Sillapawattana and Schäffer, 2017). After 54 days, acetamiprid was degraded to concentrations below the detection limit (0.01 mg/kg), meaning that the insecticide was no longer able to cause direct toxic effects. De Lima e Silva et al. (2020) showed that the LC_{50} and EC_{50} for effects on *F. candida* survival and reproduction were quite similar (0.12 and 0.10 mg acetamiprid/kg dry soil), meaning that springtails in our experiment either died and did not reproduce or did not die and could still successfully reproduce, which would allow for recovery. However, natural population dynamics could also explain the increase of springtails over time as by chance the sampling moment could coincide with a peak of abundance. Our findings are likely a combination of both factors. The largest increase in abundance was between 7 and 54 days and was highest for the highest acetamiprid dose. This can be explained by the fact that the effect of acetamiprid was also the strongest at the highest dose and many springtails died as a consequence of the application. This potentially allowed for the survivors to fill in the niche space that became available, and enable population growth (Müller, 2018). Such dynamics differ depending on developmental stage and sex, while

in the field, immigration from non-affected areas is also possible leading to increases in abundance (Martikainen et al., 1998).

The observed negative effects of acetamiprid on springtail population size indicate that field applications in potato cultivation also directly affect soil arthropod communities since the observed lethal doses correspond to a field application rate of 150 g a.s./ha. Hence, it is likely that springtails are negatively affected by acetamiprid in potato cultivation. However, it should be noted that migration between affected and unaffected communities could not occur in our closed mesocosms. Epedaphic springtails, for example, have great dispersal potential and thereby can recover quickly in natural situations (Rebek et al., 2002), which might alter recovery rates. At present, our experiment is one of the few mesocosm studies demonstrating the impact of neonicotinoids on natural soil arthropod communities. Mesocosm tests with artificial communities, typically sourced from laboratory cultures, may be used as an alternative (De Lima e Silva et al., 2021), but they often lack most of the natural interactions between the species. Moreover, the species used in laboratory tests often only occur in low abundances in natural soils as they prefer high organic matter contents and generally are opportunistic species with a short life cycle (De Lima e Silva et al., 2021).

This experiment with natural arthropod communities revealed a link between increasing acetamiprid levels and the decline in springtail abundance, along with shifts in community composition. However, our findings may underestimate the true impact as we only tested a single neonicotinoid with a short half-life in soil. In most field situations, multiple pesticides persist over longer periods (Sillapawattana and Schäffer, 2017), likely producing cumulative effects. In addition, it is proven that neonicotinoids of the N-nitroguanidine class can be more toxic to arthropods than the N-cyanoamidines (De Lima e Silva et al., 2017; Manjon et al., 2018).

5. Conclusions

Field application doses of acetamiprid significantly altered natural soil arthropod communities by strongly reducing the abundance of springtails, likely due to direct toxicity. At field application rates, the abundance of total Collembola decreased by 19%, while at the highest dose the reduction was as high as 53% 7 days after application. In addition, a small reduction in taxa richness was found, indicating the severity of toxicity. These changes initiated a turnover in community structure as well as increasing community dissimilarity with increasing acetamiprid concentration compared to the control. The strong reductions in springtails coincided with strong increases in mites, probably due to opening up niche space. This study is the first to show that a N-nitroguanidine neonicotinoid, acetamiprid, can significantly impact soil biodiversity at actual application rates. We also found some shifts in other ecological variables that are not directly affected by neonicotinoid application, such as increased plant biomass and increased soil C/N, indicating that the alteration of soil biodiversity also induced changes in ecosystem functionality. This warrants further investigation of the functional consequences of these applications to preserve the functionality of agricultural and natural soils. Similarly, it demonstrates that current neonicotinoid applications already alter the natural soil biodiversity.

CRediT authorship contribution statement

Michella Ligtelijn: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **S. Henrik Barmentlo:** Writing – review & editing, Visualization, Formal analysis. **Cornelis A.M. van Gestel:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2024.124568.

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