Assessing the exposure and effects of imidacloprid on the abundance of microarthropods in an agricultural soil community

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Abstract

Soil-living biota are essential for soil processes and functions, but in recent years soil biota have increasingly been facing multiple and interacting threats due to land use change and agricultural intensification. One of the threats that has recently come under increasing scrutiny for their impact on non-target organisms is neonicotinoids; a group of systemic neuro-active insecticides that disturbs the transmission of signals in the insect's nervous system. The direct toxicity of neonicotinoids to non-target species warrants an evaluation of their long-term impact on agricultural soils and the surrounding ecosystems.

The aim of this study was how imidacloprid combined with an environmental stressor affected abundance within a microarthropod soil community during a summer season, focusing on occurring springtails and mites in the soil core as well as the added springtail *Folsomia quadrioculata*. Soil communities in mesocosms were exposed in situ to four different concentrations of imidacloprid by injecting it directly in to the soil, ranging from realistic field levels (0.02 – 2.5 mg/kg), in addition to control. One half of the communities were also exposed to a watering treatment that consisted of 20% of the mean precipitation the previous 10 years of that week.

The overall results showed that watering did not alter the exposure regime due to high natural rainfall that year. However, indications of mobility of imidacloprid over time, leaching in depth was observed. The results also showed that springtails are sensitive to the concentration levels of imidacloprid. Moreover, they respond in a concentration-dependent manner, suggesting that the higher the dose, the more severe is the impact of imidacloprid. Soil dwelling springtails was found to have the highest reduction in abundance, especially towards the end of the experiment. The abundance of mites was not severely affected by imidacloprid, and showed little to no decrease in abundance when exposed to even the highest concentrations of imidacloprid.

Abbreviations

ACh	Acetylcholine
AIC	Akaike information criterion
ANOVA	Analysis of variance
$C_{1,2}$	Concentration
CAS	Chemical Abstract Service
CI	Confidence interval
DW	Dry weight
EU	European Union
EFSA	European Food Safety Authority
EPA	Environmental Protection Agency
FW	Fresh weight
IUPAC	International Union of Pure and Applied Chemistry
MLE	Maximum likelihood-principle
MULTICLIM	Effects of climate change in a multiple stress multispecies perspective
N	Nitrogen
n	Number of observations
NA	Not available
nAChR	Nicotine acetylcholine receptors
NMBU	Norwegian University of Life Sciences
NIVA	Norwegian Institute of Water Research
OECD	The Organisation for Economic Co-Operation and Development
P	Phosphorus
PEC	Predicted Environmental Concentration
RCN	The Research Council of Norway

UiO	University of Oslo
UV	Ultra violet
$V_{1,2}$	Volume

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1 Introduction

1.1 Agricultural interferences

Agricultural land is a critical resource for food production worldwide where farmland is responsible for the vast majority of food production. However, as the world population grows in number, wealth and consumption, so does the pressure on agricultural land and soils. Agricultural intensification has already reduced area of land used to feed one person by a third in the period 1963 to 2005 (Kastner et al., 2012). Humans have already appropriated up to one-third of the global terrestrial potential net primary production with croplands covering 12-14% of the ice-free global surface (IPCC, 2019). At the same time, loss of fertile cropland due to urbanisation, climate change, pollution and loss of biodiversity challenge crop production. This is exacerbated by factors such as urban industrialisation, agricultural inputs, global warming and loss of fertile cropland (Foley et al., 2011; Kastner et al., 2012). Together with increasing temperatures, changes in precipitation (e.g. drought or increased humidity) and biotic (e.g. pests, disease) disturbances are to be expected (IPCC, 2019). Consequently, these effects may have an effect on crop production and soil biota. Environmental conservation is not only key for future successful food production to reach the food demand but will be instrumental in preventing a loss of biodiversity, deforestation, water degradation and an increase in greenhouse gas emissions (Foley et al., 2011). Natural soil consists of a comprehensive storage of diverse biological communities and the activity and interactions of these biotas shape ecosystem processes many other ecosystems depend on (Wardle et al., 2004; Wagg et al., 2014). As the majority of processes in terrestrial ecosystems have soil as an active regulatory centre, the responses of soil fauna to environmental stressors is of growing importance (Wardle et al., 2004; Pritchard, 2011). Especially because of their crucial role in providing ecosystem services.

The increasing land use change and agricultural intensification also brings other concerns to the effects on abundance and biodiversity. The exposure to anthropogenic contaminants and their join effects to environmental changes might have a range of detrimental effects on natural soil communities when exposed to even low concentrations and over time (Helgason et al., 1998;).

1.2 Soil communities

Soil biota are essential for soil processes and functions, and soil communities are often used as bioindicators for soil conditions providing quantified measurements of physical or chemical properties of their natural environment (Stork and Eggleton, 1992; Barrios, 2007). The soil fauna composition has a large variation and a soil community is defined as healthy if it has a diverse food web and is able to keep pests and diseases under control through predation and competition (Barrios, 2007; Widenfalk et al., 2016). Soil invertebrates play an important role in soil formation and maintaining soil fertility, and are often crucial components of terrestrial ecosystems providing decomposition of organic matter, recycling of nutrients, and general maintenance of soil health (Stork and Eggleton, 1992). The invertebrate community structure and composition is strongly controlled by soil temperature and moisture (Stork and Eggleton, 1992; Jucevica and Melecis, 2006). For simplicity soil fauna are often subdivided into groups based on size (μm – mm) or functional role within soil food webs as macrofauna (e.g. earthworms and termites), mesofauna (e.g. microarthropods), microfauna (e.g. nematodes and protozoans) and microflora (e.g. bacteria and fungi) (Wardle et al., 2004; Pritchard, 2011). Microarthropods are an important and numerous group of soil fauna. They are a morphologically and functionally diverse group that inhabits air-filled pore space. These organisms have a relatively large role in soil ecosystems being responsible for nutrient cycling through a diversity of processes (Swift, Heal and Anderson, 1979; Makkonen et al., 2011).

1.2.1 Collembola (Springtails)

Collembola (Hexapoda), or springtails, are the most abundant, diverse and complex group of soil microarthropods with a global distribution inhabiting numerous niches including soil and litter (Hopkin, 1997). These organisms are often good indicators within the soil mesofauna, providing an important contribution in mineralisation of organic matter (Bardgett and Chan, 1999, Hopkin, 1997). These small, entognathous (having internal mouth parts) soil organisms (0.2–8 mm), contribute to the nutrient cycle in soil and other processes via their feeding activity (Rusek, 1998). Springtails are also major components in the soil fauna and of terrestrial ecosystems, constituting a significant contribution to the total biomass of soil invertebrates. Springtails are omnivorous and have a diverse diet defined by the specific niche they inhabit and are found to feed on fungal hyphae, bacteria, algae, protozoa, dead vegetation, living plants, soil detritus, nematodes, and other microbiota (Hopkin, 1997;

Rusek, 1998). They are an integral part of soil ecosystems, contributing to dispersion of plant litter, fungal spores and bacteria (Hopkin, 1997). By feeding in one soil area and excreting in another, their faecal pellets may also have a crucial role in increasing the availability and surface area of organic matter, engaging further microbial and fungal decomposition, and the release of essential nutrients (Rusek, 1998; Sjursen and Holmstrup, 2004; Buse, Ruess and Filser, 2014). By doing so, the springtails become an important link in the transfer of energy between food webs above and below ground, where their feeding activities are joined by increased nutrient availability, as well as being preyed upon by a wide variety of small arthropod predators (Hopkin, 1997; Rusek, 1998). Studies show that the presence of springtails also affect the increase of mineralisation of nutrients, such as nitrogen (N) and phosphorous (P), making them available for uptake by plants and hence increasing plant production. The changes demonstrate how a ecosystem respond to natural and anthropogenic environmental changes (Hopkin, 1997; Cragg and Bardgett, 2001).

1.2.2 Mites (Acari)

Together with the springtails, mites (Acari) are the other large group of microarthropods in soil. Mites are small arachnids (0.1 – 30 mm) that are distributed throughout the world and are found in almost every ecosystem (Dhooria, 2016). Mites display an enormous variation in lifestyle, ranging from saprophagous (feeding on dead or decaying organic matter) to herbivorous and from parasitic to predator (Van Leeuwen and Dermauw, 2016). Plant feeding mites play an important role as pests of different crops and controller of weeds. Soil mites are typical representatives of soil microfauna inhabiting soil pores and other soil spaces. They inhabit primarily the upper layers of soil. Soil mites have an exceptional importance in the circulation of nutrients in soil and are more resistant to desiccation in contrast to other soil microfauna, often showing a high tolerance to loss of moisture (Perdue and Crossley, 1989; Sjursen and Sømme, 2000; Dhooria, 2016). In the soil ecosystem, mites influence decomposition and soil structure by reduction of organic matter and production of faecal pellets, while predatory mites contributes to population control by feeding on other mites and smaller soil mesofauna (Dhooria, 2016). Mites are also found to inhabit resistance to xenobiotics (chemicals not produced by organisms or the environment) through adaptations in mechanisms to overcome toxic substances such as increased metabolism, behavioural changes, target-site insensitivity etc,. (Holmstrup, Maraldo and Krogh, 2007; Kardol et al., 2011; Dhooria, 2016).

1.3 Pesticides

Plant disease vectors and harmful organisms (‘pests’) pose economic threats in agricultural ecosystems. Pesticides are substances used for protection of plants against pests by either preventing, destroying or controlling the unwanted pest that causes harm or otherwise interferes during production, processing, storage and transport. Pesticides are often grouped based on the target organisms such as insecticides, fungicides, herbicides, rodenticides, bactericides, repellents and biocides. The usage of pesticides has increased in the last half-century. An analysis done by the EPA (Environmental Protection Agency) found ~3 million tonnes of pesticides were produced in 2012 (Atwood and Paisley-Jones, 2017). Of all the pesticides produced, herbicides had the highest market share followed by insecticides with a market share of 29% (Atwood and Paisley-Jones, 2017). Insecticides are classified based on their structure and mode of action, consisting of five major classes: pyrethroids, chlorinated hydrocarbons, methyl carbamates, organophosphorus compounds and neonicotinoids (EPA, 2021).

1.3.1 Neonicotinoids

Neonicotinoids, meaning “new nicotine-like insecticide”, has become one of the highest selling and most widely used group of insecticides due to their effective action against target organisms (e.g., chewing insects like plant hoppers and coleopteran pests) as well as their easy and extensive application range (Jeschke and Nauen, 2008). Neonicotinoids are divided into two groups: N-cyanoamidines (containing a cyano group) and N-nitroguanidines (containing a nitro group). N-cyanoamidines include thiacloprid and acetamiprid. N-nitroguanidines include inter alia, imidacloprid, thiamethoxam and clothianidin. Neonicotinoids are systemic, meaning they are distributed throughout the plant via the sap stream making the entire plant toxic to the target insects (van Gestel et al., 2017). Due to neonicotinoids being highly soluble molecules they also hinder transmissions of vector viruses indirectly (Jeschke and Nauen, 2008). As a result of the difference between the number of nicotinic receptors in the nervous system between vertebrates and invertebrates, neonicotinoids show a higher toxicity to insects and other arthropods (Gibbons, Morrissey and Mineau, 2015; Simon-Delso et al., 2015).

Although synthetised in the 1970s, neonicotinoids were patented and sold as a commercial product in the mid 1980s with a peak in the 1990s. The neonicotinoids was perceived as

having low risk to the environment as well as to non-target organisms, they were also considered as a milestone in agricultural research, replacing other hazardous insecticides (e.g., organophosphates) (Jeschke and Nauen, 2008). However, the substances have recently come under increasing scrutiny for their impact on non-target organisms. Their broad methods of application as seed-dressing agents, soil treatment or spraying increases the possibilities of soil dwelling invertebrates being affected by the neonicotinoids (Tomizawa and Casida, 2005; Jeschke et al., 2011).

Neonicotinoids today

In the mid-2000s, after several studies raised awareness of the effect neonicotinoids had on beneficial pollinators, the European Safety Authority (EFSA) conducted a risk assessment that lead to a moratorium on three neonicotinoids in 2013 and then a total ban including use on all outdoor field crops of certain neonicotinoids in 2018 (imidacloprid, clothianidin and thiamethoxam) (EFSA, 2018). An authorisation may be given to these neonicotinoids if it is only used as an insecticide in a permanent greenhouse, or for the treatment of seeds intended to be used only in permanent greenhouses. However, several Member States in the EU have repeatedly granted emergency authorisations for outdoor usage for some of the banned pesticides (EFSA, 2020). Neonicotinoids are also still registered globally for agricultural and non-agricultural uses, such as lice treatment both on pets and in aquatic farming (Naiel et al., 2020, Thompson et al., 2020). In the US, EPA wants to continue to allow five neonicotinoid pesticides to remain in the US marketplace (EPA, 2020).

Neonicotinoids have low sorption in soil and are known to contaminate terrestrial ecosystems (Selim, Jeong and Elbana, 2010). Depending on the pH, temperature, type of soil, moisture etc., neonicotinoids can accumulate and persist in soils ranging from 1 day to almost 19 years (Goulson, 2013; Bonmatin et al., 2015; Wood and Goulson, 2017; van Gestel et al., 2017). Even if the persistence of neonicotinoids presents a potential environmental health concern that has been previously highlighted by recent research and public health agencies, the current scientific literature is mainly focused on the impact of neonicotinoids on pollinators and some aquatic insects. Since contamination of neonicotinoids into different ecosystems can take place through multiple pathways, gathering knowledge about how it affects the soil fauna is of utmost importance. Some toxicity data on soil invertebrates is available. The Organisation for Economic Co-operation and Development (OECD) have set internationally accepted specifications for the testing of chemicals through standardised test guidelines. There are two

OECD test guidelines set for assessing the effects of chemicals on the reproductive output of soil dwelling microarthropods, specifically for soil dwelling springtails (*Folsomia candida* and *Folsomia fimetaria*) and soil dwelling predatory mites (*Hyoaspis aculeifer*) (OECD, 2016). However, natural stressors are not included in OECD tests and environmental conditions are kept optimal, whereas free-ranging species seldom have optimal conditions.

1.3.2 Combined effects of a changing climate and pesticides

Predicting and understanding the effects of multiple stressors to ecosystems is crucial yet challenging. Anthropogenic activities expose ecological systems to a wide range of stressors that can act in concert with pollutants. These stressors and their direct, indirect and/or interactive effects can vary depending on the ecosystem, species and the characteristic of the stressor (Holmstrup et al, 2010; Tilman et al, 2014). An understanding of how multiple stressors impact ecosystems, especially agricultural systems, will improve the ability to protect, manage and assess the systems as well as contributing to an understanding of fundamental ecological principles.

Recent global circulation models predicts changes in precipitation patterns and soil moisture contents the next years, an increase in rainfall in the higher latitudes and a decrease in rainfall in the lower latitudes of the northern hemisphere are to be expected (IPCC, 2007, 2014). Water is often the most limiting factor for crop growth and changes in water availability can cause severe alterations in agricultural ecosystems. Pesticides like neonicotinoids have the tendency to leach from soils if water is present and potentially pass over to a wider environment, affecting larger variety of organisms (Wood and Goulson, 2017). Studies have also shown extremes in rainfall can interact with pesticides and cause short-term shifts in microbial community function and long-term shifts in bacterial community structure indicating a redundancy in soil functions (Ng et al., 2014). Studies have also shown that prolonged drought in soil can increase pesticide soil pollution over time (Goulson, 2013), as well as affect the pesticide's toxic potential for ecological receptors (Delcour, Spanoghe and Uyttendaele, 2015; Ogungbemi and van Gestel, 2018). Reduction in soil moisture can in itself also have a negative effect on surface dwelling arthropods that inhabit important roles such as cycling nutrients, decomposition of organic matter and maintenance of soil structure (Dai, 2013).

1.5 MULTICLIM

This thesis is part of the project Effects of climate change in a multiple stress multispecies perspective (MULTICLIM), financed by the Research Council of Norway (RCN) through the large-scale Programme on Climate Research (KLIMAFORSK). The project MULTICLIM is studying the complex question of how multiple stressors (climatic stress and human-made toxicants) affect biological responses at the individual and population level in springtails by combining both laboratory, field and modelling studies to disentangle the joint responses. Within MULTICLIM, laboratory tests with a single springtail species and controlled treatments have provided valuable data that can be used as a basis for investigating how combinations of natural and chemical stressors work under realistic conditions. The advantage of field study gives the opportunity to measure and manipulate the effects of the changes in natural communities while also representing realistic scenarios.

Studies on sublethal effects of short-term imidacloprid exposure and postexposure recovery in the springtail *Folsomia quadrioculata* have been conducted under laboratory conditions, suggesting that low imidacloprid exposures can restrict reproduction, with potentially severe consequences for the population (Sengupta et al., 2021). How soil communities respond to climate change is still an understudied topic, and understanding what the combined impacts of neonicotinoids and other environmental stressors have on these organisms is of growing importance because of their crucial role in providing the services of ecosystems, especially to agricultural ecosystems.

1.6 Aim and objective

The overall aim of this study was to determine how the abundance of a soil community is affected by the neonicotinoid imidacloprid under a future climate by looking at springtails and mites. The natural soil community was manipulated by adding the springtail species *Folsomia quadrioculata*, from which there is comparable data from laboratory studies (Sengupta et al., 2021). In particular, the objectives and hypotheses tested were as follows:

H1) The effects of the water-treatment together with increasing concentrations of imidacloprid will have a negative effect on the abundance of springtails, especially in the no-watering blocks as imidacloprid degradation is reduced in dry soils.

H2) Watering will lead to increased leaching, and thus lead to lower concentrations in the surface and elevated imidacloprid levels in the deeper soil with time

H3) The abundance of *F. quadrioculata* will be reduced in a concentration-dependent manner when exposed to increasing concentrations of imidacloprid

H4) The abundance of coexisting springtail species will be reduced in a concentration-dependent manner when exposed to increasing concentrations of imidacloprid. Compared to the other springtails, the soil dwelling springtails will be greatly affected over time due to imidacloprid leaching in to the deeper soil with time

H5) Due to the mites resistance to anthropogenic compounds and desiccation, the abundance of mites will not be reduced in a concentration-dependent manner when exposed to increasing concentrations of imidacloprid, nor will they be affected by the watering regime.

2 Materials and Methods

2.1 Study species

2.1.1 *Folsomia quadrioculata*

Folsomia quadrioculata (Tullberg 1871, Isotomidae, Figure 1) is a Holarctic species dominating different types of habitats, including arctic and temperate sites (Fjellberg, 2007). *F. quadrioculata* may reach up to 2.5 mm in length, and is slightly pigmented with a light greyish colour (Fjellberg, 2007). It is a highly successful soil and litter-dwelling species found in habitat types ranging from open meadows and dense forests at temperate sites, to dry ridges and wet vegetation in the Arctic (Sømme and Birkemoe, 1999; Ponge, 2000; Chimitova, Chernova and Potapov, 2010; Sengupta, 2015). The responses to toxic exposure seen in *F.quadrioculata* in other studies (Coulson et al., 2000; Krab et al., 2010; Sengupta, 2015; Roos et al., 2020), suggests that an assessment of responses to the exposure to neonicotinoids require validation across springtail species more typical of natural environments. Making the *F. quadrioculata* a suited study organism.

Figure 1. The study species *Folsomia quadrioculata* observed in a sample. Photo credit: Mia Drazwkowski Teksum

2.1.2 Ecological groups of springtails

The main ecological types of springtails (Figure 2) used in this study has roughly been divided into three groups of species: epiedaphic (surface dwelling), hemiedaphic (litter dwelling) and euedaphic (soil dwelling) species. These groups, distinguished by certain morphological and genetic features, are based on the springtails vertical distribution in the soil column and morphological traits related to moisture preference and habitat width (Hopkin, 1997; Ponge, 2000; Makkonen et al., 2011). Surface dwelling springtails are characterised by their pigmented bodies and number of ocelli (eye spots). These organisms live mainly on top of the soil or litter surface where they are subjected to a higher predation and highly variable environmental conditions, and as a result are more adapted to soil surface conditions (Makkonen et al., 2011). Species of springtails that are litter dwelling live mostly within the litter layer and are often somewhat pigmented with a reduced number of eye spots. Lastly the soil dwelling springtails often consist of unpigmented species that live and feed in the soil, with or without visible ocelli and are less adapted to environmental fluctuations (Hopkin, 1997; Fjellberg, 2007).

Figure 2. Figure showing a pigmented surface dwelling springtail, *Hypogastrura viatica*.
Photo credit: Hans Petter Leinaas

2.1.3 Mites

Within the soil fauna, species of mites (Figure 3) are often used as bioindicators to investigate the ecological stage of soils from natural or anthropogenic ecosystems due to their high population density, species richness, low mobility and a responsiveness to a variety of soil and environmental conditions (Manu et al., 2019; Meehan et al., 2019). Families of mite species have also been found as strong indicators of agricultural disturbances (Behan-Pelletier, 1999; Gergócs and Hufnagel, 2017). Although the community composition of mites may differ between type of soils and habitat, a resistance to synthetic chemicals has been studied in economically relevant species of mites. In these laboratory studies, an exposure to neonicotinoid pesticides has been found to increase the fecundity of some mites species that are damaging pests of horticultural and field crops throughout the world (James and Price, 2002; Szczepaniec et al., 2011).

Figure 3. Figure showing two types of naturally occurring soil mites in an agricultural field, a predatory *Gamasida* (1) and three *Oribatida* (2). Photo credit: Heidi Sjursen Konestabo

2.2 Study chemical: imidacloprid

The Imidacloprid CAS-name is 1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine (C9H10ClN5O2), and the IUPAC-name is (E)-1-(6-chloro-3-pyridyl-methyl) Nnitroimidazolidin-2-ylideneamine (Figure 4). Imidacloprid, like the rest of the N-nitroguanidines, has shown to be more toxic to insects than N-cyanoamidines by binding much more strongly to insect neuron receptors than to mammal neuron receptors (Jeschke et al., 2011; Mani, Shivaraju and Kulkarni, 2014).

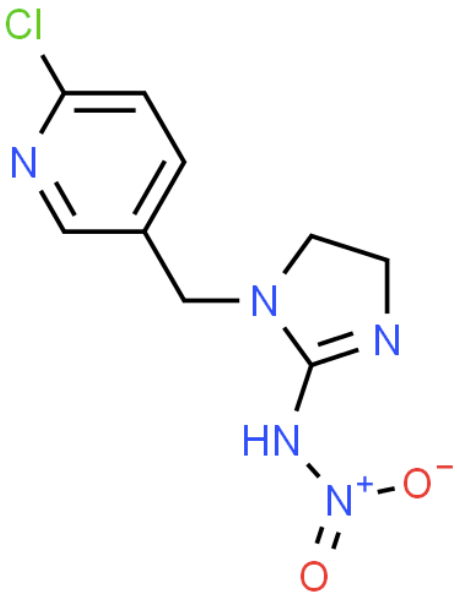

Figure 4. Molecular structure of the neonicotinoid imidacloprid. Source: Chemspider.

Like the other neonicotinoids, imidacloprid acts as an agonist on the nAChR receptors of insects, found in the synaptic neuropil regions of the insects' central nervous system, evoking the same effect as the natural neurotransmitter acetylcholine (ACh) by causing an inward current that leads to action potentials being generated (Jeschke, Nauen and Beck, 2013). Imidacloprid is a photosensitive compound in the aqueous phase and breaks down quickly in water when exposed to light at wavelengths between 200 and 300 nm ((Sharma, Toor, and Rajor 2015)). However, it can persist in soil for more than 100 days depending on the soil depth (Zheng et al., 2004; van Gestel et al., 2017; Wood and Goulson, 2017; Silva et al., 2019).

2.3 Laboratory procedures

Pure imidacloprid (CAS No. 138261-41-3, Sigma-Aldrich) was dissolved in distilled water and dilution was performed to obtain the right concentration. Due to the rapid degradation, the stock solution was prepared in a dimly lit room, and the stock and diluted solutions were kept in containers covered with aluminium foil and stored at +4 °C in a refrigerator out of UV-light. The concentrations calculated in this thesis are based upon the imidacloprid concentrations in the natural LUFA 2.2 standard soil.

To calculate the right amount of volume needed, from the stock solution, to make the final solution, the dilution equation below was used:

$$C_1V_1 = C_2V_2 \qquad \text{(Equation 1)}$$

Where C_1 is the concentration of the starting solution, V_1 is the volume of starting solution required to make a new solution, C_2 is the concentration of the new solution and V_2 is the final volume of the new solution. In these dilutions, the unknown was the volume of starting solution, V_1.

The calculations done were based upon a measured mean of soil that each mesocosm contained and the number of mesocosms needed. The mesocosms contained approximately 95 g soil and the number of mesocosms needed in the experiment was 190 units. The different concentrations of imidacloprid where then calculated by adding 2 ml of imidacloprid to 95 g soil.

Stock solution

0.1 mg imidacloprid powder was weighed in a disposable weighing boat on a Mettler Toledo AG204 Analytical Balance. The powder was transferred into a 500 mL bottle before adding 105 mL of distilled water. Imidacloprid and water were mixed using a magnet agitator set at 400 rpm. Subsequently, 24.9375 mL of the solution was diluted to 200mL with distilled water. This was done to obtain a stock solution with the highest concentration used in the experiment, 2.5 mg/kg dry soil, which would be achieved when adding 2 mL solution to a mesocosms containing 0.095 kg dry soil.

Final solutions

Concentration 2.5mg/kg: From the stock solution 100 mL was taken out with a mechanical pipette (Eppendorf 50 ml) into a 100 mL volumetric flask.

Concentration 0.5mg/kg: From the stock solution 20 mL was taken out with a mechanical pipette (Eppendorf 50 ml) into a 100 mL volumetric flask and distilled water was added, so the volume reached the line of the flask. The solution was stored in a 100 mL centrifuge tube.

Concentration 0.1mg/kg: From the stock solution 4 mL was taken out with a mechanical pipette mechanical pipette (Eppendorf 1000 µl) into a 100 mL volumetric flask and distilled water was added, so the volume reached the line of the flask. The solution was stored in a 100 mL centrifuge tube.

Concentration 0.02 mg/kg: From the stock solution 0,8 mL was transferred with a mechanical pipette (Eppendorf 1000 µl) into a 100 mL volumetric flask and distilled water was added, so the volume reached the line of the flask. The solution was stored in a 100 mL centrifuge tube.

Concentration 0 mg/kg: 100 mL of distilled water was transferred with a mechanical pipette (Eppendorf 50 ml) into 100mL centrifuge tube and stored together with the other dosages.

2.3.1. Culturing of study species

Adult individuals of *F. quadrioculata* were sampled from a stock culture at the Department of Biosciences, University of Oslo (UiO). The populations from the stock cultures had been obtained in 2007 from randomly collected and intact soil core samples from a spruce forest close to Ås, Norway. The culture were kept on moist plaster of Paris mixed with activated charcoal in 30 ml plastic boxes (d = 3.5 cm, h = 3 cm) in temperature cabinets (Sanyo MIR 553; accurate to ±0.5°C) at 15°C, a favourable temperature for the animals (Sengupta, Ergon and Leinaas, 2016). The animals in the cultures are fed with pieces of bark from trees covered with cyanobacteria and green algae and humidity conditions are kept by adding droplets of distilled water to the plaster. Stock culture boxes contain about 100 animals to minimise the risk of differences in culturing conditions such as food quality as well as competition (Sengupta, Ergon and Leinaas, 2016). To collect the animals from the stock cultures, a stereo microscope at x6 magnification was used and a thin brush damped with water. The collected animals were then kept in new 30 ml culture boxes with 20 individuals per box at +15°C (Figure 5).

Figure 5. Figure showing culture boxes with plaster of Paris. Photo credit: Mia Drazkowski Teksum

2.4 Study design

2.4.1 Study site

The field study was carried out at Kjerringjordet (59º 39’ N, 10º 45’ E) in Ås, Viken, Norway, close to Oslo (Figure 6). Kjerringjordet, situated 78 meters above sea level, is located in an agricultural area surrounded by fields of crop production, and where the study species *F. quadrioculata* is a naturally occurring species. No treatment with pesticides had been done on the site previously. A week before the experiment was started, the grass in the experimental field was cut.

Figure 6. Map showing an overview of the study site, Kjerringjordet at Ås, Viken, Norway. The red circle shows the field site where the experiment was conducted. Source: Kartverket

2.4.2 Study design

The experiment was started 16. May and continued until 5. August 2019. To minimise the effect of local variance on the experiment, a block design with replicates of intact soil cores in mesocosms was chosen. The block design consisted of 16 1x1 m blocks placed 1 m apart, in a grid pattern across the field. Eight blocks were watered twice a week, and eight blocks only received natural precipitation. Within each block, a 4x4 grid plot was established where 13 mesocosms were installed, each mesocosm separated with 12 cm between each position (Figure 7). Each block was measured accordingly to the study design (1x1 m with 1 m between each block and 12 cm between each mesocosm position within the block) by using a self-retracting metal tape measurer. The plots without mesocosms were sampled for natural abundance at different times during the experiment. The block design thus had a total of 256 sample plots all together. Positions of mesocosms and sampled plots were chosen through randomisation (Appendix A).

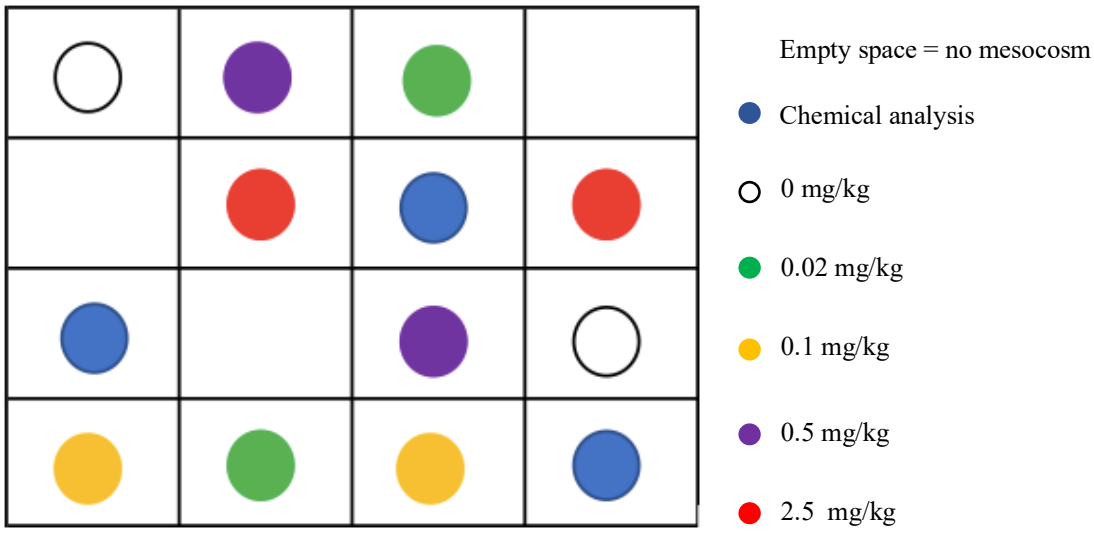

Figure 7. The figure shows an example of how a block looked like in the experimental field. The circles in each square represents the position of the mesocosms and the correspondent soil samples where each sample plot was separated by 12 cm. The sample plots consisted of correspondent soil samples with no mesocosms (empty space), mesocosms for chemical analysis (blue) and mesocosms with treatment: 0 mg/kg (white), 0.02 mg/kg (green), 0.1 mg/kg (yellow), 0.5 mg/kg (purple) and 2.5 mg/kg (red).

The mesocosms consisted of cylindrical plastic containers (h=5 cm , d= 5 cm), (Figure 8) with a bottom and top lid covered by a fine mesh net (0.3 mm) making it possible for water and gas to exchange naturally between cylinder and the environment, but preventing the animals from moving in and out of the cylinder. Each mesocosms was labelled with block letter and position number, and put back in place from where the soil core was taken. 20 adult *F. quadrioculata* were added to each mesocosms, and the mesocosms were treated with 5 different imidacloprid concentrations equivalent to 0, 0.02, 0.1, 0.5 and 2.5 mg/kg dry soil. A watering regime was also conducted to manipulate the water availability. All mesocosms, including the plots without mesocosms, was randomised within the block. The mesocosms used for chemical analysis contained no added *F. quadrioculata*.

Figure 8. The figure shows a mesocosms that has been in the ground for 81 days, covered with moss and grass growing out of the lid. Photo credit: Simen Kjellin

As a water treatment, an assumed precipitation for Ås during the experiment was simulated. The watering regime was set up in L/m2 by calculating the exact amount of water needed two times a week.. The chosen amount of water for the watering blocks were found through calculating 20% of the mean rainfall at Ås each day in May, June, July, and August from the period 2000 to 2018. The overall mean of monthly precipitation at Ås in May to August were; From the 16th of May 70.5 L/m2 and the entire months of June 77 L/m2, July 89 L/m2 and August 96 L/m2. Precipitation data was found through eKlima (now named seKlima), Norwegian Meteorological Institutes weather and climate data web page.

2.4.3 Field procedures

To sample intact soil cores including the litter layer the habitat metal, a metal soil corer (d = 5 cm) was used. This was done to get the natural structure of the soil and to preserve the remaining material in the soil core (Figure 9a). By pushing the corer approximately 4 - 10 cm down in the selected plot and by twisting the corer and lifting it the soil sample was collected. The lower half of the soil sample was adjusted by using a knife to cut off excessive soil if the sample was too deep. Each mesocosm was labelled with block letter and position number before placing the soil sample in to the mesocosms with the plant layer facing upwards. Each

mesocosm was placed back into the position where the soil column had been extracted. Grass and other plants in the sample that were too long was carefully cut with a scissor. 2 mL of the imidacloprid treatments were applied in situ in to each of the respective mesocosms (Figure 9b). To prevent the chemical to go through photodissociation (a chemical reaction in which a chemical compound is broken down by photons) aluminium foil was wrapped around the solutions, as well as plain white paper was used to shade when applying the treatment. Before carefully placing the lid onto the mesocosm 20 individuals of *F.quadrioculata* was added into each one. The mesocosms chosen for chemical analysis were treated with concentrations of imidacloprid similar as the other treated mesocosms, but without adding *F.quadrioculata*.

a)

b)

Figure 9ab. Figure showing soil corer (a) and applying of imidacloprid concentrations in situ in the field (b) using aluminium foil to prevent the chemical from going through photodissociation. Photo credit: Mia Drazkowski Teksum

Watering blocks were watered Mondays and Thursdays each week. Distilled water was used to prevent possible high concentrations of minerals from regular tap water, using a pressure sprayer. It was made sure not to step inside of the blocks while watering, and rather make a path on the outside to not disturb the soil fauna during the setup and the duration of the entire experiment.

2.4.4 Sampling over time

During the set-up of the experiment in May, 26 soil cores were sampled as start of the experiment. After 6 and 12 weeks, mesocosms were sampled from each block (106 in June, 112 in August). A number of mesocosms in each blocks were collected each sampling both from the "watering" and "no watering" blocks. Mesocosms where chosen by randomisation (Appendix A). Additional corresponding soil samples without a mesocosm was also collected from the empty position in each block. This was done to get an understanding of the natural occurrence at the experimental site. Sampled mesocosms, corresponding soil samples and samples for chemical analysis were put in labelled plastic bags and transported to the lab at UiO for weighing of fresh weight (FW). Soil samples for chemical analysis were also sampled during each sampling time, these were weighed for fresh weight and then immediately stored in a freezer (-18°C) for further analysis. Having the chemical analysis-mesocosms in a freezer was done to prevent the soil column to dry out as well as preventing breakdown of imidacloprid. Consequently, there were no extraction and counting of soil fauna in samples for chemical analysis.

2.5 Extraction and analysis of soil fauna

Each soil core and mesocosms were weighed for fresh weight with both lids, containers and plastic bags. Before extraction of soil fauna the soil column had to be taken out of the mesocosm, this was done by tapping the edge of the lid against the end of a table until the lid popped off. The soil column was then gently put into new containers in an inverted position, surface layer down, over a collecting vessel containing water saturated with benzoic acid (Figure 10). Soil organism were extracted in extraction apparatus modified after Macfadyen (1961) used to extract living organisms, particularly soil arthropods (Macfadyen, 1961). The extraction was done by creating a temperature gradient by having a cooling system at the bottom of the apparatus and a heat source from above, making the organisms move away from higher temperatures and into collecting vessels (Halbritter et al., 2020). Mesocosms, lids and plastic bags were weighed separately during the extraction time.

After each extraction all soil core samples was weighed for dry weight. The extracted soil fauna from the collecting vessels were removed by using a pipette, water saturated with dishwashing soap (to allow the microarthropods to sink to the bottom of the vessel) and Leica stereo-microscope, this process was done to collect and place the soil microarthropods into

new containers for further examination. The extracted organisms were put in glycerol for preservation and kept in a temperature room at +4°C.

Figure 10. Figure of extraction apparatus modified after Macfadyen (1961) showing containers with soil. Photo credit: Heidi Sjursen Konestabo

The springtails from each sample was counted under a Leica microscope, as well as identified and grouped by family by using identification keys (Fjellberg 1998; 2007). *F. quadrioculata* was the only species of springtail that was identified by genus. Individuals of springtails that could not be identified was sorted as "unidentified springtails". Individuals of *Symphypleona* that was too difficult to identify was sorted as "unidentified *Symphypleona*". Juvenile organisms difficult to identify by species was sorted as "juveniles". Other soil microarthropods like mites were identified by subclass Acari and pooled together, while others organisms found in the soil samples were identified by class and grouped together accordingly. The samples were analysed by sample time (from samplings done in May, June and August 2019) and each sample inspected was randomised so that chemical treatment was not known during the counting and identification.

2.6 Determination of moisture content in soil samples

Before extracting the soil fauna, the soil samples were weighed for fresh weight (FW). After extraction of soil fauna the now dry soil samples was re-weighed for dry weight (DW).
The % moisture content (given in Appendix B) was calculated using Equation 2:

$$Moisture\ content\ \% = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{DW}} \times 100 \qquad \text{(Equation 2)}$$

2.7 Imidacloprid analysis

2.7.1 Preparation of samples

Chosen soil samples for chemical analysis were moved from the freezer and put in to a refrigerator (4°C) for approximately 30 minutes before preparation. This was done to let the thawing process start but continuing to keep the samples cold, which prevented them from drying out. The samples was kept away from light sources during the thawing process to prevent breakdown of imidacloprid. The preparation process was also done without a direct light source in the working area to further prevent a possible breakdown of imidacloprid.

All preparations of the soil samples was done in a fume hood to reduce exposure of contamination. When ready, a sample was taken out of the refrigerator and by tapping the mesocosm lid to the edge of a table, popping off the lid. The soil column was then taken out of the mesocosm and into the fume hood on a plastic cutting board. Each sample was cut with a knife into three different parts; upper, middle and lower part of the soil column to analyse soil distribution. A sub sample of each part was taken out, grinded and crushed with a metal sieve and then a mortar and pestle to homogenise the soil sample without pebbles, roots and other apparent plant material. Approximately 2 g from each sub sample was weighed on a Mettler Toledo Standard ICS425 Scale in a 15 ml Falcon tube. Exact weight was noted down and the vial stored dark and frozen (-18°C) until freeze drying. Excess soil from each segment was put in individual labelled plastic bags and put back in to the freezer. Between each soil sample preparation, the cutting board, knife, metal sieve and mortar and pestle was washed with dishwasher soap and dried before cleaned with an ethanol based surface disinfectant. This was done to prevent contamination between samples.

2.7.2 Freeze drying process

Approximately 3 hours prior to the freeze drying the soil samples, a pre-treatment of elements, such as vacuum hood, product shelves and a metal plate, of Leybold-Heraeus GT2 Freeze Dryer were put in a freezer (-18°C). This was done to keep the sample temperature low enough during the process to avoid changes in the dried product appearance and characteristics. A start-up of the Leybold Vakuum GmbH vacuum pump was also initiated. The soil samples in the Falcon tubes were then stored in the product shelves on top of the metal plate, the lids of the Falcon tubes were taken off and then the samples were freeze dried for 24 hours. A process that removes ice and other frozen solvents through sublimation (removing the ice crystals from the soil) and removal of bound water molecules through the process of desorption. This way of dry storage is useful where the catalytic efficiency can be increased by 5 to 20 times preserving the chemical for further studying.

2.7.3 Chemical analysis

Soil samples were analysed to examine the measured content of imidacloprid (µg/g per sample). The analyses were performed by collaborators at the Norwegian Institute of Water Research (NIVA) and the following procedure of chemical analysis of soil samples has been described in detail in Sengupta et al., (2021) and can be found in Appendix C. Here only a shortened version of the analysis will be described.

After the freeze drying process was completed, each samples was then preserved in darkness at -18°C prior to the high-performance liquid chromatographic–mass spectroscopic analysis of the imidacloprid content in soil. The homogenised soil (10 – 30mg) samples was weighed and added to a 15-mL tube. Prior to extraction of the content, samples were spiked with solutions and filtered before a liquid chromatography was performed on an Acquity BEH C18 column (1.8 µm, 100 × 2.1 mm; Waters). This was done by using an Acquity UPLC module. The UPLC system was coupled to a Xevo TQ-Standem mass spectrometer operating with an ESI interface. Screening of imidacloprid was performed with multiple reaction monitoring in positive ionization mode. The limit of detection of 0.1 ng g^{-1} imidacloprid was estimated to be 3 times the signal-to-noise ratio using spiked control samples. Due to economic and time-related issues only a few soil samples were analysed. The bottom and middle soil layers for May, treated with 0.1, 0.5 and 2.5 mg/kg, was not analysed.

2.8 Statistical analysis

All statistical analyses were performed using the statistical software R (version 4.0.3, the R Foundation for Statistical Computing 2016) and by using RStudio (version 1.2.5033). Raw data was processed in Microsoft Excel (version 16.49) for Mac. Figures were created using both RStudio and Microsoft Excel. An overview of the packages and the usage of these packages done in RStudio are shown in Table 1.

Table 1: Overview of the packages applied in R to do the statistical analysis of the data from the experimental design and the abundance of springtails and mites. The overview does not include the default packages that comes with R.

R-package	Citation	Use
ggplot2	(Wickham., 2016)	Data visualisation
drc	(Ritz et.al.,2015)	Analysis of Dose-Response Curves
dplyr	(Wickham et.al., 2020)	Reordering dataset
nlme	(Pinheiro et.al.,2019)	Comparing linear and nonlinear mixed-effects models.
tidyr	(Wickham., 2020)	Reordering dataset
vegan	(Oksanen et al.,2019)	Diversity analysis

The primary focus of the different analyses was to test whether the imidacloprid treatment, together with the watering regime, had an effect on the abundance of springtails and mites in the sampled soil cores over time. A second focus was to measure how that effect changed over time with respect to month. When possible, all groups were treated the same before statistical analysis. The statistical significance level was set to be $p<0.05$.

2.8.1 Data for exposure regime and biological response

For the analysis, each mesocosm was treated as the unit of replication. All model fits were checked using Residual vs. Fitted-, QQ-, Scale-location- and Residual vs. Leverage-plots. Correlations and differences between explanatory parameters in each dataset were checked and visualised using scatterplots, boxplots, correlations coefficients and variance inflation factors. Datasets for imidacloprid concentrations and abundance were assessed to meet the

assumptions of normality and homogeneity of variance with Shapiro Wilk's test (Shapiro and Wilk, 1965). When testing differences between more than two groups (e.g. groups of springtails), a one-way analysis of variance (ANOVA) was performed when the assumptions of normality and homogeneity were met. If they were not met, the cause for non-normality was determined, and a Wilcoxon signed-rank test was applied. Wilcoxon analysis is a non-paracontinuous-level test, meaning that it tests whether the difference between two observations has a mean signed rank of 0, and not requiring a special distribution of the dependent variable in the analysis (Wilcoxon, 1945). This was done by creating a pooled ranking of all observed differences between the two dependent measurements, and Wilcoxon uses the standard normal distributed z-value to test for significance.

The response variables consisted of the total abundance of F.quadrioculata, springtails and mites at each treatment type (Table 2). When addressing the effects of imidacloprid on abundance, it was expressed on the basis of nominal soil concentrations. A dose-response curve was analysed applying a general model fitting function with a non-linear regression model. A 95% confidence interval was also applied using a linear mixed-effects model estimating parameters between the upper and lower limits, this was done to detect a reduction or increase in abundance. A mixed effect model was also fitted to address how the different parameters that was counted varied with the imidacloprid concentrations.. The fixed effects was set as concentrations of imidacloprid and sampling time to see how the effect varied over time, while blocks was set as random effects. The mixed-effects model was applied using the function lme, specifically to specify the random components and to get p-values and determine statistical significance. (Laird and Ware, 1982).

As there were no *F. quadrioculata* counted in the control samples in May the datasets for dose-response and mixed-effects model was manipulated by plotting 20 individuals of *F.quadrioculata* in the samples from May. This was done to make the first sampling time May, as a reference point throughout the model fitting.

Table 2. Overview of the three explanatory variables used in the statistical analysis

Explanatory variable	Biological response	Exposure regime
Concentration	A numeric variable with five values: 0, 0.02, 0.1, 0.5, 2.5	A numeric variable with five values: 0, 0.02, 0.1, 0.5, 2.5
Sampling month	A numeric variable describing days of exposure from beginning and end of the experiment	A numeric variable describing days of exposure from beginning and end of the experiment
Watering	A chategorical variable describing the different watering blocks (Yes/No)	A chategorical variable describing the different watering blocks (Yes/No)

2.8.2 Model selection

Model selection was applied to derive the best-fitted model for each data set. Model selection included the most complex model, containing all parameters relevant for the model and simpler models (Table 2). Akaike´s Information Criterion (AIC) was used to evaluate and compare the best fit model for the data by distinguishing among a set of possible models describing the relationship between concentration and groups of organisms. AIC determines the relative information value of the model using the maximum likelihood estimate and the number of independent variables in a model. The best-fit model, carrying the lowest AIC, included every parameter with no interaction effect. For the dose-response curves, the built-in model functions in the R-package drc was applied (Table 1). These models are parameterised by using unified coefficients for denoting the steepness of the dose-response curves, the lower and upper asymptotes and the limits of response (Ritz et al., 2015). Within the drc-package a four-parameter log-logistic was used, where the parameters are based on the maximum likelihood-principle (MLE), and 3-parameter Weibull. MLE estimates the parameters of a probability distribution by maximizing a likelihood function, which means that under the assumed statistical model the observed data is most probable. The Weibull distribution is a continuous probability distribution reflecting the steepness of the dose–response curve with large values corresponding to steeper curves (Ritz, 2010). A single dose-response model is then fitted based when a number of plausible candidate models have been identified and the model-averaging approach is adopted.

As ANOVA does not assume correlated errors with multiple measurements per subject, a mixed effects model will estimates the effects of one or more explanatory variables on a response variable. Mixed models uses both fixed and random effects that correspond to a hierarchy of levels with the repeated, correlated measurement occurring among all of the lower level units for each particular upper level unit (Seltman, 2014).The within-group errors are allowed to be correlated and/or have unequal variances and a fitted mixed model provides straightforward predictions for unseen levels of random-effect factors. The output of a mixed model will give you a list of explanatory values, estimates and confidence intervals of their effect sizes, p-values for each effect, and at least one measure of how well the model fits.

3 Results

3.1 Watering treatment compared to rainfall

The total amount of added water in the watering treatments during the experiment had no effect on imidacloprid concentrations or abundance compared to the natural rainfall which all treatments were exposed to (Figure 11). From the beginning of the experiment (16.05.2019) until the start of June, there was a total rainfall of 82 L/m^2, which resulted in the water treatment added being only ~4 % on the rainiest days. The total added water was only 9 % of the total precipitation and not 20 % as estimated a priori. June had a total precipitation of 126 L/m^2 where the water treatment amounted to only 11 % of the total rainfall that month. In comparison July had less rainfall with 52 L/m^2 and water treatment being 30 % of the precipitation. August had a total of 1 L/m^2 precipitation the following days before the end of the experiment, and the blocks for water treatment in August were watered with a total of 2.25 L/m^2.

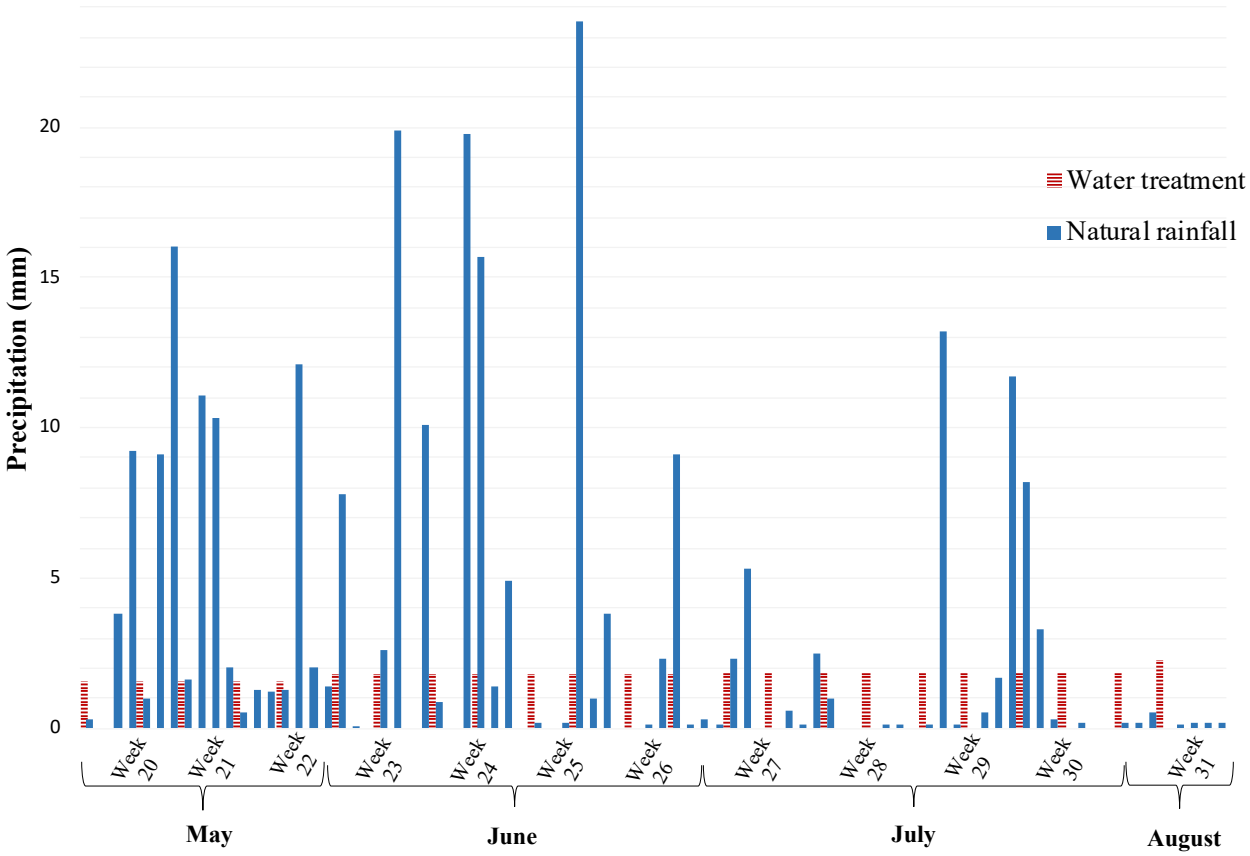

Figure 11. Histogram showing the daily amount of natural rainfall (blue bars) and the amount of water treatment (red stapled bars) that was applied every third day. Y-axis shows the amount of precipitation in mm and the x-axis shows the timeline (in weeks and months) of the experiment

No difference in moisture content in the soil samples between the watering blocks was found in June (mean moisture content in both blocks = 59%) or August (mean moisture content in both blocks = 23%) (Appendix B).

3.2 Imidacloprid exposure regime – time and soil layer

Surface layer

The nominal and measured soil concentrations of imidacloprid at start, middle and end of experiment, as well as in each soil layer are presented in Table 3. No effect was found between the watering treatment and the total abundance of all groups of springtails and mites in the control samples in June and August ($p > 0.05$). There were also no effect between the watering treatment, the abundance and the nominal concentrations of imidacloprid ($p > 0.05$) in the topsoil layer (Appendix D and E). As a consequence of the results from the watering treatment being affected by the total natural rainfall for the different concentrations of imidacloprid as well as having no clear pattern on the abundance of soil organisms during the experiment, the scope of this thesis has been narrowed to only include the effects of the different concentrations of imidacloprid.

Leaching to lower layers

An overall decrease over time in each soil layer for the measured concentrations was observed. May was found to consistently have the highest measured concentrations in the top soil layers for each nominal concentration, with the exception of one sample treated with 0.1 mg/kg imidacloprid (Table 3). A few samples in May was also found to have a higher measured concentration than the initial nominal concentration.

A reduction (50 %) was observed between May and August in the top soil layer at the nominal concentration 0.02 mg/kg. For the nominal concentration 0.5 mg/kg a relatively low reduction in concentration was observed in the top soil layer from May to June (32 %), and a slightly higher reduction from June to August (46 %). At the nominal concentration 2.5 mg/kg a reduction in concentration was seen from May to June (66 % and 65 %) with June to August having the lowest reduction of all samples observed (13 %).

Table 3. Results from the initial imidacloprid exposure in soil, with a comparison of the nominal concentrations (mg/kg) and the measured concentrations (µg/g) at start, middle and end of the experiment (May, June and August) as well as in each soil layer (top, middle and bottom). NA = not analysed

All imidacloprid concentrations measured in the top soil layers

Month	Soil layer	Nominal conc.	Measured conc.	Month	Soil layer	Nominal conc.	Measured conc.	Soil layer	Month	Nominal conc.	Measured conc.
May	Top	0	0.0011	June	Top	0	0.0003	Top	August	0	0.0006
May	Top	0	0.0012	June	Top	0	0.0006	Top	August	0	0.0005
May	Top	0.02	0.0132	June	Top	0.02	0.0156	Top	August	0.02	0.0069
May	Top	0.02	0.0288	June	Top	0.02	0.0167	Top	August	0.02	0.0111
May	Top	0.1	0.0003	June	Top	0.1	0.0013	Top	August	0.1	0.0268
May	Top	0.1	0.233	June	Top	0.1	0.0636	Top	August	0.1	0.043
May	Top	0.5	0.697	June	Top	0.5	0.341	Top	August	0.5	0.151
May	Top	0.5	0.296	June	Top	0.5	0.238	Top	August	0.5	0.102
May	Top	0.5	NA	June	Top	0.5	NA	Top	August	0.5	0.163
May	Top	0.5	NA	June	Top	0.5	NA	Top	August	0.5	0.188
May	Top	2.5	1.687	June	Top	2.5	0.434	Top	August	2.5	0.378
May	Top	2.5	1.265	June	Top	2.5	0.853	Top	August	2.5	0.287
May	Top	2.5	NA	June	Top	2.5	NA	Top	August	2.5	0.0102
May	Top	2.5	NA	June	Top	2.5	NA	Top	August	2.5	0.011

Table 3 Continued. Results from the initial imidacloprid exposure in soil, with a comparison of the nominal concentrations (mg/kg) and the measured concentrations (µg/g) at start, middle and end of the experiment (May, June and August) as well as in each soil layer (top middle and bottom). NA = not analysed

The highest imidacloprid concentrations measured in all soil layers

Month	Soil layer	Nominal con.	Measured conc.	Month	Soil layer	Nominal con.	Measured conc.	Month	Soil layer	Nominal con.	Measured conc.
May	Top	0	0.011	June	Top	0	0.0006	August	Top	0	0.0067
May	Middle	0	NA	June	Middle	0	NA	August	Middle	0	NA
May	Bottom	0	NA	June	Bottom	0	NA	August	Bottom	0	NA
May	Top	0.02	0.0288	June	Top	0.02	0.0156	August	Top	0.02	0.0111
May	Middle	0.02	0.0035	June	Middle	0.02	0.0082	August	Middle	0.02	NA
May	Bottom	0.02	0.0039	June	Bottom	0.02	0.007	August	Bottom	0.02	NA
May	Top	0.1	0.233	June	Top	0.1	0.0636	August	Top	0.1	0.043
May	Middle	0.1	NA	June	Middle	0.1	NA	August	Middle	0.1	NA
May	Bottom	0.1	NA	June	Bottom	0.1	NA	August	Bottom	0.1	NA
May	Top	0.5	0.697	June	Top	0.5	0.341	August	Top	0.5	0.151
May	Middle	0.5	NA	June	Middle	0.5	NA	August	Middle	0.5	0.128
May	Bottom	0.5	NA	June	Bottom	0.5	NA	August	Bottom	0.5	0.18
May	Top	2.5	1.687	June	Top	2.5	0.853	August	Top	2.5	0.378
May	Middle	2.5	NA	June	Middle	2.5	0.787	August	Middle	2.5	0.0072
May	Bottom	2.5	NA	June	Bottom	2.5	0.767	August	Bottom	2.5	0.0047

Residues of the nominal concentrations (with the exception of the controls and 0.02, 0.1, 0.5 mg/kg, Table 3) was found in the bottom soil layers in June and August for the measured concentrations. Comparing the measured with the nominal concentration 2.5 mg/kg, the highest measured concentration for a bottom soil layer was found in June, having a hight reduction (70%) from the initial exposure. Within the same nominal concentration, the reduction in a bottom soil layer between June and August was also found to be high (94 %) (Table 3). The highest measured concentration in a bottom soil layer was seen in August at the nominal concentration 0.5 mg/ kg imidacloprid (64 % reduction in concentration).

3.3 Biological response to imidacloprid exposure

Below only the results form the final (best) model from the model selection are presented. The overall variance in the abundance of springtail species and groups differed among treatments, as shown by the standard deviation values listed in Table 4, with two significant numbers and reduction in abundance shown with a 95% confidence interval (CI).

Table 4. Table showing the overall variance in the abundance of springtail species and groups differed among treatments. Results are given from a mixed effect model. Fixed effects are sampling time * imidacloprid concentrations. Random effect is set as block. Significant numbers are highlighted with grey.

						Random effect = Block	
Response	Fixed effect	Value	DF	t-value	p-value	StdDev	Residual
F. quadrioculata	June x 0.02	- 0.553	210	- 1.333	0.183	0.506	0.829
	June x 0.1	- 0.335	210	- 0.815	0.420		
	June x 0.5	- 1.039	210	- 2.506	0.013		
	June x 2.5	- 1.732	210	- 4.306	< 0.001		
	August x 0.02	- 0.210	210	- 0.506	0.613		
	August x 0.1	- 0.338	210	- 0.815	0.416		
	August x 0.5	- 1.167	210	- 2.813	0.005		
	August x 2.5	- 1.732	210	- 4.175	< 0.001		
Surface dwelling	June x 0.02	0.235	210	0.654	0.513	0.239	0.718
	June x 0.1	- 0.168	210	- 0.468	0.639		
	June x 0.5	0.015	210	- 0.044	0.964		
	June x 2.5	- 0.645	210	- 1.795	0.074		
	August x 0.02	- 0.302	210	- 0.842	0.401		
	August x 0.1	- 0.031	210	- 0.086	0.931		
	August x 0.5	- 0.246	210	- 0.685	0.493		
	August x 2.5	- 0.641	210	- 1.756	0.081		

Table 4 Continued. Table showing the overall variance in the abundance of springtail species and groups differed among treatments. Results are given from a mixed effect model. Fixed effects are sampling time x imidacloprid concentrations. Random effect is set as block. Significant numbers are highlighted with grey.

						Random effect = Block	
Response	Fixed effect	Value	DF	t-value	p-value	StdDev	Residual
Litter dwelling	June x 0.02	0.336	210	- 0.948	0.343	0.503	0.718
	June x 0.1	- 0.384	210	- 1.086	0.278		
	June x 0.5	0.941	210	- 2.657	0.008		
	June x 2.5	- 1.943	210	- 5.515	<0.001		
	August x 0.02	- 0.080	210	0.226	0.821		
	August x 0.1	- 0.154	210	- 0.436	0.662		
	August x 0.5	- 0.713	210	- 2.012	0.045		
	August x 2.5	- 2.019	210	- 5.700	<0.001		
Soil dwelling	June x 0.02	- 0.773	210	- 1.579	0.115	0.690	0.979
	June x 0.1	- 1.746	210	-3.567	<0.001		
	June x 0.5	- 2.068	210	- 4.223	<0.001		
	June x 2.5	- 2.569	210	- 5.246	<0.001		
	August x 0.02	- 0.316	210	- 0.654	0.519		
	August x 0.1	- 0.769	210	- 1.571	0.117		
	August x 0.5	- 1.831	210	- 3.740	<0.001		
	August x 2.5	- 2.885	210	- 5.246	<0.001		
Mites	June x 0.02	0.094	210	0.313	0.754	0.638	0.605
	June x 0.1	- 0.271	210	- 0.895	0.371		
	June x 0.5	- 0.212	210	- 0.702	0.483		
	June x 2.5	0.119	210	0.394	0.693		
	August x 0.02	- 0.128	210	- 0.424	0.671		
	August x 0.1	- 0.067	210	- 0.223	0.823		
	August x 0.5	- 0.220	210	- 0.728	0.467		
	August x 2.5	- 0.170	210	- 0.561	0.574		

3.3.1 Effects of imidacloprid on Folsomia quadrioculata

Overall, the abundance of *F. quadrioculata* had a concentration-dependent increase over time (Figure 12). A higher number of *F.quadrioculata* was counted in the mesocosms with added individuals compared to the corresponding control samples. The highest increase in abundance was seen through the entire experiment at 0.02 and 0.1 mg/kg, specifically. One outlier was found at 0.5 mg/kg in June and 0.1 mg/kg in August with a large increase in abundance (Figure 12). Due to the large variation of the data set, a dose-response curve based solely on *F.quadrioculata* was not viable. However, an increase and reduction in number of individuals with a 95 % CI was obtained, showing which concentration of imidacloprid affected *F. quadrioculata* the most throughout the experiment.

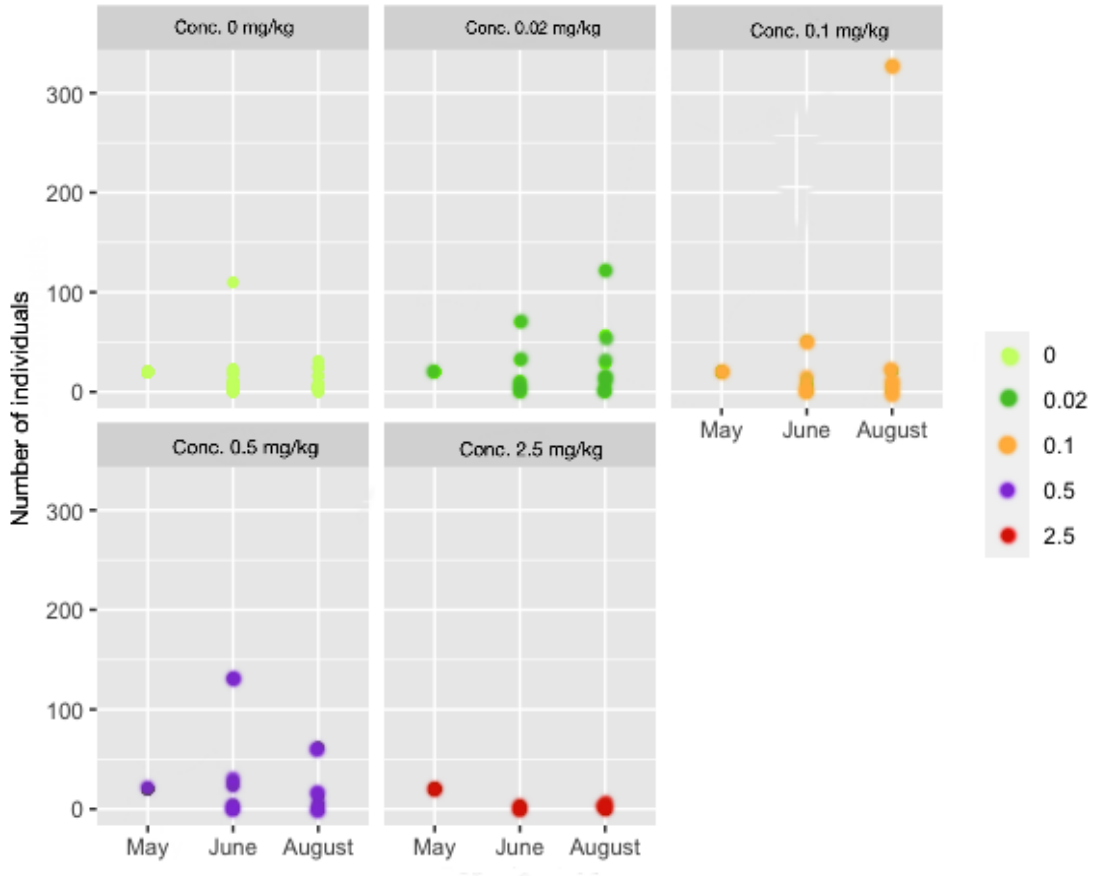

Figure 12. Scatterplots showing responses in abundance at each imidacloprid concentration for *F. quadrioculata* over time. Each plot represents concentrations of imidacloprid and the number of individuals counted per sample. Number of individuals in May is manipulated to be the added n = 20. Y-axis for all plots show the total number of individuals counted, x-axis show the time in months. Number of individuals per concentration are shown in different colours. Specific values are presented in Table 4.

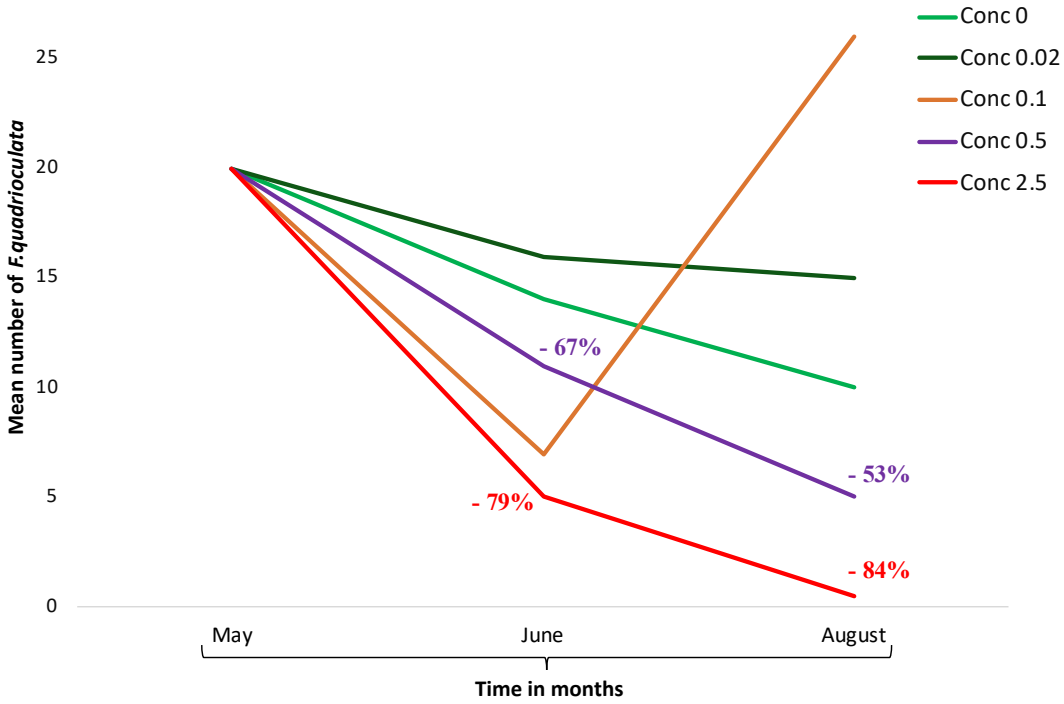

Figure 13. Line chart presenting the mean number of abundance between each treatment per month of *F.* quadrioculata from start of the experiment in May. Number of individuals at start of the experiment is manipulated to be the added n = 20. Y-axis show the total number of individuals, x-axis show the time in months. Significant numbers in abundance reduction are shown in %.

The concentrations 0.5 and 2.5 mg/kg was found to have the highest impact on the abundance of *F. quadrioculata* (Table 4). For June a significant effect on abundance of imidacloprid concentrations was found at 0.5 (p-value = 0.013) and 2.5 mg/kg (p-value< 0.001) (Table 4 and Figure 13). A significant reduction in abundance was observed at 0.5 mg/kg (95% CI = -0.85, -0.26) and 2.5 mg/kg (95% CI = -0.90,-0.53). A significant effect of imidacloprid concentrations on the total abundance was also found in August at 0.5 (p-value = 0.005) and 2.5 mg/kg (p-value< 0.001). A significant reduction was observed at 0.5 mg/kg (95% CI = -0.79, -0.54) and 2.5 mg/kg (95% CI = -0.93, -0.64) (Figure 13).

3.3.2 Effects of imidacloprid on springtail communities

Overall, the total number of springtails was reduced by the increasing concentrations of imidacloprid (Table 4). At the highest imidacloprid concentration 2.5 mg/kg, a specifically consistent and significant decrease in the total number of springtails in June and August was observed.

Surface dwelling springtails

The highest density in abundance of springtails was found in June at all concentrations (with the exception of 2.5 mg/kg), specifically in the control samples and the samples with low concentrations, 0.02 mg/kg imidacloprid (Figure 14). For June a close to significant effect of imidacloprid concentrations was only found at 2.5 mg/kg (p-value =0.07) and having no significant reduction in abundance (Table 4). For August an effect of imidacloprid was also only observed at 2.5 mg/kg (p= 0.08). A significant reduction in abundance was observed at 0.02 mg/kg (95% CI = -0.66, -0.81) and 0.5 mg/kg (95% CI = -0.76, -0.17), having a higher reduction in abundance compared to June (Figure 15). One outlier with a high number of individuals was seen in June at 0.02 and 0.5 mg/kg (Figure 14).

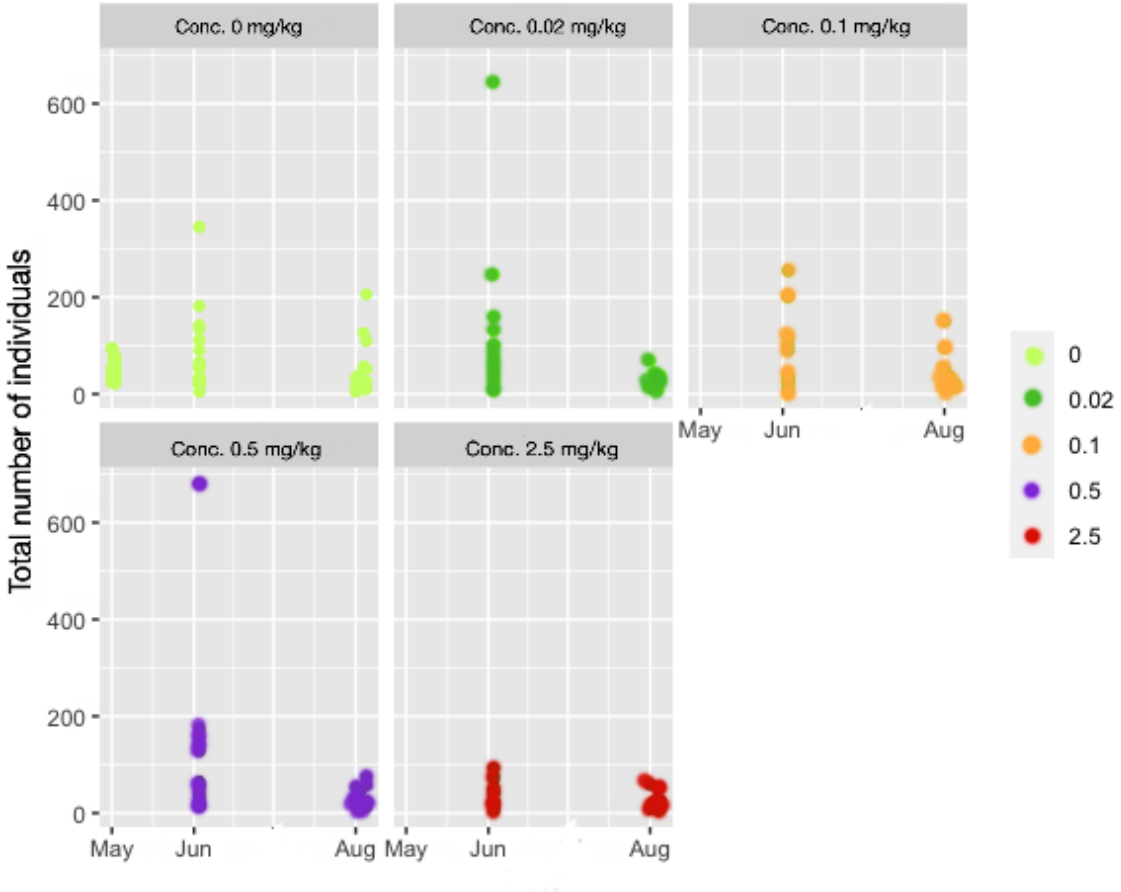

Figure 14. Scatterplots showing responses in abundance at each imidacloprid concentration for surface dwelling springtails over time. Each plot represents concentrations of imidacloprid and the number of individuals counted per sample. Y-axis for all plots show the total number of individuals counted, x-axis show the time in months. Number of individuals per concentration are shown in different colours. Specific values are presented in Table 4.

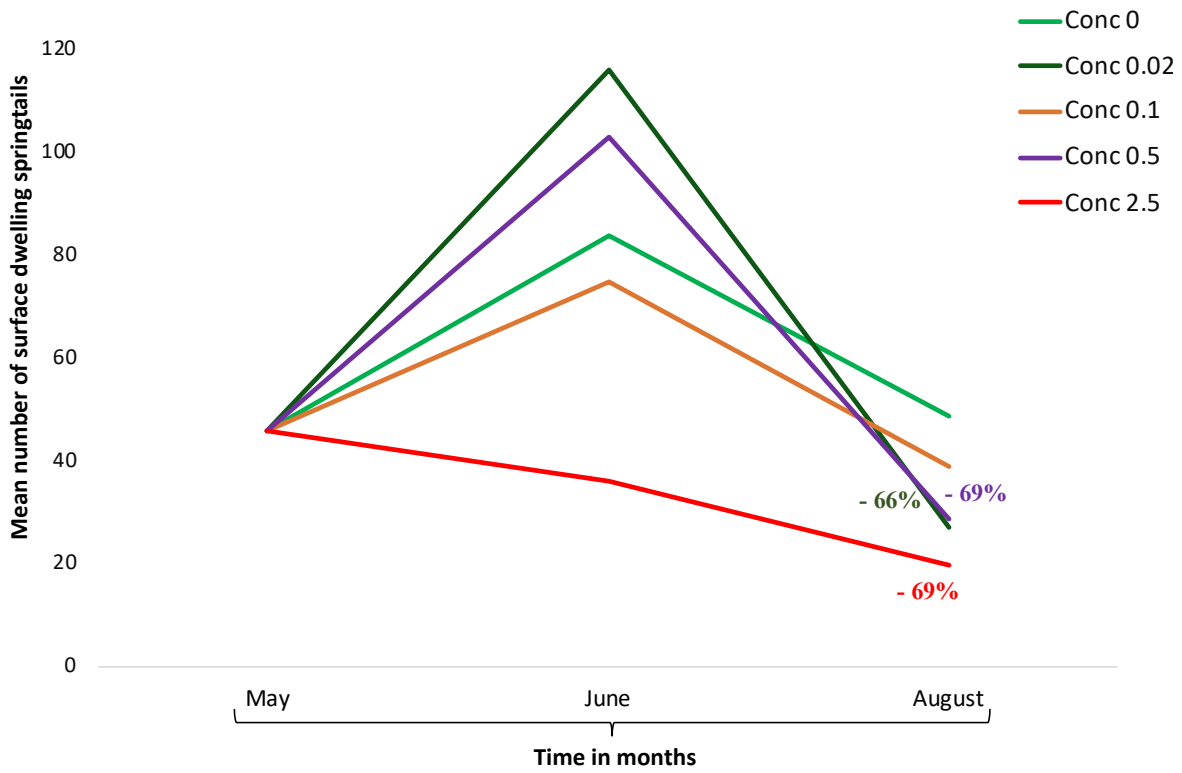

Figure 15. Line chart presenting a mean number of abundance at each treatment (0, 0.02, 0.1, 0.5 and 2.5 mg/kg) per month of surface dwelling springtails from start of the experiment in May (control mean n = 46). Y-axis show the total number of individuals, x-axis show the time in months. Close to significant numbers in abundance reduction are shown in %.

Litter dwelling springtails

A higher abundance of litter dwelling springtails was found in June, with the exceptions of a few outliers in August at the control groups and 0.02, 0.1 and 0.5 mg/kg imidacloprid (Figure 16). However, there was a negative relationship between increasing concentrations of imidacloprid and the overall abundance within the concentrations 0.5 and 2.5 mg/kg dry soil (Table 4).

Within the samplings in June, an effect of imidacloprid was found at 0.5 mg/kg ($p= 0.008$) and at 2.5 mg/kg ($p\text{-value} < 0.001$). A significant reduction in abundance was only observed at 2.5 mg/kg (95% CI = -0.94, -0.77) (Figure 17). Compared to June, August was found to have an overall higher reduction in abundance, specifically at 0.5 mg/kg ($p\text{-value}= 0.045$, 95% CI = -0.72, -0.11) and 2.5 mg/kg ($p\text{-value}< 0.001$, 95% CI = -0.95, -0.80).

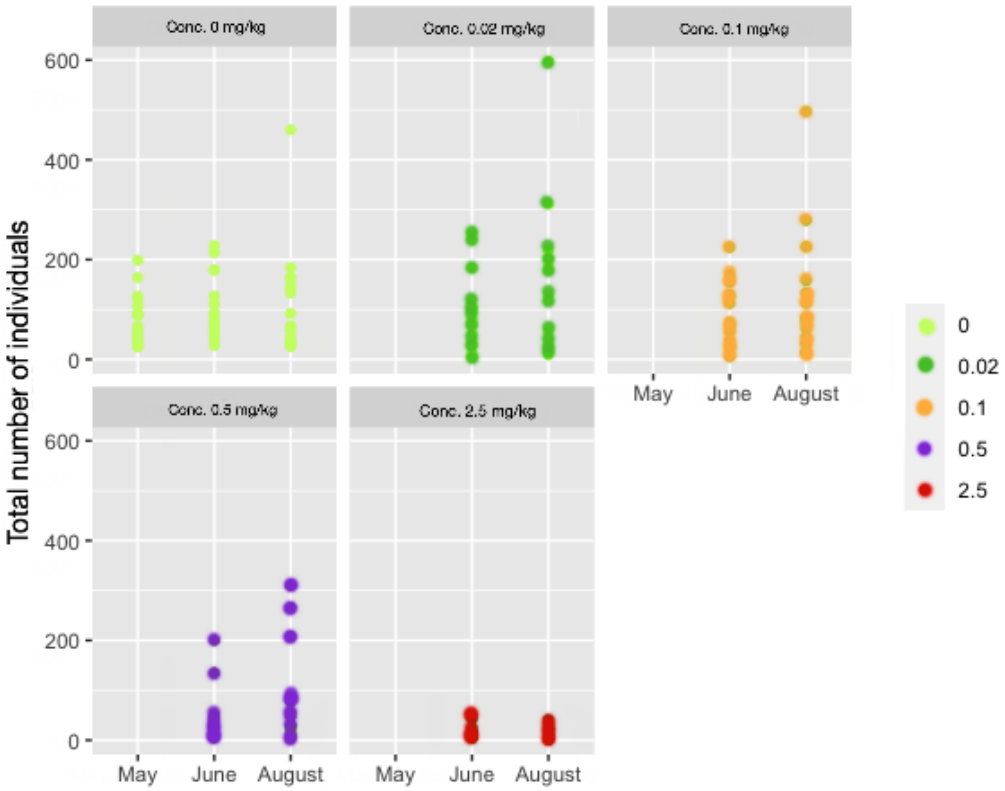

Figure 16. Scatterplots showing responses in abundance at each imidacloprid concentration for litter dwelling springtails over time. Each plot represents concentrations of imidacloprid and the number of individuals counted per sample. Y-axis for all plots show the total number of individuals counted, x-axis show the time in months. Number of individuals per concentration are shown in different colours. Specific values are presented in Table 4.

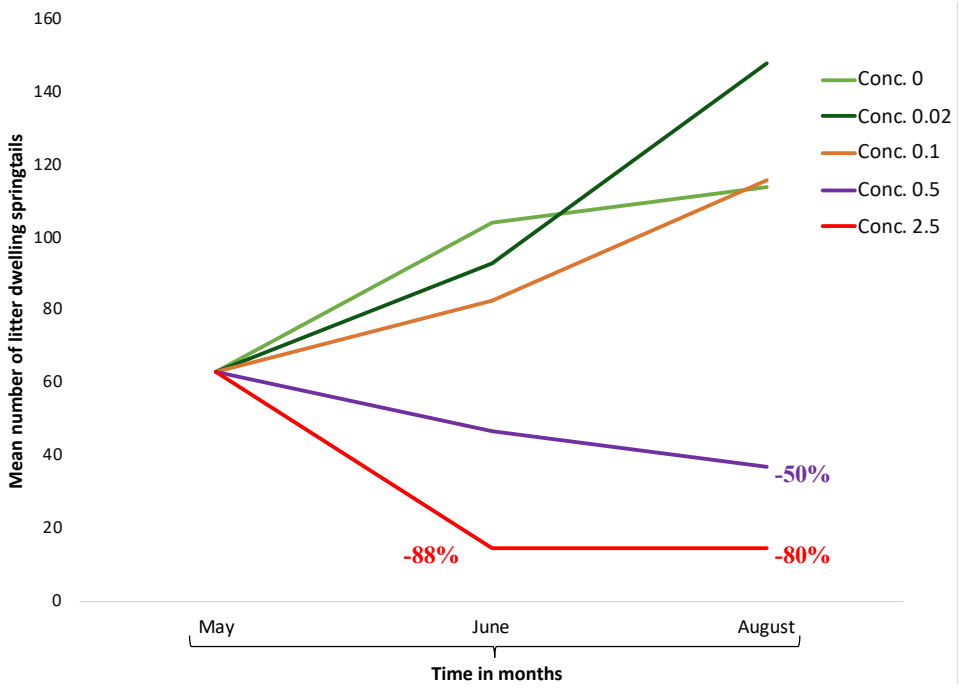

Figure 17. Line chart presenting a mean number of abundance at each treatment (0, 0.02, 0.1, 0.5 and 2.5 mg/kg) per month of litter dwelling springtails from start of the experiment in May (control mean n = 63). Y-axis show the total number of individuals, x-axis show the time in months. Close to significant numbers in abundance reduction are shown in %.

Soil dwelling springtails

The highest density in abundance of soil dwelling organisms was found in the control samples as well at 0.02 mg/kg, with the exception of an outlier at concentration 0.1 mg/kg in August (Figure 18). Overall, a lower abundance of organisms was observed with the increasing concentrations of imidacloprid in both the sampling in June and August. However, August was found to have a higher number of soil dwelling springtails (Figure 18 and 19). For June a reduction in abundance when exposed to imidacloprid was found at 0.1 mg/kg (p-value< 0.001, 95% CI = -0.94, -0.35). Effects were also found within the concentrations 0.5 mg/kg (p-value< 0.001, 95% CI =- 0.95, -0.73) and 2.5 mg/kg (p-value< 0.001, 95% CI = -0.97, -0.83) (Table 4).

In August at significant decrease in abundance of soil dwelling organisms was observed at both concentrations 0.5 mg/kg (p-value< 0.001, = -0.96, - 0.80) and at 2.5 mg/kg (p-value< 0.001, 95% CI = -0.98, - 0.90).

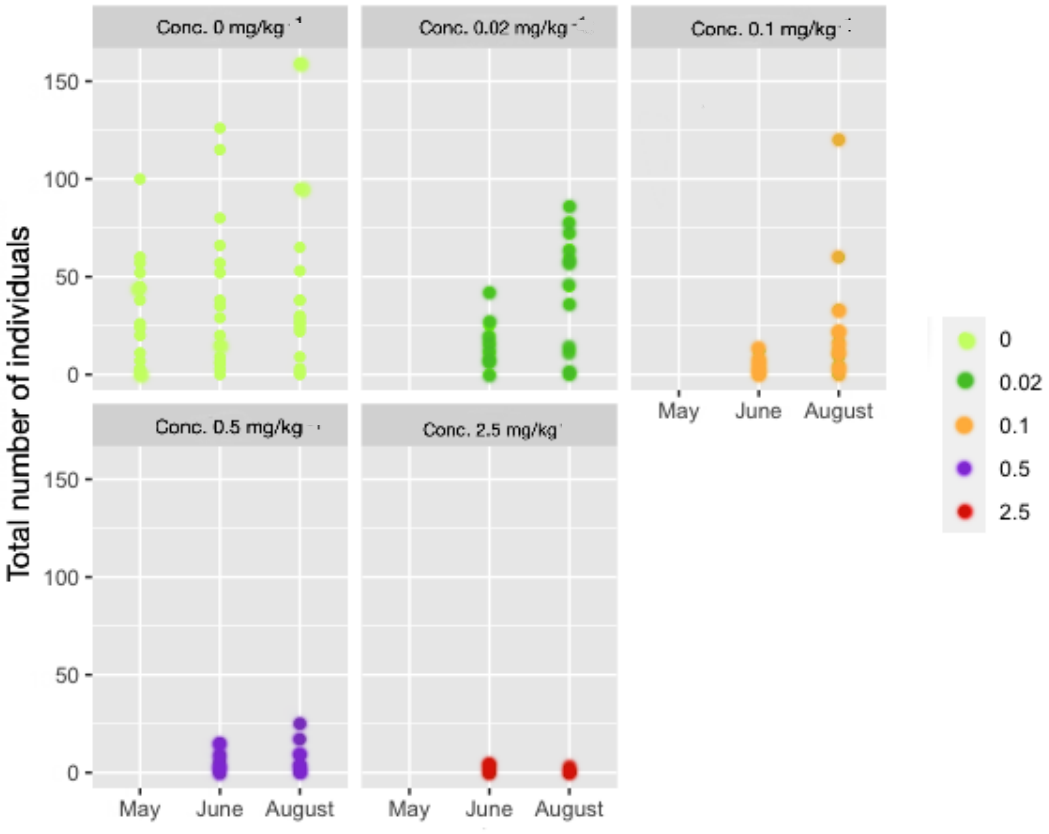

Figure 18. Scatterplots showing responses in abundance over time for the soil dwelling springtails within the different imidacloprid concentrations. Each plot represents concentrations of imidacloprid and the number of individuals counted per sample. Y-axis for all plots show the total number of individuals counted, x-axis show the time in months. Number of individuals per concentration are shown in different colours. Specific values are presented in table 4.

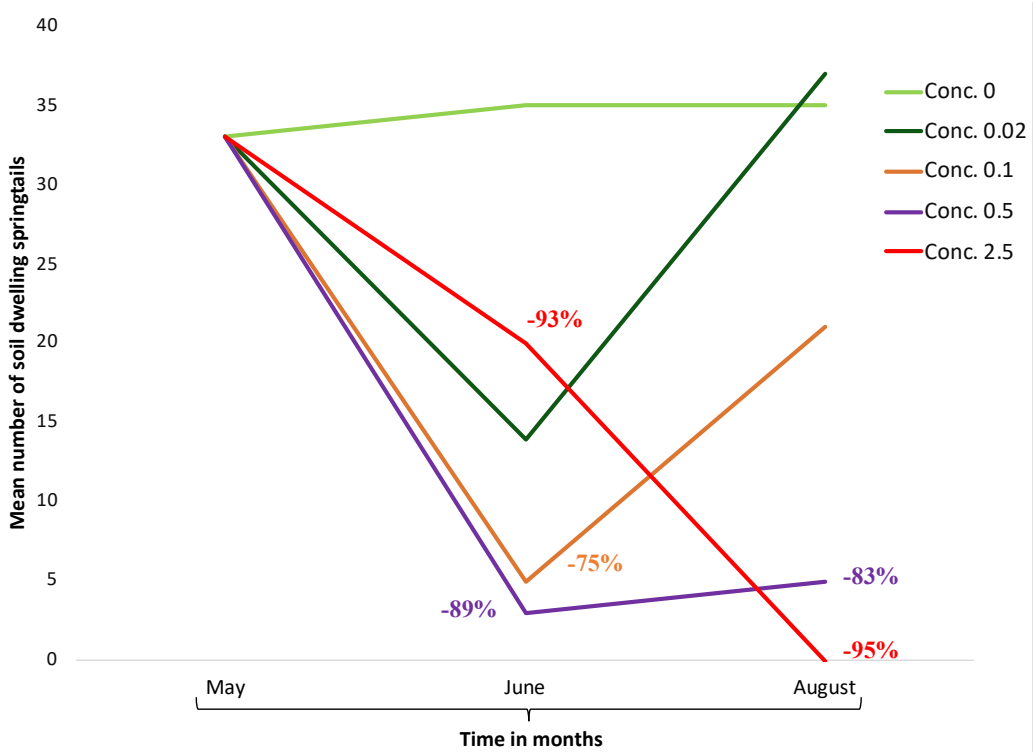

Figure 19. Line chart presenting the total number of abundance between each treatment per month of soil dwelling springtails from start of the experiment in May (control mean n =33) and within different treatments of imidacloprid (0, 0, 0.02, 0.1, 0.5 and 2.5 mg/kg). Significant change in abundance is shown in %. Y-axis show the total number of individuals, x-axis show the time in months

3.3.3 Effect of imidacloprid on mites

The effect of imidacloprid were calculated for mites at the different concentrations, and the imidacloprid regime was found to have no detectable effect on the total abundance of mites (Figure 20). A higher lower abundance of mites were in June. However, the abundance increased in August, and all p-values at each concentration in each month were found to be above a significant value ($p > 0.05$) (Table 4 and Figure 20 and 21).

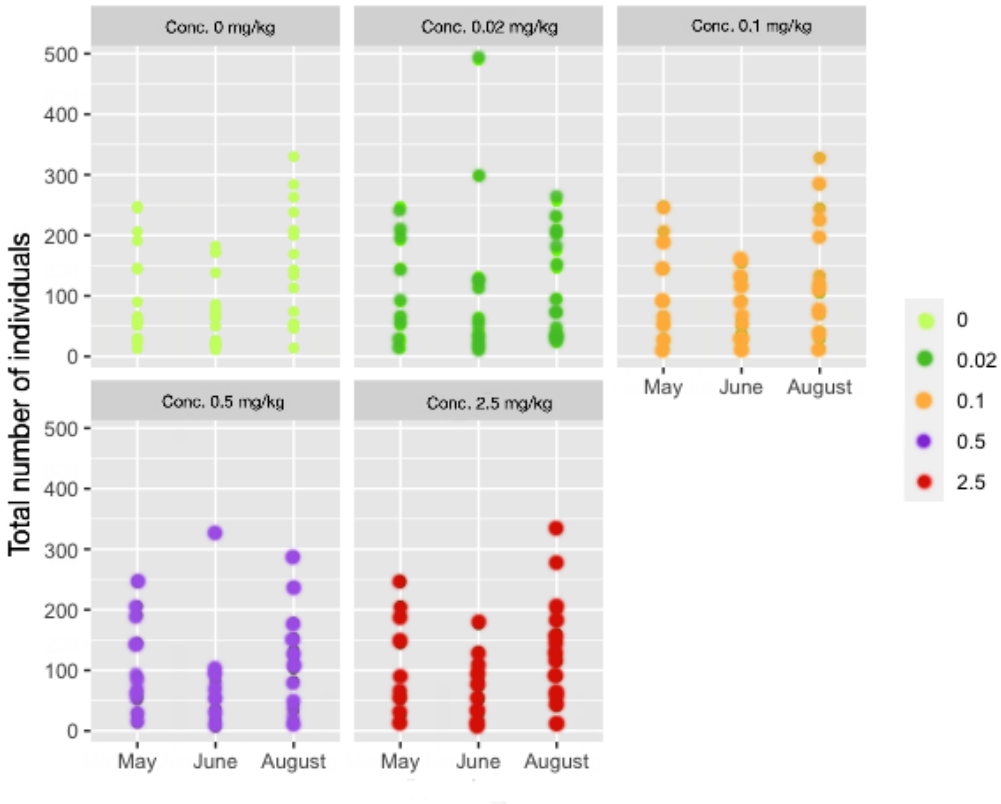

Figure 20. Scatterplots showing responses in abundance over time for mites within the different imidacloprid concentrations. Each plot represents concentrations of imidacloprid and the number of individuals counted per sample. Y-axis for all plots show the total number of individuals counted, x-axis show the time in months. Number of individuals per concentration are shown in different colours. Specific values are presented in table 4.

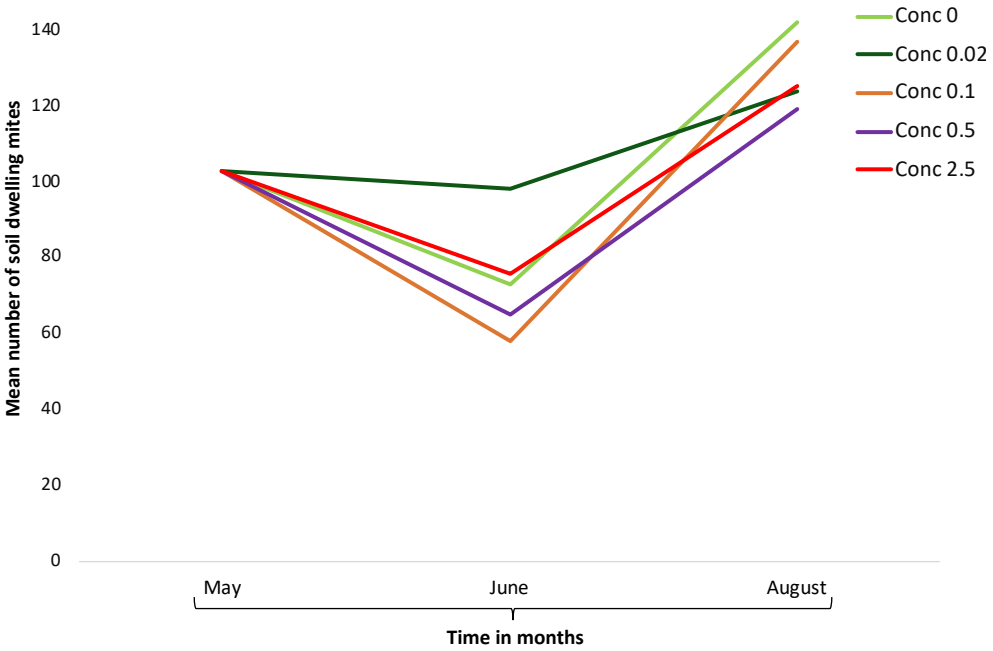

Figure 21. Line chart presenting the total number of abundance between each treatment per month of mites from start of the experiment in May (mean n = 103) and within different treatments of imidacloprid (0, 0, 0.02, 0.1, 0.5 and 2.5 mg/kg). Significant change in abundance is shown in %. Y-axis show the total number of individuals, x-axis show the time in month

3.4 Species composition of the soil community

A total of 55 289 invertebrates were collected, counted and classified from the soil cores. Across samples, the invertebrates collected represented 12 unique soil and other taxa (Appendix F). The distribution of springtails differed between samples where three of the springtail families *Isotomidae* (typically litter dwelling species), *Onychiuridae* (typically soil dwelling dwelling) and *Hypogastruidae* (typically surface dwelling), where widely distributed in the study site and found in almost all samples counted. *Isotomidae* being the most dominant, where the counted *F. quadrioculata* consisted of 3 % of the total *Isotomidae*. *Hypogastruidae* was present in all 208 samples counted. Springtails (57 %) and mites (39%) where the most common groups of animals found across all samples (Figure 22). In this study a total of 10 families of springtails were identified.

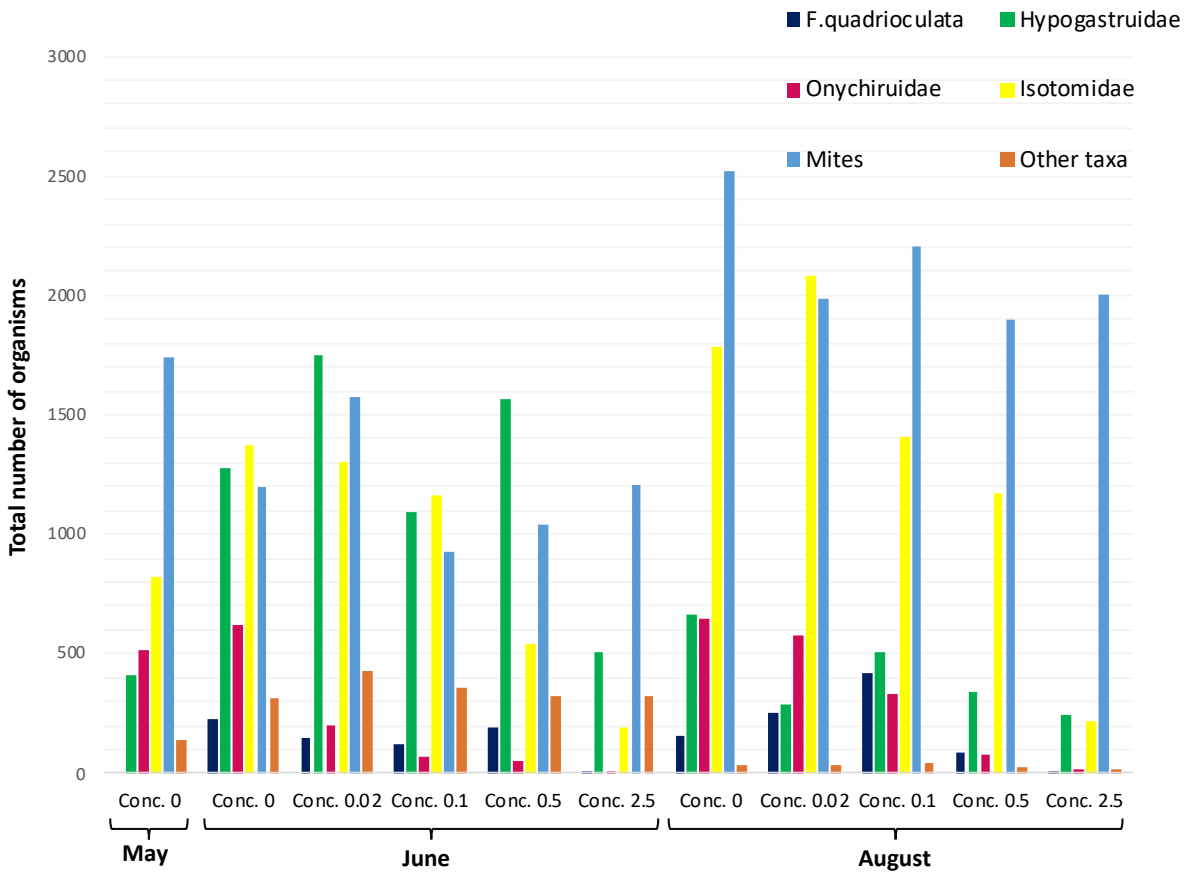

Figure 22. Bar chart showing the total abundance of the most abundant springtail groups including *F. quadrioculata*, mites and other taxa of soil invertebrates sampled during the experiment. The abundance of organisms are shown per imidacloprid concentration (mg/kg dry soil) for each month. X-axis shows concentration and time and y-axis shows total number of organisms. Families of other taxa are pooled together and all organisms are represented in individual colours.

4 Discussion

The overall aim of this study was to determine how the abundance of species is affected by the neonicotinoid imidacloprid under a future climate with reduced precipitation and increased drought. This was done through analysing the change in total abundance of groups of springtails and mites from May to August after exposure to imidacloprid, with half the blocks watered in addition to the natural precipitation. Due to a rainy start on the summer season, the watering treatment was difficult to assess as the added water constituted only a small percentage of the total natural rainfall, and as a consequence it had no prominence on the experiment. The overall results from the exposure to imidacloprid show that there is a negative relationship between the abundance of springtails over time with increasing insecticide concentration, and no effect on the abundance of mites.

4.1 Exposure regime

The present study aimed to create a field realistic situation by having intact soil fauna in closed mesocosms to extrapolate lab to field results as well as adding two environmental stressors. Assessing the effects of toxic compounds in soil, especially under naturally varying environmental conditions, can be a challenging procedure. However, by having controlled systems that simulate realistic field conditions, the efforts of measure and understanding exposure is made easier. Recreating natural soil fauna in mesocosms is an appropriate test system to investigate the impact of pesticides on the abundance of a soil community and the biodiversity, representing both the complexity of a biological community as well as the ecological realism (Schäffer et al., 2008).

4.1.1 Watering treatment

A simulated decrease in precipitation in addition to imidacloprid exposure was expected to have a negative impact on the overall abundance of springtails and mites, with the assumption that an altered water content in soil may influence the persistency of pesticides and hence the toxicity to soil fauna. As imidacloprid degradation is reduced in dry soil condition compared to wet soil (Bonmatin et al., 2015), it was expected that the imidacloprid concentrations were higher in the blocks without additional watering. Imidacloprid is known to leach more rapidly through soil columns, and persistence is highest under dry conditions especially in soils with high organic matter (Bonmatin et al., 2015). Contrary to the expectations (H1) there were no

effect observed from the watering treatment due to the increased natural rainfall during the experiment. Additionally, the amount of water added in the blocks with watering treatment had no effect on the soil conditions in the experiment, especially on the first six experimental weeks. During these first six weeks of the experiment, an extreme weather warning was announced twice by the Norwegian Meteorological Institute. There were several days of heavy rainfall in the beginning of the experiment. The watering treatment that was added in May amounted to only 9 % of the natural rainfall instead of the expected 20%, while in June the watering treatment was 11% of the natural rainfall. Although the natural rainfall decreased towards the end of the experiment, with a drier period in the last 6 experimental weeks and where watering treatment amounted to 30% of the natural rainfall in July, the timeline to see an effect of drought would probably have been too small. As a consequence, the watering treatment did not have an effect on the soil moisture.

Watering on the abundance of organisms

The lack of effect of watering was contrary to the expectations (H1) on the abundance of the soil community inspected. A lower abundance of *F.quadrioculata* and groups of springtails was expected at the blocks having no water treatment, especially in the samples with higher imidacloprid concentrations, but no difference was observed in neither. The watering treatment on mites showed no effect on abundance and are thus in line with the expectations (H5). As there were no difference in water content in the samples (Appendix B), the possibility of there not being any changes in soil humidity as a result of the watering treatment and the amount of natural rainfall is high. Although this hypothesis has not been tested. As a consequence it is reasonable to conclude that the watering did not have an effect on the abundance of the soil microarthropods. It is also reasonable to believe, based upon the calculation on the watering treatment, that a difference between the treatment blocks would have been observed if the rainfall had been within the average ranges of precipitation.

The extent of a chemicals toxicity, absorption, distribution, metabolism and excretion, is highly dependent on the exposure route (Fu et al., 2013) and a soil pesticide dynamic and its toxic potential can be affected by reduced soil moisture content (van Gestel, 2012; Ogungbemi and van Gestel, 2018; Braúlio Hennig et al., 2020). Water availability is critically important to the survival of springtails, and as a chemical's bioavailability is affected by environmental conditions, the uptake of a toxicant may be severely modified by climatic drivers (van Gestel and Diepen, 1997; Biswas et al., 2018). Since water affects an important

role in mobility of chemicals, the moisture of soil will be subsequent for the mobility of imidacloprid (Schroll et al., 2006). However, since the findings in the present study are not consistent with the expectations sat by a priori, the lack of the watering treatment will not be further discussed.

4.1.2 Imidacloprid analysis

As imidacloprid is known to have moderate mobility in wet soil, the concentration in the top soil layer was expected to be reduced throughout the experiment. The expected assumptions of imidacloprid behaviour, as found in other studies (Selim et al., 2010; Yadaw and Watanabe, 2018), included a higher measured concentration in the top soil layers at the start of the experiment and a decrease in concentration over time. Another behaviour was the persistency of imidacloprid in soil where the compound's residues was predicted to remain at low levels for more than 60 days (Sharma and Singh, 2014; Simon-Delso et al., 2015). A low variation in the data made it difficult to run statistical analysis to obtain a possible significant effect, and as result the analysed samples did not support the expectations set to the decreasing concentrations and the persistency of concentrations and soil depth. However, the overall concentrations measured at each imidacloprid treatment in August in the top soil layers were lower compared to June and May. May had the highest measured concentrations, specifically at the nominal concentration 2.5 mg/kg, with a 75% decrease in top soil layer concentration from May to August. These results may indicate a leaching in to the soil column, as imidacloprid residues were present in all the analysed soil layers. Imidacloprid has moderate mobility in soil, and even if mobility is dependent on moistures and types of soils, the concentrations of imidacloprid is found to increase with soil depth (Selim et al., 2010). The measured samples at the highest imidacloprid treatments (0.5 and 2.5 mg/kg) had relatively high concentrations in both the middle and lower soil layers. Approximately 30% of the initial exposure had leached down to the bottom soil layers with ~10% of initial concentration remaining in the soil. Additionally, the results from the analysis also showed an indication of persistency, with residues of imidacloprid in soil layers 82 days after application. Thus, the results are in line with the expectations (H2). An increase in soil depth also causes a decrease in degradation efficiency of imidacloprid. Due to the sunlight not being able to reach down in to the soil depth and hence the photocatalytic degradation will be absent (Sharma, Toor and Rajor, 2015).

The decrease in concentration of imidacloprid is also dependent on abiotic and biotic transformations, specifically by microbial activity and abiotic conditions such as light, temperature, moisture, soil type (the texture of the soil and organic matter content) and pH. However, since neonicotinoids can bind to soil particles and the same abiotic factors will also be important for the compounds ability to persist and degrade (Jeschke and Nauen, 2008; Wood and Goulson, 2017), it is natural to conclude that the indications seen in this study can contribute to the findings on other studies on imidacloprid persistency in soil. Depending on the factors previously mentioned, imidacloprids sorption increases with soil organic matter content (Bonmatin et al., 2015), and imidacloprid has a relatively low absorption rate in agricultural soil (Nemeth-Konda et al., 2002).

Imidacloprid was found in 7% of the top agricultural soil samples taken from different locations in the EU at concentrations up to 0.06 mg/kg, which exceeds the predicted environmental concentration (PEC) (de Lima et al, 2019). In this present study, the imidacloprid concentrations in the bottom soil layer, after being exposed to 0.5 and 2.5 mg/kg, was 0.18 and 0.01 µg/g after 81 days. Imidacloprid and other neonicotinoids are found in agricultural soils over more than a year after application, both with seed treatment and soil spraying (Wood and Goulson, 2017). The potential for this compound to accumulate in soil indicates that if applied more than once per year in agricultural fields its concentration might increase and eventually reach high values of environmental concentrations for several species of soil invertebrates. Degradation on soil surfaces can play an important role in the environmental dissipation of imidacloprid and the compound exhibits a low soil mobility with a negligible leaching potential (Selim, Jeong and Elbana, 2010). The indication of mobility and persistency in soil found in this study correlates with results from other studies (Kreutzweiser et al., 2008; Bonmatin et al., 2015; Simon-Delso et al., 2015).

4.2 Biological response

Understanding how anthropogenic stressors will influence the abundance of soil microarthropods, biodiversity and patterns of toxicological fate is inherently difficult to study. The way an ecological community reacts to changes in their environment is a complex combination of numerous responses to the same environmental variation. The important advantage of conducting a field study is that the effects of changes will be measured in natural communities. Ecological theory predicts that a continued stress may result in poorer

assemblages by filtering out the most sensitive species that again may lead to a shift in the occupation of the functional space (Chase, 2007; Peguero et al., 2019). According to Pisa et al (2015), insecticides have a significant impact on animal metabolism, affecting detoxification, intermediary and energetic metabolism pathways, and therefore reducing biomass gain.

4.2.1 The response of *Folsomia quadrioculata*

F.quadrioculata abundance varied over the season depending on the imidacloprid exposure. A lower abundance increase with increasing concentrations was observed and thus, it is in line with the expectations (H3). This relationship was especially seen for the higher concentrations ranging within 0.5 and 2.5 mg/kg in both the sampling months June and August. In the control and low exposure concentrations (0.02 – 0.1 mg/kg) the abundance was more constant with only a slow reduction towards the end of the experiment, indicating that the imidacloprid levels were below the threshold for a longer period. Studies have shown that a chronic low exposure to insecticides, often have the same effects in springtails as an acute exposure (Jager et al., 2006; Liu et al., 2012). The results in the present study suggests that long-term exposures to imidacloprid concentrations can have strong negative effects on the abundance of *F. quadrioculata* in the field, even at low concentrations.

Sengupta et al. (2021) analysed the toxicity of imidacloprid to the life stages of *F. quadrioculata* in laboratory experiments using dietary exposure over a timeline of 14 days. They found that even the lowest concentrations causing toxic effect was 0.12 mg/kg dry weight feed, that negatively affected the egg production. Concentrations at low environmental concentration values (EC50) of imidacloprid caused a temporary stop of egg production. With these results, the study suggested that *F. quadrioculata* reduces egg production after initiation of exposure to imidacloprid in favour of channelling resources for maximising adult survival. Although the study is not inherently similar to the present study, it does suggest adverse negative effects when exposed to imidacloprid over time. In the present study the indications of *F. quadrioculata* being tolerant to low concentrations of imidacloprid, having no significant increase or decrease in abundance, may support Sengupta et al. (2021) claims on a temporarily stop in egg production. However, in the field *F. quadrioculata* will be exposed to several biotic and abiotic factors that can affect the organism's responses compared to laboratory surroundings. Despite that, studies shows that imidacloprid cause impairment of movement in other soil arthropods and earthworms (Desneux, Decourtye and Delpuech, 2006;

Dittbrenner et al., 2011), that may lead to a reduced foraging ability which again increases susceptibility for predation (Sengupta et al., 2021). However, other studies have shown that the effect of concentrations in soil may be lower than those in food, probably because of the lower availability of chemicals in the high organic food compared to the low organic soil (de Lima e Silva, 2020). Similar studies done on imidacloprid suggests that when exposed to concentrations above 0.12 mg/kg during long periods, depending on the application mode, litter dwelling springtail species, like *F.quadrioculata,* may become extinct (de Lima e Silva et al., 2017).

In environmental risk assessment it is common to use the most sensitive species when assessing a compounds toxicity, mainly to enable protection of a broader range of species (OECD, 2016). However, based upon the findings in this present study, *F.quadrioculata* may be a good indicator when questions such as invertebrate sensitivity to imidacloprid are asked. Especially when exposure of imidacloprid can be correlated to what ecological niche the soil microarthropods inhabit. F.quadrioculata it is a species with a wide geographical distribution found across a broad climatic gradient (Hopkin, 1997). *F.quadrioculata's* geographical range may also reflect the wide variation human impact has on their environment and as followed a more ecological relevant species The present study found a reduction of 84% of the abundance of *F.quadrioculata* at 2.5 mg/kg between May and August, after being exposed for over 80 days, which suggests adverse effects on population recruitment and viability.

4.2.2 The responses of the springtail community

As expected (H4), the overall springtail abundance was reduced in a concentration-dependent manner, having a negative effect to increasing concentrations to imidacloprid. The findings were thus in line with other studies and done on the abundance, reproduction and overall fitness and survival when exposed to neonicotinoids (Peck, 2009; de Lima e Silva et al., 2021). However, the responses in abundance differed with treatments and distribution with soil depth and in line with the expectations (H4), the toxicity of imidacloprid had a higher effect on the most sensitive species in the litter layers and soil depth. Results in the present study found soil dwelling springtails to be the most sensitive to imidacloprid especially when compared to the litter and surface dwelling springtails, who only showed an intermediate sensitivity at the highest concentrations. The abundance of springtails in the control groups were more or less constant throughout the experiment. The distribution of chemicals in soil is

heterogeneous, and as an important biological aspect chemicals will often accumulate in the topsoil layer leading to a depth-related concentration gradient (van Gestel, 2012). Depending on the habitat and mobility of organisms, and the fact that springtail species show different strategies to tolerate climatic and environmental stress, they may be more or less exposed to chemicals present in their environment (Hopkin, 1997; van Gestel et al., 2017).

Over time the litter dwelling springtails, compared with the surface dwelling, had a higher percentage of reduction in abundance. A possible component of a higher abundance of surface dwelling springtails can be the degradation efficiency of imidacloprid being affected with the soil depth decreasing light intensity (Sharma, Toor and Rajor, 2015). Pesticides can directly or indirectly influence populations of surface dwelling arthropods (Desneux et al., 2006). Springtail species that are more surface active are more exposed to changes in their environment and as a result more adapted to rapid intensive disturbances (Zaller et al., 2016; Saifutdinov et al., 2020). Due to a constant changing environment, arthropods that live in the upper litter often have the tendency to move about to forage and avoid predation, and thus are more likely to be less affected by chemicals by being less sensitive (Peck, 2009). However, studies have also imidacloprid to be toxic to reproduction for surface dwelling springtail species when exposed to imidacloprid through soil, and over time (De Lima e Silva et al., 2021). A reduction in abundance was seen at the surface dwelling springtails at 0.02 mg/kg imidacloprid, indicating adverse effects to a chronic low exposure. In this present study the species of springtails was kept in their natural habitat during the duration of the experiment, since the litter dwelling species spend their life cycle part in the soil and part on the surface, it is possible they have spent more time on the soil surface and in this way avoided contact with imidacloprid. These findings are also consistent with the responses of *F. quadrioculata* in this present study, although a less mobile species it lives both within the litter and soil, and reductions in abundance was also only found at the concentrations 0.5 and 2.5 mg/kg.

In this present study, there were a large variation in density of soil arthropods over short distances. The few outliers found in June at 0.5 mg/kg with a very high density of surface dwelling springtails could be explained by top-down processes. The sudden abundance of springtails can be due to a higher absence in predatory mites and other predatory soil invertebrates, and as a consequence lead to an increase in surface dwelling springtails (Bitzer et al., 2005; Frampton and van den Brink, 2007). Although the mites were not identified by species, a total abundance of mites were found to be lower in June at all concentrations. The

highest abundance of surface dwelling springtails belonged to *Hypogastruidae*, which are highly pigmented and mobile species. However, this hypothesis has not been tested in the present study.

In line with the available previous studies (Sverdrup et al., 2002; Schnug et al., 2014; Ogungbemi and van Gestel, 2018), and H4, soil dwelling organisms was found to be more affected by the neonicotinoid imidacloprid. The decrease in abundance within soil dwelling springtails was considerately higher than the other groups of springtails, having the largest reduction in abundance at the highest concentrations of imidacloprid. The results were also in line with the analysis done of imidacloprid in the soil column, and also consistent with previous studies on the behaviour of imidacloprid in soil (Selim et al., 2010). The expectancy of a decrease in imidacloprid degradation efficiency due to soil dept is high, and may be reflected in the responses of the soil dwelling springtails. Species of soil dwelling springtails tend to be less mobile than the surface and litter dwelling species (Hopkin, 1997), and with imidacloprid leaching down in to the soil column, the organisms would be more exposed and have difficulties avoiding contaminated areas. The exposure would specifically be through the cuticle by direct diffusion of water and/or through diet by feeding on contaminated organic matter in the soil (Hopkin, 1997; Sagnik et al., 2021; de Lima e Silva et al., 2021). A reduction in abundance of soil dwelling species may also lead to a slowed down decomposition rate being that the litter and surface dwelling species are responsible for mineralisation of litter and regulation of microbial communities (Hopkin, 1997; Potapov et al., 2016).

The overall decrease in abundance of springtails may be influenced by the fact that most springtails lay their eggs in the soil to avoid predation and desiccation (Hopkin, 1997), by doing so the eggs in contaminated areas will be in constant contact with the toxic compound possibly affecting egg production and survival. Toxicant exposure strongly affects reproduction in springtails (van Gestel et al., 2017), and a decrease in abundance may be linked to the impact imidacloprid has on reproduction, affecting embryos, gametes and egg-laying success and the overall organisms fitness (de Lima e Silva et al., 2021; Sengupta et al., 2021). However, this hypotheses has not been tested in the present study. But, regardless of the ecological niche between these groups of springtails, the neonicotinoid were toxic and caused a loss in abundance over time at even low concentrations.

Effects on populations over time

The resistance of a population to a disturbance may be reflected in different proxies such as abundance, long-term abundance and relative abundance in disturbed parts of a habitat (Van Straalen et al., 2005; van Gestel, 2012; Prinzing, Kretzler and Beck, 2016). The metabolic pathways for imidacloprid presents many similarities between insects and soil dwelling arthropods, having serious and profound effects as toxic endpoints (Simon-Delso et al., 2015). One of the factors responsible for a reduction in abundance is mortality, where a decrease can be connected to a number of components that are not only lethal effects but the inability to avoid contaminated areas (Lique et al., 2008).

Nevertheless, there is a link between increasing concentrations of imidacloprid and the overall abundance of springtails. Similar to other studies done on non-target pollinators, an exposure to imidacloprid caused a decrease in abundance at even low concentrations. A decrease in abundance will have severe effects of the soil fauna composition. By reducing the variation and diversity of species the soil food web may not be able to keep pests and diseases under control and therefore no longer be regarded as a healthy soil community (Barrios, 2007; Widenfalk et al., 2016). By comparing the results in this present study and other studies done on *F.quadrioculata*, surface, litter and soil dwelling springtails, it is clear that imidacloprid has strong negative effects on abundance when exposed to increasing concentrations over longer periods of time. Similar to other studies done on non-target pollinators (Desneux et al., 2006; Dittbrenner et al., 2011; Schnug et al., 2014; de Lima e Silva et al., 2017), an exposure to imidacloprid caused a decrease in abundance at even low concentrations. Long-term exposure to imidacloprid can have important impacts on the soil ecosystem, with possible reduction of the offspring or the quality of the ecosystem services provided by these groups of organisms. This may especially be the case for springtails.

4.2.3 Mites

No decrease in the overall abundance of mites was found in this present study and the findings were thus consistent with the expectations (H5), and in line with the few available previous studies which have found mites to be tolerant to certain insecticides (Szczepaniec et al., 2011; Prinzing, Kretzler and Beck, 2016; de Lima e Silva et al., 2017). The nervous system of the mites may be different from that of the insect target species, having n-AChr subunits with lower affinity for substances like imidacloprid, which again requires higher

dosages of these compounds to produce any toxic effects, but as stated in de Lima et al., (2017) this is an assumption that needs further investigation.

An overall lower abundance of mites was found in June at all concentrations, which can indicate that some species of mites were particularly more susceptible to imidacloprid. Previous studies have found differences in pesticide sensitivity and consequently affecting the diversity of species of soil mites (Barbar, 2017; Kakoki et al., 2019). Resistance to a stressor might differ among species due to different physiological mechanisms, where the effect of a disturbance to a rare species may be intensified (Prinzing, Kretzler and Beck, 2016). The response to a disturbance may also be reflected in a species susceptibility where surface dwelling organisms are more exposed to a changing environment, and some species may also profit from a disturbance as their competitors decrease (Van Straalen et al., 2005; Dhooria, 2016; Prinzing, Kretzler and Beck, 2016). However, the species and vertical distribution of mites in this present study have been pooled together making it difficult to consider responses on intraspecific differences between species of mites. Nevertheless, the results in this present study show that the overall abundance of mites were not specifically affected by increasing concentrations of imidacloprid over time.

As the ecosystem service of mites, springtails and pesticides are vital for efficient agriculture, especially with a rapidly expanding world population, it is necessary to investigate whether the insecticide is compatible with the ecosystem services of these organisms. Understanding the effects of imidacloprid has on beneficial soil microarthropods is essential of making evidence based decisions. The results from the present study give insight in how microarthropods is affected by field realistic concentrations of imidacloprid.
A better understanding of the negative effects imidacloprid have on populations in soil communities will be achieved by combining the results from this present study and the knowledge from studies that have assessed the effects on the individual level. In view of regulatory bodies like EPA are considering the continued usage of imidacloprid, the uncertainty of the long-term effects of imidacloprid, and other neonicotinoids, only emphasises the importance of understanding realistic exposure in the field.

5 Conclusion

The overall aim of this study was to examine the effects of imidacloprid combined with an environmental stressor in a microarthropod soil community during a season. However, results in this present study showed that an added watering regime will have no impact on the joint responses to imidacloprid and climatic variables in an already rainy season. Nevertheless, the effects of climate change may lead to unpredictable weather and a focus on the effect on drier seasons, soil conditions and the joint responses with neonicotinoids and soil organisms will continue to be of a high priority.

The results of the neonicotinoid tested in this study were toxic to all species of springtails, with an overall response of a reduction in abundance. This response may reflect the residues of imidacloprid concentrations that persists in the soil column over time, specifically in the lower soil depth. Moreover, the concentrations of imidacloprid that persist from a single application indicate a real threat to populations of springtails. The effect of imidacloprid is severe, and the sensitivity to the concentration levels used in this present study show how springtail communities respond in a concentration-dependent manner, suggesting that the higher the concentration, the more severe is the impact of imidacloprid. Nevertheless, there is a link between the abundance of soil microarthropods and long-term exposure to increasing imidacloprid concentrations. Although arthropods presents different sensitivity to neonicotinoids even at high tested concentrations, the sensitivity to imidacloprid may be linked to exposure route, the persistency of imidacloprid in the environment and genetic affinity. The data presented in this study indicate that communities of soil microarthropods in agricultural soils are already at high risk of exposure to concentrations of imidacloprid, potentially affecting important soil ecosystem services.

The direct toxicity of neonicotinoids to non-target species warrants an evaluation of their long-term impact on agricultural soils and also the surrounding ecosystems. Although the toxic effect of imidacloprid do not inherently explain the difference in sensitivity among the groups of springtails observed in this thesis, it helps by giving an understanding of the possible effects this neonicotinoid might have on non-target species. An addition of an ecological approach can be very useful when distinguishing the differences in sensitivity in an endpoint

The uncertainty connected to the effects of neonicotinoids on soil microarthropods emphasise the importance of understanding the realistic exposure of these pesticides.
Since pesticides are vital for the needed growth in agriculture, the importance of understanding the potential risks they pose on non-target organisms, especially on the species providing ecosystems services to agriculture, will continue to be of exceeding interest.

6 Future studies

A challenge of the present study was to obtain an effect of the watering regime, specifically simulating drought. Future studies should continue the focus on predicted climatic changes following pesticide application and the implication it may have on agroecosystems. Although fieldwork was essential for the present study, it is advantageous to interpret and obtain similar data in the light of laboratory experiments, supplemented with exposure regimes on water content and imidacloprid. This could increase the knowledge on the causality of effects in agricultural soil communities.

While the results of the present study contribute to the knowledge about neonicotinoids, the low number of samples for analysing imidacloprid content in the soil also resulted in few replicates per concentrations of imidacloprid for each soil layer. As a consequence it reduced the opportunities for statistical analysis of the obtained data. The present study did therefore partly present indications that were not backed by statistical significance, and nominal concentrations may overestimate a compounds real toxicity. Future investigations are necessary to validate the kinds of conclusions that can be drawn from this study.

Although important pollinators such as bumblebees have received much needed attention in the scientific literature and media regarding neonicotinoids, these compounds are shown to be toxic to other invertebrates such as important soil biota and aquatic insects. Studies done on the functional group and composition of springtails and mites in agricultural soils are scarce, and there is still insufficient knowledge on the species richness in their response to disturbances in agroecosystems. While the results in the present study show the toxicity of imidacloprid to soil microarthropods, a single compound applied once during a growth season in the field is not a reality. Future research is needed to delimitate the mixtures of pesticides in soil. Different pesticides are often added in different interval of applications, or one pesticide may be applied several times during the same season. These mixtures and applications only contributes to the complexity of the response soil microarthropods have to toxic compounds and adds to the concern of imidacloprids negative effects. In addition, a development of a better comprehensive analysis of imidacloprid and soil invertebrates might prove an important area for future research. Specific information such as mode of application (foliar versus seed treatment and soil spraying), use of natural soil and a better understanding of ecological

niches of the soil invertebrates may be key components in understanding the effects of imidacloprid and other neonicotinoids in the environment.

Considering the influential role springtails have in soil ecosystems future studies should include the effects of neonicotinoids on species interactions across biological communities as well as recolonisation and recovery of species after exposure. This could broaden the understanding of soil ecosystems and allow predictions of effects of multiple stressors on soil processes.

The generalisation of the results of the abundance of mites in this present study requires some cautions and additional experiments to clarify the threshold tolerance of imidacloprid and the direct effects the chemical has on species of mites. Future studies should consider including intraspecific responses of mites and the susceptibility of imidacloprid within species as a way of minimising pesticide impact.

References

Atwood, D., and Paisley-Jones, C. (2008). US EPA - Pesticides Industry Sales and Usage 2008 - 2012 (pp. 1–24)

Atwood, L. W., Mortensen, D. A., Koide, R. T., and Smith, R. G. (2018). Evidence for multi-trophic effects of pesticide seed treatments on non-targeted soil fauna. Soil Biology and Biochemistry, 125, 144–155. https://doi.org/10.1016/j.soilbio.2018.07.007

Barbar, Z. (2017). Evaluation of three pesticides against phytophagous mites and their impact on phytoseiid predators in an eggplant open-field. Acarologia, 57(3), 529–539. https://doi.org/10.24349/acarologia/20174170

Barrios, E. (2007). Soil biota, ecosystem services and land productivity. Ecological Economics, 64(2), 269–285. https://doi.org/10.1016/j.ecolecon.2007.03.004

Behan-Pelletier, V. M. (1999). Oribatid mite biodiversity in agroecosystems: Role for bioindication. Agriculture, Ecosystems and Environment, 74(1–3), 411–423. https://doi.org/10.1016/S0167-8809(99)00046-8

Biswas B, Qi F, Biswas JK, Wijayawardena A, Khan MAI, Naidu R. The Fate of Chemical Pollutants with Soil Properties and Processes in the Climate Change Paradigm—A Review. Soil Systems. 2018; 2(3):51. https://doi.org/10.3390/soilsystems2030051

Bitzer, R. J., Rice, M. E., Pilcher, C. D., Pilcher, C. L., & Lam, W. K. F. (2005). Biodiversity and community structure of epedaphic and euedaphic springtails (Collembola) in transgenic rootworm Bt corn. Environmental Entomology, 34(5), 1346–1376. https://doi.org/10.1093/ee/34.5.1346

Bonmatin, J. M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess. M., Long. E., Marzaro. M., Mitchell. E. A, Noome., Simon-Delso, D. A., A .Tapparo. (2015). Environmental fate and exposure; neonicotinoids and fipronil. Environmental Science and Pollution Research, 22(1), 35–67. https://doi.org/10.1007/s11356-014-3332-7

Braúlio Hennig, T., Ogliari Bandeira, F., Dalpasquale, A. J., Cardoso, E. J. B. N., Baretta, D., and Lopes Alves, P. R. (2020). Toxicity of imidacloprid to collembolans in two tropical soils under different soil moisture. Journal of Environmental Quality, 49(6), 1491–1501. https://doi.org/10.1002/jeq2.20143

Buse, T., Ruess, L., & Filser, J. (2014). Collembola gut passage shapes microbial communities in faecal pellets but not viability of dietary algal cells. Chemoecology, 24(2), 79–84. https://doi.org/10.1007/s00049-013-0145-y

Chase, J. M. (2007). Drought mediates the importance of stochastic community assembly. Proceedings of the National Academy of Sciences of the United States of America, 104(44), 17430–17434. https://doi.org/10.1073/pnas.0704350104

Chimitova, A. B., Chernova, N. M., and Potapov, M. B. (2010). Springtail (Collembola) populations in cryogenic soils of the Vitim Plateau. Entomological Review, 90(8), 957–967. https://doi.org/10.1134/S0013873810080014

Coulson, S. J., Leinaas, H. P., Ims, R. A., and Søvik, G. (2000). Experimental manipulation of the winter surface ice layer: The effects on a High Arctic soil microarthropod community. Ecography, 23(3), 299–306. https://doi.org/10.1111/j.1600-0587.2000.tb00285.x

Cragg, R. G., and Bardgett, R. D. (2001). How changes in soil faunal diversity and composition within a trophic group influence decomposition processes. Soil Biology and biochemistry, 33(15), 2073–2081.https://doi.org/10.1016/S0038-0717(01)00138-9

Dai, A. (2013). Increasing drought under global warming in observations and models.

Nature Climate Change, 3(1), 52–58. https://doi.org/10.1038/nclimate1633
Delcour, I., Spanoghe, P., and Uyttendaele, M. (2015, February 1). Literature review: Impact of climate change on pesticide use. Food Research International. Elsevier Ltd. https://doi.org/10.1016/j.foodres.2014.09.030
Desneux, N., Decourtye, A., and Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. Annual Review of Entomology. https://doi.org/10.1146/annurev.ento.52.110405.091440
Dhooria, M. S. (2016). Fundamentals of applied acarology. Fundamentals of Applied Acarology (pp. 1–470). Springer Singapore. https://doi.org/10.1007/978-981-10-1594-6
Dittbrenner, N., Moser, I., Triebskorn, R., and Capowiez, Y. (2011). Assessment of short and long-term effects of imidacloprid on the burrowing behaviour of two earthworm species (Aporrectodea caliginosa and Lumbricus terrestris) by using 2D and 3D post-exposure techniques. Chemosphere, 84(10), 1349–1355. https://doi.org/10.1016/j.chemosphere.2011.05.011
Douglas, M. R., Rohr, J. R., and Tooker, J. F. (2015). Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield. Journal of Applied Ecology, 52(1), 250–260. https://doi.org/10.1111/1365-2664.12372
Douglas, M. R., & Tooker, J. F. (2016). Meta-analysis reveals that seed-applied neonicotinoids and pyrethroids have similar negative effects on abundance of arthropod natural enemies. PeerJ, 2016(12). https://doi.org/10.7717/peerj.2776
De Vries, F. T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M. A., Bjørnlund, L., Bardgett, R. D. (2013). Soil food web properties explain ecosystem services across European land use systems. Proceedings of the National Academy of Sciences of the United States of America, 110(35), 14296–14301. https://doi.org/10.1073/pnas.1305198110
EFSA European Food Safety Authority. (2018). Peer review of the pesticide risk assessment for bees for the active substance imidacloprid considering the uses as seed treatments and granules. EFSA Journal 2018;16(2):5178. https://doi.org/10.2903/j.efsa.2018.5178
EFSA European Food Safety Authority. (8. 12. 2020). Pesticides: EFSA to examine emergency use of neonicotinoids. EFSA: https://www.efsa.europa.eu/en/news/pesticides-efsa-examine-emergency-use-neonicotinoids
El-Naggar, J. B., and Zidan, N. E. H. A. (2013). Field evaluation of imidacloprid and thiamethoxam against sucking insects and their side effects on soil fauna. Journal of Plant Protection Research, 53(4), 375–387. https://doi.org/10.2478/jppr-2013-0056
EPA (2019) Insecticides | CADDIS Volume 2 | US EPA. Retrieved from https://www.epa.gov/caddis-vol2/insecticides (Accessed: 3 June 2021).
EPA (2020) Proposed Interim Registration Review Decision for Neonicotinoids | US EPA. Retrieved from https://www.epa.gov/pollinator-protection/proposed-interim-registration-review-decision-neonicotinoids (Accessed: 27 June 2021).
Fjellberg, A. (1998). The Collembola of Fennoscnadia and Denmark: Part I: Poduromorpha. Fauna Entomologica Scandinavica.
Fjellberg, A. (2007). The Collembola of Fennoscandia and Denmark: Part II: Entomobryomorpha and symphypleona. Fauna Entomologica Scandinavica, 42, 1–270. https://doi.org/10.1163/ej.9789004157705.i-265
Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., … Zaks, D. P. M. (2011). Solutions for a cultivated planet. Nature, 478(7369), 337–342. https://doi.org/10.1038/nature10452

Frampton, G. K., & van den Brink, P. J. (2007). Collembola and macroarthropod community responses to carbamate, organophosphate and synthetic pyrethroid insecticides: Direct and indirect effects. Environmental Pollution, 147(1), 14–25. https://doi.org/10.1016/j.envpol.2006.08.038

Fu, C., Liu, T., Li, L., Liu, H., Chen, D., and Tang, F. (2013). The absorption, distribution, excretion and toxicity of mesoporous silica nanoparticles in mice following different exposure routes. Biomaterials, 34(10), 2565–2575. https://doi.org/10.1016/j.biomaterials.2012.12.043

Gergócs, V., and Hufnagel, L. (2017). Comparing the natural variation of oribatid mite communities with their changes associated with anthropogenic disturbance. Environmental Monitoring and Assessment, 189(4). https://doi.org/10.1007/s10661-017-5897-3

van Gestel CA, van Diepen AM. The influence of soil moisture content on the bioavailability and toxicity of cadmium for Folsomia candida Willem (Collembola: Isotomidae). Ecotoxicol Environ Saf. 1997 Mar;36(2):123-32. doi: 10.1006/eesa.1996.1493.

van Gestel, C. A. M. (2012). Soil ecotoxicology: State of the art and future directions. ZooKeys. https://doi.org/10.3897/zookeys.176.2275.

van Gestel, C. A. M., de Lima e Silva, C., Lam, T., Koekkoek, J. C., Lamoree, M. H., and Verweij, R. A. (2017). Multigeneration toxicity of imidacloprid and thiacloprid to Folsomia candida. Ecotoxicology, 26(3), 320–328. https://doi.org/10.1007/s10646-017-1765-8

Gibbons, D., Morrissey, C., and Mineau, P. (2015). A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environmental Science and Pollution Research, 22(1), 103–118. https://doi.org/10.1007/s11356-014-3180-5

Goulson, D. (2013, August). An overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology. https://doi.org/10.1111/1365-2664.12111

Halbritter, A. H., De Boeck, H. J., Eycott, A. E., Reinsch, S., Robinson, D. A., Vicca, S., … Zurba, K. (2020). The handbook for standardized field and laboratory measurements in terrestrial climate change experiments and observational studies (ClimEx). Methods in Ecology and Evolution, 11(1), 22–37. https://doi.org/10.1111/2041-210X.13331

Helgason, T., Daniell, T. J., Husband, R., Fitter, A. H., and Young, J. P. W. (1998, July 30). Ploughing up the wood-wide web? [4]. Nature. https://doi.org/10.1038/28764

Holmstrup, M., Bindesbøl, A. M., Oostingh, G. J., Duschl, A., Scheil, V., Köhler, H. R., Spurgeon, D. J. (2010, August). Interactions between effects of environmental chemicals and natural stressors: A review. Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2009.10.067

Holmstrup, M., Maraldo, K., and Krogh, P. H. (2007). Combined effect of copper and prolonged summer drought on soil Microarthropods in the field. Environmental Pollution, 146(2), 525–533. https://doi.org/10.1016/j.envpol.2006.07.013

Hopkin, S. P. (1997). Biology of the springtails (Insecta: Collembola). Biology of the springtails (Insecta: Collembola). Oxford University Press.

IPCC (2007) Climate Change 2007 Synthesis Report, Intergovernmental Panel on Climate Change. Retrieved from https://www.ipcc.ch/site/assets/uploads/2018/03/ar4_wg2_full_report.pdf (Accessed 22.June 2021)

IPCC (2014) Climate Change 2014, Climate Change 2014: Synthesis Report.Retrived from: https://www.ipcc.ch/report/ar5/syr/ (Accessed 22 June 2021)

IPCC (2019) 'IPCC SR: Climate Change and Land', An IPCC Special Report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.Retrieved from:

https://www.ipcc.ch/site/assets/uploads/2019/11/SRCCL-Full-Report-Compiled-191128.pdf (Accessed 22 June 2021)
Jager, T., Crommentuijn, T., van Gestel, C. A. M., & Kooijman, S. A. L. M. (2007). Chronic exposure to chlorpyrifos reveals two modes of action in the springtail Folsomia candida. Environmental Pollution, 145(2), 452–458. https://doi.org/10.1016/j.envpol.2006.04.028
James, D. G., and Price, T. S. (2002). Fecundity in twospotted spider mite (Acari: Tetranychidae) is increased by direct and systemic exposure to imidacloprid. Journal of Economic Entomology, 95(4), 729–732. https://doi.org/10.1603/0022-0493-95.4.729
Jeschke, P., and Nauen, R. (2008, November). Neonicotinoids - From zero to hero in insecticide chemistry. Pest Management Science. https://doi.org/10.1002/ps.1631
Jeschke, P., Nauen, R., Schindler, M., and Elbert, A. (2011). Overview of the status and global strategy for neonicotinoids. Journal of Agricultural and Food Chemistry, 59(7), 2897–2908. https://doi.org/10.1021/jf101303g
Jeschke, P., Nauen, R., and Beck, M. E. (2013, September 2). Nicotinic acetylcholine receptor agonists: A milestone for modern crop protection. Angewandte Chemie - International Edition. https://doi.org/10.1002/anie.201302550
Jucevica, E., and Melecis, V. (2006). Global warming affect Collembola community: A long-term study. Pedobiologia, 50(2), 177–184. https://doi.org/10.1016/j.pedobi.2005.10.006
Kakoki, S., Kamimuro, T., Ikenoue, Y., Inokuchi, M., Tsuda, K., & Sakamaki, Y. (2019). The response of three species of phytoseiid mite (Acari: Phytoseiidae) to synthetic pyrethroid pesticides in the laboratory and the field. Experimental and Applied Acarology, 77(1), 27–41. https://doi.org/10.1007/s10493-018-0334-z
Kardol, P., Reynolds, W. N., Norby, R. J., & Classen, A. T. (2011). Climate change effects on soil microarthropod abundance and community structure. Applied Soil Ecology, 47(1), 37–44. https://doi.org/10.1016/j.apsoil.2010.11.001
Kastner, T., Rivas, M. J. I., Koch, W., & Nonhebel, S. (2012). Global changes in diets and the consequences for land requirements for food. Proceedings of the National Academy of Sciences of the United States of America, 109(18), 6868–6872. https://doi.org/10.1073/pnas.1117054109
Krab, E. J., Oorsprong, H., Berg, M. P., & Cornelissen, J. H. C. (2010). Turning northern peatlands upside down: Disentangling microclimate and substrate quality effects on vertical distribution of Collembola. Functional Ecology, 24(6), 1362–1369. https://doi.org/10.1111/j.1365-2435.2010.01754.x
Kreutzweiser, D. P., Good, K. P., Chartrand, D. T., Scarr, T. A., Holmes, S. B., & Thompson, D. G. (2008). Effects on litter-dwelling earthworms and microbial decomposition of soil-applied imidacloprid for control of wood-boring insects. Pest Management Science, 64(2), 112–118. https://doi.org/10.1002/ps.1478
Laird, N. M. and Ware, J. H. (1982) ‘Random-Effects Models for Longitudinal Data. Published by : International Biometric Society Stable. Biometrics, 38(4), pp. 963–974.
Van Leeuwen, T., & Dermauw, W. (2016). The Molecular Evolution of Xenobiotic Metabolism and Resistance in Chelicerate Mites. Annual Review of Entomology. Annual Reviews Inc. https://doi.org/10.1146/annurev-ento-010715-023907
de Lima e Silva, C., Brennan, N., Brouwer, J. M., Commandeur, D., Verweij, R. A., & van Gestel, C. A. M. (2017). Comparative toxicity of imidacloprid and thiacloprid to different species of soil invertebrates. Ecotoxicology, 26(4), 555–564. https://doi.org/10.1007/s10646-017-1790-7.
de Lima e Silva. (2020) Impacts of neonicotinoids to soil invertebrates (Doctoral dissertation,

Department of Ecological Sciences, Vrije Universiteit Amsterdam, The Netherlands). Retrieved from the University of Oslo Library.
de Lima e Silva, C., van Haren, C., Mainardi, G., de Rooij, W., Ligtelijn, M., van Straalen, N. M., & van Gestel, C. A. M. (2021). Bringing ecology into toxicology: Life-cycle toxicity of two neonicotinoids to four different species of springtails in LUFA 2.2 natural soil. Chemosphere, 263. https://doi.org/10.1016/j.chemosphere.2020.128245
Liu, Y. R., Zheng, Y. M., & He, J. Z. (2012). Toxicity of profenofos to the springtail, Folsomia candida, and ammonia-oxidizers in two agricultural soils. Ecotoxicology, 21(4), 1126–1134. https://doi.org/10.1007/s10646-012-0867-6
Lique, A., Raveton, M., Lempérière, G., & Ravanel, P. (2008). Phenylpyrazoles impact on Folsomia candida (Collembola). Soil Biology and Biochemistry, 40(9), 2351–2357. https://doi.org/10.1016/j.soilbio.2008.05.014
Macfadyen, A. (1961). Improved Funnel-Type Extractors for Soil Arthropods. The Journal of Animal Ecology, 30(1), 171. https://doi.org/10.2307/2120
Makkonen, M., Berg, M. P., van Hal, J. R., Callaghan, T. V., Press, M. C., & Aerts, R. (2011). Traits explain the responses of a sub-arctic Collembola community to climate manipulation. Soil Biology and Biochemistry, 43(2), 377–384. https://doi.org/10.1016/j.soilbio.2010.11.004
Mani, M., Shivaraju, C., & Kulkarni, N. S. (2014). The grape entomology. The Grape Entomology (pp. 1–195). Springer India. https://doi.org/10.1007/978-81-322-1617-9
Manu, M., Honciuc, V., Neagoe, A., Băncilă, R. I., Iordache, V., & Onete, M. (2019). Soil mite communities (Acari: Mesostigmata, Oribatida) as bioindicators for environmental conditions from polluted soils. Scientific Reports, 9(1). https://doi.org/10.1038/s41598-019-56700-8
Meehan, M. L., Song, Z., Lumley, L. M., Cobb, T. P., & Proctor, H. (2019). Soil mites as bioindicators of disturbance in the boreal forest in northern Alberta, Canada: Testing taxonomic sufficiency at multiple taxonomic levels. Ecological Indicators, 102, 349–365. https://doi.org/10.1016/j.ecolind.2019.02.043
Naiel, M A. E., Shehata, A. M., Negm, S. S., Abd El-Hack, M. E., Amer, M. S., Khafaga, A. F., Bin-Jumah, M., Allam, A.A. (2020). The new aspects of using some safe feed additives on alleviated imidacloprid toxicity in farmed fish: a review. Reviews in aquaculture, 2020-11, Vol.12 (4), p.2250-2267. https://doi-org.ezproxy.uio.no/10.1111/raq.12432
Nemeth-Konda, L., Füleky, G., Morovjan, G., & Csokan, P. (2002). Sorption behaviour of acetochlor, atrazine, carbendazim, diazinon, imidacloprid and isoproturon on Hungarian agricultural soil. Chemosphere, 48(5), 545–552. https://doi.org/10.1016/S0045-6535(02)00106-6
Ng, E. L., Bandow, C., Proença, D. N., Santos, S., Guilherme, R., Morais, P. V., … Sousa, J. P. (2014). Does altered rainfall regime change pesticide effects in soil? A terrestrial model ecosystem study from Mediterranean Portugal on the effects of pyrimethanil to soil microbial communities under extremes in rainfall. Applied Soil Ecology, 84, 245–253. https://doi.org/10.1016/j.apsoil.2014.08.006
OECD (2016), Test No. 232: Collembolan Reproduction Test in Soil, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264264601-en.
OECD (2016), Test No. 226: Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, https://doi.org/10.1787/9789264264557-en.
Ogungbemi, A. O., & van Gestel, C. A. M. (2018). Extrapolation of imidacloprid toxicity

between soils by exposing Folsomia candida in soil pore water. Ecotoxicology, 27(8), 1107–1115. https://doi.org/10.1007/s10646-018-1965-x
Peck, D. C. (2009). Long-term effects of imidacloprid on the abundance of surface- and soil-active nontarget fauna in turf. Agricultural and Forest Entomology, 11(4), 405–419. https://doi.org/10.1111/j.1461-9563.2009.00454.x
Peguero, G., Sol, D., Arnedo, M., Petersen, H., Salmon, S., Ponge, J. F., … Peñuelas, J. (2019). Fast attrition of springtail communities by experimental drought and richness–decomposition relationships across Europe. Global Change Biology, 25(8), 2727–2738. https://doi.org/10.1111/gcb.14685
Perdue, J. C., & Crossley, D. A. (1989). Seasonal abundance of soil mites (Acari) in experimental agroecosystems: Effects of drought in no-tillage and conventional tillage. Soil and Tillage Research, 15(1–2), 117–124. https://doi.org/10.1016/0167-1987(89)90068-8
Ponge, J. F. (2000). Vertical distribution of collembola (Hexapoda) and their food resources in organic horizons of beech forests. Biology and Fertility of Soils, 32(6), 508–522. https://doi.org/10.1007/s003740000285
Potapov, A. A., Semenina, E. E., Korotkevich, A. Y., Kuznetsova, N. A., & Tiunov, A. V. (2016). Connecting taxonomy and ecology: Trophic niches of collembolans as related to taxonomic identity and life forms. Soil Biology and Biochemistry, 101, 20–31. https://doi.org/10.1016/j.soilbio.2016.07.002
Prinzing, A., Kretzler, S., & Beck, L. (2000). Resistance to disturbance is a diverse phenomenon and does not increase with abundance: The case of oribatid mites. Ecoscience, 7(4), 452–460. https://doi.org/10.1080/11956860.2000.11682617
Pritchard, S. G. (2011, February). Soil organisms and global climate change. Plant Pathology. https://doi.org/10.1111/j.1365-3059.2010.02405.x
Ritz, C. (2010). Toward a unified approach to dose-response modeling in ecotoxicology. Environmental Toxicology and Chemistry. SETAC Press. https://doi.org/10.1002/etc.7
Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose-response analysis using R. PLoS ONE, 10(12). https://doi.org/10.1371/journal.pone.0146021
Roos, R. E., Birkemoe, T., Asplund, J., Ľuptáčik, P., Raschmanová, N., Alatalo, J. M., Klanderud, K. (2020). Legacy effects of experimental environmental change on soil micro-arthropod communities. Ecosphere, 11(2). https://doi.org/10.1002/ecs2.3030
Rusek, J. (1998). Biodiversity of Collembola and their functional role in the ecosystem. Biodiversity and Conservation, 7(9), 1207–1219. https://doi.org/10.1023/A:1008887817883
Saifutdinov, R. A., Sabirov, R. M., & Zaitsev, A. S. (2020). Springtail (Hexapoda: Collembola) functional group composition varies between different biotopes in Russian rice growing systems. European Journal of Soil Biology, 99. https://doi.org/10.1016/j.ejsobi.2020.103208
Schäffer, A., Van Den Brink, P., Heimbach, F., Hoy, S., De Jong, F., Römbke, J., Roß-Nickoll, M. (2008). Semi-field methods are a useful tool for the environmental risk assessment of pesticides in soil. Environmental Science and Pollution Research, 15(3), 176–177. https://doi.org/10.1065/espr2008.01.477
Schnug, L., Jensen, J., Scott-Fordsmand, J. J., & Leinaas, H. P. (2014). Toxicity of three biocides to springtails and earthworms in a soil multi-species (SMS) test system. Soil Biology and Biochemistry, 74, 115–126. https://doi.org/10.1016/j.soilbio.2014.03.007
Schroll, R., Becher, H. H., Dörfler, U., Gayler, S., Grundmann, S., Hartmann, H. P., & Ruoss, J. (2006). Quantifying the effect of soil moisture on the aerobic microbial mineralization of selected pesticides in different soils. Environmental Science and Technology, 40(10), 3305–3312. https://doi.org/10.1021/es052205j

Selim, H. M., Jeong, C. Y., & Elbana, T. A. (2010). Transport of imidacloprid in soils: Miscible displacement experiments. Soil Science, 175(8), 375–381. https://doi.org/10.1097/SS.0b013e3181ebc9a2

Seltman, H. J. (2014).Chapter 15 Mixed Models. Experimental Design And Analysis,357-378

Sengupta, S., Ergon, T., & Leinaas, H. P. (2016). Genotypic differences in embryonic life history traits of Folsomia quadrioculata (Collembola: Isotomidae) across a wide geographical range. Ecological Entomology, 41(1), 72–84. https://doi.org/10.1111/een.12270

Sengupta, S., Ergon, T., & Leinaas, H. P. (2017). Thermal plasticity in postembryonic life history traits of a widely distributed Collembola: Effects of macroclimate and microhabitat on genotypic differences. Ecology and Evolution, 7(19), 8100–8112. https://doi.org/10.1002/ece3.3333

Sengupta, S., Leinaas, H. P., van Gestel, C. A. M., Rundberget, J. T., & Borgå, K. (2021). A Multiple Life-History Trait–Based and Time-Resolved Assessment of Imidacloprid Effects and Recovery in the Widely Distributed Collembolan Folsomia quadrioculata. Environmental Toxicology and Chemistry, 40(1), 139–147. https://doi.org/10.1002/etc.4897

Sharma, S., & Singh, B. (2014). Persistence behaviour of imidacloprid and its metabolites in soil under sugarcane. Environmental Monitoring and Assessment, 186(4), 2281–2288. https://doi.org/10.1007/s10661-013-3536-1

Sharma, T., Toor, A. P., & Rajor, A. (2015). Photocatalytic degradation of imidacloprid in soil: Application of response surface methodology for the optimization of parameters. RSC Advances, 5(32), 25059–25065. https://doi.org/10.1039/c5ra02224j

Silva, V., Mol, H. G. J., Zomer, P., Tienstra, M., Ritsema, C. J., & Geissen, V. (2019). Pesticide residues in European agricultural soils – A hidden reality unfolded. Science of the Total Environment, 653, 1532–1545. https://doi.org/10.1016/j.scitotenv.2018.10.441

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Chagnon, M., Downs, C., … Wiemers, M. (2015). Systemic insecticides (Neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. Environmental Science and Pollution Research, 22(1), 5–34. https://doi.org/10.1007/s11356-014-3470-y

Sjursen, H., & Sømme, L. (2000). Seasonal changes in tolerance to cold and desiccation in Phauloppia sp. (Acari, Oribatida) from Finse, Norway. Journal of Insect Physiology, 46(10), 1387–1396. https://doi.org/10.1016/S0022-1910(00)00061-5

Sjursen, H. and Holmstrup, M. (2004) 'Cold and drought stress in combination with pyrene exposure: Studies with Protaphorura armata (Collembola: Onychiuridae)', Ecotoxicology and Environmental Safety, 57(2), pp. 145–152. https://doi.org/10.1016/S0147-6513(02)00144-6.

Sømme, L., & Birkemoe, T. (1999). Demography and population densities of Folsomia quadrioculata (Collembola, Isotomidae) of Spitsbergen. Norwegian Journal of Entomology, 46, 35–45.

Stork, N. E., & Eggleton, P. (1992). Invertebrates as determinants and indicators of soil quality. American Journal of Alternative Agriculture, 7(1–2), 38–47. https://doi.org/10.1017/S0889189300004446

Van Straalen, N. M., Donker, M. H., Vijver, M. G., & Van Gestel, C. A. M. (2005). Bioavailability of contaminants estimated from uptake rates into soil invertebrates. Environmental Pollution, 136(3), 409–417. https://doi.org/10.1016/j.envpol.2005.01.019

Sverdrup, L. E., Jensen, J., Krogh, P. H., & Stenersen, J. (2002). Studies on the effect of soil

aging on the toxicity of pyrene and phenanthrene to a soil-dwelling springtail. Environmental Toxicology and Chemistry, 21(3), 489–492. https://doi.org/10.1002/etc.5620210303
Swift, M. J., Heal, O. W., & Anderson, J. M. (1979). Studies in ecology volume 5. Decomposition in terrestrial ecosystems. Studies in Ecology (pp. 66–71).
Szczepaniec, A., Creary, S. F., Laskowski, K. L., Nyrop, J. P., & Raupp, M. J. (2011). Neonicotinoid insecticide imidacloprid causes outbreaks of spider mites on elm trees in urban landscapes. PLoS ONE, 6(5). https://doi.org/10.1371/journal.pone.0020018
Thompson, D. A., Lehmler, H. J., Kolpin, D. W., Hladik, M. L., Vargo, J. D., Schilling, K. E., Field, R. W. (2020, June 1). A critical review on the potential impacts of neonicotinoid insecticide use: Current knowledge of environmental fate, toxicity, and implications for human health. Environmental Science: Processes and Impacts. Royal Society of Chemistry. https://doi.org/10.1039/c9em00586b
Tilman, D., Isbell, F. and Cowles, J. M. (2014) 'Biodiversity and Ecosystem Functioning', Annu. Rev. Ecol. Evol. Syst, 45, pp. 471–493. https://doi.org/10.1146/annurev-ecolsys-120213-091917.
Tomizawa, M., & Casida, J. E. (2005). Neonicotinoid insecticide toxicology: Mechanisms of selective action. Annual Review of Pharmacology and Toxicology. https://doi.org/10.1146/annurev.pharmtox.45.120403.095930
Wagg, C., Bender, S. F., Widmer, F., & Van Der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences of the United States of America, 111(14), 5266–5270. https://doi.org/10.1073/pnas.1320054111
Wardle, D. A., Bardgett, R. D., Klironomos, J. N., Setälä, H., Van Der Putten, W. H., & Wall, D. H. (2004, June 11). Ecological linkages between aboveground and belowground biota. Science. https://doi.org/10.1126/science.1094875
Widenfalk, L. A., Malmström, A., Berg, M. P., & Bengtsson, J. (2016). Small-scale Collembola community composition in a pine forest soil – Overdispersion in functional traits indicates the importance of species interactions. Soil Biology and Biochemistry, 103, 52–62. https://doi.org/10.1016/j.soilbio.2016.08.006
Wilcoxon, F. (1945). Individual Comparisons by Ranking Methods. International Biometric Society. Biometrics Bulletin, 1(6), 80–83. Retrieved from http://www.jstor.org/stable/3001968
Wood, T. J., & Goulson, D. (2017). The environmental risks of neonicotinoid pesticides: a review of the evidence post 2013. Environmental Science and Pollution Research, 24(21), 17285–17325. https://doi.org/10.1007/s11356-017-9240-x
Yadav, I. C., & Watanabe, H. (2018). Soil erosion and transport of Imidacloprid and Clothianidin in the upland field under simulated rainfall condition. Science of the Total Environment, 640–641, 1354–1364. https://doi.org/10.1016/j.scitotenv.2018.06.008
Zaller, J. G., König, N., Tiefenbacher, A., Muraoka, Y., Querner, P., Ratzenböck, A., Koller, R. (2016). Pesticide seed dressings can affect the activity of various soil organisms and reduce decomposition of plant material. BMC Ecology, 16(1). https://doi.org/10.1186/s12898-016-0092-x
Zheng, W., Liu, W. P., Wen, Y. Z., & Lee, S. J. (2004). Photochemistry of insecticide imidacloprid: Direct and sensitized photolysis in aqueous medium. Journal of Environmental Sciences, 16(4), 539–542.

Appendices

Appendix A: Imidacloprid concentrations in mesocosms

Table A1. Overview of the randomisations of concentrations and mesocosms in the experimental blocks. SampleID reflects block, watering treatment and position in the block and treatment of imidacloprid. E.g.; Block (C) + yes-watering (V) + position (7), imidacloprid = CV72.5. U = corresponding soil samples. K = chemical analysis. L = loggers.

SampleID	Block no.	Conc.	Sample time	Sample no.
AVU1	A	0.00	1	2
AVU2	A	0.00	2	7
AVU3	A	0.00	3	5
AVK1	A	0.00	1	8
AVK2	A	0.00	2	1
AVLK3	A	0.00	3	6
AV2-0	A	0.00	2	3
AV2-0.02	A	0.02	2	15
AV2-0.1	A	0.10	2	16
AV2-0.5	A	0.50	2	9
AV2-2.5	A	2.50	2	13
AV3-0	A	0.00	3	11
AV3-0.02	A	0.02	3	14
AV3-0.1	A	0.10	3	10
AV3-0.5	A	0.50	3	12
AV3-2.5	A	2.50	3	4
AU1	A	0.00	1	1
AU2	A	0.00	2	8
AU3	A	0.00	3	4
AK1	A	0.00	1	12
AK2	A	0.00	2	9
ALK3	A	0.00	3	7
A2-0	A	0.00	2	5
A2-0.02	A	0.02	2	14
A2-0.1	A	0.10	2	10
A2-0.5	A	0.50	2	13
A2-2.5	A	2.50	2	2
A3-0	A	0.00	3	6
A3-0.02	A	0.02	3	16
A3-0.1	A	0.10	3	3
A3-0.5	A	0.50	3	15
A3-2.5	A	2.50	3	11

SampleID	Block no.	Conc.	Sample time	Sample no.
BVU1	B	0.00	1	9
BVU2	B	0.00	2	15
BVU3	B	0.00	3	7
BVK1	B	0.02	1	16
BVK2	B	0.02	2	6
BVLK3	B	0.02	3	4
BV2-0	B	0.00	2	11
BV2-0.02	B	0.02	2	8
BV2-0.1	B	0.10	2	12
BV2-0.5	B	0.50	2	2
BV2-2.5	B	2.50	2	10
BV3-0	B	0.00	3	1
BV3-0.02	B	0.02	3	13
BV3-0.1	B	0.10	3	3
BV3-0.5	B	0.50	3	5
BV3-2.5	B	2.50	3	14
BU1	B	0.00	1	10
BU2	B	0.00	2	11
BU3	B	0.00	3	2
BK1	B	0.02	1	16
BK2	B	0.02	2	3
BLK3	B	0.02	3	6
B2-0	B	0.00	2	5
B2-0.02	B	0.02	2	7
B2-0.1	B	0.10	2	15
B2-0.5	B	0.50	2	8
B2-2.5	B	2.50	2	14
B3-0	B	0.00	3	12
B3-0.02	B	0.02	3	4
B3-0.1	B	0.10	3	9
B3-0.5	B	0.50	3	13
B3-2.5	B	2.50	3	1

Table A1 Continued. Overview of the randomisations of concentrations and mesocosms in the experimental blocks. SampleID reflects block, watering treatment and position in the block and treatment of imidacloprid. E.g.; Block (C) + yes-watering (V) + position (7), imidacloprid = CV72.5. U = corresponding soil samples. K = chemical analysis. L = loggers.

SampleID	Block no.	Conc.	Sample time	Sample no.	SampleID	Block no.	Conc.	Sample time	Sample no.
CVU1	C	0.00	1	10	DVU1	D	0.00	1	16
CVU2	C	0.00	2	15	DVU2	D	0.00	2	11
CVU3	C	0.00	3	14	DVU3	D	0.00	3	9
CVK1	C	0.10	1	1	DVK1	D	0.50	1	6
CVK2	C	0.10	2	9	DVK2	D	0.50	2	2
CVLK3	C	0.10	3	12	DVLK3	D	0.50	3	8
CV2-0	C	0.00	2	4	DV2-0	D	0.00	2	14
CV2-0.02	C	0.02	2	8	DV2-0.02	D	0.02	2	3
CV2-0.1	C	0.10	2	7	DV2-0.1	D	0.10	2	10
CV2-0.5	C	0.50	2	3	DV2-0.5	D	0.50	2	15
CV2-2.5	C	2.50	2	2	DV2-2.5	D	2.50	2	13
CV3-0	C	0.00	3	11	DV3-0	D	0.00	3	4
CV3-0.02	C	0.02	3	16	DV3-0.02	D	0.02	3	12
CV3-0.1	C	0.10	3	6	DV3-0.1	D	0.10	3	5
CV3-0.5	C	0.50	3	13	DV3-0.5	D	0.50	3	1
CV3-2.5	C	2.50	3	5	DV3-2.5	D	2.50	3	7
CU1	C	0.00	1	7	DU1	D	0.00	1	8
CU2	C	0.00	2	13	DU2	D	0.00	2	12
CU3	C	0.00	3	1	DU3	D	0.00	3	15
CK1	C	0.10	1	9	DK1	D	0.50	1	5
CK2	C	0.10	2	15	DK2	D	0.50	2	3
CLK3	C	0.10	3	5	DLK3	D	0.50	3	13
C2-0	C	0.00	2	6	D2-0	D	0.00	2	2
C2-0.02	C	0.02	2	4	D2-0.02	D	0.02	2	6
C2-0.1	C	0.10	2	3	D2-0.1	D	0.10	2	10
C2-0.5	C	0.50	2	11	D2-0.5	D	0.50	2	4
C2-2.5	C	2.50	2	2	D2-2.5	D	2.50	2	7
C3-0	C	0.00	3	10	D3-0	D	0.00	3	14
C3-0.02	C	0.02	3	12	D3-0.02	D	0.02	3	11
C3-0.1	C	0.10	3	16	D3-0.1	D	0.10	3	9
C3-0.5	C	0.50	3	8	D3-0.5	D	0.50	3	1
C3-2.5	C	2.50	3	14	D3-2.5	D	2.50	3	16

Table A1 Continued. Overview of the randomisations of concentrations and mesocosms in the experimental blocks. SampleID reflects block, watering treatment and position in the block and treatment of imidacloprid. E.g.; Block (C) + yes-watering (V) + position (7), imidacloprid = CV72.5. U = corresponding soil samples. K = chemical analysis. L = loggers.

SampleID	Block no.	Conc.	Sample time	Sample no.	SampleID	Block no.	Conc.	Sample time	Sample no.
EVU1	E	0.00	1	14	FVU1	F	0	1	2
EVU2	E	0.00	2	6	FVU2	F	0	2	7
EVU3	E	0.00	3	2	FVU3	F	0	3	4
EVK1	E	2.50	1	12	FVLK3	F	0	3	6
EVK2	E	2.50	2	13	FV2-0	F	0	2	5
EVLK3	E	2.50	3	16	FV2-0.02	F	0.02	2	14
EV2-0	E	0.00	2	10	FV2-0.1	F	0.1	2	1
EV2-0.02	E	0.02	2	9	FV2-0.5	F	0.5	2	10
EV2-0.1	E	0.10	2	11	FV2-2.5	F	2.5	2	3
EV2-0.5	E	0.50	2	7	FV3-0	F	0	3	9
EV2-2.5	E	2.50	2	8	FV3-0.02	F	0.02	3	12
EV3-0	E	0.00	3	5	FV3-0.1	F	0.1	3	13
EV3-0.02	E	0.02	3	15	FV3-0.5	F	0.5	3	11
EV3-0.1	E	0.10	3	1	FV3-2.5	F	2.5	3	8
EV3-0.5	E	0.50	3	4	FU1	F	0	1	3
EV3-2.5	E	2.50	3	3	FU2	F	0	2	13
EU1	E	0.00	1	6	FU3	F	0	3	4
EU2	E	0.00	2	16	FLK3	F	0	3	10
EU3	E	0.00	3	10	F2-0	F	0	2	8
EK1	E	2.50	1	3	F2-0.02	F	0.02	2	5
EK2	E	2.50	2	11	F2-0.1	F	0.1	2	6
ELK3	E	2.50	3	7	F2-0.5	F	0.5	2	2
E2-0	E	0.00	2	9	F2-2.5	F	2.5	2	11
E2-0.02	E	0.02	2	1	F3-0	F	0	3	1
E2-0.1	E	0.10	2	5	F3-0.02	F	0.02	3	7
E2-0.5	E	0.50	2	8	F3-0.1	F	0.1	3	14
E2-2.5	E	2.50	2	15	F3-0.5	F	0.5	3	9
E3-0	E	0.00	3	14	F3-2.5	F	2.5	3	12
E3-0.02	E	0.02	3	12					
E3-0.1	E	0.10	3	4					
E3-0.5	E	0.50	3	13					
E3-2.5	E	2.50	3	2					

Table A1 Continued. Overview of the randomisations of concentrations and mesocosms in the experimental blocks. SampleID reflects block, watering treatment and position in the block and treatment of imidacloprid. E.g.; Block (C) + yes-watering (V) + position (7), imidacloprid = CV72.5. U = corresponding soil samples. K = chemical analysis. L = loggers.

SampleID	Block no.	Conc.	Sample time	Sample no.	SampleID	Block no.	Conc.	Sample time	Sample no.
GVU1	G	0	1	10	HVU1	H	0.00	1	5
GVU2	G	0	2	8	HVU2	H	0.00	2	3
GVU3	G	0	3	9	HVU3	H	0.00	3	1
GVLK3	G	(0,5)	3	11	HVLK3	H	0.00	3	10
GV2-0	G	0	2	3	HV2-0	H	0.00	2	13
GV2-0.02	G	0.02	2	14	HV2-0.02	H	0.02	2	9
GV2-0.1	G	0.1	2	13	HV2-0.1	H	0.10	2	4
GV2-0.5	G	0.5	2	2	HV2-0.5	H	0.50	2	14
GV2-2.5	G	2.5	2	4	HV2-2.5	H	2.50	2	7
GV3-0	G	0	3	7	HV3-0	H	0.00	3	6
GV3-0.02	G	0.02	3	12	HV3-0.02	H	0.02	3	12
GV3-0.1	G	0.1	3	5	HV3-0.1	H	0.10	3	2
GV3-0.5	G	0.5	3	6	HV3-0.5	H	0.50	3	8
GV3-2.5	G	2.5	3	1	HV3-2.5	H	2.50	3	11
GU1	G	0	1	9	HU1	H	0.00	1	5
GU2	G	0	2	2	HU2	H	0.00	2	14
GU3	G	0	3	6	HU3	H	0.00	3	4
GLK3	G	0	3	10	HLK3	H	0.00	3	1
G2-0	G	0	2	14	H2-0	H	0.00	2	2
G2-0.02	G	0.02	2	12	H2-0.02	H	0.02	2	11
G2-0.1	G	0.1	2	3	H2-0.1	H	0.10	2	12
G2-0.5	G	0.5	2	7	H2-0.5	H	0.50	2	10
G2-2.5	G	2.5	2	4	H2-2.5	H	2.50	2	9
G3-0	G	0	3	5	H3-0	H	0.00	3	3
G3-0.02	G	0.02	3	11	H3-0.02	H	0.02	3	7
G3-0.1	G	0.1	3	13	H3-0.1	H	0.10	3	13
G3-0.5	G	0.5	3	8	H3-0.5	H	0.50	3	6
G3-2.5	G	2.5	3	1	H3-2.5	H	2.50	3	8

Appendix B: Moisture content in soil samples

Table B1. Table showing FW (g), DW (g). moisture content (%), percentage change and if the samples have been treated with the water regime (Yes/No). SampleID reflects block, watering treatment and position in the block. E.g.; Block (C) + yes-watering (V) + position (7) = CV7.

SampleID	Month	Water treatment	Conc. imidacloprid	FW (g)	DW (g)	Moisture content (%)	Change (%)
E6	May	No	0.0	105.7	80.6	31	24
AV2	May	No	0.0	98.3	68.6	43	30
EV14	May	No	0.0	66.4	45.4	46	31
D8	May	No	0.0	76.1	56.1	35	26
B12	May	No	0.0	107.2	78.4	36	27
DV16	May	No	0.0	127.6	92.0	38	28
C7	May	No	0.0	125.6	90.3	39	28
H5	May	No	0.0	87.9	62.7	40	29
HV5	May	No	0.0	79.1	55.8	41	29
BV9	May	No	0.0	113.3	85.1	33	25
G9	May	No	0.0	89.6	64.7	38	28
CV10	May	No	0.0	146.5	109.4	33	25
F3	May	No	0.0	103.0	72.3	42	30
FV2	May	No	0.0	69.4	47.4	46	32
A1	May	No	0.0	118.5	88.4	33	26
GV10	May	No	0.0	85.6	60.8	40	29
GV3	June	No	0.0	132.8	81.2	63	39
A5	June	No	0.0	106.5	70.0	51	34
G14	June	No	0.0	157.8	104.3	51	34
F8	June	No	0.0	135.7	86.3	57	36
C6	June	No	0.0	112.1	72.8	53	35
E9	June	No	0.0	150.9	104.9	43	30
B5	June	No	0.0	133.9	89.9	48	33
H2	June	No	0.0	133.9	81.5	64	40
D2	June	No	0.0	134.0	89.6	49	33
FV5	June	Yes	0.0	96.2	61.2	57	36
BV11	June	Yes	0.0	160.7	108.0	48	33
DV14	June	Yes	0.0	176.1	108.2	62	38
AV3	June	Yes	0	118.6	72.9	62	38
EV10	June	Yes	0	148.5	96.1	54	38
CV4	June	Yes	0	94.5	58.6	61	35
AV11	June	No	0.02	115.6	71.8	60	38
B7	June	No	0.02	104.6	67.2	55	37
A14	June	No	0.02	111.9	71.4	56	36

Table B1 Continued. Table showing FW (g), DW (g). moisture content (%), percentage change and if the samples have been treated with the water regime (Yes/No). SampleID reflects block, watering treatment and position in the block. E.g.; Block (C) + yes-watering (V) + position (7) = CV7.

SampleID	Month	Water treatment	Conc. imidacloprid	FW (g)	DW (g)	Moisture content (%)	Change (%)
G12	June	No	0.02	123.3	80.1	53	36
C4	June	No	0.02	125.5	93.9	33	35
E1	June	No	0.02	86.7	48.9	77	25
H11	June	No	0.02	129.5	80.7	60	43
D6	June	No	0.02	115.2	71.1	62	38
FV14	June	Yes	0.02	143.0	90.1	58	38
AV15	June	Yes	0.02	155.1	97.4	59	37
EV9	June	Yes	0.02	142.9	89.4	59	37
HV9	June	Yes	0.02	113.5	64.8	75	37
GV14	June	Yes	0.02	110.3	64.4	71	43
CV8	June	Yes	0.02	133.6	83.9	50	42
BV8	June	Yes	0.02	110.1	64.4	70	37
DV3	June	Yes	0.02	127.9	76.7	66	41
G3	June	No	0.1	139.8	88.1	58	40
D10	June	No	0.1	105.3	66.4	58	37
A10	June	No	0.1	109.9	66.6	65	37
B15	June	No	0.1	128.1	81.8	56	39
F6	June	No	0.1	104.8	62.9	66	36
H12	June	No	0.1	112.1	63.9	75	40
C3	June	No	0.1	116.0	76.2	52	43
E5	June	No	0.1	147.2	99.3	48	34
BV12	June	Yes	0.1	150.7	100.7	49	32
DV10	June	Yes	0.1	106.1	65.7	61	33
FV1	June	Yes	0.1	104.8	68.8	52	38
CV7	June	Yes	0.1	113.2	72.8	55	35
HV4	June	Yes	0.1	133.5	81.8	63	39
AV16	June	Yes	0.1	135.3	85.5	58	37
GV13	June	Yes	0.1	109.8	63.0	74	42
EV11	June	Yes	0.1	131.3	82.7	58	37
B8	June	No	0.5	153.8	98.9	55	35
F2	June	No	0.5	129.2	82.4	56	36
C11	June	No	0.5	121.8	77.8	56	36
G7	June	No	0.5	147.0	96.0	53	34
A13	June	No	0.5	121.7	75.7	60	38
D4	June	No	0.5	171.6	112.0	53	35
E8	June	No	0.5	112.8	77.4	45	31

Table B1 Continued. Table showing FW (g), DW (g). moisture content (%), percentage change and if the samples have been treated with the water regime (Yes/No). SampleID reflects block, watering treatment and position in the block. E.g.; Block (C) + yes-watering (V) + position (7) = CV7.

SampleID	Month	Water treatment	Conc. imidacloprid	FW (g)	DW (g)	Moisture content (%)	Change (%)
H10	June	No	0.5	71.1	38.6	84	46
BV2	June	Yes	0.5	96.5	61.0	58	37
FV10	June	Yes	0.5	128.9	82.3	56	36
EV7	June	Yes	0.5	121.5	73.6	64	39
AV9	June	Yes	0.5	144.0	90.7	58	37
CV3	June	Yes	0.5	124.8	86.1	44	31
GV2	June	Yes	0.5	113.0	68.0	66	40
HV14	June	Yes	0.5	118.2	76.1	55	35
DV15	June	Yes	0.5	170.6	110.1	54	35
B14	June	No	2.5	115.8	73.5	57	36
H9	June	No	2.5	115.4	70.9	62	38
C2	June	No	2.5	109.9	68.1	61	38
G4	June	No	2.5	125.9	79.3	58	37
D7	June	No	2.5	157.0	103.9	51	34
F11	June	No	2.5	88.6	54.9	61	38
E15	June	No	2.5	142.3	83.7	69	41
A2	June	No	2.5	128.5	85.5	50	33
EV8	June	Yes	2.5	125.9	77.6	62	38
CV2	June	Yes	2.5	123.6	80.8	52	34
DV13	June	Yes	2.5	170.4	110.1	54	35
HV7	June	Yes	2.5	119.6	73.4	62	38
GV4	June	Yes	2.5	142.7	89.0	60	38
FV3	June	Yes	2.5	108.0	72.7	48	32
BV10	June	Yes	2.5	140.5	91.6	53	35
AV13	June	Yes	2.5	126.1	78.0	61	38
B10	August	No	0.0	97.92	81.48	20	20
E14	August	No	0.0	93.65	80.59	16	16
H3	August	No	0.0	104.53	87.49	19	19
A6	August	No	0.0	84.24	72.5	16	16
C10	August	No	0.0	96.9	81.77	18	18
D14	August	No	0.0	94.36	73.98	27	27
F1	August	No	0.0	88.69	74.24	19	19
G5	August	No	0.0	91.93	74.51	23	23
AV11	August	Yes	0.0	114.7	94.47	21	21
DV4	August	Yes	0.0	106.42	85.93	24	24
GV7	August	Yes	0.0	113.27	82.95	37	37

Table B1 Continued. Table showing FW (g), DW (g). moisture content (%), percentage change and if the samples have been treated with the water regime (Yes/No). SampleID reflects block, watering treatment and position in the block. E.g.; Block (C) + yes-watering (V) + position (7) = CV7.

SampleID	Month	Water treatment	Conc. imidacloprid	FW (g)	DW (g)	Moisture content (%)	Change (%)
FV9	August	Yes	0.0	99.46	85.16	17	17
EV5	August	Yes	0.0	94.53	78.86	20	20
CV11	August	Yes	0.0	114.16	88.38	29	29
HV6	August	Yes	0.0	108.79	88.86	22	22
BV1	August	Yes	0.0	73.29	62.37	17	17
D11	August	No	0.02	148.03	124.2	19	19
A16	August	No	0.02	93.94	82.39	14	14
F7	August	No	0.02	76.82	61.6	25	25
C12	August	No	0.02	95.23	79.7	19	19
G11	August	No	0.02	129.02	105.03	23	23
H7	August	No	0.02	45.49	29.51	54	54
B4	August	No	0.02	129.32	107.82	20	20
E12	August	No	0.02	90.23	77.37	17	17
DV12	August	Yes	0.02	134.88	111.76	21	21
GV12	August	Yes	0.02	85.13	69.73	22	22
AV14	August	Yes	0.02	106.73	81.1	32	32
FV12	August	Yes	0.02	96.65	77.05	25	25
HV12	August	Yes	0.02	96.11	73.02	32	32
BV13	August	Yes	0.02	133.89	115.3	16	14
CV16	August	Yes	0.02	94.91	80.35	18	15
EV15	August	Yes	0.02	110.71	91.83	21	17
E4	August	No	0.1	99.72	87.84	13	12
D9	August	No	0.1	90.47	73.85	22	18
H13	August	No	0.1	78.81	67.07	17	15
F14	August	No	0.1	92.98	76.72	21	17
G13	August	No	0.1	111.61	91.79	22	18
B9	August	No	0.1	80.79	68.48	18	15
C16	August	No	0.1	113.76	95.47	19	16
A3	August	No	0.1	97.44	84.58	15	13
AV10	August	Yes	0.1	104.66	83.94	25	20
EV1	August	Yes	0.1	128.02	105.24	22	18
HV2	August	Yes	0.1	72.01	61.68	17	14
FV13	August	Yes	0.1	129.64	108.73	19	16
GV5	August	Yes	0.1	63.86	53.31	20	16
BV3	August	Yes	0.1	101.35	83.47	21	18

Table B1 Continued. Table showing FW (g), DW (g). moisture content (%), percentage change and if the samples have been treated with the water regime (Yes/No). SampleID reflects block, watering treatment and position in the block. E.g.; Block (C) + yes-watering (V) + position (7) = CV7.

SampleID	Month	Water treatment	Conc. imidacloprid	FW (g)	DW (g)	Moisture content (%)	Change (%)
CV6	August	Yes	0.1	101.45	86.25	18	15
DV5	August	Yes	0.1	88.18	70.18	26	20
B13	August	No	0.5	84.31	70.31	20	17
A15	August	No	0.5	71.18	60.65	17	15
H6	August	No	0.5	39.97	20.05	99	50
G8	August	No	0.5	107.17	87.18	23	18
C8	August	No	0.5	87.03	68.86	26	21
D1	August	No	0.5	107.1	87.24	23	18
E13	August	No	0.5	72.39	62.12	16	14
F9	August	No	0.5	93.2	78.55	19	16
GV6	August	Yes	0.5	56.43	45.47	24	19
EV4	August	Yes	0.5	102.44	81.93	25	20
FV11	August	Yes	0.5	86.8	68.71	26	21
DV1	August	Yes	0.5	112.26	92.31	21	18
BV5	August	Yes	0.5	98.38	84.11	17	14
AV12	August	Yes	0.5	101.88	85.11	20	16
CV13	August	Yes	0.5	84.07	71.7	17	15
HV8	August	Yes	0.5	81.06	64.24	26	21
C14	August	No	2.5	80.67	68.87	17	15
G1	August	No	2.5	107.96	87.33	23	19
F12	August	No	2.5	100.52	83.2	21	17
B1	August	No	2.5	136.34	113.48	20	17
E2	August	No	2.5	102.13	88.97	15	13
D16	August	No	2.5	91.9	71.49	28	22
A11	August	No	2.5	73.35	64.14	14	12
H8	August	No	2.5	65.01	53.47	21	18
EV3	August	Yes	2.5	89.55	71.49	25	20
GV1	August	Yes	2.5	94.24	83.01	13	12
HV11	August	Yes	2.5	108.42	85.48	27	21
AV4	August	Yes	2.5	118.99	91.73	30	23
FV8	August	Yes	2.5	111.34	89.69	24	19
CV5	August	Yes	2.5	56.49	46.78	21	18
BV14	August	Yes	2.5	121.92	105.06	16	14
DV7	August	Yes	2.5	101.41	80.21	26	21

Appendix C: Imidacloprid analysis of soil

The analysis of imidacloprid in soil done at NIVA, as explained in detail in Sagnik Sengupta et al (2021):

Subsamples of the spiked food were preserved in darkness at –20 °C prior to highperformance liquid chromatographic–mass spectroscopic analysis of imidacloprid content. The homogenized food (10–30 mg) sample was weighed and added to a 15-mL tube. Prior to extraction, 50 μL of an internal standard (1 ng μL–1 of d4-imidacloprid) was spiked into the sample. Two milliliters each of water and acetonitrile (MeCN) were added to the tube. The sample present in the tube was mixed by vortexing for 1 min, followed by sonification for 30 min at room temperature. Approximately 1 g of NaCl (salt) was added to the tube, which was then shaken. The salt dissolved in the water, which then separated from the MeCN. The MeCN fraction was removed and evaporated until dryness by heating (60 °C) under nitrogen. The remaining content was then dissolved in 0.5 mL 10% MeCN in water. All the samples were filtered with 20-μm Spin-X centrifuge nylon filter (Corning) and finally transferred into a liquid chromatography vial ready for ultraperformance liquid chromatographic (UPLC)-mass spectroscopic analysis.

Liquid chromatography was performed on an Acquity BEH C18 column (1.8 μm, 100 × 2.1 mm; Waters), using an Acquity UPLC module (Waters). Separation was achieved using linear gradient elution at 0.5 mL min–1 starting with MeCN:water (10:90 v/v, water containing 0.1% formic acid), rising to 100% MeCN over 9 min. An isocratic elution with 100% MeCN was maintained for 2 min before the eluent was switched back to 10% MeCN. The UPLC system was coupled to a Xevo TQ-S tandem mass spectrometer operating with an ESI interface (Waters Micromass). Screening of imidacloprid was performed with multiple reaction monitoring in positive ionization mode; imidacloprid 256 > 175, 256 > 209 and deuterated 260 > 179, 260 > 213. The limit of detection of 0.1 ng g–1 imidaclo prid was estimated to be 3 times the signal-to-noise ratio using spiked control samples.

Appendix D: Effects of the watering treatment

Table D1. Overview of the statistics on the watering regime found in each springtail group and mites. The effect of the watering on abundance and together with concentrations of imidacloprid is compared with the control groups. Model generated by mixed effects model, lme in RStudio.

	Value	Std. Error	DF	t-value	p-value	AIC	LogLik
F.quad						**819.190**	**-403.595**
Watering	-0.371	0.297	221	-1.245	0.214		
Watering*Conc.	-0.008	0.166	221	-0.053	0.957		
Upper						**569.085**	**-274.543**
Watering	-0.074	0.207	217	-0.358	0.720		
Watering*Conc.	0.058	0.096	217	0.600	0.548		
Middle						**626.591**	**-303.295**
Watering	0.449	0.269	217	1.665	0.097		
Watering*Conc.	0.089	0.106	217	0.840	0.401		
Lower						**795.910**	**-387.955**
Watering	0.689	0.106	217	1.803	0.072		
Watering*Conc.	-0.055	0.153	217	-0.361	0.718		
Mites						**504.646**	**-242.323**
Watering	0.244	0.211	217	1.151	0.250		
Watering*Conc.	0.002	0.079	217	0.031	0.974		

Appendix E: Figures

Overview of the boxplots on the abundance of organisms between the watering blocks

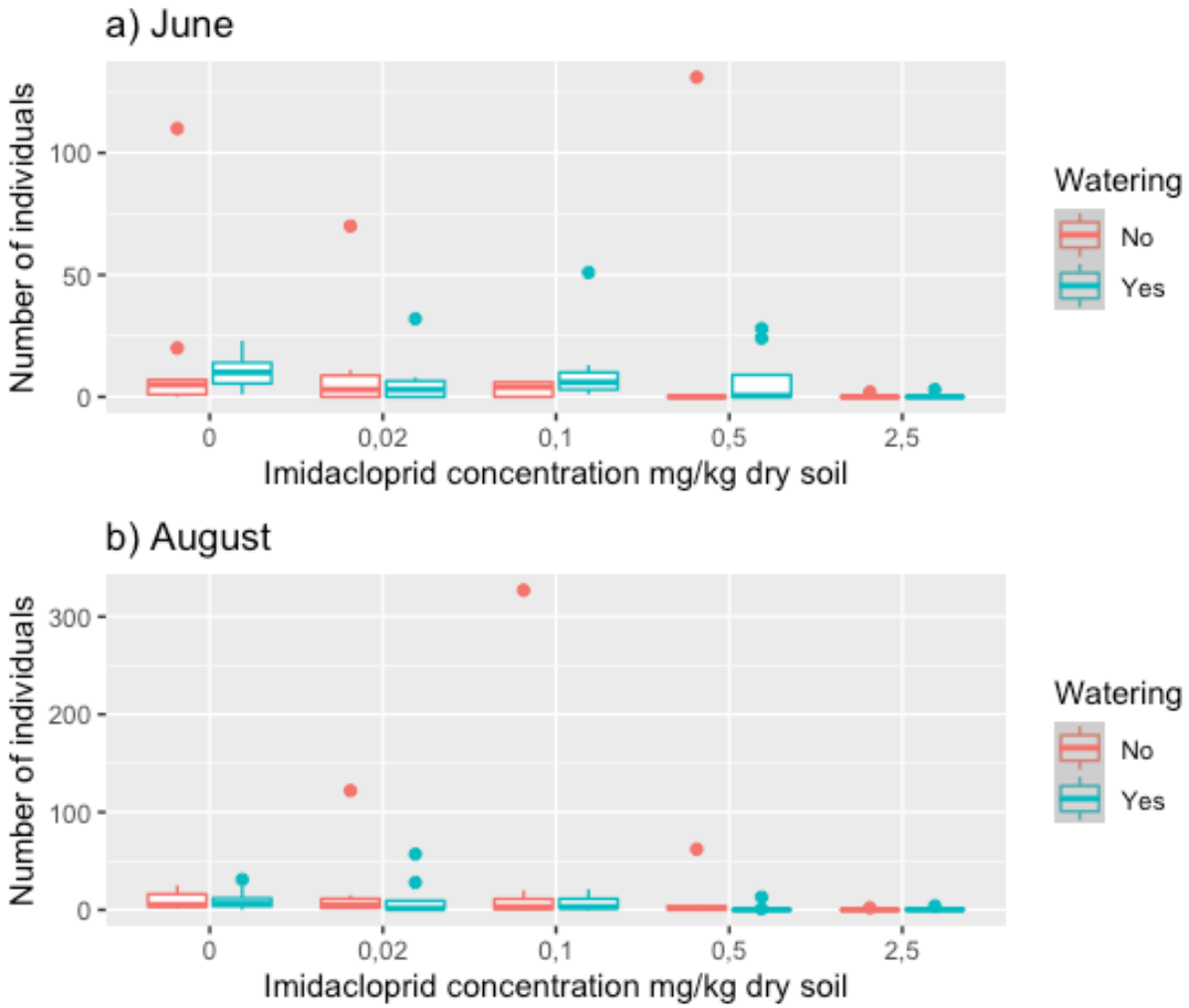

Figure E1 ab). Box plots showing the abundance of *F. quadrioculata* in a) June and b) August. The abundance is shown at each concentration and water treatment blocks. Watering blocks are shown in blue (Yes) and no-watering blocks are shown in red (No). Y-axis show the number of individuals and x-axis shows the concentrations. The middle line represents median individuals. The bottom and top lines represents the first and third quantiles. Whiskers represent the range of the data and outliers are marked as individual points.

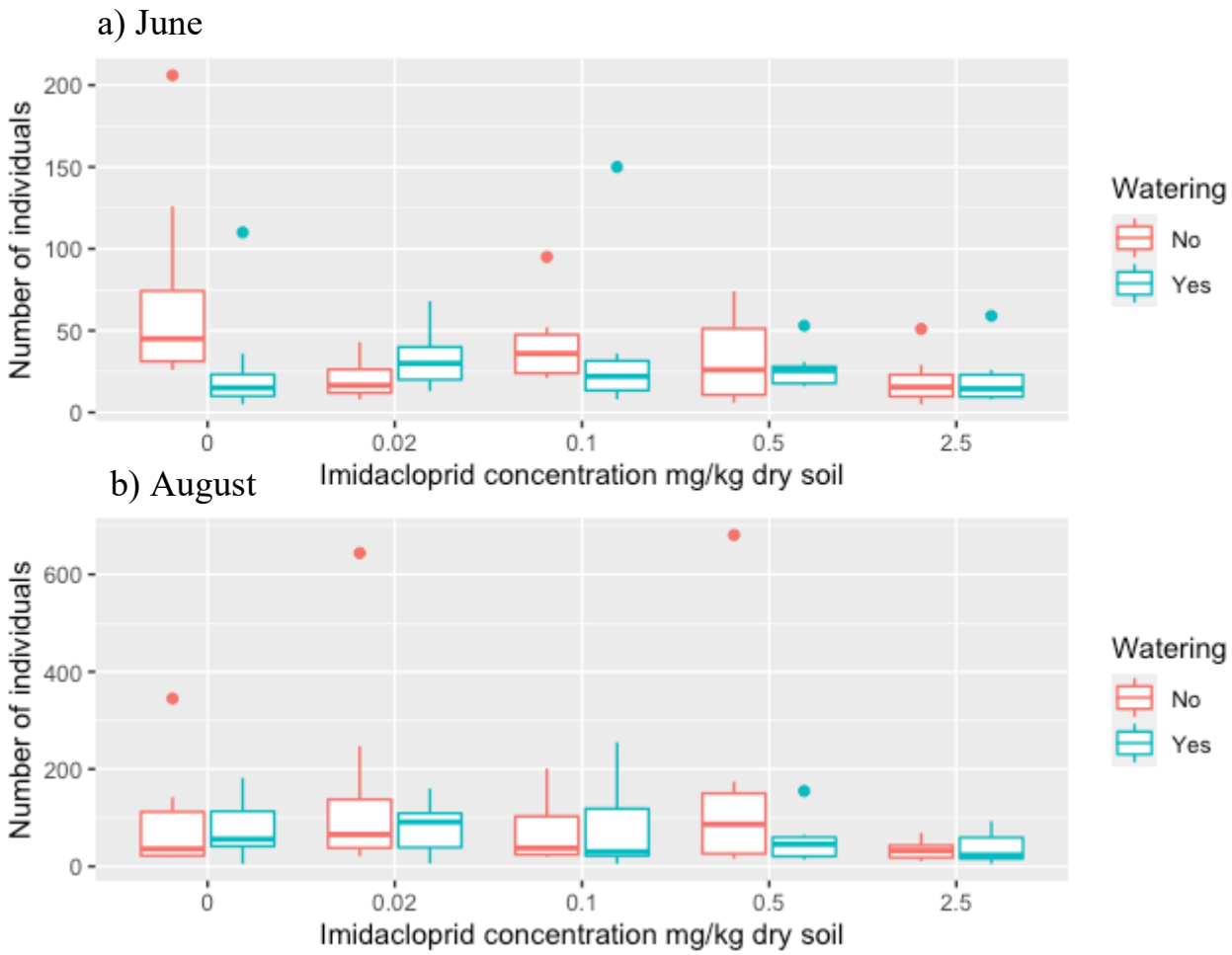

Figure E2 ab). Box plots showing the abundance of surface dwelling springtails in a) June and b) August. The abundance is shown at each concentration and water treatment blocks. Watering blocks are shown in blue (Yes) and no-watering blocks are shown in red (No). Y-axis show the number of individuals and x-axis shows the concentrations. The middle line represents median individuals. The bottom and top lines represents the first and third quantiles. Whiskers represent the range of the data and outliers are marked as individual points.

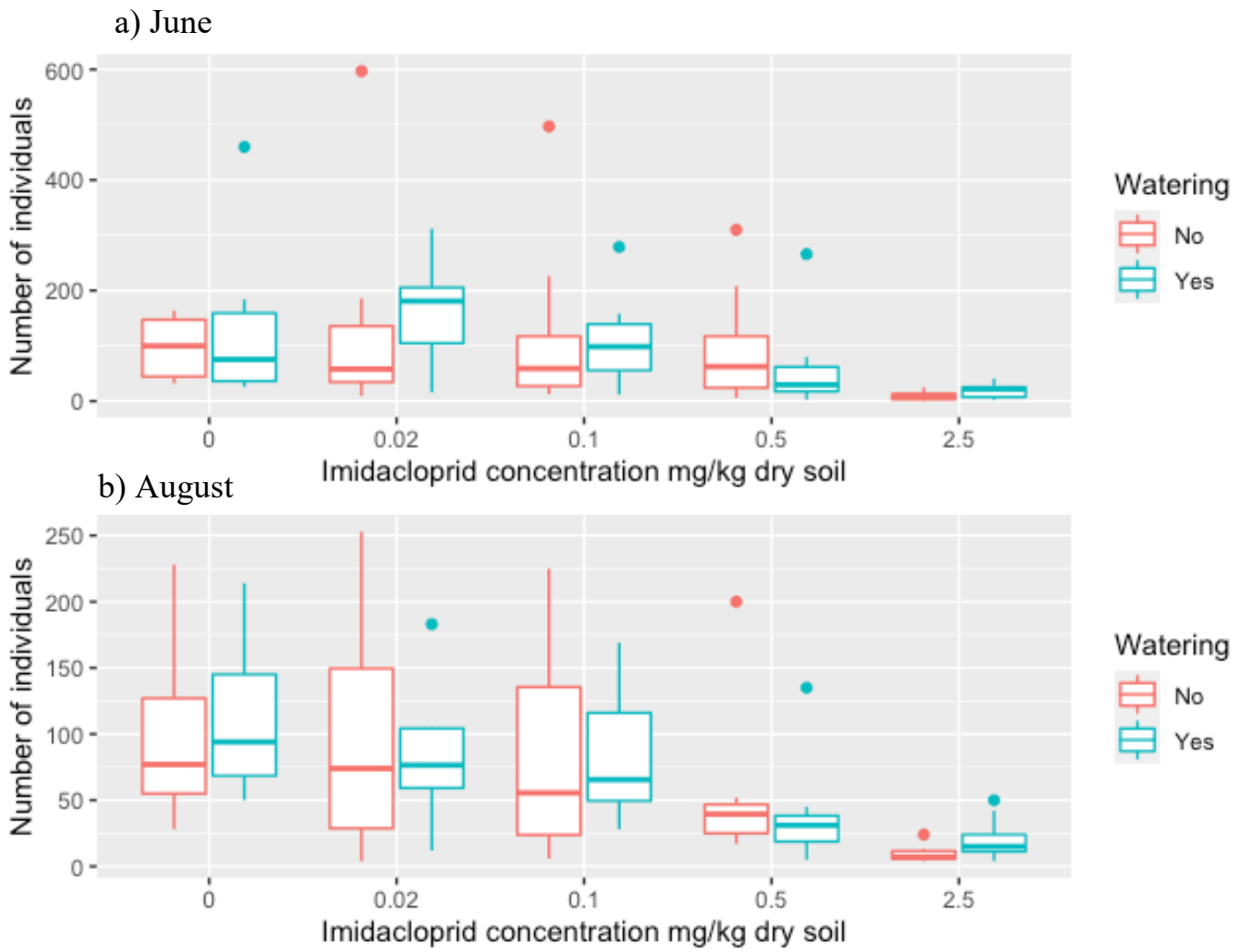

Figure E3 ab). Box plots showing the abundance of litter dwelling springtails in a) June and b) August. The abundance is shown at each concentration and water treatment blocks. Watering blocks are shown in blue (Yes) and no-watering blocks are shown in red (No). Y-axis show the number of individuals and x-axis shows the concentrations. The middle line represents median individuals. The bottom and top lines represents the first and third quantiles. Whiskers represent the range of the data and outliers are marked as individual points.

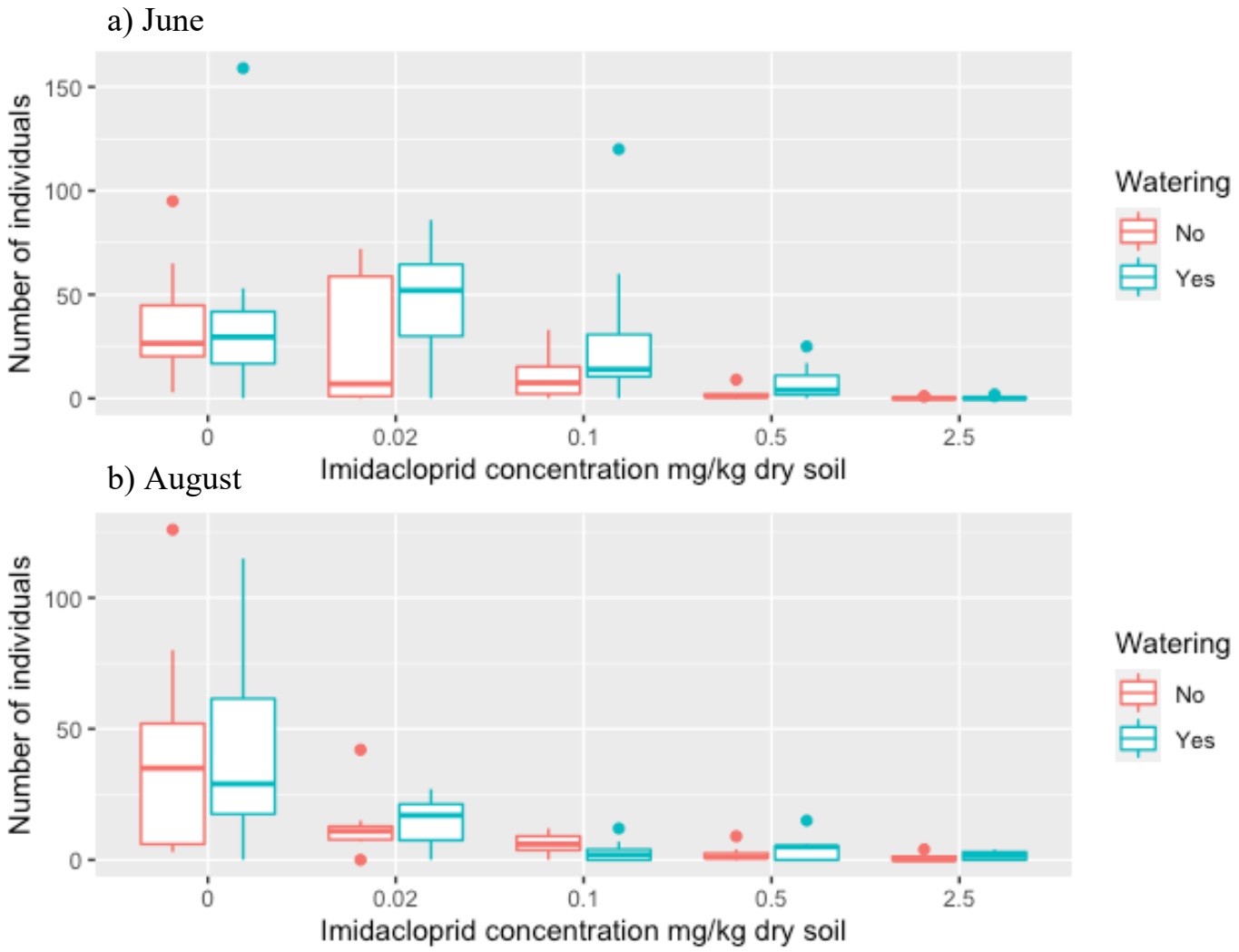

Figure E4 ab). Box plots showing the abundance of soil dwelling springtails in a) June and b) August. The abundance is shown at each concentration and water treatment blocks. Watering blocks are shown in blue (Yes) and no-watering blocks are shown in red (No). Y-axis show the number of individuals and x-axis shows the concentrations. The middle line represents median individuals. The bottom and top lines represents the first and third quantiles. Whiskers represent the range of the data and outliers are marked as individual points.

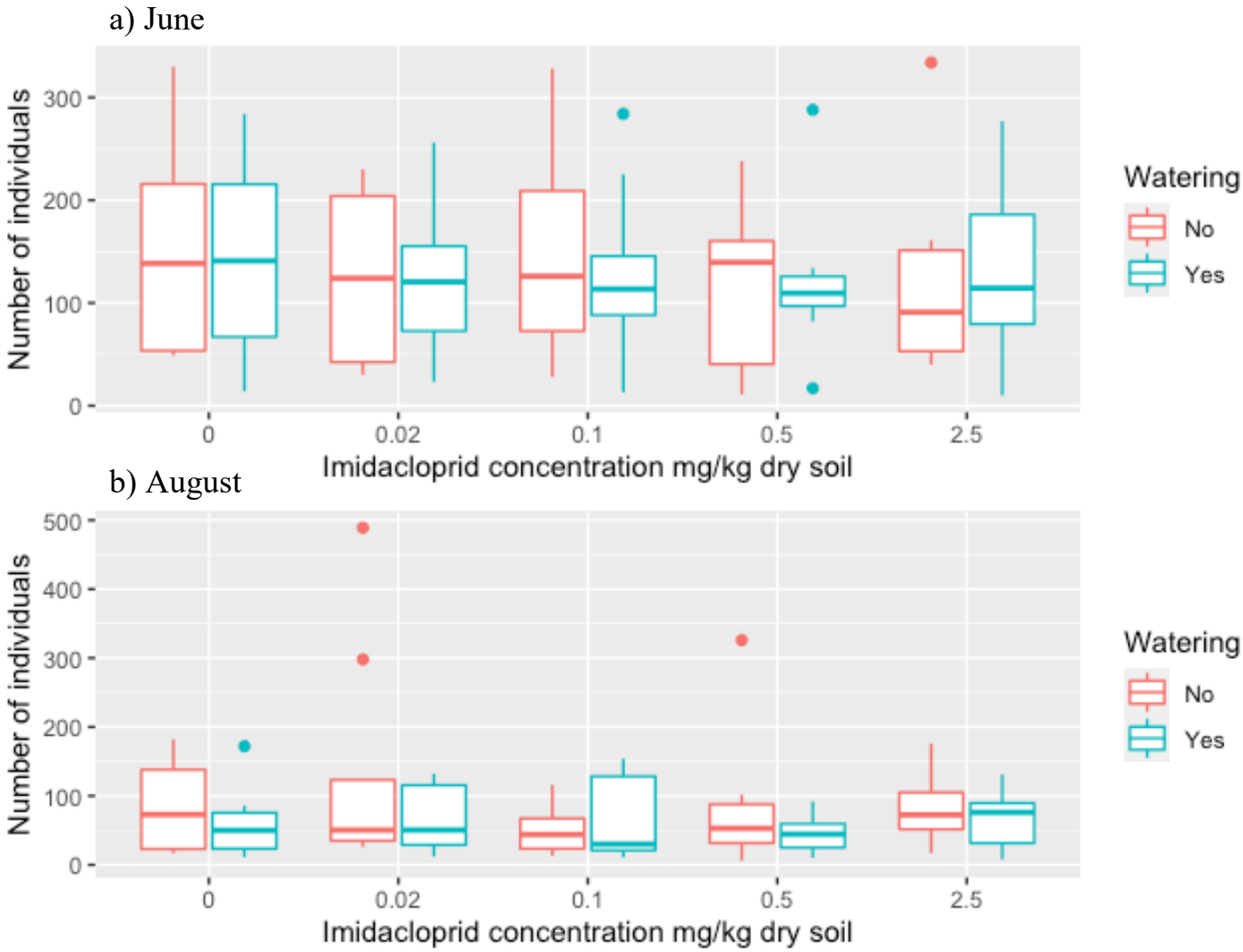

Figure E5 ab). Box plots showing the abundance of mites in a) June and b) August. The abundance is shown at each concentration and water treatment blocks. Watering blocks are shown in blue (Yes) and no-watering blocks are shown in red (No). Y-axis show the number of individuals and x-axis shows the concentrations. The middle line represents median individuals. The bottom and top lines represents the first and third quantiles. Whiskers represent the range of the data and outliers are marked as individual points.

Appendix F: Sampled organisms

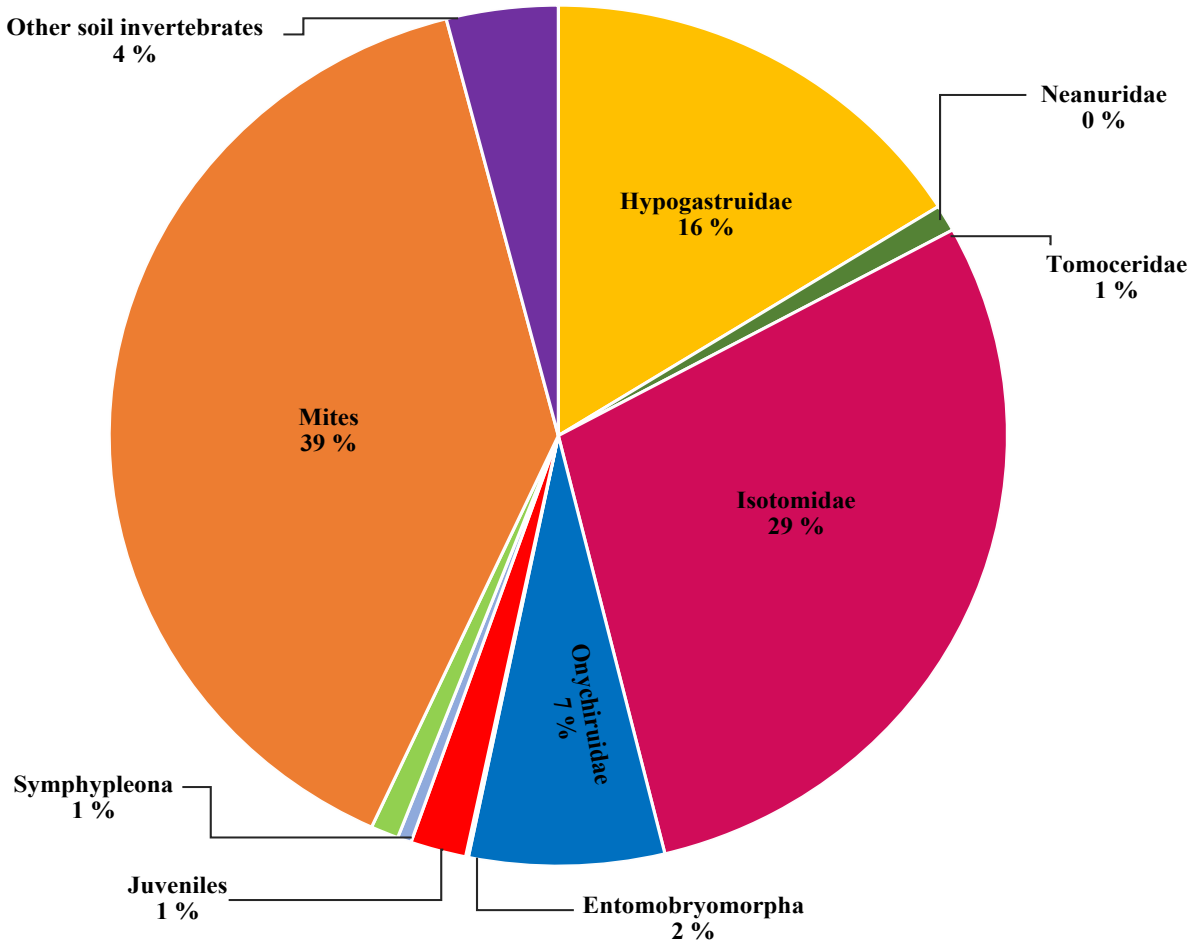

Figure F1. Pie chart showing an overview of the total abundance of organisms found and counted during the experiment. In this chart all treatment samples and months are pooled together.

Table F1. Overview of the other sampled taxa in the experiment. The organisms are organised by species order, sample time (Month) and the total number of organisms sampled per concentration of imidacloprid (mg/kg).

Species	Month	Total number organisms per conc. imidacloprid (mg/kg)				
		0	*0.02*	*0.1*	*0.5*	*2.5*
Enchytraeide	*May*	54	-	-	-	-
	June	332	383	318	294	303
	August	5	1	1	1	2
Coleoptera	*May*	6	-	-	-	-
	June	5	7	4	4	2
	August	3	2	2	1	3
Acridimorpha	*May*	11	-	-	-	-
	June	3	1	0	0	0
	August	3	0	0	0	0
Larvae	*May*	33	-	-	-	-
	June	56	16	13	12	8
	August	43	20	12	20	6
Aranea	*May*	3	-	-	-	-
	June	6	1	1	4	1
	August	21	1	27	0	0
Diptera	*May*	4	-	-	-	-
	June	5	5	2	2	0
	August	4	2	1	0	1

Table F1 Continued. Overview of the other sampled taxa in the experiment. The organisms are organised by species order, sample time (Month) and the total number of organisms sampled per concentration of imidacloprid (mg/kg).

Species	Month	Total number organisms per conc. imidacloprid (mg/kg)				
		0	*0.02*	*0.1*	*0.5*	*2.5*
Myriapoda	*May*	14	-	-	-	-
	June	10	6	4	3	7
	August	5	0	2	5	2
Nematocera	*May*	4	-	-	-	-
	June	3	2	1	2	2
	August	2	2	0	0	1
Megadrilacea	*May*	2	-	-	-	-
	June	0	0	0	2	1
	August	0	0	0	0	0
Hymenoptera	*May*	0	-	-	-	-
	June	0	0	7	1	0
	August	0	1	0	0	0
Unidentified invertebrate	*May*	10	-	-	-	-
	June	12	7	5	16	1
	August	22	6	1	3	2