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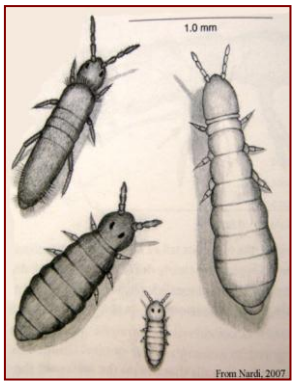

Tanya Marcela González Martínez

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor José Paulo Sousa, Professor Auxiliar do Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia da Universidade de Coimbra.

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Summary

To identify potential risks derived from changing climatic regimes has become a major concern worldwide. Alterations of rain patterns are expected to modify the environmental responses of biological communities in soil, often due to alterations in moisture levels, a key factor for soil microarthropods. Pesticide use imposes great disturbances to soil, altering its functional dynamics. Since environmental conditions such as rain and temperature regimes can interfere with chemical speciation and/or chemical's persistence in soil, soil organisms might be affected in a different way in contaminated soil under different climatic scenarios. Lambda-cyalothrin is a pyrethroid insecticide widely used to control insect pests for public health and cultivated lands. Annual agricultural use of L-cyalothrin has increased over the last years, while insecticide residuals have been detected in irrigation and storm-runoff water, and associated sediments as well. The potential risk of this pesticide to aquatic organisms is known to be high, but its effects on terrestrial communities remain practically unknown. Moreover, to date, the combined effect of changes on rain patterns and L-cyhalothrin application has not been investigated. Aiming to fill this gap, a semi-field experiment was performed using Terrestrial Model Ecosystems (TMEs). The effect of different doses of the commercial formulation of Judo® insecticide, containing L-cyalothrin as active ingredient (a.i.), was evaluated in soil fauna communities of a pasture field free of pesticide applications for more than 5 years. Doses of 0, 7.5 and 37.5 g of a.i./ha were investigated, equivalent to 0, 1 and 5 times the recommended dose, respectively. Three replicates per test dose were exposed to different rain regimes to reach moistures corresponding to 30, 50 and 70% of the water-holding capacity of the field soil. After 2 and 8 weeks of insecticide application, soil samples were collected to characterize soil fauna communities (microarthropods, nematodes, enchytraeids and earthworms). In this study only the results of mites and Collembola are shown. These data, although with high variability, suggest that toxicity levels derived from L-cyalothrin applications may be influenced by rain regime and that composition of soil mesofauna communities may be a good indicator of the influence of pesticides along time under changing climatic conditions.

Acknowledgements

The completion of this project depended largely on the participation of others. It would not have been possible without the guidance, insight and support provided by Dr. Prof. José Paulo Sousa, my academic advisor at the University of Coimbra. I would like to recognise also the collaboration of all members of the Soils Laboratory at the Department of Life Sciences of the University, including Sara, Filipe, Karla, Joana, Toni and all others for sharing advice, literature, knowledge and practical support. Also, to Andressa, Osmar and Tiago, who carried out the practical work of this project from October 2011 to March 2012. A special mention goes to Tiago, whose invaluable assistance, kindness and good mood were invaluable and determinant for the conclusion of this project.

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"Le véritable voyage n'est pas d'aller vers d'autres paysages, mais d'avoir d'autres yeux"

Marcel Proust.

Contents

1 Introduction

Soils are a dynamic natural medium, made up of mineral and organic matter that supports the vegetation layer, and inhabited by many other living forms. The complexity of physical, chemical and biological interactions taking place belowground, and their influence on life aboveground has long been valued by agricultures. Although soil is an essential component of ecosystems, a wider appreciation and understanding of its dynamics is relatively recent.

The soil is a complex networking system that provides crucial services for the environment at all spatial scales. Soil affairs support nutrient cycling and primary production; regulate climate and greenhouse gases fluctuations and control erosion and flooding. Belowground interactions result in a balance between the processes of growth and decay that maintains the food web and the general flux of energy. Humus generated by soil organisms' activity during the processes of decay is required to power the processes of growth (Nardi 2007). Nutrients from fertilizers are lost soon from soil without humus. Pore spaces created by the same ground organisms hold the moisture and air that characterizes a well-structured and fertile productive soil. Healthy soil, good nutrition and good health go hand in hand (Nardi 2007); so is the fauna that contribute to soils health.

When looking at soil organism communities, it is important to differentiate among structural and functional properties. The first one refers mainly to biodiversity (also called *biocoenosis*) in terms of the ecological description of living populations, described at species level through number of species (richness), abundance, dominance and biomass. Function refers to the biological processes that occur from the interaction of soil's different components, such as nutrient cycling, community respiration, organic matter breakdown, etc. (Odum 1985); these processes that are of global importance because soil biota represents a large part of the world's biodiversity. The most dominant groups of soil organisms in terms of abundance and biomass are bacteria and fungi, followed by protozoans and nematode. Then come soil invertebrates, which are usually classified according to their size and trophic level they belong to. Microarthropods such as mites (Order *Acari*) and springtails (Order *Collembola*) come next in numbers, followed by oligochaetes such as enchytraeids,

tardigrades (Phyllum *Tardigrada*) and earthworms (Family *Lumbricidae*). Macroarthropods (e. g. insects) and other macrofauna live mainly in the uppermost layers, the soil surface, and the litter, which have different trophic organization in the food web (Schaeffer, et al. 2011, Nardi 2007) (Figure 1).

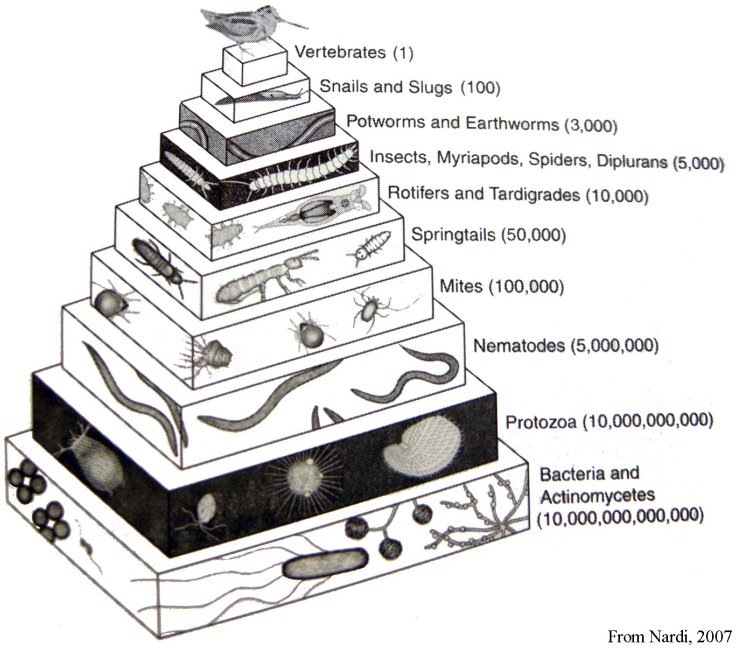

Figure 1. Approximate abundances of organisms from different taxa that live in a square meter of soil arranged according to their sizes **(Nardi 2007)**.

Humans have transformed between one third and one half of the earths' surface through urbanization, farming, logging, and grazing activities, leading to a general disturbance of the soil layer. As a result, the cycling of water and chemical elements has been altered. The great issues that our environment faces at both local and global scales are linked to those organisms living underground. In this framework, knowledge of the role that soil organisms play in maintaining structural and functional processes, and how climatic conditions shape these features becomes increasingly relevant.

1.1 Climate change, altered rainfall patterns and pesticide risk in soils

Global warming is predicted as a straight effect of the rising levels of CO_2 in the atmosphere, a consequence of the massive use of carbon fuels. General circulation models predict that global mean surface air temperature will increase altering the

spatial distribution, frequency and intensity of rainfall ((Bradley, et al. 1987); (Schneider 1993)). Changes in the precipitation regime have not been spatially nor temporally uniform. The IPCC (2001) reports that in the mid- and high latitudes of the Northern Hemisphere a decadal increase of 0.5±1% mostly occurs in autumn and winter, whereas in the sub-tropics precipitation generally decreases by about 0.3% per decade. Although the precise distribution or magnitude of such changes is not known yet, climate change has been demonstrated to be an important driving force on natural systems (Parmesan and Yohe 2003) (IPCC 2001).

The incidence of droughts has increased over recent years, while the climate has already become warmer and drier. Climatic factors play a key role for the fate and distribution of chemicals in soils, for instance the speed of metabolization, transport or volatilization of a chemical depends on temperature and moisture (Filsner et al 2008). However, whether the combined influence of climatic and anthropogenic stressors would enhance or decrease the effects of each factor, and how this interaction can modify the structural and functional shape of the soil community, remains largely unclear. In most cases the application of chemicals cause shifts in population densities of different organisms but not total extinction. How chemicals affect soil organisms depends on their behavior (avoiding, for example, the contaminated substrate and/or agglomerating in more favorable areas) as well as organism's physiological tolerance. Changes in behavior and/or tolerance capacity may alter ecological parameters that are relevant when measuring populations (e. g. population growth, richness, abundance, and composition and structure of the population). These shifts will shape species interactions differently, therefore altering ecosystem processes. Repeated exposure of a system to disturbances beyond its capacity at a higher rate than the pace the systems naturally recovers to its original condition often leads to losing either the number or quality of functions, or both. Not surprisingly, soil exposure to chemicals may result in loss or reduction of a number of vital processes and further degradation of soil features (Filsner et al 2008).

The recurrent and heavy use of chemical products for plant protection (PPPs, i.e. pesticides) in agricultural landscapes has been identified as one of the many factors that may lead to spatial and temporal changes in soil communities (Schaeffer, et al. 2011). Such situation has raised the necessity of evaluating pesticides' potential on altering soil structure and reducing its functional properties. To assess whether pesticides may led to spatial and temporal changes in soil communities in agricultural

landscapes, current European Union regulations for the authorization of PPPs require an evaluation of the effects of pesticides in soil structure and soil functions (Schaeffer, et al. 2011) done by assessing how the communities of soil organisms are altered after being exposed to those chemicals. In the eventual scenario of an interaction of the negative effects on soil due to climate change (mainly extreme conditions such as droughts and floods) with pesticide use, the topic could be included among the required measures to take for mitigating climate change effects.

1.2 Lambda-cyhalothrin pesticide

Lambda-cyhalothrin or L-cyhalothrin is a pyrethroid insecticide. Synthetic pyrethroids are the most widely class of insecticides used in European agriculture (Frampton and van den Brink, Collembola and macroarthropod community responses to carbamate, organophosphate and synthetic pyrethroid insecticides: Direct and indirect effects 2007). Pyrethroids are synthetic chemicals with a similar structure to vegetal pyrethrins, which have a natural insecticide action. L-cyhalothrin is a colorless to beige, solid substance, with a mild odor (WHO 1990). It is used in a wide range of domestic and industrial products coming in different presentations such as wettable powders, pellets, emulsifiable concentrates, solutions, impregnated materials, and microencapsulates. These products include agricultural insecticides for food and non-food crops; insecticides used indoors and outdoors for homes, hospitals and other buildings; greenhouse, ornamental plant, and lawn insecticides; products for insect control on cattle; termite treatments; and even aerially-applied insecticides, among others (NPIC 2001). Signal words for products containing lambda-cyhalothrin range from “caution” (low toxicity) to “danger” (high toxicity). This non-volatile chemical is considered to have low water solubility (5 x 10-3 mg/L). Its half-life (time required for 50% of the compound to degrade) in different environmental conditions is shown on Table I. The potential risk of lambda-cyalothrin is known to be moderate to high for aquatic organisms such as fish and aquatic invertebrates, but its adsorption to soil and sediments reduces exposure and lese the potential risk for aquatic organisms. Existing reports on terrestrial organisms intoxicated with this pesticide include bees, birds and some mammals (NPIC 2001). However, the effect of this pesticide on soil terrestrial communities remains practically unknown.

Table I. Half-life of L-cyhalothrin under exposition to different environmental conditions. Half-life is understood as the time required for 50% of the compound to degrade **(NPIC 2001)**.

Sunlight exposition in water	30 days approx.
Sunlight exposition in soil	<30 days approx.
Representative soil half-life	30 days (with values ranging from 28-84 days)
Hydrolization in water at pH 9	7 days (no hydrolysis at lower 5 and 7 pH values)
On plant surfaces	5 days

1.3 Microcosms and the TME approach

An appropriate tool to investigate potential impacts of potential chemical and environmental stressors on terrestrial compartments, at the biological organization level of ecosystems, is to look at an isolated subset of the original system that can maintain most of its elements (Koolhaas, et al. 2004). The impact of stressors on the integrity of a system is related to its structure and function and therefore should be assessed for both structural and functional properties (Koolhaas, et al. 2004). There are different experimental approaches to study such properties that range from single species tests to natural communities and ecosystems in the field, and from controlled laboratory conditions to highly variable open natural environments; including different ecological levels and processes and ranging not only in complexity level but also in the time, effort and experience needed to operate logistics Figure 2 (Schäffer, et al. 2008).

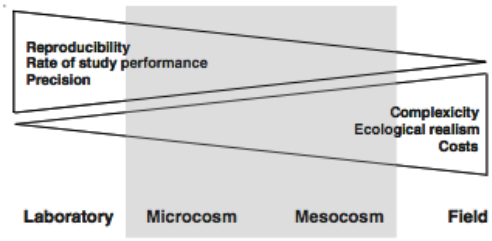

Figure 2. Comparison of test systems **(Schäffer, et al. 2008)**

Odum (1985) defined a microcosm as "a replicable, artificially bounded subset of a given naturally occurring environment with several trophic levels". Microcosms can be a natural or artificially system with specific size, time and mass boundaries and, thus biotic-abiotic interactions at the interior and in relation to its environment are restricted, which makes them a good tool for experimental assessment. The microcosm principle has been used in soil ecotoxicology to assess the potential

impacts of a chemical stressor on terrestrial compartments that illustrate the biological organization level of ecosystems. The impact of stressors on the integrity of a system is related to its structure and function and therefore should be assessed for both structural and functional properties (Koolhaas, et al. 2004).

Terrestrial Model Ecosystems (TMEs) are controlled and reproducible systems that attempt to simulate the processes and interactions of components in a portion of the terrestrial environment (Knacker, et al. 2004). The degree of control of the variables depends on the design of the experimental units, ranging from terrestrial microcosms studied on-site under field conditions where control of environmental parameters such as temperature, moisture and light is low, to greenhouse or laboratory-based systems.

Many measurements can be obtained from TMEs, each of them being an actual environmental value. Selection of those specific points of assessment depends on the study priorities but should represent "quantitative expressions of an impact caused by a stressor" (Knacker, et al. 2004); special care must be taken on staying at a defined level of biological organization to avoid the uncertainties caused by trying to extrapolate the results. Selection of measurement parameters (also called "endpoints") is normally done based on the fate and effect of the model chemical as well as the structure and function of the terrestrial compartment; endpoints should combine functional and structural aspects of the ecosystem (Table II) such as cycling of essential elements, biological diversity and its activity, biodegradation of organic matter, or biodegradation and accumulation of contaminants (Knacker, et al. 2004). Depth of the soil profile investigated and the size of the sample should be indicated as well.

Table II. Parameters or effect endpoints to be measured in a TME experimental approach, classified by the type of ecosystem aspect they assess.

Functional	Nutrients and residues in leachate, soil and in plants Soil enzyme activity Microbial substrate induced respiration (SIR) Bacterial growth Feeding activity of soil organisms (biocenosis) Organic matter decomposition
Structural	Abundances, diversity and community structure of microarthropods, nematodes, enchytraeids, lumbricids Plant biomass Plant diversity

1.4 Assessing the state of the soil ecosystem after disturbance by characterizing soil populations and their functional traits.

Understanding the role of biodiversity in ecosystem processes has implications for how human activities should respond to biodiversity loss and climate change. To establish the state of a certain ecosystem it is necessary to look at proper indicators that can be useful when comparing how much of the structure and function have changed after perturbation. A key step to assess pesticides risk and the effects of changing rain patterns on soil structure and function is to investigate their combined effects on soil biota. As mentioned before, soil provides habitat for an overwhelming amount and variety of organisms, which in turn influence soil formation, soil's physical and chemical properties, and the nature of the vegetation that grows on it. Changes in the patterns of precipitation and temperature are expected to alter soil processes, thus shaping vegetal and soil communities in different ways; the effects are most likely to impact humidity-sensitivity organisms by altering their abundances, richness and species composition. Since many population processes vary with population density, assessing the combined effects of chemicals and rain events on population densities of soil inhabitants contributes to increase the knowledge of the impact of chemicals on the functional properties of soil ecosystems.

On the other hand, processes such as the number of trophic groups and ecosystem process rates, the patterns of use of environmental resources and other ecological interactions depend on the morphological characteristics of the organisms (Petchey and Kevin 2006). Such features are known as "functional traits". Trait-based approaches are widely used in ecological and evolutionary research; Darwin initially used the term as a proxy of organismal performance but it has expanded to explain complex processes at higher organizational levels (Violle, et al. 2007). The way these traits vary over environmental gradients is essential to determine the specific features that could be ecologically more meaningful.

However, as soil processes vary largely in time and space, to identify or predict possible relationships between the diversity and function of soils is not an easy task. Also, although the species taxonomical level is the most appropriate when using natural communities for bioindication purposes, it requires a high amount of labour, experience, formal knowledge and a great amount of time that is not often available. It seems that the more practical approach is to include in the studies of species

composition, functional groups as indicators for processes given that, although some species can dominate the function of the whole system, when they are gone, another with functionally redundant capabilities, can take over in the process (Schaeffer, et al. 2011). The number and type of traits used for a given classification depends entirely on the objectives of research. Traits selection must be carefully justified in ecological terms considering that small amounts of very high quality information, that is often difficult to collect, may give more insight about the functional properties, while large amounts of soft quality material provides indirect information (Petchey and Kevin 2006).

1.5 General diversity indices

To properly evaluate the composition of a biological community it is important to go beyond the simply counts of the total number of species present (or biomass, for the case of modular organisms), which is also defined as *richness* (commonly denoted as *S*). Different indices have been widely used to incorporate information not only about how common are each species with respect to the other members of the same community, but also to know how "equitable" are their abundances distributed along the structure of such community (i.e. how many individuals belong to each species). Richness (*S*) and equitability (*E*) are combined in different indices to determine community diversity (Begon, Towsend and Harper 2006).

In 1949 Simpson described the probability that a second individual drawn from a community could be of the same species as the first (Seaby and Henderson 2006). In other words, Simpson's index calculates the proportion (p_i, for the *i*-th species) of individuals or biomass per species that contributes to the total abundance in the sample, thus it gives an idea of how dominant is each one of the species in the community. Simpson index can be calculated as:

$$Simpson = \frac{1}{D}$$

where *D* states for *dominance* and it is expressed as (Vandevalle, et al. 2010):

$$D = \sum_{i=1}^{S} p_i^2$$

with *S* the total number of species in that community (i.e. richness) and p_i the proportion of the *i*-th species in a sample (i.e $p_i = N_i/N$, and $N = \sum N_i$, where N_i is

the number of individuals of the *i*-th species). This measure combines individual's abundance (*N*) and the species richness (*S*); *D* increases with equitability for a given richness, and for a given equitability *D* increases with richness. Now, if the maximum theoretical value of D is the total number of species in the community (D_{max}=*S*), then *D* can be expressed as a proportion of this value when assuming that all individuals are evenly distributed among the species. Thus $E = \frac{D}{D_{max}}$

Another index that is used very frequently is Shannon –Wiener Index, with a similar form of the previous index, but with a different array of p_i values (Seaby and Henderson 2006):

$$Shanon, H = -\sum_{i=1}^{s} p_i ln\, p_i$$

To analyse the pattern of distribution of the individuals between species, i.e. the *equitability* or *evenness*, Pielou Index (J) compares the Shannon H against the distribution of individuals between those species that would maximise diversity. The maximum value that H can take is the logarithm of the richness. Therefore the index is:

$$Pielou, J = \frac{H}{\log(S)}$$

However, as it is not possible to know the real total amount of species in the sampled habitat, calculations must assume that the total number of observed species over all samples equals to *S.* For this reason heterogeneity of the sampled community and the sampling effort will play an important role when interpreting results from *J* Index.

Biodiversity involve more than just species richness. The number of species that a community can hold is determined by the size of the niche and the extent to which they overlap in relation to the available resources (Begon, Towsend and Harper 2006). Heavy disturbances act as a selective pressure that favours the most tolerant species, because over time they end up not only shaping the organization of a community, but also modifying the path of the process in which their members participate, thus altering the functional properties of the ecosystem. As biodiversity is a potential modulator of ecosystem's processes (Loreau, et al. 2001), comparing the functional diversity can explain and predict the impact of organisms on ecosystems. The number of functional groups may be a good indicator for community composition (Petchey and Kevin 2006), and can be used to judge how strong are processes and

functions under the action of certain perturbation. The higher is the amount of species running the process, the weaker the risk of instability of the system.

Functional diversity (FD) concept can be described as "the overall difference among species in a community in terms of their traits", for example, two assemblages with a similar amount of species may be more or less functionally diverse depending on how similar or dissimilar are the species' traits among the composition of species for a given community (Lepš, et al. 2006). It involves understanding of communities and ecosystems based on what organisms do, rather than on their evolutionary history (Petchey and Kevin 2006). The FD concept gains biological importance because it is linked to how species share the niche space available in a community, which has important consequences on the general functioning of ecosystems. Measuring FD requires appropriate information about the functional traits of organisms, weighted according to their relative functional importance, and a statistical measure of trait diversity (Petchey and Kevin 2006). One approach to calculate FD is through the estimation of the "Rao" index of diversity adapted to calculate dissimilarity among species traits per sample. The Rao coefficient is a generalized form of the Simpson index by reflecting the probability that two members from the same group, randomly chosen, will be different. In terms of species trait diversity, this represents the probability that these two individuals will be from a different morphospecies, so it includes the evenness on the distribution of traits in the community (or sample) (Petchey and Kevin 2006). Rao index can be calculated in three steps, whose rationale is described in Lepš, et al. (2006) in the following way. If the proportion of *i*-th species in a community is p_i and dissimilarity of species *i* and *j* is d_{ij}, the Rao coefficient has the form:

$$FD, Rao = \sum_{i=1}^{s} \sum_{j=1}^{s} d_{ij}\, p_i p_j$$

where *s* is the number of species in the community and *dij* varies from 0 (when two species share the exact same traits) to 1 (if the two species have completely different traits). If d_{ij}=1, for any pair of species *i* and *j*, then *FD* is the Simpson index of diversity expressed as 1 minus Simpson index of dominance *D*, i.e. $1 - \sum_{j=1}^{s} p_{ij}^2$. Thus, the first step in *FD* calculation was the computation of the "dissimilarity matrix" using the algorithm: $[\sum i(x_{1,i} - x_{2,i})]/2$, where *i* is the category index; a resulting value of 1 means that the two species compared do not share any trait. The

second step is to introduce a "*species x plot matrix"* showing how many morphospecies (in rows) were found in each sample (columns); this is also called "absolute abundance matrix" and needs to be converted to a "relative abundance matrix", so the total abundance for every sample should sum 1. Finally, the sum of the products of the dissimilarity matrix among species multiplied by each relative abundance matrix produces a Rao *FD* for each functional trait, per sample, and the average of them gives the final compound Functional Diversity Index (Lepš, et al. 2006).

Another functional index that can be calculated is the *mean trait value per community* (mT), computed as "the average of trait values in the community, weighted by the relative abundance of the species carrying each value", so it is also known as the *community weighted mean value* of a trait (CWM) (Díaz, et al. 2007). Since it is strongly determined by the functional trait values of the more abundant species (Díaz, et al. 2007), it is often understood as "defining the dominant functional attribute in a community or the proportion of a given functional group" (Vandevalle, et al. 2010), and can be expressed as:

$$mT = \sum_{i=1}^{S} p_i \, x_i$$

where x_i is the trait value of the i-th species (Vandevalle, et al. 2010). To calculate this index all traits must be in a binary form, so if the trait is present it adopts a value of 1 and absence is 0. For this reason, when traits have categorical values (i.e. more than two possible responses, such as, for example, flowers colours or size categories) this information can be included by creating an extra matrix of "dummy coding" that records the presence (1) or absence (0) of all possible outcomes for each trait category

Averaged trait values over a community (mT) and FD metrics can respond strongly to environmental changes and are therefore promising as biodiversity indicators (Vandevalle, et al. 2010). The quantification of ecosystem's biodiversity through functional traits approach requires two things: 1) a well defined environmental gradient, and 2) to select the functional indicators that have proper biological meaning for the system, according to the type of design and ecological hypothesis underlying the response of the organism to the chosen gradient. It is also important no to forget about taking in consideration a possible overlapping between the taxonomic and functional components.

1.6 Springtails as bioindicators

Mites, springtails and enchytraeids are the organism groups usually recommended to study anthropogenic stress (Ref). Their size, between 0.2 and 20 mm, ranks in the middle of soil fauna body sizes when compared to other soil invertebrate, for which they are often known as "mesofauna". Although all of them are highly abundant in agricultural and forest soils of temperate areas, springtails are the better-studied order among this group, perhaps because they are known for being prone to experience readjustments driven by drought events (Ref); therefore a good candidate to compare the effects of the water stressors at different magnitudes.

Springtails are tiny but highly abundant arthropods, members of the Order Collembola, whose size ranges in between 0.12 and 10 mm. They are thought to be among the most abundant arthropods on Earth with a long evolutionary history. Their most distinguished feature is the jumping organ or *furca* that makes many of them capable of hopping many times their body length as an escape mechanism. Additionally, all of them have a ventral tube that helps them to maintain fluid balance and also function as a sticky appendage in slippery surfaces (Nardi 2007). The basic body plan of a springtail is pictured in Figure 3.

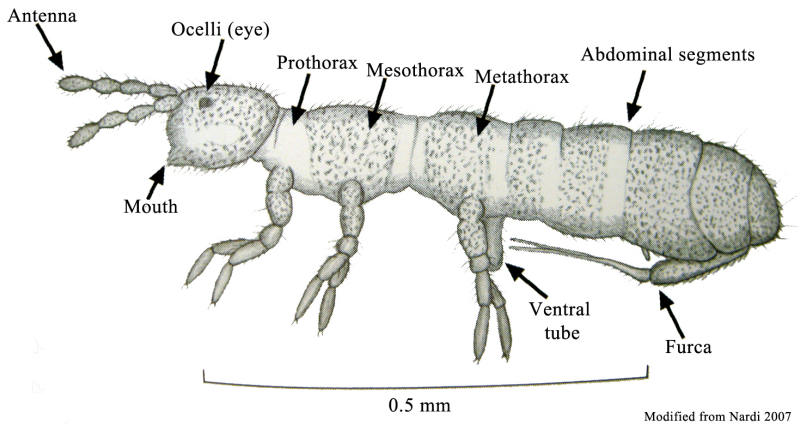

Figure 3. General body plan of an edaphic springtail (Order Collembola). Adapted from Nardi **(2007)**.

Around 6500 species have been described globally, although as far as 50,000 are expected to live in our planet (Hopkin 1997). Its long evolutionary history –with oldest fossils dating back from the Devonic period about 400 million years ago, is reflected in the wide range of morphological and livelihood characteristics that allow them for adaptation to the most different environments. Collembola have a very wide

distribution, they appear to be present in all continents, from common locations in agricultural lands of temperate and tropical areas, to localities with extreme climatic conditions such as Antarctica, the Himalayas or de Australian deserts (Hopkin 1997). Their scope of niches include the soil column, where they can reach down to 150 cm depth the litter layer; the surface of plants and trees high up in the forest canopies and even in highly humid environments such as the surface of fresh water, the sea shore and on the snow, being the latest where springtails were probably first described by Aristotle as "snow flies" that aggregate in swarms to form reddish carpets over the white surface (Hopkin 1997). Despite their relatively low biomass, springtails have strong influence on the structuration and functions of some soils, for example by creating humus through their faeces, which after soil microbes processing allows release of essential nutrients for plant roots uptake (Hopkin 1997). They also contribute to decomposition and soil respiration by grazing on fungal hyphae, and interactions with mycorrhizae on roots may stimulate plant growth and control of microbes and fungi population sizes.

Taxonomy of Collembola is somehow complicated and perhaps under studied. Linnaeus placed them as a part of the Aptera, the wingless insects, under the genus *Podura*, but no original specimens remain in his collections. John Lubbock, contemporary of Charles Darwin, used the term "Collembola" for the first time in 1862. However, interest on these animals blossom up only with the beginning of the 20th Century, mainly after Börner found a modern classification of the group in 1913. Currently, Class Collembola is considered to have a monophyletic origin, although its position as a part of the insects is debated. Based on cladistics principles, modern phylogeny of springtails recognises three Orders: Arthropleona, Neelipleona and Symphypleona with 15, 1 and 2 Families, respectively, that are monophyletic groups (see Table 3.1 in Hopkin 1997). The great majority of phylogenies have been made based on anatomical comparisons, internal and external.

The modern approach of study puts animal morphology in an ecological, physiological and behavioural context, regarding these aspects as evolutionary adaptations for a changing environment, an approach sometimes called "ecomorphology". Such scheme is useful for giving a broad indication of Collembola groups, especially when lacking of taxonomic knowledge. A division by ecomorphological groups was proposed by Gisin as early as 1943, in which three "life forms" were recognised: *eudaphic*, for species that are permanently soil-dwellers;

hemiedaphic, for those found in superficial soil layers and litter; and *epiedaphic* or *atmobiotic* for surface-living species and inhabiting the vegetation stratus (Hopkin 1997). The "life-form concept" implies that some key corporal structures can, for their presence, development and form, give an idea of the living habits of the individuals, so these traits, of functional nature, allow them to be better adapted for living in a specific depth along the soil column. Most members of Entomobryoidea and Symphypleona typically represent epiedaphic species with their pigmented body, sometimes patterned, and often covered with hairs and scales; and their furca, antennae and ocelli (eyes) well developed. These features contrast with the pale species inhabiting lower soil-layers, with furca, antennae and ocelli poorly developed, as is the case for Onychiuridae. Some examples of the general appearance of springtails classified according the life-form concept are shown in Figure 4. The introduction of a simplified eco-morphological index, that does not require the classification of organisms to species level, allows a wider application of these methodologies (Parisi, Menta, et al. 2005), in special when advanced taxonomical knowledge is missing. However it is important to keep in mind that classifications of this nature always depend on the specific experimental approach.

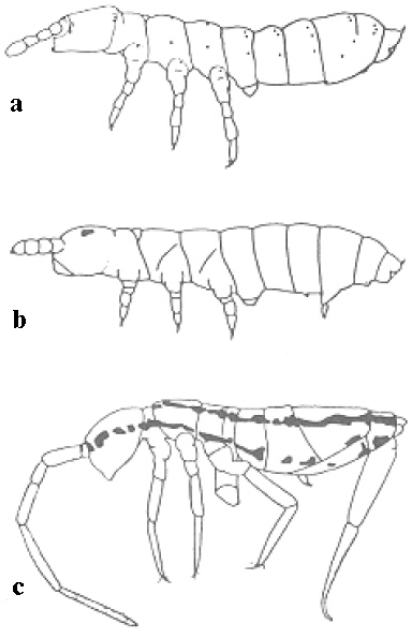

Figure 4. Ecomorphological types of Collembola are related to their habitat. a) Euedaphic b) Hemiedaphic c) Epiedaphic or atmobiotic. From Gardi et al. **(2002b)**.

1.7 Indices for soil quality

Mesofauna groups are useful biological indicators of soil quality when assessing environmental impacts. They are very abundant, their role in soil formation and transformation is well recognized, the area covered during their life cycle is representative of the site under examination and their life histories permit insights into soil ecological conditions (Parisi, Menta, et al. 2005).The wide variation in life traits of collembolan species can provide a functional tool for assessing the effects of land disturbances (Vandevalle, et al. 2010).

Collembolan life-forms have already been included in Parisi's 'Qualità Biologica del Suolo' (QBS) (Parisi 2001), a soil quality index based on the range of morphological adaptations of arthropods to the edaphic conditions. Parisi et al. (2005) describe the calculation of the QBS index in the next way. The main idea behind the QBS index is that the number of microarthropod groups well adapted to the soil they inhabit is dependent to the soil quality. It tests the degree of adaptation of soil microarthropods using eco-morphological traits assuming that to define the biological forms present in a sample means to recognize the different adaptation levels to soil environment. Thus, each form can correspond to an eco-morphological score (EMI) proportionate to its adaptation level, being the highest EMI the most eudaphic form, and the lowest the most epiedaphic ones. The score results from choosing the characteristics (i.e. traits) considered the most connected to the adaptation level of collembolan species.

Originally, Parisi (2001) considered seven traits: size of the body, number of ommatidia (ocelli), length antennas, furca, and legs, presence of hairs and/or scales, body pigmentation, and presence of specialized structures involved in sensing environmental stimuli. For each trait category he assigned a numerical value from 0 to 6 according to the possible outcomes of each category. Later on, this classification was simplified and adapted to five traits and values from 0 to 4 (e.g. an absent furca is "4", while a reduced or short one is "2", and a fully developed is "0"), to use it in the comparison of the quality and structure levels of authoctonous forest versus introduced Eucaliptus plantations in Portugal (Nunes dos Santos 2008); the coding applied for this study is shown in table 8 of Vandevalle et al. (2010). In both versions of the classification, however, codes that corresponds to the most eudaphic characteristics always gets the highest value. The corresponding EMI is obtained by

summing the five values to get a final number that can go from 1 to 20. Parisi (2001) applied the same rationale to all microarthropods in soil assigning an EMI value to each taxonomical group, which was in agreement with its degree of specialization to live in soil, being an EMI=20 the most eudaphic and EMI=1 the most epidaphic (see Table 1 in (Parisi et al. 2005). Some groups have a single value (e.g. Acari is 20, Isopoda is 10), while others display a range of EMI values because they have different levels of adaptation to soil. The later is the case for Collembola. Whenever two EMIs are present in the same group, the higher one determines the final score. The final QBS of a sample is the sum of the EMIs of all collected groups (Parisi et al. 2005).

The QBS index of Parisi (2001) was developed for using it mainly in open areas such as grasslands. A particular form of the QBS index, called $ICQS_c$ for its Portuguese name "Índice de Classificação da Qualidade do Solo for Collembola" (Quality Soil Classification Index for Collembola) (Nunes dos Santos 2008), was adapted to compare the Portuguese forested areas referred in Vandevalle, et al. 2010, by considering the sum of relative abundance of individuals of on *i*-th species per sample and its corresponding EMI values, multiplied by the total number of species per each sample (Nunes dos Santos 2008):

$$ICQS_c = \sum P_i \, EMI_i \, S$$

where P_i is the proportion of individuals of the *i*-th species (i.e. the relative abundance) in the sample, EMI_i is the ecomorphological code for the *i*-th species, and S is the total number of species in the sample (i.e. richness). Unlike the QBS, this formula weights the contribution of the abundances of each species to the overall EMI value of the traits of the sample. Different weightings can produce very different classifications (Petchey and Kevin 2006). The clasisfication produced through the apllication of the $ICQS_c$ matched that find by Sousa et al. (2000) using a classic taxonomic approach (Nunes dos Santos 2008).

QBS is a good tool to for environmental assessment because the average values change with disturbances pressure and land-use types (Parisi, Menta, et al. 2005). For example, they increase as arable land pressure is reduced (Gardi, Menta and Parisi 2002a), and are higher for well-established wooded areas compared to shrubs, and for organic farming compared to traditional (Parisi, Menta, et al. 2005). As QBS index does not require a species-level diagnosis, it is therefore a potential appropriate tool for large-scale monitoring that involves large number of samples

(Parisi, Menta, et al. 2005). Yet, the present state of knowledge concerning the impact of disturbance on life-history traits and functional diversity of collembolans remains limited, partly due to a lack of empirical data for many species (Vandevalle, et al. 2010).

2 Objectives

The aim of this study was to investigate the combined effect of pesticide application at different concentrations and variable amounts of rainwater, on the richness and abundance of soil mesofauna, along two time-periods, using a TME approach. Based on the idea that species less tolerant to high amounts of pesticide and/or low levels of soil humidity will be less abundant, soil community composition is expected to be different under different conditions of moisture and pesticide concentration in soil. However, many rain events or higher amounts of rainwater may leach the pesticide from soil surface to deeper layers, therefore reducing its toxicity.

Working hypothesis:

- Toxicity of the pesticide will be lower as higher the rain frequency due to pesticide leaching.
- Species less tolerant to soil contamination will not be present at the highest pesticide concentration.

3 Materials and methods

3.1 Field site

All soil-cores containing biota were extracted from a selected field site located on the grounds of Coimbra Agricultural School (ESAC), near the Mondego River, at Bencanta, Coimbra city, Portugal (40°13' N, 8°27' W). The area, 27 m above the sea level, is plot of 50*50 m selected inside a fenced-off field. The study area is surrounded by several drainage channels emptying into the main river. However, the actual site is separated from maize fields by wide boundaries (25-30 m) and was not used for cultivation since 1996. In the area, the long term average precipitation is 985 mm, while long term mean average air temperature is 16°C. Climatological and precipitation records were obtained from the local meteorologic station. Pedological characteristics of the soil were determined by the Portuguese Agricultural and Environmental Chemistry Laboratory of the Ministry of Agriculture, Sea, Environment and Territorial Planning (Laboratório de Química Agrícola e Ambiental, Ministério da Agricultura, Mar, Ambiente e Ordenamiento do Territorio; Registry number: SF355/2012, reception date: 26-04-2012), from 15 cm depth of composite samples from the field site (Table III).

Table III. Environmental and pedological characteristics (0-15 cm layer) of the field site where TMEs were obtained.

Long term average precipitation[a]	985 mm
Long term mean average air temperature[a]	16°C+-2
Soil type	Alluvisol/fluvisol (brown earth)
pH H_2O (1:5, v:v)	7.9
ph KCl (1M KCl, 1:5, v:v)	7.2
WHC (water-holding capacity)[b]	47.06 %
Bulk density[b]	1.7 g/cm^3
Organic matter[c]	3.2 %
Organic carbon, CO [d]	1.9 %
Total N[e]	2114 mg/Kg
Mineral N[f]	39 mg/Kg
Sand	65.3 %
Silt	18.59 %
Clay	16.12 %
Textural class [g]	Sandy loam
Site use	Arable land

a Obtained from local climatological records of ESAC meteorological station(s).
b Natal da Luz, T., 2012, pers. comm. 30th July. WHC measured according to ISO 1999; bulk density obtained through water volume increase after dry pre-weighed soil addition; organic matter by loss on ignition ay 500 ºC for 6 h.
d Calculated by multiplying total C by 1.724 (Haug 1993).
e By Kjeldahl method (Moreira, Sousa and Canhoto 2008).
f By distillation method (Moreira, Sousa and Canhoto 2008).
g By pipetting (LNEC (Laboratório Nacional de Engenharia Civil) 1970).

3.2 TME conditions

TMEs used in this work were "open enclosures" (soil surface and plants not separated from the surrounding atmosphere) and intact (soil cores were extracted from the field without disturbing the soil layers), as the conditions firstly followed by the guidelines explained in the 36-month and four-country, international *TME-project,* designed for the Research and Technological Developments of the European Union, whose results are full described in a special issue of the *Ecotoxicology* journal (Issue 13) (Knacker, et al. 2004). The structural and functional endpoints measured in this project were previously ring-tested and field-validated for carbendazim, a fungicide considered as a model chemical, to ensure quality of data derived from this type of TMEs.

Intact soil-cores were taken from the field site in October 31st, 2011. One month before it, weeds and grassland vegetation were clipped to an uniform height of approximately 2 cm. Soil-cores were carefully extracted using a hydraulic excavator and a stainless-steel borer tube (Figure 5**.A**) in which interior was the actual encasement of the TME, made of a high-density polyethylene (HDP) plastic tube of 40-cm long and 17.5 cm diameter. Special care was taken trying to avoid any disturbance of the natural soil layering such as soil compaction in order to preserve the original soil community. The bottom of extracted soil-cores was covered with a very fine plastic mesh (2mm) and a drilled plastic plate also made of high-density polyethylene. The whole column, from the top vegetation layer to the bottom dish, encased in the HDP tube, represents one TME (Figure 5**.B**). Cores were then transferred into the greenhouse in an upright position to be placed into moveable carts specifically design for this purpose. The bottom plate was connected through a polyvinylchloride tube to a 1000 ml wide neck polyethylene Nalgene bottle to collect leachates during the experiments (Figure 5**.C**). Soil-cores extraction was done 8 weeks before the application of the pesticide on December 28th, 2011.

TMEs were assigned randomly to each cart maintaining similar conditions of temperature, light and soil moisture as similar as possible for each soil-core. Temperature of each TME was measured on real time using electronic sensors connected to data loggers. Inside the greenhouse air temperature was

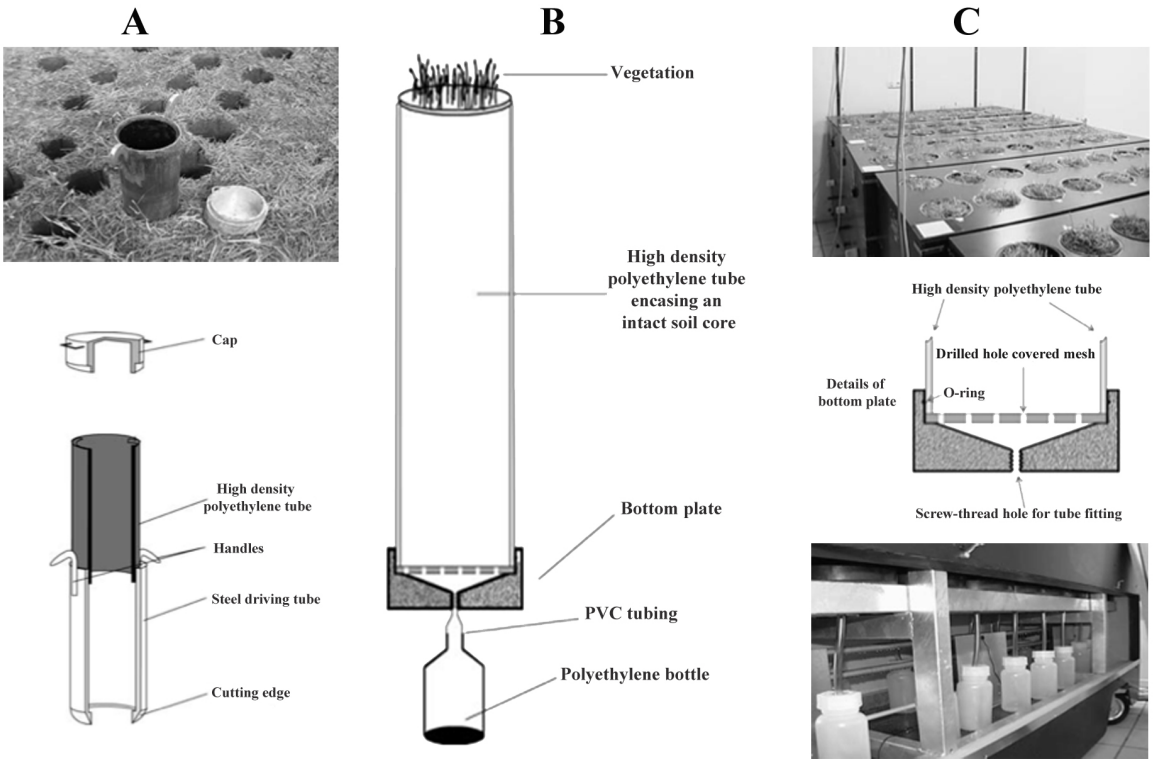

Figure 5. Terrestrial model ecosystem (TME) design. A) Borer for soil-cores extraction. B) A single TME is composed by a soil core, from the top vegetation layer to the bottom plate, encased in a HDP tube. C) Each TME is placed in movable carts where controlled environmental conditions are simulated and leachates are collected from each TME.

23°C (Min = 9°C, Max = 37°C), relative humidity 50–80%, and day/night cycle of 16/8 h. During installation, TMEs that appeared to have holes or cracks in the soil surface were discarded. Experimental trials begun by applying the pesticide after eight weeks of acclimatization. Along this period TMEs were irrigated manually with artificial rainwater 2 to 3 times per week to ensure that by the time of the beginning of the experimental period all TMEs stabilized to the equivalent moisture of 50% of the water holding capacity of the soil. Thus, the amount of water used changed during the course of the experiments to maintain a constant moisture condition in soil. Two weeks before pesticide application, electronic sensors were set for each TME and connected to a general data logger to record moisture on real time. No plants were sown in the TMEs, and only original native vegetation was maintained.

3.3 Treatments design

Treatments were design to cover all possible combinations of pesticide application and rain regime simulations, at two periods of exposure. Therefore the design of the experiment was made with the following application doses of pesticide: no-pesticide

(0x), the recommended dose (RD = 7.5 g/ ha) and five times the recommended dose (5x). Conditions to assess the effect of different amounts of rain were set as the equivalents to drought, average normal rain and heavy precipitation; such situations were recreated by adding the necessary amount of water to maintain 30, 50 and 70%, respectively, of the specific water holding capacity (WHC), previously calculated for this soil type. Artificial rainwater was applied in each TME. This was prepared by diluting 10 ml of a stock solution to a volume of 1 l in demineralized water. The stock solution was prepared by adding (NH4)2SO4 (925 mg), NaCl (386 mg), CaCO3 (200 mg), MgSO4 (180 mg), KCl (37 mg), KH2PO4 (14 mg), NaNO3 (40 mg), HNO3 (3.5 M) (2.0 ml) and HCl (1.0 M) (1.0 ml) to demineralized water at a final volume of 1 l. TME columns were manually irrigated. Moisture and temperature were monitored on real time each 6 h using data loggers (ecoTech, model EnviLog GP5W-Shell, Germany) to assure the maintenance of the described settings. Samples were collected 2 and 8 weeks after chemical application. Three replicas were set per period and condition.

Sampling dates: Two and eight weeks, with three replicas per period and condition. Thus 54 samples (3 pesticide concentrations x 3 moisture conditions x 3 replicas x 2 times) were assessed in total (Table IV).

Table IV. Experimental design was a combination of L-cyhalothrin pesticide application at different doses (0, 1 and 5 times the recommended dose), and different frequencies of rain-simulations measured through the water holding capacity (WHC) of the soil. This design was done for each of the two experimental periods (two and eight weeks).

Two experimental periods (2 and 8 weeks)	Free of pesticide (0x)			Recommended dose (1x RD)			Five times the RD (5x RD)		
Low precipitation (drought, 30% WHC)	B	C	D	B	C	D	B	C	D
Normal precipitation (average, 50% WHC)	B	C	D	B	C	D	B	C	D
High precipitation (flood, 70% WHC)	B	C	D	B	C	D	B	C	D

3.4 Pesticide application and recommended dose

The chemical was applied as a commercial formulation called Judo® (provided by SAPEC AGRO, S. A., Setúbal, Portugal) whose active ingredient concentration is 100g of L-cyhalothrin per liter. The recommended dose (RD) in product's label was 75 mL of insecticide per hectare. The product was prepared following fabricant's recommendations by diluting proper amounts of it for each treatment (considering 3.2

ml of insecticide per each 100 ml of solution for the RD) in half of the necessary final amount of demineralized water (which was 1 L in total per TME). After homogenizing by shaking, the dilution was sprayed on the surface of each TME and immediately after, each one was sprayed with the same amount of water.

3.5 Microarthropods sampling

Many samples were taken from the same TMEs to assess the different endpoints of the system. For soil fauna all the samples were soil-destructive. This report will focus only on those procedures concerning the assessment of microarthropod populations (mesofauna). For this purpose a split corer of 5 cm diameter and 12–16 cm length was used to extract one soil core of 5 cm height from the topsoil layer of each TME treatment. Half of the TME were sampled 2 weeks after pesticide application and the other half after 8 weeks.

Soil samples were extracted in a Berlesse apparatus for 10 days using a temperature of extraction of 45ºC. Microarthropods were collected and preserved in 80% ethanol. Arthropods recovered were identified and sorted out to order level by counting them manually through direct stereomicroscope observation (up to 40x magnification) using thin paintbrushes for their manipulation. A complete record of the arthropods found in all TMEs per sampling period can be reviewed in Annex **Error! Reference source not found.**.

Individuals from Collembola were grouped in morphospecies based on the traits of Parisi described by Vandevalle et al. (2010). However, aiming to simplify and speed up the process, the same approach was used in this work but considering only three traits: **eyes** (merely presence or absence, not number of omatidia), **furca** (presence and degree of development), and **pigmentation**; all values ranging from zero to four (Table V). Therefore, the resulting ecomorphological index (EMI, i.e. the sum of the values per trait) for each morphoespecies varied from 0 to 12. Annex **Error! Reference source not found.** refers to the Collembolan morphospecies found in each TME.

3.6 Collection of data and diversity analysis

The number of individuals counted per sample was extrapolated to the total area of the TME (each TME has a diameter of 16.5 cm) and abundances were used for all data calculations.

Table V. Coding for Collembolan traits used to construct a composite life-form morphotype, calculated by adding individual trait scores.

Trait	Codification
Ocelli	Present = 0 Absent = 4
Furca	Absent = 4 Reduced or short = 2 Fully developed = 0
Pigmentation	Absent (white color) = 4 Colored but no patterns = 2 Colored and with patterns = 0

Effects of L-cyhalotrin application, at the recommended dose and five times that concentration, on mites and springtails mean abundances were assessed at different moisture regimes after two and eight weeks of exposure. For each sampling period, the null hypothesis considered that counts of arthropods were independent of insecticide treatment and was tested using analysis of variance (One-way ANOVA for each treatment, and Two-way ANOVA with treatment and moisture as factors). Collembola biodiversity was estimated applying different indices to the morphospecies found at each experimental period. Shannon (H), Simpson (D), richness (S), and equitability of Pielou (J), were calculated using "Species Diversity and Richness" software (Seaby and Henderson 2006).

Functional diversity (FD) and mean trait value (mT), were calculated through the Microsoft Excel file called "FunctDiv.exl" designed by Lepš, et al. (2006), which contains macros for these calculations and uses the Rao coefficient. Since the Collembola morphotypes analyzed in this work are "categorical (i.e. nominal) traits", a matrix with *dummy* variables was created in which columns with all the possible combinations of codes for each trait were included (see Table V to review the values in which each trait could be classified) for each morphospecies (in rows).

Soil biological quality index (QBS) and the quality index for soil classification for Collembolan morphospecies ($ICQS_c$) were estimated. All indices within each

sampling period were tested using analysis of variance (one-way ANOVA) to evaluate the null hypothesis, which considered that each index was independent of insecticide or moisture treatments (for theoretical and ad hoc groups of moisture).

3.7 Multivariate analysis

The TME experiment provided large data sets comprising information about temporal changes in the structure and function of control and pesticide treated replicates. Univariate statistical tool allows analysis for only a few taxa or other endpoints, while multivariate methods summarize all information on the investigated populations simultaneously, and in doing so evaluate the effects of contaminants at the community level (van den Brink and ter Braak 1999). Principal Component Analysis (**PCA**) is a common type of factor analysis that uses linear models similar to the linear model underlying regression analysis, with the differences that the explanatory variables (called here "sampled scores") are not explicitly manifested but hidden, and the regression coefficients of the linear model are called "species weights". The sample scores and species weights are displayed in an ordination diagram as the first, horizontal axis; the scores and weights together explain a particular fraction of the total variance of the data set. A second set of sample scores and species weights for this second group of hidden variables, is displayed as the second, vertical axis of the ordination diagram. After extracting more and more hidden variables, PCA eventually accounts for all the variance of a data set (van den Brink and ter Braak 1999).

Effects of L-cyhalothrin and moisture content treatments on Collembola community were analyzed using PCA for treatment at each experimental period separately. PCA was performed using CANOCO software, version 4.5 (Ter Braak and Smilauer 2009). The input data about species consisted of a matrix with the number of morphotypes by the number of samples (TMEs) for each treatment and experimental period. Abundance data were log transformed, centered and standardized, and the scaling used was adjusted to focus on inter-sample distances; default values were chosen for all remaining options.

To know which variables contributed the most to the observed pattern among objects, e.g. which species contribute most to the separation of sampling units, **ANOSIM** (analysis of similarity) and **SIMPER** (analysis of similarity percentages) methods were used to associate objects based on similarities or dissimilarities between them, using the distance between objects on the plot to represent their

relative dissimilarity and the scores for objects on axes as variables. These analyses were performed using PRIMER software version 5.2.6 (PRIMER-E, Ltd. 2001).

SIMPER method determines which morphospecies (variables) are contributing most to the overall degree of dissimilarity among the sampling units (each TME in this case), by computing the Bray-Curtis dissimilarity for a each pair of sampling units (i. e. the differences between the units for each species, summed over all the species). For this, SIMPER calculates the proportional contribution of each morphospecies to i) the dissimilarities between all pairs of sampling units in different groups and ii) the similarities between all pairs of sampling units within each group; both expressed in percentages. It then calculates the average of these percentages and its standard deviation (SD), to compare the ratio of each average/SD; those morphospecies with a large ratio are the ones that best discriminate between groups. There are no formal hypotheses with this method, just a list of species in order of their percentage of contributions to dissimilarities between groups, or similarities within groups.

ANOSIM is a hypothesis testing procedure analogous to an ANOVA that uses Bray-Curtis dissimilarities to compare between- and within-group variation. The H_0 being tested by ANOSIM is that the average of the rank dissimilarities between all possible pairs of objects in different groups is the same as the average of the rank dissimilarities between pairs of objects in the same groups. It produces a tests statistic called *R* that ranges between +1 to -1; differences' values greater than zero suggest objects are more dissimilar between groups than within groups, values of zero indicate that the null hypothesis is true, and negative values mean that dissimilarities within groups are greater than between groups.

Multivariate techniques are often used to reduce multivariate time series to fewer dimensions, but since the time vector is often not a straight line in the multivariate diagrams, these diagrams can be highly cluttered, making them difficult to interpret. The Principal Response Curves (**PRC**) overcomes this problem analyzing time series resulting from mesocosm experiments, in which treatments are contrasted with a control (van den Brink, den Besten, et al. 2008). This method is based on the Redundancy Analysis (RDA) ordination technique, which is a constrained form of PCA that includes the effect of environmental variables. PRC was especially designed for the analysis of data from model ecosystem experiments. The result of PRC analysis is a diagram showing time on the *x*-axis as the dependent variable, and the

responses (i.e. the first principal component of the treatment effects) on the *y*-axis. The effects of the pesticide in the different sampling dates are shown in one line for each test treatment that deviates from the control base line. To draw these lines a species weight diagram is used, in which the weight of each species is shown with the response in the diagram. Multiplying the weight 'b_k' of species 'k' by the canonical coefficient 'C_{dt}' of each treatment at each sampling time gives the fitted change of this species compared to the control; taking the exponent of this quotient returns the relative abundance compared to the control and the relative abundance times 100 gives the abundance in percent of the control (Koolhaas, et al. 2004). The significance of the effects of the treatments on the species composition in PRC diagram is tested by Monte Carlo permutation tests with whole time series in the partial RDA from which the PRC was obtained, using an F-type test statistic based on the eigenvalue of the component. The analysis was performed using the software package CANOCO 4.56 (Ter Braak and Smilauer 2009).

4 Results

4.1 Groups of samples according to soil moisture

Three groups were planned to analyze the effect of different soil moistures on mesofaunal population parameters. These theoretical groups were named: “Low” comprising replicates with 30% of WHC, “Medium” for replicates with 50% WHC, and “High” for replicates with 70% WHC. However, great variability was found in daily measurements of soil moisture from data loggers’ records. When real-time moisture measurements in each TME were plotted together for each experimental period (Figure 6), it was revealed that the theoretical moistures were not reached in all cases. Due to this fact, a K-means clustering of multivariate analysis was applied to the average of soil moisture records for each experimental period separately. Minimizing variance within clusters while maximizing it between them, three new groups were formed, designated simply as “Low2”, “Medium2” and “High2”. Moreover, differences in average moistures recorded per group were significantly different when analyzed with one-way ANOVA ($p \leq 0.05$, for all cases in both periods). However, the effective amounts of water added to each TME along the corresponding experimental courses were carefully reviewed (whenever data were available) to ensure coherent placement of the samples in each category. All samples matched the K-means clustering, except for one on the two-weeks period that corresponds to a low moisture treatment without pesticide (sample “ct23B”), which received about 1.6 times more watering than the average for its theoretical category (“Low”) within 2-weeks smples. For that reason such sample was considered in the “High2” category. Grouping for original – theoretical, soil moisture (called here just “Moisture”) and the new “ad hoc” groups based on K-means clustering (generally denoted as “Moisture2”) were compared in subsequent data analysis. See annex **Error! Reference source not found.**to review the members belonging to each group according to their respective type of categorization.

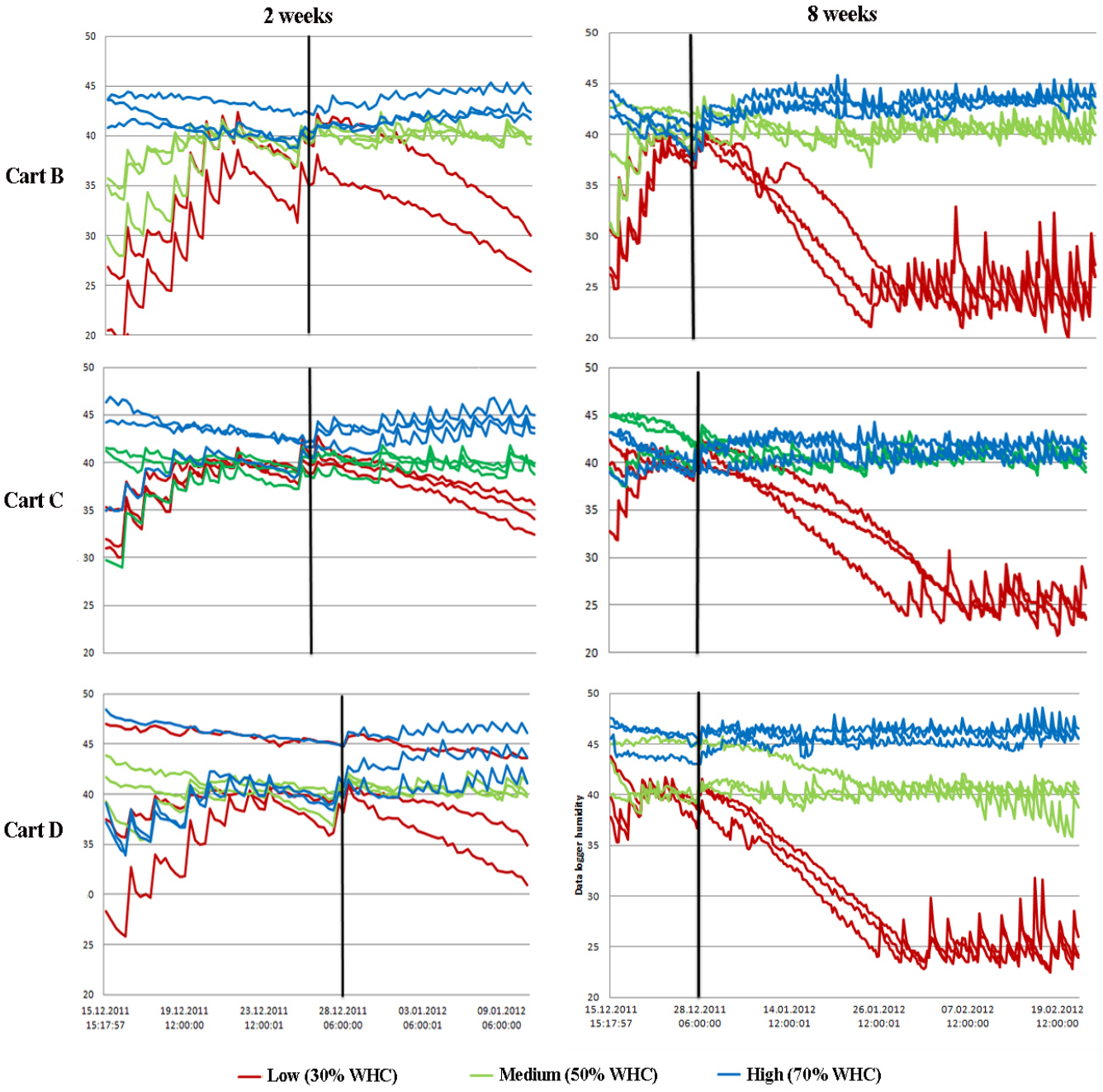

Figure 6. Soil moisture recorded on real time during the two experimental periods (2 and 8 weeks). Each TME represents a different treatment to test combined conditions of moisture (Low = 30% WHC; Medium = 50% WHC; and High = 70% WHC) and pesticide dose (no pesticide, control conditions = Ct; recommended dose = RD; 5 times the recommended dose = HD), which were randomly placed in TMEs distributed into three carts inside of a green house. Records begin on November 15th, 2011, exactly four weeks after TME cores collection (October 31st). Pesticide application (thick black line) occurred 6 weeks later, on December 28th.

4.2 Effect of L-cyhalothrin pesticide on microarthropods abundances (mites and springtails) according to soil moisture.

A total of 4591 mites, 2400 springtails and 505 other microarthropods (e.g. enchytraeids, colleoptera and diptera larvae, hemyptera, etc.) were manually counted in the samples of all TMEs. After extrapolating the number of organisms found in the soil core (diameter = 5 cm) to the total area of the TME (diameter = 16.5 cm), 49995.99 mites, 26136 springtails and 5499.45 other microarthropods were recorded per TME. All subsequent analysis was done with extrapolated values. In samples from two TMEs no organisms were found. These TMEs were treatments corresponding to the RD of pesticide, one set for low (L83C) and one for medium moistures (L85D) (see **Error! Reference source not found.** for details).

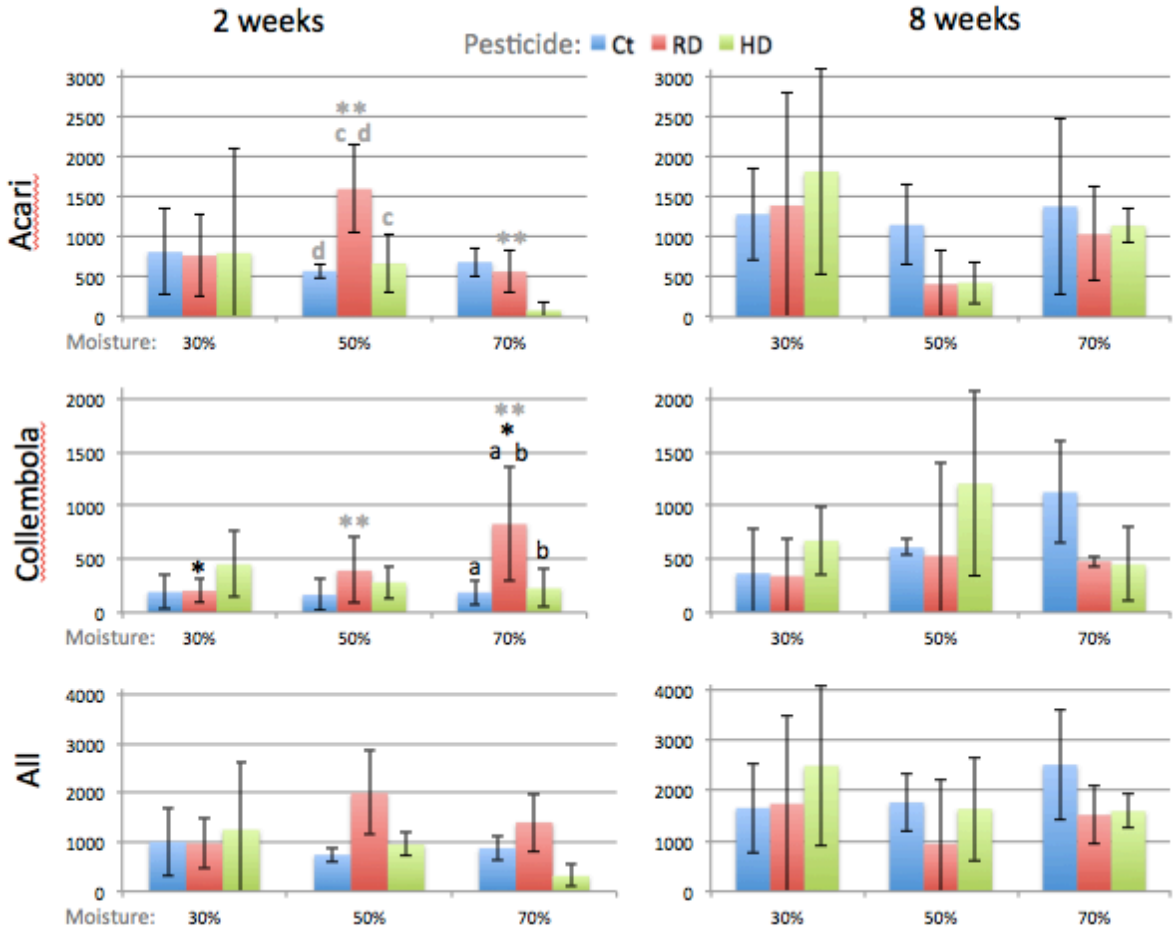

Figure 7. Average abundances found for mites (Acari; first row), collembola (second row) and both (third row) in each TME considering theoretical treatments of moisture and pesticide treatment. Statistical significant differences found through two-way ANOVA and Duncan post-hoc test are shown considering p≤0.05 (*, a, b) and p≤0.1 (**, c, d). * denote differences among moisture and letters denote differences among pesticide doses.

Figure 7 shows the average numbers of microarthropods found in the TMEs when organizing data by groups of theoretical moisture and pesticide treatment, at both experimental periods. At two weeks, the highest collembolan densities (±SD) were found at 70% of the water holding capacity (WHC) of soil, compared to 30% ($p \leq 0.05$), and to 50% ($p \leq 0.1$). For pesticide treatments, there were differences in abundances only at the condition of highest moisture, where the highest abundance was for TMEs treated with the recommended dose of pesticide (RD) compared to control and to the highest concentration of the chemical ($p \leq 0.05$). There were no significant differences on pesticide treatment at other conditions of soil moisture. For acarii, significant differences were found only between 50 and 70% of the WHC of soil, having higher amount of mites at 50% ($p \leq 0.05$). An effect of the pesticide was observed only at the middle condition of soil moisture (50% of the WHC), with the highest amount of springtails recorded for the TMEs treated with the RD compared to 5x RD and control ($p \leq 0.1$).

Because the theoretical groups of moisture did not match the moisture recorded for each TME on real time, microarthropods abundances were tested for the new groups of moisture rearranged *ad hoc* (Figure 8). Average amount of mites were significantly higher when moisture was low compared to medium ($p \leq 0.05$) and high ($p \leq 0.1$) moisture. On the other hand, springtails showed an increment of abundance with higher moisture conditions compared to the medium one ($p \leq 0.1$), but did no significantly changed from low to medium ($p = 0.107$) or to high moisture ($p = 0.79$).

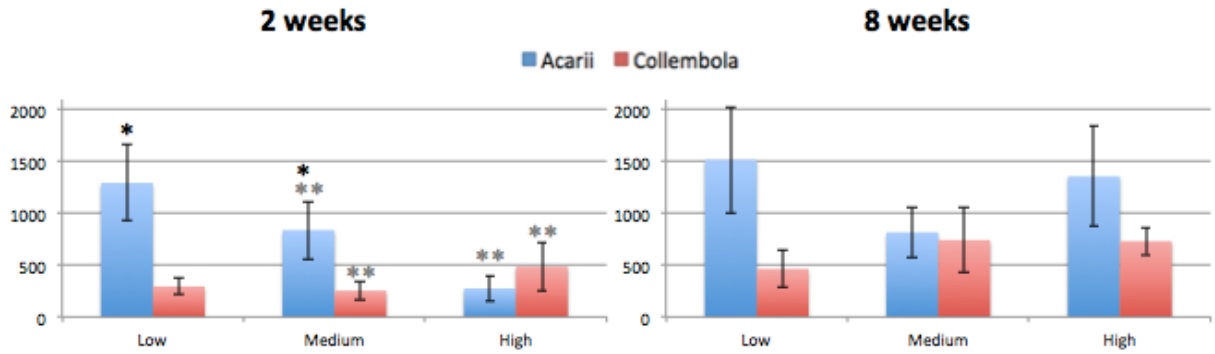

Figure 8. Average abundances (±SD) of mites (Acari) and collembola considering *ad hoc* groups of moisture. Statistical significant differences found through one-way ANOVA and Duncan post-hoc test are shown considering $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

The effect of the pesticide was also compared in *ad hoc* groups of moisture through a factorial ANOVA (Figure 9). In this analysis, abundances of mites show significant differences at two weeks between high and low concentrations of pesticide ($p \leq 0.5$), and between high and control conditions when moisture was low ($p \leq 0.05$).

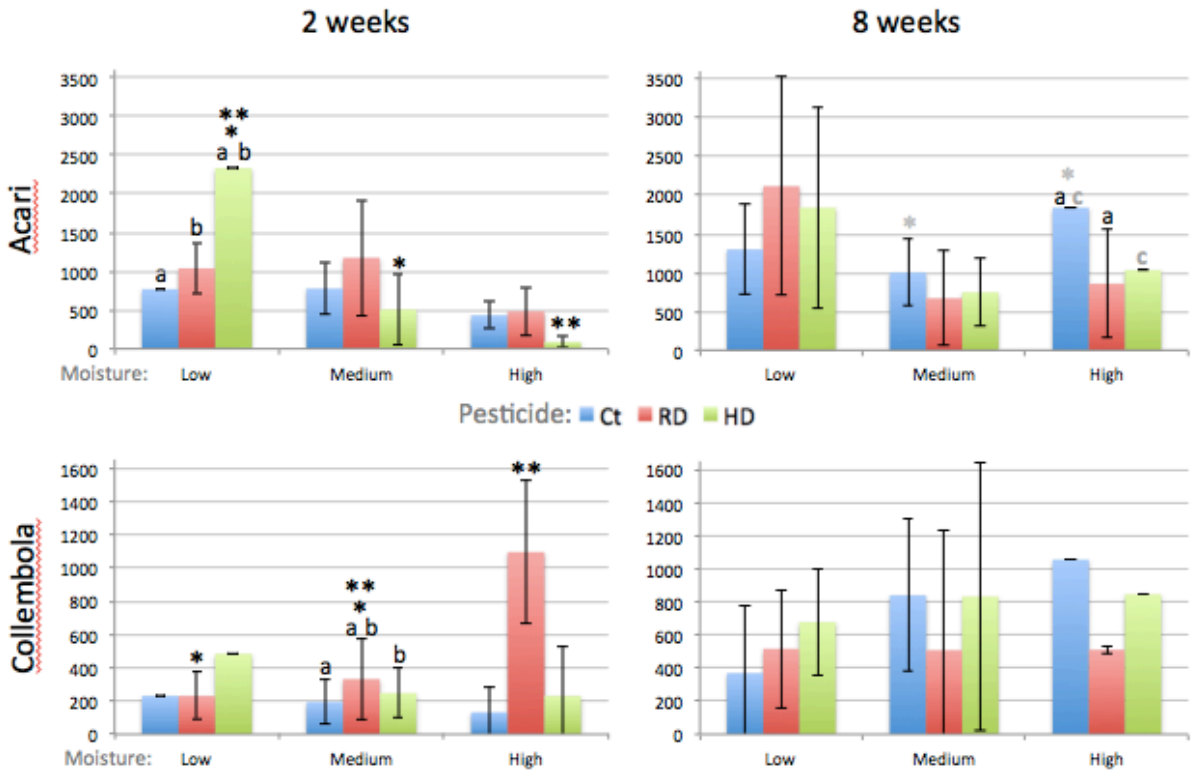

Figure 9. Comparison of the effect of pesticide treatment on average abundances (±SD) of mites (Acari; first row) and collembola (second row) arranged by *ad hoc* groups of moisture. Statistical significant differences found through two-way ANOVA and Duncan post-hoc test are shown among different moistures (*,**) and pesticide doses (a, b, c) at different p levels(black for $p \leq 0.05$ and gray for $p \leq 0.01$

The average number of mites was significantly higher at low moisture conditions ($p \leq 0.05$) compared with medium and high moisture conditions. At eight weeks more mites were present without pesticide for high moisture conditions comparing to one ($p < 0.05$) and five ($p < 0.01$) times the RD, and also comparing to medium moisture ($p < 0.01$). Although results are not conclusive, results suggest that acari prefer dry conditions. On the other hand, springtails are more abundant in high moisture, although significant differences were found only at two-week period, with the highest number of collembola found at high moisture ($p \leq 0.05$). Among pesticide treatments more springtails were found in the RD ($p < 0.05$) but only at medium moisture.

4.3 Collembola morphospecies

A total of 2400 springtails (26400 per TME) were identified in samples using a three-traits classification, producing a total of 16 different morphotypes or morphospecies, named MFN1 to MFN16.

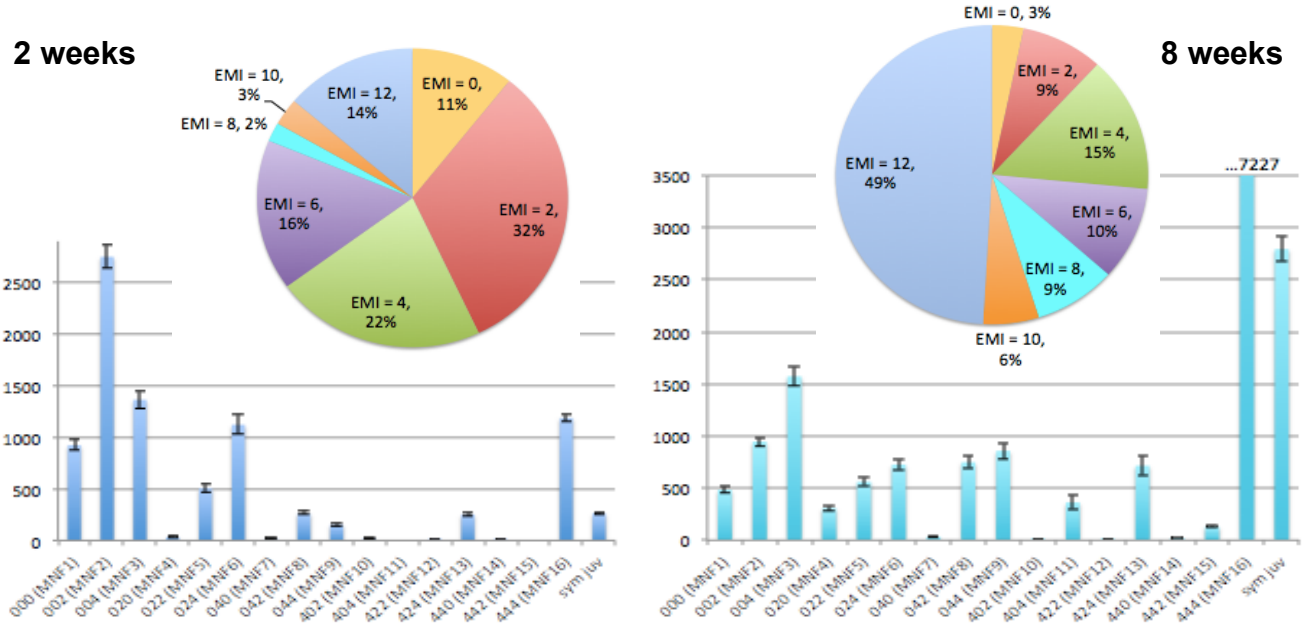

Figure 10. Structural diversity of Collembola morphospecies in TMEs. Graphs show total amounts of springtails per each morphospecies (MFN) at two and eight-week samples of pesticide treated replicates. Labels of morphospecies show its name and a three-digits code that corresponds to the morphotype classification according to its trait categories. Note that at eight weeks, a great amount of individuals of morphospecies MFN16 were registered, but the graph is adjusted to allow visualization of other types. Pie charts above show proportions of ecomorphological indices (EMI) found per experimental period.

Ecomorphological indices ranged from 0 to 12. Figure 10 shows the total amounts of Collembola morphospecies recorded, with their respective code of traits (see Table V) in the bars' graph and the proportional distributions of EMIs. Worth is to note that general structure of Collembola communities at each experimental period is different. For example, at two weeks the most abundant morphospecies are those with an EMI=2, while at eight weeks morphospecies with EMI=12 take 49% of total percentage. The only morphospecies with that EMI is MFN16, which is by far the most abundant one at eight weeks (7227 individuals). All 16 morphotypes were present in samples of 8-weeks experimental period, but in samples from 2-weeks only 13 morphotypes were recorded (the missing morphospecies were MFN8, MFN11 and MFN15). Juveniles that look similar to sympleonidae (named: "sym juv") were highly

abundant, especially in samples from 8 weeks, where they account for up to 16% of the total abundance. However, only adult forms were not taken into account for further analysis.

Finally, three samples from the eight-week experimental period did not have springtails. These samples were from a TME with the control treatment of pesticide and low theoretical humidity (Ct83B), a TME with the RD of pesticide and low theoretical soil humidity (L83C) and a TME with the RD and medium theoretical soil humidity (L85D). Springtails were found in all samples from two-weeks TMEs.

4.4 Effect of moisture and L-cyhalothrin pesticide on functional diversity patterns of Collembla morphospecies.

Most of the responses for the groups made with theoretical moisture were similar to those classified based to *ad hoc* moisture (i.e. soil moisture recorded on real time), except for the mT index. As variability among data was high, statistical differences are reported up to p=0.1.

One-way ANOVAs showed that at two weeks, **average abundance** in RD treatment was significantly higher than control (p=0.019), but no significant differences were found compared to HD (5xRD). When analysing abundances according to moisture, there was a tendency for ad hoc groups of moisture to find higher numbers correlated with the highest moistures., At eight weeks the tendency appears inverted, with the highest abundance of collembola morphospecies at both high and low pesticide treatments, and the lowest ones in low moisture conditions. However, no significant differences were found for any of these treatments (Figure 11, upper panel).

A factorial ANOVA, with the combined effects of pesticide (Ct=Control, RD=Recomended Dose, or HD=High Dose) and moisture (L=Low, M=Medium, or H=High), on springtails average abundances, showed no significant differences when comparing RD and HD with all other conditions, for both theoretical and *ad hoc* groups of moisture ($p \leq 0.05$), at two and eight weeks. No significant differences among any comparisons were found for eight weeks (Figure 11, second row). Patterns for **Shannon** and **Simpson** indices were similar when analysing the effects of pesticide and mositure separetely through one-way ANOVAs. At two weeks, Shannon was significantly higher in RD treatments compared to control (p=0.05) and tend to show a difference with HD treatments (p=0.06). No significant differences were found when comparing moisture conditions, although there is a tendency of reducing the lowest concentrrations at the higher moistures. This trend appears to be inverted at eight weeks, for ad hoc moisture, where indices were significantly diferent at high conditions from low ($p<0.05$)

and medium (p=0.05 for Shannon, and p<0.05 for Simpson). Although theoretical moisture followed the same trend, no statistically significant differences were found (Figure 12).

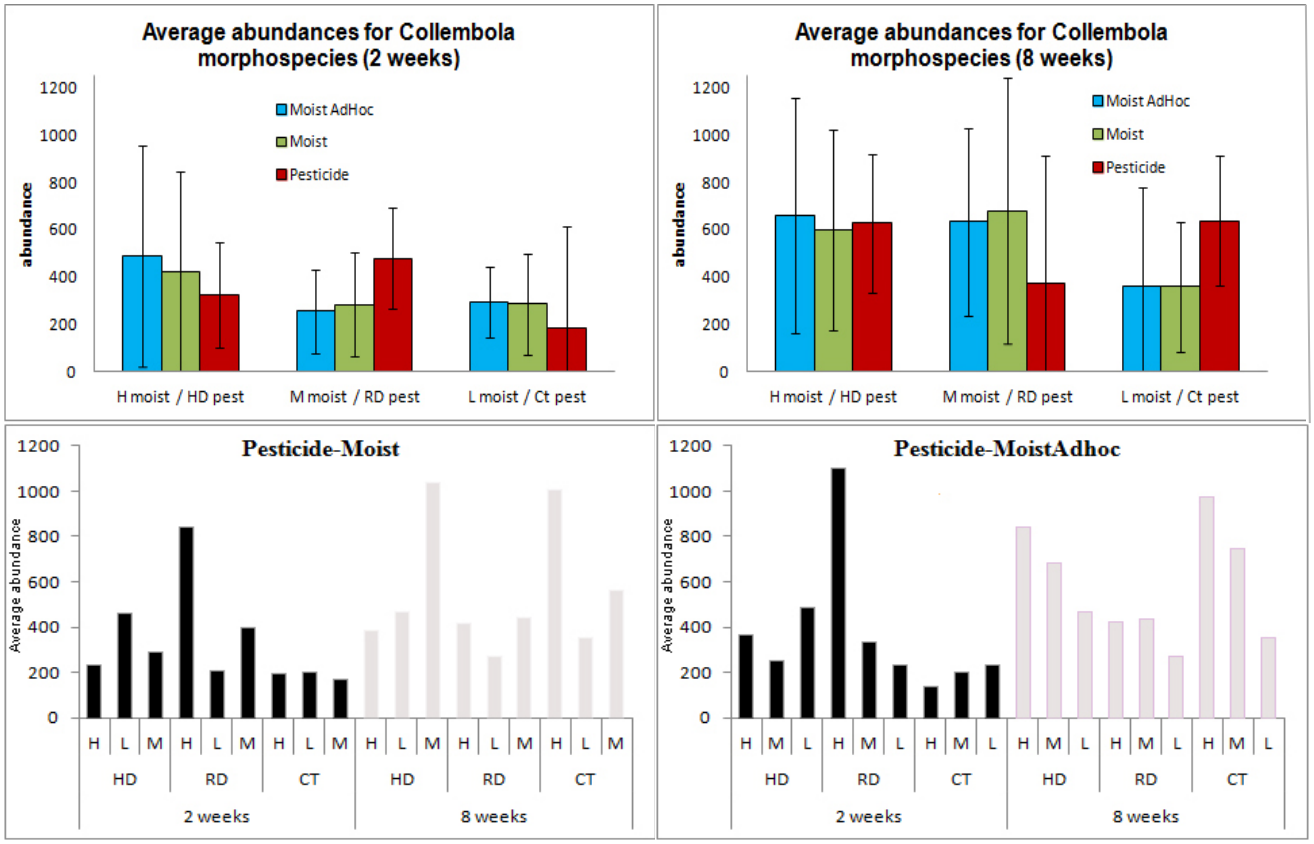

Figure 11. Average abundances (±SD) of Collembola morphospecies in treatments of different pesticide doses or moistures (using theoretically and ad hoc groups), separately (first row), and combined (second row) at two and eight weeks.

The factorial analysis of variance (ANOVA) to evaluate the combined effect of pesticide treatment and soil moisture content, did not show differences for both Simpson and Shannon indeces. At eight weeks, although not significant, there were some trends on Simpson index among Ct+L and Ct+H (p=0.06) and RD+L and HD+L (p=0.09) for theoretical groups of moisture. For *ad hoc* moistures the differences in Simpson index were shown when comparing Ct+H with Ct+M, RD+H and HD+H (p<0.05); whereas for Shannon index the differences were on comparing Ct+H with Ct+L (p<0.05) (Figure 13).

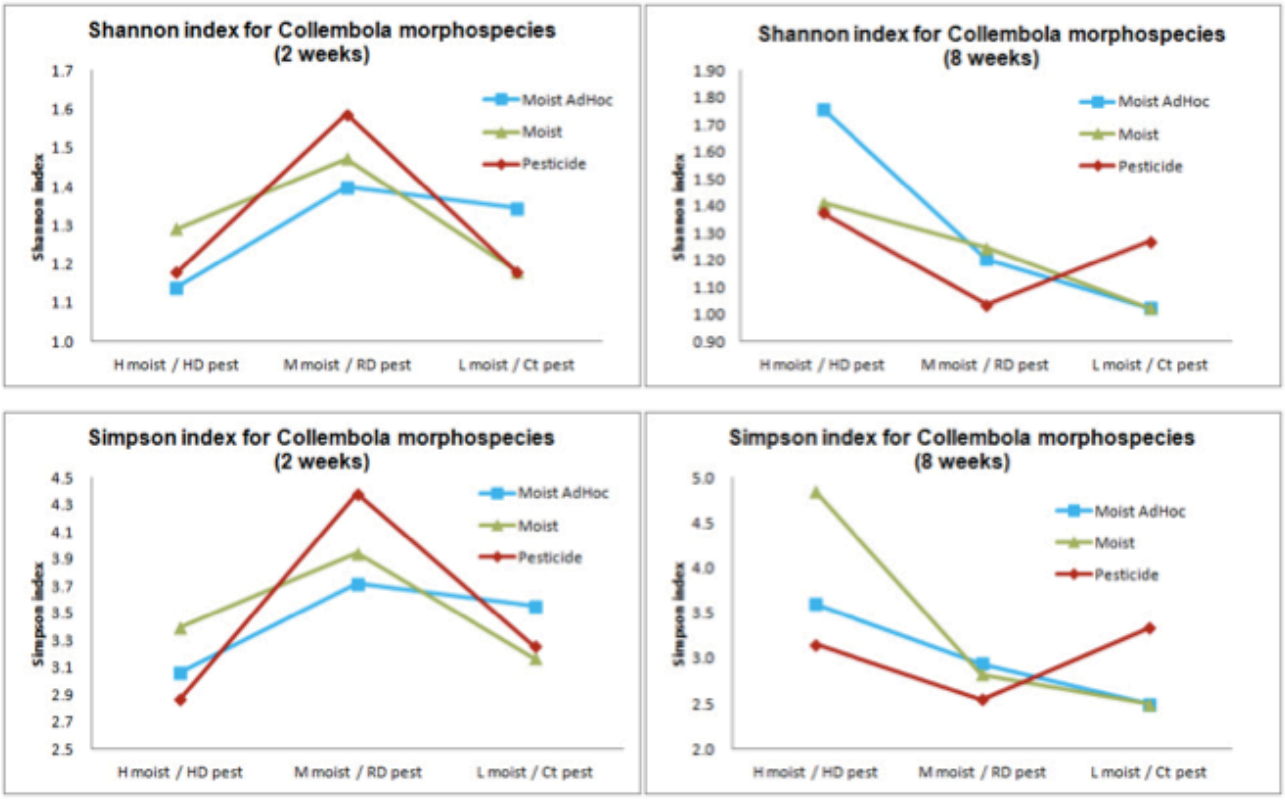

Figure 12. Shannon and Simpson indices for Collembola morphospecies in treatments of different pesticide doses or moistures (using theoretically and ad hoc groups), separately (first row), and combined (second row) at two and eight weeks.

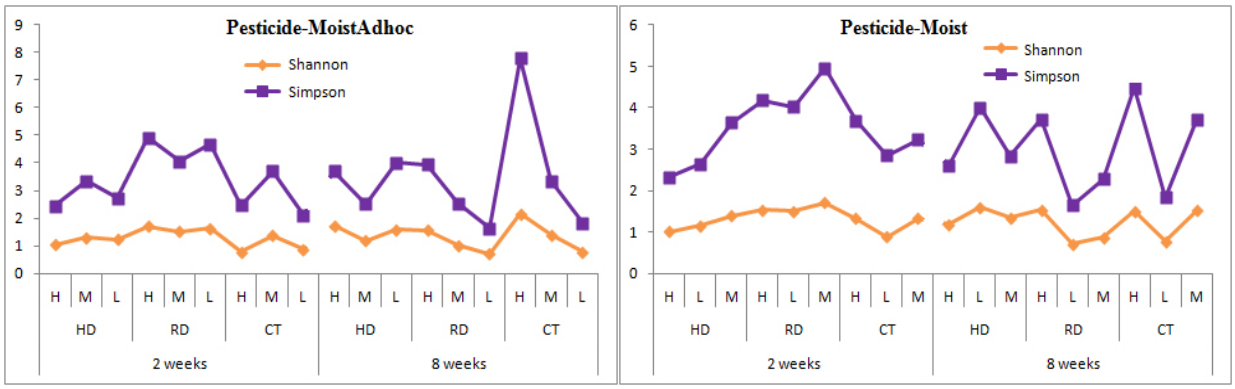

Figure 13. Shannon and Simpson indices combining treatments with different pesticide doses (Ct, RD and HD)and soil moistures (L – 30%, M – 50%, H – 70% of WHC) at two and eight weeks.

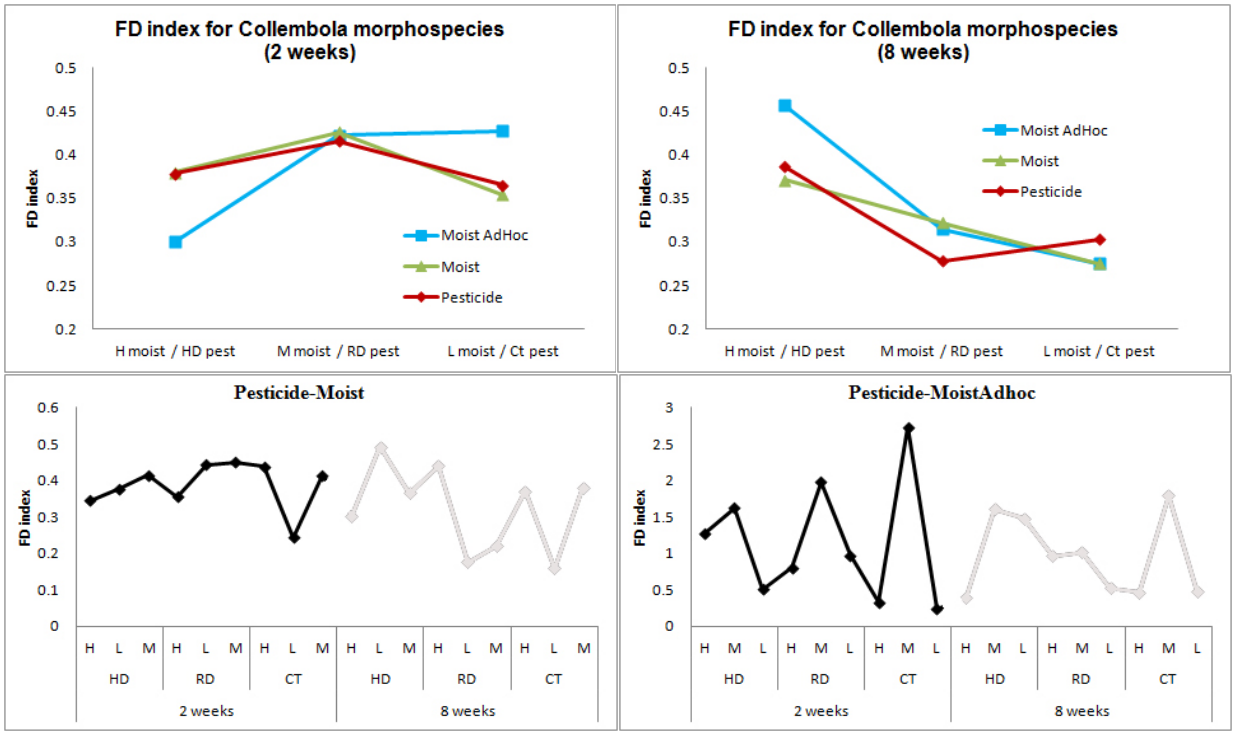

Figure 14. Functional Diversity index for Collembola morphospecies in treatments of different pesticide doses or moistures (using theoretically and ad hoc groups)separately (first row), and combined (second row) at two and eight weeks.

One-way ANOVAs for **Functional Diversity** index (**FD**) did not show significant differences between treatments of different pesticide doses at two and eight weeks. Regarding moisture conditions, differences were found only for ad hoc groups in both sampling dates. At two weeks FD index was higher at high moisture compared to medium and to low conditions, however, this difference was not significant ($p=0.06$). At eight weeks the index was significantly higher at high conditions compared to low ($p=0.05$) (Figure 14, first row). The factorial analysis of variance (ANOVA) for FD index for two weeks did not find significant differences between treatments considering theoretical moisture groups. For *ad hoc* moisture groups the significant differences were found when comparing Ct+H with Ct+M ($p<0.05$) and Ct+L with HD+L ($p<0.05$). At eight weeks no significant differences were found between treatments ($p > 0.05$) (Figure 14**,** second row).

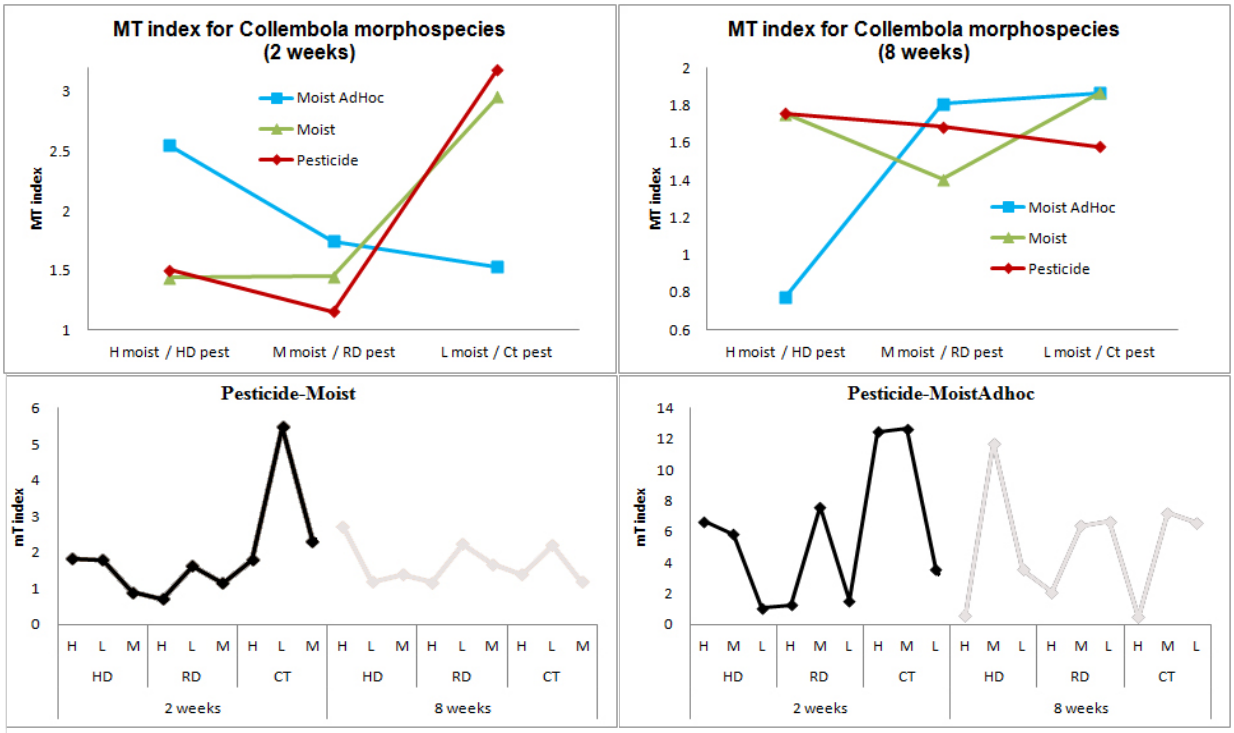

Figure 15. Mean trait value (mT) of Collembola morphospecies for each treatment at two and eight weeks analysed for different moistures or pesticide dose separately (first row) or together (second row) at two and eight weeks.

Analysis of variance for one factor showed that **Mean trait** values (**mT**) at two weeks were higher in control conditions compared to RD and HD ($p<0.05$) of pesticide (red line), and also at low theoretical moisture (green line) compared to medium and high ($p\leq0.05$) conditions. No significant differences were found when comparing groups of ad hoc moisture (blue line) for two weeks, not at any pesticide treatment or moisture condition at eight weeks (Figure 15, second row).

Two-way ANOVAs for two-weeks data of mT, showed significant differences comparing the Ct+L (theoretical moisture conditions) with Ct+M, Ct+H, RD+L, and HD+L ($p<0.05$). For ad hoc moisture significant differences were found when comparing Ct+H vs. Ct+M, RD+H and HD+H ($p<0.05$) No significant differences were found at eight weeks (Figure 15, second row).

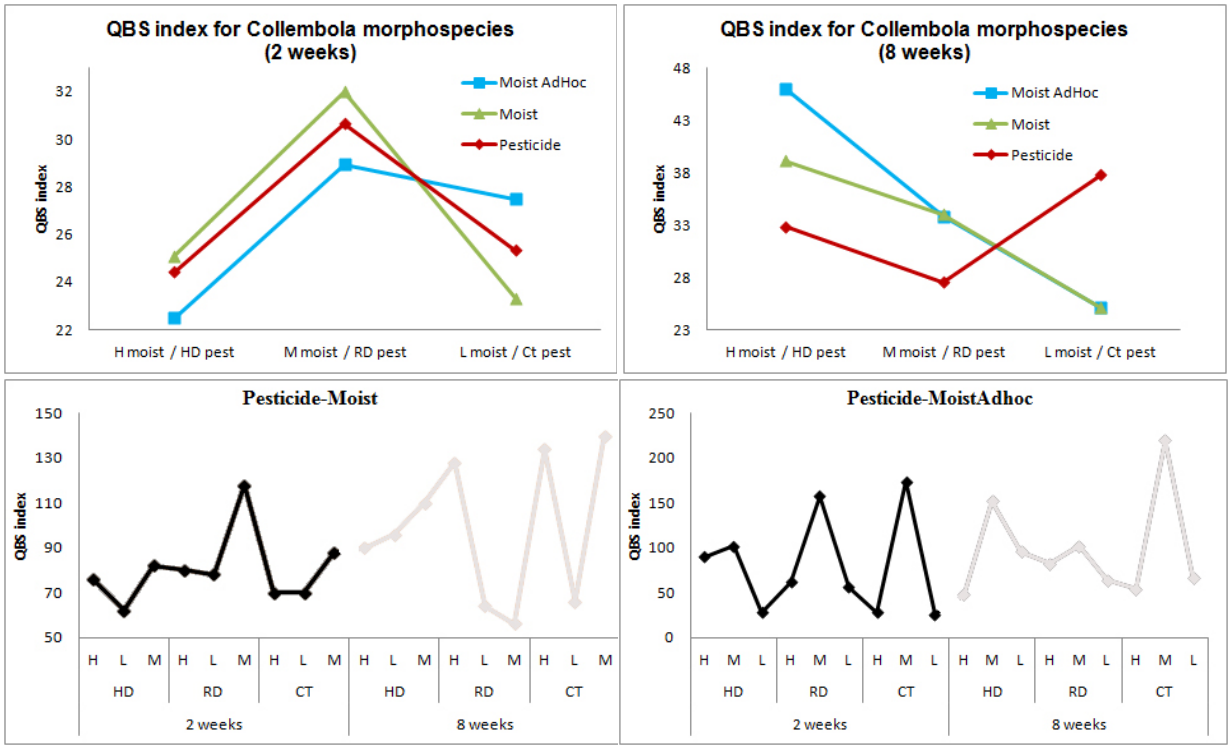

Figure 16. Biological Soil Quality Index (QBS) of Collembola morphospecies at two and eight weeks analysed for different moistures (L – 30%, M – 50%, H – 70% of WHC) or pesticide doses (Ct, RD and HD) separately (first row) or together (second row) at two and eight weeks.

The trend for **Soil Quality Index** (**QBS**) at two weeks were similar for all treatments and no significant differences were found. At eight weeks significant statistical difference were found comparing low and high ad hoc moisture conditions ($p \leq 0.05$). No significant differences were found with multifactorial analysis. Trends are shown in Figure 16**.**

No significant differences were detected for the **Classification Index of Soil Quality for Collembola** (**ICQS$_c$**). Notwithstanding, the graphs show a tendency for the lowest index values at HD treatments and high moisture for two weeks. For eight weeks there is a tendency to decrease with low moisture and RD pesticide dose. No significant differences were found in multifactorial ANOVAs either. Trends are shown in Figure 17**.**

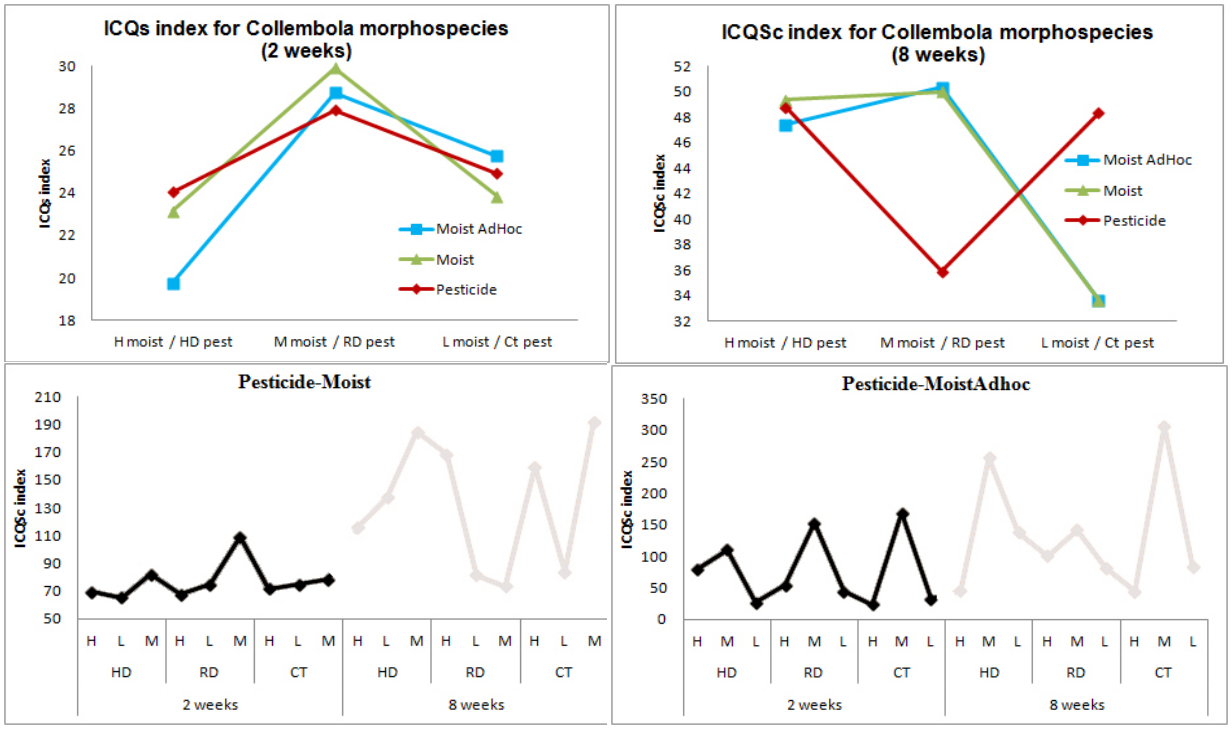

Figure 17. Classification Index of Soil Quality for Collembola morphospecies at two and eight weeks analysed for different moistures (L – 30%, M – 50%, H – 70% of WHC) or pesticide doses (Ct, RD, HD) separately (first row) or together (second row) at two and eight weeks.

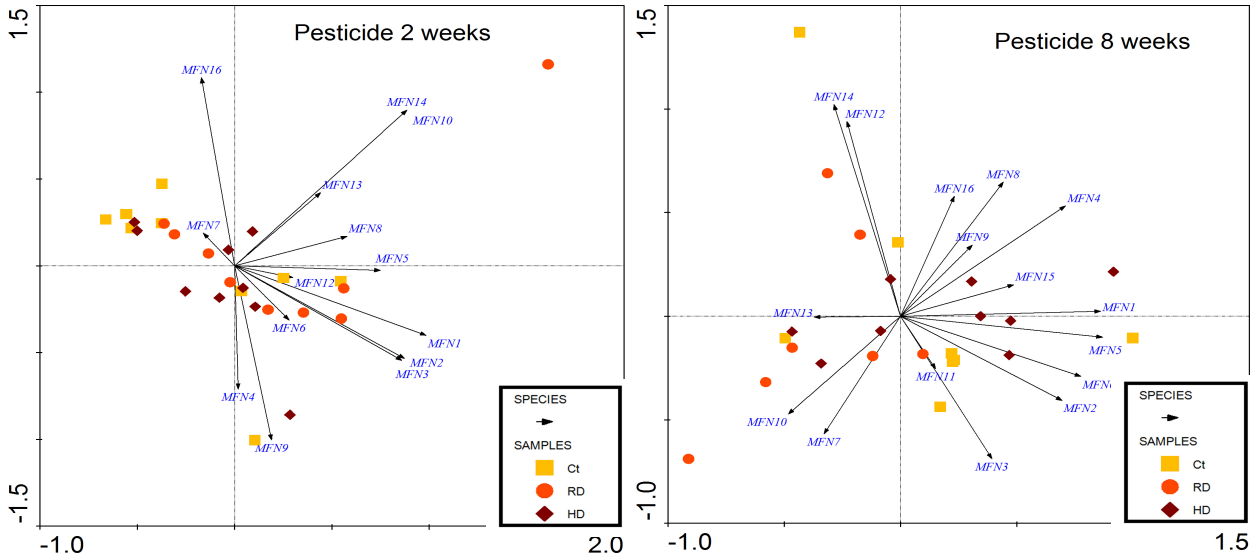

Figure 18. PCA diagrams for the distribution of samples (squares, circles or lozenges) at two and eight weeks, classified by L-cyhalothrin dose (Ct, RD, HD). Full lines with arrowheads represent the morphotypes.

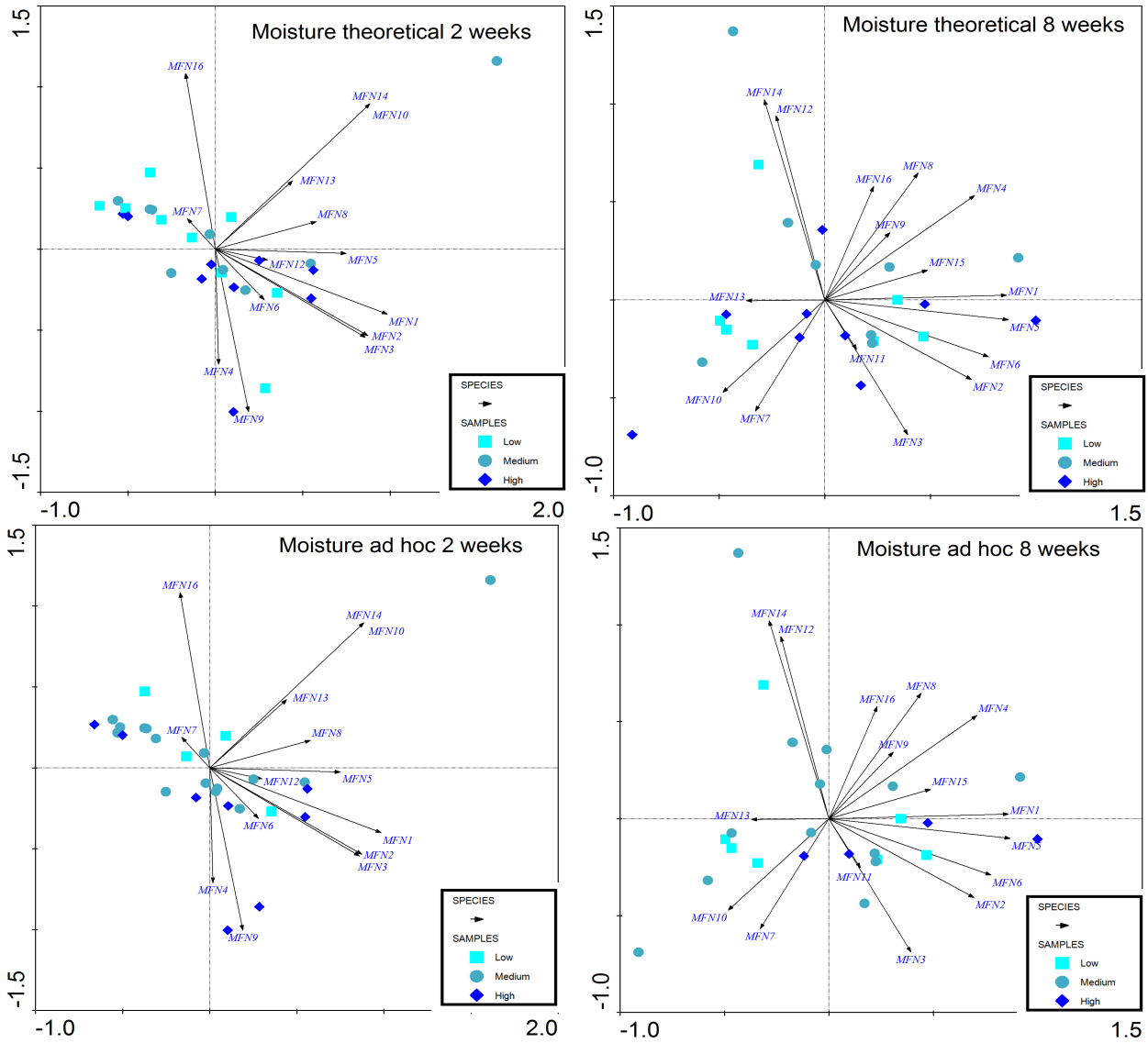

Figure 19. PCA diagrams showing the distribution of samples (sqaures, circles or lozenges) at two and eight weeks, classified by theoretical or *ad hoc* soil moisture content. Full lines with arrowheads represent the morphotypes.

4.5 Multivariate analysis of the effects of moisture and pesticide dose on Collembola morphospecies

Further analysis using data on the effects of L-cyhalothrin dose and soil moistures was performed applying multivariate statistics. Data on abundances of collembolan morphospecies in samples from TMEs were analyzed at two and eight weeks. A DCA provided a linear response with a length of the gradient of 2.2 for two-weeks and 2.18 for eight-weeks. These data (i.e. abundances of collembolan morphospecies) were analyzed through **PCAs** to explore the structure of the whole data set, considering the morphospecies presented in each TME and the number of individuals per each morphospecies in the respective TMEs (i.e. morphospecies

abundances). Axis 1 (vertical axis) and 2 (horizontal axis) explained 20.8% and 16%, respectively, of the variation for two weeks, and 22.1% and 15.7% for eight, respectively, for eight weeks.Samples were then classified by pesticide dose or by soil moisture (considering theoretical and *ad hoc* categories). There were no clear separation of samples considering pesticide dose (Figure 18). Only a slight trend of RD samples showed a tendency to follow the direction of morphotypes MFN1, MFN2 and MFN3, at two weeks, and a biplot line on the direction of MNF3 arrow at eight weeks could separate most of RD samples on one side. The classification of samples according to its soil moisture did not show groups of points (i.e. samples) clearly separated, although for both ways of grouping samples (theoretical and *ad-hoc* soil moisture), at eight weeks low and high moisture points are mainly distributed in the negative side of vertical axis (Figure 19). Some general inferences can be made concerning the morphotypes traits in the PCA plot. At two weeks, most of the morphospecies with ocelli (except for MFN7 at the left, and MFN8 at the upper quadrant) and furca are grouped at the bottom-right area of the plot (negative values of axis 2 and positive values of axis 1); the trend of the points corresponding to "pigmentation" trait is not very clear. At eight weeks, morphospecies with eyes are still at the right side of the plot (except for MFN7 at the left, and MFN8 and MFN9 in the upper quadrant), while many of the morphotypes with furca are located at the bottom-right quadrant (positive values of axis 1 and negative values of axis 2; Figure 20). Some morphospecies share similar relationships on the plot at two and at eight weeks (e.g. MFN3 and MFN2 or MFN6 and MFN5). On the other hand, at two weeks the opposite directions of arrowheads of MFN16 and MFN9 share the same traits for furca and pigmentation, but while the former is blind, the later is not. A trend similar to this late occurred between MFN7 (no furca and with patterns) and MFN6 (reduced furca and white).

Analysis of similarity (**ANOSIM**) among morphotypes between pesticide doses, moistures, or both did not show significant differences. Simper analysis revealed that in all cases, considering pesticide doses and moistures in both experimental periods, the MFN16 was the morphotype that contributed most (the highest percentage) to the distance between samples, generally followed by MFN2, MFN3 and MFN1. On the other hand, the MFN6 morphotype, was identified as the lowest contributor (the lowest percentage) to the distance between samples in both periods. MFN8 and MFN13 morphotypes were also important for dissimilarity between samples at two and eight weeks, respectively.

Finally, Figure 21 shows the **PRC** analysis evaluating the variance explained by time and pesticide dose of test treatments in all test moistures and sampling dates. PRC analysis revealed a decrease of Collembola morphotypes in HD treatment, at two and eight weeks. For the RD treatments, a different pattern was found showing a decrease even higher than that of the HD treatments at two-week samples, and a pronounced increase over the control.

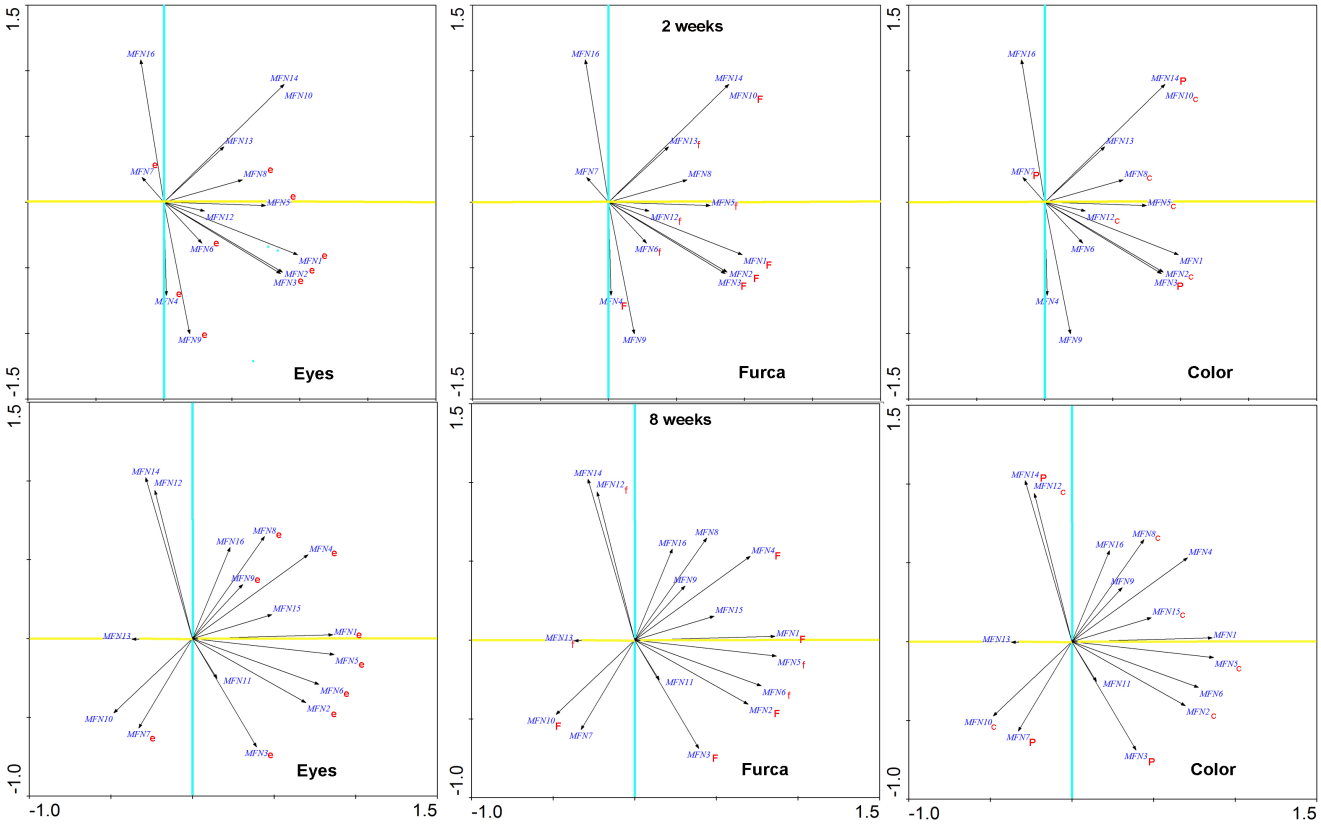

Figure 20. PCA diagrams showing morphospecies distribution at two weeks (upper panels) and eight weeks (lower panels), considering traits for each morphospecies (red letters). First axis (blue) explains 20.8% of the variation at two weeks, and 22.1% at eight weeks.

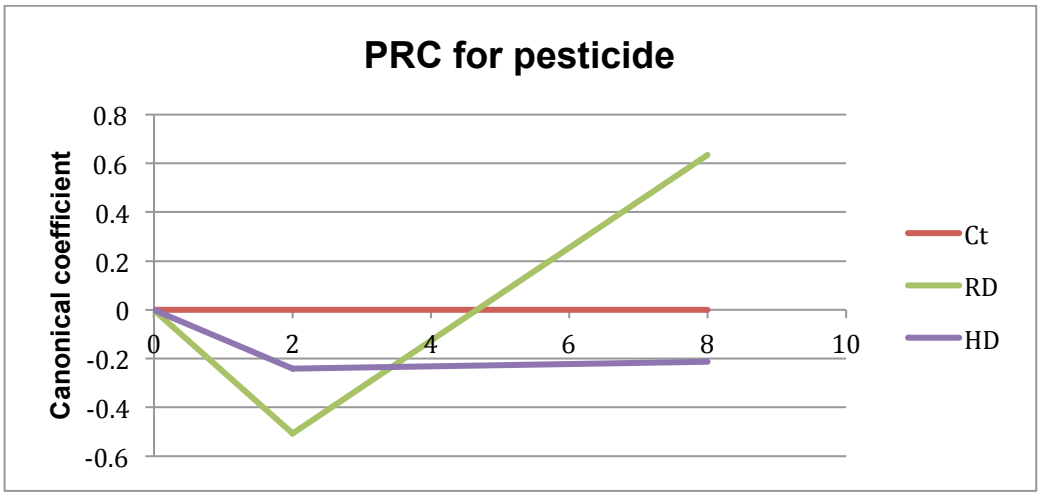

Figure 21. Principal Response Curves (PRC) diagram for the Collembola data set, showing the effect of L-cyhalothrin on Collembola morphotypes in TMEs over time.

5 Discussion

Although soil is often considered a stable environment, the surface layers are subject to wide fluctuations in temperature and moisture (Patrick 2001) and there are still major gaps of knowledge on the function of ecosystems (Filsner et al 2008). Considering behavior not only as a test endpoint itself but as an important driver of ecosystem functioning is not a widespread approach. The activity of organisms, from feeding to digging, is critical for vital ecosystem functions, such as organic matter breakdown, plant nutrition or water drainage in the case of soil ecosystems (Filsner et al 2008).

It is recognized that changes in precipitation regime can affect soil organisms and the functions they provide because soil moisture strongly influence their reproduction and development rates (Kardol, et al. 2011). The sensitivity of soil microarthropods in response to changes in moisture is a pattern observed in numerous studies across diverse ecosystems (e.g. (Frampton, Van den Brink and Philip 2000)). However, the patterns may not be quite clear in all cases. Kardol et al. (2011), for example, did not detect any effects on microarthropod abundance when analyzing changes on rain regimes in two seasons of the year, along five years, but noticed that in total microarthropod richness was lower in dry than in wet treatments at least in one season. In general, collembolan abundance and richness are positively related to soil moisture content (Kardol, et al. 2011).

Soil moisture content may have a large influence on microarthropod abundance, since the activities of most soil animals are determined by soil water status (Patrick 2001). In general, mites are more resistant to desiccation than springtails, and observation that was corroborated in our study at least at two-week samples. In fact, when average abundances of mites significantly decreased with increasing real moisture, springtails increased. The present study, however, showed large variation between samples. Although regrouping the samples attempted to solve this problem, variability was evidenced by the large standard deviations showed in Figure 7 to Figure 9. Similarly large variations have been reported in other studies (e.g. (Koolhaas, et al. 2004)). It is probable that this variation was related with changing factors typical from soil systems (e.g. natural homogeneity of soil matrix). In addition, according to Koolhaas et al. (2004), species of mites and springtails tend to

cluster, a fact that may also contribute to increase variation between samples. On the other hand, the TMEs reflect the natural variation of the field site. Probably, having more samples per treatment could have reduced variability; however, increasing the number of replicates would considerably intensify the logistic effort as well.

The effects of pesticides on Collembola in field and semi-field (using TMEs) conditions were investigated using the fungicide carbendazim in an international ring-test. Such study showed that springtail communities were scattered and therefore no effects on species diversity could be found (Koolhaas, et al. 2004). However, analysis using principal response curves (PRC) revealed some significant effects of carbendazim pesticide on Collembola communities in some tests with TMEs and in the field (Schaeffer, et al. 2011). In our experience, values for diversity indices generally did not show a clear decrease with increasing L-cyhalothrin dose; furthermore, PRC analysis showed inconsistent effects of the pesticide dose on Collembola communities. This may be attributed to the large variation between replicates, possibly overshadowing the effect of L-cyhalothrin treatment. Another factor that could explain the inconsistent PRC analysis was the difficulties controlling soil moisture of the TMEs. This brought more variability to the systems, though that the PRC analyses were performed considering only the treatments according to its pesticide dose (the different moistures of TMEs were not taken into consideration for PRC analyses).

Some studies suggests that, depending on the concentration and application regimes, Collembola resurgences may occur after insecticide application, altering diversity and composition of the community of springtails (e.g. (Filser, et al. 2008) (G. K. Frampton 2000)), and that synthetic pyrethroids can even favor increased collembolan abundance due to differences in susceptibility of Collembola and their predators against the pesticide (Frampton and van den Brink 2007).

Some reports suggest that synthetic pyrthroid insecticides (such as L-cyhalothrin) usually do not affect the abundance of springtails in arable agriculture or forestry field studies (Frampton and van den Brink 2007). However, in some cases it is unclear whether the pesticide treatments were appropriately replicated. Studies from the literature reported that Collembola abundance increased after applications of synthetic pyrethroids in forest plots and arable crops (e.g. (G. K. Frampton 1999)), responses of individual Collembola species reported only the total abundance of springtails.

6 Final considerations

The characterization of collembola communities based on a trait approach to evaluate the effect of the pesticide L-cyalothrin under different climate conditions did not provide conclusive results most probably due to the variability of data. This fact suggests the need of improving soil moisture monitoring in order to be able to apply treatments correctly. Using more samples/replicates may increase robustness of data (therefore reducing its variability), provided that the number of replicates and the design of the whole experiment are workable in practical and logistic terms.

Moreover, to understand better the reasons behind the observed response, a finer analysis of the community composition may be achieved by using few more trait types for mesofaunal identification of morphospecies, choosing among those that better explain the functional attributes of each member of the community, e.g. feeding habilities and/or ecophysiological tolerance traits. However, considering the effort input required for such labor in terms of time, knowledge and technical requirements (e. g. microscope augmentation, etc), it would be important to work with the fewer possible number of traits.

The characterization of other communities of organisms with different routes of exposure towards soil contaminants (e.g. nematoda, enchytraeids) may give less variable data providing a more realistic view on the effect of L-cyalothrin on soil fauna communities. It is probable that, after a pesticide application, the type of communities affected from soil fauna are highly dependent on the mode of action of the contaminant that is being investigated. However, further research is needed (especially focused on soil communities other than Collembola) to confirm this assumption.

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8 Annex

8.1 Raw data 2-weeks

8.2 Raw data 8-weeks

8.3 Diversity scores per sample at 2-weeks

8.4 Diversity scores per sample at 8-weeks

8.1 Raw data 2-weeks

Sample name	Moist. theo.	Moist. ad hoc	Pesticide	Acarii	Others	Collembola real	Collembola transf	MFN 1	MFN 2	MFN 3	MFN 4	MFN 5	MFN 6	MFN 7	MFN 8	MFN 9	MFN 10	MFN 11	MFN 12	MFN 13	MFN 14	MFN 15	MFN 16	sym juv
Ct23B	L	H	CT	28	0	2	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0
Ct23C	L	M	CT	127	13	30	330	22	77	0	0	88	44	0	0	11	0	0	0	0	0	0	55	33
Ct23D	L	L	CT	70	2	21	231	0	0	22	0	0	0	0	0	0	0	0	0	66	0	0	143	0
Ct25B	M	M	CT	53	5	30	330	33	154	11	0	11	33	0	55	0	0	0	11	11	0	0	11	0
Ct25C	M	M	CT	61	5	4	44	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	22	11
Ct25D	M	M	CT	45	3	12	132	22	44	0	0	0	0	11	0	0	0	0	0	0	0	0	55	0
Ct27B	H	M	CT	57	1	5	55	0	22	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0
Ct27C	H	H	CT	51	6	22	242	55	99	33	22	0	0	0	0	22	0	0	0	0	0	0	0	11
Ct27D	H	M	CT	82	8	25	275	22	22	88	0	33	0	0	0	11	0	0	0	44	0	0	55	0
L23B	L	L	RD	73	23	30	330	77	99	11	0	0	66	0	44	0	0	0	0	33	0	0	0	0
L23C	L	L	RD	116	1	12	132	44	0	11	0	0	11	0	11	0	0	0	0	0	0	0	22	33
L23D	L	M	RD	23	3	14	154	11	0	22	0	0	11	0	0	0	0	0	0	0	0	0	88	22
L25B	M	M	RD	153	0	32	352	22	55	66	0	0	33	0	22	22	0	0	0	0	0	0	77	55
L25C	M	M	RD	195	7	66	726	176	275	88	0	33	0	0	22	0	22	0	0	11	11	0	88	0
L25D	M	M	RD	94	0	10	110	0	0	11	0	0	11	0	22	0	0	0	0	0	0	0	44	22
L27B	H	M	RD	69	6	29	319	11	165	99	0	0	0	0	0	0	0	0	0	0	0	0	22	22
L27C	H	H	RD	64	16	127	1397	198	308	319	0	77	462	0	0	0	0	0	0	0	0	0	33	0
L27D	H	H	RD	24	4	72	792	88	121	88	0	143	242	0	44	0	0	0	0	0	0	0	44	22
H23B	L	L	HD	212	50	44	484	22	264	22	0	0	0	0	0	0	0	0	0	88	0	0	88	0
H23C	L	M	HD	0	0	12	132	0	11	0	0	0	33	0	0	0	0	0	0	0	0	0	77	11
H23D	L	H	HD	9	1	68	748	55	308	319	0	0	0	0	0	66	0	0	0	0	0	0	0	0
H25B	M	M	HD	23	3	42	462	22	330	88	0	0	0	0	0	0	0	0	0	0	0	0	22	0
H25C	M	M	HD	75	0	17	187	0	77	11	0	0	22	0	0	11	0	0	0	0	0	0	44	22
H25D	M	M	HD	87	9	19	209	22	44	22	0	11	33	11	0	0	0	0	0	0	0	0	66	0
H27B	H	H	HD	7	3	3	33	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	0
H27C	H	H	HD	17	13	34	374	0	275	22	0	22	0	0	33	11	0	0	0	0	0	0	11	0
H27D	H	H	HD	0	0	26	286	11	0	11	11	88	121	0	0	0	0	0	0	0	0	0	44	0

8.2 Raw data 8-weeks

Sample name	Moist. theo.	Moist. ad hoc	Pesticide	Acarii	Others	Collembola real	Collembola transf	MFN 1	MFN 2	MFN 3	MFN 4	MFN 5	MFN 6	MFN 7	MFN 8	MFN 9	MFN 10	MFN 11	MFN 12	MFN 13	MFN 14	MFN 15	MFN 16	sym juv
Ct83B	L	L	CT	59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ct83C	L	L	CT	137	41	74	814	11	55	143	0	0	0	0	11	44	0	352	0	0	0	22	176	0
Ct83D	L	L	CT	159	15	27	297	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	209	66
Ct85B	M	M	CT	55	3	48	528	11	0	0	11	0	0	0	33	22	0	0	11	11	11	0	385	33
Ct85C	M	M	CT	142	14	59	649	33	0	132	0	33	154	0	0	22	0	0	0	0	0	0	187	88
Ct85D	M	M	CT	121	2	61	671	0	44	66	0	55	165	0	11	22	0	0	0	11	0	0	253	44
Ct87B	H	M	CT	80	0	64	704	11	143	33	0	0	66	11	11	0	0	11	0	0	0	11	275	132
Ct87C	H	M	CT	58	7	150	1650	0	0	0	88	11	0	0	0	0	0	0	0	0	0	22	1342	187
Ct87D	H	H	CT	244	7	96	1056	77	143	165	77	187	187	0	22	22	0	0	0	0	0	11	77	88
L83B	L	L	RD	128	30	29	319	0	0	77	0	0	0	0	0	0	0	0	0	0	0	0	220	22
L83C	L	L	RD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L83D	L	L	RD	257	20	65	715	0	0	11	0	0	0	0	88	44	0	0	0	0	11	11	330	220
L85B	M	M	RD	77	7	141	1551	0	33	0	11	0	0	0	143	99	0	0	0	451	0	0	550	264
L85C	M	M	RD	37	11	5	55	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	11	22
L85D	M	M	RD	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L87B	H	H	RD	33	50	45	495	11	88	143	0	0	0	0	11	0	0	0	0	33	0	0	209	0
L87C	H	M	RD	130	9	39	429	0	11	22	0	0	0	22	0	11	11	0	0	165	0	0	165	22
L87D	H	H	RD	123	2	48	528	33	44	77	0	0	22	0	11	11	0	0	0	11	0	0	132	187
H83B	L	L	HD	150	42	44	484	0	22	110	0	0	0	0	0	66	0	0	0	22	0	0	88	176
H83C	L	L	HD	293	20	96	1056	44	22	66	11	99	55	0	0	0	0	0	0	0	0	0	352	407
H83D	L	L	HD	59	9	45	495	121	33	11	11	55	0	0	11	0	0	0	0	0	0	0	187	66
H85B	M	M	HD	55	1	202	2222	22	88	66	66	55	55	0	33	33	0	0	0	0	0	44	1320	440
H85C	M	M	HD	52	7	64	704	33	99	0	11	0	22	0	22	385	0	0	0	0	0	0	132	0
H85D	M	M	HD	12	13	66	726	22	44	0	0	0	0	0	253	0	0	0	0	0	0	0	297	110
H87B	H	M	HD	127	8	30	330	11	11	0	0	11	0	0	0	0	0	0	0	0	0	11	110	176
H87C	H	M	HD	94	2	17	187	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	143	33
H87D	H	H	HD	94	3	77	847	44	55	407	22	55	0	0	88	77	0	0	0	11	0	0	77	11

8.3 Diversity scores per sample at 2-weeks

Sample	Morphospecies richness S	Collembola abundance	Shannon H	Simpson D	FD	MT	QBS	ICQSc
Ct23B	1	22	0	1	0.00	12	12	12
Ct23C	7	330	1.783	5.429	0.50	0.9	32	30.67
Ct23D	3	231	0.8788	2.12	0.23	3.6	26	32
Ct25B	9	330	1.682	3.719	0.40	0.4	52	34.2
Ct25C	3	44	1.04	2.774	0.30	5	18	20
Ct25D	4	132	1.237	3.182	0.55	1.5	18	24
Ct27B	2	55	0.673	1.957	0.48	4	14	16
Ct27C	6	242	1.551	3.951	0.33	0.5	16	11.9
Ct27D	7	275	1.767	5.158	0.51	0.9	40	43.68
L23B	6	330	1.635	4.741	0.47	0.6	28	22.4
L23C	6	132	1.633	4.624	0.50	0.9	28	22.22
L23D	5	154	1.253	2.678	0.36	3.3	22	30
L25B	8	352	1.968	6.67	0.49	0.9	38	42.52
L25C	9	726	1.702	4.256	0.43	0.4	52	31.64
L25D	5	110	1.471	3.949	0.43	2.2	28	35
L27B	5	319	1.189	2.684	0.27	0.8	18	13.33
L27C	6	1397	1.562	4.287	0.37	0.6	28	23.06
L27D	8	792	1.867	5.558	0.43	0.6	34	31
H23B	5	484	1.232	2.729	0.51	1.1	28	26.36
H23C	4	132	1.075	2.426	0.38	3.2	20	28.36
H23D	4	748	1.135	2.749	0.24	1.2	14	10.48
H25B	4	462	0.8461	1.818	0.20	0.7	18	11.05
H25C	6	187	1.543	3.913	0.49	1.1	32	28.67
H25D	7	209	1.767	5.186	0.55	0.9	32	42
H27B	2	33	0.6365	1.846	0.44	4	12	16
H27C	6	374	0.9811	1.799	0.20	0.5	36	18.35
H27D	6	286	1.39	3.341	0.39	1	28	35.08

8.4 Diversity scores per sample at 8-weeks

Sample	Morphospecies richness S	Collembola abundance	Shannon H	Simpson D	FD	MT	QBS	ICQSc
Ct83B	0	0	0	0	0.00	0	0	0
Ct83C	8	814	1.553	3.672	0.36	1	50	61.41
Ct83D	3	297	0.7743	1.823	0.11	5.6	16	22.48
Ct85B	9	528	1.113	1.843	0.23	1.3	54	85.69
Ct85C	7	649	1.712	4.898	0.44	1.2	34	42.82
Ct85D	9	671	1.75	4.384	0.47	1	52	63.16
Ct87B	10	704	1.695	4.164	0.51	0.9	52	69.58
Ct87C	5	1650	0.6621	1.477	0.14	2.8	28	45.23
Ct87D	11	1056	2.163	7.781	0.46	0.4	54	44.55
L83B	3	319	0.7838	1.862	0.26	5	16	19.85
L83C	0	0	0	0	0.00	0	0	0
L83D	7	715	1.342	3.064	0.27	1.7	48	61.6
L85B	7	1551	1.541	3.97	0.36	1.7	40	59.9
L85C	3	55	1.055	2.872	0.30	3.3	16	13.33
L85D	0	0	0	0	0.00	0	0	0
L87B	6	495	1.38	3.363	0.47	1.2	34	44.27
L87C	8	429	1.474	3.288	0.35	1.4	46	68.49
L87D	9	528	1.75	4.512	0.49	0.9	48	55.74
H83B	6	484	1.567	4.2	0.38	1.5	36	37.14
H83C	8	1056	1.543	3.615	0.51	1.2	30	56.95
H83D	8	495	1.659	4.22	0.58	0.9	30	43.44
H85B	11	2222	1.398	2.517	0.33	1	54	100.2
H85C	7	704	1.345	2.797	0.35	1	36	51.19
H85D	5	726	1.295	3.171	0.41	2.1	20	33.57
H87B	6	330	1.155	2.511	0.38	1.9	28	48.57
H87C	3	187	0.6779	1.62	0.13	5.6	14	22.57
H87D	10	847	1.74	3.711	0.40	0.6	48	45