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Hazard assessment of organic wastes: effects on soil microarthropod communities

Dissertação de Mestrado em Ecologia,
orientada pelo Professor Doutor José Paulo Sousa e co-orientada pelo Doutor Henrique Azevedo Pereira,
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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor José Paulo Sousa e Doutor Henrique Pereira (Universidade de Coimbra)

Ana Daniela Cascão Alves

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Abstract

The action of four types of organic wastes with potential interest to be used as agricultural soil amendments from different sites on microarthropod communities, namely of collembola, were studied under field (*in situ*) and Terrestrial Models Ecosystems (TME) conditions. Municipal sewage sludge (SS2), mixed municipal solid waste compost (MMSWC), agricultural wastes compost (AWC) and pig slurry digestate (PSD) were the selected organic wastes. SS2 was applied in three (6, 12, and 24 ton dry matter/ha) and five (6, 12, 24, 40 and 90 ton dry matter/ha) different doses in the field and TME's respectively, while the remaining residues (MMSWC, AWC and PSD) were calculated in order to correspond to the same amount of organic matter (OM) per unit area of SS2. The action of successive applications (two applications within a year range) were studied along a year and a half on field essay and a potential recovery of microarthropods communities after 4 months of application of organic wastes was studied on TME's. Soil microarthropods were sampled with soil cores on field and in TME's and sorted by different groups. Collembolans were identified by morphotyping the collected individuals. Differences in communities were accessed by performing a one-way-ANOVA, diversity indices were calculated, and to compare communities composition between different residues at each sampling period, a PCA/CA with PERMANOVA and Simper was performed; to access the differences of communities over time with a possible recover, a PRC was made. Despite the variation in abundance over the residuals, in general, no significant differences were found, neither by the action of successive residues application, nor by the potential recover of communities. The main conclusion of this study is that the organic wastes applied here, can be used like soil amendments, without affecting soil microarthropods communities by exposure to contaminants from limed soils.

Resumo

Foram estudados os efeitos de quatro tipos de resíduos orgânicos com um potencial interesse no uso de melhoramento do solo na comunidade de microartrópodes, nomeadamente em colêmbolos, em ensaios de campo e em Modelos de Ecossistemas Terrestres (TME’s). Lamas residuais urbanas (SS2), composto da fração orgânica de resíduos sólidos urbanos com recolha indiferenciada (MMSWC), composto de resíduos agrícolas (AWC) e excrementos de porco (PSD) foram os resíduos orgânicos selecionados. O resíduo SS2 foi aplicado em três (6,12 e 24 toneladas/hectare de matéria seca) e cinco (6, 12, 24, 40 e 90 toneladas/hectare de matéria seca) diferentes doses, no ensaio de campo e nos TMEs respetivamente, ao passo que os restantes resíduos (MMSWC, AWC e PSD) foram aplicados em quantidade de maneira a que fosse colocado o mesmo valor de matéria orgânica (MO) que no resíduo SS2. No ensaio de campo foi estudada a ação de aplicações sucessivas dos resíduos ao longo de um ano e meio (duas aplicações com espaçamento de um ano), nos TMEs foi estudada a possível recuperação da comunidade de microartrópodes de solo após 4 meses da aplicação dos resíduos. Os microartrópodes de solo foram recolhidos com cilindros de recolha de solo, tanto no campo como nos TMEs, e posteriormente, foram separados e classificados em diferentes grupos. Os colêmbolos recolhidos foram também identificados por morfotipagem. Para aceder às diferenças das comunidades foi

realizada uma ANOVA de uma via, foram ainda calculados índices de diversidade e, para comparar a composição das comunidades entre os diferentes resíduos em cada tempo de amostragem foi feito um PCA/CA, complementado com Simper e PERMANOVA. Por fim, para aceder ao comportamento das comunidades ao longo do tempo e visualizar uma possível recuperação, foi feito um PRC. Apesar da abundância ter variado entre resíduos, em geral não foram encontradas diferenças significativas das comunidades, nem na aplicação sucessiva de resíduos, nem na sua potencial recuperação. A conclusão geral deste estudo é que o uso dos residuos orgânicos usados neste estudo podem ser usados como corretivos de solo sem que estes afetem as comunidades de microartrópodes pela exposição de contaminantes.

1. Introduction

The Landfill Directive (council directive 1999/31/EC) aims to prevent or reduce negative effects on the environment, including pollution on surface water, groundwater, and soil, caused by organic wastes, requiring a diversion of wastes sent to landfills.

In this scenario, the reuse of these wastes on agricultural soil is an increasingly important management option with some advantages: it is a cheaper alternative that allows a reduction in the use of fertilizers. Furthermore, some studies have shown that there are some benefits in using organic wastes as agricultural soil amendments, like: the improvement in soil fertility by adding nutrients that allow the crop grows and production, the improvement of soil structure due to the incorporation of OM into soil humus, the improvement of water retention capacity, and the reduction of erosion risks (Albiach *et a.l*, 2001; Schowanek *et al*., 2004).

The Directive 86/278/EEC outlined the environmental safety - namely soil protection - in the use of organic residues as soil amendment on agriculture. While organic wastes generally show no adverse effects on soil fertility or biological activity (Debosz *et al*., 2002; Petersen *et al.*, 2003), there are associated risks like heavy metals, the increase of soil salinity, NH_3 emissions, contamination with pathogenic microorganisms and nitrate pollution of ground waters (Alvarenga *et al*., 2015; Düring and Gäth, 2002). Moreover, the European Directive 91/76/EEC (European Council Directive, 1991), which concerns the protection of waters against pollution caused by nitrates from agricultural sources, also limits the use of wastes in soil. This practice can reach different environmental modules, like soil, ground waters and surface waters, and therefore causing major impacts on human health, climate change, biodiversity, and food safety (COM, 2006). Considering this, it is very important to evaluate the potential risks and to perform bioassays using different organic wastes as agricultural soil amendments, since they provide a more truthful response to the overall composition of the matter.

The different organic wastes and their concentration may influence positive or negatively soil organisms due to their toxicity, pathogenic agents, or just by modifying the composition of the soil, because soil organisms, microflora and plants are directly exposed to contaminants in sludge-amended soils.

The suitability of soils for sustainable production of healthy crops and trees depends on the presence of soil fauna - plants, invertebrates and microorganisms have coevolved over several hundred million years within soils - and any change occurring in soil properties is likely to affect them (Lavelle *et al*., 2006). Soil invertebrates have an important role on ecosystem services, improving water and nutrient cycling, production of healthy crops and trees, primary production, and increasing field water holding capacity (Lavelle *et al.*, 2006).

According to their role in the soil processes, organisms can be grouped into “chemical engineers” - includes bacteria and fungi - that are responsible for the decomposition of organic matter into nutrients available to plants, and some bacteria form symbioses with animals, in particular with earthworms, that help in nitrogen recycling; “biological regulators” - comprising protists, nematodes and microarthropods (enchytraeids, mites, and springtails) - which control the abundance of populations in the soil food webs, and can be herbivores, fungal feeders, or predators (Turbé *et al*., 2010). For last, the “soil ecosystem engineers” - includes earthworms, termites, ants, and other macrofauna - have the ability to ensure the process denominated by bioturbation, constructing structures and pores by moving through the soil (Turbé *et al*., 2010). Due to their role in the ecosystem, soil organisms are good bioindicators, i.e., species that due to their importance in the ecosystem and their sensitivity to anthropogenic pressures can give us a quick and cheap access to the quality of soil, in a simple, measurable and quantifiable way (Harrington *et al.,* 2010). Collembola (springtails), small arthropods around 0.2 to 4mm, belonging to mesofauna, are among the most accepted in this role (Parisi *et al.,* 2005, Bispo *et al.,* 2009). They can be found on litter or in the pore space of the upper 10 to 15 cm of soil, being a very diverse taxon (Lavelle and Spain, 2001). Springtails have an important role in nutrient cycling, since they are saprophagous and feed mainly on fungi and bacteria, thus affecting decomposition rates (Jeffery *et al*., 2010). These organisms have been used in ecotoxicological studies as model organisms (ex: Axelsen and Kristensen, 2000; Crouau, 2002; Domene *et al.*2010; Krogh and Pederson, 1997), and have shown to be sensitive to different land use types (Bandyopadhyaya *et al.*, 2002; Sousa, 2006). However, the taxonomy of springtails is not easy, requires taxonomic identification experience, and many specific materials in order to accurately determine the species or even the genus. This problem is common to other organisms, leading to a pursuit for alternative approaches to the traditional taxonomic classification. Parisi (2005) purposed an index of soil quality based on eco-morphological traits of soil microarthropods (QBS- *Qualità Biologica del Suolo*), that has been successfully adapted and used for collembolans, and could give us the possibility to offer with less effort a higher discrimination of the data (Vandewalle *et al.,* 2010).

For this work, the effects of different organic wastes with various concentrations on soil microarthropods communities in the field were assessed. Nevertheless, due to climate variability and other external factors, Terrestrial Model Ecosystems (TME) were used to assess the differences without external/environmental factors. TME's are controlled, reproducible systems that allow simulation of the processes and relations of components in a fraction of terrestrial environment (Sheppard, 1997); and soil communities of springtails showed to be constant within 1 year using this semi-field tool (Scholz-Starke *et al.,* 2013). TME's can be used to evaluate the effects of contaminants in soil, as they allow not only the evaluation of their effects, but also the communities' recovery potential (Scholz-Starke *et al.,* 2013). Additionally, there are several studies using TME's to assess the effects of contaminants in different concentrations, allowing for Ec_x calculations (Förster *et al.*, 2011; Moser *et al.,* 2007; Scholz-Starke *et al*., 2013).This work is integrated in a broader project "ResOrgRisk - Environmental risk assessment of the use of organic residues as soil amendments", PTDC/AAC-AMB/119273/2010, funded by "Fundação para a Ciência e Tecnologia" (FCT). The general objectives of this work are (1) to assess the effects of the application in different concentrations of organic wastes on soil microarthropods (with focus on Collembola communities), using an eco-morphological trait approach to classify the specimens, through field essays with successive applications over a year and a half; (2) to determinate if multiple applications affect the communities positively or negatively; and (3) assess the short and long term effects through semi-field set ups to verify if there is a recovery potential of those soil communities.

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2. Materials and Methods

2.1. Organic wastes

Previous chemical analysis of the organic wastes used in this work was made as described in Alvarenga *et al.*, 2015, including heavy metals concentrations, organic contaminants concentrations, and pathogenic microorganisms concentrations. After that, four residues were selected (table II.1), those which do not have much toxicity, but exhibit metals and also with results that are not as good as there are no doubts about their use, and not as bad as for their use to be considered completely unsuited.

Table II.1- Organic wastes used in this study and their respective description (Alvarenga *et al.*, 2015): (1) organic wastes used in the field experiment and in TME's, (2) organic wastes used only in the field experiment, (3) organic wastes used in TME's only.

Treatments	Description
SS2 (1)	-Untreated dewatered municipal sewage sludge; -From a small village in Alentejo region; -Mechanically dehydrated by centrifugation -15% dewatered matter content
MMSWC (1)	-Mixed municipal solid waste compost; -From Setúbal; -Mechanically segregated and biologically treated; -Applied on vineyards in Alentejo region
AWC (2)	-Agricultural compost; -From Serpa (Alentejo region); -61% of sheep manure; -21% of olive mill waste; -10%of olive leaves; -8% of meat flour; -Used in soils of the farm
PSD (3)	-Pig slurry from several pig farms; -From Serra de Aires e Candeeiros, in Ribatejo region; -Treated by anaerobic digestion; -Dehydrated and stabilized over time

2.2. Experimental procedure

2.2.1. Field experiment

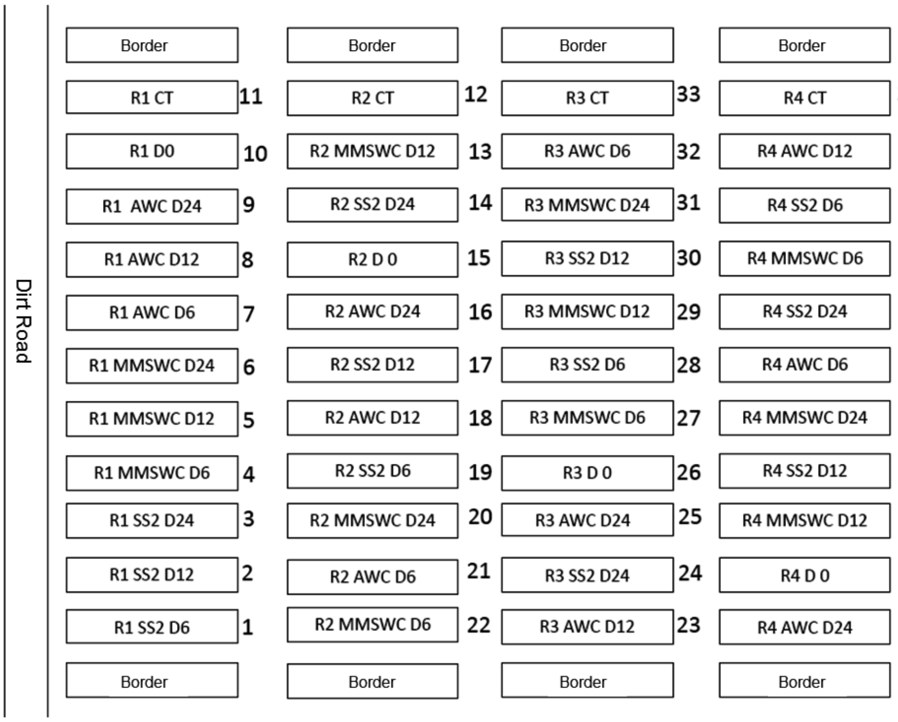
2.2.1.1. Experimental design

To the field experiment, three of four residuals of this study were used (SS2, MMSWC and AWC). The field assay was performed on nearby Beja (N 38 01.704 ; W 7 52.210).

Due to differences in organic matter content of different residues and to the fact that organic matter amount can affect soil communities (Axelsen, 2000), , SS2 was applied in three (6, 12, and 24 ton dry matter/ha) different doses, while the remaining residues (MMSWC and AWC) were calculated in order to correspond to the same amount of organic matter (OM) per unit area of SS2 which is the residue with an higher OM percentage (74.3±0.1). The different residues and its different doses were applied with four replicates each, and compared with two controls (CT and D0) also with four replicates each. Both controls didn't have residues, but one of them was plowed, D0 and it will be the main control in field assay.

In order to have a better view of the results, the codes of residues doses will be D6, D12 and D24, that is, the concentration of ton dry matter/ha corresponding to SS2.

Summarizing, nine treatments (SS2 D6, SS2 D12, SS2 D24, MMSWC D6, MMSWC D12, MMSWC D24, AWC D6, AWC D12 and AWC D24) and two controls (D0 and CT) were studied. The field experimental design is presented in Figure II.1.



Border		Border		Border		Border	
R1 CT	11	R2 CT	12	R3 CT	33	R4 CT	34
R1 D0	10	R2 MMSWC D12	13	R3 AWC D6	32	R4 AWC D12	35
R1 AWC D24	9	R2 SS2 D24	14	R3 MMSWC D24	31	R4 SS2 D6	36
R1 AWC D12	8	R2 D 0	15	R3 SS2 D12	30	R4 MMSWC D6	37
R1 AWC D6	7	R2 AWC D24	16	R3 MMSWC D12	29	R4 SS2 D24	38
R1 MMSWC D24	6	R2 SS2 D12	17	R3 SS2 D6	28	R4 AWC D6	39
R1 MMSWC D12	5	R2 AWC D12	18	R3 MMSWC D6	27	R4 MMSWC D24	40
R1 MMSWC D6	4	R2 SS2 D6	19	R3 D 0	26	R4 SS2 D12	41
R1 SS2 D24	3	R2 MMSWC D24	20	R3 AWC D24	25	R4 MMSWC D12	42
R1 SS2 D12	2	R2 AWC D6	21	R3 SS2 D24	24	R4 D 0	43
R1 SS2 D6	1	R2 MMSWC D6	22	R3 AWC D12	23	R4 AWC D24	44
Border		Border		Border		Border	

Figure II.1: Field experimental design.

2.2.1.2. Sampling

Soil samples were taken with a core sampler (5cm diameter), that removes the first 5 cm of the soil layer (ISO, 2005); and then transferred directly into individual plastic bags. Sampling took place throughout a year and a half (since 2013 October until 2015 April) and the samples were collected in four sampling times: 4 weeks after the first application of organic wastes, on the same day the seeding was done, in November 2013 (T1); the second sampling was made in March 2014 (T2). A new application of the treatments was made and after four weeks a new sampling was done (T3, October 2014); the last sampling took place in April 2015 (T4). A total of 176 samples were collected - 3 soil cores were taken at each plot (Figure II.2).

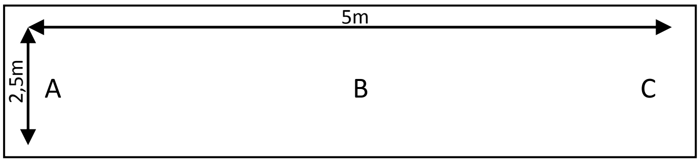


Figure II.2- Sampling design in each plot; A, B and C represent the core sampling.

2.2.2. TME's

2.2.2.1. Experimental design

In the TME experiment, three of four residuals of this study were used (SS2, MMSWC and PSD). The TME's are intact soil core with 40 cm deep and 16,5 cm of diameter. A total of 96 TME's were collected and placed in the TME carts that are within the temperature 10 to 15 degrees to simulate the temperatures that exist below the soil surface. The external temperature and humidity have remained relatively constant, averaging 23.92±1,2ºC and 51.6±7,1% of relative humidity, respectively. This experiment took place from January to April.

Due to differences in organic matter content of different residues and to the fact that organic matter amount affect soil communities (Axelsen, 2000), SS2 was applied in five (6, 12, 24, 40 and 90 ton dry matter/ha) different doses, while the remaining residues (MMSWC and PSD) were calculated in order to correspond to the same amount of organic matter (OM) per unit area of SS2, that is the residue with an higher OM percentage (74.3±0.1).

In order to have a better view of the results, the codes of residues doses will be D6, D12, D24, D40 and D90, that is, the concentration of ton dry matter/ha corresponding to SS2.

The different residues and its different doses were applied with three replicates each, and compared with a control (CT) without a treatment (but it was plowed) also with three replicates.

Summarizing, 15 treatments (SS2 D6, SS2 D12, SS2 D24, SS2 D40, SS2 D90, MMSWC D6, MMSWC D12, MMSWC D24, MMSWC D40, MMSWC D90, PSD D6, PSD D12, PSD D24, PSD D40, PSD D90) and one control (CT), were studied.

2.2.2.2. Sampling

TME's were collected on land in Coimbra, near to the *Faculdade de Ciências e Tecnologias da Universidade de Coimbra* at coordinates 40º10'59.8"N 8º24'57.7"W, and a relatively small area in the field was used, in order to have the lowest variability possible. A three week acclimatization period to the laboratorial conditions was done before the incorporation of the organic wastes (January). The first sampling period took place four weeks (a month) after the application of the residuals (T1-February), and the second, three months after the application (T2- April) collecting 48 samples at each time. A total of 96 samples were collected and the sampling method was destructive.

Each treatment was replicated in 3 soil cores, taken from each TME plot. A core sampler (5cm diameter) was used to remove the first 5 cm of the soil layer (ISO, 2005); the soil samples were transferred directly into individual plastic bags.

2.3. Extraction and sorting of microarthropods

All the collected soil samples, from the field and from the TME'S, were processed under the same conditions: the samples were taken to the laboratory within a short time after collection, at University of Coimbra, where soil microarthropods were extracted following ISO (2005) protocol, during 7 days the samples were placed in a MacFadyen extractor at 45ºC. At the end of the extraction, samples were sorted and soil microarthropods were separated in different groups. Collembolans were further identified and classified in morphotypes.

2.4. Collembola classification into morphotypes

Collembolans were morphotyped using an adaptation from the classification described in Vandewalle et al. (2010). A score was given to each specimen according a combination of five morphological traits (from 0 to 4): presence/absence of ocelli, antennae size, furca development, presence/absence of scales and hairs and pigmentation (Martins da Silva *et al.*, 2015). Each morphotype corresponds to a different combination of individual scores, and higher scores mean that the organisms are more adapted to live below the soil surface/in the soil, possessing lower dispersal ability (edaphic); those who are more adapted to live in the soil surface (epigeous) have a lower score and higher dispersal ability. Intermediate scores belonged to Hemi-edaphic individuals. Scores may range from 0 (morphotype 00000) to 20 (morphotype 44444).

Table II.2- Morphotypes present in the field study and respective scores.

	Morphotype	Score
Ep1	m02000	2
Ep2	m02002	4
Ep3	m04000	4
Ep4	m04002	6
Ep5	m02040	6
Ep6	m04004	8
Ep7	m04040	8
Ep8	m02042	8
He1	m02044	10
He2	m04042	10
He3	m44004	12
He4	m04242	12
Ed1	m04442	14
Ed2	m04244	14
Ed3	m44044	16
Ed4	m44244	18
Ed5	m44444	20

Table II.3- Morphotypes present in TME's study and respective scores.

	Morphotype	Score
Ep1	m02000	2
Ep2	m04002	6
Ep3	m02042	8
Ep4	m04004	8
He1	m04042	10
He2	m04242	12
He3	m44004	12
Ed1	m04442	14
Ed2	m42044	14
Ed3	m44044	16
Ed4	m44244	18
Ed5	m44444	20

2.5. Statistical analysis

2.5.1. Diversity descriptors

The biodiversity indices (Margalef, Shannon-Wiener, and Pielou (Magurran, 2004)) were calculated for each sampling time, both for Collembola and mesofauna data, using PRIMER 5. For these calculations, in Collembola data, each morphotype was considered a “species”; for mesofauna data, each group was considered like “species” too. Before putting the data in the program, they were treated by adding replicas belonging to the same type of residues.

2.5.2. Collembola Functional diversity

Using the scores attributed to Collembolans as traits, functional diversity (FD) and mean trait value (mT) were calculated for each sampling time, using the “FD” package in R 3.2.2. Once again, before putting the data in the R program, they were treated by adding replicas belonging to the same type of residues.

For TME’s data of Collembola, in T2, SS2 D40 was excluded from the analysis due to the absence of collembolans.

2.5.3. Comparison of communities abundance (and richness in Collembolans) between different concentrations of each residue at each sampling time.

To assess if there were significant differences in Collembola richness and abundance, and in mesofauna abundance, for the same kind of organic waste but at different concentrations, a one way ANOVA was performed, after verifying that there was no variation of homocedascity (Bartlett's test). When variation was detected, data was log transformed (log (x+1)). Both analysis were made in STATISTICA 7.

2.5.4. Comparison of community composition between different residues at each sampling time

2.5.4.1. Field

To assess the differences between treatments in the same sampling time, a Principal Components Analysis (PCA) or a Correspondence Analysis (CA) were used. First, a DCA (Detrend Correspondence Analysis) was performed to test if data had a linear or unimodal distribution. This was made for each sampling period, and for both Collembola and mesofauna data. If the length of the gradient on axis 1 was lower than 3, it was considered that the data had a linear response, and a PCA was chosen for the remaining analysis; if it was higher than 3, it was considered that the data had a unimodal distribution, so CA was used instead. CA was always used for Collembola data, except in T4. Mesofauna data had a linear response, so a PCA was always chosen. All tests were performed using Canoco for Windows 4.5.

To further explore the data, a PERMANOVA with main-test and Bray-Curtis coefficient was done to see if there were differences between treatments; and, if p value was significant ($p<0.05$) i.e, if there were differences, a PERMANOVA with "pair wise" test was done. In addition a Simper was performed to verify which species (morphotypes) or orders were more influenced by each treatment, allowing to access the dissimilarity between treatments. Both analyses were done using PRIMER 6 & PERMANOVA+ and samples without individuals were excluded due to the program.

2.5.4.2. TME's

In the TME study, an analysis between each type of organic waste at different concentrations was made. As in the analysis of the field experiment data, a PERMANOVA with main-test and Bray-Curtis coefficient was done. In addition, a Simper was performed to show which species (morphotypes) or groups were more influenced by each treatment, allowing the assessment of the similarity between treatments. Lastly, PCoA was used to see which morphotypes were more affected in each treatment. All analyses were done using PRIMER 6, and samples without individuals were excluded in order for the program to run.

2.5.5. Comparison of community composition on each residue over time

The effects of each organic waste on soil communities over time were assessed by multivariate analysis, using Principal Response Curves (PRC) (Van den Brink and Ter Braak, 1999).

This method is particularly appropriate when the focal point is checking the changes observed over time in the communities of each treatment in comparison to the community in the control treatment; due to this, every score of treatment *d* at time *t* has a cdt response pattern (Cdt = RegCoef*TAU/SD), coefficients were plotted for each respective time point, the resulting PRC diagram displays a curve of the community. In the field experiment, T4 data was not included in this analysis, to complete the one-year cicle (2013 October to 2014 November).

A PRC was done for each type of organic waste and respective concentrations for both data (Collembola and mesofauna), but only PRC with a significative *p* value is showed. PRC analysis were done using Canoco 4.5, and Cdt was calculated in an excel file.

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3. Results

3.1. Field Results

3.1.1. Richness and abundance (and ANOVA's results)

3.1.1.1. Mesofauna

In this study, a total of 35697 soil organisms were collected and 23 different groups were identified in the four sampling periods. There was a clear dominance of the group Acari with a total of 29873 individuals collected, followed by Collembola with 4616 organisms.

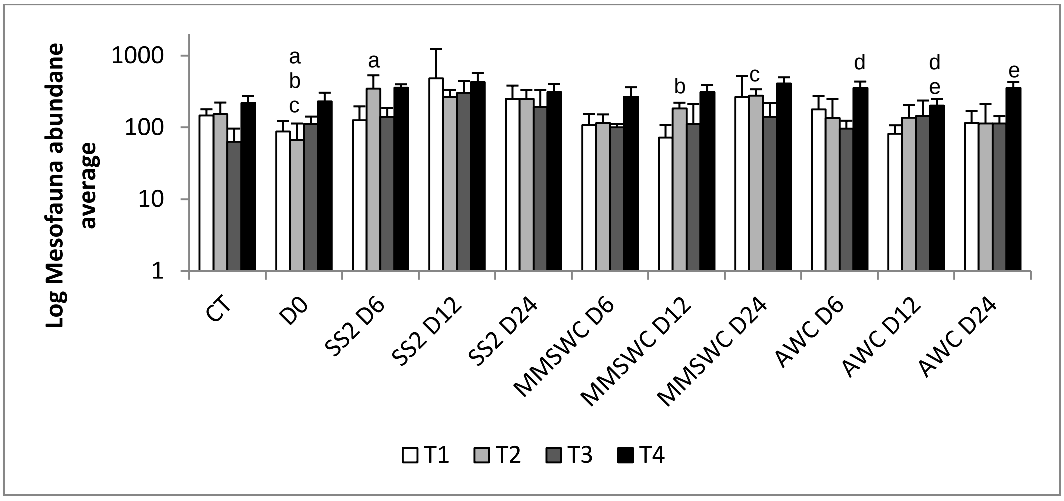


Figure III.1- Average (and standard deviation – vertical lines) in Log scale abundance of all organisms for each treatment and sampling period in the field work. Similar letters on the top of the bars are the treatments which there are significant differences. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; AWC: agricultural wastes compost.

Higher mesofauna abundance was found in T4, followed by T2. Both samplings took place in the same season in two consecutive years (figure III.1).

A one way ANOVA (annex table V.I) followed by a Tukey's test (Zar, 1996) was done to assess the differences between treatments, showing that in T2 presents significant differences between SS2 D0 and SS2 D6 ($p<0.05$), whilst

D6 is the treatment with a higher abundance, and D0 the treatment with lower abundance. In MMSWC T2, D0 is significantly different from MMSWC D12 ($p<0.05$) and MMSWC D24 ($p<0.01$). AWC T4 shows differences between D6 and D12 ($p<0.05$), and between D12 and D24 ($p<0.05$).

3.1.1.2. Collembola

In the field experiment, a total of 4616 Collembolans were collected and 17 morphotypes were identified at the four sampling periods. There was a clear dominance of the morphotype m04042 with 2710 individuals collected. On the other hand, m02040, m04040, and m02044 had only 1 representative individual each. The highest abundance was found at T4, while T1 had the lowest abundance (table III.1).

Table III.1- Collembola abundance for each treatment and sampling period.

	T1	**T2**	**T3**	**T4**
Ct	39	22	18	127
D0	9	12	52	227
SS2 D6	41	37	129	447
SS2 D12	17	50	281	304
SS2 D24	83	142	87	183
MMSWC D6	17	19	27	282
MMSWC D12	2	51	106	371
MMSWC D24	19	85	92	350
AWC D6	14	37	4	237
AWC D12	5	28	84	148
AWC D24	8	48	77	198

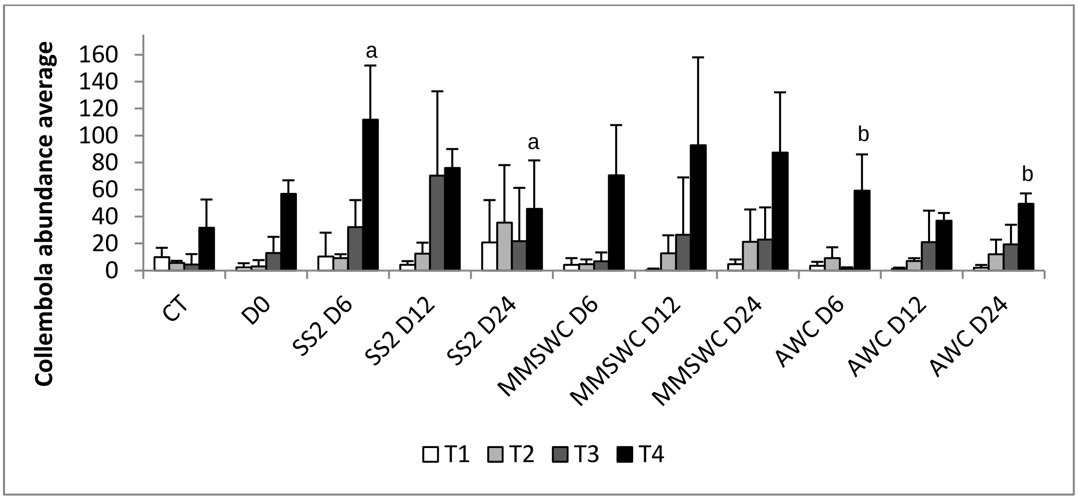


Figure III.2- Average (+ standard deviation) abundance of individuals for each treatment and sampling period in the field work. Similar letters on the top of the bars are the treatments which there are significant differences. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; AWC: agricultural wastes compost.

After performing a One Way ANOVA (Annex Table V.2) followed by a Tukey's test, it revealed that at T4, in SS2 D6 is significant different from SS2 D24 ($p<0.05$), and that SS2 D6 has a higher Collembola abundance; AWC (T3) has significant differences between these same doses ($p<0.05$), but in this case, AWC D24 has a larger abundance than AWC D6.

As in mesofauna data, higher Collembola abundance was found in T4 (figure III.2).

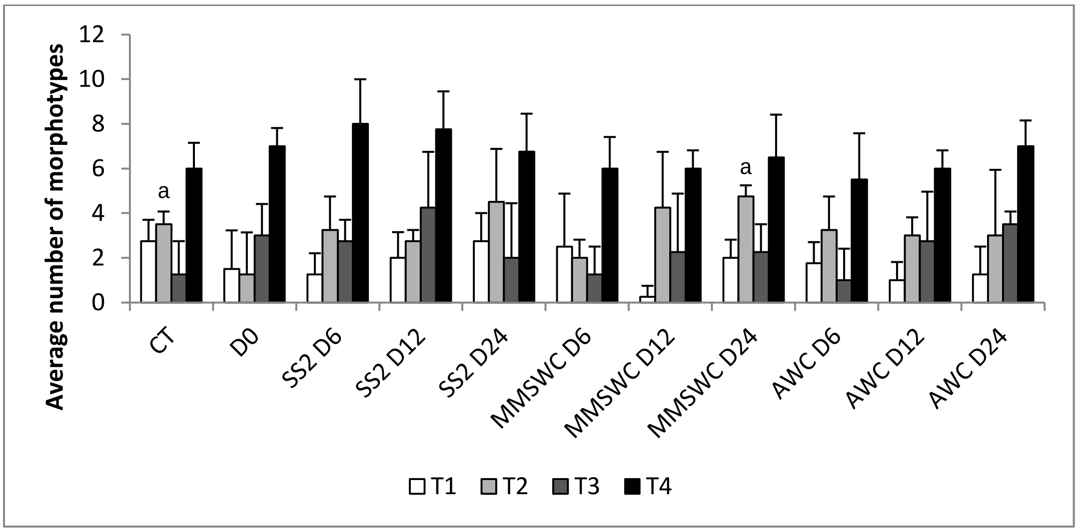


Figure III.3- Average number (+ standard deviation) of morphotypes for each treatment and sampling period in the field work. Similar letters on the top of the bars are the treatments which there are significant differences. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; AWC: agricultural wastes compost.

For morphotype richness, one way ANOVA (Annex Table V.3) reveals differences in MMSWC (T2) between D0 and MMSWC D24 ($p<0.05$), whilst MMSWC D24 has a higher number of morphotypes present.

A higher number of morphotypes were identified in T4, as shown in Figure III.3. T2 follows T4 on the number of morphotypes found (both sampling periods took place in the same season of two consecutive years).

In general, for both Collembola and mesofauna, the sampling periods with more significant differences were T2 (March 2014) and T4 (April 2015), both done 5/6 months after the application of the organic wastes.

MMSWC was the organic waste with more differences when compared to D0: in mesofauna abundance at T2, D0 showed differences with MMSWC D12 and MMSWC D24, and in Collembola richness there were significant differences between D0 and MMSWC D24.

3.1.2. Diversity descriptors

The diversity indices Pielou, Margalef, Shannon - and, for Collembolans, mean-trait value (mT) and functional diversity (FD) - are expressed in tables III.2 and III.3.

3.1.2.1. Mesofauna

Table III.2- Mesofauna diversity indices by Time: **T1**- October 2013; **T2**- March 2014; **T3**- November 2014; **T4**- April 2015. S-W- Shannon Wiener index The highest values are presented in red and the lowest in green. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; AWC: agricultural wastes compost.

	Indices	**Ct**	**D0**	**SS2 D6**	**SS2 D12**	**SS2 D24**	**MMSWC D6**	**MMSWC D12**	**MMSWC D24**	**AWC D6**	**AWC D12**	**AWC D24**
T1	**S-W**	0,35	0,21	0,67	0,28	0,52	0,27	0,20	0,14	0,14	0,10	0,19
	Margalef	0,94	0,68	0,64	0,66	0,87	0,82	1,24	0,72	0,61	0,35	0,98
	Pielou	0,18	0,13	0,42	0,16	0,27	0,15	0,09	0,08	0,08	0,09	0,10
T2	**S-W**	0,26	0,45	0,22	0,39	0,73	0,20	0,31	0,31	0,34	0,26	0,37
	Margalef	0,78	0,90	1,11	0,57	0,72	0,49	0,76	1,00	0,79	0,48	0,49
	Pielou	0,15	0,25	0,10	0,24	0,40	0,15	0,17	0,15	0,19	0,19	0,27
T3	**S-W**	0,48	0,49	0,64	0,65	0,46	0,50	0,66	0,53	0,17	0,51	0,57
	Margalef	1,08	1,15	1,11	1,27	1,05	1,67	0,82	0,95	0,84	0,94	0,82
	Pielou	0,25	0,24	0,31	0,28	0,22	0,21	0,37	0,27	0,10	0,26	0,32
T4	**S-W**	0,52	0,70	0,76	0,76	0,63	0,71	0,72	0,80	0,87	0,60	0,59
	Margalef	1,18	0,88	0,96	1,21	1,12	1,72	1,12	1,62	0,96	0,90	1,52
	Pielou	0,23	0,36	0,36	0,33	0,29	0,28	0,33	0,31	0,42	0,31	0,24

Generally, AWC had the lowest values of diversity indices in all sampling times (table III.2).

3.1.2.2. Collembola

Table III.3- Diversity descriptors for each sampling period. **FD**- functional diversity; **mT**- mean trait value. **T1**- October 2013; **T2**- March 2014; **T3**- November 2014; **T4**- April 2015; S-W: Shannon Wiener index The highest values are presented in red and the lowest in green.

	Indices	Ct	D0	SS2 D6	SS2 D12	SS2 D24	MMSWC D6	MMSWC D12	MMSWC D24	AWC D6	AWC D12	AWC D24
T1	**S-W**	1,58	1,52	0,23	1,20	0,79	1,71	0,00	1,44	1,63	1,33	1,21
	Margalef	1,36	1,82	0,54	1,41	1,36	2,12	0,00	1,70	1,89	1,86	1,44
	Pielou	0,88	0,95	0,21	0,75	0,40	0,88	ND	0,80	0,91	0,96	0,88
	FD	1,27	0,22	0,03	0,39	0,19	0,25	0,00	1,04	0,26	0,30	0,63
	mT	10,26	6,67	9,80	9,76	11,33	7,29	20,00	9,79	5,57	6,40	11,00
T2	**S-W**	1,98	1,27	1,26	1,64	1,17	1,11	2,01	1,70	1,88	1,84	1,23
	Margalef	2,26	1,21	1,38	1,79	1,61	1,02	2,54	1,80	2,22	2,10	1,55
	Pielou	0,95	0,91	0,70	0,79	0,53	0,80	0,84	0,77	0,86	0,88	0,63
	FD	0,76	0,49	0,61	0,38	0,28	0,19	1,03	0,47	0,68	0,80	0,43
	mT	14,27	10,33	9,35	11,92	12,35	8,42	9,61	10,21	9,78	12,57	10,96
T3	**S-W**	0,90	1,10	0,49	0,56	0,66	0,42	1,13	0,78	1,39	1,04	0,75
	Margalef	0,69	1,52	1,23	1,42	1,34	0,61	1,07	1,11	2,16	1,35	1,38
	Pielou	0,82	0,56	0,25	0,26	0,34	0,38	0,63	0,44	1,00	0,53	0,39
	FD	0,59	0,22	0,07	0,16	0,07	0,14	0,54	0,28	0,40	0,26	0,18
	mT	9,22	9,04	9,80	9,77	10,09	9,48	11,32	10,33	11,00	10,86	10,47
T4	**S-W**	1,68	1,77	1,17	1,14	1,51	1,39	1,14	0,84	1,24	1,46	1,48
	Margalef	2,06	1,47	1,80	1,92	1,92	1,42	1,69	1,71	1,46	1,60	2,08
	Pielou	0,70	0,81	0,47	0,46	0,63	0,63	0,48	0,35	0,56	0,67	0,60
	FD	0,55	0,85	0,26	0,22	0,25	0,52	0,38	0,18	0,51	0,76	0,56
	mT	12,36	12,75	10,57	10,28	10,00	12,49	11,36	10,34	11,35	12,35	10,56

Just like in mesofauna (Table III.2), there are some discrepancies between indices on Collembolans (table III.3), but AWC D6 (T3) is the treatment with highest values along with the CT and D0. However it is important to notice this is also the treatment (SS2 D6) with the lowest number of individuals (4) in T3 (but the each of these individuals represent a different morphotype). On the other hand, MMSWC D12 at T1 has the lowest indices values (0, 00) since there were only 2 individuals present and both belong to the same morphotype (m44444).

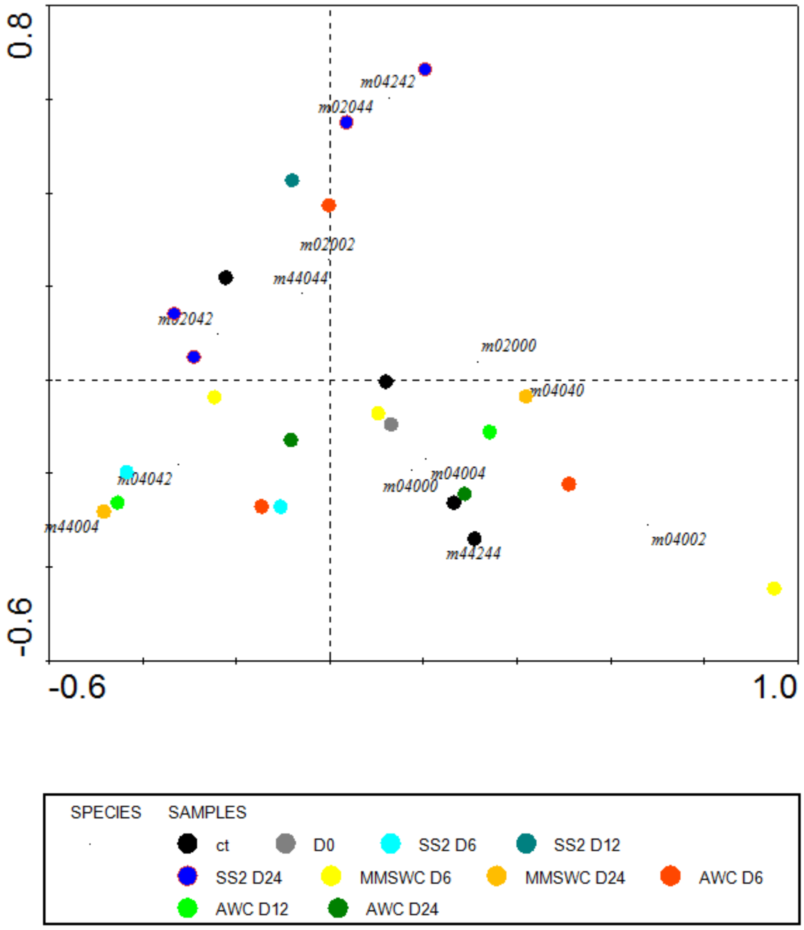
CT shows the highest Functional Diversity (FD) at T1, while the lowest FD value was found at MMSWC D12 (T1) due to the reason previously explained (Table III.3).

3.1.3. Comparison of community composition between different residues at each sampling time

3.1.3.1. Ordination analysis

At T1 on Collembola CA the morphotype m44444 was excluded in the analysis due to being rare in samples, and consequently it was originating a large bias in the diagram (Figure III.4 (A))

(A)



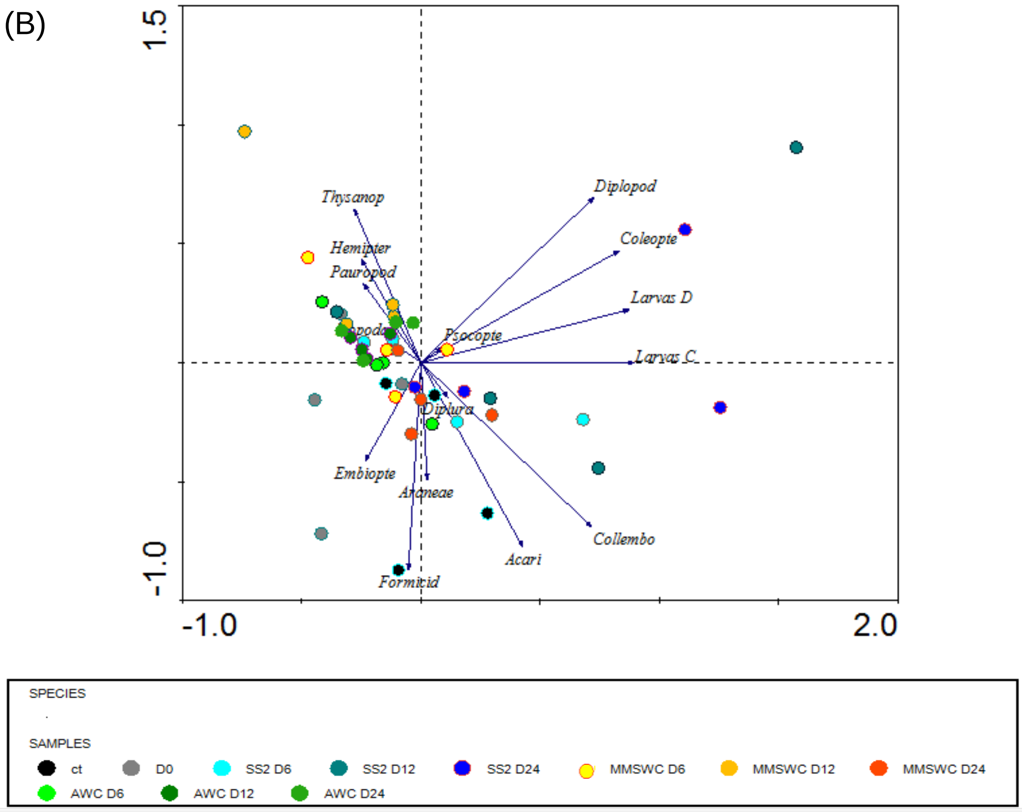


Figure III.4 CA/PCA results at T1 of Collembola (A) and Mesofauna (B) in the field work. PCA was centered by species and data was log transformed. In collembola, axis 1 explains 17.4% of total variation, while axis 2 explain 16.9%. In Mesofauna axis 1 explains 20.2% of total variation, while axis 2 explains 11.5%. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; AWC: agricultural wastes compost.

SS2, either in Collembolans or mesofauna, it was the residue that demonstrates a higher separation from the other residues, and there was a separation in the Collembola plot corresponding to Axis 2 which explains almost as much of the total variation (16.9%) as Axis 1 (17.4%) (Figure III.4(A)).

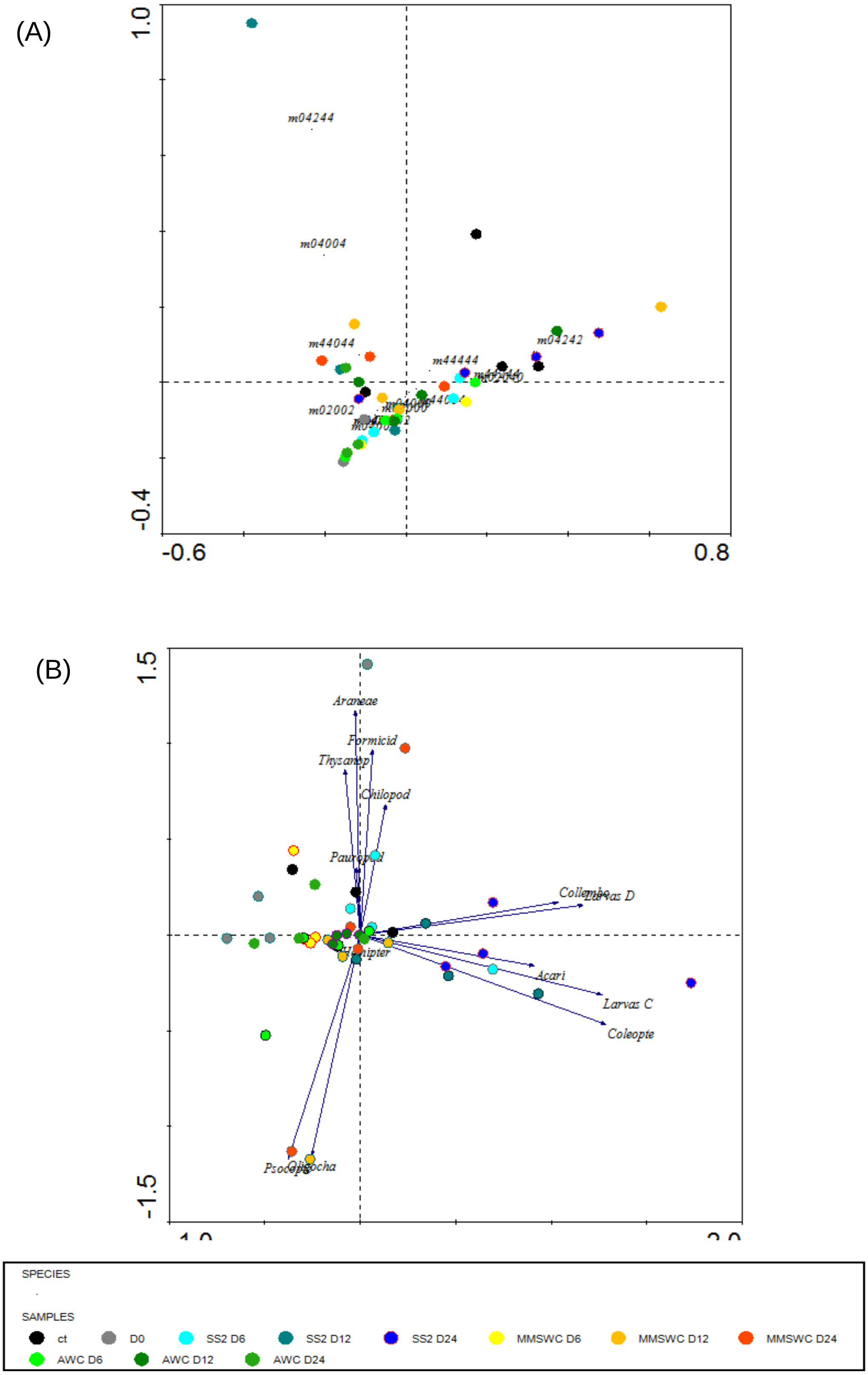
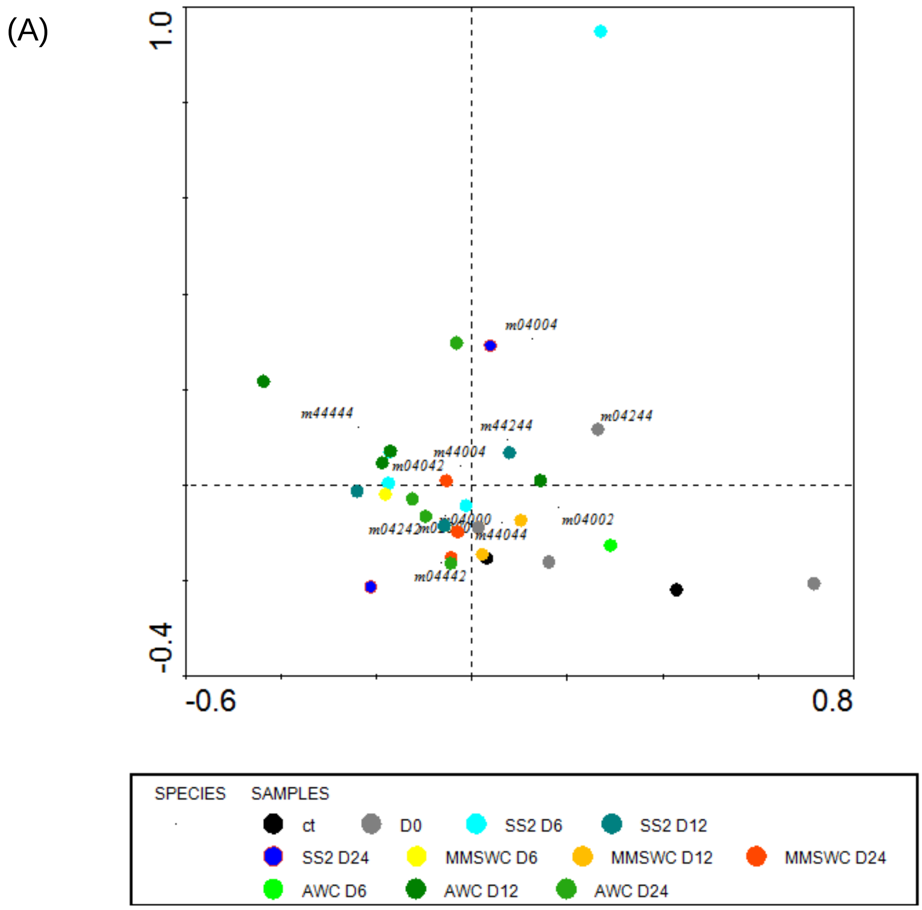


Figure III.5- CA/PCA results at T2 of Collembola (A) and Mesofauna (B) in the field work. PCA was centered by species and data was log transformed. In Collembola, axis 1 explains 18.9% of total variation, while axis 2 explains 15.6%. In Mesofauna axis 1 explains 19.2% of total variation, while axis 2 explains 15.7%. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; AWC: agricultural wastes compost.

In T2 it is noticeable a separation of SS2 residue, like in T1, the separation is mainly done on Axis 1 (Figure III.5).

In T3 on Collembola CA (Figure III.6 (A)) the morphotype m02002 was excluded from the analysis due to the fact that its number of collembolans in samples was scarce (3), and consequently it was originating a large bias in the diagram.



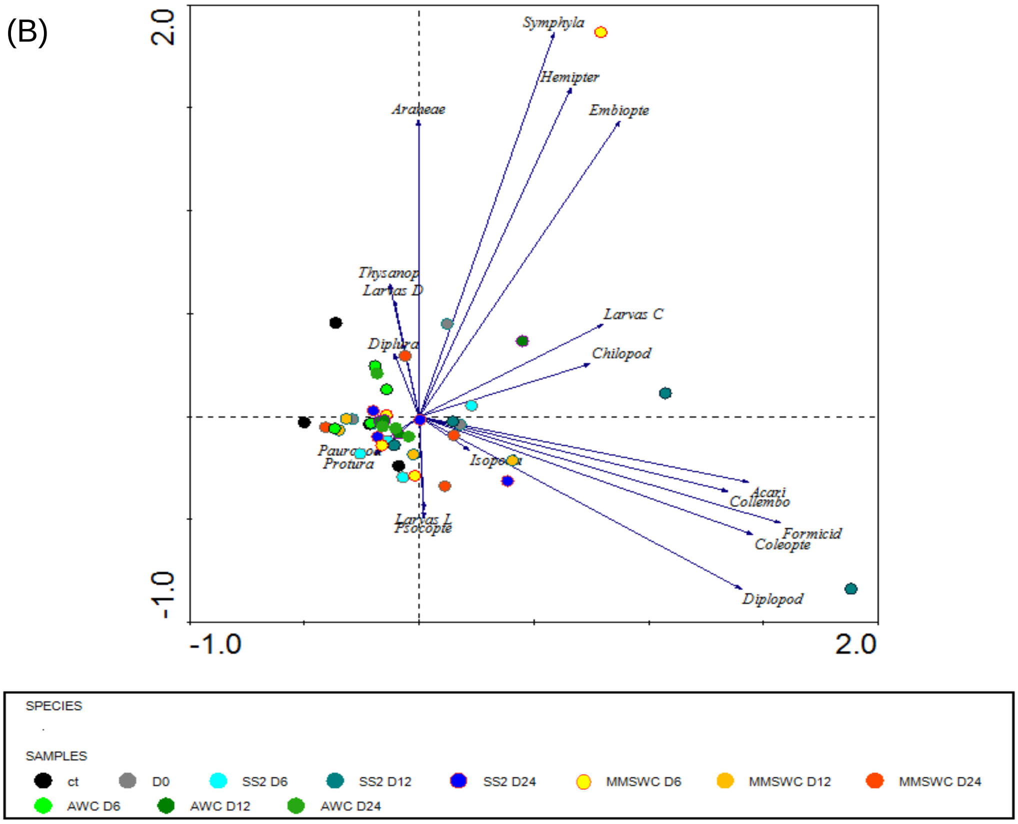


Figure III.6- CA/PCA results at T3 of Collembola (A) and Mesofauna (B) in the field work. PCA was centered by species and data was log transformed. In collembola, axis 1 explains 20.2% of total variation, while axis 2 explains 17.5%. In Mesofauna axis 1 explains 17.1% of total variation, while axis 2 explains 12.8%. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; AWC: agricultural wastes compost.

In T3 it is not noticeable any concrete separation between the different residues, nor in the mesofauna, nor in the Collembolans (Figure III.6).

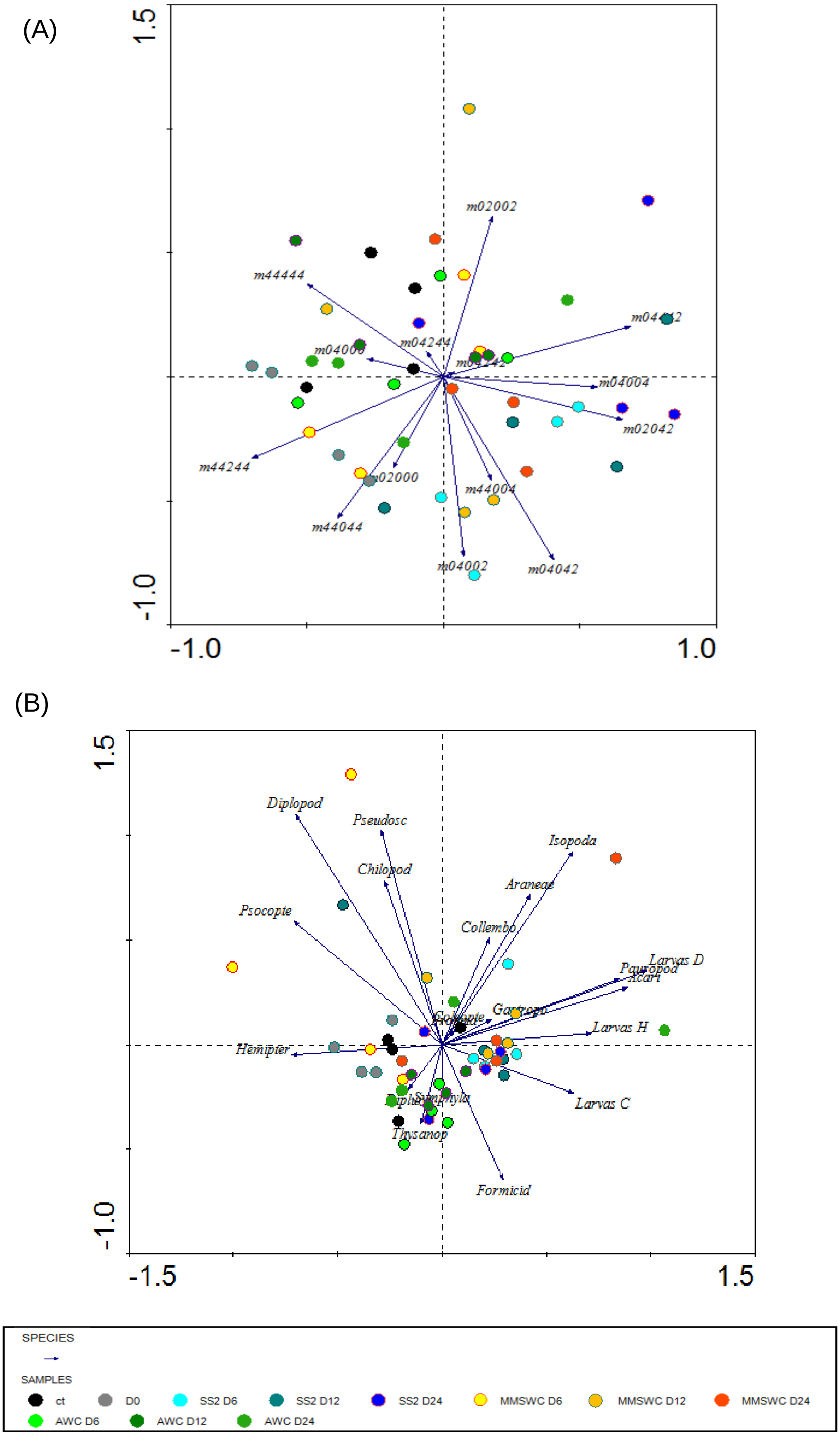


Figure III.7- PCA results at T4 of Collembola (A) and Mesofauna (B) in the field work. PCA was centered by species and data was log transformed. In

Collembola, axis 1 explains 15.8% of total variation, while axis 2 explains 13.6%. In Mesofauna axis 1 explains 12.3% of total variation, while axis 2 explains 10.4%. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; AWC: agricultural wastes compost.

In T4, in the Collembola plot it is possible to see two different groups, the SS2 residue and the AWC residue (Figure III.7).

In general, SS2 and AWC were the organic wastes that appeared separated from the other sludges in almost every period, both for Collembola and mesofauna. SS2 was the sludge that presented a higher detachment from the others and, in general, the species follow it.

3.1.3.2. PERMANOVA/SIMPER

3.1.3.2.1. Mesofauna

PERMANOVA showed significant differences ($p<0.05$) between treatments at T1 ($p=0.004$), T2 ($p=0.001$) and T4 ($p=0.006$). T3 is closely to be significant, presenting a significance of $p=0.077$.

Generally, at the SS2 residue in T1, the group that contributed more to dissimilarity was Coleoptera larvae (average contribution 30.26%), while at MMSWC and AWC residues in the same period, Collembola was the group presenting a higher contribution to dissimilarity between treatments (average contribution of 28.13%) (Annex Table V.5).

Generally in all the residues in T2, the groups that contributed more to dissimilarity were Collembola and Acari (average contribution of 35.44% and 26.50% respectively). (Annex Table V.6).

Generally, in T3, Collembola was the group which had contributed more to dissimilarity between treatments (average contribution of 36.99%) (Annex Table V.7).

In T4, Formicidae and Diptera larvae were the groups that cause more dissimilarity between treatments (average contribution of 24.23 % and 17.04% respectively) (Annex Table V.8).

3.1.3.2.2. Collembola

PERMANOVA showed no significant differences between treatments in none of the sampling periods; however T4 p value is the closest to 0.05 ($p=0.053$).

Generally, in T1, m04042 was the morphotype that contributed more to dissimilarity between treatments (average contribution of 28.43%) (Annex Table V.9).

In T2, m04042 and m04002 were the morphotypes that cause more dissimilarity between treatments. (average contribution of 26.39% and 22.12% respectively) (Annex Table V.10).

In T3, m04042 was the morphotype that contributed more to dissimilarity between treatments (average contribution of 33.98%) (Annex Table V.11) (Annex Table V.11).

In T4, m44444 was the morphotype that cause more dissimilarity between treatments (average contribution of 15.17%) (Annex Table V.12).

Data interpretation must be carefully done because the program (Primer 6 & PERMANOVA+) does not allow the inclusion of empty samples, so it’s possible that more differences can occur.

3.1.4. Response of community composition for each organic waste type over time - Principal Response Curves (PRC)

In the following sections, only the PRC diagrams that showed significant effects and those that were almost significantly of the treatments are presented.

3.1.4.1. Mesofauna

All treatments

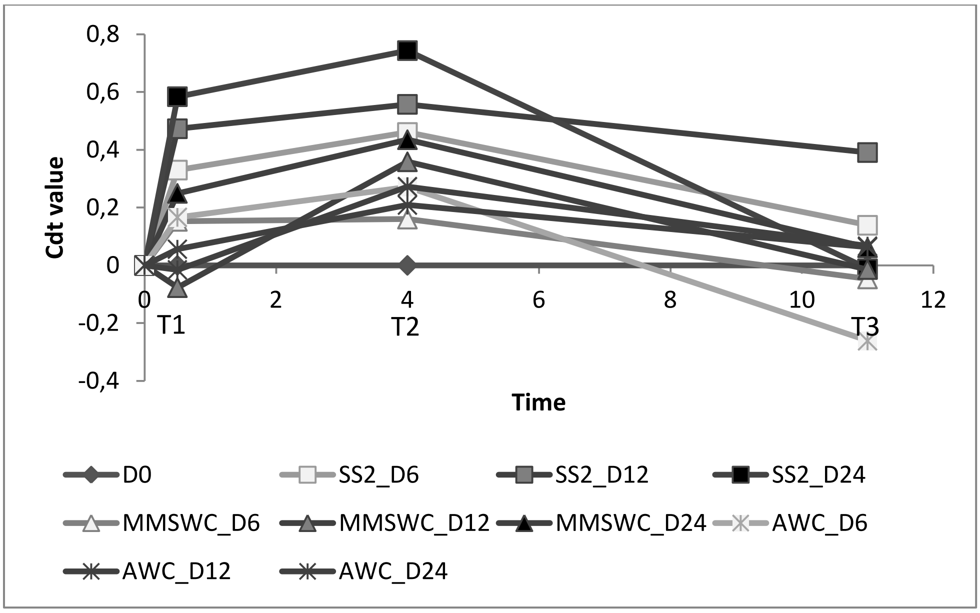


Figure III.8- PRC diagram for the principal component of the field communities when exposed to different organic wastes with different concentrations at three sampling periods. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; AWC: agricultural wastes compost.

The Figure III.8 shows the behavior of the communities on different treatments over time. SS2 D24 was the one that had an higher increase of Cdt value in T2, while in T3 it diminished and there was an approximation to 0.

Of the total variance in the dataset, 7.4% is explained by time, 58% by residuals and 34.6% is explained by treatment (Monte-Carlo test: F=23.93; p=0.0040).

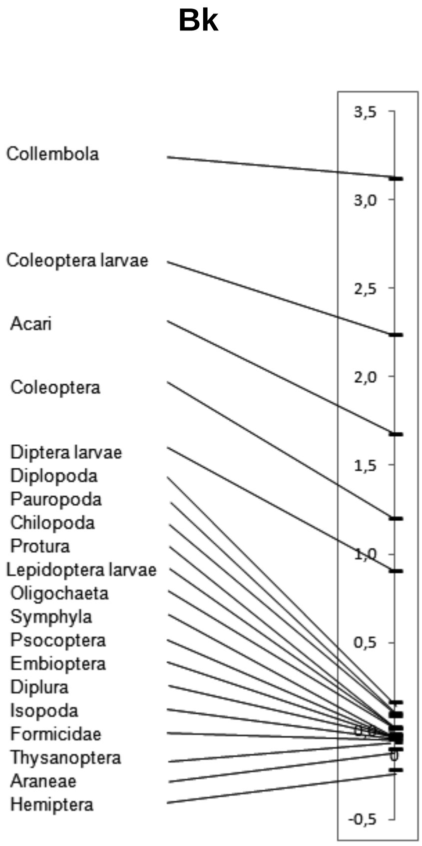


Figure III.9- "Species" weights for the principal component of the field communities when exposed to different residues in different concentrations.

According to the "species" weights (bk) of the PRC (figure III.9), the group that contributed the most to the observed differences was Collembola. Its positive score indicates that they were the most sensitive to the different treatments.

3.1.4.2.. Collembola

AWC

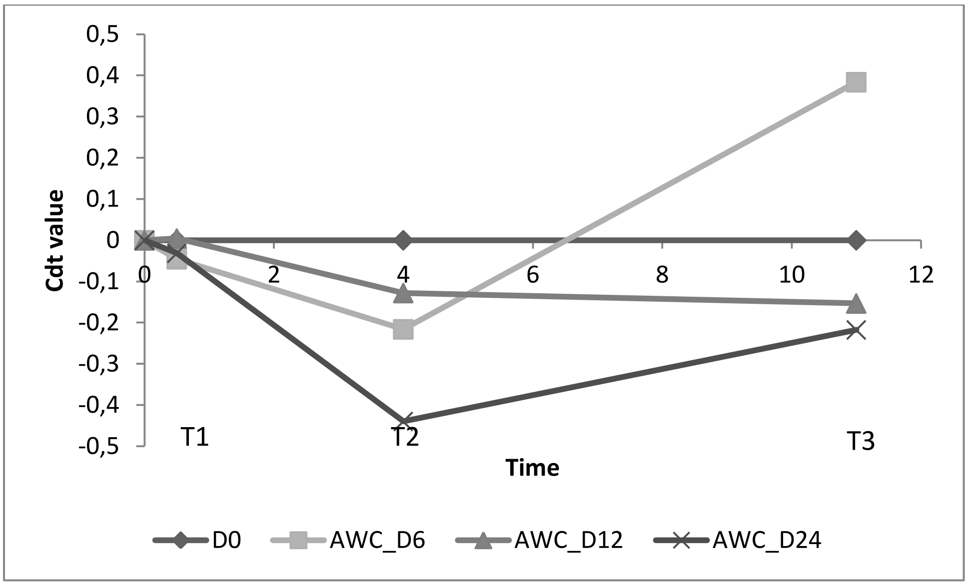


Figure III.10- PRC diagram for the principal component of the field communities when exposed to different concentrations of AWC, considering three sampling periods. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; AWC: agricultural wastes compost.

Of the total variance in the dataset, 70% is explained by time, 59.1% by residuals, and 23.9% by treatment (Monte-Carlo test: F=9.249; p=0.0560).

Figure III.10 shows that, contrary to mesofauna in general, the Collembolans maintained the Cdt value in T1, but decreased the Cdt value in T2.

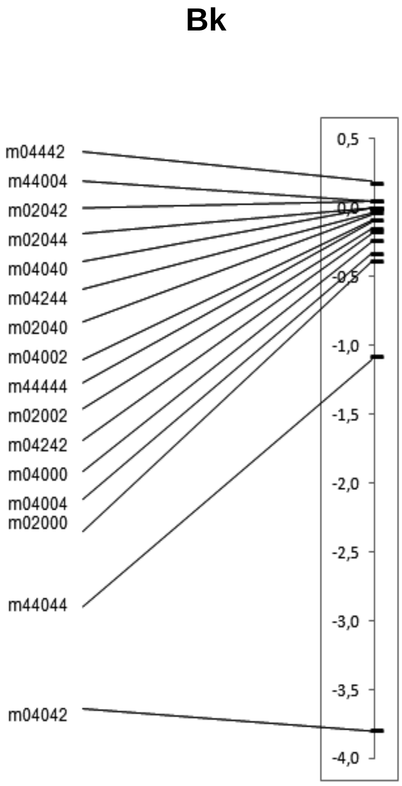


Figure III.11- “Species” weights for the principal component of the field communities when exposed to different AWC concentrations.

According to the “species” weights (bk) of the PRC (figure III.11), the morphotype that contributed the most to the observed differences was m04442. Its positive score indicates that they were the most sensitive to AWC.

3.2. TME Results

3.2.1. Richness and abundance (and ANOVA's results)

3.2.1.1. Mesofauna

In this study, a total of 9378 soil organisms were collected and 7 different groups were identified for both sampling periods. There was a clear dominance of the group Acari with a total of 7605 individuals collected, followed by Collembola with 1505 organisms collected.

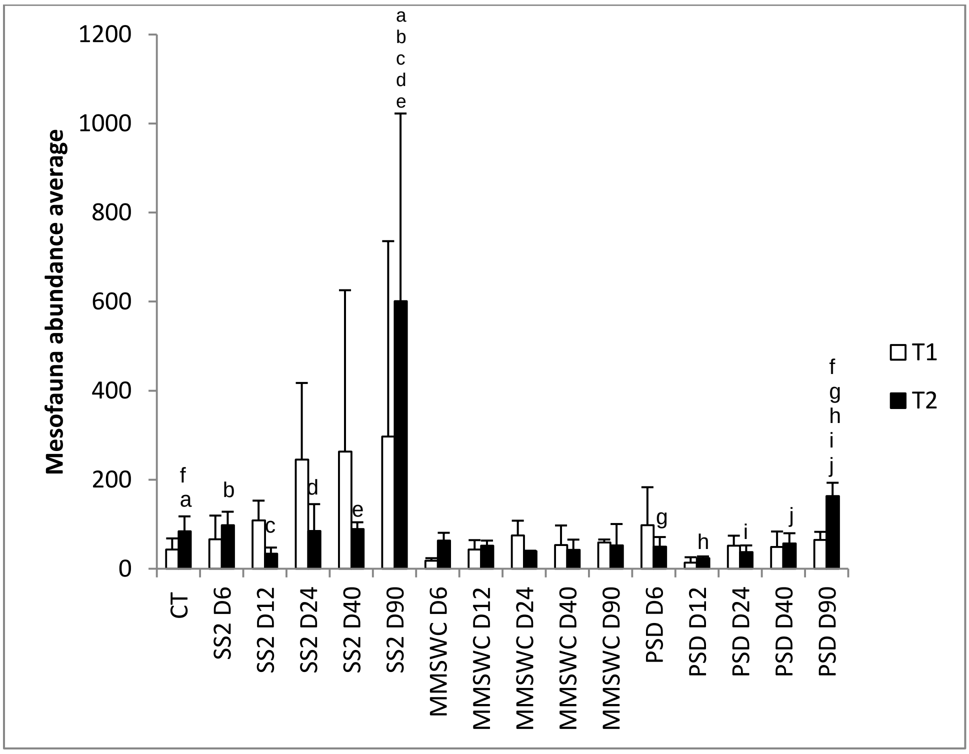


Figure III.12- Average (+ standard deviation) abundance of mesofauna organisms on TME for each treatment and sampling period. Similar letters on the top of the bars are the treatments which there are significant differences. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; PSD: pig slurry digestate.

In microarthropods in general, the greatest abundance was found in SS2 D90 (Figure III.12)

A one way ANOVA (Annex Table V.13) followed by a Tukey's test (Zar, 1996) was performed to assess the differences between treatments, showing that there are significant differences at T2 in SS2 D90 and remaining doses of SS2 and the same to PSD D90, because D90 in both residues (SS2 and PSD) has a significantly higher number of organisms than the others.

3.2.1.2. Collembola

In this study, a total of 1505 Collembolans were collected and 12 morphotypes were identified at the two sampling periods. There was a clear dominance of the morphotype m02024 with a total of 1177 individuals. On the other hand, m02000 and m04442 had only 1 representative individual each.

When comparing the two sampling periods, in terms of abundance, T1 shows the highest number of individuals (Table III.4 and Figure III.13).

Table III.4- Collembola abundance for each treatment and sampling period.

	T1	**T2**
CT	39	6
SS2 D6	2	6
SS2 D12	174	7
SS2 D24	361	28
SS2 D40	27	0
SS2 D90	18	9
MMSWC D6	11	7
MMSWC D12	45	10
MMSWC D24	57	7
MMSWC D40	72	44
MMSWC D90	99	13
PSD D6	1	6
PSD D12	5	11
PSD D24	82	40
PSD D40	24	33
PSD D90	20	4

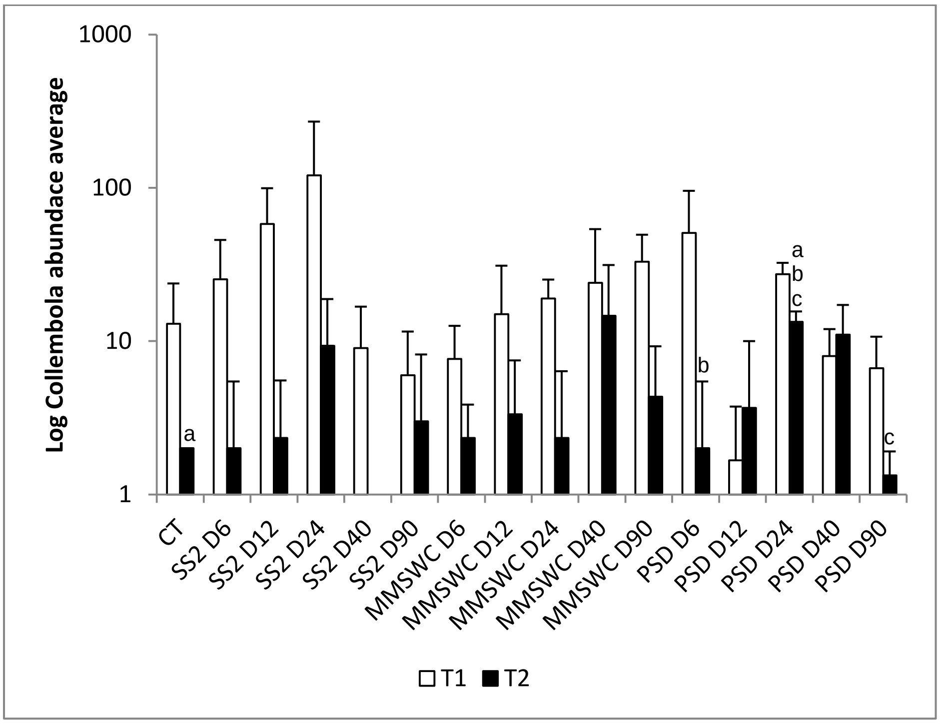


Figure III.13- Average (+ standard deviation) abundance in log scale of collembolans abundance for each treatment and sampling period on TME. Similar letters on the top of the bars are the treatments which there are significant differences. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; PSD: pig slurry digestate.

A One Way ANOVA followed by a Tukey's test (Zar, 1996) was done to assess the differences between treatments, showing that at T2 in PSD organic waste, D24 is significantly different from CT ($p<0.05$), D6 ($p<0.05$), and D90 ($p<0.05$) (Annex Table V.14). D24 has also a higher abundance than CT, D6 and D90.

At T1 (four weeks after the OW application), the application of SS2 seems to result in a notable increase of Collembola abundance, from SS2 D6 to SS2 D24, and then, a large drop in the average number of individuals. In the case of MMSWC, the average number of individuals seems to have a constant pattern. The lowest dose of PSD (D6) seems to cause a large decrease in the number of individuals over time (Figure III.13).

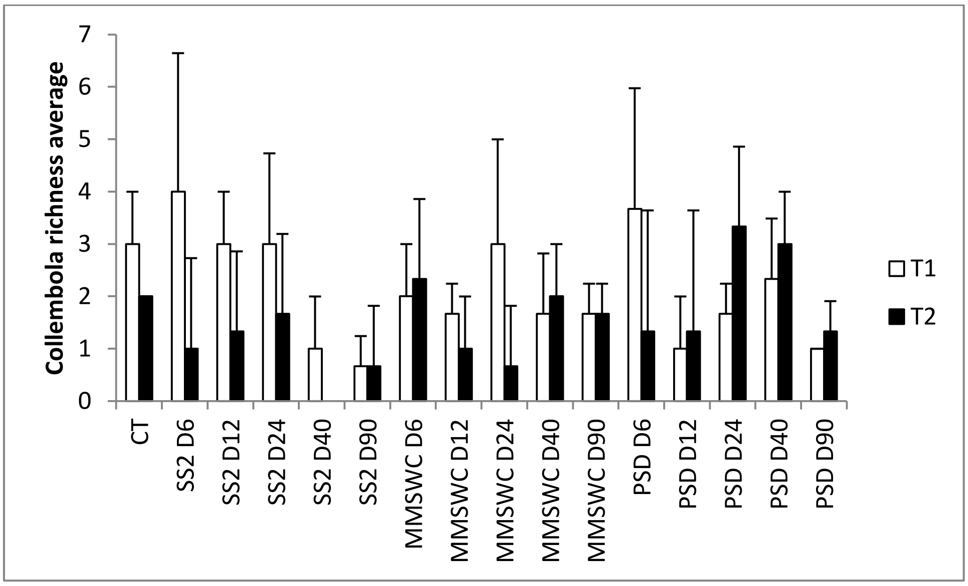


Figure III.14- Average number (+ standard deviation (SD)) richness of collembolans morphotypes for each treatment and sampling period on TME. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; PSD: pig slurry digestate.

The one way ANOVA did not reveal any significant differences in collembola richness between different concentrations of treatments (Annex Table V.15).

3.2.2. Diversity descriptors

The calculated Pielou, Margalef, and Shannon-Wiener diversity indices and in the case of Collembola, mT and FD, are expressed in tables III.5 and III.6.

3.2.2.1. Mesofauna

Table III.5- TME Mesofauna diversity descriptors for each treatment and sampling period.. **T1**- February; **T2**- April. The highest values are presented in red and the lowest in green. **S-W**: Shannon-Wiener. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; PSD: pig slurry digestate.

	Indices	**CT**	**SS2 D6**	**SS2 D12**	**SS2 D24**	**SS2 D40**	**SS2 D90**	**MMSWC D6**	**MMSWC D12**	**MMSWC D24**	**MMSWC D40**	**MMSWC D90**	**PSD D6**	**PSD D12**	**PSD D24**	**PSD D40**	**PSD D90**
T1	**S-W**	0,89	0,74	1,05	0,72	0,15	0,10	0,90	0,73	0,87	0,77	0,77	1,06	0,78	0,76	0,58	0,33
	Margalef	1,03	0,57	1,04	0,30	0,15	0,15	0,75	0,62	1,29	0,39	0,58	1,23	1,08	0,59	0,60	0,19
	Pielou	0,50	0,54	0,54	0,65	0,22	0,14	0,65	0,53	0,42	0,70	0,55	0,51	0,48	0,55	0,42	0,48
T2	**S-W**	0,50	0,21	0,38	0,53	0,00	0,04	0,25	0,68	0,94	0,64	0,77	0,37	0,89	0,95	0,53	0,23
	Margalef	1,08	0,35	0,43	1,08	0,00	0,27	0,57	0,99	1,26	0,21	1,58	1,20	1,17	1,27	0,39	1,13
	Pielou	0,26	0,19	0,34	0,27		0,04	0,18	0,38	0,48	0,93	0,35	0,19	0,50	0,49	0,48	0,11

At both times, lower values of mesofauna diversity were found in SS2 in high concentrations (D24 and D40); on the other hand, higher values of diversity descriptors were founded in MMSWC (Table III.5).

3.2.2.2. Collembola

Table III.6- TME Collembola diversity descriptors for each treatment and sampling period. **FD**- functional diversity; **mT**- mean trait value. **T1**- February; **T2**- April. The highest values are presented in red and the lowest in green. **S-W**: Shannon-Wiener.

	Indices	**CT**	**SS2 D6**	**SS2 D12**	**SS2 D24**	**SS2 D40**	**SS2 D90**	**MMSWC D6**	**MMSWC D12**	**MMSWC D24**	**MMSWC D40**	**MMSWC D90**	**PSD D6**	**PSD D12**	**PSD D24**	**PSD D40**	**PSD D90**
T1	**S-W**	1,09	1,54	0,45	0,14	0,16	0,00	0,73	0,54	0,96	0,15	0,14	1,16	0,67	0,23	0,86	0,00
	Margalef	1,09	1,62	0,97	0,85	0,30	0,00	0,96	0,79	0,99	0,47	0,22	1,19	0,62	0,45	0,63	0,00
	Pielou	0,68	0,74	0,25	0,08	0,23		0,53	0,39	0,60	0,13	0,20	0,59	0,97	0,21	0,78	
	FD	0,62	0,41	0,10	0,04	0,03	0,00	0,31	0,27	0,49	0,03	0,01	0,54	0,04	0,12	0,84	0,00
	mT	11,28	10,58	10,07	10,09	10,15	10,00	9,83	10,62	11,93	10,06	9,94	10,89	9,20	10,20	11,83	10,00
T2	**S-W**	1,10	0,87	0,80	0,59	0,00	0,35	1,55	0,67	0,60	0,91	0,69	1,24	1,24	1,71	1,11	1,04
	Margalef	1,12	1,12	1,03	0,60		0,46	2,06	0,43	0,51	0,79	0,78	1,67	1,25	1,36	1,43	1,44
	Pielou	1,00	0,79	0,72	0,54		0,50	0,96	0,97	0,86	0,65	0,63	0,90	0,89	0,96	0,62	0,95
	FD	0,97	0,68	0,36	0,34		0,01	0,95	0,84	0,03	0,14	0,36	0,89	0,98	0,68	0,41	0,87
	mT	12,67	10,33	14,00	9,07		8,22	11,43	14,00	11,43	9,86	11,69	12,00	13,09	12,25	11,33	12,50

The diversity indices of the collembolans varies a lot between themselves and between treatments (Table III.6). There is no pattern in between the different indices, but like in the mesofauna diversity (Table III.5), it’s clear that SS2 D90 is the treatment with the lowest values in almost all diversity indices, with only a morphotype present at T1, and 2 morphotypes at T2. The organic waste that has higher values is the MMSWC (Table III.6), in accordance with the mesofauna diversity results (Table III.5).

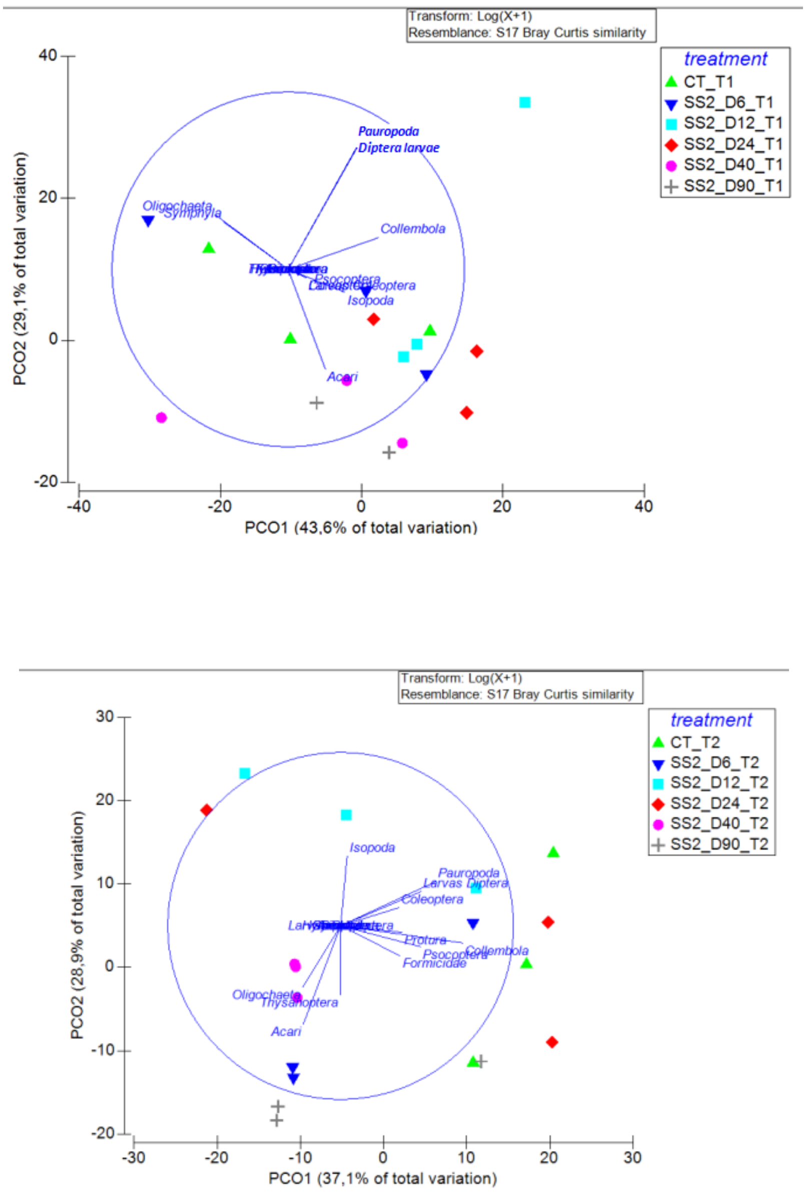
3.2.3. Comparison of communities at different times - PCoA &. PERMANOVA+SIMPER.

3.2.3.1. Ordination analysis

3.2.3.1.1. Mesofauna

SS2

(A)



(B)

Figure III.15- SS2 PCoA (principal coordinates analysis) plots based on log abundance of mesofauna at T1 (A) and T2 (B) on TMEs. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; PSD: pig slurry digestate.

In T2, the SS2 D24 and the SS2 D6 are the treatments with the lowest distance from CT and the species generally tend to accompany them (Figure III.15)

MMSWC

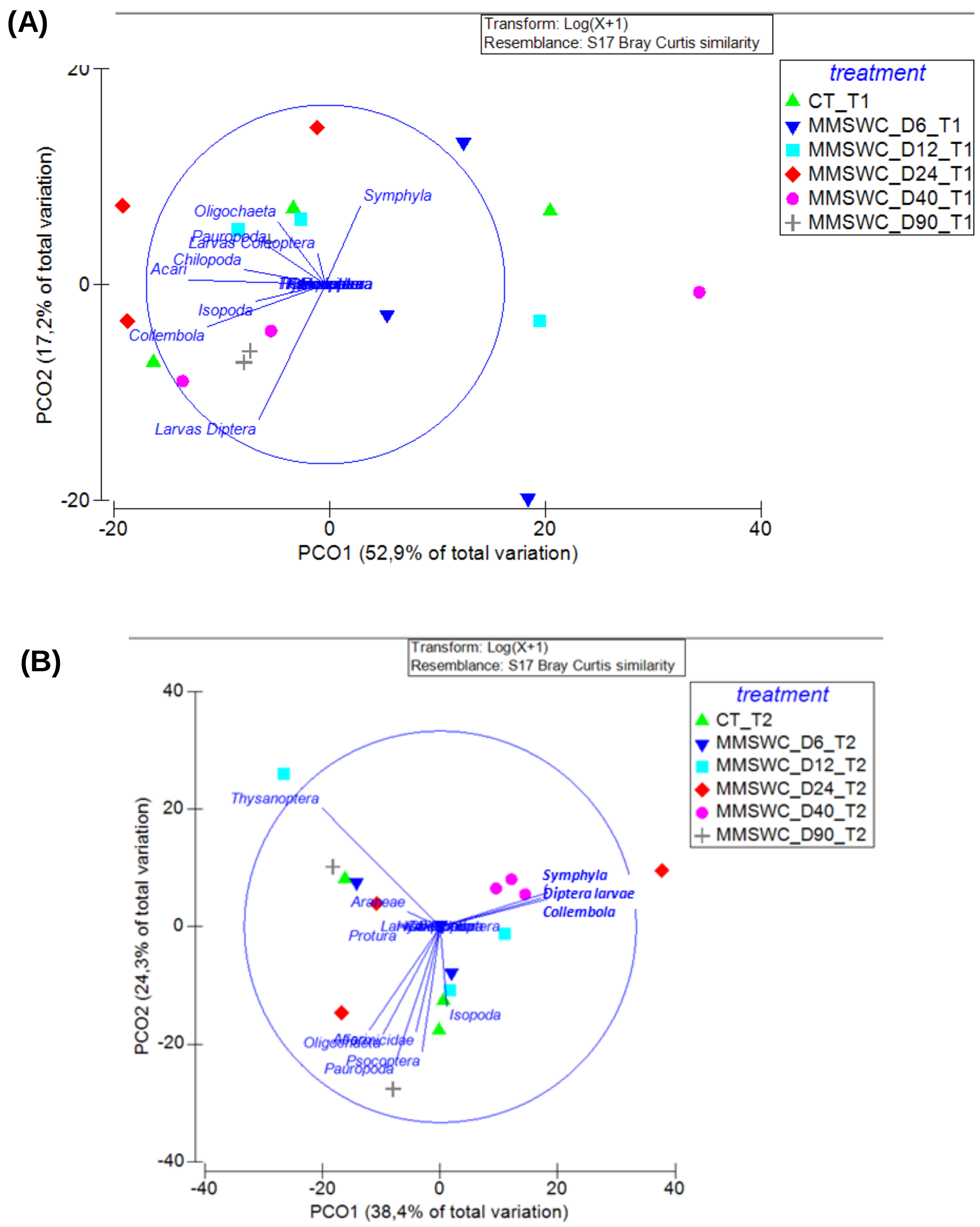


Figure III.16- MMSWC PCoA (principal coordinates analysis) plots based on log abundance of mesofauna at T1 (A) and T2 (B) on TMEs. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; PSD: pig slurry digestate.

At T1 there exists an high concentration of samples (points) on the left side (Figure III.16 (A)). These points are related to higher doses, and also it is visible that the species have a tendency be correlated to them.

PSD

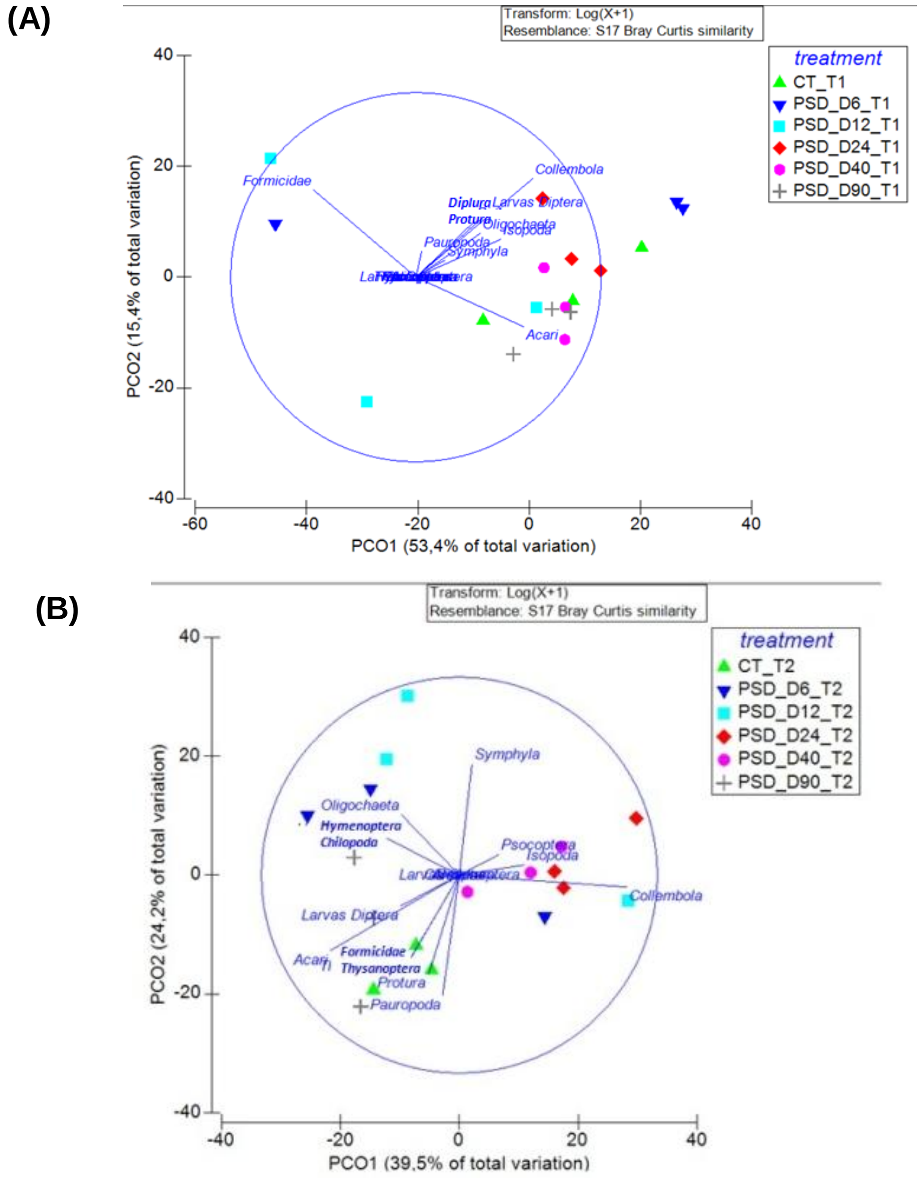


Figure III.17- PSD PCoA (principal coordinates analysis) plots based on log abundance of mesofauna at T1 (A) and T2 (B) on TMEs. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; PSD: pig slurry digestate.

In T1 there exists an high concentration of samples (points) on the right side (Figure III.17 (A)). These points are related to higher doses (D20, D40 and D90) but are also related with CT, and also, it is visible that the species have a tendency to follow them. On the other hand, in T2 a higher number of species has a tendency to follow CT and the D90 which are near to each other (Figure III.17 (B)).

3.2.3.1.2. Collembola

SS2

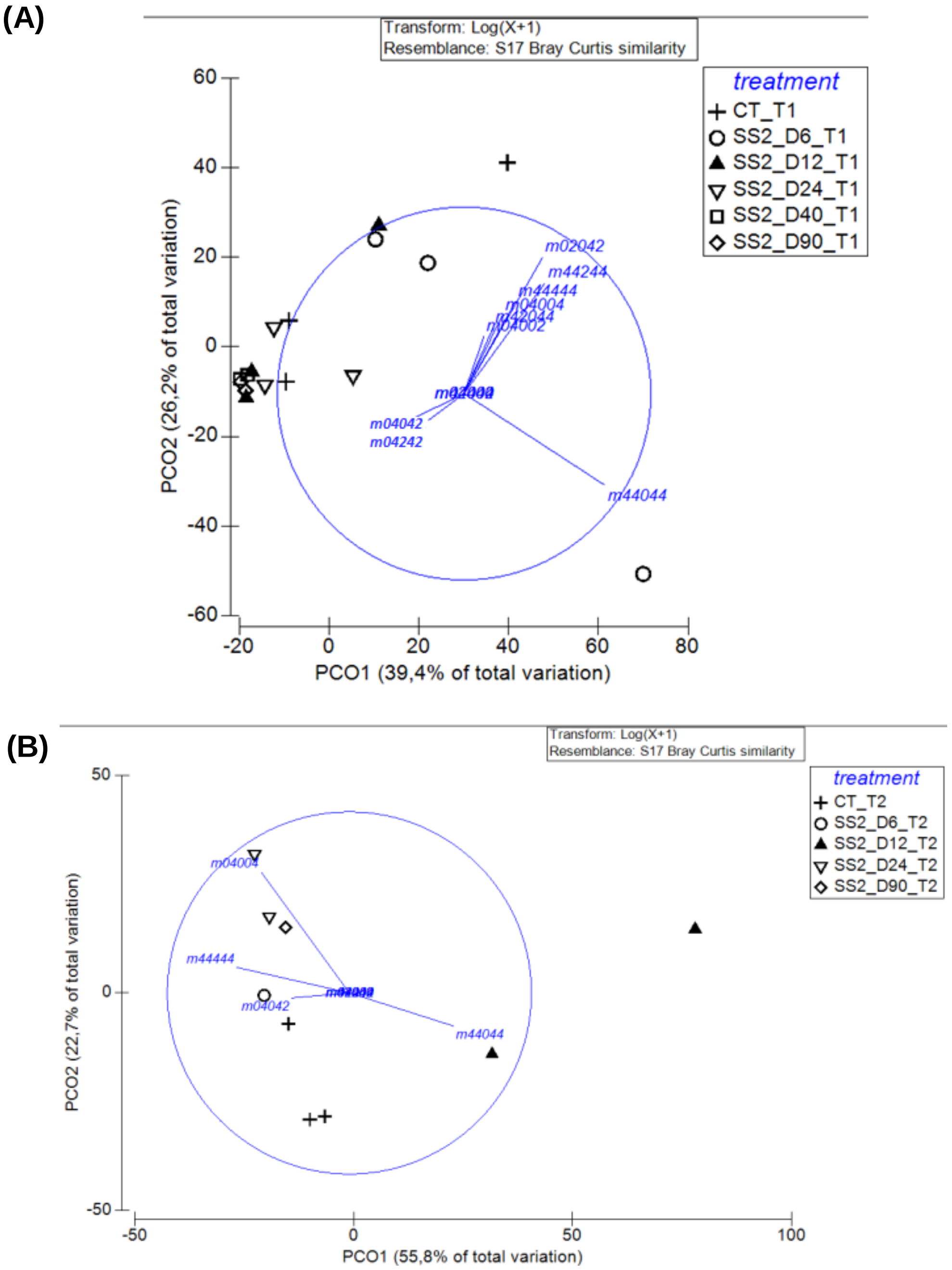


Figure III.18- SS2 PCoA (Principal Coordinates Analysis) plots based on log Collembola abundance at T1 (A) and T2 (B) on TMEs. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; PSD: pig slurry digestate.

In Figure III.18 (B) in T2, it is possible to observe that species have a tendency to follow the treatments with higher doses of SS2.

MMSWC

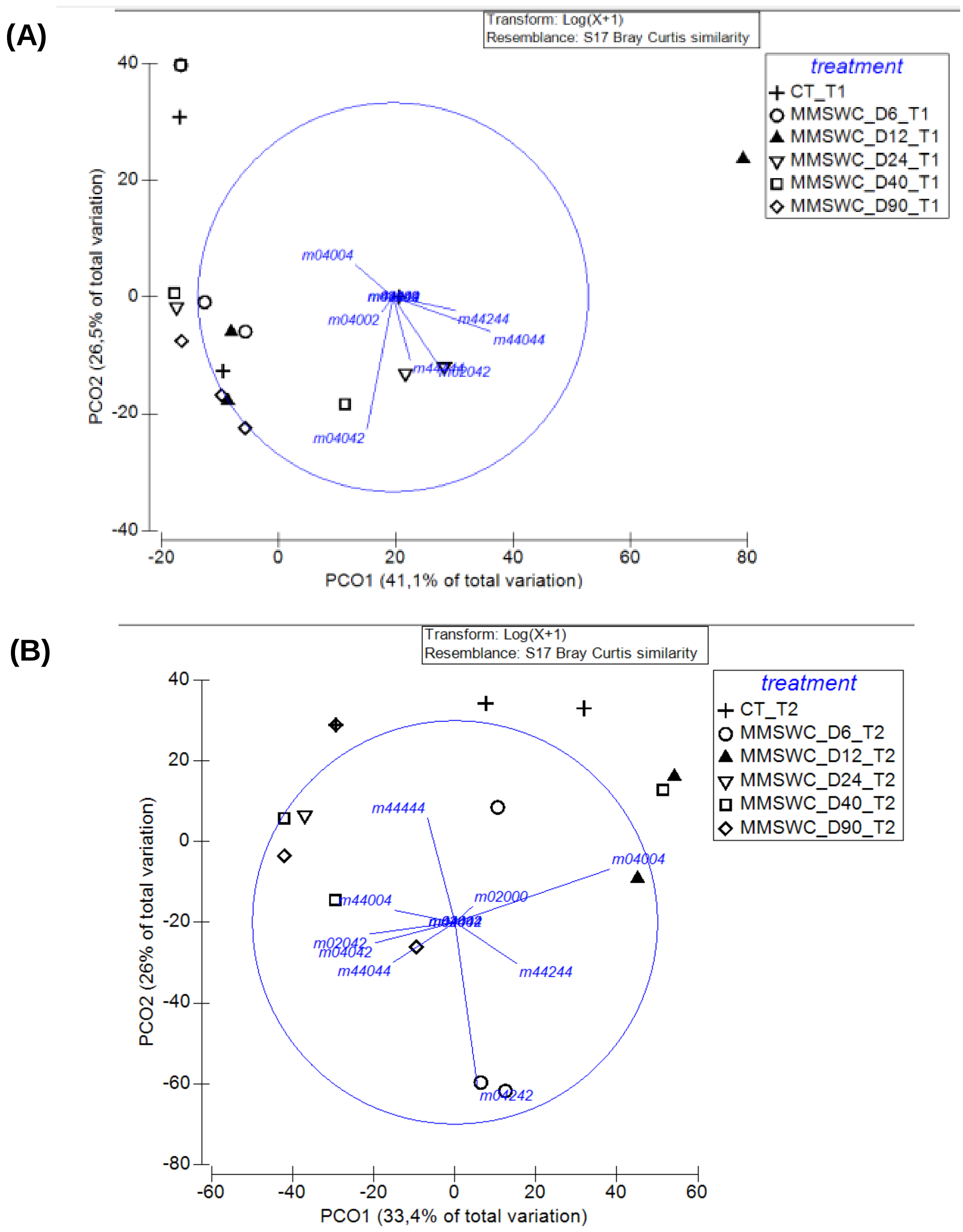


Figure III.19- MMSWC PCoA (principal coordinates analysis) plots based on log Collembola abundance at T1 (A) and T2 (B) on TMEs. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; PSD: pig slurry digestate.

In T1, the samples are concentrated in the left side of the plot, but the species have a tendency to follow the treatment of MMSWC D24 which is in the left side (Figure III.19 (A)).

PSD

(A)

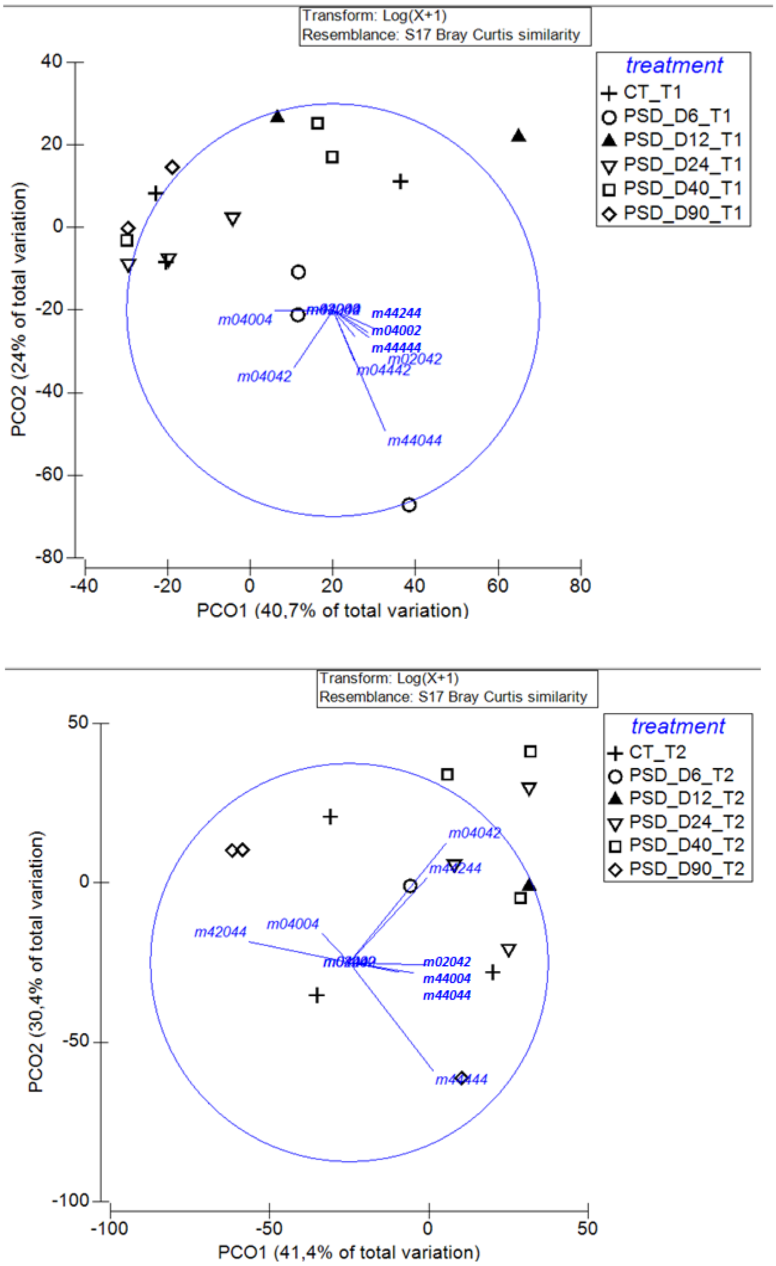


Figure III.20- PSD PCoA (Principal Coordinates Analysis) plots based on log Collembola abundance at T1 (A) and T2 (B) on TMEs. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; PSD: pig slurry digestate.

In T1 the species don’t generally follow the treatments, but they have a tendency to follow PSD D6 (Figure III.20 (A)).

3.2.3.2. Permanova & Simper

3.2.3.2.1. Mesofauna

In mesofauna, significant differences ($p<0.05$) were only found at T2 in treatments with SS2 and PSD.

Generally, in T1, Collembola (average contribution of 32.34%) and Acari (average contribution of 32.52%) were the groups that cause more dissimilarity between treatments (Annex Table V.16). In T2, Collembola and Acari were also the groups that contributes more to dissimilarity with an average contribution of 23.85% and 25.45% respectively (Annex Table V.17).

3.2.3.2.2. Collembola

For both sampling periods (T1 and T2), p-value for PSD was lower than 0.05, which means that there are significant differences between doses of this type of organic waste.

In T1 and T2, m04042 was the morphotype that caused more dissimilarity between treatments (average contribution of 35.66% and 34.62% respectively) (Annex Table V.18 and Table V.19).

Data interpretation must be carefully done because the program (Primer 6 & PERMANOVA +) does not allow the inclusion of empty samples, so it’s possible that more differences can occur.

3.2.4. Principal Response Curves (PRC)- Response of community composition on each treatment over time.

In the following sections, only the PRC diagrams that showed significant effects and those that were almost significantly of the treatments are presented.

Mesofauna

PSD

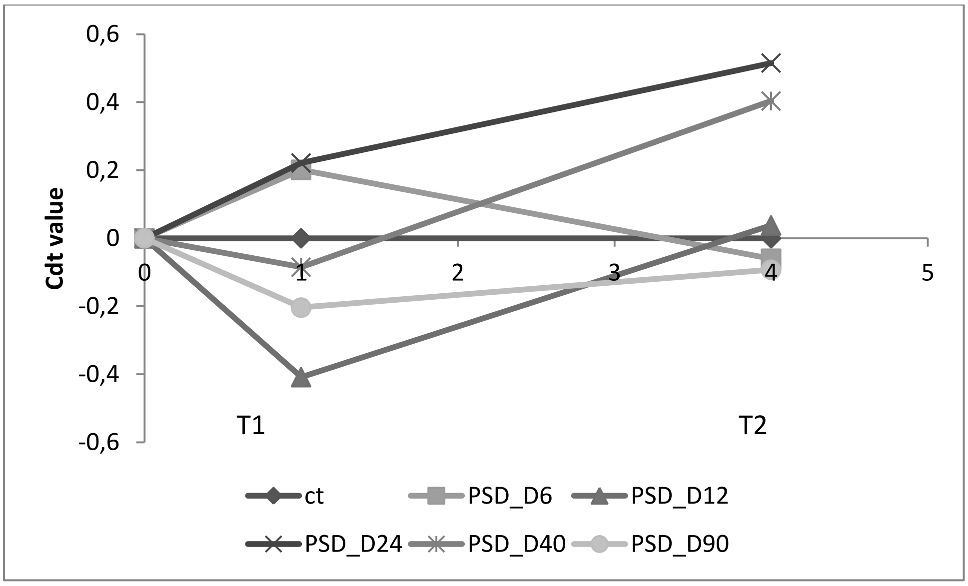


Figure III.21- PRC diagram for the principal component of the TMEs communities when exposed to different concentrations of PSD, considering two sampling periods. SS2: sewage sludge; MMSWC: mixed municipal solid waste compost; PSD: pig slurry digestate.

Of the total variance in the dataset, 9.3% is explained by time, 50.1% by residuals, and 40.6% by treatment (Monte-Carlo test: F=6.495; p=0.0520).

PRC diagram (Figure III.21) shows that in PSD D6 and D24 the Cdt value increases in T1, but on D6 in T2 there is a recovery contrary to D24 which increased. The remaining treatments, on the other hand, had a decrease and after that they had a recovery.

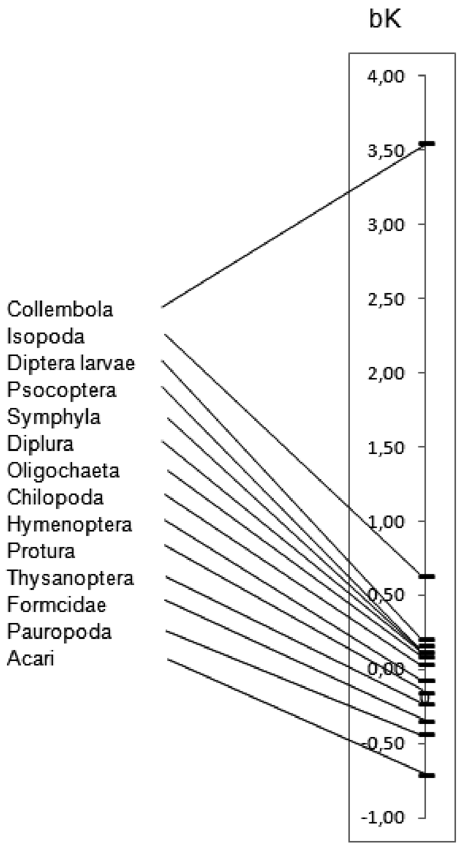


Figure III.22- “Species” weights for the principal component of the TMEs communities when exposed to different PSD concentrations.

According to the “species” weights (bk) of the PRC (figure III.22), the order that contributed the most to the observed differences was Collembola. Its positive score indicates that they were the most sensitive to PSD.

4. Discussion

The field work took place during a year and a half, and the variations of mesofauna and collembola abundance between different doses of residues were assessed. The first objective of this work was to see if there are differences on soil microarthropod abundances in different concentrations of the different residues. Despite an increase of microarthropods abundance being noted over time (including CT and D0), overall there are no significant differences between D0 and other doses, however in T2, the residues SS2 D6, MMSWC D12 and D24 present significant differences towards D0. The increase of microarthropods abundance at T4, particularly of Collembolans, occurred probably due to the second application of the organic wastes, made 4 weeks before the T3 sampling time. A reason for the increasing of microarthropods abundance at this time is plausible: climatic conditions. This probably had to do with differences in precipitation and temperature between seasons (and between years), noting that the higher abundances and Collembola richness appear during the spring season (T2 and T4).

Some pollutants may directly exert negative effects on soil organisms and even kill them, either by direct acute toxicity or by promoting an indirect effect due to the contamination of their food supply (Edwards 2002). Long term effects may not be promoted by organic wastes/pollutants, depending on a number of factors: the overall toxicity, the persistence on soil (e.g. heavy metals), the resilience of the local community and the potential of some soil organisms to develop resistance to a determinate organic waste after successive exposures (Edwards, 2002).This can explain why in time T4 an increase of microarthropods abundance occurred, as the organisms may have created resistance to the organic waste after successive applications (Tranvik *et al.*, 1993)- However, Ferreira da Silva (2016) stated that abundance may increase in the first application of organic waste, but after a few successive applications the abundance has a tendency to decrease. Other studies with springtails have shown different sensitivity responses of collembolans in the presence of organic wastes, depending on the type of waste and its concentration (which did happen in this study between some doses of certain residues), causing differences in reproduction and survival rates, with reproduction being more affected than the survival rate (Domene, 2007).

Although some studies have shown that TME’s allows predicting of soil communities behavior on the field (Knacker *et al.,* 2004), in this study the field assessment and TME study are contradictory in the case of Collembola. On the field experiment, after 5 months of residues application an increase of Collembola abundance occurred whilst, in TME’s after 3 months there was a decrease of their abundance. TME’s allows overcoming the environmental factors and to have controlled conditions. Due to this, a test with TME’s was performed, showing the effects of OW after 4 weeks and after 3 months of the application. The abundance of microarthropods generally increased after 3 months, but the Collembola group had a decrease of abundance, even in the control samples, maybe because of the TME’s closed system, or of the natural food chain (presence of predators, like acari, that weren’t taken into consideration in this study). Despite the presence of these visual differences, they were not significant.

According to Antoniolly *et al.* (2012), heavy metals Cu and Zn have a negative effect in Collembola population probably due to the pH reduction caused by these metals and, on the other hand, Cd at 1mg Kg^{-1} of soil concentration causes an increase of soil Collembola. This is in accordance to the results found in the TME's study for PSD, the treatment with less increase at T1 in Collembola abundance at higher doses, which is the residue with a higher concentration of Cu (183,3±0,9 mg/kg) and Zn (1691,6±172,1 mg/kg) (Alvarenga *et al*., 2015). In addition, Tranvik *et al.,* (1993) supported that some eu-edaphic species of Collembolans create resistance to Cu and Zn.

From the organic wastes used in this study, SS2 has the higher percentage of OM (74.3 ± 0,1 %), while MMSWC is the one that has a lower percentage of OM (39.5 ± 2,2 %) (Alvarenga *et al*., 2015; table 1). PCA plots demonstrate that SS2 is the organic waste which has a greater separation from the others, and adding to this species keep up with it, maybe because SS2 is the sludge with higher OM values, or just to the type of residues, causing an increase of fungi in the soil. According to Jørgensen *et al* (2003), Collembola are not very selective in relation to the fungi they eat, which could be an explanation on the fact that SS2 was actually the treatment where the largest increase of Collembola abundance (until D24; figure III.15) and soil microarthropods occurred (until D90; figure III.14).

In this study, according to the PRC results, there are no differences in each dose of organic waste type and its re-application on Collembola and in other microarthropods over time. The figure III.10 shows us that after the application of OW, the community has a tendency to increase after a few weeks, but there is a deviation of the community after 5 months. However, statistical analysis revealed that the exposure to organic wastes did not cause any significant effects on soil microarthropods.

Organic wastes used in soil improvement for agriculture increase the amount of organic matter, and there are some benefits in using organic wastes on soil, and some studies conclude that such benefits rely on the high organic matter (OM) and nutrient content of organic materials (Alvarenga *et al*. 2015), and some field experiments have shown that soil biota is stimulated when sludge is added to soil at agricultural rates (Krogh and Pedersen, 1997; Petersen *et al.*, 2003, Axelson, 2000). Despite the presence of a higher number of pathogenic microorganisms in SS2; the high EC, Na and Ni in AWC; the high EC, Na, Ca, Cd, Pb in MMSWC and the high Zn, Cu, Cd in PSD; the different doses of each organic waste, apparently, do not cause any significant effects on soil microarthropods, and their accumulation, due to successive applications, does not demonstrate any differences either. The main conclusion of this study is that the organic wastes applied here, can be used like soil amendments, without affecting soil microarthropods communities, namely collembolans, by exposure to contaminants from limed soils.

References

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5. Annex:

Field:

Table V.1- Bartlett test and one way ANOVA values to assess mesofauna **abundance** differences between different concentrations per sewage sludge type at each time.

		Non transform data				Log transform data			
		Bartlett	p(Bartlett)	F	p	Bartlett	p(Bartlett)	F	p
SS2	T1	23,29825	**0,000035**	0,901464	0,468928	6,932565	0,074079	0,9060	0,466856
	T2	5,703022	0,126988	4,78879	**0,020334**				
	T3	7,440867	0,059097	2,81904	0,084036				
	T4	4,185245	0,242143	3,0295	0,071096				
MMSWC	T1	15,81952	**0,001235**	1,85690	0,190706	1,646246	0,648950	2,934	0,076645
	T2	1,042506	0,790968	15,5346	**0,000196**				
	T3	10,16383	**0,017224**	0,27739	0,840643	9,482720	**0,023516**	0,2259	0,876553
	T4	0,234735	0,971796	3,4069	0,053215				
AWC	T1	5,137590	0,161995	2,27545	0,132006				
	T2	2,265697	0,519124	0,58629	0,635456				
	T3	6,412637	0,093172	0,64742	0,599439				
	T4	0,959281	0,811103	5,4858	**0,013165**				

Table V.2- Bartlett test and one way ANOVA results to assess collembola **abundance** differences between concentrations of each sewage sludge type at each time.

		Non transform data				Log transform data			
		Bartlett	p(Bartlett)	F	p	Bartlett	p(Bartlett)	F	p
SS2	T1	16,35651	**0,000958**	0,830203	0,502480	2,260538	0,520123	1,20574	0,349586
	T2	20,41091	**0,000140**	1,672816	0,225452	5,586309	0,133567	2,82405	0,083698
	T3	7,058994	0,070041	1,68345	0,223262				
	T4	5,973815	0,112891	4,1679	**0,030775**				
MMSWC	T1	5,072993	0,166530	1,25818	0,332495				
	T2	10,49498	**0,014795**	1,409907	0,287935	0,761223	0,858718	2,54429	0,105200
	T3	8,527967	**0,036272**	0,512303	0,681392	0,678130	0,878335	0,32062	0,810378
	T4	6,441055	0,092016	0,55624	0,653810				
AWC	T1	3,491914	0,321812	0,56376	0,649177				
	T2	5,957608	0,113691	1,09453	0,389020				
	T3	11,50374	**0,009292**	1,43387	0,281514	1,410833	0,702997	3,82616	**0,039130**
	T4	7,771843	0,050970	1,7332	0,213332				

Table V.3- Bartlett test and one way ANOVA results to assess differences in morphotype **richness** between concentrations of sewage sludge types at each time.

		Bartlett	p(Bartlett)	F	p
SS2	T1	1,009557	0,798940	1,02439	0,416281
	T2	4,938248	0,176375	2,46099	0,112778
	T3	2,899526	0,407377	0,92308	0,459202
	T4	1,945611	0,583772	0,5397	0,664101
MMSWC	T1	6,462801	0,091141	1,55652	0,251020
	T2	6,947113	0,073603	4,30233	**0,028068**
	T3	2,307473	0,511091	0,68276	0,579440
	T4	2,784196	0,426108	0,5238	0,674085
AWC	T1	1,753784	0,625043	0,27027	0,845632
	T2	3,924825	0,269697	0,90110	0,469094
	T3	3,880108	0,274705	2,04505	0,161284
	T4	3,412205	0,332329	1,2857	0,323881

Table V.4- Collembola PERMANOVA p value with significant differences between treatments at each time. Empty cases are which ones that don’t have significant differences.

	treatments	CT	D0	SS2 D6	SS2 D12	SS2 D24	MMSWC D6	MMSWC D12	MMSWC D24	AWC D6	AWC D12	AWC D24
T1	CT							0,035			0,026	
	D0					0,028						
	SS2 D6										0,025	
	SS2 D12											
	SS2 D24							0,026			0,027	
	MMSWC D6											
	MMSWC D12								0,049			
	MMSWC D24											
	AWC D6											
	AWC D12											
	AWC D24											
T2	CT				0,029	0,026	0,023		0,025			
	D0					0,03						
	SS2 D6					0,021						0,037
	SS2 D12						0,03				0,031	
	SS2 D24						0,021	0,032	0,024			
	MMSWC D6								0,032			
	MMSWC D12										0,048	
	MMSWC D24											
	AWC D6											
	AWC D12											

	AWC D24											
T4	CT			0,029						0,027		
	D0			0,03				0,026				
	SS2 D6						0,018			0,043		
	SS2 D12											
	SS2 D24											
	MMSWC D6									0,019	0,03	
	MMSWC D12									0,032		
	MMSWC D24											
	AWC D6										0,036	
	AWC D12											
	AWC D24											

SIMPER tables:

Table V.5- Mesofauna contribution to dissimilarity by treatments at T1 (>15%).

		Group	**Contribution %**
SS2	D6	Coleoptera Larvae	36,21
		Collembola	24,9
	D12	Larvas Coleoptera	28,78
		Acari	20,98
	D24	Coleoptera Larvae	25,78
		Collembola	24,23
		Acari	15,14
MMSWC	D6	Collembola	28,89
		Coleoptera Larvae	18
	D12	Collembola	38,64
		Acari	15,2
	D24	Collembola	27,39
		Acari	25,28
AWC	D6	Collembola	26,39
		Acari	23,54
	D12	Collembola	33,31
		Acari	17,1
		Psocoptera	16,91
		Embioptera	16,63
	D24	Collembola	24,67
		Acari	18,53

Table V.6- Mesofauna contribution to dissimilarity by treatments at T2 (>15%).

		group	contribution %
SS2	D6	Acari	28,58
		Collembola	25,22
	D12	Collembola	25,37
		Acari	24,43
		Coleoptera	16,73
		Larvas Coleoptera	16,13
	D24	Collembola	25,76
		Larvas Diptera	17,43
		Larvas Coleoptera	16,79
		Acari	15,9
MMSWC	D6	Collembola	35,93
		Acari	26,66
	D12	Collembola	33,92
		Acari	26,39
	D24	Collembola	35,47
		Acari	29,49
AWC	D6	Collembola	35,47
		Acari	19,87
	D12	Collembola	36,72
		Acari	24,28
	D24	Collembola	44,85
		Acari	22,36

Table V.7- Mesofauna contribution to dissimilarity by treatments at T3 (>15%).

		group	contribution %
SS2	D6	Collembola	44,61
	D12	Collembola	32,81
	D24	Collembola	40,82
MMSWC	D6	Collembola	30,3
	D12	Collembola	36,77
	D24	Collembola	33,45
AWC	D6	Collembola	45,45
	D12	Collembola	34,73
	D24	Collembola	34,33

Table V.8- Mesofauna contribution to dissimilarity by treatments at T4 (>15%).

		group	contribution %
SS2	D6	Collembola	24,06
		Larvas Diptera	19,17
		Formicidae	15,04
	D12	Formicidae	26,3
	D24	Formicidae	21,98
		Larvas Coleoptera	15,04
MMSWC	D6	Formicidae	16,57
	D12	Larvas Diptera	18,34
	D24	Larvas Diptera	15,74
		Isopoda	15,47
AWC	D6	Formicidae	39,32
	D12	Formicidae	20,87
	D24	Formicidae	20,35
		Larvas Diptera	15,79

Table V.9- Collembola contribution to dissimilarity by treatments at T1 (>10%).

		morphotypes	contribution %
SS2	D6	m04042	47,54
		m04004	20,59
		m04000	15,90
	D12	m02042	28,41
		m04004	18,11
	D24	m02042	22,67
		m04242	21,69
MMSWC	D6	m04042	20,96
		m04004	16,97
		m04002	16,14
	D12	m44444	48,07
		m04004	20,09
	D24	m04042	23,72
		m44444	21,61
		m04004	17,53
AWC	D6	m04042	21,48
		m04004	21,32
		m04000	19,53
	D12	m04004	33,63
		m04042	21,79
		m04000	19,00
		m02000	15,15
	D24	m04004	29,75
		m04042	21,88
		m44444	17,13

Table V.10- Collembola contribution to dissimilarity by treatments at T2 (>15%).

		morphotypes	contribution %
SS2	D6	m04042	26,8
		m04000	18,27
		m04002	17,94
	D12	m04042	17,94
		m04002	15,68
		m44044	15,39
	D24	m04242	22,77
		m04002	19,24
MMSWC	D6	m04002	22,35
		m04042	19,81
		m44004	17,06
		m04242	16,96
		m04442	15,87
	D12	m04002	21,78
	D24	m04042	17,8
		m44004	16,27
AWC	D6	m04002	22,8
		m44004	16,14
		m04042	15,44
	D12	m04002	21,54
		m44004	16,01
	D24	m04042	43,04

Table V.11- Collembola contribution to dissimilarity by treatments at T3 (>15%).

		morphotypes	contribution %
SS2	D6	m04042	37,76
		m04002	20,55
	D12	m04042	36,22
		m04002	17,05
		m02000	15,55
	D24	m04042	30,65
		m04002	17,24
MMSWC	D6	m04042	37,28
		m04002	32,66
	D12	m04042	29,2
		m44044	25,35
		m04002	23,5
	D24	m04042	33,45
		m04002	24,06
		m44044	15,34
AWC	D6	m04042	38,22
		m04002	26,19
	D12	m04042	33,78
		m04002	25,92
	D24	m04042	29,33
		m04002	23,83
		m44044	16,05

Table V.12- Collembola contribution to dissimilarity by treatments at T4 (>15%).

		morphotypes	contribution %
SS2	D6	m44444	14,05
		m04042	13,89
		m44244	11,89
		m44004	11,14
		m44044	10,41
	D12	m44444	13,94
		m44244	11,88
		m04042	11,46
		m44004	10,88
		m04000	10,4
	D24	m44044	15,96
		m44244	14,48
		m44444	12,45
		m04042	10,02
MMSWC	D6	m44444	17,86
		m44244	14,5
		m44044	14,09
		m44004	12,49
		m04000	11,27
	D12	m44444	15,37
		m04042	13,67
		m44244	12,3
		m44004	12,17
		m44044	11,12
		m04002	10,88
	D24	m44444	14,64
		m44244	13,33
		m04042	13,25
		m44044	12,67

		m04002	11,2
AWC	D6	m44044	17,21
		m44444	16,08
		m44244	15,94
		m04002	15,23
		m04042	10,11
	D12	m44244	18,51
		m44444	18,42
		m44044	13,78
		m04002	11,52
		m04000	10,59
	D24	m44444	17,51
		m44244	15,11
		m44044	14,52

TME's:

Table V.13- Bartlett test and one way ANOVA values to assess mesofauna **abundance** differences between different concentrations per sewage sludge type at each time.

		Non transformed data				Log data			
		Bartlett	p(Bartlett)	F	p	Bartlett	p(Bartlett)	F	p
SS2	T1	16,36767	0,005869	0,611587	0,693313	3,085432	0,543632	1,8630	0,188191
	T2	28,92410	0,000024	4,55277	0,014676	5,977338	0,308430	7,431	**0,002183**
MMSWC	T1	41,53397	0,000000	7,64547	0,001935	3,678868	0,451207	1,0222	0,454252
	T2	12,72677	0,026078	0,74360	0,605762	11,41671	0,043716	1,093	0,412719
PSD	T1	8,524221	0,129614	1,34221	0,311769				
	T2	5,494580	0,358541	14,0836	**0,000114**				

Table V.14- Bartlett test and one way ANOVA values to assess collembola **abundance** differences between different concentrations per sewage sludge type at each time.

		Non tramsformed data				log data			
		Bartlett	p(Bartlett)	F	p	Bartlett	p(Bartlett)	F	p
SS2	T1	23,74420	0,000243	1,416464	0,286768	1,537529	0,908699	2,46777	0,092790
	T2	-0,049040	1,000000	1,326969	0,317172				
MMSWC	T1	6,622118	0,250294	0,89877	0,512509				
	T2	8,124602	0,087119	1,265166	0,340074				
PSD	T1	21,39602	0,000682	2,72460	0,071896	9,134163	0,103831	2,36954	0,102532
	T2	5,822911	0,212769	5,07766	**0,009904**				

Table V.15- Bartlett test and one way ANOVA values to assess collembola **richness** differences between different concentrations per sewage sludge type at each time.

		Bartlett	p(Bartlett)	F	p
SS2	T1	4,763949	0,445362	2,32000	0,107877
	T2	-2,41495	1,000000	1,03704	0,439635
MMSWC	T1	3,766338	0,583524	0,97500	0,471121
	T2	-0,060613	1,000000	1,25556	0,343782
PSD	T1	1,940466	0,746708	2,37037	0,102444
	T2	2,095839	0,718137	1,02791	0,444146

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Table V.16- mesofauna contribution to dissimilarity by treatments at T1 (>15%).

		group	contribution %
SS2	D6	Collembola	34,44
		Acari	23,34
	D12	Collembola	26,65
		Diptera larvae	21,29
	D24	Collembola	39,48
		Acari	28,7
	D40	Acari	34,44
		Collembola	33,47
	D90	Acari	54,34
MMSWC	D6	Acari	31,42
		Collembola	23,35
	D12	Collembola	31,77
		Acari	18,02
	D24	Acari	19,63

		Collembola	17,38
	D40	Collembola	32,14
		Acari	24,69
	D90	Collembola	34,83
		Acari	16,33
PSD	D6	Collembola	29,18
		Acari	24,54
		Diptera larvae	15,65
	D12	Collembola	34,15
		Acari	25,68
	D24	Collembola	28,45
		Acari	25,19
		Isopoda	15,71
	D40	Acari	26,06
		Isopoda	22,7
		Collembola	20,7
	D90	Acari	29,25
		Collembola	26,92
		Isopoda	15,24

Table V.17- Mesofauna contribution to dissimilarity by treatments at T2 (>15%).

		groups	contribution %
SS2	D6	Thysanoptera	20,96
		Collembola	20,79
		Pauropoda	17,43
		Protura	16,72
	D12	Acari	16,81
		Pauropoda	16,59
		Protura	15,84
		Collembola	15,09
	D24	Collembola	22,36
	D40	Collembola	24,41

		Pauropoda	18,95
		Protura	18,08
	D90	Acari	41,84
		Thysanoptera	23,76
		Collembola	23,31
MMSWC	D6	Protura	21,31
		Thysanoptera	20,62
		Pauropoda	20,15
	D12	Thysanoptera	23,34
		Protura	16,73
		Collembola	15,08
	D24	Larvas Diptera	16,83
		Collembola	16,24
		Acari	15,1
	D40	Collembola	23,66
		Acari	17,75
		Pauropoda	16,44
		Protura	15,74
	D90	Acari	21,36
		Thysanoptera	16,62
PSD	D6	Collembola	19,96
		Pauropoda	15,1
	D12	Acari	21,79
		Collembola	18,94
	D24	Collembola	24,1
		Acari	19,67
	D40	Collembola	24,74
		Pauropoda	16,25
		Protura	15,6
	D90	Pauropoda	17,97
		Protura	15,83
		Acari	15,44

Table V.18- Collembola contribution to dissimilarity by treatments at T1 (>15%).

		morphotype	contribution %
SS2	D6	m04042	25,63
		m02042	15
	D12	m04042	41,81
	D24	m04042	46,67
	D40	m04042	35,51
	D90	m04042	36,16
		m04004	16,7
		m44444	16,56
		m02042	15,29
		m44244	15,29
MMSWC	D6	m04042	28,71
		m02042	16,32
		m04004	15,46
	D12	m04042	37,11
	D24	m04042	26,96
		m44044	24,12
	D40	m04042	37,51
		m02042	15,22
	D90	m04042	44,07
		m02042	17,9
PSD	D6	m04042	31,68
		m02042	17,34
	D12	m04042	36,19
		m02042	22,27
	D24	m04042	43,13
		m02042	16,48
	D40	m04042	29,21
		m02042	21,58
		m44444	19,77
		m04004	15,18
	D90	m04042	33,91
		m04004	17,67
		m44444	16,98
		m02042	15,72
		m44244	15,72

Table V.19- Collembola contribution to dissimilarity by treatments at T2 (>15%).

		morphotype	contribution %
SS2	D6	m04004	71,29
	D12	m44044	44,39
		m44444	20,76
		m04004	17,42
		m04042	17,42
	D24	m04004	69,09
		m04042	22,81
	D90	m04004	71,46
		m44444	19,03
MMSWC	D6	m04242	22,83
		m44444	19,57
		m04004	17,39
		m04042	17,39
		m44244	16,3
	D12	m44244	30,94
		m04004	28,17
		m04042	20,45
		m44444	20,45
	D24	m44004	53,45
		m04042	18,99
	D40	m04042	30,47
		m04004	25,66
		m02042	23,42
	D90	m04042	34,19
		m04004	23,29
		m44044	23,16
		m44444	19,37
PSD	D6	m04004	44,44
		m44244	33,33
	D12	m02042	39,75
		m44244	30,75
	D24	m44044	25,75
		m04042	20,26
		m02042	17,57
		m04004	16,79

	D40	m04042	39,43
		m44244	20,46
	D90	m04042	34,38
		m44444	28,13
		m04004	23,44