Effects of Collembola of Soil aggregate formation and stabilization: A trait-based approach

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Master thesis:

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Abstract

Soil aggregation is the key ecological process mediated by diverse soil fauna, which has become a central issue in soil functional ecology. Having implication in ecological sustainability, it has received much attention in recent years but focuses were mostly on larger soil animals, while the highly abundant group of soil invertebrates (e.g., Collembola) have been largely neglected. Despites, they are thought to be the drivers of ecosystem process due to their high abundance in most of the ecosystem types. Their most important effects within soil ecosystems through their feeding behaviour on saprotrophic fungi, however, precise mechanistic evidence on how they interact with fungi and thus effects on soil aggregation is still lacking. Being aware of this clear research gaps on how Collembola interacts with saprotrophic fungi and thus influence on soil aggregate formation and stabilization, we aimed to investigate more precisely on how Collembolan trophic interaction with a highly abundant soil borne fungi (*Chaetomium globosum*) and their different functional behaviour influences on soil aggregate formation and stabilization. This study has been initiated through re-creating soil-fungal-collembolan systems in laboratory mesocosm. Collembolan trophic interactions were unrevealed by using a combination of fatty acids (FAs) composition and natural variation in C and N stable isotope ratios. Further, different functional behaviour of Collembolan (e.g., their ability to reduce fungal growth, fungal spore dispersal capability, ability to transport micrometric particles) were measured through laboratory experimentation. Results showed that, different Collembola species increases macroaggregate formation significantly with their different trophic and functional behaviour, although their effects on soil aggregates stability were rather limited.

Keywords: Soil aggregation, Collembola, Saprotrophic fungi, Phospholipid Fatty Acids, Stable Isotope, Trophic Interaction, Behavioural traits.

CHAPTER-I: INTRODUCTION

1. Introduction

Soils, the most crucial and critical component of the earth's biosphere (Ellert et. al., 1996; Coleman et. al., 2004), which provides essential ecosystem services such as food production, water filtration and carbon sequestration. Soil leads in developing ecosystem properties (Ellert et. al., 1996) through serving as the ground for major ecosystem processes (i.e., primary production, decomposition and nutrient cycling). However, about 24% of the global land areas are in a state of degradation (IFPRI, 2012); much of which is caused by declines in the soil physical structure, modifying the transport of water, nutrients and gases, and habitats for the soil biota. An increasing global concern about soil degradation has been evident in literatures (Lal and Stewart, 1990; Six et. al., 2000; Chan et. al., 2003; Piotrowski et. al., 2004; Bronick and Lal, 2005); where, soil structure is being considered a burning issue globally because its degradation leading to a continuous threat to crop yield, environmental quality and sustainability (Lal and Stewart, 1990; Lal, 1991; Bronick and Lal, 2005).

Soil structure is often referred to the different micro and macro-aggregates of soils organo-mineral complexes and corresponding pore spaces (Hartge and Stewart, 1995; Rillig and Mummey, 2006). Soil aggregates are the association of primary soil particles which adheres more tightly to one another comparing to the surrounding soil particles (Follett et al., 2009). Generally, soil aggregation is the process of being coupled together of mineral primary particles, where the organic matter serves as the principle binding agent to form and stabilize aggregates (Tisdall and Oades, 1982). This process is additionally influenced by the soil texture, soil microorganisms, plant roots, inorganic binding agents, predominant environmental conditions and the diverse soil animals (Dexter and Horn, 1988; Rilling et. al., 2015). While the soil organisms are being highly acknowledged as important for soil aggregation (Maaß et.al., 2015), precise mechanistic understanding of how the high diversity of the soil organisms interacting in complex food webs influences the formation and stabilization of aggregates is still lacking. Highly abundant groups of soil dwelling micro-arthropods, such as Collembolan (commonly known as Springtails) have been largely

neglected; despite they are thought to have important effects on the ecosystem processes as drivers (Hopkin, 1997).

As they feed in fungi, their grazing effects on saprotrophic fungi indirectly influences on the decomposition process of organic materials (Hopkin, 1997), even they are able to alter soil fungal community structure and composition through their selective grazing on fungi (Visser, 1985). Moreover, known for having important role in soil aggregation (e.g., Maaß et. al., 2015), an indirect role of Collembolan is well expected through their functional behaviour in modifying of fungal growth and biomass, hyphae branching, transporting micrometric particles, or even fungal spore dispersion. However, it is still poorly understood how Collembolan interacts with saprotrophic fungi, even direct mechanistic evidence about their trophic interactions in the complex soil food webs, and their influence on soil structure and functioning is still largely underrepresented in scientific literatures.

Trophic interaction of micro-arthropods in soil food webs play a key role in carbon and nutrient dynamics of ecosystem and might be possible to detect through the analysis of C and N stable isotope ratios, where $\delta^{15}N$ ($^{15}N/^{14}N$) can be used to indicate the trophic levels of organisms in food chains, and $\delta^{13}C$ ($^{13}C/^{12}C$) can be used to indicate relative contributions to the diet of different potential basal resources (primary carbon sources) that present within a trophic network (Pereira et. al., 2010). Moreover, recent advancement on Fatty Acids (FAs) biomarker's application have already proven to be used as a powerful tool in order to investigate food web ecology. Some specific fatty acids can be used as biomarkers to assess feeding preferences of the soil animals (Chamberlain et. al., 2005, Haubert et. al., 2009). Consumer-resources relationships especially for the below ground ecosystems are still poorly understood because of the very recent advancement of the fatty acids biomarkers application (Ruess and Chamberlain, 2010). However, more detailed information on feeding strategies of soil animals is attainable by the combined application of nitrogen and carbon isotope ratios with fatty acids (FA) analysis (Chamberlain et. al., 2005, Haubert et. al., 2009). So far, this present study aims to investigate on how different Collembolan species with their different trophic behaviour within soil ecosystem influences on soil aggregate formation and stabilization. Further, we also aimed to investigate the influence of their different functional behavioural traits on soil aggregate formation and stabilization.

1.1. Research Objectives

Broadly, this present research aims to investigate the effects of Collembola on soil physical structure and microbial functioning.

More specifically, this study

Aims to investigate the trophic interactions between organisms and their role on soil aggregate formation and stabilization. Further, this work aims to investigate the influences of different functional behavioural traits of collembola on soil aggregation.

1.2. Research Hypothesis

We aimed to investigate the trophic interaction of Collembolans with different functional behaviour and their role on soil aggregate formation and stabilization considering the following possible direct and indirect effects as hypothesis:

Direct effects

1. Collembolan increases soil aggregation via modification of microbial communities of soils.
2. Different Collembola species with different feeding strategies have different influences on soil aggregation.

Indirect effects

3. Collembola reduces saprotrophic fungal growth through grazing on fungal hyphae, thus restricting to the positive influence of fungi on soil aggregation.
4. Collembola may disperse fungal spores rapidly, thus plays a positive influence on soil aggregation through multiplying fungal isolates rapidly.
5. Soil aggregation is influenced by Collembolan ability to transport micrometric particles.
6. Collembolan activity effects on soil aggregate physical stability.

CHAPTER-II: MATERIALS AND METHODS

2. Overview of the Methodologies

A wide range of methodologies have been used to carry out the overall research. Initially, to investigate the ecological role of key species of soil-arthropods (e.g., Collembola) on soil structure and functioning, we conducted Mesocosm soil pot experiment under laboratory-controlled conditions with combination of four different collembolan species (*Falsomia candida*, *Protaphorura fimata*, *Ceratophysella denticulata* and *Sinella curviseta*) and saprotrophic fungi (*Chaetomium globosum*). In order to understand the trophic interactions of Collembolans, Fatty Acids (FAs) and stable isotope analysis (SIA) have been carried out. Further, we followed to a set of simple experiments for measuring some important functional behavioural traits of Collembolans to investigate effects on soil aggregation. Physical stability in water of some selected fractions of macro-aggregates (those were harvested after mesocosm experiment) were measured following a slow-wetting technique in order to understand Collembolan effects on soil aggregate stability. The overall experimental methodologies including some general preparatory methods have been described in this chapter.

2.1. General Methods

2.1.1. Study organisms

Collembola (commonly known as Springtails), an ideal bioindicator (Stork and Eggleton, 1992; and Zeppelini et. al., 2009), one of the highly abundant group of micro-arthropods usually found in soil ecosystem throughout the world. However, they may present in every habitat types including Antarctica (Block, 1884) to high altitude of Himalayas (Yosii, 1966). Till today, 8279 species of Collembola have been enlisted globally (Janssens, 2016). Collembolans play their role in soil ecosystems (Palacios-Vargas, 1985) through feeding on organic materials and influence in their decomposition and soil mineralization (Culik and Filho, 2003). Many Collembolan species feeds on fungi while some others feed on nematodes, rotifers, algae, bacteria, protozoa and even other collembola also. There is a long history of studying their interactions with fungi (Poole, 1959; Mills and Sinha, 1971), their grazing on saprotrophic

fungi indirectly influences on the decomposition process (Hopkin, 1997). In order to understand their ecological role, we studied on four species of fungal feeding collembola (Figure-1; Table-1) those usually prefer soil habitat.

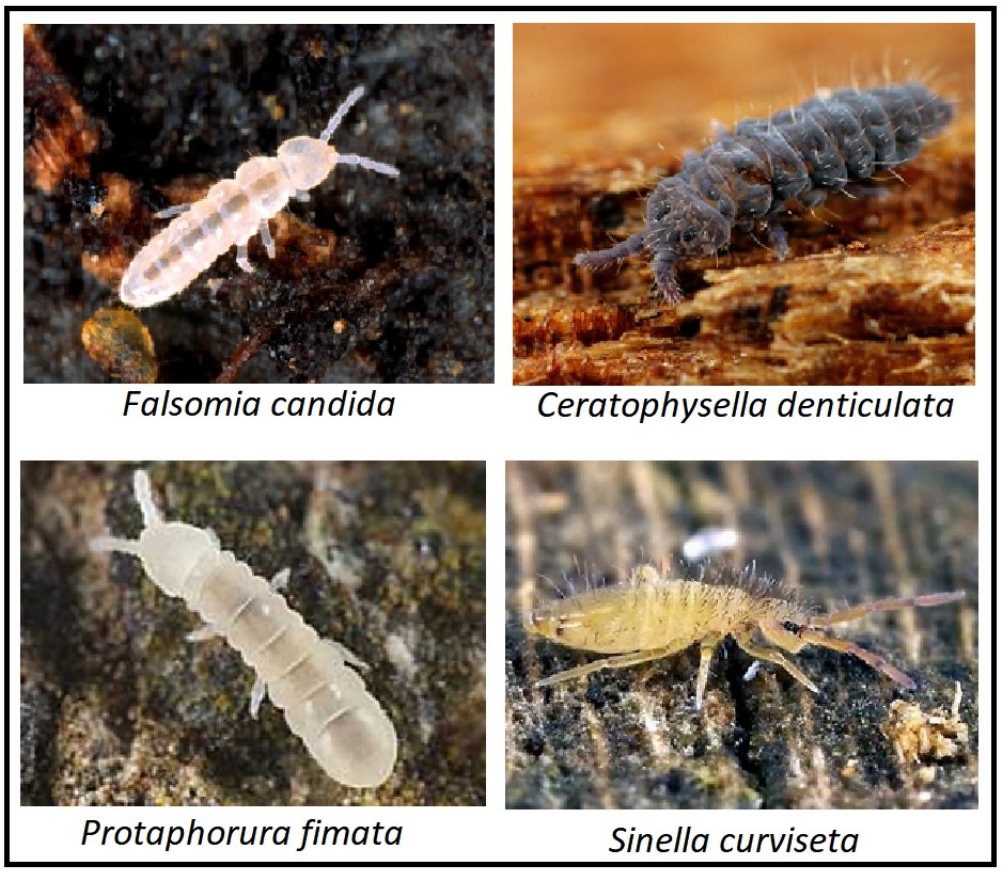


Figure-1. Different species of Collembola (Source: Class COLLEMBOLA Lubbock, 1870; link: http://www.janvanduinen.nl/collembolaengels_b.html

Table-1. Information on different Collembolan species used for present experiment (Sources: The ***SoilBioStore*** Trait database; Bandow et.al., 2013; Denis 1929; Bagnall, 1941).

Species	Body length (mm)	Habitat preference	Moisture preference	Antenna	Mode of reproduction
Falsomia candida	2.5	Hemiedaphic	Mesophilic	Medium	Generally parthenogenetic
Ceratophysella denticulata	1.2	Hemiedaphic	Meso-hydrophilic	short	Bisexual
Protaphorura fimata	2.5	Euedaphic	Mesophilic	short	Bisexual
Sinella curviseta	2	Epedaphic	Mesophilic	Long	Sexual

2.1.2. Collembola culture

Several Collembolan species i.e., *Falsomia candida*, *Protaphorura fimata*, *Ceratophysella denticulata* and *Sinella curviseta* (Source: Department of Bioscience, Arhus University, Denmark) were cultured in petri-dishes (8.9 cm diameter) with a mixture of plaster of Paris and activated charcoal (3:1). As we planned to conduct Mesocosm experiment, we relied on these four species because of their habitat (soil) preference (Table-1) and having their previous evidences on laboratory culturing (e.g., Greenslade, P. and Vaughan, G. T., 2003; Joseph et. al., 2015; Reader, N.,1994; Draney, M.L., 2000).

Collembolan individuals were first counted and placed into the plaster of Paris using an entomological exhauster. Dried Baker's Yeast were supplied into the culture boxes once per week as food, and deionized (DI) water were added to keep the moisture of the plaster of Paris. Lids were placed on the petri-dishes in such a manner so that it protects collembolan jumping out from the culture boxes as well as facilitates a little aeration also. The whole systems were placed in the incubation chamber at room temperatures (about 20°C).

2.1.3. Fungal culture

Chaetomium globosum (Source: Gottingen University culture collection) was sub-cultured on Potato Dextrose Agar (PDA) culture media. Initially, PDA culture media were prepared (11.7g PDA powder in 0.3L Nanopure water, then autoclaving for 20 minutes at 121°C) into petri-dishes (8.9 cm diameter) in sterile condition. Small agar core (about 4cm^2) with growing *Chaetomium globosum* from old culture were placed on the PDA culture media into petri-dishes. In order to prevent contamination, petri dish systems were made non-vented by closing lids and covering it with biofilm, further incubated at room temperature.

2.2. Mesocosm experiment and experimental design

Soil-fungal-Collembolan system were recreated into Mesocosm pots in laboratory condition where seven different type of treatment were set up i.e., Soils with fungi only (represented by "**F**"), Soils with fungi and Collembola *Ceratophysella denticulata* (represented by"**FCD**"), Soils with fungi and Collembola *Falsomia candida* (represented by "**FFC**"), Soil with fungi and Collembola *Protaphorura fimata* (represented by "**FPF**"), Soils with fungi and Collembola *Sinella curviseta* (represented by "**FSC**"), Soils with fungi and mixed Collembolan species (where all of the four species were mixed together, represented by "**FMIX**") and one control treatment with only soils which is represented by "**C**" throughout this manuscript. A total of 56 mesocosm pots were set up making 8 replicates of each of the 7 treatments combination including control one (Figure-2). We have been adopted to a two-step inoculation protocol where fungi added to the microcosm soil pots first, then Collembola added two weeks later of fungal inoculation.

Agricultural soil was harvested from a University wheat field in Gottingen (conventional tillage site), air dried and sieved to 1 mm size to crush the previously formed aggregates, further soils were conditioned with dry sands (dried overnight at 105°C and sieved between 50 µm to 2mm mesh) in order to minimize effects of clay materials. Finally, dry leaf and root litter (wheat and maize) were mixed uniformly with the soils to prepare the mesocosm soil mixture. Mesocosm glass pots were filled with 120g (dry mass) of soil mixture with the final proportion of 60% sands, 40% soils and 0.5g (dry mass) of litter as well in each pot, autoclaved them with lids for 2 hours at 121°C to make them sterile, further dried under air flow and placed them

into incubation chamber at 20°C. The mesocosm's soil water holding capacity (WHC) adjusted to 60% because it is considered as the optimal for both C and N mineralization (Linn and Doran, 1984). Moreover, water is a limiting factor for microbial biological activity below 60% WHC, especially within soil aggregates (Linn and Doran, 1984).

Replicates	x8	x8	x8	x8	x8	x8	x8
Microcosm							Control
Soil Mixture	120g	120g	120g	120g	120g	120g	120g
Fungi (C. globosum)	√	√	√	√	√	√	---
Collembola	CD	FC	PF	SC	MIXED	---	---

Figure-2. Experimental design of the mesocosm pot experiment. Block letter represents the Collembolan species name (i.e., **CD** = *Ceratophysella denticulata*; **FC**= *Falsomia candida*; **PF**= *Protaphorura fimata*; **SC** = *Sinella curviseta*; **MIXED** = mixture of all the four species).

2.2.1. Fungal Inoculation (Food for Collembola)

Agar cores of about 4 cm^2 were taken from petri-dishes of PDA (Potato Dextrose Agar) with growing *Chaetomium globosum* in a 50 ml falcon tube. Fungal dilution was prepared by smashing and shaking it with 20 ml demineralized water. Further, 2 ml of this fungal dilution were added drop by drop to the soil surface of microcosm pots except the control pots. However, in case of the control pots, the same protocol was adopted with only demineralized water and agar, no fungal dilution. The whole procedure was done in sterile condition to avoid contamination.

2.2.2. Collembolan Inoculation

Collembolans were inoculated to the microcosm pots after two weeks of fungal inoculation, where the same dry mass of Collembola were inoculated in each mesocosm pot in order to minimize the biomass effect on C and N mineralization. As this experiment carried out with four different species of collembola which have different body sizes, the number of

individuals for each species to be inoculated into microcosm pots has been estimated by using an allometric regression that links body length to body mass, which is:

$$W = aL^b$$

Where, parameters, W = body dry mass (mg); L = body length (mm); and for Taxon Collembola, a = 0.1533, b = 2.3 (Ganihar, 1997).

The number of individuals per species per mesocosm (120g soil pots) as estimated shown in the Table-2. In case of the mixed species treatment where the four different collembolan species were mixed all together, the number of individuals were also estimated species wise first and then coupled them together in order to make an equivalence of collembolan biomass with the other treatment.

Table-2. Number of individuals per mesocosm pots as estimated by using the allometric regression equation.

Single species microcosm treatment				**Mixed species treatment**
Collembolan species	Average Body length (mm)	Dry body mass of a single individual (mg)	Number of Individuals per mesocosm pot	Number of Individuals per species per mesocosm pot
C. denticulata	1.2	0.08656847	319	80
F.candida	2.5	0.468279229	59	15
P. fimata	2.5	0.468279229	59	15
S. curviseta	2	0.28029275	99	25

Collembolan were counted and transferred from Plaster of Paris to microcosm soils using an entomological exhauster. All the mesocosm pots were incubated at 20°C, soil moisture content was checked periodically and the water holding capacity were adjusted to 60% by spraying demineralized water throughout the whole incubation period. To assure the collembolan are live and functioning well in all mesocosm pots, gas exchange (elevated CO_2) were measured twice following the titrimetric method during the whole incubation period.

After 2.5 months long incubation, the live collembolans were extracted out from the microcosm pots using an entomological exhauster and preserved in separate petri-dishes at 4°C. To assess the aggregates formation in each mesocosm, soils were harvested, dried at 4°C, sieved to separate the dry distribution of different aggregate fractions (e.g., >5mm, 5-3mm, 3mm-250µm and 250µm-50µm). Therefore, the different aggregate fractions were weighted and stored at 4°C in glass vials for further analysis.

2.3. Exploring the trophic interactions of Collembola

2.3.1. C and N Stable Isotope Analysis

A total of 28 soil samples which comprises of 4 replicates from each of the 7 treatments were taken from microcosm experiment pots. Soil samples were dried for 24 hours at 60°C and milled to make powder (Pollierer et. al., 2009) in order to homogenizing the soil elements, then an appropriate amount of powdered soil from each sample was transferred into the tin capsules (size: 5.9 x 9.0 mm), then weighted and stored in a desiccator.

In addition to the mesocosm soil, C and N stable isotope composition of Collembola was analysed in order to understand their feeding preferences. A total of 16 collembolan sample (comprises of 4 replicates from each of the 4 Collembolan species treatment) were transferred into tin capsules (size: 3.2 x 4.0 mm) and dried at 60°C for 48 hours, then weighted and stored in a desiccator. Depending on the different body sizes of Collembola, each tin capsule was consisted of 3 to 5 individuals which facilitates in obtaining sufficient material for ^{15}N and ^{13}C analysis.

Samples were analysed with a system combined with an elemental analyser (Flash 2000; Thermo Fisher Scientific, Cambridge UK) and a mass spectrometer (Isotopenmassenspektrometer; Thermo Electron, Bremen Germany). Then, stable isotopes (^{13}C and ^{15}N) were measured by a computer-controlled system. Isotopic signature expressed as δ, and their abundance (δX) usually expressed as:

$$X\ (‰) = (R_{sample} - R_{standard})/R_{standard} \times 1000$$

Where, R_{sample} and $R_{standard}$ represent the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of samples and standard respectively. PDB (PD belemnite) and the atmospheric nitrogen served as the primary

standard for ^{13}C and ^{15}N respectively; and for the internal calibration Acetanilide (C_8H_9NO) was used.

2.3.2. Fatty Acid Analysis

2.3.2.1. Sampling and processing the specimens

A total of 28 mesocosm soil samples that comprises 4 replicates from each of 7 treatments were taken and stored at -20°C until the starting of Fatty Acids (FAs) analysis.

2.3.2.2. Phospholipid Fatty Acids (PLFA) analysis from Soils

2.3.2.2.1. Lipid extraction and separation

An amount (4g) of each mesocosm soil samples (frozen at -20°C) were taken and FAs were extracted using the procedure described by Bligh and Dyer (1959) and modified by Frostegård et. al. (1993) by adding 18.5 ml Bligh/Dyer solvent (chloroform, methanol, citrate buffer mixed together with a ratio of 1:2:0.8; pH adjusted to 4.0) and shaking for 2 hours on a sample shaker. Further vortexed and centrifuged at 2500 rpm for 10 min at 10°C. Supernatant was then transferred using Pasteur pipette and samples were washed again with 5 ml Bligh/Dyer solvent, vortexed and centrifuged again at 2500 rpm for 10 min at 10°C. Supernatant was transferred again like before. Extracts of both steps were combined, and 6.25 ml chloroform and 6.25 ml citrate buffer were added, samples shaken properly and centrifuged at 2500 rpm for 10 min. The bottom organic phase of the solvent was collected using Pasteur pipette, then 7ml of each sample from this organic fraction were transferred into 10ml tubes and noted as defined volume for calculation. The organic fraction of each sample was then dried in the vacuum evaporator (SpeedVac; RVC 2-25, CHRIST®, Buddeberg, Mannheim) for about 30 minutes at 30°C. Products were stored immediately at -20°C until further processing.

2.3.2.2.2. Lipid fractionation

Organic phases were transferred to a silica acid column (0.5 g silicic acid, 3 ml; HF BOND ELUT) and the lipids were eluted with 5 ml chloroform (NLFAs fraction), 10ml Acetone (Glycolipids fraction) and 5ml methanol (PLFAs fraction). The chloroform phase and the Acetone phase were discarded. The methanol phase (PLFAs fraction) was dried by using a vacuum evaporator

(SpeedVac; RVC 2-25, CHRIST®, Buddeberg, Mannheim) for about 90 minutes at 30°C. Products were kept at -20°C until further processing.

2.3.2.2.3. Alkaline Methanolysis

Products of PLFAs fraction were dissolved in 1 ml methanol–toluene solvent (1:1) and 30 µl internal Standard C19:0 (5.77 mg Methyl-nondecanoate in 25 ml Isooctane) was added. Basic methanolysis of lipids was conducted in 1ml 0.2M methanolic KOH (2.8 g KOH in 250 ml methanol) with incubation in water bath for 15 min at 37°C. The Fatty Acids Methyl Esters (FAMEs) were extracted with 2 ml hexane–chloroform solvent (4:1), 0.3ml 1M acetic acid and 2 ml demonized water. Samples were vortexed and centrifuged at 2500 rpm for 10 min. The top organic phase was transferred to new tubes with Pasteur pipette, and FAMEs were reextracted with 2 ml hexane–chloroform solvent. Extraction solvents of both steps were combined and dried in the vacuum evaporator (SpeedVac; RVC 2-25, CHRIST®, Buddeberg, Mannheim) for about 35 minutes at 30°C. Products were dissolved in 100 µl isooctane and vortexed for a very short time (few seconds), then transferred into 200 µl glass vials, caped properly and stored at -20°C until analysis by gas chromatography.

2.3.2.2.4. Identification and measuring Fatty Acids

Fatty acid methyl esters (FAMEs) were analysed by gas chromatography (GC) using a Perkin Elmer CLARUS 500 GC with a flame ionization detector (FID). The FAMEs of all samples were identified by chromatographic retention times comparison with a standard mixture composed of 37 different FAMEs ranging from C11 to C24 (Sigma-Aldrich, St Louis, USA); and quantified using the Perkin Elmer Software TotalChrom Navigator (PE Nelson-Version 6.3.2, 2008).

2.4. Collembolan's behavioural traits Measurement

2.4.1. Measuring Collembolans ability to reduce fungal growth and disperse fungal spores

2.4.1.1. Collembolans Ability to reduce fungal growth

An effect of Collembolan grazing on fungi is reducing fungal biomass by eating fungal hyphae, but any sophisticated quantitative measurement about their grazing effect on fungal growth and further effects on soil aggregation is underrepresented in the scientific literatures. The ability of Collembolans to reduce fungal growth was assessed quantitatively through measuring the area of fungal hyphae spreading on a transparent surface.

Petri-dishes were prepared with Potato Dextrose Agar (PDA) culture media, then agar cores of same size with growing fungi (*Chaetomium globosum*) were taken from old culture media and placed them on petri dishes. Collembolans were added to the petri dishes and lids were closed with biofilms to avoid further contamination, then incubated at room temperature. Experimental set up was designed with five treatment sets including control one e.g., four treatment sets with four different Collembolan species (*Ceratophysella denticulata*, *Falsomia candida, Protaphorura fimata* and *Sinella curviseta*) and one as control without Collembola. A total of 20 Petri-dishes were set up that comprises 4 replicates of each treatment. In order to minimize the Collembolan biomass effect, different number of individuals per species depending on their body size were estimated using the allometric equation as explained in the section 2.2.2. Images were taken once every day with Smartphone camera (SM-A510F) from a fixed altitude of 25cm with same specification. The ability of different Collembolan species to reduce fungal growth was measured in terms of the area changes of fungal hyphae spreading as effect of collembolan grazing. Images (Figure-3) were analysed to measure the area changes of fungal hyphae spreading using ImageJ software (ImageJ Image Processing and Analysis with Java 1.8.0_112).

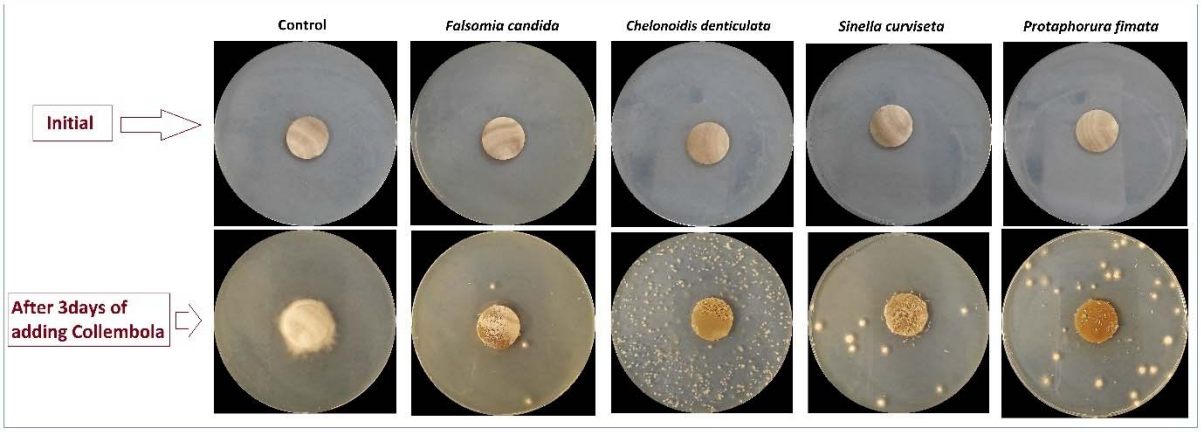


Figure-3. Collembolan grazing on *Chaetomium globosum* (cultured with Potato Dextrose Agar).

2.4.1.2. Collembolans Ability to disperse fungal spores

The ability of different Collembolan species to disperse fungal spores were investigated in laboratory-controlled situation. The relevant experimental design and set up was the same as explained in the section 2.4.1.1. The simple technique involved in comparing spore dispersal capability related to the counting of scattered spots of fungal isolates growing on agar media around the centre of the peri-dishes (i.e., feeding station) shown in the Figure-4, which facilitates us in making a proxy to quantify the fungal spores dispersed by Collembolan activity. Images were taken before and after adding Collembola to growing fungi on Potato Dextrose Agar (PDA), then new scattered spots of fungal isolates were simply counted. In order to minimize bias, the coloured spots other than *Chaetomium globosum* whitish isolates were discarded.

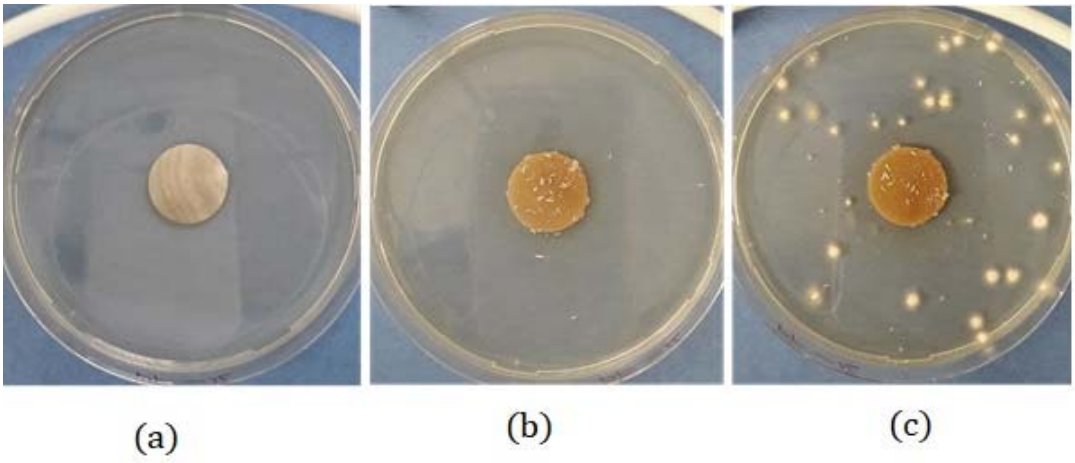


Figure-4. Collembolan ability to disperse fungal spores. (a) initial fungal growing condition before adding Collembola; (b) One day after adding Collembola; (c) Scattered spots of newly growing fungal isolates around the feeding station found after 3 days of adding Collembola.

2.4.2. Measuring Collembolans ability to transport micrometric particles

To measure the ability of different collembolan species to transport micrometric particles, we have been adopted to the Arena experiment as described in Maaß et. al. (2017) with colour sand particles. Colour sands were collected, dried and sieved to get two different fractions (ranging from 125- 250 µm and 250-300 µm). Petri-dishes were filled with a 5 mm thick layer of a mixture of Plaster of Paris and activated charcoal (3:1) and dried. A layer of particles (Source station) of 0.5 cm diameter circle at the middle of petri-dishes were created on the plaster of Paris surface using colour sand particles. For the experiment with larger sized particle fractions (250-300 µm), 10 mg of colour sands were put at the source station, while 5 mg sands for the experiment with smaller sized particles fractions (125- 250 µm) were put at the source station; which corresponds to the amount of particle needed to evenly cover the Source stations. Experiments were set up without providing any additional food.

Four different Collembolan species (*Ceratophysella denticulata*, *Falsomia candida, Protaphorura fimata* and *Sinella curviseta*) having different body size were used in this experiment. We added 25 individuals for each Petri-dish except the controls, and 5 replicates for each combination were set up which resulting a total of 50 samples including controls. To avoid the distribution of particles by airflow, we placed all the total system in such a place

that was not subjected to any disturbances, moreover, lids were closed very carefully to all the Petri-dishes. Further, incubated at room temperature for 7 days, and images were taken for each Petri-dishes once a day with Smartphone camera at an altitude of 25 cm. In order to facilitate counting the number of particles transported by Collembola through image analysis, four concentric circles of 1, 2, 3 and 4 cm diameter were placed around the source station and the number of particles was counted in each ring (Figure-5).

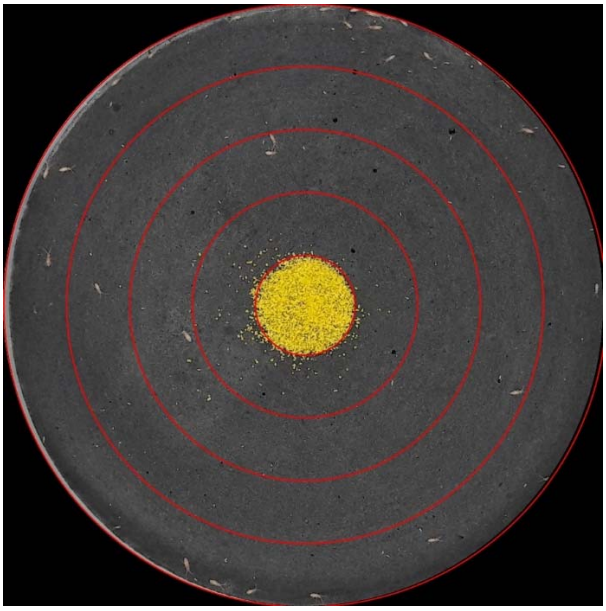


Figure-5. The concentric rings of 1,2,3,4cm diameter placed around the feeding station, and the displaced particles in each ring outside feeding station.

2.5. Measuring aggregate's physical stability in water

2.5.1. Slow wetting technique

In order to measure the aggregates physical stability, macroaggregates fractions (3-5mm) were sieved and initially dried for 24 hours at 40°C, then weighted between 5 to 10 g of dry aggregates. The method proposed by Le Bissonnais (1996) was used, which composed of three tests (fast wetting, slow wetting, and shaking after pre-wetting). However, the slow wetting test was performed because it is less disruptive comparing to the other tests, and

especially provide the behaviour of soils structural stability of those are not subjected to heavy disruptive events. A sponge was submerged in bucket of water in such a way so that the bucket water level reaches up to 0.5 cm below the top surface of sponge. Filter papers were disposed on the sponge, then dry soil aggregates were placed on the filter paper once it becomes wet. Due to the hygroscopic nature, soil aggregates absorb water from the filter paper. Once all the aggregates on filter paper becomes wet, then the aggregates were transferred to a 50µm sieve which is immersed in ethanol to prevent the further breakdown of aggregates, and 5 cycles of helicoidal movement applied to the sieve very gently. After washing in ethanol, the soil aggregates were dried in vials under the air flow in order to remove the ethanol. Further, dried in the woven for 24 hours at 40°C.

2.5.2. Measuring aggregates stability

Stable soil aggregates were then subjected to sieve through six sieves column (2.00, 1.00, 0.5, 0.2, 0.1 and 0.05 mm) and weighted again with seven different aggregate diameter size classes (i.e., >2mm, 2-1mm; 1- 0.5mm; 0.5 -0.2mm; 0.2 - 0.1mm; 0.1 – 0.05mm; and <0.05 mm) after disaggregation as explained in Le Bissonnais (1996).

To measure the stable aggregate, the Mean Weight Diameter (MWD) of aggregates was calculated by the following formula:

$$MWD = \frac{\sum_{i=1}^{i=7} Mi.Di}{\sum_{i=1}^{i=7} Mi}$$

Where, total number of diameter classes is 7 (i= 1- 7), ***Di*** represents the central diameter of each class size, where ***Di*** for two extreme classes (i.e., >2mm and <50µm) are 3.5 and 0.025 respectively (according to the norm NF X 31–515 2005). ***Mi*** is the mass (g) of stable soil aggregates after applying slow wetting technique within each diameter class.

As initially the mesocosm soils used in this study were mixed with sands between 50µm -2mm size, the aggregates fraction between 1 – 2 mm contained some course sands those were corrected by removing the mass of coarse sands. To avoid over estimation, the MWD was also corrected for the primary particle of the same size, i.e. fine sand particles (50µm < fine sand <1mm), according to the method described in Lal and Schukla (2004), as follows:

$$\%\ stable\ aggregate\ on\ each\ sieve = \frac{(weight\ retained) - (weight\ of\ the\ sand\ fraction)}{(\text{total sample weight}) - (\text{weight of sand})}\ \text{X}\ 100$$

2.6. Statistical Analysis

Data on soil aggregation including different Collembolan behavioural trait effects, C and N isotopic signature values acquired from stable isotope analysis, and water stable aggregates quantity of different sets of treatment were subjected to analysis of variances (ANOVA) in order to determine significant difference between different treatment sets. Normality of residuals and the equality of the variances were tested using ***Shapiro–Wilk test*** and ***Levene test*** respectively, then followed by a ***Post-hoc test*** (Turkey HSD post-hoc test) to see the pairwise differences between treatment sets. In case of the non-normal residuals and/or inequality of the variances, we relied on the ***Kruskal-Wallis*** non-parametric test, following a pairwise comparison between groups using paired ***t-test***. The statistical analysis was performed using ***R package*** (version R 3.5.0.).

For soils Fatty Acids (FAs) percentage values were subjected to arcsine-square root transformation prior analysis in order to achieve normal distribution of data. For fatty acids profile using Discriminant Function Analysis (DFA), dimensions of the data were reduced by using nonmetric multidimensional scaling (NMDS). Differences between the individual fatty acids in each treatment were subjected to multivariate analysis. Analysis was conducted using STATISTICA 10 (Stat Soft Inc. 2011, Tulsa, USA).

CHAPTER-III: RESULTS

3.1. Collembolan's influence on Soil aggregation

3.1.1. Dry distribution of soil aggregates

Results indicate that, there is significant differences between treatments in terms of aggregates dry distribution with different size classes between control and other treatments (Figure-6). In case of bigger aggregate fractions (above 5 mm size), the control treatment indicating lower amount of aggregates than the treatments with fungi and Collembola (Figure-6a, 6b). On the other hand, control treatment showing higher quantities of aggregates of relatively smaller fractions (<3mm) than other treatments with fungi and Collembola (Figure-6c, 6d).

Figure-6(a) indicates that, there is no significant (TukeyHSD; P>0.05) difference between control (C) and the treatment with only fungi (F) in case of bigger aggregates formation. Pairwise difference was not significant (TukeyHSD; P>0.05) also between treatments with different Collembolan species, which means all the Collembolan species used in this present study (*Ceratophysella denticulata*, *Falsomia candida, Protaphorura fimata* and *Sinella curviseta*) have almost same influence on relatively bigger aggregate (>5mm) formation. However, their mixed effects (FMIX) of four Collembolan species together have less influence on bigger aggregate formation comparing to the other treatments with single Collembolan species (Figure-6a); but a reverse effect has been evident in case of relatively smaller aggregates (<3mm) as shown in the Figure-6c and 6d. In terms of inter-species performance comparison, *Falsomia candida* and *Sinella curviseta* have shown the higher influences on soil aggregate formation in case of the relatively bigger (>3mm) aggregate fractions (Figure-6a and 6b), while the mixed species treatment has shown higher influences on relatively smaller (<3mm) aggregate formation comparing to the other treatment with single Collembolan species (Figure-6c and 6d).

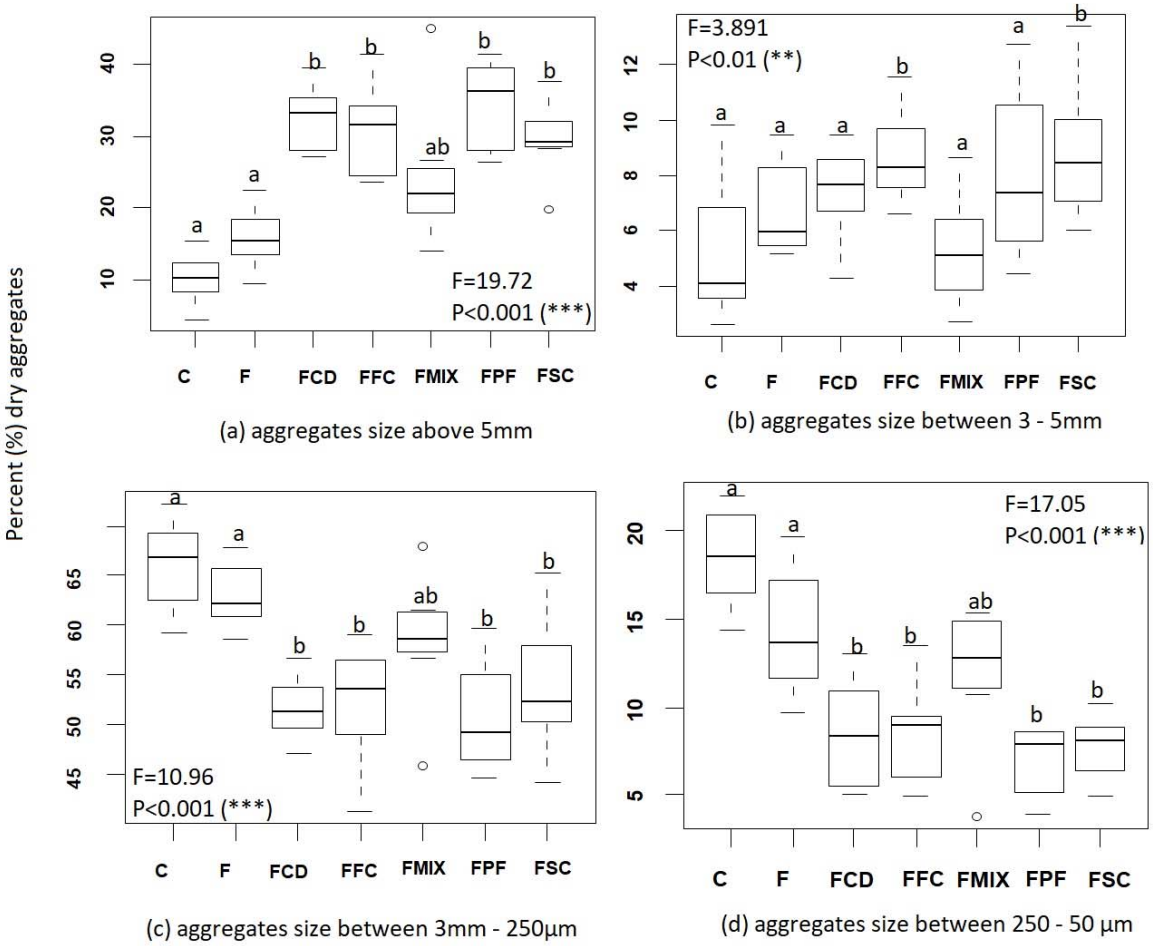


Figure-6. Dry distribution of soil aggregates with different aggregates class size. (a) Aggregates fraction sized above 5mm (>5mm); (b) Aggregates fraction sized between 5 – 3mm; (c) Aggregate fractions sized between 3mm - 250µm; (d) Aggregate fractions sized between 250 – 50µm. Block letters along the horizontal axis indicates the different treatment name (i.e., **C** = control treatment; **F** = treatment with only fungi; **FCD** = treatment with fungi and Collembola *C. denticulata*; **FFC** = treatment with fungi and Collembola *F. candida*; **FPF** = treatment with fungi and Collembola *P. fimata*; **FSC** = treatment with fungi and Collembola *S. curviseta*; **FMIX** = treatment with fungi and mixture of four Collembolan species), and the corresponding number along the vertical axis represents the percentage of aggregate's dry distribution. Letters above bars indicates significant difference (P≤ 0.05) between treatment means.

3.2. Collembolan's functional behaviour (Traits)

3.2.1. Effects of Collembolan grazing on fungal growth

Collembolan grazing have distinct and suppressing effect on *Chaetomium globosum* growth. All of the four Collembolan species studied have shown a rapid decrease in radial expansion of fungal hyphae coverage (Figure-7). The fungi *Chaetomium globosum* have shown a linear tendency to expanse its hyphal coverage over time in control treatment. On the other hand, the different Collembolan species showing different grazing effect on fungal growth performances, where, Collembola *C. denticulate* and *P. fimata* grazing showing highest decreasing effect on fungal growth at all time comparing to the other species. Figure-7 indicating significance difference between different Collembolan species and control treatment in terms of decreasing fungal hyphae coverage after 2 days of adding collembola (chi-squared = 15.678, P < 0.01).

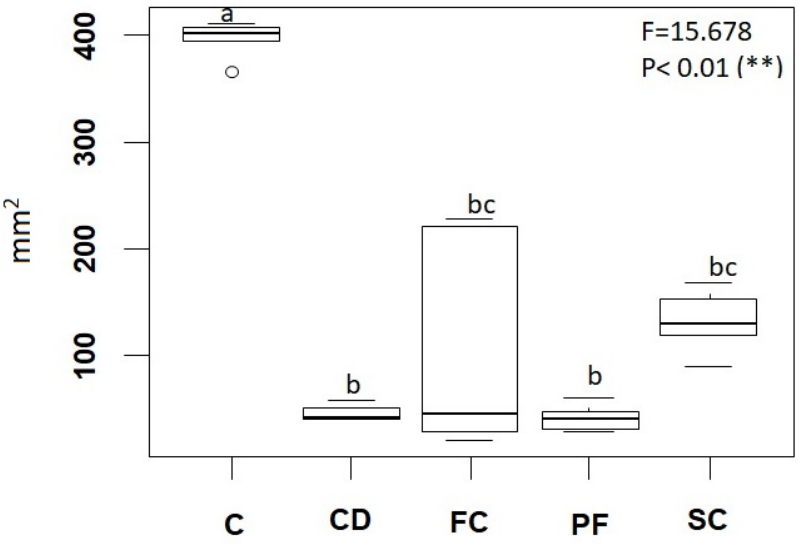


Figure-7. Effect of Collembolan grazing on radial expansion of *C. globosum* hyphal coverage. Vertical axis represents the area of fungal hyphae coverage (in mm^2), Letters along the horizontal axis denotes the treatment group with different Collembolan species (i.e., **C** = Control; **CD** = *C. denticulate*, **FC**= F. candida; **PF**= P. fimata; **SC**= S. curviseta). Different letters above bars indicates significant difference ($P \leq 0.05$) between treatment means.

3.2.2. Fungal spore dispersal by Collembolan activity

Collembolan activity on fungi showed significant (F = 35.93, P < 0.001) ability to disperse fungal spores (Figure-8). No scattered spots of fungal isolates were found around the feeding stations in control treatment, but several new scattered spots of fungal isolates were counted from the treatment samples after 3 days of adding Collembola which represents as a proxy of the spores dispersed. There is also a significant difference between species in terms of their ability to disperse fungal spores (Figure-9), where the highest mean of scattered spots of fungal isolates found in the treatments with *P. fimata.* Rest of the three Collembolan species *(C. denticulate, F. candida, S. curviseta)* did not shown any significant differences (Tukey HSD, P > 0.05) in terms of their ability to disperse fungal spores.

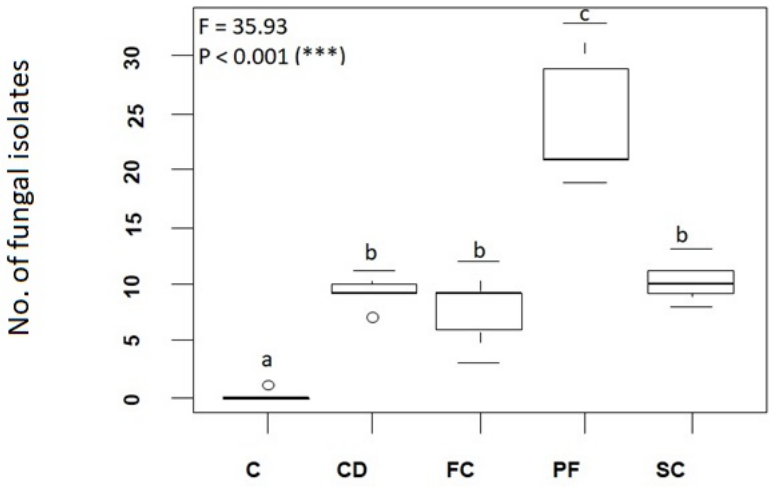
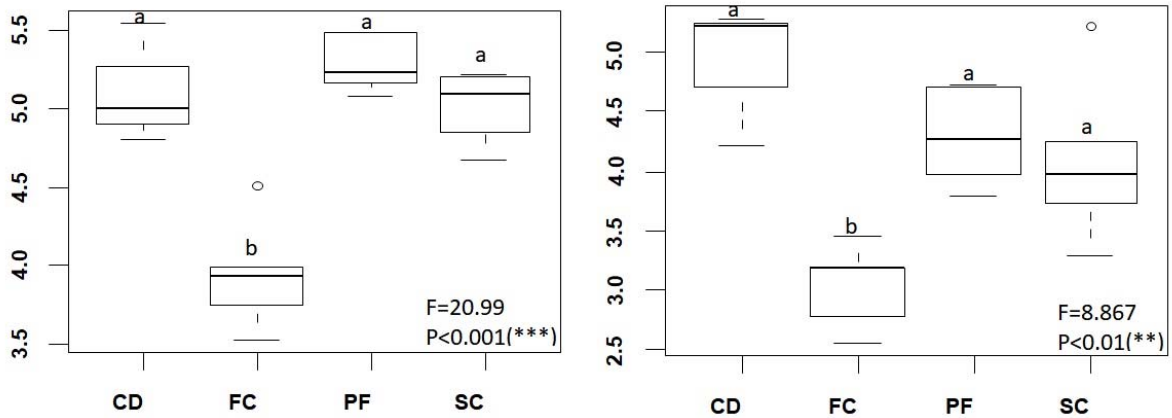


Figure-8. Collembolan ability to disperse fungal spores. Different Collembolan species denoted with block letters (i.e., **C** = Control; **CD** = *C. denticulate*, **FC**= *F. candida*; **PF**= *P. fimata*; **SC**= *S. curviseta*) along the horizontal axis and corresponding number of scattered spots of fungal isolates growing around feeding station of treatment dishes were plotted along the vertical axis. Different letters above bars indicates significant difference (P ≤ 0.05) between treatment means.

3.2.3. Ability to transport micrometric particles

For both size classes of the sand particles, result shows significant difference between species (for particles size class 125-250µm: F= 20.99, P < 0.001; for particles size class 250-300 µm: F= 8.867, P < 0.01) as well as between rings (for particles size class 125-250 µm: F= 61.98, P < 0.001; for particles size class 250-300 µm: F=23.55, P < 0.001) which is the distance the particles were transported by Collembola. *Falsomia candida* has shown less performance in both particle size classes comparing to other Collembolan species in terms of their ability to transport micrometric particles (Figure-9). No significant difference has been found between *C. denticulate*, *P. fimata* and *S. curviseta* in terms of their ability to transport particles; although they have shown their higher ability to transport particles of both size classes. However, the highest performance in transporting particles of size class of 125-250 µm is shown by *P. fimata*; and *C. denticulata* as well in case of particle size class of 250-300 µm (Figure-9a, 9b).



(a) Particles size class: 125-250 µm

(b) Particles size class: 250-300 µm

Figure-9. Comparison between different Collembolan species in terms of their ability to transport particles. (a) Comparison based on particle size class of 125-250 µm; (b) Comparison based on particle size class of 250-300 µm. Different Collembolan species denoted with block letters (i.e., **CD** = *C. denticulate*, **FC**= *F. candida*; **PF**= *P. fimata*; **SC**= *S. curviseta*). Different letters above bars indicates significant difference (P ≤ 0.05) between different species ability to transport particles.

Amount of sand particles transported by Collembola have shown significant difference between different rings (i.e., the horizontal distance) for both size classes of particles (Figure-10). Collembolans have shown their ability to transport particles to a very short distance which also depends on the particle size. Results shows that, relatively smaller sized particles (i.e., particles size between 125-250 µm) are being more dispersed by Collembola comparing to the relatively larger sized particles (i.e., particles size between 250-300 µm).

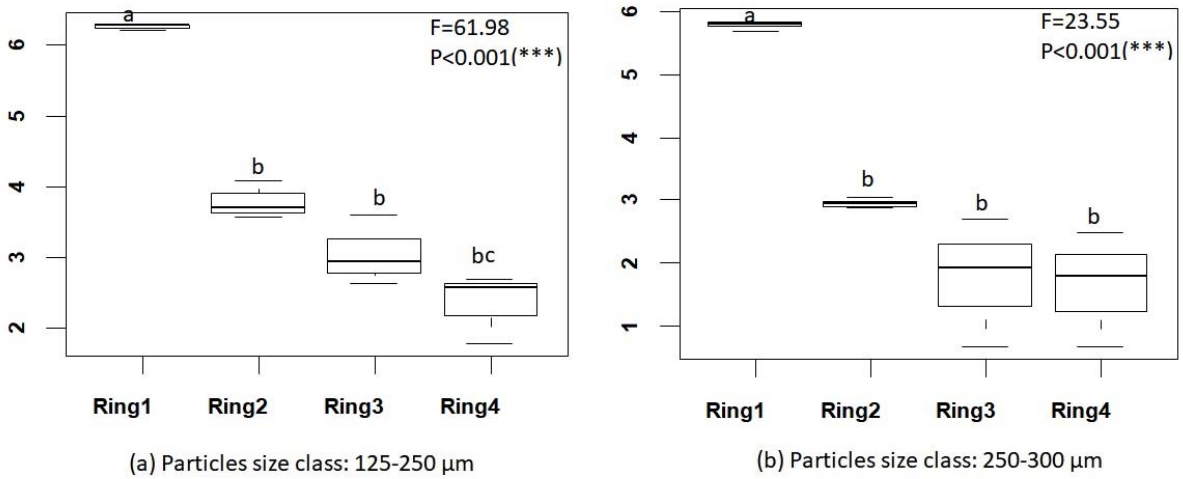


Figure-10. Amount of sand particles transported to different rings (horizontal distance) by Collembola. (a) particle size class of 125-250 µm; (b) particle size class of 250-300 µm. Different horizontal distances are indicated by the rings with different diameter (i.e., Ring1 = 1 cm diameter, Ring2 = 2cm diameter, Ring3 = 3cm diameter, Ring4 = 4cm diameter).

3.3. Trophic Resources for Collembola

3.3.1. Stable isotope signature of potential food resources and Collembola

Mean $\delta^{13}C$ values in different microcosm treatment soils are ranging from -12.58 ‰ to -15.61 ‰, where the lowest value (-12.58 ± 2.80 ‰) in soils with mixed Collembolan species and the highest value is in the soils with *Sinella curviseta which is* -15.61 ± 1.69 ‰; and the mean $\delta^{15}N$ values are ranging from 5.17 ‰ to 6.46 ‰ (Table-3).

Table-3. Mean signature values with standard error (± sd) for C and N stable isotope found in different microcosm treatment soils.

Microcosm Soils Types	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**
C	-13.79 ± 0.85	6.46 ± 1.11
F	-12.81 ± 2.55	5.60 ± 0.93
FCD	-14.00 ± 0.87	6.18 ± 0.35
FSC	-15.61 ± 1.69	5.17 ± 1.65
FFC	-14.06 ± 2.73	5.80 ± 0.31
FPF	-14.03 ± 2.16	5.42 ± 0.69
FMIX	-12.58 ± 2.80	5.91 ± 0.94

In contrast to the soil samples, mean $\delta^{13}C$ and $\delta^{15}N$ values are also varying with regards to the different Collembolan species, where $\delta^{13}C$ ranging from -19.80‰ to -21.85‰ and $\delta^{15}N$ ranging between 2.01‰ to 5.62‰ (Table-4). *Falsomia candida* have shown as to be the most enriched in $\delta^{13}C$ signature ($\delta^{13}C$ = -19.80 ‰) and at the same time most depleted in $\delta^{15}N$ signature ($\delta^{15}N$= 2.01 ‰). On the other hand, *Sinella curviseta* is the most depleted in $\delta^{13}C$ but highly enriched in $\delta^{15}N$ signature ($\delta^{13}C$= -21.85 ‰, $\delta^{15}N$= 2.01 ‰). There is no significant difference in $\delta^{13}C$ signature values between Collembolan species (chi-squared = 6.9044, p-value = 0.07501); however, the $\delta^{15}N$ signature values differs significantly between Collembolan species (F= 6.416, P= 0.0077) as shown in the Figure-11.

Table-4. Mean signature values with standard error (± sd) of C and N stable isotope ($\delta^{13}C$ and $\delta^{15}N$) in different Collembolan species.

Collembolan species	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**
Ceratophysella denticulata	-21.80 ± 0.25	5.11 ± 0.79
Falsomia candida	-19.80 ± 0.98	2.01 ± 1.44
Protaphorura fimata	-21.61 ± 4.70	2.91 ± 2.03
Sinella curviseta	-21.85 ± 0.38	5.62 ± 0.82

It is evident that, Collembola did changes slightly on isotope composition of substrate (soil $\delta^{13}C$ ranges between -12.58 ‰ to -15.61 ‰; Collembola $\delta^{13}C$ ranges between -19.80‰ to -21.85‰). Therefore, it is indicated that, different Collembola species rely on the similar basal resources in the mesocosms. Collembolan $\delta^{15}N$ signature values (Table-4; Figure-11) indicates that, different Collembola species occupy different trophic levels as the $\delta^{15}N$ values differs significantly (Figure-11), which ultimately indicating different feeding preferences of different Collembolan species.

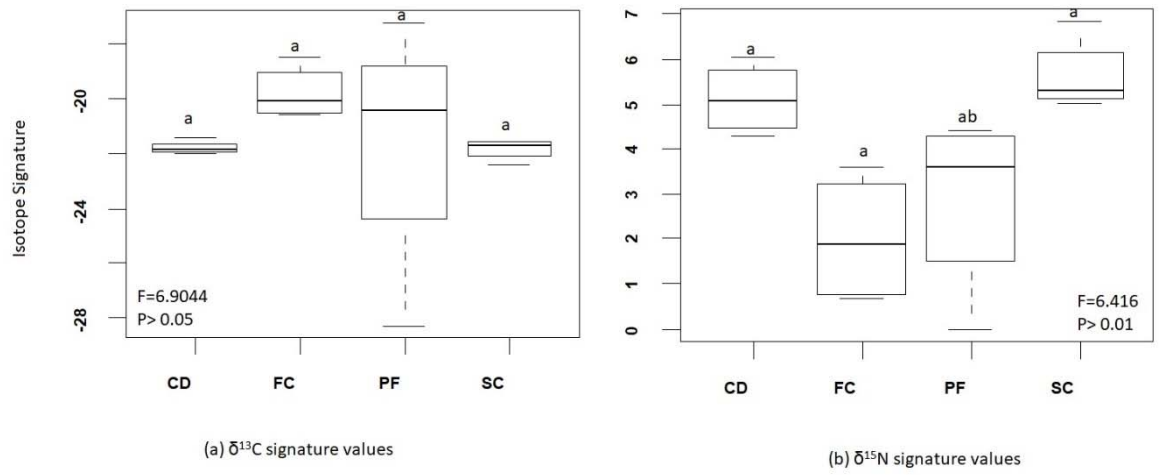


Figure-11. Differences in $\delta^{13}C$ and $\delta^{15}N$ signature between different Collembolan species.

3.3.2. Origin of potential trophic resources for Collembola (PLFAs composition)

A total of 36 fatty acids were extracted from the microcosm soils with a chain length ranging between C14 and C24 in Phospholipid fractions. Some of them were not included in further analysis because of their presence only in few replicates (less than 1%), and only 25 fatty acids (Figure-12; supplementary table-1) were taken into further analysis. 9 fatty acids (i15:0, a15:0, 15:00, i16:0, i17:0, cy17:0, cy19:0, 16:1w7 and 17:00) out of 25 left belongs to bacterial origin (Frostegård and Bååth, 1996; Kujur and Patel, 2014); two (16:1W5, 18:2w6,9) fatty acid represents fungal origin (Frostegård and Bååth, 1996; Kujur and Patel, 2014; Ruess et. al., 2007); three (22:0, 24:0 and 16:0) represents plant origin (Volkman et. al., 1980), where 16:00 also found mostly in wheat (Canada Nutrient File, 2010; Food code: 4456). Total amount of PLFA (nmol/gDW) from different microcosm treatment soils differ much (lowest amount in Control treatment soils: 30.16 ± 7.77; and the highest amount found in soils from the treatment with *Falsomia candida* which is: 80.42 ± 18.74).

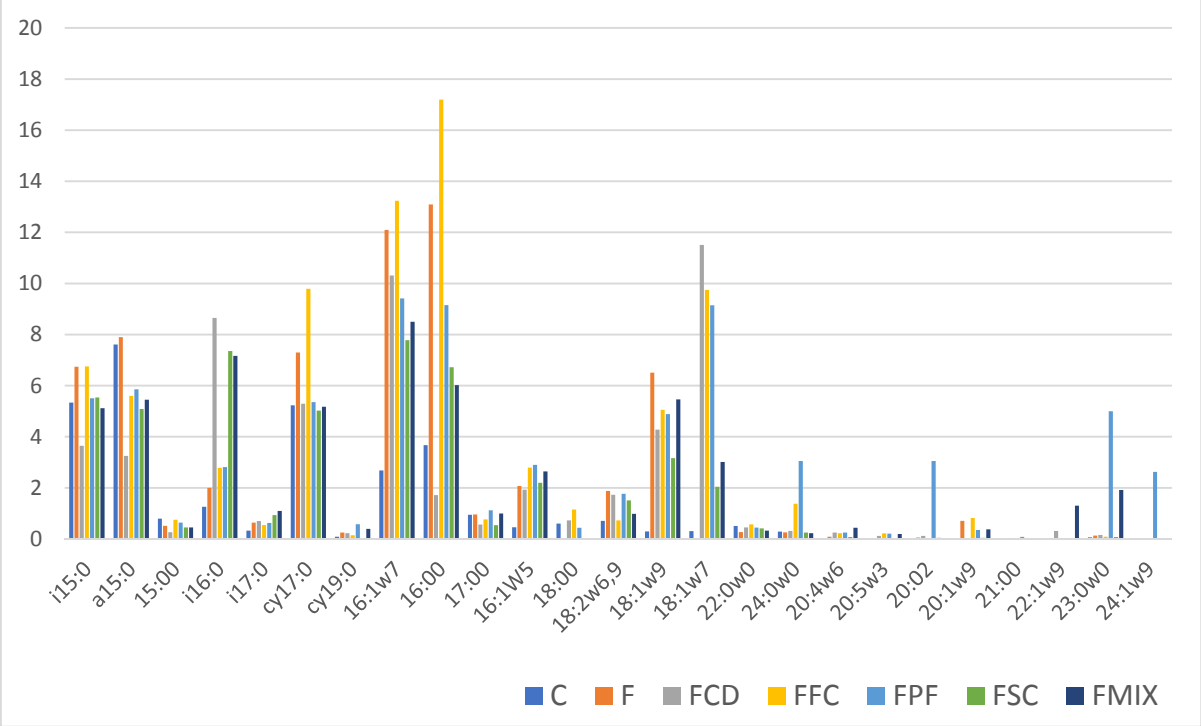


Figure-12. Amount (nmol/gDW ± S.D.) of different Phospholipid Fatty Acids (PLFA) in soils from different microcosm treatments.

The discriminant function analysis (DFA) results shows significant (p-value <0.0039) differences in PLFA composition between different microcosm treatment soils (Figure-13).

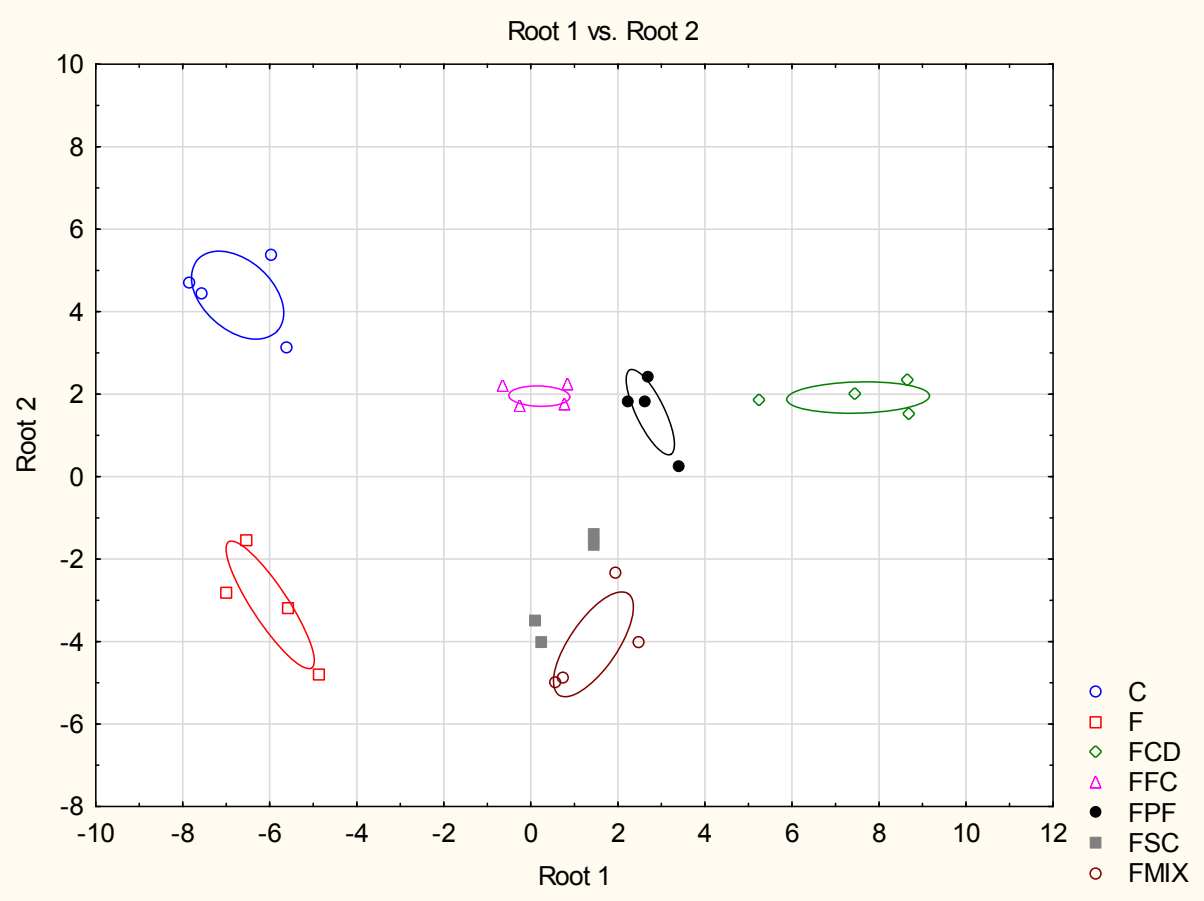


Figure-13. Discriminant Function Analysis (DFA) of the PLFA composition of soils from different microcosm treatment. Ellipse represents confidence intervals at p < 0.0039. Here, "**C"** represents Control treatment; "**F"** for treatment with only fungi; **"FCD"** for treatment with fungi and Collembola *Ceratophysella denticulata*; **"FFC"** for treatment with fungi and Collembola *Falsomia candida*; **"FPF"** for treatment with fungi and Collembola *Protaphorura fimata*; **"FSC"** for treatment with fungi and Collembola *Sinnella curviseta*; **"FMIX"** for treatment with fungi and a mixture of four Collembolan species.

Table-5. Squared Mahalanobis Distances between different groups of microcosm treatment soils makes reliability of discrimination for PLFA composition. (***P<0.0001; **P<0.01; *P<0.05).

Type	C	F	FCD	FFC	FPF	FSC	FMIX
C	-	68.47	213.62**	76.09	107.37*	109.14*	140.26 ***
F		-	211.50**	75.27	105.13*	58.25	66.52
FCD			-	71.89	33.69	72.18	78.70*
FFC				-	21.93	38.16	52.18
FPF					-	29.67	5.45
FSC						-	5.45
FMIX							-

3.4. Aggregates physical stability

Result shows significant difference (Chi-squared= 14.044; $P < 0.05$) in Mean weight diameter (MWD) distribution between water stable aggregates from different microcosm treatment soils (Figure-14; Table-6). The highest MWD belongs to the aggregates from mesocosm treatment with fungi (F). The MWD of water stable aggregates from the treatment with only fungi have shown a significant difference ($P < 0.05$) in paired t-test; and rest of the other treatments didn't show any significant difference with the control treatment.

Table-6. Mean weight diameter (MWD) distribution of water stable aggregates from different microcosm treatment soils (with ± sd).

Mean weight diameter (MWD) distribution of water stable aggregates							
Treatment	**C**	**F**	**FCD**	**FFC**	**FPF**	**FSC**	**FMIX**
MWD	0.48 ± 0.04	0.66 ± 0.03	0.85 ± 0.23	0.57 ± 0.03	O.57 ± 0.03	0.59 ± 0.04	0.52 ± 0.03

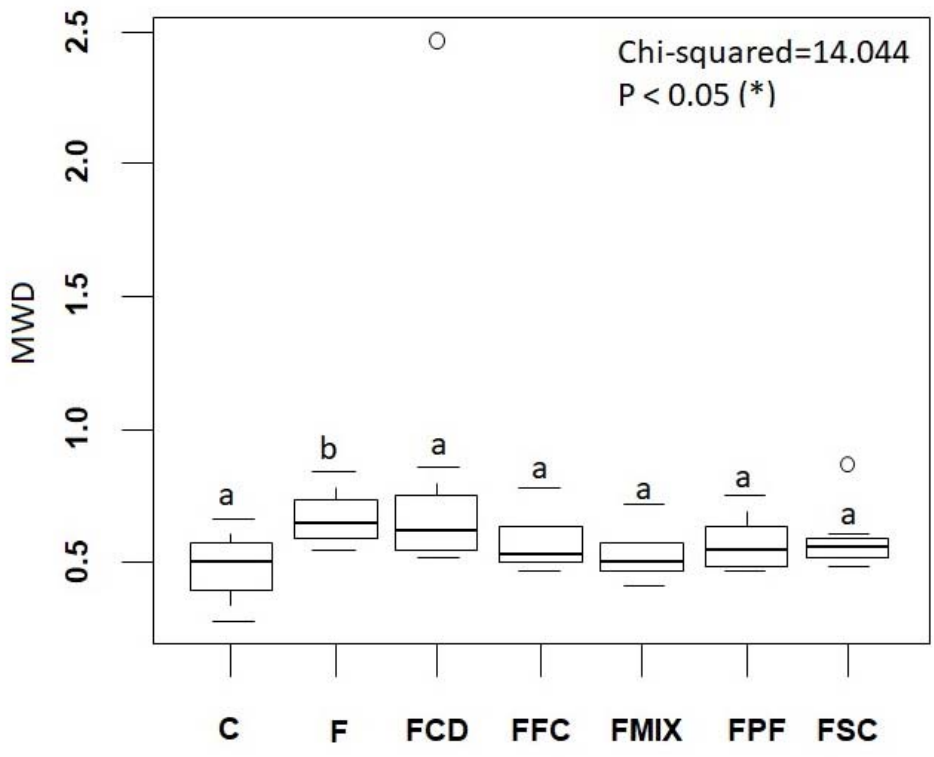


Figure-14. Difference in Mean weight diameter (MWD) of water stable aggregates from different microcosm treatment soils. “C” represents Control treatment; “F” for treatment with only fungi; “FCD” for treatment with fungi and Collembola *Ceratophysella denticulata*; “FFC” for treatment with fungi and Collembola *Falsomia candida*; “FPF” for treatment with fungi and Collembola *Protaphorura fimata*; “FSC” for treatment with fungi and Collembola *Sinnella curviseta*; “FMIX” for treatment with fungi and a mixture of four Collembolan species. Letters above bars indicates significant ($p < 0.05$) difference between treatment.

CHAPTER-IV: DISCUSSION

4.1. Trophic interactions between Collembolans

Feeding preferences of different Collembolan species has been revealed by using C and N stable isotope ratios in combination with soils fatty acids composition. In general, $\delta^{15}N$ values usually indicates the trophic level of an organism, whereas $\delta^{13}C$ values give insight about the basal resources (Tiunov, 2007; Perkins et al., 2014). The $\delta^{13}C$ signature values are generally negative in terrestrial ecosystems and range between −35 and−10‰, whereas $\delta^{15}N$ signature values usually ranges between −10 and +15‰ (Potapov et. al., 2018). The present study of mesocosm soils with different combination of saprotrophic fungi and Collembola have shown almost similar basal resources conditions where the $\delta^{13}C$ values ranging between -12.58 to -15.61 ‰. This reasons for such less negative $\delta^{13}C$ values of the substrate (mesocosm soils) is the soils used for this microcosm experiment comes from the Wheat field (C4 plant) and the litter that used was also a mixture of wheat and maize, which ultimately leading to soils with less negative $\delta^{13}C$ value ranging between -12.58 ‰ to -15.61 ‰. The plants differ much in C assimilation even within ecosystem. In general, this difference is between C4 and C3 plants in terms of their carbon fixation pathways. The underlying fact as explained by Farquhar et al. (1989) is that, “the C4 plants use enzymes (PEP carboxylase) that fractionates carbon isotope much less than C3 plants those use RuBisCO (ribulose bisphosphate carboxylase oxygenase)”, which ultimately leading to have them higher $\delta^{13}C$ value in microcosms soils. Plants litters, organic matters coming from these C4 plants have generally higher C isotope value which ranges between −9‰ to −17‰ with a mean value of −13‰ (Farquhar et al., 1989).

However, $\delta^{13}C$ values in the mesocosm soils (Table-3) also indicating the Collembola have changed slightly the decomposition processes via modifying microbial composition comparing to the Control treatment, which is better understood with the total PLFA composition of different treatment. All the mesocosm treatments with Collembolan have shown a significant increase in total PLFA composition (nmol/gDW) of soil comparing to Control treatment (Supplementary Table-1), which partially fulfil the hypothesis-1. The $\delta^{15}N$ in Collembola differs greatly (Table-4) which indicating their difference in feeding preferences. Taking in consideration an enrichment of $\delta^{15}N$ by 3 ‰ per trophic level

(Minagawa & Wada 1984), two different feeding guilds have been distinguished (Table-7), this statement also partially fulfils the hypothesis -2.

Table-7. Schematic representation of Collembolan feeding guilds and possible trophic resource preferences, indicated by their C and N isotope ratios.

Collembola	**$\delta^{13}C$**	**$\delta^{15}N$**	**Feeding guilds**	**Trophic resources**
Ceratophysella denticulata	-21.80 ± 0.25	5.11 ± 0.79	'Browser' or secondary decomposers	feeding most likely microorganisms, fungi etc.
Sinella curviseta	-21.85 ± 0.38	5.62 ± 0.82		
Falsomia candida	-19.80 ± 0.98	2.01 ± 1.44	'Grazers' or Closer to primary decomposers	Mainly feeding on litter/detritus with adhering bacteria and fungi also.
Protaphorura fimata	-21.61 ± 4.70	2.91 ± 2.03		

Ceratophysella denticulata and *Sinella curviseta* feeding selectively on microorganisms (e.g. 'browsers' or 'secondary decomposers'), predominantly feeding on microorganisms and fungi. PLFA composition also shown higher content of fungal marker fatty acid 18:2w6,9 (Supplementary Table-1). *Folsomia candida* and *Protaphorura fimata* grazing on soil/litter mixed with microorganisms (e.g. 'grazers', closer to 'primary decomposers'), most likely on litter/detritus with adhering fungi and bacteria. High proportion of Hexadecanoic acid (16:0), Docosanoic acid (22:00) and Tetracosanoic acid (24:0) found in PLFA composition also indicating the same possibility. Fiera (2014) have identified Collembolan feeding strategies in Romanian beech forest ecosystem stable isotope signature and fatty acids compositions in soils, where it has been speculated that different Collembolan species can occupy different feeding guilds. However, Collembola feed very unselectively which is indicated by their gut contents analysis as it represents a random selection of the components of their environment

(Fiera, 2014). However, the opportunistic feeding behaviour of many Collembolan species may be the reason of their success (Hopkin 1997). Collembola *Protaphorura fimata* have shown a different feeding behaviour comparing to the other species. Some animal fatty acids (20:2, 18:0) was found highly comparing to other species. Previous study also found *Protaphorura fimata* to occupy different feeding guilds (Feira, 2014).

4.2. Collembolan functional behaviour (Traits)

4.2.1 Collembolan ability to reduce fungal growth

Collembolan contribution to ecosystem is little comparing to other larger soil animals due to their small size, however, they contribute to the most types of ecosystems. Collembolan effects usually occurs through their grazing on fungi which indirectly influences on the decomposition process (Hopkin, 1997). Most of the Collembolan species graze on fungal hyphae, but it is still nearly unknown that how different Collembolan species can reduce fungal growth through grazing on fungal hyphae. The present study showed evidence for direct quantitative measurement on different Collembolan ability to reduce fungal growth with significant difference (Figure-7). *Protaphorura fimata* and *Ceratophysella denticulata* have reduced radial expansion of fungal hyphae coverage very rapidly comparing to comparing to *Falsomia candida* and *Sinella curviseta* (Figure-7). The possible explanation of this mechanism would be the higher feeding preferences on fungal hyphae of these two species (*Protaphorura fimata* and *Ceratophysella denticulata*) comparing to the others.

4.2.2. Collembolan ability to disperse fungal spores

While the Collembolans are being evident to have a negative effect on fungal growth, it is important to know how they contribute in maintaining fungal population. One important mechanism is their ability to disperse fungal spores by their activity. Previous studies have acknowledged that, Collembolans are able to disperse arbuscular mycorrhizal fungi (AMF) inoculum to uncolonized parts of soil both horizontally and vertically (Fitter and Sanders, 1992; Klironomos and Moutoglis, 1999; Gange, 2000; Dromph, 2001). Previous evidence has shown that sores can also be dispersed through Collembolan fecal pellets (Moore et al. 1987; Willium et al. 1998). It has been evident by present study that, fungal spores are being

attached to their cuticle during grazing on fungal hyphae and being dispersed by their movement. Whipps and Budge (1993) already showed that “Collembolan are able to carry viable spores on the cuticle or in the gut”. The comparison between species used highlighted that, *Protaphorura fimata* have shown to be highly capable in terms of their ability to disperse fungal spores, while rest of the three species (*Ceratophysella denticulata*, *Falsomia candida* and *Sinella curviseta*) has been evident with the same and lesser capability comparing to Protaphorura *fimata* (Figure-8).

4.2.3. Collembolan ability to transport micrometric particles

While relatively larger soil animals (e.g., earthworms, termites, ants) are being studied in terms of transport of particles (Anderson, 1988; Benckiser and Schnell, 2007), the highly abundant group of Collembolan are being neglected due to their small size. First evidence was revealed by Maaß et. al. (2015) on Collembolan in terms of transport of microplastic particles. Assuming their effect on soil aggregation by micrometric soil particles, study have been carried out to understand their ability to transport micrometric particles. It has also been evident by this present study that, Collembolan are able to transport micrometric sized particle only because of their small body size. In comparison between the four Collembolan species used for this experiment, *Falsomia candida* has shown to be the least capable to transport micrometric particles comparing to rest three species (Figure-10), although *Falsomia candida* is the slow moving Collembola among the all species studied. This is also indicating that the particles transport capability is related to their movement. In terms of the distance that particles transported by Collembola, it has been evident that Collembola can transport particles at a distance to few centimetres, and the smaller the particles the higher distance transported.

4.3. Effects of Collembolan on soil aggregation

A significant increasing effect of Collembolan has been observed on soil macro-aggregation (Figure-6a, 6b), while in case of the micro-aggregate formation no such significant was evident (Figure-6c, 6d). One of the possible reason behind this discrepancy is that the smaller aggregate fractions (3 mm - 50µm) contains a high amount of non-aggregated soils as we initially set up our microcosms experiment with soils sieved fractions between 1mm - 50µm sized. Soil macro-aggregation in the microcosm treated with only fungi didn't differ significantly from the control treatment that has been shown in all cases (micro and macro-aggregation). This statement also did supports the previous studies (Caruso et al. 2011; Lussenhop, 1992) that Collembola increases soil aggregation. Therefore, it has been proved the hypothesis-1 that, Collembolan activities have changed mesocosm soils microbial compositions which affects soil physical structure (aggregation). Siddiky et.al. (2012) have shown the positive influence of Collembola on soil aggregation in such cases where they co-occur with arbuscular mycorrhizal fungi (AMF), although, the Collembola do not prefer feeding on AMF hyphae (Gange, 2000).

In comparison between different Collembolan species, no significant difference was found between species in case of the aggregates size above 5 mm (Figure-6) although all species have increased aggregation, however, for the aggregates size between 3 – 5 mm, *Falsomia candida* and *Sinella curviseta* have shown significant increases in soil aggregate formation comparing to *Ceratophysella denticulata* and *Protaphorura fimata*. As overall comparison in terms of total dry distribution (%) of soil macro-aggregates, *Ceratophysella denticulate* and *Protaphorura fimata* possess higher increasing effect on soil macro-aggregation (Supplementary Table-2). However, stable isotope signature indicating that these two species inhabit two different feeding guilds as discussed in the section 4.1. (Figure-11b). Although, all the four Collembolan species feeds on fungi, however, *Protaphorura fimata* predominantly feeding on primary decomposers e.g., fungi, litter/detritus with adhering bacteria; while *Ceratophysella denticulata* feeding most likely on secondary decomposers e.g., microorganisms, fungi etc.

The effects of Collembolan feeding strategies including their functional behavioural traits on soil aggregation is summarized in the Table-8. It has been evident that, Collembolan those are predominantly feeding on litter/detritus (with less grazing effect on fungal growth) have

increased soil aggregation via decomposition of materials (e.g., *Falsomia candida*). However, having the same feeding guild with higher effect on reducing fungal growth *Protaphorura fimata* has shown less influence on soil aggregation, this species reducing fungal hyphae branching which anchor the soil particles. Comparing to *Ceratophysella denticulata, Protaphorura fimata* have a little higher effect of soil aggregation, this may be because of their higher capability of dispersing fungal spores and also higher capability of transporting particles. Therefore, Collembolan ability to disperse fungal spores as well as transport particles have been indicating positive influence on aggregation which proves the hypothesis -4 and 5. The possible mechanism behind this is that, the higher spore dispersal capability can increase fungal colonization that anchors soil particles through their hyphae branching into soils.

Ceratophysella denticulata feeding most likely on soil microorganism (e.g., those decompose materials) showed less amount of soil aggregates (Figure-6b) while it is showing higher capability of transporting micrometric particles (Figure-9) and least grazing effect on fungal growth (Figure-7). Therefore, it has been evident that, primarily, soil aggregation is being influenced according to their feeding guilds, the primary litter/detritus feeding Collembola have higher influence than the secondary decomposers those feed on mostly microorganisms, which ultimately proves our hypothesis 2. In comparison with different functional behaviour, Collembolan ability to reduce fungal growth (e.g., Collembolan grazing on fungal hyphae) have shown a decreasing effect on soil aggregation due to reducing fungal hyphae branching which anchors soil particles. Thus, it proves the hypothesis 3.

The mechanism behind Collembolan ecological role of enhancing soil aggregation is the combined effects of their trophic and other functional behaviour (e.g., transporting micrometric particles, fungal spore dispersal, grazing on fungal hyphae etc.).

Table-8. Summary of effects of Collembolan trophic and functional behaviour (traits) on soil aggregation. Different " + " sign indicating different trait's performance.

Collembola	**Feeding guilds**	**Trophic resources**	**Functional behaviour**			**Soil aggregation**
			Grazing on fungi	Fungal spore dispersal	Transporting particles	
Falsomia candida	'Grazers' or Closer to primary decomposers	Predominantly feeding on litter/detritus with adhering fungi and bacteria.	+	+	+	+++
Protaphorura fimata			+++	+++	+++	++
Ceratophysella denticulata	'Browser' or secondary decomposers	feeding most likely microorganisms, fungi etc.	+++	+	+++	+
Sinella curviseta			+	+	+++	+++

4.4. Effects of Collembolan on aggregates physical stability

Soils aggregate stability have shown a significant difference between different mesocosm treatments (Figure- 14; Table -6). Aggregates from the treatment with only fungi (F) increases stability in water significantly comparing to the Control treatment although the effects are rather limited. The other treatments with different Collembolan species did not show any statistically significant difference (Figure-14) between them. However, Collembolan activity has increased aggregates stability (Table-6) a very little with no statistical significance. The possible mechanisms behind the Collembolan role on soil aggregates stability as follows:

1. Collembolan grazing on fungi reduces fungal hyphae branching which anchors the soil particles. Thus, decreasing aggregation and aggregate stability. However, aggregates show higher stability with only fungi, no Collembolan activity (Figure-14).

2. Collembolans are capable to disperse fungal spores by carrying through their fecal pellets (Moore et al. 1987; Willium et al. 1998) and carrying viable spores attaching with their cuticle or even through the gut (Wiggins and Curl 1979; Whipps and Budge 1993). We also came up with the same evidence that, Collembola disperse fungal spores which increase newly isolated fungal colony to unlocalized part into soils. However, another argument that we have been evident that, Collembola can reduce fungal hyphae very rapidly comparing to spore dispersal, which effect may suppress fungal growth all the time as Collembolan are highly abundant in nature.

Conclusion

Collembolans are able to influence positively on soil structure and functioning by modifying soil's biological, physical and chemical properties. They increase soil macro-aggregation, while the plant litter/detritus feeders' species have more influence on soil aggregation. Individual Collembola have a little influence on soil structure and functioning, however, it is not such small at the ecosystem level because of their high abundance in most of the ecosystems. Not only on soil aggregate formation but also, they have influence on their stabilization too, although the effects are rather limited. The ecological role that Collembola plays varies with their different functional behaviours as well.

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Supplementary Materials

Table-1. Amount (nmol/gDW ± S.D.) of different Phospholipid Fatty Acids (PLFA) in soils from different microcosm treatments.

Fatty acids	**Origin**	**Types of treatments**						
		C	**F**	**FCD**	**FFC**	**FPF**	**FSC**	**FMIX**
i15:0	**Gram (+) Bacteria**	5.33 ± 0.62	6.74 ± 0.44	3.64 ± 0.14	6.75 ± 1.06	5.51 ± 0.68	5.53 ± 074	5.11 ± 0.84
a15:0		7.61 ± 0.48	7.89 ± 0.34	3.25 ± 0.09	5.59 ± 0.82	5.85 ± 0.92	5.08 ± 0.57	5.44 ±1.13
15:00		0.79 ± 0.11	0.51 ± 0.18	0.26 ± 0.15	0.74 ± 0.11	0.64 ± 0.46	0.45 ± 0.16	0.45 ± 0.17
i16:0		1.26 ± 0.73	1.99 ± 0.71	8.64 ± 2.92	2.78 ± 0.40	2.81 ± 2.12	7.35 ± 3.32	7.16 ± 2.72
i17:0		0.32 ± 0.32	0.63 ± 038	0.7 ± 0.41	0.54 ± 0.54	0.62 ± 0.38	0.93 ± 0.33	1.09 ± 0.38
Gram (+) total:		**15.32 ± 1.10**	**17.77 ± 1.03**	**16.50 ± 2.78**	**16.42 ± 2.73**	**15.44 ± 3.83**	**19.35 ± 4.89**	**19.27 ± 3.63**
cy17:0	**Gram (-) bacteria**	5.22 ± 1.80	7.29 ± 0.88	5.29 ± 0.73	9.79 ± 0.92	5.35 ± 0.45	5.02 ± 1.26	5.17 ± 0.40
cy19:0		0.08 ± 0.09	0.25 ± 0.18	0.23 ± 0.23	0.14 ± 0.09	0.57 ± 054	0.02 ± 0.02	0.39 ± 0.39
16:1w7		0.94 ± 0.25	0.95 ± 024	0.57 ± 0.03	0.76 ± 0.14	1.12 ± 0.48	0.54 ± 0.09	0.99 ± 0.42
17:00		2.68 ± 2.68	12.09 ± 2.36	10.31 ± 1.27	13.23 ± 0.59	9.41 ± 0.79	7.78 ± 1.34	8.5 ± 1.52
Gram (-) total:		8.93 ± 3.15	20.59 ± 2.77	16.39 ± 1.86	23.93 ± 1.50	16.46 ± 117	13.36 ± 2.67	15.06 ± 2.09
Bacteria total		**24.25 ± 2.27**	**38.37 ± 1.92**	**32.89 ± 3.78**	**40.35 ± 4.20**	**31.90 ± 4.49**	**32.72 ± 7.53**	**34.33 ± 5.21**

16:1W5	**Fungi**	0.00	2.07 ± 0.18	1.92 ± 0.22	2.79 ± 0.47	2.9 ± 0.51	2.2 ± 0.27	2.64 ± 0.61
18:2w6,9		0.71 ± 0.41	1.88 ± 0.32	1.73 ± 0.98	0.72 ± 0.51	1.76 ± 091	1.50 ± 0.63	0.98 ± 0.41
Total Fungi:		**0.71 ± 0.41**	**3.95 ± 0.41**	**3.65 ± 0.95**	**3.52 ± 0.89**	**4.66 ± 1.42**	**3.70 ± 0.54**	**3.62 ± 0.43**
18:1w7, 18:1w9t	**Plant and/or fungal**	0.31 ± 0.31	0.00	11.50 ± 0.90	9.74 ± 5.85	9.14 ± 3.19	2.04 ± 2.04	3.01 ± 3.01
18:1w9, 18:3w3, 18:2w6t		0.30 ± 0.30	6.51 ± 0.95	4.27 ± 0.65	5.05 ± 2.47	4.88 ± 2.02	3.16 ± 109	5.46 ± 1.76
Plant and/or fungal mixture total:		**0.61 ± 0.61**	**6.51 ± 0.95**	**15.78 ± 1.54**	**14.81 ± 8.31**	**14.03 ± 5.20**	**5.20 ± 3.12**	**8.47 ± 4.77**
22:0w0	**Plants**	0.50 ± 0.05	0.26 ± 0.15	0.45 ± 0.08	0.56 ± 0.09	0.44 ± 0.12	0.41 ± 0.04	0.32 ± 0.11
24:0w0		0.28 ± 0.06	0.25 ± 0.09	0.31 ± 0.06	1.38 ± 1.12	3.047 ± 2.76	0.24 ± 0.04	0.22 ± 0.08
16:00		3.67 ± 2.49	13.09 ± 1.01	1.71 ± 0.08	17.19 ± 2.56	9.15 ± 2.27	6.72 ± 2.32	6.01 ± 3.52
Plants total		4.46 ± 2.46	13.61 ± 1.20	2.48 ± 0.19	19.14 ± 3.72	12.64 ± 4.21	7.38 ± 2.33	6.56 ± 3.41
18:00	**Animals**	0.00	0.00	0.72 ± 0.42	1.15 ± 0.70	0.44 ± 0.44	0.00	0.00
20:4w6		0.00	0.08 ± 0.04	0.25 ± 0.06	0.22 ± 0.05	0.25 ± 0.09	0.07 ± 0.01	0.44 ± 0.20
20:5w3		0.02 ± 0.02	0.02 ± 0.01	0.11 ± 0.04	0.21 ± 0.06	0.20 ± 0.12	0.03 ± 0.03	0.20 ± 0.20
20:2		0.01	0.05 ± 0.04	0.11 ± 0.04	0.02 ± 0.01	3.05 ± 1.80	0.04 ± 0.04	0.01 ± 0.01
Animals total:		**0.03 ± 0.01**	**0.17 ± 0.07**	**1.21 ± 0.80**	**1.61 ± 0.80**	**3.94 ± 1.77**	**0.15 ± 0.04**	**0.64 ± 0.30**

20:1w9	**Others**	0.00	0.70 ± 0.53	0.00	0.82 ± 0.76	0.34 ± 0.32	0.04 ± 0.03	0.37 ± 0.20
21:00		0.00	0.02 ± 0.02	0.00	0.03 ± 0.03	0.08 ± 0.07	0.01 ± 0.01	0.01 ± 0.01
22:1w9		0.01 ± 0.01	0.01 ± 0.01	0.31 ± 0.27	0.02 ± 0.02	0.01 ± 0.01	0.03 ± 0.01	1.3 ± 1.28
23:0w0		0.06 ± 0.02	0.13 ± 0.04	0.15 ± 0.03	0.09 ± 0.03	4.99 ± 4.89	0.07 ± 0.01	1.91 ± 1.80
24:1w9		0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	2.62 ± 2.58	0.01 ± 0.01	0.01 ± 0.0
Others total:		**0.01 ± 0.03**	**0.87 ± 0.47**	**0.47 ± 0.24**	**0.98 ± 0.79**	**8.05 ± 5.01**	**0.17 ± 0.03**	**3.60 ± 3.28**
GRAND TOTAL:		**30.16 ± 7.77**	**63.49 ± 69**	**56.50 ± 7.96**	**80.42 ± 18.74**	**75.25 ± 22.64**	**49.33± 3.63**	**57.23 ± 17.92**