FEEDING BEHAVIOUR OF *FOLSOMIA CANDIDA* AS INFLUENCED BY DIET-SWITCHING IN THE PRESENCE OF LIVE MAIZE ROOTS

by

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To my parents Krishna Rao and Naga Lakshmi for always supporting and encouraging me....

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ABSTRACT

Collembola form the largest subset of the decomposer community in the soil ecosystem. They are known to feed on soil fungi, mycorrhizae, plant detritus and other root derived products. A recent study revealed that one species of Collembola, *Protaphorura fimata,* completely switched from decomposer to herbivore when live roots were present in the soil. The current study investigated the occurrence of diet-switching in another species of Collembola: *Folsomia candida* Willem. The switch from plant detritus to live roots was tested by examining the dietary preferences of *F. candida* under controlled environment conditions using stable isotope techniques. Tests were carried out in microcosms where *F. candida* were offered live maize roots (C_4 plant) in C_3 soil, along with ^{15}N enriched ryegrass litter. Results demonstrated the presence of a partial diet-switch from detritus to live maize roots for *F. candida*. Additional tests suggested that the diet-switch towards maize roots was a response to both improved food quality and greater food availability. The presence of live roots improved the body growth of *F. candida,* and the incorporation of C from live roots into Collembola tissue increased with root biomass suggesting *F. candida* acted as an omnivore when live roots were present.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ISO – International Standards Organization

OECD - Organization for Economic Co-operation and Development

C – carbon

N – nitrogen

m – metre

cm = centimetre

mm – millimetre

Kg - kilogram

g – grams

mg – milligram

RUBISCO –ribulose 1,5-bisphosphatase

PEPcase – phosphoenol pyruvase enzyme

WHC – water holding capacity

L – litres

mL – millilitres

mol. – moles

ca. - approximately

‰ – per mil (per 1000)

e.g. – example

i.e – that is

ad lib – ad libitum

µ - micron

h – hours

s - sec

° C – degrees centigrade

δ – delta

spp. – species pluralis

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CHAPTER 1   INTRODUCTION

1.1 Background

Soils house a variety of microorganisms and invertebrates which are involved in the decomposition process and nutrient cycling (Swift et al., 1979). These soil biota are divided into three broad categories based on their size, namely: microfauna, mesofauna and macrofauna. Soil microfauna are known to be involved in the nutrient cycling process, whereas the meso- and macrofauna serve as catalysts to speed up the process. Two groups of mesofauna, mites and Collembola are considered particularly important due to their worldwide distribution, large numbers (Filser, 2002; Petersen and Luxton, 1982; Takeda and Ichimura, 1983), their role in both litter decomposition and soil microstructure formation (Rusek, 1998). Mites and Collembola constitute a large and vital component of the ecosystem as they influence nutrient flow through their direct and indirect effects (Bakonyi, 1989). The former group is important in undisturbed habitats like forest soils, while the latter is especially important in agricultural soils (Filser, 2002).

Collembola also improve microbial activity, decomposition and mineralization (Reichle, 1977) by their combined activities of comminution, dispersal and grazing (Moore et al., 1987; Seastedt, 1984). Collembola are known to feed on fungi (Bakonyi, 1989; Ponge, 1991), bacteria, pollen, plant litter and algae (Drift and Jansen, 1977; Rusek, 1998; Scheu, 2002). Feeding on surfaces of live roots & root-derived products was observed, but the conclusions were based on visual examinations in rhizotrons (Gunn and Cherrett, 1993). The organisms appeared to have grazed on all root parts (main and lateral roots, root tips, root hairs and transparent roots), including some decaying roots. However, they also suggested that most soil animals obtain C via a fungal pathway and that root feeding is uncommon due to slow turnover of root C. Several species of Collembola are also considered omnivorous (Beare et al., 1995; Filser, 2002) or indiscriminate feeders (Andren and Schnurer, 1985). Previous studies on feeding preferences of Collembola, using microscopic and gut content analysis, suggested fungi

and plant material as the predominant food source. Direct observations on aspidistra plants showed some species of Collembola feed on the stigmas of the flowers (Kato, 1995). The role of detrital decomposition in soil C dynamics is well established, but the mesofaunal incorporation of C from roots itself or indirectly via recent photosynthate-C deposited in the rhizosphere and its surroundings is poorly understood or is only just being unraveled. Many studies have suggested a close relationship between rhizodeposition and soil fauna (Sticht et al., 2006; Ostle et al., 2007; Brüggemann et al., 2011). Even though many studies have observed root feeding, there is no conclusive evidence for such feeding activity. Larsen et al. (2007) used Collembola to study the release of low-molecular weight compounds from roots into decomposer system and showed Collembola derived 54 – 79 % C from roots or root exudates. However, Endlweber et al. (2009), with the help of ^{13}C and ^{15}N stable isotope ratios, were the first to show that the springtail species *Protaphorura fimata* Gisin completely acted as herbivore by switching to live maize roots obtaining its C and N entirely from living plant roots, rather than from plant residues covered or not by fungi and other microorganisms. In a study on the effect of drought in the uptake of recent photosynthetic C by Collembola and mites, Seeber et al. (2012) observed a strong link between these organisms and living plants. They proposed that Collembola might have obtained C both by direct plant herbivory or from fungi by a mycorrhizal pathway. In contrast, the role of Collembola in determining the fate of recent photosynthetic C in or near the rhizosphere region of the Sitka spruce could not be confirmed by Churchland et al. (2012).

Given that the ability of Collembola in general to feed on live plant roots remains undetermined, hence the current study aimed to examine how widespread live root feeding by Collembola may be and their role in C cycling, by examining the diet of the springtail species *Folsomia candida* Willem 1902, using the stable isotope technique.

1.2 Decomposition in ecosystems

Decomposition is the process of converting complex organic molecules into smaller and simpler forms. It involves both chemical and physical processes to reduce

litter into its constituent chemical elements. It is a two-stage process where litter is first fragmented into smaller pieces, mostly by soil meso- and macrofauna, and then further reduced into inorganic molecules by bacteria, fungi and nematodes (Swift et al., 1979; Tugel et al., 2000). Some fractions of residue resist decomposition through either physical protection within soil aggregates or formation of organo-mineral complexes. Soil animals act as catalysts and enhance the decomposition rates, whereas soil microbes are the true decomposers, as they possess the enzymes necessary for complex organic molecules breakdown (Petersen and Luxton, 1982). This decomposition process is critical for energy (C) and nutrient cycling in ecosystems. Decomposition rate is also partly controlled by abiotic factors: (1) type of organic material, (2) amount of nitrogen (N), and (3) environmental factors (Tenney and Waksman, 1929). As result, litter decomposition rates vary significantly (Swift and Anderson, 1989; Aerts, 1997). Although soil microfauna are responsible for mineralization and nutrient recycling, soil macro and mesofuana are known to only stimulate or enhance plant growth by direct and indirect effects like microbial feeding, dispersal of microbial structures, microbial composition modification and increased nutrient availability (Griffiths and Bardgett, 1997; Kreuzer et al., 2004; Cole and Bardgett, 2005). Bacteria are the primary decomposer group preyed upon by protozoa and nematode species, whereas fungi, the secondary decomposer group, are the prey for collembolans and mite species (Scheu et al., 2005). Mites can sometimes feed on dead plant material but collembolans until recently are known to primarily feed on fungi and to a small extent, on bacteria and other sources (Rusek, 1998). Thus any changes in the fungal composition will also change the feeding activities of the collembolans.

1.3 Collembola

Collembolans represent an abundant and widespread group of microarthropods on the earth where they play an important ecological role. They are distributed throughout the globe from the Arctic to the Antarctic, where no invertebrates survive and from the littoral zone to the highest peaks in the Himalayas (Filser, 2002; Seastedt, 1984). Despite their high population densities they contribute to less than 1% of the total soil faunal biomass in most agricultural systems, due to their small size (Petersen, 1994). Their

numbers range from a few thousand to several million per square meter with a species richness of 1-3 to 50-60, depending on the ecosystem (Petersen and Luxton, 1982; Rusek, 1998). They are colloquially called springtails due to the presence of *furca* or a *springing organ* on the last abdominal segment which helps them to jump many times greater than their body size. A pair of fused structures on the first abdominal segment comprises the *ventral tube (or collophore)*, important in fluid exchange with the external medium and in sticking to slippery surfaces (Hopkin, 1997). Adults can attain a maximum body length of 3mm. Their body is divided into a head with a pair of antennae, a thorax bearing three pairs of legs and an abdomen with six segments (Fountain and Hopkin, 2005). Because their mouthparts are entognathous, they are no longer considered as insects and occasionally, are placed in a separate class under Arthropoda along with Diplura and Protura (Hopkin, 1997).

Collembola influence the microbial biomass of soil and respond to soil disturbance (Bakonyi, 1989). Respiration from soils with Collembola is higher than soils without them (Petersen, 1994; Filser, 2002). Collembolans are now widely used in eco-toxicological studies to study the effect of various chemicals on soils (Environment Canada, 2007). Of many other springtail species, *F. candida* is more commonly used due to its short reproductive cycles and ease of maintenance in the laboratory.

1.3.1 *Folsomia candida*

The springtail species *F. candida* belongs to the family Isotomidae of the order Entomobryomorpha in the class Collembola (Environment Canada, 2007). This species occurs worldwide but is less abundant in cultivated soils than in forests and is commonly called a "compost" springtail as it is often found in compost heaps or areas with high organic matter (Römbke et al., 2006). It is the most intensively studied springtail species. The following description is from Hopkin (1997), who reviewed its life cycle, and from personal observations the laboratory. It is a euedaphic, unpigmented, eyeless species reaching 1.5 to 3 mm in length when fully matured. Populations of *F. candida* exclusively contain female individuals and reproduce parthenogeeitically. Adult females lay 30-50

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eggs in communal heaps, which take ca. one week to hatch. Although they lack eyes, more eggs are laid in constant darkness compared to light/dark cycles, which implies they possess internal photoreceptors. Eggs hatch to become nymphs and develop into adults in 3 – 4 weeks at 20° C. They do not undergo metamorphosis; their nymphs or the juveniles develop into adults directly and only differ in size (Environment Canada, 2007). Adults can molt up to 48 times in their lifetime and start laying eggs after their sixth molt (Fountain and Hopkin, 2005). Their ease of maintenance with common laboratory equipment, rapid growth and short life cycle makes them ideal organisms for many laboratory experiments (Hanlon and Anderson, 1979; Briones et al., 1999; Chamberlain et al., 2005; Chamberlain et al., 2006b). The International Standards organization (ISO, 1999) proposed a set of guidelines to use this species as a "standard organism" to assess the quality of polluted soils. It has also been used as a bio-indicator of soil quality in forest (Kaneda and Kaneko, 2002) and agricultural soils (Nelson et al., 2011).

1.4 Collembola feeding behavior

Although there can be millions of individuals/m^2 of soil, little is known about the feeding behavior of Collembola (Anderson and Healey, 1972) due to their small body size (Briones et al., 1999) and their ability to feed on a wide variety of materials. Collembola feeding activities recycle nutrients stored in fungal tissue (Cragg and Badgett, 2001; Kaneda and Kaneko, 2002) or by physical breakdown of organic material (Seastedt, 1984; Persson, 1989; Verhoef and Brussard, 1990; Filser 2002). The process of breakdown is accomplished in different ways (Hopkin, 1997):

1. Feeding on dead or living vegetation and expelling it as partially decomposed faecal pellets which increases its suitability and surface area for microbial attack.
2. Their direct grazing on fungi causes dead tissues to decompose faster which may either arouse or deter the growth of certain fungal species.
3. They are mobile in soil and distribute fungal spores and incompletely fragmented plant material to areas not accessible to certain microorganisms.

Release of C and N from fungal tissues enhances nutrient availability to the plant, thus they are directly involved in nutrient cycling in soil. Although some Collembola were shown as preferential fungal feeders (Visser and Whittaker, 1977), some feed on nematodes (Lee and Widden, 1996) and plants (Endlweber and Scheu, 2006) when they are offered a choice in the laboratory. Most Collembola feed on plant residues (Briones et al., 1999; Berg et al., 2004; Chahartaghi et al., 2005; Albers et al., 2006) and re-release nutrients in the form of faecal pellets (Schrader et al., 1997).

Collembola that forage for food must, like other animals (Pyke, 1984) reach a balance between food types that are readily available and food types that provide better nutrients. Feeding in the root zone of plants might occur by chance or preferentially if roots have higher nutrient value than the surrounding litter. If herbivory increases Collembola fitness, live plants are likely to become an important collembolan food source. Quantifying the diet of an organism is a fundamental aspect of its ecology (Sih and Christensen, 2001).

The foraging decisions of animals are influenced by the constraints of diet availability and quality. The opportunistic feeding behavior said to characterize many species of Collembola suggest that most will feed on available resources, rather than on spatially distributed food resources that require an intensive search (Anderson and Healey, 1972), which might be justified for nutrient rich food such as yeast (for Collembola). Many techniques have been used to try and determine the food sources of Collembola: direct microscopy gut content analysis, direct observation of springtails with video cameras, color marking techniques and radiotracers (Briones et al., 1999). There are positive and negative aspects to all techniques: some are time consuming and the results may depend on the observer's interpretation (DeNiro and Epstein, 1978), some food material may already have been degraded by enzymes, materials may have been ingested accidentally and only a small fraction of the ingested material may be assimilated into the tissues (Albers et al., 2006). All these factors make it challenging to identify the different elements making up the diet of Collembola (Hopkin, 1997).

As already identified the main sources of food are fungi, lichens, detritus, decomposing vegetation, pollen and algae (Hopkin, 1997). Relatively recent findings indicate that diet-switching may be part of the feeding strategy of Collembola (Endlweber et al., 2009). Here diet-switching with regards to Collembola refers animals which are usually litter or organic matter feeders, largely depends on plants, when available to meet their nutritional needs. Heterotrophs such as Collembola generally reflect the isotopic signature of their diet. With the help of stable isotope technique, it is possible to examine the consumed resource (Albers et al., 2006), as well as elaborate the long-term trophic relations between animals and the interactions between Collembola, soil microorganisms and plant remains. Briones et al. (1999) were the first to use $\delta^{13}C$ technique with Collembola to show preferential feeding in *F. candida* and *Proisotoma minuta* on C_4 litter over soil taken from a field trial cropped with C_3 plants (C_3 soil). Laboratory studies using the stable isotopic composition of lipids showed that collembolans are capable of feeding on different sources when switched from yeast to other sources artificially (Chamberlain et al., 2006b). Using this technique, Endlweber et al., (2009) were the first to demonstrate that *P. fimata* acted as an herbivore and preferentially foraged on live maize roots by switching from ryegrass litter sources to satisfy its C and N requirements. This suggests a herbivore component to at least some of the Collembola, even though they have been primarily classified as decomposers in the soil food web.

1.4.1 Diet-switching

Populations of *P. fimata* readily feed on litter residues incorporated into soil but were shown to favor live plant roots over litter when given the choice between both (Endlweber et al., 2009). The authors speculated that the active diet-switch may have reflected the easy availability of the roots, the surrounding root hairs and root derived materials, in contrast to the slow decomposition rate and high fiber content of the litter. The preference for the plant C over the litter C could also have been in response to the higher food quality of live maize roots over litter.

Although, Larsen et al. (2007) showed Collembola derived a significant amount of C from roots or root exudates (54 – 79%), Endlweber et al. (2009) appears to be the only documented case who showed complete diet-switching of collembolans to live roots. Based on this study and the evidence in the literature of a close association between many collembolan species and the rhizosphere, it may be possible to generalize the behavior across the subclass. Collembolans show "species-specific" feeding habits and these differ among closely related taxa and sometimes even between the populations and life stages within the same species. However, the relative importance of herbivory as a feeding strategy , among Collembola, remains difficult to estimate. The feeding strategy is complex and could be affected by a range of factors including temperature and moisture content or even seasonality. McMillan (1975) studied seasonal variation in types of material ingested by collembolan species belonging to family Onychuridae. In this study more fungal hyphae and plant material was observed in the guts during summer and autumn months respectively in both *Onychiurus spp. and Tullbergia callypygos*. Another study showed that the guts of Collembola contained fungal hyphae during winter and autumn while plant matter dominated in spring and summer (Takeda and Ichimura, 1983).

Regardless, because animals tend to forage optimally, a switch from detritus to live roots would have to provide some advantage either in food abundance or quality. Hence, the relative availability of live roots and detritus, as well as their impact on performance of the organisms, should also be taken into consideration when investigating the feeding behavior of collembolans. Ultimately, if a broader occurrence of active diet-switching in the presence of live plant roots can be shown for other Collembola, it may be necessary to extend the classification of Collembola from decomposer to herbivore or omnivore.

1.5 Stable isotopes in soil food web studies

The heterotrophic C pathway above the soil surface, during the initial stages of decomposition, has been extensively studied because most of the above ground litter inputs can be easily manipulated (Berg and McClaugherty, 2003). Belowground

interactions between microorganisms are often neglected due to the difficulty in investigating these events. Recent technical and methodological advancements in the use of isotopic techniques have facilitated the study of the C pathway below ground (Staddon, 2004). Carbon is the main source of energy for most living systems; hence it is appropriate to study the changes in C isotopes. Stable isotopes of C are less fractionated (than N, another commonly studied isotope) as ^{13}C propagates through trophic levels (DeNiro and Epstein, 1978). The relative proportion of three of the C isotopes (^{14}C, ^{13}C and ^{12}C) differs in natural organic materials, making it possible to trace the source and fate of C in the ecosystems. ^{12}C is the lightest and most abundant isotope, followed by ^{13}C and then ^{14}C. The quantity of the heavier ^{13}C to lighter ^{12}C isotope is expressed as δ^{13}C for natural abundance and ^{13}C atom ‰ when samples are enriched with a ^{13}C rich compound. δ^{13}C is expressed relative to a standard (the content of ^{13}C in Vienna-PeeDee belemnite (V-PDB), a now depleted limestone formation in North Carolina (Vitorello, 1989). The isotopic composition of a substance can be measured using Isotope Ratio Mass Spectrometer (IRMS) and is expressed in *per mil excess* or ‰. The expression to determine δ^{13}C is given in the following equation.

$$\delta^{13}C = \frac{R_{sample} - R_{standard}}{R_{standard}} \; x \; 1000 \; ‰$$

R_{sample} is the isotopic ratio of the sample and $R_{standard}$ is for the standard or reference substance. The same equation can also be used to calculate δ of other elements, such as N, and the standard for N is atmospheric N (Tiunov, 2007). Naturally occurring materials and organisms have different isotope ratios (Staddon, 2004). Stable isotopes of both C and N help unveil complex trophic interactions in soil communities and have been reviewed by Scheu (2002). For example, C_4 plants have isotopic ratios in the range of -6 to -19‰, whereas the ratios for C_3 plants range from -24 to -34‰ (Smith and Epstein, 1971). This difference is due to the discrimination of ^{13}C in different biological pathways (Ehleringer, 1991). The low values in C_3 plants are due to discrimination of ^{13}C versus ^{12}C by the RUBISCO enzyme which selectively activates the more mobile and lighter ^{12}C and incorporates it into plant cells. In C_4 plants the primary CO_2 acceptor, PEPcase does

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not discriminate between ^{13}C and ^{12}C (Stevenson and Cole, 1999). Naturally occurring stable isotopes are reliable to use, provided there is a sufficient difference between the C pools being studied (Staddon, 2004), and the samples can be directly obtained from the trial location. The $\delta^{13}C$ difference between C_3 and C_4 plants has been extensively used to study the fate of decomposing soil organic matter and the soil C cycle in ecosystems (Balesdent et al., 1987; Balesdent et al., 1988; Balesdent et al., 1990; Angers et al., 1995; Wanniarachchi et al., 1999, Lynch et al., 2006). It is also possible, with this technique, to study the substrate utilization by soil biota. The $\delta^{13}C$ of consumers is the weighted average of the C isotopes in the diet constituents (Chamberlain et al., 2006b) as heterotrophs generally reflect the isotopic signature of ingested material (Peterson and Fry, 1987). Although this method has many advantages, it is often limited due to difficulty in finding materials with sufficient differences in $\delta^{13}C$ values of C pools (Staddon, 2004).

Nitrogen isotopes are less conservative tracers (i.e. more fractionated) than C isotopes, making the isotopic technique less convenient for studying N flows than C flows (Tiunov, 2007). Fractionation refers to the change in isotope signature while the isotopes pass through food webs, as animals tend to preferentially incorporate the heavier ^{15}N isotope over ^{14}N (DeNiro and Epstein, 1981). As a result, unlike C isotopes, N isotopes are enriched by a certain amount between every trophic level. However, the $\delta^{15}N$ of organisms is also dependent on the diet and hence, the N isotope configuration of their tissue can give additional information on food sources (DeNiro and Epstein, 1981).

1.6 Study of Collembola feeding ecology using isotope technique

The feeding behavior of the soil mesofuana can be studied using the ^{13}C natural abundance technique (Staddon, 2004). The origin of C and N incorporated into collembolan tissue can be analyzed by offering two food sources with distinct isotope signatures (Briones et al., 1999; Scheu and Folger, 2004; Chamberlain et al., 2006a; Endlweber et al., 2009). In the case of Collembola, the isotopic techniques have more advantages than direct examination because of their small body size and the short time

period required for incorporating dietary C into their tissue (DeNiro and Epstein, 1978). Briones et al. (1999) showed that the C signature of *P. minuta* and *F. candida* changed from that of yeast to maize when the diet was artificially switched. Scheu and Folger (2004) investigated the fitness and isotope fractionation of *Heteromurus nitidus* fed on single and mixed diets of fungi and algae. They showed Collembola fed with a mixed diet had higher survival rates than those fed with a single diet, probably by benefiting from the increased resources of two diet sources. Chamberlain et al. (2006a) used fatty acid distribution patterns coupled with $\delta^{13}C$ values to determine a feeding preference of *P. minuta* and *F. candida* fed with fungi and nematodes. Chamberlain et al. (2006c) analyzed $\delta^{13}C$ of microbial phospholipid fatty acids (PLFAs) and showed the *F. candida* increased litter availability to the soil microbial community in laboratory microcosms. In a grassland ecosystem, Ostle et al. (2007) noted that microarthropods acquired their C from root derived products and potentially directly from roots. Approximately 45% of the ^{13}C from grassland (enriched with $^{13}CO_2$) was incorporated into collembolans compared to 22% and 28% for mites and earthworms respectively. Sticht et al. (2006) used a "Free Air CO_2 Enrichment" (FACE) technique in winter wheat to study the influence of elevated CO_2 levels on collembolan density and abundance. These researchers found $\delta^{13}C$ of Collembola was highly enriched under elevated CO_2 levels and was closest to roots. Higher plant density increased both fine root and microbial biomass and thus, *Protaphorura* density (Salamon et al., 2004). The study suggested that Collembola densities are dependent on food quality rather than quantity. Soil mesofuana, including Collembola belonging to family Entomobryidae, were shown to feed on ^{32}P labelled clover roots in a pasture system (Baylis et al., 1986). Studies based on isotopic analysis have provided strong evidence that root derived C resources form a significant portion of the food source for soil animals which is often underestimated.

1.7 Objectives

The study investigated the impact of live plant roots on the feeding behavior of *F. candida*. It consisted of two main objectives:

1. Although Collembola are generally described as decomposers, Endlweber et al. (2009) suggested that some species may improve their fitness by switching their diet from plant residues to live roots, when available. Endlweber et al. (2009) demonstrated the existence of such an active diet-switch from plant residues to live roots for *P. fimata.* The first objective of this study was to determine if such a diet-switch occurs in other Collembola species by examining *F. candida* for its presence. This is addressed in Chapter 2.

2. The optimal foraging theory suggests that organisms forage on food resources in a manner that maximizes their net energy intake, subsequently increasing their performance (i.e. growth, survival and reproduction). According to the theory, foraging Collembola should switch their diet to more profitable sources of C and N, if the choice will maximize fitness. The profitability of the food source selected depends on its quality (higher N content) and availability (abundance, distribution in soil).

    The second objective was to determine if the performance of *F. candida* is improved in the presence of live roots and, if the diet-switch was confirmed, whether the utilization of live roots is a response to food source quality (Chapter 3) or availability (Chapter 4).

CHAPTER 2   DIET-SWITCHING OF *FOLSOMIA CANDIDA* IN THE PRESENCE OF LIVE MAIZE ROOTS

2.1 Introduction

To understand the role of the global C cycle, it is of paramount importance to understand the fate of C in terrestrial ecosystems. Of the total C fixed by plants about 80 to 90% enters soil (Pollierer et al., 2007), either through litter fall or root derived products thereby fuelling underground food webs. With rapid climate change, there is also a growing interest in the role of soil organisms (Fitter et al., 1999) as soil fauna are considered to be regulators of the soil C cycle (Johnson et al., 2005). Even though the soil food web entirely depends on plant-derived products, the exact food source of most soil animals is still largely unknown. Leaf litter is considered as the general food source of the underground decomposer community. The contribution of root-derived products has been largely ignored or underestimated excluding a few studies. There is some evidence that roots and root exudates contribute to the diet of soil animals, either directly or indirectly through the microbial community associated with the roots (Pollierer et al., 2007). Salaman et al. (2004) suggested that the roots and/or associated microflora of the grass *Trisetum flavescens*, among other plants, could be an attractive food source for many species of Collembola. Similarly, Gunn and Cherrett (1993) and Albers et al. (2006) showed that roots and root derived products provided a source of nutrition to soil animals. Roots contain a wide variety of products which can form an abundant source of food for soil arthropods. These include carbohydrates, organic acids, amino acids and other secondary metabolites, sloughed off cells, root tips and other rhizosphere derived microfauna and microbes (Ostle et al., 2007).

Euedaphic Collembola reach high densities, particularly in the rhizosphere region of plant roots where abundant food materials are available in the form of root exudates and mucilages (Endlweber and Scheu, 2006). Collembolans have fast tissue turnover (Garrett et al., 2001) and are closely linked to the rhizosphere C flow by grazing on mycorrhizal fungi or root derived products (Ostle et al., 2007). This evidence for

potential direct root feeding, supplemented by Larsen et al. (2007) and Ostle et al. (2007), was confirmed by Endlweber et al. (2009). The feeding behaviour can be a response to the quality or quantity of food available for Collembola (Scheu and Folger, 2004). By employing stable isotopes, it is possible to follow the C movement between food resources and soil animals, and trace the food source (Albers et al., 2006). Since the N cycle is closely linked to the C cycle, analyses of N along with C can provide additional understanding of C changes (Lynch et al., 2006; Wardle, 2013).

Previous studies suggested that Collembola are fungal feeders (Moore et al., 1987; Bakonyi, 1989; Scheu and Simmerling, 2004) with occasional preference for organic matter. Certain Collembola species are nourished by feeding on specific fungal taxa (Jørgensen et al., 2005). Collembola also feed on resources that are in immediate proximity, rather than the ones which require extensive search (Ponge, 2000). δ^{15}N ratios of Collembola show that their trophic guilds range from primary decomposers to herbivores (feeding on plant tissues), implying a wide resource base (Chahartaghi et al., 2005). By feeding on mixed diets of ectomycorrhizal fungi, conidial fungi and algae, *Heteromurus nitidus* increased fitness and reproduction when compared to single diets (Scheu and Folger, 2004). Similarly, Scheu and Simmerling (2004) observed increased growth and reproduction in *Folsomia candida* and *Protaphorura armata* when exposed to different combinations of seven saprophytic fungi. Root grazing of collembolans has also been reported by Brown (1985) and Fitter and Garbaye (1994). Presence of trehalase and cellulase in their guts show their ability to digest fungal hyphae and plant cell walls respectively (Berg et al., 2004; Chahartaghi et al., 2005). A number of other studies also have mentioned about live plant feeding in Collembola (Hurej et al., 1992; Rusek, 1998: Milcu et al., 2006). All the above studies suggest that switching to resources that are more palatable may be a strategy in Collembola feeding behavior to ingest higher quality food material (Endlweber et al., 2009) or to create resource partitioning when more than one species is present (Cortet et al., 2003; Jørgensen et al., 2003; Eisenhauer et al., 2011).

Even though, few studies mentioned earlier either reported or studied root feeding, they were largely based on direct observations or gut content analysis from

which a conclusive evidence cannot be derived. However, Endlweber et al. (2009) successfully, demonstrated the existence of an active diet-switch from plant residues to live roots using stable isotope technique in the Collembola species *P. fimata*. The objective of this experiment is to determine whether the existence of such a diet-switch extends to other Collembola species such as *F. candida*. Based on the observations of Endlweber et al. (2009), it may be hypothesized that *F. candida* satisfies both its C and N requirements from litter residues but switches preferentially to living plant roots when they are present. The potential dietary switching was accomplished by growing *F. candida* in microcosms containing C_4 plants (Maize – *Zea mays* L.) in C_3 soil mixed with ^{15}N enriched ground C_3 ryegrass (*Lolium perenne* L.) litter.

2.2 Materials and methods

2.2.1 *Folsomia candida* colony maintenance

An initial colony of *F candida* was obtained from Potato Research Centre, Agriculture and Agri-Food Canada, Fredericton, NB in May 2011. The colony of *F. candida* was maintained according to the standard rearing procedure adapted from "Test for measuring survival and reproduction of springtails exposed to contaminants in soils" (Environment Canada, 2007; Nelson et al., 2011). Key elements of the procedure are summarized below.

Folsomia candida maintenance colonies were reared in tightly closed plastic boxes 22.5 x 16.5 x 7.5 cm^3 lined with 9:1 mixture of plaster of Paris and charcoal (425µ, Fischer Scientific), here onwards referred as plaster mix. To prepare the substrate, 120gms of plaster of Paris and 15gms of charcoal were weighed into a 1 L glass bottle and then 130mL of water was added to the contents before shaking for at least 30sec. This slurry was then transferred to plastic boxes and left to dry. The substrate was then washed thoroughly and wiped to remove any charcoal floating on the top. The boxes were placed in a temperature-controlled incubator (BOD model L127, Sheldon manufacturing, Cornelius, Oregon) at 20° C. Collembola were fed with baker's yeast *ad*

lib. every 3 days and the old yeast was removed to prevent mold formation. The substrate was also sprayed with deionized water every 3 days or whenever it appeared dry, simultaneously aerating the colonies. The colonies were transferred to a new plaster mix every two months to prevent overcrowding and induce oviposition. Collembola raised on commercial baker's yeast have δ^{13}C signatures close to C_4 plants (-11 ‰ δ^{13}C). The design of the diet-switch experiment required a colony of *F. candida* with the isotope signature similar to C_3 plants. This was achieved by initiating another colony where *F. candida* were raised on yeast cultured on a modified Sabourad's media consisting of a mixture of beet sugar and soya peptone (Chamberlain et al., 2004). The procedure followed for the production of the yeast is detailed in Appendix 1.

To obtain Collembola that were age synchronized for the experiment, groups of 200 – 300 adults were transferred into new boxes to induce egg laying. Once the eggs had hardened, generally a week after laying, they were transferred into new egg boxes. Egg cards consisting of pieces of filter paper coated with plaster mix or plaster pieces placed at the bottom of the boxes at the same time as the adult *F. candida* facilitated the transfer of the eggs. Eggs were observed every day for emergence, and, 48 h after first nymph emergence, egg cards were removed. Nymphs left in the rearing boxes were thus age synchronized within 2 days of emergence and were used when they attained the required age for the experiment.

2.2.2 Experimental procedure

The experimental design was a modification of the procedure described by Endlweber et al. (2009). Collembola were grown in soil microcosms in a randomized block factorial experiment in the presence (+) and absence (-) of maize and ryegrass litter which gave a total of four treatments (Table 2.1). Blocks were established on January 21st, Febuary 19th, March 11th and March 18th, 2013. Each treatment was replicated thrice within each block. The experimental units were microcosms made up of PVC pipes (10 cm diameter x 25 cm height) containing 1 Kg of air-dried soil (C:N – 12.5) collected from the surface 15 cm of an organic field trial (Truro, NS) with no prior history of corn

or other C_4 crop production. Soil texture was a loamy sand to sandy loam and is an ortho-humic ferric podzol (Truro soil association). Soil was sieved using a sieve shaker (Ro-Tap, Mantor, OH, USA) with a 2mm sieve and defaunated at -80° C for 24 h (Mebes and Filser, 1998). Microcosms were sealed at the bottom with a layer of household mosquito net and another layer of a wire mesh (22 μ) to prevent *F. candida* escaping the microcosms. Each microcosm was filled with soil to approximately 15 cm, with the remaining 10 cm to the top of the PVC microcosm acting as a barrier to *F. candida* emigration. Prior to placing soil into microcosms, two grams of finely ground ryegrass material (<0.5 mm, 2.5 ± 0.1 % N, C/N ratio 18.7 ± 0.4) enriched with ^{15}N ($\delta^{15}N$ 17516.5 $\pm$ 113 ‰, the procedure for enriching ryegrass is detailed in Appendix II), was uniformly mixed into the soil in half of the microcosms. Maize treatments received two seeds thinned after emergence to retain one healthy seedling per microcosm. C:N ratio of maize plant roots was 54.5. To inoculate the soil with microorganisms, 200 g fresh soil was collected from the same field where the soil was obtained and mixed with 2 L distilled water. The soil suspension was allowed to settle for 3 h and 30 mL of the supernatant was added to the soil in the microcosms. After the maize plants germinated, 15 day-old *F. candida* (numbers) were added to each microcosm and were randomly placed in growth chambers (Conviron, Controlled Environments LTD., Winnipeg, MB), maintained at 20° C and light/dark cycles of 16:8 h with a light intensity of 400 $\mu E \ m^{-2} \ s^{-1}$. Soil was maintained at field capacity by adding water every alternate day until the initial total weight was achieved. This initial total weight was adjusted for plant growth. Weed seedlings were removed from the microcosms, as required.

Folsomia candida were recovered from all treatments after 8 weeks. Plant roots, along with the complete soil column, were pulled out of the microcosms. The loose soil was placed in large plastic containers and the rest carefully washed off the roots. Microcosms were also rinsed into the plastic containers to transfer the adhering soil. Small quantities of soil in suspension in water were transferred into 1 L Mason jars and stirred so the Collembola would float to the surface due to their hydrophobic integument. They were immediately skimmed off and transferred into glass vials for freeze-drying.

Maize roots, after being washed were carefully examined for any remaining *F. candida* and separated from shoots and dried at 65° C for 24 h. Dried root and soil samples were powdered in a ball mill (Retsch, Haan, Germany). Samples of freeze-dried Collembola (1 mg), ground soil (25 mg) and dried root (2mg) were packed into tin capsules for isotope analysis. Stable isotope ratios of all samples were analyzed in a coupled system of an elemental analyzer (Costech ECS4010, Costech Analytical, Valentia, USA) and isotope ratio mass spectrophotomer (IRMS) (Delta V mass spectrophotometer, Thermo scientific, Bermen, Germany) at the Stable Isotope laboratory, University of Saskatchewan, Saskatoon, SK. This instrument provides the direct measurement of the $\delta^{13}C$ and $\delta^{15}N$ of the samples using V-PDB limestone and atmospheric nitrogen as standards, respectively. For internal calibration purposes, International standards IAEA-N1, IAEA-N2, IAEA-CH6 and USGS-24 were used. The fraction of C and N incorporated into Collembola from maize and ryegrass litter sources were calculated using the two source mixing model described by Gearing (1991) and employed by Endlweber et al. (2009):

$$F_x = \frac{\delta R_m - \delta R_y}{\delta R_x - \delta R_y} x \ 100$$

Where, F_x is the fraction of C (or N) from source X, $\delta^{13}R_m$ is the isotopic ratio of the Collembola exposed to mixture, $\delta^{13}R_x$ is the isotopic ratio of the source X, and $\delta^{13}R_y$ is the isotopic ratio of the source Y. R is the respective isotope signature of C or N

Table 2.1 Treatments used to determine if *Folsomia candida* switches diet in the presence of plant roots (+ and – denotes presence or absence of a factor).

Treatment	Maize	Ryegrass litter
T1	+	+
T2	-	+
T3	+	-
T4	-	-

Figure 2.1 Growth chamber holding microcosms used in diet-switch experiment with maize and ryegrass litter as factors arranged in a randomized block design.

2.3 Statistical analysis

Analysis of variance (ANOVA) was conducted for plant biomass, Collembola tissue C and N concentrations and isotope signatures using Proc GLM procedures with maize and ryegrass litter as two factors in SAS 9.3 (SAS Institute, Cary, NC). In a randomized block design, blocks acts as replicates; however, in the current experiment three replicates were used within each block and were analyzed as such without deriving averages. If the difference between treatment means was significant, they were separated by Tukey's HSD test at $P < 0.05$. Results were also checked to ensure whether the statistical assumptions of normality and constant variance were met.

2.4 Results

2.4.1 Maize plants

Addition of ^{15}N enriched ryegrass litter caused a slight increase in maize root biomass and a slight decrease in shoot biomass. However, these differences were not significant ($F_{1,19} = 0.38$, $P < 0.54$ for shoot biomass and $F_{1,19} = 0.02$, $P < 0.88$ for root biomass, Table 2.2). Tissue C and N concentrations of maize roots were measured in both treatments. Addition of ryegrass litter had no influence on either of the above two variables ($F_{1,19} = 0.38$, $P < 0.55$ for C and $F_{1,19} = 2.93$, $P < 0.1$ for N, Table 2.2). Enriched ryegrass litter did not affect $\delta^{13}C$ signature ($F_{1,19} = 0.49$, $P < 0.49$), but its presence increased the $\delta^{15}N$ of maize roots significantly ($F_{1,19} = 137.6$, $P < 0.0001$, Table 2.2).

Plant root and shoot biomass and their C and N concentrations were also measured, both in the presence and absence of Collembola. Presence of Collembola did not influence plant performance. Root biomass was highest when both ryegrass litter and Collembola were present together, whereas the shoot biomass was highest in the absence of both. However, root and shoot biomass did not differ significantly between the treatments with and without Collembola ($F_{3,30} = 1.71$, $P < 0.18$ and $F_{3,30} = 3.01$, $P < 0.05$ respectively, Table 2.3). Although C and N concentration of maize plants (both root and shoots) did not differ among the treatments, N content was slightly higher in plants when Collembola alone were present without ryegrass litter (Table 2.3).

Table 2.2 Maize Shoot and root biomass, root C and N concentrations, and $\delta^{13}C$ and $\delta^{15}N$ isotope signatures in the presence of ryegrass litter and *Folsomia candida,* and *Folsomia candida* only without ryegrass litter.

Treatment	Shoot biomass (g)	Root biomass (g)	Root C (%)	Root N (%)	Root $\delta^{13}C$ (‰)	Root $\delta^{15}N$ (‰)
Ryegrass Litter + *F. candida*	4.2±1.9	1.4±0.6	39.7±1.1	0.9±0.07	-13.2±0.2	1036±369[a]
F. candida only	4.6±1.6	1.4±0.5	39.9±1.3	0.9±0.08	-13.2±0.4	12.2±1.7[b]
P -value	0.54[n.s]	0.89[n.s]	0.54[n.s]	0.11[n.s]	0.49[n.s]	0.0001***

[n.s] Treatments means (± S.D, n= 9) are not significantly different from each other at *P* < 0.05
***Significant at *P* < 0.0001.

Table 2.3 Maize shoot and root biomass, their C and N concentrations in the presence of ryegrass litter and *Folsomia candida, Folsomia candida* only without ryegrass litter, ryegrass litter without *Folsomia candida,* and control treatment without *Folsomia candida* and ryegrass litter.

Treatment	Shoot biomass (g)	Root biomass (g)	Root C (%)	Root N (%)	Shoot C (%)	Shoot N (%)
Ryegrass litter + *F. candida*	4.2±1.9	1.4±0.6	39.6±2.6	0.82±0.13	42.9±0.4	0.95±0.23
F. candida only	4.6±1.6	1.4±0.5	39.8±3.0	0.88±0.25	42.9±0.5	0.99±0.37
Ryegrass only	5.5±0.7	1.8±0.22	39.6±2.5	0.76±0.07	38.0±13.0	0.79±0.30
No *F. candida* and no ryegrass	5.8±0.8	1.5±0.26	39.6±2.0	0.82±0.15	42.9±0.6	0.9±0.25
P - value	0.05[n.s]	0.18[n.s]	0.21[n.s]	0.29[n.s]	0.26[n.s]	0.24[n.s]

[n.s] treatment means (±S.D, n = 9) are not significantly different at *P* < 0.05.

2.4.2 Collembola

There was population growth of *F. candida* in all treatments (Fig. 2.2). The number of *F. candida* recovered differed significantly ($F_{3,41} = 8.56$, $P < 0.0002$) between treatments. The highest number was recovered from microcosms with maize and ryegrass litter (212 ± 123), followed by microcosms with maize alone (200 ± 115), ryegrass litter only (128 ± 79) and soil alone without maize or ryegrass litter (94 ± 67). Due to the extremely small size of Collembola nymphs, 100% recovery was not possible from soil in water suspension.
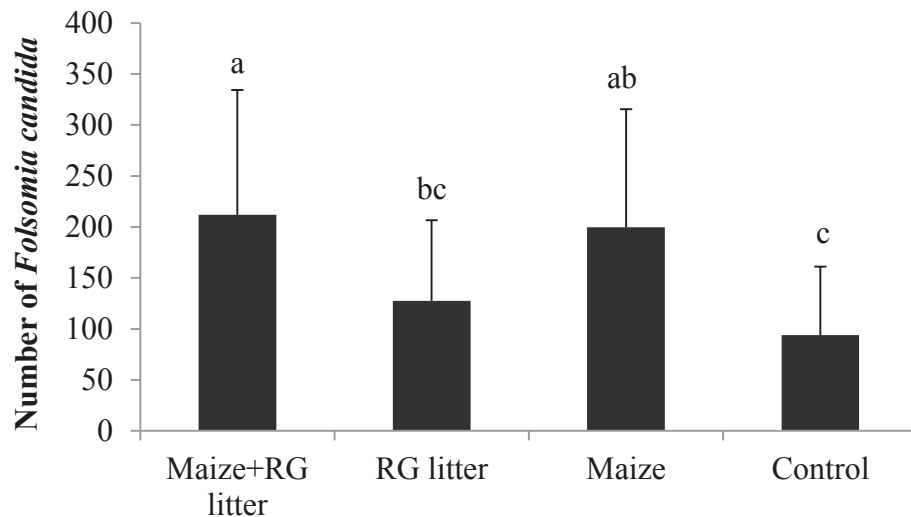
Figure 2.2 Number (mean ± S.D, n = 12) of *Folsomia candida* recovered per microcosm containing soil and maize ^{15}N enriched ryegrass (RG) litter, ^{15}N enriched ryegrass litter without maize, maize without ^{15}N enriched ryegrass litter and control without maize or ^{15}N enriched ryegrass litter. Mean separation was done using Tukey's HSD at $P < 0.05$. Treatments means sharing the same letter are not significantly different from each other.

The presence of maize and/or ryegrass litter had no significant effect on the tissue C content of *F. candida* (Table 2.4). However, $\delta^{13}C$ of Collembola tissue was influenced significantly by the presence of maize, but not ryegrass litter ($F_{3,42} = 18.01$, $P < 0.0001$, Fig. 2.3). $\delta^{13}C$ of *F. candida* in both maize plant treatments differed significantly from the non-maize treatments. The $\delta^{13}C$ signature of *F. candida* exposed to maize and

ryegrass litter combined (-19.28±3.12‰) was between the values of maize plant source tissue (-13±0.4‰) and ryegrass litter tissue (-29±0.01‰). It did not differ significantly from Collembola which were exposed only to maize. In treatments without maize, $\delta^{13}C$ reflected the signature of typical C_3 sources: in this case ryegrass litter in the soil (-23.15 ‰) or soil alone (-23.66 ‰), which did not differ significantly. The C isotopic signature for *F. candida* exposed to maize and ryegrass litter and the signatures for maize and litter as C sources applied to the two source mixing model indicated that 59.7±0.2% of its C originated[1] from live maize roots.

Nitrogen content of Collembola tissue did not differ in the four treatments ($F_{3,38} = 0.83$, $P < 0.48$, Table 2.4). $\delta^{15}N$ of Collembola was lowest for the control without maize plants or ryegrass litter (9.9±3.1‰) and maize plants alone (12.91±2.4‰) and these treatments did not differ. However, addition of ^{15}N enriched ryegrass litter increased ($F_{3, 42} = 83.53$, $P < 0.0001$) the $\delta^{15}N$ signature of Collembola to 518.7±146.2‰, when maize was absent and to 498±154.7‰, when maize roots were present. These latter two were not different from each other. The amount of N derived by Collembola from maize roots/ryegrass litter calculated using the two source mixing model showed that Collembola derived 3±0.01% N from ryegrass litter and 97.2±0.01% from maize roots.

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[1] The above mixing model requires two sources to calculate the amount of C and N incorporated into animal tissue. The model has the limitations that it assumes both the sources contribute the same amount of C and N to the animals, and there are only two diet sources at any time.
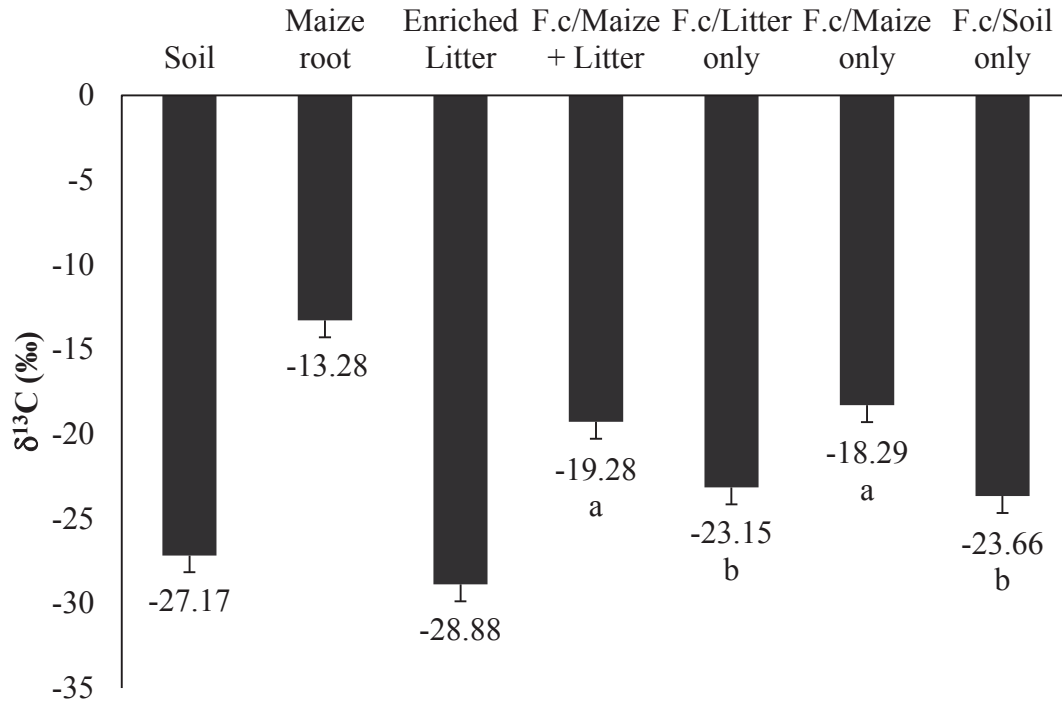
Figure 2.3 $\delta^{13}C$ (mean ± S.D, n = 12) values of *Folsomia candida* (F.c) grown in maize and ^{15}N enriched ryegrass litter, ^{15}N enriched ryegrass litter without maize, maize without ^{15}N enriched ryegrass litter and control without maize or ^{15}N enriched ryegrass litter. $\delta^{13}C$ (mean ± S.D) values of soil, maize root and enriched ryegrass litter are also given. Mean separation was done using Tukey's HSD at $P < 0.05$. Treatment means sharing same letter are not significantly different from each other.

Table 2.4 *Folsomia candida* tissue C and N concentration in the presence of maize plant and ryegrass litter, ryegrass litter without maize plant, maize plant without ryegrass litter and a control treatment without maize plant and without ryegrass litter.

Treatment	*F. candida* C (%)	*F. candida* N (%)
Maize + ryegrass litter	46.7±3.2	10.3±0.8
Ryegrass litter only	55.2±9.4	9.8±4.4
Maize only	47.1±3.4	11.0±0.7
Control	44.9±3.4	11.2±0.9
ANOVA *P*-value	0.48[n.s]	0.7[n.s]

[n.s] Treatment means (±S.D, n = 12) are not significantly different from each other at $P < 0.05$.
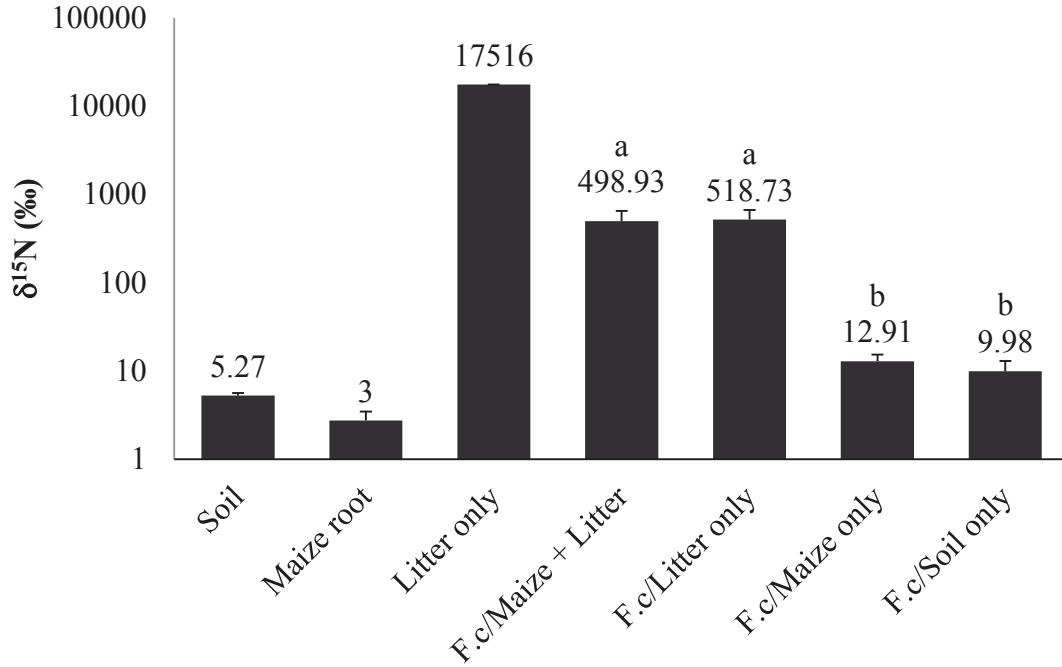
Figure 2.4 $\delta^{15}N$ (mean ± S.D, n = 12) values of *Folsomia candida* grown in maize and ^{15}N enriched ryegrass litter, ^{15}N enriched ryegrass litter without maize, maize without ^{15}N enriched ryegrass litter and control treatment without maize or ryegrass litter. $\delta^{15}N$ (mean ± S.D) values of soil, maize root and enriched ryegrass litter were also given. Y-axis is given in log scale (base-10). Mean separation was done using Tukey's HSD at $P < 0.05$. Treatments means sharing same letter are not significantly different from each other.

2.5 Discussion

Results extend the active diet-switch observed by Endlweber et al. (2009) with *P. fimata* to another collembolan species, *F. candida*. However, in contrast to our hypothesis, when given a choice, *F. candida* did not completely switch their diet from ryegrass litter to maize but instead, obtained their C and N in varying proportions from both sources, showing a partial diet-switch. The $\delta^{15}N$ value was greater than expected due to the high $\delta^{15}N$ signature of the ryegrass litter used in this study compared to that in Endlweber et al. (2009). Collembola tissue C and N concentrations did not differ between treatments, but the fraction of C and N derived from maize and ryegrass litter varied greatly. Ryegrass litter in combination with maize accounted for slightly less than half of

F. candida tissue C. *Folsomia candida* derived essentially the same proportion of its C from maize irrespective of the presence or absence of ryegrass litter. In a similar experimental system, *P. fimata* foraged exclusively on live maize roots, either in the presence or absence of ryegrass litter both for their C and N requirements (Endlweber et al., 2009). Larsen et al. (2007) obtained values closer the present than to Endlweber (2009) for C incorporation from roots (54%) by *P. minuta.* They exposed Collembola to wheat plants and dual labelled ryegrass in their microcosms and exposed them to the same amount of time, 8 weeks. Carbon incorporation values for two other collembolan species, *P. armata* and *F. fimerata* exposed to a mixture of wheat roots and enriched litter were 59 and 77% respectively (Larsen et al. 2007).

It may be possible to improve the accuracy of the estimated C fractionation between food sources if one uses corrected C source values in the equation of the two source mixing model. Gearing (1991) suggested to replace the signature of the food source itself by the use of δR_x and δR_y for the same animal species feeding on only one source of food at a time. These data are rarely used because they are usually not available. Based on this correction, a slightly higher (72%) fraction of *F. candida*'s C originated from maize roots. Although, both *P. fimata* and *F. candida* both are euedaphic species, the former species switched exclusively to maize for its nutritional requirements, it appears not to hold true for *F. candida.* The total diet-switch to maize observed with *P. fimata* suggests that the plant may have provided more of the nutritional requirements for that species than for *F. candida*. The current results show maize roots had a significant influence on the diet of *F. candida,* but ryegrass litter also contributed to a great extent. Leaf litter (organic matter), once presumed to be the primary food source for many soil biota including Collembola had a ^{13}C signature similar tree roots (Pollierer et al., 2007). The current results also confirms the findings of Larsen et al. (2007) who showed that Collembola in the presence of live wheat roots and ryegrass applied as green manure, derived a significant amount of their energy source from live roots. However, roots remained more preferred food source over litter because leaf litter possesses more

recalcitrant compounds like polyphenols and high fiber content which deter soil organisms.

Similar to previous studies, this experiment showed that *F. candida* benefited by the availability of plants and ryegrass litter. Live plant roots seem to have a beneficial effect on Collembola as indicated by a tendency for greater abundance of springtails. There were twice as many Collembola in the presence of maize plants than in ryegrass litter only treatments. Availability of more food in the form of plants and ryegrass litter compared to the soil control might have caused increased Collembola densities, as observed in *F. candida* (Usher et al., 1971), *Orchesella cincta* (Joosse and Testerink, 1977) and *P. fimata* (Endlweber and Scheu, 2007). On the contrary, *F. candida* abundance was not influenced by fine root biomass in a greenhouse soil system with the evergreen *Chamaecyparis obtusa* (Fujii et al., 2013). Higher densities of Symphypleona and Isotomidae were observed in the presence of legumes than in grasses, probably because of the greater N content of legume roots (Salamon et al., 2004).

The δ^{15}N signature of *F. candida* in the presence of maize plants alone differed by 6.33 δ units from maize roots, suggesting a shift of two trophic levels (DeNiro and Epstein, 1981; Haubert et al., 2005), implying *F. candida* could have obtained their N from fungi, but not from feeding on plant roots directly. When maize roots were absent δ^{15}N signature of *F. candida* increased by 4.7 δ units suggesting a shift of only one trophic level. Tissue C content of *F. candida* in the presence of live maize roots could also account for direct consumption of recent photosynthetic C deposited in the rhizosphere region by plant roots.

Presence of various materials in the guts of Collembola indicates that they feed unselectively and the contents are characteristic of the particular environment in which they occur (Hopkin, 1997). Based on visual observations in rhizotrons, Gunn and Cherrett (1993) found that the feeding activity of soil meso- and macro-invertebrates could be separated into 5.5% root materials, 0.5% decaying material and 80% feaces/detritus/soil surfaces. Ponge (2000) also noted feeding on a wide variety of material, implying a low

degree of food specialization in Collembola. This was attributed to the availability of excess and variable food resources (Anderson and Healey, 1972). In the majority of collembolan samples analyzed for enzyme activity, Thimm et al. (1998) and Berg et al. (2004) found positive scores for cellulase, chitin and trehalase which are needed for digesting plant, fungal and microbial cell contents, respectively. The presence of these enzymes support the suggestion that collembolans are omnivores or indiscriminate feeders. Although dietary specialization of Collembola to avoid niche overlap has been reported, this study challenges that theory by showing that they feed on different types of food materials which was also reported by Scheu and Simmerling (2004). This opportunistic feeding in Collembola life history (Petersen and Luxton, 1982) can be an important reason behind their success in various environments. Feeding on many resources also explains their role as important drivers of the decomposition process.

The results of the current experiment are on par with the root biomass of *Trifolium. repens* or *L. perenne* which was also not influenced by the presence of Collembola and enriched ryegrass litter in similar microcosm experiments (Kreuzer et al., 2004). The presence of ^{15}N enriched ryegrass litter increased the ^{15}N signature of maize but not the plant tissue N concentration. Endlweber et al. (2009) observed an increase in shoot biomass in the presence of ryegrass litter whereas the current study did not find any differences in shoot biomass whether or not the ryegrass litter was present. This ryegrass litter had higher N and optimum C:N ratios compared to the same in Endlweber et al. (2009), but the increase in shoot biomass was not clearly understood. Complete root herbivory by *P. fimata* on maize roots might have increased the fine root hairs which could have in turn improved the N-uptake and subsequently shoot biomass. Results from the current experiment with respect to plant biomass, tissue C and N content are similar with those reported by Larsen et al. (2007). These authors also did not find any difference in shoot biomass but observed differences in δ^{15}N, which shows that the majority of maize N was derived from added ryegrass litter in both studies.

2.6 Conclusion

Folsomia candida showed a partial diet-switch towards maize roots from ryegrass litter acting as an omnivore. The current study highlights the importance of underground root systems in the diet of another collembolan species along with *Protophorura fimata* studied earlier. These findings support the results of Endlweber et al. (2009), suggesting the role of roots in the soil food web has been greatly underestimated. The studies that show leaf litter to be the primary source of food for Collembola and other soil animals should be reconsidered to get a better understanding of their feeding ecology. If these results can be confirmed for other soil biota, our understanding of the functioning of underground systems will be changed. This will have major implications for the study of carbon fluxes and functioning of decomposer system. Different combinations of root availability and root quality could affect the level of diet-switching observed with *F. candida,* but this could not be tested under this experimental design.

CHAPTER 3   IMPACT OF LIVE ROOTS ON PERFORMANCE OF *FOLSOMIA CANDIDA*

3.1 Introduction

The performance (survival, growth and reproduction) of Collembola and *Folsomia candida* in particular is generally associated with plant derived products and their associated fungi (McMillan, 1975; Booth and Anderson, 1979; Chen, 1995; Kaneko et al., 1995; Kaneda and Kaneko, 2002; Fountain and Hopkin, 2005). Food choice studies with Collembola have only partially determined the food source due to the difficulties associated with studying underground soil animals. The contribution of living roots to food webs received relatively little attention initially but the prevalence of microarthropods in soil with roots over soil without roots has suggested that they may play an important role (Gunn and Cherrett, 1993; Pollierer et al., 2007). The results of a study on the effects of plant diversity on the density of Collembola by Salamon et al. (2004) suggested food quality rather than quantity determines the abundance of Collembola. There is also evidence that exudates of living roots may serve as a source of food for Collembola (Pollierer et al., 2007). A stable isotope analysis by Endlweber et al. (2009) demonstrated that *Protaphorura. fimata* switched diet from plant detritus to live plant roots when given a choice between the two in soil microcosms. In the previous experiment (see chapter 2), a similar but partial diet-switch from ryegrass litter to maize roots was also demonstrated for *F. candida.* This preference for live plant roots could result from a food quality higher in plant roots than in ryegrass litter (Endlweber et al., 2009), greater abundance of roots than ryegrass litter or a combination of both. This experiment is aimed at determining the role of food quality in triggering the diet-switch by measuring the corresponding expected positive impact on the performance of *F. candida*. The potential role of live root abundance in influencing diet-switching is considered in Chapter 4.

Nutritional studies on insects and ruminants documenting the impact of food quality on their performance and feeding behavior (Slansky and Rodriguez, 1987;

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Slansky Jr et al., 1993; Van Soest, 1994) led to the development of theories regarding optimal diet selection and optimal foraging (Cruz-Rivera and Hay, 2000). According to the optimal foraging theory, organisms forage on food resources in a particular environment to maximize their energy intake and thus performance (Schoener, 1971; Charnov, 1976). Foraging Collembola would switch diet from plant detritus to live roots if roots helped maximize their fitness. Body growth, reproduction and survival are three common indicators of animal performance. We hypothesized that *F. candida* demonstrate a partial diet-switch towards live plant roots because roots may be a food source of higher quality than plant litter and predicted a performance gain for the springtails as a result of the switch. To test this prediction we exposed *F. candida* individuals to soil microcosms with and without maize plants and compared their body growth, reproduction and survival. The prediction was tested in two types of soil and with or without supplementary yeast food.

3.2 Materials and methods

The potential impact of live root quality on the performance of *F. candida* was assessed by measuring its effect on body growth (Experiment 1), reproduction (Experiment 2) and survival (Experiment 1 and 2). The experiments were carried out separately for logistical reasons. *Folsomia candida* used in the experiments were obtained from a colony maintained at 20° C in incubators and fed with baker's yeast as described in Chapter 2.

3.2.1 Experiment 1: Effect of live maize roots on body length and survival

The experiment was set up as a split-plot factorial design with three factors: soil type, plant roots and food supplement, each at two levels (Table 3.1). The two types of soil used consisted of a field soil collected from a certified organic field trial (see section 2.2.2 for soil characteristics) and OECD soil, also referred as artificial soil (OECD, 1984; ISO, 1999) made up of sphagnum peat, kaolin and industrial quartz mixed in the ratio

10:20:70. Plants were either present or absent and consisted of maize plants. The food supplement was either absent or present and consisted of yeast.

The experiment was carried out in microcosms consisting of 20 mL glass vials measuring 6.8 cm in length and 1.8 cm internal diameter at opening. To each microcosm 8 g of soil was added and one pre-soaked maize seed was sown for all treatments with maize as a factor. Baker's yeast (0.5 g/microcosm) was added to the treatments with yeast and replenished every 5 days. All treatments were replicated 16 times to allow destructive sampling of microcosms on days: 5, 10, 15 and 20 after springtail release (t_0). Soil was maintained at 100% water holding capacity (WHC) throughout the experimental period by adding 5 mL deionized water initially and thereafter 1 mL every alternate day. Once the maize was germinated, usually 4 days after sowing, each microcosm received 100 three-day old *F. candida* and this was recorded as day t_0. Microcosms were randomly placed in an incubator (BOD Model L127, Sheldon Manufacturing, Cornelius, Oregon) maintained at 20° C and illuminated with a 40 W light bulb. The position of microcosms was shuffled every other day when the microcosms were removed for watering. The 21-day-long experiment was carried out twice, starting on 5th and 12th July 2012.

Table 3.1 Treatments used in experiments 1 and 2 to determine the impact of live root presence on the body growth and reproduction of *Folsomia candida* (+ and – denotes presence or absence of a factor).

Treatment	Soil type	Maize	Yeast
T1	Field soil	+	+
T2	Field soil	+	-
T3	Field soil	-	+
T4	Field soil	-	-
T5	OECD soil	+	+
T6	OECD soil	+	-
T7	OECD soil	-	+
T8	OECD soil	-	-

To recover *F. candida* at each sampling date, deionized water was added to each microcosm and the contents were carefully transferred into 125 mL Mason jars. Collembola floating on the surface due to their hydrophobic integuments were recovered with the help of a moist fine tipped paint brush and transferred onto a plaster mix for further analysis. The size of *F. candida* was measured under a stereomicroscope (Leica M80, Leica microsystems, Buffalo Grove, IL) coupled with Leica image analysis suite v3.7. Body length measurements were taken before addition to microcosms and after recovery from microcosms. Plants were carefully taken from the microcosms and washed thoroughly in Mason jars to remove soil particles adhered to the surface. This suspension was also examined for the presence of *F. candida*. Roots were separated and dried at 65°C for 24 h and weighed on a balance (Scientech 80A, Precision Weighing Balances, Bradford, MA., precision =1 mg).

Figure 3.1 Experimental set up of performance test showing microcosms (test vials) filled with field or OECD soil, maize and/or yeast, arranged in randomized order.

Survival of adult *F. candida* was measured by counting the number of live individuals in each microcosm for each treatment replicate on each sampling day.

3.2.2 Experiment 2: Effect of live maize roots on reproduction

Treatments were the same as in experiment 1 (Table 3.1) and the experimental procedure was similar. However, each microcosm received one 15 day-old *F. candida* and the test was carried out for 49 days. This test was carried for a longer period to allow sufficient time for *F. candida* to produce eggs and nymphs to develop from eggs. Newly emerged *F. candida* were provided with yeast *ad lib* for ten days and maintained without food supplement for the next five days before their transfer into test microcosms. The experiment was carried out twice starting on 21st August and 15th September 2012. On each sampling day, *F. candida* were recovered by destructively sampling test microcosms by flooding them with deionized water. This suspension was transferred to 125mL Mason jars to facilitate removal of *F. candida.* This slurry was gently stirred to dislodge any *F. candida* adhering to the substrate and the nymphs floated to the surface are transferred to petri plates. This process was repeated until no more nymphs were visible. Maize roots were carefully washed and examined for *F. candida* on the roots. Water used to wash maize roots was also examined for nymphs by gently stirring it. Roots were separated and dried at 60° C for 24 h and weighed. The largest springtails were recovered first and counted as adults (body length > 1 mm). The remaining springtails were counted as nymphs. Individual nymphs were then transferred to 10 cm diameter petri plates with a black plaster mix layer at the bottom to permit easier counting against the dark background. Plates were placed on ice to reduce movement and dispersal of springtails.

Survival of adult *F. candida* was measured as in section 3.2.1.

3.3 Properties of treatment soils

The two test soils were analyzed for total C, N, pH and WHC. Soils were finely ground and total C and N content were measured (0.5 g sample) on a Vario MAX CN analyser (Elementar, Hanau, Hesse). To determine pH, 8 g of field soil or OECD soil was mixed with 40mL of 0.01M $CaCl_2.2H_2O$, shaken for 30 min at 5min intervals and allowed to settle for an hour. pH of the supernatant solution was measured with a

standard pH electrode (Denver instruments LTD., Bohemia, NY). All measurements for total C, N, pH and WHC were analyzed for three replicates of each sample. WHC was determined based on "Biological test method: Test for measuring survival and reproduction of springtails exposed to contaminants in soil" (Environment Canada, 2007). Briefly, 25 g of each soil was transferred onto a moist pre-weighed filter paper (W_1) (Fisher Scientific, Fisher brand Q2, Cat. # 09-790-4D) placed in a sealed funnel mounted on a volumetric flask. To this soil sample 100 mL of deionized water was added in quantities of 25 mL every 30 min. The bottom seal was removed after 30 min to drain the water and the sample was left for three hours to saturate. Wet soil along with the filter paper was weighed and recorded as W_2. Soil samples were dried at 105° C in a hot air oven for 24 h and then weighed (W_3). WHC of the samples was determined by using the following formula

$$WHC = \frac{(W_2 - W_1) - W_3}{W_3} X \ 100$$

3.4 Statistical analysis

Experiment 1 was conducted in two batches and the data was analyzed as a split plot factorial design using batch as outside blocking factor, sampling day as whole plot and maize, yeast and soil type as sub-plot treatments. Even though the samplings occurred repeatedly over four sampling periods, split plot design was used as the observations were recorded on different experimental units. The experiment was completely randomized within each split plot and the analysis was carried out using Proc Mixed procedure in SAS 9.3 (SAS Inc, Cary, NC). As the initial length of the insects cannot be controlled, body length was also subjected to ANCOVA to increase the precision of the results,. Means were separated using LSD for body length and Tukey's HSD for maize root biomass at $P < 0.05$. Data for maize root mass and body length of *F. candida* were checked to ensure statistical assumptions of normality and constant variance were met. Normality for maize root dry weight was corrected by excluding one outlier. Data for survival in the experiments 1 and 2, and reproduction for experiment 2

deviated from normality and constant variance even after applying transformations; hence the results were reported after analyzing the data with Friedman's non-parametric test in Minitab 16 (Minitab Inc, State College, PA). Wherever the highest order interaction was significant in non-parametric analysis, Mann-Whitney's pair wise comparison of means for all the possible combinations was performed to separate the means.

3.5 Results

3.5.1 Properties of treatment Soils

There were significant differences in the properties of the two types of soil used in the tests (Table 3.2). The pH and N content of the field soil were significantly higher and the C:N ratio lower than OECD soil. Yeast had higher C and N content than maize roots. The mean soil pH for field and OECD soil differed significantly (5.7 and 6.1 respectively) but remained in the favorable range for this particular species (van Straalen and Verhoef, 1997). Soil pH is not an important factor regulating growth of *F. candida* as long as it is within the optimum range (Kaneda and Kaneko 2002).

Table 3.2 Chemical and physical properties of OECD and field soils, and C and N content of yeast and maize roots used in estimating the performance of *Folsomia candida* with and without live plant roots.

Soil Type	pH	Total C (mg/g)	Total N (mg/g)	C/N ratio	WHC (%)
Yeast	n.d[1]	455±9[a]	56±6[a]	8.2±1[a]	n.d[1]
Maize roots	n.d[1]	402±6[b]	7±0.1[b]	54.5±1[b]	n.d[1]
Field soil	6.1±0.1[a2]	28±2[c]	2±0.1[b]	12.5±1[a]	60
OECD soil	5.7±0.1[b]	45±12[c]	0.7±0.1[c]	64.2±19[b]	55

values reported in the table are mean ± S. D.
treatment means within a column sharing different letters are significantly different at $P <$ 0.05.
[1]not determined.

3.5.2 Experiment 1: Body length

There was a significant interactive effect of maize, yeast, soil type and time elapsed on the changes in body length of *F. candida* (ANOVA, $F_{3, 210} = 4$, $P < 0.0085$) (Table 3.3). The mean body length was smallest in treatments without yeast (0.55 – 0.60 mm to 0.86 - 1.16 mm) and significantly longer in all treatments with yeast (0.69 – 0.78 mm to 1.41 – 1.48 mm) (Fig. 3.2). In yeast containing treatments, maize and soil type had a limited and inconsistent impact on body length throughout the duration of the test. Some of the shortest body lengths were recorded in the presence of maize (T6). However, in non-yeast treatments (T2 and T6), maize had a significant positive impact on the body growth of *F. candida* (Fig. 3.2). Body length on day 5 was similar across treatments, but from day 10 onward it was significantly longer in treatments with maize than in treatments without maize. It is also interesting to note that the combination maize-field soil resulted in longer mean body length than the combination maize-OECD soil, but that in the absence of maize, field soil resulted in shorter mean body length than OECD soil.

Table 3.3 Results of ANOVA evaluating the variation in the mean body length of *Folsomia candida* over four sampling days when exposed to the presence/absence of maize, yeast and two types of soil (*P*-values for type 3 fixed effects)

Effect	Num DF	Den DF	F Value	Pr > F
Maize	1	210	84.69	<.0001
Yeast	1	210	2462.30	<.0001
Soil	1	210	5.35	0.0217
Maize*yeast*soil	1	210	27.70	<.0001
Maize*yeast*soil*day	3	210	4.00	0.0085

The mean dry weight of maize roots increased consistently throughout the 20 days of Experiment 1 (Fig. 3.3 & 3.4). Interaction between soil type and yeast significantly influenced root biomass (ANOVA, $F_{1, 114} = 4.94$, $P < 0.028$). Mean dry weight of roots also differed significantly between soil types and yeast or no yeast treatments in some of the sampling days, but there was no consistent pattern. Maize in both OECD soil

treatments (T5 and T6) produced higher root mass than in field soil treatments on day 5. Root biomass in yeast and field soil treatment (T1) differed from T5 only. Dry weight of maize roots in field soil (T2) is similar to T5 but significantly different from OECD soil (T6). However, there was no difference between the four maize treatments on days 10 and 15. At the end of the trial, the effect was reversed with field soil recording higher root mass than the OECD soil. Even though maize in T2 had higher root biomass than T1, the difference is not significant (Fig 3.3). Roots in T5 differed significantly only from T1 but not from T2. T6 only differed with respect to both T1 and T2.
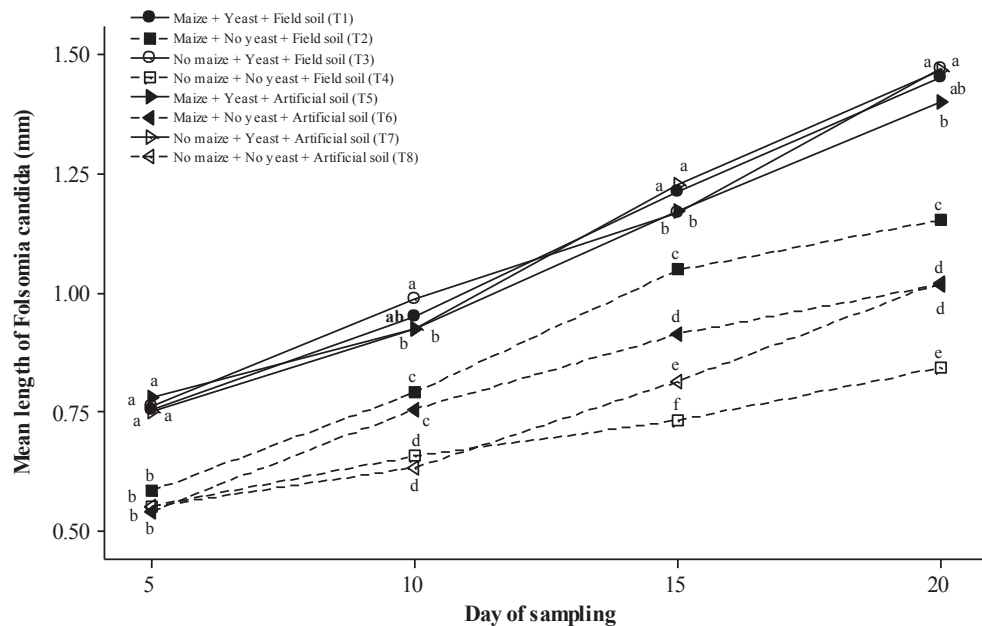
Figure 3.2 Increase in body length of *Folsomia candida* (n=4) in eight different treatments with field and OECD soils. Mean separation was done using LS means option in SAS 9.3 and given for each sampling date separately. Treatments means sharing same letter are not significantly different from each other at *P* < 0.05. Presence of maize is represented with closed symbols and absence of maize is represented with open symbols, similarly treatments with yeast are represented with solid lines and treatments without yeast are represented with broken lines.

Treatment effect had no influence on survival rate of *F. candida* (Friedman test, *df* = 31, *P* < 0.325). Survival was 87.5% or greater in all treatments except for yeast in OECD soil where it was 75%. The average survival rate for four sampling periods ranged from 93.8% in the treatment with maize and field soil (T2) to 100% in the treatment with maize, yeast and field soil (T1) and also for the OECD soil only treatment (T8). Overall survival of *F. candida* throughout the experiment, for all the treatments, was 96.5%.
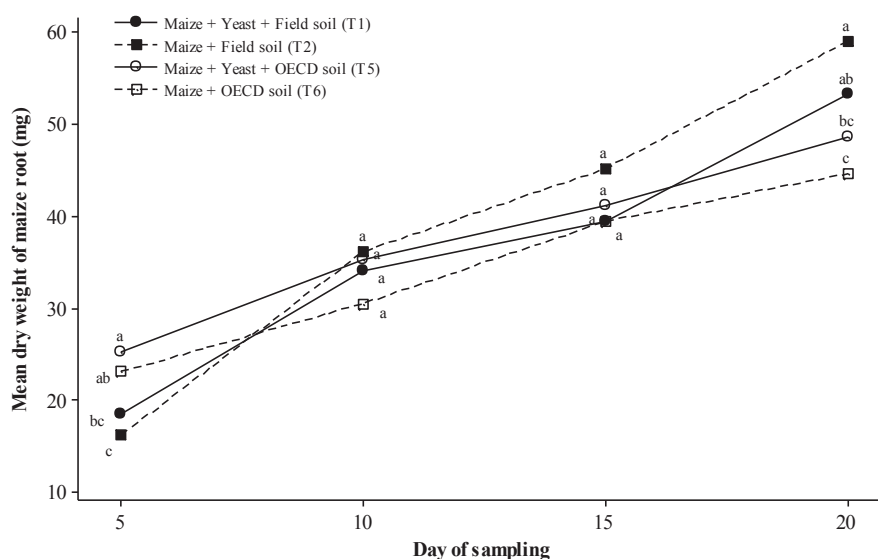
Figure 3.3 Changes in the mean dry weight (g) of maize roots in soil microcosms across the four sampling dates of Experiment 1. Mean separation was done using Tukey's HSD in SAS 9.3 and given for each sampling date separately, means sharing same letters within each sampling day are not significantly different from each other at *P* < 0.05. Field soil is represented with closed symbols and OECD soil is represented with open symbols. Similarly treatments with yeast are represented with solid lines and treatments without yeast are represented with broken lines.

Figure 3.4 Maize root development on field soil during the four sampling dates of experiment 1. From left to right – maize plants on 5th, 10th, 15th and 20th day of sampling.

3.5.3 Experiment 2: Reproduction

Nymph production on any sampling day was significantly higher (Friedman test, $df = 31$, $P < 0.003$) in treatments that included yeast, compared to treatments without yeast. However, contrary to the effect on body length, the number of nymphs produced remained constant over the four sampling periods, even in the presence of yeast. There was no observable trend in the number of nymphs produced within yeast and non-yeast treatments throughout the experimental period (Fig 3.5).

In general, treatments with OECD soil produced slightly more nymphs than field soil and this is true for both yeast and non-yeast treatments. Among the treatments to which yeast was added, OECD soil (T5 and T7) produced the highest level of reproduction on day 28, but gradually decreased to little over 12 nymphs at the end of the experiment. On day 28, T3 (yeast in field soil) showed the lowest reproduction rate and it gradually increased, finishing slightly greater than T1 and T7. With regard to the non-yeast treatments T2, T4, T6 and T8, the number of nymphs produced never exceeded two in any of the sampling dates. On average, throughout the experiment, the greatest number of nymphs was produced with maize and yeast in OECD soil (T5) on day 28

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(14.5/microcosm), followed by yeast in OECD soil (T5) (14/microcosm), while OECD soil (T7) on Day 28 recorded the lowest number of nymphs per microcosm (0.75).
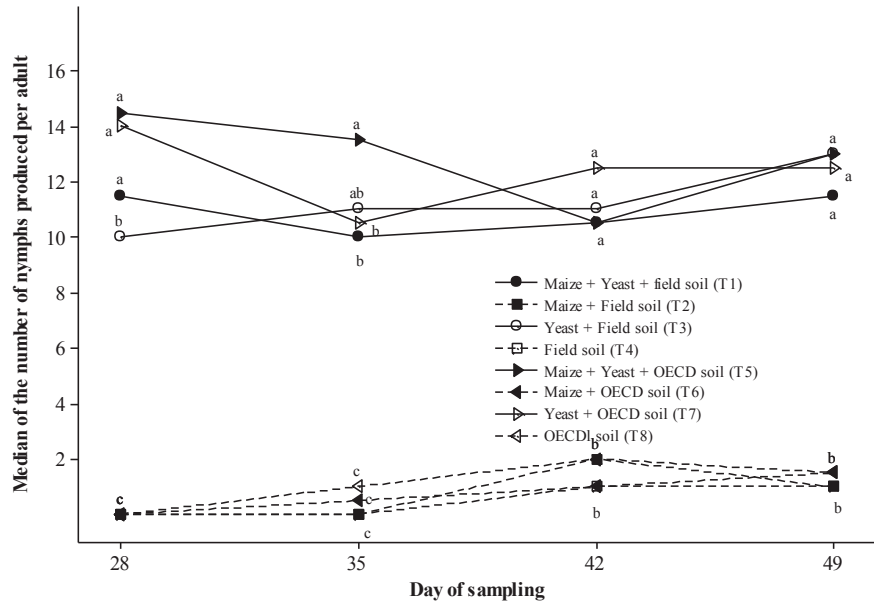
Figure 3.5 Mean number of nymphs produced (per adult) by *Folsomia candida* feeding on different combinations of maize, yeast and two types of soil. Mean separation was carried using Mann-Whitney's pair wise comparison at $P < 0.05$ and given for treatments within same sampling date. Treatments sharing same letters are not significantly different from each other. Closed symbols represent treatments with maize and open symbols represent treatments with no maize. Similarly treatments with yeast are represented with solid lines and treatments without yeast are represented with broken lines.

Mean weight of maize roots increased consistently throughout the first 20 days of this experiment, similar to that in Experiment 1 (Fig. 3.2), but was followed by a parabolic growth pattern before leveling off (Fig. 3.6). There was no significant interaction of yeast and soil type on root biomass among the treatments studied (ANOVA, $F_{1, 117} = 0.08$, $P < 0.77$).

Similar to Experiment 1, survival was not influenced by the treatment effect (Friedman test, $df = 31$, $P < 0.105$). However, the survival of adult *F. candida* decreased slightly as time progressed. For all of the sampling dates, survival rates in field soil treatments were higher than OECD soil treatments. OECD soil (T8) on Day 28 and maize

in field soil (T2) on Day 42 recorded the lowest survival rates (62.5%) with most of the treatments with both field and OECD soils recording 100% survival.
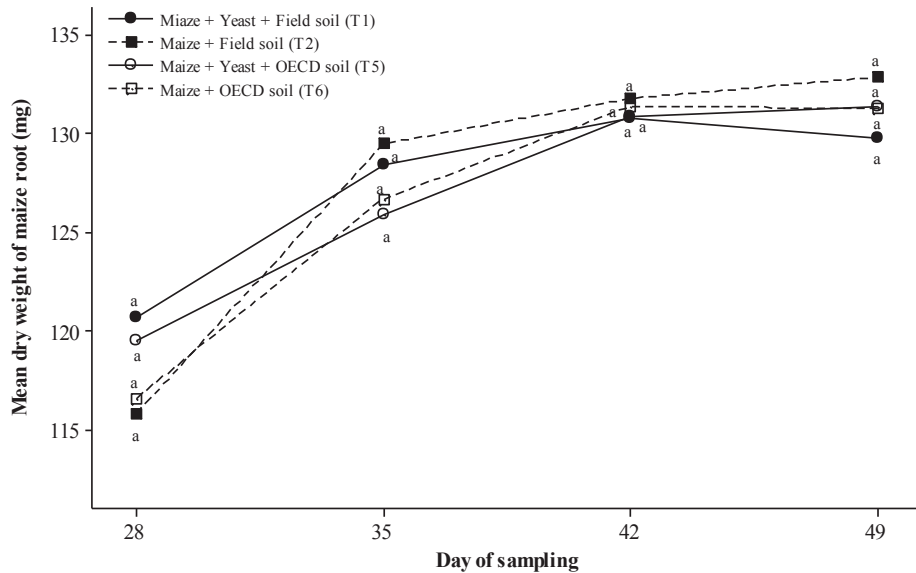
Figure 3.6 Changes in the mean dry weight (g) of maize roots in soil microcosms across the four sampling dates of Experiment 2. Mean separation was done with Tukey's HSD in SAS 9.3 at $P < 0.05$ and given for each sampling date separately. Treatment means sharing same letters within a sampling day are not significantly different from each other. Field soil is represented with closed symbols and OECD soil is represented with open symbols. Similarly treatments with yeast are represented with solid lines and treatments without yeast are represented with broken lines.

3.6 Discussion

3.6.1 Impact on body growth

Folsomia candida had a higher rate of body growth in the presence of live maize roots than in their absence. However, this positive impact of live roots was masked by the greater impact of yeast in microcosms receiving a yeast supplement. Although live maize roots represented the source of highest quality food in microcosms without yeast, yeast itself became the source of high quality food when present. *Folsomia candida* body

growth in the current experiment (1.41 to 1.48 mm) was similar to that reported by San Miguel et al. (2008) in the presence of yeast after 20 days (1.34 ± 0.16mm). This is not surprising considering that yeast is an ideal food for *F. candida* and many other Collembola species (van Amelsvoort and Usher, 1989a) as it possesses higher N content than maize roots. In similar experiments, it was shown that feeding on high quality food resulted in increased body growth (van Amelsvoort and Usher, 1989b). The key role of food quality in body growth and population regulation of animals is well documented but less is known for below ground animal species. Essentially, the current results demonstrate that live roots may improve the performance of Collembola such as *F. candida,* but its impact was far less than that of higher quality food source such as yeast.


Figure 3.7 Maize root development during the four sampling dates on OECD soil for experiment 1. From left to right – maize plants on 5th, 10th, 15th and 20th day of sampling.

3.6.2 Impact on reproduction

The absence of changes in reproduction in response to the different treatments may have resulted from the application of a starving period before the individuals were added to test microcosms. The starving break may have induced a shift of resources to growth and survival at the expense of reproduction. The allocation of internal resources to survival by starved springtails could have affected reproduction (Kirk, 1997; Gutow et

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al., 2007). Starved individuals will have low energy levels, which can lead to reduced growth and reproduction as they replenish the internal levels before any further growth can occur (Boersma and Kreutzer, 2002). Another reason for reduced reproduction could be increased feeding activity on maize to meet their energy requirements. This can cause an increase in the amount of energy spent on feeding activity, with a subsequent decrease in growth and secondary production (Sterner and Hessen, 1994).

Although reproduction of *F. candida* in the current experiment could not be linked to food quality, some relationship has been established in other studies. For example, van Amelsvoort and Usher (1989a) established a complex relation between food quality and fecundity and showed that there was early reproductive activity in presence of high quality foods. In the current experiment, individual *F. candida* were used, but collembolan are known to reach maximum fitness when they are living in aggregate colonies (Green, 1964). Collembola present in groups of 10 produced more eggs than individual organisms (Draheim and Larink, 1995). *Folsomia candida* also shows inter-individual variability with respect to reproduction (Crouau and Cazes, 2003).

3.6.3 Effect on survival

Survival of *F. candida* was not significantly affected by any of the eight treatments in both experiments. Food quality did not have a clear effect on survival rate. The lowest survival rate for *Folsomia candida* was observed in a treatment with yeast, a high quality food supplement, whereas individuals in OECD soil without maize and yeast survived until the end of the experiment. Survival was the performance indicator least affected by food quality. This observation is in line with the findings of Nelson et al. (2011) in their study of *F. candida* as a bio-indicator of soil health.

This may result from the well-known ability of Collembola to survive long periods of time, even in the absence of food. This has been attributed in part to the allocation of their internal reserves to survival until a food source can be located (Dombos and Stimmann, 2001). These researchers showed that springtails deprived of

food took as many as 103 days to excrete the assimilated rubidium, whereas well-fed individuals lost it within 46 days. Collembola can survive without any food sources by utilizing their lipid and glycogen reserves (Testerink, 1981; Verhoef and Li, 1983). *Bonetogastrura balazuci* survived for 560 days without food on a mixture of clay and plaster (Fountain and Hopkin, 2005).

3.6.4 Root biomass and soil types

The positive impact of roots on collembolan body growth in treatments without yeast could not have been caused by differences in root availability. Measurements, taken in the course of the tests on body growth and reproduction, confirmed that there were no differences in root biomass between treatments. The increased performance observed with *F. candida* was therefore not caused by differences that might have existed between treatments in the availability of live plant roots but could only be attributed to food quality advantages provided by the presence of live roots.

The N content of the field soil was higher than that of the OECD soil; hence, the N content of respective maize plants may have been higher, which could have contributed to the increased body length of *F. candida* in these treatments. This would be in agreement with Sadaka-Laulan and Ponge (2000) who reported that *Onychiurus sinensis* preferentially fed on litter with a higher N content and had higher body length when feeding on high N litter compared with litter containing lower N content. Booth and Anderson (1979) showed Collembola benefitted by having higher moulting rate and fecundity when the N level of a substrate on which the fungi were cultured was increased. Two amphipod species *Gammarus mucronatus* and *Elasmopus levis* were able to feed on low quality food (low in protein, N and organic C), but with decreased fitness compared to feeding on high quality foods. However when a high quality food was offered, they immediately switched to it (Cruz-Rivera and Hay, 2000). Decreasing food quality (food with low N and P) also decreased survival, growth and fecundity in planthopper species, *Prokelisia dolus* and *P. marginata* (Huberty and Denno, 2006).

3.7 Conclusion

The results of this experiment support the hypothesis that the presence of plants and their underground root systems play a key role in determining the fitness and performance level of Collembola in ecosystems. More specifically, these results have shown that the body growth of *F. candida* was positively affected by the presence of live roots in the soil irrespective of soil type. This was only surpassed when a high quality resource, such as yeast, was continuously supplied. It supports the diet-switch from prior incorporated ryegrass litter to a higher quality food source such as living maize roots, observed in Chapter 2. The absence of improved survival or reproduction on maize also suggests that maize may not provide all the nutritional requirements of *F candida*.

CHAPTER 4   IMPACT OF LIVE ROOT BIOMASS ON DIET-SWITCHING BY *FOLSOMIA CANDIDA*

4.1 Introduction

It is not uncommon for consumers to preferentially choose higher quality foods to increase their fitness (Stephens, 1986). In situations where high quality food is not available or accessible consumers have different options to maintain or improve their fitness (Real and Caraco, 1986; Pennings et al., 1993; Bernays et al., 1994). During such cases, soil animals may disperse to find optimal food sources or increase their intake of low quality food to satisfy their nutritional requirements (a phenomenon called compensatory feeding) (Price et al., 1980). Although many studies have focused on the relative effects of food quality and quantity in both terrestrial and aquatic systems (Slansky and Scriber, 1985; Slansky Jr et al., 1993; Cruz-Rivera and Hay, 2000; Bukovinszky et al. 2012), only a few studies have examined their impact on Collembola (Booth and Anderson, 1979; Bengtsson et al., 1988).

In a previous experiment (Chapter 2), *Folsomia candida* was shown to exhibit a partial diet-switch from ryegrass residues towards live plant roots when present. By the end of that prior diet-switch experiment microcosms with maize were largely occupied by maize roots, suggesting that springtails possibly switched their diet to live maize roots because of their relative availability. Another experiment (Chapter 3) found a greater increase in the body growth *F. candida* in the presence of live maize roots than in their absence suggesting that the diet-switch was at least in part a response to food quality. In these previous two experiments, root availability (quantity) was similar among treatments that included maize. On the basis of those experiments we hypothesized that the diet-switch to live maize roots by *F. candida* is due to their high quality compared to the added ryegrass litter and that there will be no differences in feeding behavior when different quantities of root biomass are available. The objective of this experiment was to determine if live maize root biomass plays a role in determining the level of diet-switching by *F. candida.* This was accomplished by exposing springtails to different

amounts of live maize roots and monitoring the movement of C and N using stable isotopes.

4.2 Materials and methods

The impact of root availability on the feeding behavior of *F. candida* was assessed by exposing springtails to three levels of live maize root biomass in microcosms (see section 2.2.2 for detailed characteristics of microcosms, soil and ryegrass litter). Maize plants were grown in microcosms filled with 1 Kg soil into which 2 g of ^{15}N enriched ryegrass litter was mixed homogeneously at the same time as maize was sown in each treatment. The three treatments consisted of three maize sowing dates (Table 4.1). All three treatments were replicated thrice. On the last day of maize sowing, May 20th, 100 individuals of age-synchronized *F. candida* (15 day-old) were released into each microcosm. These Collembola and their progeny were recovered on 10th June 2013, three weeks after the last sowing by washing the roots in water and collecting *F. candida*. Roots were separated from shoots, dried at 65° C and ground in a ball mill. Freeze-dried Collembola, ground root and soil samples were packed separately into tin capsules and sent for isotope analysis (see section 2.2.2 for detailed procedure on sample processing).

Table 4.1 Treatments based on the maize sowing date, used to study the influence of root abundance on feeding behavior of *F. candida* (+ indicates the presence of factor).

Treatment	Maize	ryegrass litter	Date of maize sowing
T1	+	+	April 8, 2013
T2	+	+	April 29, 2013
T3	+	+	May 20, 2013

4.3 Statistical analysis

Results were analyzed using SAS 9.3 (SAS Inc, Cary, NC) using Proc GLM procedures for number of Collembola recovered, concentration of tissue C and N, and

isotope signatures of $\delta^{13}C$ and $\delta^{15}N$. The concentration of soil and maize C and N, plus isotope signatures were also analyzed. If the differences between means were significant they were separated using Tukey's HSD test. Data was checked to ensure statistical assumptions of normality and constant variance were satisfied. Normality test of $\delta^{15}N$ showed one abnormal observation; it was treated as an outlier and excluded from the analysis. The amount of C and N contributed by maize and ryegrass litter to Collembola tissue contents were also determined using a two-source mixing model as described in section 2.2.2.

4.4 Results

4.4.1 Maize

As expected, by the end of the experimental period, T1 (maize planted on April 8th) had developed the greater root biomass, followed by T2 (April 29th) and T3 (May 30th) (Fig. 4.1; Table 4.2). Root biomass differed by 330 mg between T1 and T2 and by almost twice (730 mg) between T2 and T3. Root quality (C and N content), did not differ between treatments. The presence of enriched ryegrass litter did not influence N content of the maize tissue, but the $\delta^{15}N$ was influenced greatly. The $\delta^{15}N$ signature of maize roots in T2 was higher than in T1 than T3. Notably, the $\delta^{13}C$ signature of roots did not differ between treatments.
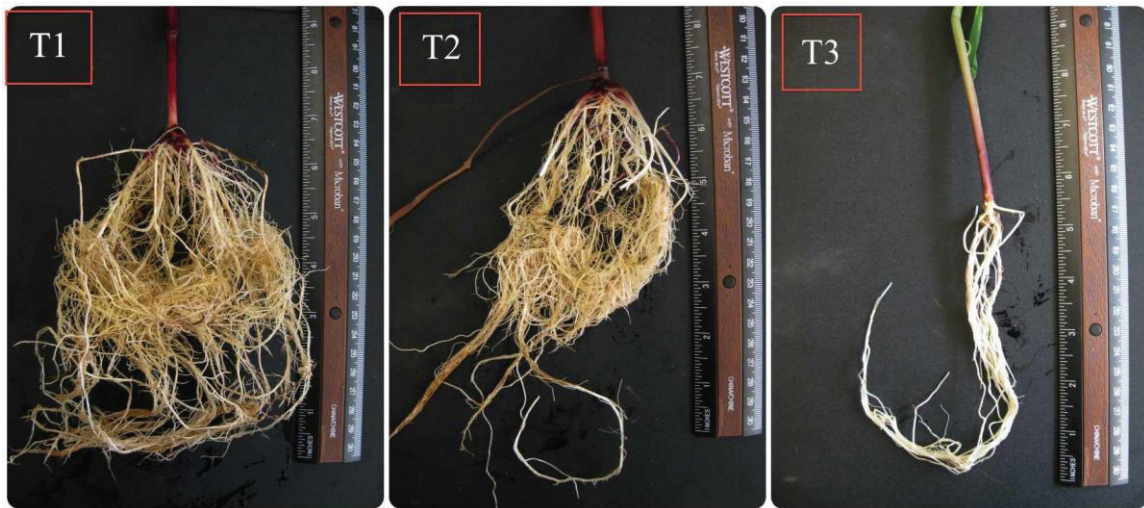
Figure 4.1 Typical maize root development after 63 (T1), 42 (T2) and 21 (T3) days of growth in microcosms.

Table 4.2 Physical and chemical characteristics (mean ± S.D of dry weight, C, N, $\delta^{13}C$ and $\delta^{15}N$) of maize roots after 63 (T1), 42 (T2), 21 (T3) days of growth in microcosms.

Treatment	Dry biomass (mg)	C %	N %	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰
T1	1066.7±177[a]	40.4±1.06	1.8+0.41	-13.4±0.13	180±141.3[b]
T2	736.7±119[b]	42.3±5.56	1.2±0.26	-13.4±0.09	830±43.7[a]
T3	106.7±25[c]	37.2±1.05	1.1±0.14	-12.9±0.41	114±24.7[b]
P-value	0.0002*	0.24[n.s]	0.07[n.s]	0.07[n.s]	0.0001***

[n.s]not significnt
***Significant at $P < 0.0001$.
Treatment means (±S.D, n = 3) within a column sharing different letters are significantly different from one another.

4.4.2 Soil

Soil in all treatments differed with respect to all four variables (C, N, $\delta^{13}C$ and $\delta^{15}N$) studied (Table 4.3). Carbon content and $\delta^{13}C$ of soil in treatments T2 and T3 did not differ significantly from each other, but differed from T1, this treatment being higher

in C content and δ^{13}C among the three. Soil in T3 showed the highest N content and it differed significantly from T1 soil, but not T2. As found also for maize roots and noted above, the δ^{15}N of T2 soil was significantly higher than the remaining two treatments. Even though δ^{15}N value of soil was higher in T3, it did not differ from T1 statistically.

Table 4.3 Soil characteristics and ANOVA *P*-values for treatment effects for soil C, N, δ^{13}C, δ^{15}N at harvest 63 (T1), 42 (T2) and 21 (T1) days (after maize planting) to study the influence of root biomass on Collembola feeding behavior.

Treatment	C %	N %	δ^{13}C ‰	δ^{15}N ‰
T1	2.4±0.06[a]	0.19+0.01[a]	-26.5±0.02[a]	18.1±8.4 [a]
T2	2.2±0.04[b]	0.20±0.01[ab]	-26.8±0.11[b]	77.7±2.1[b]
T3	2.1±0.06[b]	0.22±0.01[b]	-26.9±0.14[b]	31.8±3.8 [a]
P - value	0.001**	0.02*	0.02*	0.0003***

*Significant at $P < 0.05$, **significant at 0.001, ***significant at $P < 0.0001$. Treatment means (±S.D, n = 3) within the column sharing different letters are significant different from one another.

4.4.3 *Folsomia candida*

The mean number of *F. candida* recovered from the microcosms did not differ among the treatments ($F_{2,6} = 1.73$, $P < 0.254$), although numerically, the lowest recovery was obtained in T3 (63±0.3) and the highest in T1 (78±0.8). Enrichment in ^{13}C of *F. candida* increased proportionately to the availability of live maize roots. ($F_{2,6} = 6$, $P < 0.037$, fig 4.2). *Folsomia candida* in T1 were most enriched at -18.28±1.7‰, followed by T2 (-20.17±1.5‰) and then T3 (-22.76±1.4). δ^{13}C signatures of *F. candida* in T1 and T3 were significantly different from each other but not from T2.

The δ^{15}N of *F. candida* feeding on plants and ryegrass litter differed significantly ($F_{2,6} = 13.62$, $P < 0.0059$, Fig 4.3). As was found for soil and roots also, δ^{15}N enrichment among *F. candida* was highest in T2 (371±113‰), followed by T3 (138±40‰) and then T1 (63±50‰). The latter two (maize in T1 and T3) were similar with respect to the δ^{15}N

signature, but were different from the T2. Collembola tissue N content in the three treatments remained unaffected ($F_{2,6} = 21.87$, $P < 0.052$) but tissue C behaved differently ($F_{2,6} = 13.62$, $P < 0.0029$). Collembola tissue C content followed the same pattern as $\delta^{13}C$ signature. *Folsomia candida* in T1 and T3 showed highest (mean ± S. D) (47±1.5%) and lowest (42±1.7%) C content and in T2, it was intermediate (44±1.2%) between these two.
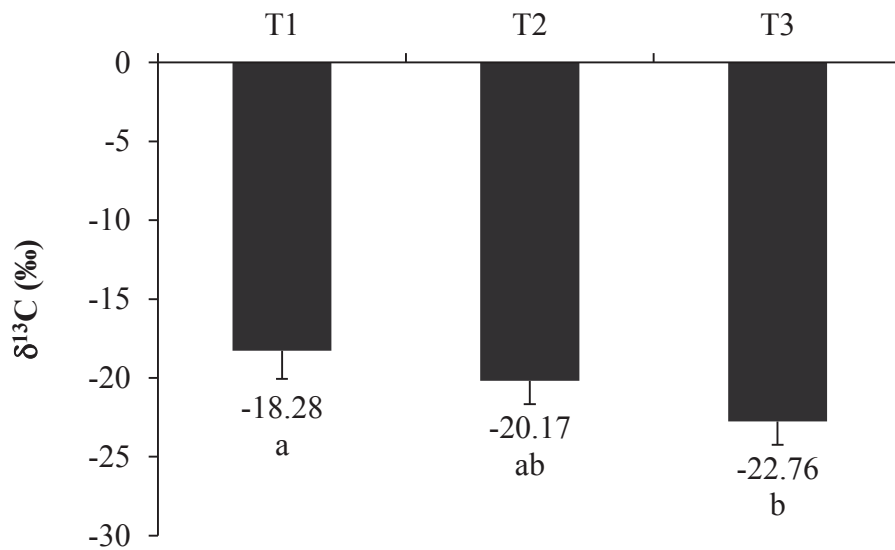
Figure 4.2 $\delta^{13}C$ isotope values (mean ± S. D) of *Folsomia candida* recovered from treatments with both maize plants and ryegrass litter recovered after 63 (T1), 42 (T2) and 21 (T3) days after sowing maize plant seeds.

The amount of C and N incorporated into *F. candida* from maize and ryegrass litter (calculated using the two-source mixing model, see Section 2.2.2) varied significantly ($F_{2,6} = 6$, $P < 0.037$) between the three treatments. *Folsomia candida* incorporated 66±0.1% C from microcosms with the greatest biomass of live maize roots (T1) but only 38±0.1% from microcosms with the lowest biomass of live roots (T3). C incorporation from maize roots in T2 (54±0.1%) was intermediate between T1 and T3. In the case of N, *F. candida* obtained more than 99% of its N from maize in treatments T1 and T3. They obtained a slightly lower but significantly different proportion of N (97±0.0%) from maize roots in T2 than in T1 or T3. ($F_{2,6} = 13.62$, $P < 0.0059$).
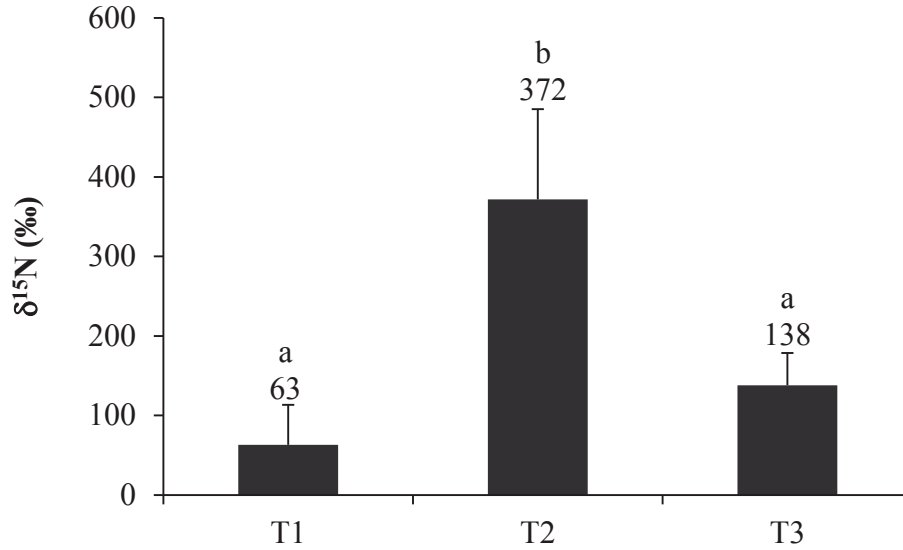
Figure 4.3 $\delta^{15}N$ isotope values (mean ± S. D, n = 3) of *Folsomia candida* recovered from treatments with both maize plants and ryegrass litter recovered after 63 (T1), 42 (T2) and 21 (T3) days after sowing maize plant seeds.

4.5 Discussion

Results established the importance of root availability in determining the occurrence of the diet-switch behavior with *F. candida*. Collembola exposed to a higher biomass of maize roots had a more substantial diet-switch from ryegrass litter to live maize roots (greater enrichment of ^{13}C) than those exposed to a lower root biomass. Although feeding on maize roots was shown to slightly improve the performance of *F. candida*, the level of diet-switching seemed to be in direct response to root biomass. The positive response of *F. candida* to the availability of live maize roots is in line with the opportunistic feeding behavior attributed to Collembola by Anderson and Healey (1972) and Peterson and Luxton (1982).

When Chamberlain et al. (2006b) switched the diet of *F. candida* from yeast to nematodes, *F. candida* showed $\delta^{13}C$ similar to that of the switched diet, as well as increased body weight. These authors expected the positive impact of the switch to be due to the higher quality of the nematode diet, but did not find any relation between diet

quality (measured as C:N ratio) and turnover rates. There are cases however when the quality of the new food source is inferior to the previous food source, compensatory feeding on the new diet would still make it possible for the organism to achieve greater fitness. Organisms can consume food materials that contain moderate amounts of N but in larger quantities to compensate for low quality (Sterner and Robinson, 1994; Cruz-Rivera and Hay, 2000; Boersma and Kreutzer, 2002). *Folsomia candida* grown in the presence of a continuous supply of yeast and maize performed better over yeast than maize roots (see Chapter 3) indicating that maize roots are lower in food quality than yeast. Nonetheless, they provided enough nutrients to sustain the growth of the springtail and were preferred over prior incorporated ryegrass litter in this experiment. Indeed, even when roots were only relatively marginally abundant (T3) and ryegrass litter most recently applied, *F. candida* were shown to have still derived 38% of their tissue C from maize roots. Consequently, it would seem that when maize roots are readily available, *F. candida* may have to forage on maize more actively than on ryegrass litter but less than on yeast to satisfy their nutritional needs. Whether it is with the Daphnia species studied by Bukovinszky et al. (2012) or Collembola the impact of food quantity and quality on diet is strongly interrelated and each species has to adjust its feeding behavior accordingly. Strategies can differ. The planthopper *Prokelisia dolus*, fed low N fertilized *Spartina* grass, compensated by increasing the rate of ingestion of the phloem tissue. However, in the same study a different species, *P. marginata* dispersed to a higher quality plant instead of adopting compensatory feeding behavior (Huberty and Denno, 2006). The amphipod *Ampithoe longimana* maintains its growth and fecundity by compensatory feeding on low quality diets in excess quantities (Cruz-Rivera and Hay, 2000). The relative roles of food availability and quality described in this research for *F. candida* cannot be extrapolated to other Collembola species. Similar studies with other collembolan species will be required to gain a comprehensive understanding of the feeding behavior and diets of the group.

The minor increase in soil C content, in relation to increased root biomass (Zak et al., 1993) could have resulted from the release of rhizodeposits from the roots (Jones et

al., 2009; Paterson et al., 2009). The presence of fine maize root hairs in the soil samples is also more likely in microcosms with heavier root biomass Similarly, changes in soil $\delta^{13}C$ were small, but the presence of maize roots for a longer time or in greater volume could cause slightly greater ^{13}C enrichment of soil C (Balesdant et al. 1988).

For ^{15}N, highest enrichment occurred in T2, consistent with the higher $\delta^{15}N$ ratios of both soil and maize roots. Higher $\delta^{15}N$ in T2 for all the three materials analyzed (soil, maize roots and Collembola), could be a result of high amount of N mineralized from ryegrass during that particular period. Where as in T1, released N might have been lost either through leaching of denitrification since the soils were water saturated. Presence of ^{15}N enriched ryegrass litter increased the $\delta^{15}N$ values of both soil and maize roots. However, maize roots had 10 fold higher $\delta^{15}N$ than soil. This suggests, plant roots took up N mineralized into soil from ryegrass litter. This N mineralization could also be a consequence of Collembola feeding on ryegrass litter and thus, releasing N forming nutrient rich patches (Endlweber and Scheu, 2006) for maize uptake. However, only 1% N from ryegrass litter was incorporated into Collembola tissue, so the role of Collembola in N mineralization in the current study is very unlikely.

The abundance of Collembola was slightly lower in microcosms with a relatively light root biomass than in microcosms with a heavier root biomass, but not statistically different. Whether or not *F. candida* population growth is linked to root availability could not be determined in this experiment, because the time available between release into the microcosms and recovery for egg production and nymph emergence was too short.

4.6 Conclusion

We have shown the existence of a partial diet-switch from ryegrass litter to live maize roots for the Collembola *F. candida* in Chapter 2. Results of this experiment have demonstrated that the C_4-C fraction, incorporated by *F. candida* exposed to different amounts of live maize root biomass in combination with ryegrass litter, varied proportionately with the availability of roots. Contrary to our hypothesis, root quantity

was the determinant factor for the diet-switch with *F. candida.* Because our experimental design was adapted from that of Endlweber et al. (2009), it is likely that the maize root biomass occupied a proportion of their microcosms similar to ours and, therefore, root availability may also play a key role in the diet-switch of *P. fimata*. These results also highlight the importance of considering food availability when designing experiments on diet-switching.

CHAPTER 5   CONCLUSION AND SUMMARY

5.1 *Folsomia candida* partially switches diet in the presence of live maize roots

The study demonstrated the existence of a partial and ctive diet-switching from plant litter to live plant roots to a second species of Collembola: *Folsomia candida.* When live maize roots were present in microcosms along with ryegrass litter, the $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of *F. candida* were closer to those of maize roots than ryegrass litter. When ryegrass litter was present alone, its C was derived exclusively from litter and *F. candida* ^{13}C signature was slightly enriched compared to that of the litter. According to the two source mixing model, the majority of *F. candida* tissue C came from live maize roots. Maize root presence also increased *F. candida* populations to almost twice than when feeding on ryegrass litter alone. Although presence of *F. candida* did not cause any significant influence of root or shoot parameters, other factors like fine root numbers and distribution, gene expression might have changed by feeding activities, which were not studied in the current experiment.

5.2 Live maize roots quality affects the performance of *Folsomia candida*

The study revealed the positive influence of live maize root quality on the performance of *F. candida*. The performance indicators studied were: body growth, reproduction and survival. The body length of *F. candida* increased more in the presence of maize roots than control treatments; survival and reproduction were unaffected. Presence of dry yeast masked the influence of maize roots as *F. candida* fed with yeast producing even higher body length than on live roots or ryegrass. The continuous replenishment and higher N content of yeast is likely responsible for this effect. N is considered as the limiting element for growth and development of organisms due to its role in protein synthesis (Huberty and Denno, 2006). Ryegrass litter in the previous diet-switch experiment possessed more N content than roots, but *F. candida* preferred maize roots. Factors such as root availability, rhizodeposition or mycorrhizae associated with roots could have influenced preference towards roots. Survival of *F. candida* was not

affected by the quality of the food resources available. This last result is in agreement with other studies.

5.3 Diet-switching by *Folsomia candida* directly related to root biomass

The study demonstrated that the level of diet-switching by *F. candida* was directly related to live maize root availability. *Folsomia candida* exposed to maize roots in microcosms showed a higher diet-switch towards large maize root biomasses than small biomasses. This is in line with the characteristic opportunistic feeding habits of many Collembola species. Collembola experience various conditions of food availability, depending on the ecosystems they inhabit and must adapt their feeding behavior based on the available resources to maintain or improve their fitness.

5.4 Recommendations

The stable isotope method has so far identified the presence of diet-switching from plant litter (decomposer) to live roots (herbivory) for two species of Collembola. Preference for live roots using this technique was demonstrated for *P. fimata* and *F. candida.* This phenomenon of diet-switching should be tested further to study its prevalence in other Collembola species. Feeding by *F. candida* on maize roots did not cause any deleterious effects on plant performance of maize in terms of tissue biomass or C and N content. However, a few studies have shown a synergistic effect on plant performance when Collembola were present. Hence, the effect of feeding on other plant species should be examined to gain a better understanding of the links between Collembola and plant performance. Examining root characteristics like fine root abundance and distribution, gene expression influenced by feeding activities of Collembola can also provide better understanding of the impact of root feeding. Diet-switching under varying climatic conditions (for eg. temperature) should also be considered as Collembola are abundant in most ecosystems. While using Collembola in such microcosm studies, care should be taken to provide sufficient moisture for growth and development. There are a number of methods to efficiently recover Collembola from

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soils and these methods differ in their recovery rates for different species. Careful selection of the extraction method can be key for good recovery of Collembola. Performance tests with maize and *F. candida* showed that the root system could play a role in determining fitness of Collembola. The effect of root biomass on *F. candida* diet-switching demonstrated that the quantity of roots available is also an important factor in determining their diet. Coupling experiments to measure the effect of diet quality and quantity provided additional information about the diet-switching process. Collembola, *F. candida* in particular, are aggregated in nature; hence, Collembola should be used in groups to study their performance in the presence of various diets. This study suggests *F. candida* does not act exclusively as a decomposer species, but can be herbivorous depending on the food availability; thus it may be better characterized as omnivorous. Further studies using different species of Collembola are needed to confirm the role of diet-switching in their feeding behavior.

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APPENDIX A: LABELLING YEAST WITH C3 SIGNATURE

Collembola raised on commercial baker's yeast yield a C signature close to C_4 plants. In procedures designed to determine a potential diet-switch to C_4 food sources, such as described in Endlweber et al. (2009), the Collembola cannot posses the same signature. The following method is adapted and modified from the procedure given in Chamberlain et al. (2004). In order to obtain *F. candida* with a C_3 isotope signature, springtails were raised on yeast prepared from a modified Sabourad's media obtained by mixing 3% beet sugar and 1% soya peptone (both are C_3 carbon source) in 250 mL flasks. This solution was autoclaved for 20 min at 121°C, cooled and inoculated with 1 mL stock yeast *(Saccharomyces cerevisiae)* solution obtained from the microbiology laboratory Dalhousie Agricultural Campus, Truro, Nova Scotia. These flasks were held at 25°C in incubators (Sheldon Manufacturing, Cornelius, Oregon) and the contents were freeze-dried at the end of 7th day. Yeast obtained by this procedure had a ^{13}C value of -24.8‰ and the Collembola raised on the yeast had a signature of -24.2‰.
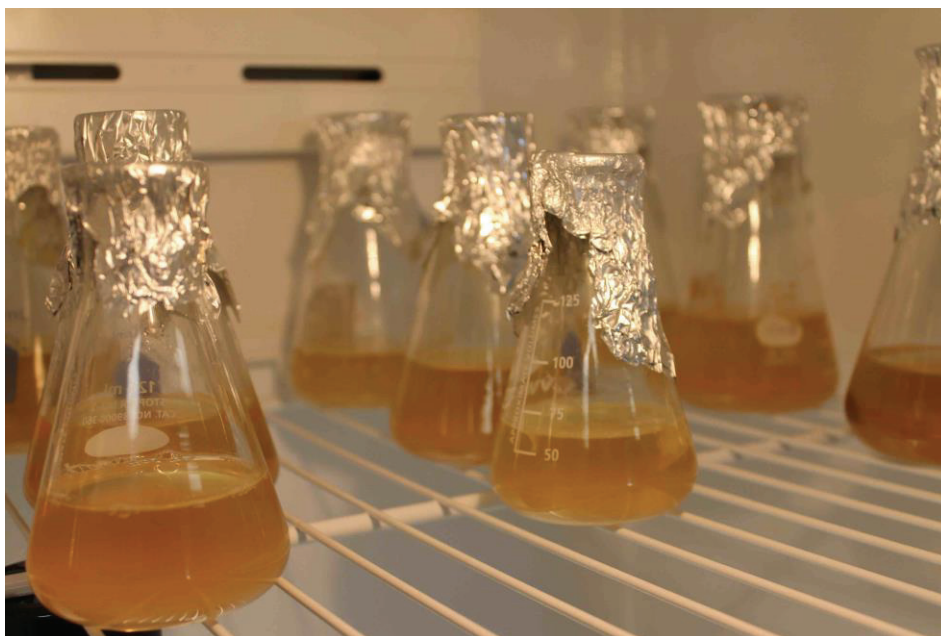
Fig. Conical flasks filled with Sabourad's media and inoculated with baker's yeast placed in incubator used in the production of C_3 yeast.

APPENDIX B: ENRICHING RYEGRASS WITH ^{15}N FERTILIZER

Ryegrass (C_3 plant) residue used in this experiment was enriched with ^{15}N to distinguish it from the background soil used to grow maize plants which also had C_3 signature. Detailed procedure to enrich ryegrass is given below.

1. Ryegrass seeds (*Lolium perenne*) seeds were germinated on a moist filter paper enclosed in a petri dish by placing it a growth room for 3 days at 24°C.

2. Three germinated seedlings were planted in a 12 cm^2 pot (2.9 L) with sand and perlite (1:1 volume basis) as growing media.

3. Pots were transferred to greenhouse to maintain optimum light and temperature.

4. Plants were watered every alternate day with 40cm^3 nutrient solution (0.40 mol. m^{-3} KH$_2$PO$_4$, 0.15 mol. m^{-3} K$_2$HPO$_4$, 1.0 mol. m^{-3} K$_2$SO$_4$, 3.0 mol. m^{-3} CaCl$_2$, 0.50 mol m^{-3} MgS0$_4$, 0.10 mol m^{-3} NaCl, 0.025 mol m^{-3} H$_3$BO$_3$, 0.50310^{-3} mol. m^{-3} CuSO$_4$, 0.002 mol. m^{-3} MnSO$_4$, 0.50310^{-3} mol. m^{-3} Na$_2$MoO$_4$, 0.002 mol. m^{-3} ZnSO$_4$, and 0.02 mol. m^{-3} FeSO$_4$).

5. 5mM ^{15}NH$_4$^{15}NO$_3$ solution was prepared by dissolving 4 g of the ^{15}NH$_4$^{15}NO$_3$ (10% atom) in 100ml water and then making up the solution to 1 L. This is same as 2% solution.

6. Enriching procedure was done by a pulse Enriching technique in three stages.

   a. On the day of transplanting 330 mL solution in aliquots of 50 mL was added to the pots

   b. 15 days after germination, another 330mL of the solution in small aliquots was added.

   c. Add the remaining solution after 30 days after transplanting.

7. Plants are harvested 45 days after sowing and analyzed for ^{15}N enrichment.

Fig. Pots with sand as growth media and ryegrass seedling which were supplied with $^{15}NH_4{}^{15}NO_3$ solution and other nutrients to label ryegrass with ^{15}N.