

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

by

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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 occurrence of this diet-switch among Collembola was tested by examining the dietary preferences of *Folsomia candida* using the stable isotope technique under controlled environment conditions. Tests were carried out in microcosms where *F. candida* were offered live maize roots in C₃ soil, along with ¹⁵N enriched ryegrass litter. A partial diet-switch towards from detritus to live maize roots was confirmed for *F. candida*. The diet-switch seemed to have been a response to both improved food quality and greater food availability. The switch to live maize roots was shown to improve the body growth of *F. candida*, although survival and reproduction were unaffected. The occurrence of the diet switch was correlated to root abundance (biomass).

LIST OF ABBREVIATIONS USED

ISO – international standards organization

OECD - organisation for economic co-operation and development

C – carbon

N – nitrogen

m – metre

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

c.a. approximately

‰ – per mil (per 1000)

chap – chapter

e.g. – example

i.e – that is

ad lib – ad libitum

cm = centimetre

mm – millimetre

μ - micron

h – hours

° C – degrees centigrade

δ – delta

Ch - chapter

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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 assumed to be involved in the nutrient cycling process, whereas the meso- and macrofauna serve as catalysts. 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), role in litter decomposition and in soil microstructure formation (Rusek, 1998). These microarthropods (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) and plant roots (Gunn and Cherrett, 1993). Several species of Collembola are 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 other species of Collembola feeding on the stigmas of the flowers (Kato, 1995). The role of detrital decomposition in soil C dynamics is well established, but the incorporation of recent photosynthate-C deposited in the rhizosphere itself and its surroundings by the mesofauna is only just being unravelled. Many studies have suggested a close relationship between rhizodeposition and soil fauna (Sticht et al., 2006; Ostle et al., 2007;

Brüggemann et al., 2011). Endlweber et al. (2009), with the help of ^{13}C and ^{15}N stable isotopes, showed that the springtail species *Protaphorura fimata* Gisin behave like an herbivore and obtains its C and N 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 and 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 could not be confirmed in Sitka spruce by Churchland et al. (2012). Given the uncertainties of the feeding behavior of Collembola, the current study aims to examine the feeding preferences of Collembola and their role in C cycling, by examining the diet of the springtail species *Folsomia candida* Willem 1902, using stable isotope techniques.

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 from decomposition 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 mesofauna stimulate or enhance plant growth by direct and indirect effects like microbial feeding, dispersal of microbial structures, microbial composition modification and increased nutrient availability (Cole and Bardgett, 2005; Griffiths and Bardgett, 1997; Kreuzer et al., 2004). 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 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 millions 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, thorax bearing three pairs of legs and abdomen with six segments (Fountain and Hopkin, 2005). Because their mouthparts are entognathous, they are no longer referred to 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 ecotoxicological 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 and the following description is from Hopkin (1997), who reviewed its life cycle, and from personal observations in 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 parthenogenitically. Adult females lay 30-50 eggs in communal heaps which take c.a. 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 moult up to 48 times in their lifetime and start laying eggs after their sixth moult (Fountain and Hopkin, 2005). Their ease of maintenance with common lab equipment, speedy 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 “standard organism” to assess the quality of polluted soils. It has also been proposed as a bio-indicator of soil quality in forest (Kaneda and Kaneko, 2002) and agricultural soils (Nelson et al., 2011).

1.4 Collembola feeding behaviour

Although there can be millions of individuals/m² 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.

Leaching 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 type that provide better nutrients. Feeding in the root zone of plants might occur by chance or preferentially if roots have a 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 also 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, colour marking techniques and radiotracers (Briones et al., 1999). There are drawbacks 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 be already degraded by enzymes, materials may be 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). Heterotrophs such as Collembola generally reflect the isotopic signature of their diet. With the help of stable isotope techniques, it is possible to examine the consumed resource (Albers et al., 2006), as well as elaborate on the long-term trophic relations between animals and the interactions between Collembola, soil microorganisms and plant remains. Briones et al. (1999) first used $\delta^{13}\text{C}$ to show preferential feeding by two Collembolan species *F. candida* and *Proisotoma minuta* on C₄ litter over soil taken from a field trial cropped with C₃ plants (C₃ 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). Endlweber et al., (2009) was the first to use stable isotope techniques to

demonstrate that *P. fimata* acted as a 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 *Protophthora 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 quality of one food type over the other.

This appears to be the only documented case of complete diet-switching to live roots with Collembolans. However, Larsen et al. (2007) showed a partial switch to live roots from litter sources in Collembola. Based on these studies and the evidence of a close association between many 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. The relative importance of herbivory as a feeding strategy however, 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 callipygos*. 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, foraging animals tend to do so optimally, suggesting that a switch from detritus to live roots would 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 behaviour of Collembolans. Ultimately, if a broader occurrence of active diet switching in the presence of plants 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) 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 either as $\delta^{13}\text{C}$ for natural abundance or ^{13}C atom% when samples are enriched with a ^{13}C rich compound. $\delta^{13}\text{C}$ is expressed relative to the content of ^{13}C in Vienna-PeeDee belemnite (V-PDB), a now depleted limestone formation in North Carolina (Vitarello, 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 $\delta^{13}\text{C}$ is given in the following equation.

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

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, C₄ plants have isotopic ratios in the range of -6 to -19‰, whereas the ratios for C₃ plants range from -24 to -34‰ (Smith and Epstein, 1971). This difference is due to the discrimination of ¹³C in different biological pathways (Ehleringer, 1991). The low values in C₃ plants are due to discrimination of ¹³C versus ¹²C by the RUBISCO enzyme which selectively activates the more mobile and lighter ¹²C and incorporates it into plant cells. In C₄ plants the primary CO₂ acceptor, PEPcase does not discriminate between ¹³C and ¹²C (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 $\delta^{13}\text{C}$ difference between C₃ and C₄ plants has been extensively used to study the fate of decomposing the soil organic matter and 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values of C pools (Staddon, 2004). Carbon isotopes are more conservative tracers (i.e. less fractionated) than N isotopes, making the isotopic technique less convenient for studying N 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 ¹⁵N isotope over ¹⁴N (DeNiro and Epstein, 1981). As a result, unlike C isotopes, N isotopes

are enriched by a certain amount between every trophic level. However, the $\delta^{15}\text{N}$ 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 techniques

The feeding behavior of the soil mesofauna can also be studied using the ^{13}C natural abundance technique (Staddon, 2004). The origin of C and N incorporated into Collembola tissues can be analysed 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 demonstrated that 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 $\delta^{13}\text{C}$ values to determine a feeding preference of *P. minuta* and *F. candida* fed with fungi and nematodes. Chamberlain et al. (2006c) analyzed $\delta^{13}\text{C}$ 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 or roots directly. Approximately 45% of the ^{13}C from grassland (enriched with $^{13}\text{CO}_2$) was incorporated into Collembolans compared to 22% and 28% for mites and earthworms respectively. Sticht et al. (2006) used “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 $\delta^{13}\text{C}$ of Collembola was highly enriched under elevated CO_2 levels and was closer to roots. Gunn and Cherrett (1993) reported that roots served as a major food source of diplurans

and Collembolans in rhizotrons under natural conditions. The organisms 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. 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 mesofauna, 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

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 the existence of such a diet-switch could be extended to other Collembola species by examining *F. candida* for its presence.
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 (energy value) 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 (Part 1) or availability (Part 2).

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. Until recently, the contribution of root-derived products has been largely ignored or underestimated. There is 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). 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). Salaman et al. (2004) suggested that roots and/or their associated microflora of *Trisetum flavescens* could be an attractive food source for Collembola. Gunn and Cherrett (1993) and Albers et al. (2006) showed that roots and root derived products provided a source of nutrition to soil animals.

Euedaphic Collembola reaches 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 channel (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 quantify the amount of food in their diet (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). $\delta^{15}\text{N}$ ratios of Collembola show that their trophic guilds range from primary decomposers to herbivores, 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 *F. candida* and *P. 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). Many other studies have recorded 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).

Endlweber et al. (2009) was the first to demonstrate the existence of an active diet switch from plant residues to live roots using 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 C₄ plants (Maize – *Zea mays* L.) in C₃ soil mixed with ¹⁵N enriched ground C₃ rye grass (*Lolium perenne* L.) (ryegrass) 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 cm³ 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 bakers yeast *ad lib.* every 3 days and the old yeast was removed to prevent mould formation. The substrate was also sprayed with deionised 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 bakers yeast have $\delta^{13}\text{C}$ signatures close to C_4 plants ($-11\text{‰ } \delta^{13}\text{C}$). The design of the diet-switch experiment required a colony of *F. candida* with the isotope signature of C_3 plants. Hence, these *F. candida* colonies were raised on yeast cultured on a modified Sabourad's media containing 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 the first nymph emergence egg cards were removed. Nymphs left in the rearing box 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 rye grass litter which gave a total of four treatments (Table 2.1). Blocks were established on January 21st, February 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 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 litter (<0.5 mm, 2.5 ± 0.1 % N, C/N ratio 18.7 ± 0.4) enriched with ^{15}N ($\delta^{15}\text{N}$ 17516.5 ± 113 ‰, the procedure for enriching rye grass 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. 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, 100 15 day-old *F. candida* 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 $\mu\text{E m}^{-2} \text{s}^{-1}$. Soil 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. Weeds 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*, 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 spectrophotometer (IRMS) (Delta V mass spectrophotometer, Thermo scientific, Bremen, Germany) at the Stable Isotope laboratory, University of Saskatchewan, Saskatoon, SK. This instrument provides the direct measurement of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the samples using 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):

$$F_x = \frac{(\delta^{13}R_m - \delta^{13}R_y)}{(\delta^{13}R_x - \delta^{13}R_y)} \times 100$$

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 with in 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. The amount of tissue C and N incorporated from maize/ryegrass litter into Collembolan tissues calculated using the two source mixing model was subjected to ANOVA. In ANOVA, C and N derived from maize in T3 or ryegrass litter T2 was compared with corresponding C and N from maize or ryegrass litter in T1.

2.4 Results

2.4.1 Maize Plants

Addition of ^{15}N labelled ryegrass litter caused a slight increase in maize root biomass and a slight decrease in shoot biomass. However, these differences were not significant ($P < 0.54$ for shoot biomass and $P < 0.88$ 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 ($P < 0.1$ for C and $P < 0.55$ for N, Table 2.2). Labelled ryegrass litter did not affect $\delta^{13}\text{C}$ signature ($P < 0.49$), but its presence increased the $\delta^{15}\text{N}$ of maize roots significantly ($P < 0.0001$, 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ (‰)	Root $\delta^{15}\text{N}$ (‰)
Ryegrass Litter + <i>F. candida</i>	4.2±1.9 ^a	1.4±0.6 ^a	39.7±1.1 ^a	0.9±0.07 ^a	-13.2±0.2 ^a	1036±369 ^a
<i>F. candida</i> only	4.6±1.6 ^a	1.4±0.5 ^a	39.9±1.3 ^a	0.9±0.08 ^a	-13.2±0.4 ^a	12.2±1.7 ^b
ANOVA F-value	0.38	0.02	0.38	2.93	0.49	137.36*

Treatments means (\pm S.D, n= 9) within a variable (column) sharing same letter are not significantly different from each other.
*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 + <i>F. candida</i>	4.2±1.9	1.4±0.6	39.6±2.6	0.82±0.13	42.9±0.4	0.95±0.23
<i>F. candida</i> 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 <i>F. candida</i> 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
ANOVA F- value	3.01*	1.71*	1.61*	1.30*	1.39*	1.47*

*Treatment means (\pm S.D, n = 9) are not significantly different at $P < 0.05$.

2.4.2 Collembola

There was population growth in all treatments (Fig. 2.2). The number of which was *F. candida* recovered differed significantly ($F_{3,41} = 8.56$, $P < 0.0002$) between treatments. The highest number of *F. candida* was recovered from microcosms with plants and ryegrass litter (212 ± 123), followed by microcosms with plants alone (200 ± 115), ryegrass litter only (128 ± 79) and then no 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.

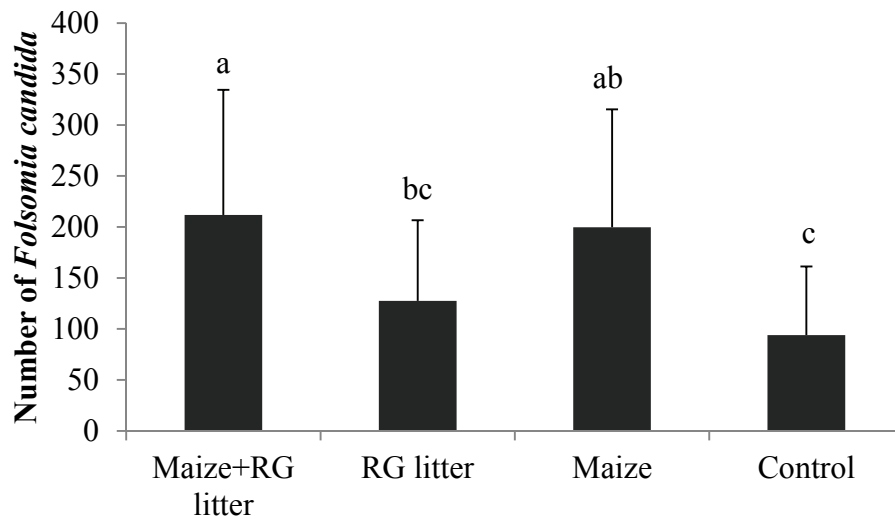


Figure 2.2 Number (mean \pm S.D, $n = 12$) of *F. candida* recovered per microcosms containing soil and maize with ryegrass litter, maize without ryegrass litter, ryegrass litter without maize and control treatment without maize or ryegrass litter. Mean separation was done using Tukey's HSD at $P < 0.05$. 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, $\delta^{13}\text{C}$ 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). $\delta^{13}\text{C}$ of *F. candida* in both maize plant treatments differed significantly from the non-maize treatments. The $\delta^{13}\text{C}$ signature of *F. candida* exposed to maize and

ryegrass litter ($-19.28 \pm 3.12\text{‰}$) was between the values of maize plant source tissue ($-13 \pm 0.4\text{‰}$) and ryegrass litter tissue ($-29 \pm 0.01\text{‰}$) when both maize and ryegrass litter were present. It did not differ significantly from when Collembola was exposed only to maize. In treatments without maize, $\delta^{13}\text{C}$ reflected the signature of typical C_3 sources: in this case ryegrass litter in the soil. The two source mixing model showed that *Folsomia candida* obtained the majority of its C from live maize roots; $59.7 \pm 0.2\%$ from maize when present along with ryegrass litter and $61.1 \pm 0.2\%$ when just maize was present ($F_{1,13} = 0.04$, $P < 0.83$).

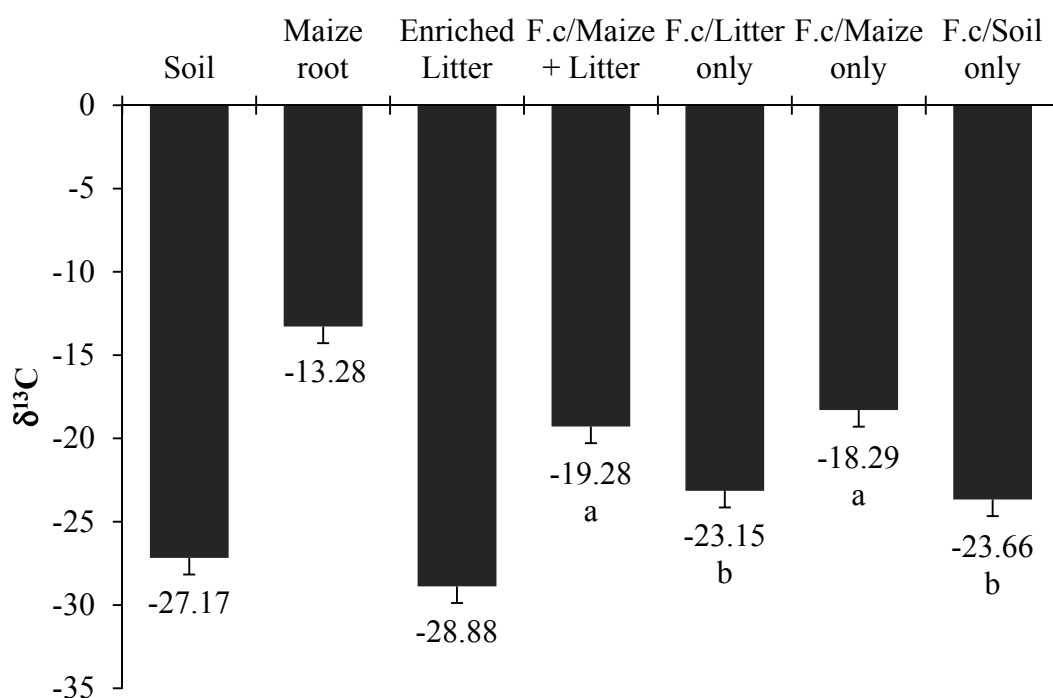


Figure 2.3 $\delta^{13}\text{C}$ (mean \pm S.D, $n = 12$) values of *Folsomia candida* (F. c) grown in maize and ^{15}N enriched ryegrass litter, ^{15}N enriched ryegrass litter without maize, maize without ^{15}N enriched ryegrass litter and control with out maize or ^{15}N enriched ryegrass litter. $\delta^{13}\text{C}$ (mean \pm S.D) values of soil, maize root and enriched ryegrass litter are also given. Mean separation was done using Tukey's HSD at $P < 0.05$. Treatments means sharing same letter are not significantly different from each other.

Nitrogen content of Collembola tissue in the four treatments did not differed significantly ($F_{3,38} = 0.83$, $P < 0.48$, Table 2.4). The addition of ^{15}N labelled ryegrass litter

significantly increased the $\delta^{15}\text{N}$ signature of Collembola tissue N ($F_{3,42} = 83.53$, $P < 0.0001$). $\delta^{15}\text{N}$ of Collembola was lowest for the control without maize plants or ryegrass litter ($9.9 \pm 3.1\text{‰}$) and maize plants alone ($12.91 \pm 2.4\text{‰}$) and these treatments did not differ. In contrast, in both treatments with ryegrass litter, with or without maize, $\delta^{15}\text{N}$ of Collembola was substantially enriched at $518.7 \pm 146.2\text{‰}$ and slightly, but not significantly, lower ($498 \pm 154.7\text{‰}$). The amount of N derived by Collembola tissue from maize roots/ryegrass litter calculated using the two source mixing model showed that Collembola obtained same amount of N from ryegrass litter regardless of the presence ($3 \pm 0.01\%$) or absence of live maize roots ($2.9 \pm 0.01\%$) ($F_{1,18} = 0$, $P < 0.96$). The majority of N was derived from maize plants, $97.2 \pm 0.01\%$ when ryegrass litter was present and $92.8 \pm 0.18\%$ when ryegrass litter was absent, but these differences were not significant ($F_{1,17} = 0.66$, $P < 0.42$).

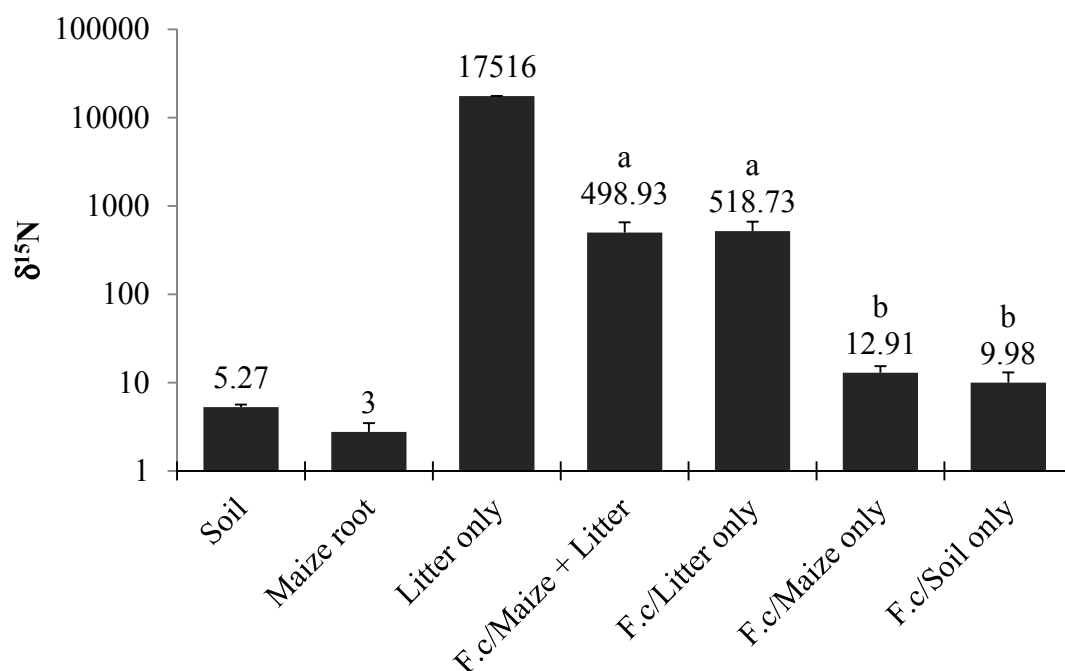


Figure 2.4 $\delta^{15}\text{N}$ (mean \pm S.D, $n = 12$) values of *F. candida* grown in maize and ^{15}N enriched ryegrass litter, ^{15}N enriched ryegrass litter without maize, maize without ^{15}N enriched ryegrass litter and control treatment with out maize or ryegrass litter. $\delta^{15}\text{N}$ (mean \pm 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.

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

Treatment	<i>F. candida</i> C (%)	<i>F. candida</i> N (%)
Maize + ryegrass litter	46.7±3.2 ^a	10.3±0.8 ^a
Ryegrass litter only	55.2±9.4 ^a	9.8±4.4 ^a
Maize only	47.1±3.4 ^a	11.0±0.7 ^a
Control	44.9±3.4 ^a	11.2±0.9 ^a

*Treatment means (±S.D, n = 12) sharing same letter with in the variable are not significantly different from each other at $P < 0.05$.

2.5 Discussion

Similar to the previous studies, this experiment also showed that *F. candida* benefited by the availability of plants and ryegrass litter. However, in contrast to our hypothesis, when given a choice, *F. candida* did not switch their diet entirely from ryegrass litter but instead, obtained their C and N in varying proportions from both sources, showing a partial diet-switch. *Folsomia candida*, in the presence of both live maize roots and ryegrass litter assimilated both resources, as indicated by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as well as greater than expected $\delta^{15}\text{N}$ due to high $\delta^{15}\text{N}$ signature of ryegrass litter compared to Endlweber et al. (2009). Collembola tissue C and N concentrations did not

differ between treatments, but the amount of C and N derived¹ from maize and ryegrass litter varied greatly. Ryegrass litter in combination with maize, accounted for less than half of *F. candida* tissue C. Irrespective of the presence or absence of ryegrass litter, C derived by *F. candida* from maize remained almost the same. Carbon incorporated from wheat roots in a system with wheat plants and dual labelled ryegrass was close to values reported here (54 to 79%) for *P. fimata*, *P. armata* and *F. fimata* (Larsen et al. 2007). However, *P. fimata* depended 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). If one uses corrected C source values, it may be possible to improve the accuracy of the two source mixing model. Gearing (1991) suggested the use δR_x and δR_y of same species feeding on one source of food. Based on this estimate, a slightly higher (72%) fraction of *F. candida*'s C, but only 1% N, would have been derived from maize. A significant 99% N derived from ryegrass litter shows its important in their diet. Although, *P. fimata* 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 requirements for that species than for *F. candida*. Maize roots had a significant influence on the diet of *F. candida*, but ryegrass litter also contributed to some extent. Surprisingly, Leaf litter (organic matter), assumed as the primary food source for many soil biota including Collembola had ¹³C signature close to root (Pollierer et al., 2007). This confirms the findings of Larsen et al. (2007) who

¹The above mixing model requires at least two sources to calculate the amount of C and N incorporated into animal tissue. In the treatment 1, maize and ryegrass litter are present hence they were used to calculate the amount of C and N incorporated. When only maize plant was present, soil was used as the second source. This model also has strong limitations in that both the sources can contribute same amount of C and N to the animals and it is assumed there are only two diet sources at any time.

showed that rye grass, when applied as green manure along with live wheat roots, contributed to the energy source of Collembola. 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. There were twice as many Collembola in the presence of maize plants than in 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 *Chamaecyparis obtuse* (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). This indicated that population densities of Collembola are influenced by a number of factors, including soil conditions, type, humus, water content, and biotic factors like plant type and microbial communities.

The $\delta^{15}\text{N}$ signature of *F. candida* in the presence of maize plants differed by 6.33 δ units from maize roots, suggesting a shift of two trophic levels (DeNiro and Epstein, 1981; Haubert et al., 2005). This can be attributed to fungal grazing (mycorrhizal) that in turn fed on the roots either directly or on the rhizodeposits (Haubert et al., 2005). 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. Based on visual observations in rhizotrons, Gunn and Cherrett (1993) showed that the feeding activity of soil meso- and macro-invertebrates constituted up to 5.5% of root materials, 0.5% decaying material and 80% faeces/detritus/soil surfaces. Ponge (2000) also noted that they showed a wide variety of material and show low degree of food specialization and 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. These observations further strengthen the idea 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 (Scheu and Simmerling, 2004). Food preferences of Collembola cannot be generalized between various species, as *F. candida* acted as an omnivore. The presence of various materials in the guts of Collembola indicate that they feed unselectively and the contents are characteristic of the particular environment in which they occur (Hopkin, 1997). 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.

Similar to the results of the current experiment, the root biomass of *T. repens* or *L. perenne* was not influenced by the presence of Collembola and enriched ryegrass litter in similar microcosm experiments (Kreuzer et al., 2004). Also, the presence of ^{15}N labelled ryegrass litter increased the ^{15}N signature but not the plant tissue N concentration. This surge can be attributed to root uptake of ^{15}N that was mineralized from ryegrass litter. $\delta^{15}\text{N}$ of maize roots was three times higher than values reported by Endlweber et al. (2009). These increases may be due to a higher $\delta^{15}\text{N}$ signature of ryegrass litter employed in the present treatments. In the above study, the authors observed an increased shoot biomass in the presence of ryegrass litter; however, in the current experiment there was no difference 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. There might be other soil or environmental factors that could have caused this effect. 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 differences between shoot biomass but observed differences in $\delta^{15}\text{N}$, which shows that the majority of N was derived from added ryegrass litter in both studies.

2.6 Conclusion

Folsomia candida ingested both plant roots and ryegrass litter resources, but in different proportions. The presence of enriched ryegrass litter increased $\delta^{15}\text{N}$ and C incorporated into *Folsomia candida*, but not the amount of N. When *F. candida* was given a choice to feed on maize roots, Collembola density and C and N incorporated from live maize roots into Collembola tissue was increased. *Folsomia candida*, feeding on both maize roots with and without ryegrass litter present, also had C signatures close ($\sim 5\text{-}6\%$ less enriched) to that for maize roots. 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 *F. 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). As discussed earlier, a recent stable isotope analysis by Endlweber et al. (2009) demonstrated that *P. 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 have resulted from a food quality higher in plant roots than in ryegrass litter (Endlweber et al., 2009), a greater abundance of roots than ryegrass litter or a combination of both. This experiment measured the potential impact of such an increased food quality on the performance of *F. candida*. The potential role of live root abundance in influencing diet switching is considered in Chapter 4.

Studies on nutrition in insects, ruminants and several terrestrial animals documented their performance and feeding behavior as impacted by food quality (Slansky and Rodriguez, 1987; Slansky Jr et al., 1993; Van Soest, 1994) which 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). This theory suggests, foraging *Collembola* would switch diet from plant detritus to live roots if roots helped maximize their fitness. Growth, reproduction and survival are three common indicators of animal performance. We hypothesized that *F. candida* demonstrated a partial diet-switch towards live plant roots because they are 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 and survival (Experiment 1), and reproduction (Experiment 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 bakers 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 trail (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. Bakers 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 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 seed was germinated, usually 4 days after sowing, each microcosm received one - 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 40W 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 and charcoal base 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 any Collembola presence. Roots were separated and dried at 65°C for 24 h and weighed on a balance (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 than the previous experiment 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. Later 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 on a balance (precision = 1 mg). 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, Germany). To determine pH, 8 g of field soil or OECD soil was mixed with 40mL of 0.01M CaCl₂.2H₂O, 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. To bring the WHC of each soil substrate to the desired level, 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 soil sample 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

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

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). Body length was also subjected to ANCOVA to increase the precision of the results, as the initial length of the insects cannot be controlled. 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). pH and N content of the field soil were significantly higher and the C:N ratio was lower than OECD soil. Yeast had higher C and N content than maize roots.

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.

<i>Soil Type</i>	<i>pH</i>	<i>Total C</i> (mg/g)	<i>Total N</i> (mg/g)	<i>C/N ratio</i>	<i>WHC</i> (%)
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.

¹not determined.

²treatment means within a column sharing different letters are significantly different at $P < 0.05$.

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 (e.g. 0.55 – 0.60 mm to 0.86 – 1.16 mm) and significantly longer in all treatments with yeast (e.g. 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 without. 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 *F. 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 mass 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, Days, 10 and 15, there was no difference between the four maize treatments. 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 dry weight 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.

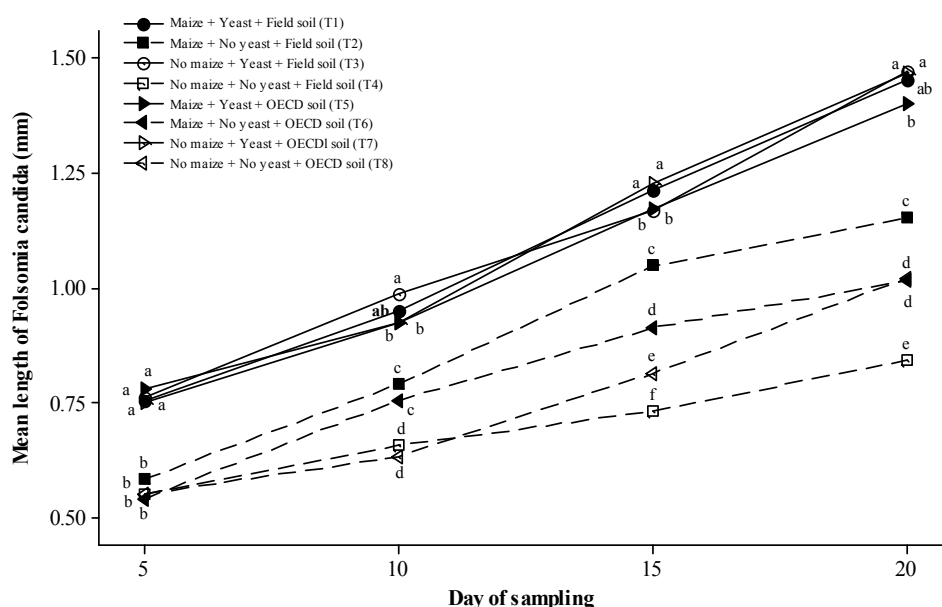


Figure 3.2 Increase in body length of *F. candida* (n=4) on eight different treatments with field and OECD soils expressed in mm. Mean separation was done using LS means option in SAS 9.3 and given for each sampling date separately. Treatments means sharing same letter are not significantly different 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.

The number of *F. candida* individuals that survived during each sampling was also recorded. 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%.

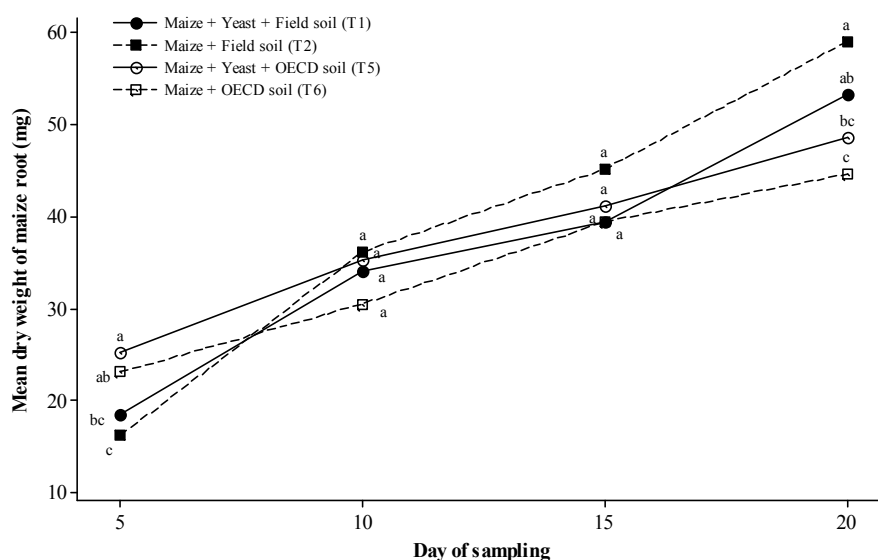


Figure 3.3 Changes in the mean dry weight (g) of maize roots in soil microcosms across the four sampling dates of Experiment 1. Mean separation was done using Tukey's HSD in SAS 9.3 and given for each sampling date separately, means sharing same letters within each sampling day are not significantly 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 during the four sampling dates on field soil for experiment 1 to measure body length of *F. candida*. 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 the 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, finally 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 yeast in OECD soil (T6) on day 28

(14/microcosm), followed by maize and yeast in OECD soil (T5) (14/microcosm), while OECD soil (T7) on Day 28 recorded the lowest number of nymphs per microcosm (0.75).

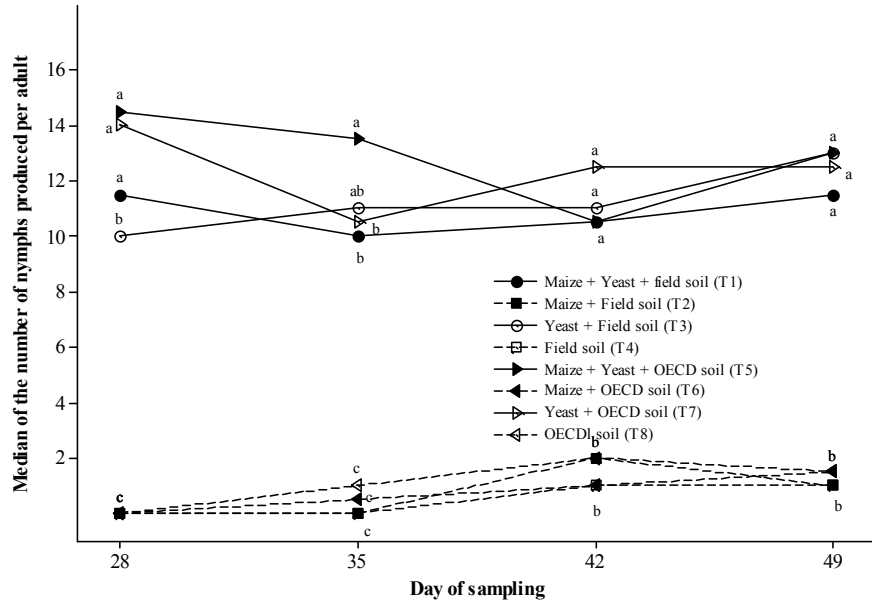


Figure 3.5 Mean number of nymphs produced (per adult) by *Folsomia candida* feeding on different combinations of maize, yeast and types of soil. Mean separation was carried using Mann-Whitney's pair wise comparison at $P < 0.05$ and given for treatments within same sampling date. Treatments sharing same letters are not significantly different 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 levelling 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.

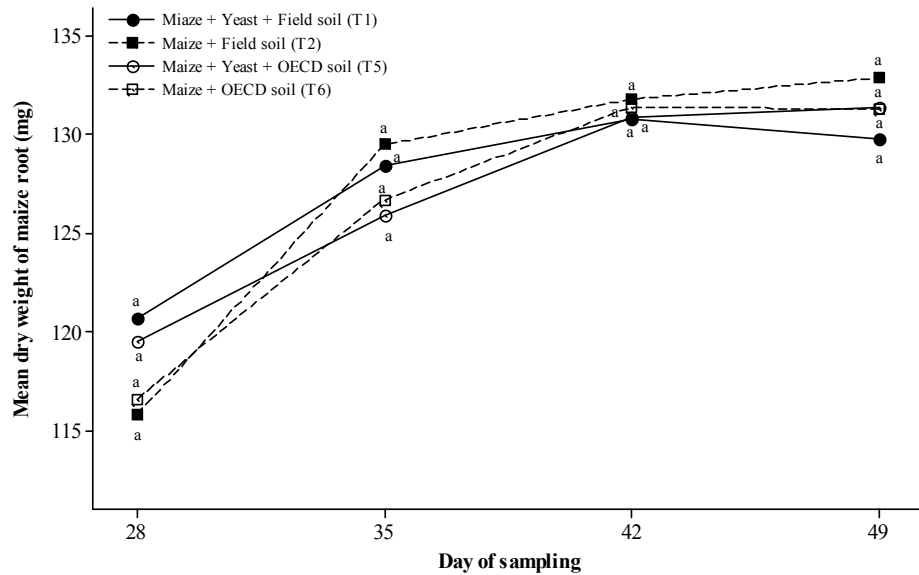


Figure 3.6 Changes in the mean dry weight (g) of maize roots in soil microcosms across the four sampling dates of Experiment 2. Mean separation was done with Tukey's HSD in SAS 9.3 at $P < 0.05$ and given for each sampling date separately. Treatment means sharing same letters within a sampling date are 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 a yeast supplement in similar microcosms. 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. San Miguel et al. (2008) showed that *F.*

candida grown in yeast treatments for 20 days had similar size ($1.34 \pm 0.16\text{mm}$) to that found in the current experiment (1.41 to 1.48 mm). 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 present our 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 Photographs showing maize root development during the four sampling dates on OECD soil for experiment 1 to measure body length and survival. From left to right – maize plants on 5th, 10th, 15th and 20th day of sampling.

3.6.2 Effect on survival

Survival of *F. candida* was not significantly affected by any of the eight treatments in both experiments. Food availability did not have a clear effect on survival rate. *Folsomia candida* in a treatment with yeast had the lowest survival rate, whereas individuals in OECD soil without maize and yeast survived until the end of the

experiment. The ability of Collembola to survive over long periods of time, even in the absence of food, is well known and 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 much 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). This suggests that survival is the performance factor least effected by food quality, in line with the findings of Nelson et al. (2011) who studied the role of *F. candida* as a potential bio-indicator of soil health.

3.6.3 Impact on reproduction

Only the presence of yeast, regardless of other treatment factors, had a clear impact on the level of reproduction by *F. candida*. However, even in the presence of yeast, reproduction rate failed to increase over time. A possible explanation is 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 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). In *F. candida*, 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. Collembolans 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). Hence, reproduction in the current experiment cannot be linked clearly to the quality of food itself.

3.6.4 Role of root biomass

Root biomass measurements, taken in the course of the tests on body growth and reproduction, indicated essentially no differences 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 advantages provided by the presence of live roots.

Field soil possessed more N content than OECD soil; hence, the N content of respective maize plant may be higher, which could have contributed to the increased body length. *Onychiurus sinensis* preferentially fed on litter with high N content and had higher body length, compared with litter containing lower N content (Sadaka-Laulan and Ponge, 2000). Booth and Anderson (1979) showed Collembola benefitted by having higher moulting rate and fecundity when the N level of 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).

3.6.5 Impact of soil types

The performance of *F. candida* was not only affected by the presence of live roots, but also by the different properties of the two soil types used. . In the absence of maize, body growth of *F. candida* was better on the OECD soil than in the field soil but

in the presence of maize, it was better in field soil. The higher C:N ratio of the OECD soil seemed to have favored the growth of *F. candida*. Survival was slightly higher in field soil than in OECD soil, but the differences were minor. However, the presence of maize provided an even better environment for the growth of *F. candida*. 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). Furthermore, work by Kaneda and Kaneko (2002) on the influence of soil quality on growth of *F. candida* also showed that pH is not an important factor regulating growth as long as it is within the optimum range.

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 INFLUENCE OF LIVE ROOT BIOMASS ON DIET SWITCHING BY *FOLSOMIA CANDIDA*

4.1 Introduction

In general, consumers preferentially chose higher quality foods to increase their fitness (Stephens, 1986). High quality food is not always available or accessible (Real and Caraco, 1986), during such situations, soil animals will increase their intake of low quality food to satisfy their nutritional requirements, a phenomenon called as compensatory feeding (Price et al., 1980). Some animals switch to foods that are of similar or higher in quality to better balance their nutritional requirements (Pennings et al., 1993; Bernays et al., 1994). Although many studies have focused on the effects of food quantity in both terrestrial and aquatic systems (Slansky and Scriber, 1985; Slansky Jr et al., 1993; Cruz-Rivera and Hay, 2000), only a few studies have examined the influence of both food quantity and quality 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. In another experiment (Chapter 3), a greater increase in the body growth *F. candida* in the presence of live maize roots than in their absence suggested that the diet-switch was at least in part a response to food quality although survival and reproduction were not affected. In these previous two experiments, root availability (quantity) was similar among treatments that included maize. We hypothesized that feeding on live maize roots by *F. candida* is due to their high quality compared to the surrounding rye litter and there will be no differences in feeding behavior when different quantities of root biomass is available. The objective of this experiment was to determine if live maize root biomass plays a role in determining the level of diet-switching taking place with *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, 3 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. 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 $\delta^{15}\text{N}$ showed one abnormal observation; it was treated as outliers and excluded from the analysis. The amount of C and N contributed by maize and rye grass 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 heavier 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 as much (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 $\delta^{15}\text{N}$ was influenced greatly. The $\delta^{15}\text{N}$ signature of maize roots in T2 was higher than in T1 than T3. Notably, the $\delta^{13}\text{C}$ signature of roots did not differ between treatments.

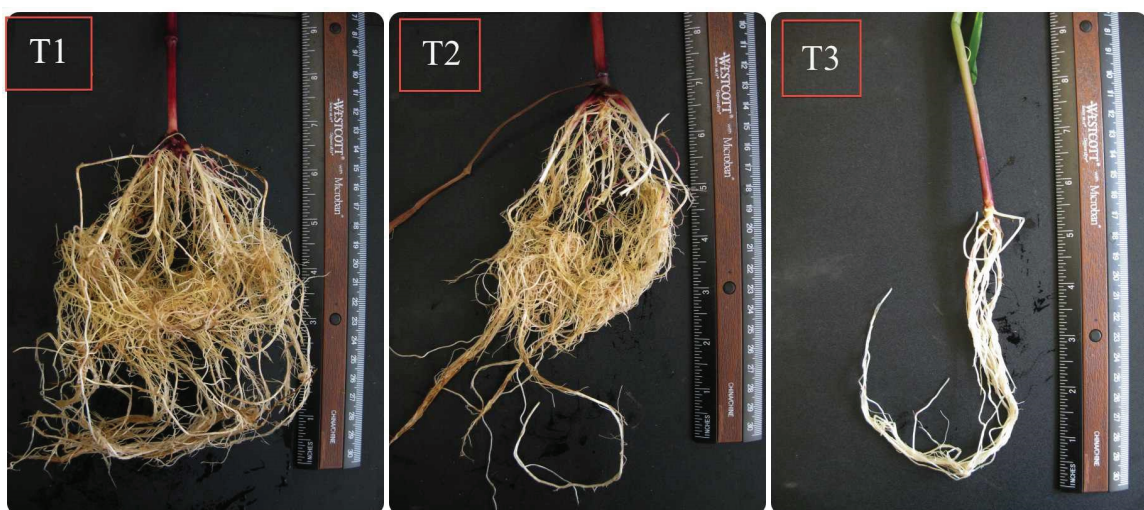


Figure 4.1 Typical maize root development after 21, 42 and 63 days of growth in microcosms of treatments T1, T and T3.

Table 4.2 Physical and chemical characteristics (mean \pm S.D of dry weight, C, N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of maize roots after 63 (T1), 42 (T2), 21 (T3) days of growth in microcosms and ANOVA F-values for treatment effects in the experiment to study influence of root biomass on Collembola feeding behavior.

Treatment	Dry biomass (mg)	C %	N %	$\delta^{13}\text{C}$ ‰	$\delta^{15}\text{N}$ ‰
T1	1066.7 \pm 177 ^a	40.4 \pm 1.06 ^a	1.8 \pm 0.41 ^a	-13.4 \pm 0.13 ^a	180 \pm 141.3 ^b
T2	736.7 \pm 119 ^b	42.3 \pm 5.56 ^a	1.2 \pm 0.26 ^a	-13.4 \pm 0.09 ^a	830 \pm 43.7 ^a
T3	106.7 \pm 25 ^c	37.2 \pm 1.05 ^a	1.1 \pm 0.14 ^a	-12.9 \pm 0.41 ^a	114 \pm 24.7 ^b
ANOVA F-value	46.05*	4.11	4.11	4.11	61.94*

*Significant at $P < 0.001$.

Treatment means (\pm S.D, n = 3) within a column sharing same letter are not different from one another.

4.4.2 Soil

Soil in all treatments differed with respect to all four variables (C, N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) studied (Table 4.3). Carbon content and $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ among the three. Soil in T3 showed highest N content and it differed significantly from T1 soil, but not T2. As found also for maize roots and noted above, the $\delta^{15}\text{N}$ of T2 soil was significantly higher than the remaining two treatments. Even though $\delta^{15}\text{N}$ value of soil was higher in T3, it did not differ from T1 statistically.

Table 4.3 Soil characteristics and ANOVA F-values for treatment effects for soil C, N, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ 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 %	$\delta^{13}\text{C}$ ‰	$\delta^{15}\text{N}$ ‰
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
ANOVA F-value	63.66**	7.65*	8.06*	63.06**

*Significant at $P < 0.05$, **significant at $P < 0.001$.

means were calculated based on three treatments.

Treatment means (\pm S.D, n = 3) within the column sharing same letter are not 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 ($-18 \pm 1.7\text{‰}$), followed by T2 ($-20 \pm 1.5\text{‰}$) and T3 (-22 ± 1.4). $\delta^{13}\text{C}$ signatures of *F. candida* in T1 and T3 were significantly different from each other but not from T2.

The $\delta^{15}\text{N}$ 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, $\delta^{15}\text{N}$ enrichment

among *F. candida* was highest in T2 ($371 \pm 113\%$), followed by T3 ($138 \pm 40\%$) and then T1 ($63 \pm 50\%$). The latter two (maize in T1 and T3) were similar with respect to the $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ signature. *Folsomia candida* in T1 and T3 showed highest (mean \pm S. D) ($47 \pm 1.5\%$) and lowest ($42 \pm 1.7\%$) C content and in T2, it was intermediate ($44 \pm 1.2\%$) between these two.

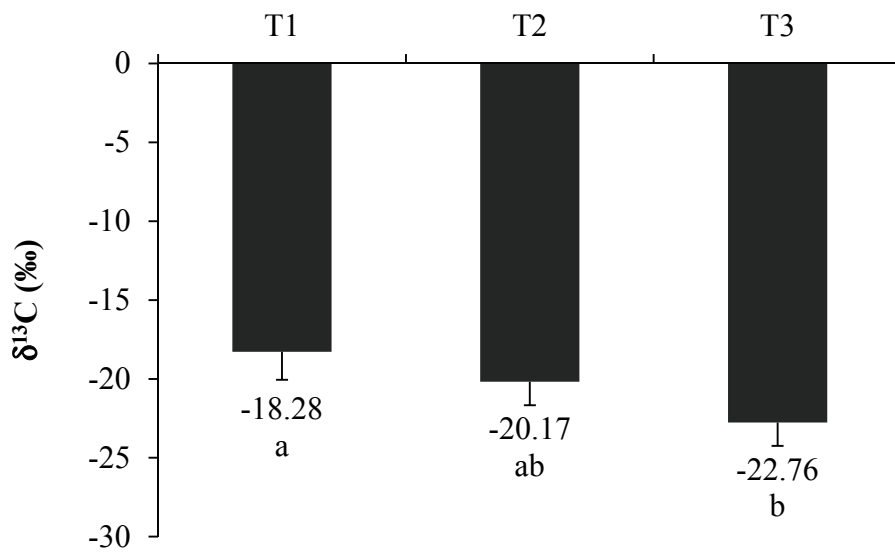


Figure 4.2 $\delta^{13}\text{C}$ isotope values (mean \pm S. D) of *Folsomia candida* recovered from treatments with both maize plants and rye grass litter recovered after 63 (T1), 42 (T2) and 21 (T3) days after sowing maize plant seeds.

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 $66 \pm 0.1\%$ C from microcosms with the heaviest biomass of live maize roots (T1) but only $38 \pm 0.1\%$ from microcosms with the lightest biomass of live roots (T3). C incorporation from maize roots in T2 ($54 \pm 0.1\%$) 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 ($97 \pm 0.0\%$) from maize roots in T2 than in T1 or T3. ($F_{2,6} = 13.62$, $P < 0.0059$).

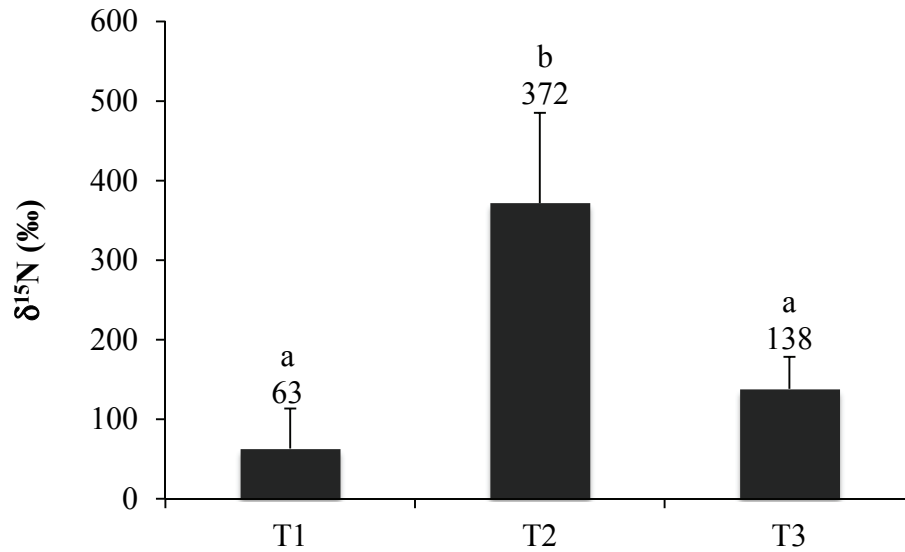


Figure 4.3 $\delta^{15}\text{N}$ isotope values (mean \pm S. D, $n = 3$) of *Folsomia candida* recovered from treatments with both maize plants and rye grass litter recovered after 63 (T1), 42 (T2) and 21 (T3) days after sowing maize plant seeds.

4.5 Discussion

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. However, 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. This establishes the importance of root availability in the occurrence of the diet-switch feeding behavior with *F. candida*. For ^{15}N , highest enrichment occurred in T2, consistent with the higher $\delta^{15}\text{N}$ ratios of both soil and maize roots. Higher $\delta^{15}\text{N}$ in T2 for all the three materials analyzed (soil, maize roots and

Collembola), could be a result of error during experimental set up or analysis of samples. The positive response of *F. candida* to the availability of live maize roots is in line with the opportunistic feeding behavior of Collembola (Anderson and Healey, 1972; Peterson and Luxton, 1982). When Chamberlain et al. (2006b) experimentally switched the diet of *F. candida* from yeast to nematodes, *F. candida* showed $\delta^{13}\text{C}$ 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. The positive impact of the switch could have resulted from compensatory feeding on the new diet. It may be possible that, food materials that contain moderate amounts of N is consumed by organisms in larger quantities to compensate for low quality (Sturner 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 favored yeast over maize roots (see Chapter 3) indicating that maize roots are not the highest quality foods in their diets. Nonetheless, they provide enough nutrients to sustain their growth and were more preferred over prior incorporated ryegrass litter, at least when present in adequate quantities. Indeed, even when roots were only relatively marginally abundant (T3) and ryegrass litter most recently applied, *F. candida* were shown here to have still derived as much as 38% of their tissue C from maize roots. Consequently, it would seem that when maize roots are readily available, Collembola may have to forage on maize more actively than on yeast but less than on ryegrass litter to satisfy their nutritional needs.

Bukovinszky et al. (2012) noted that the impact of food quantity and quality on Collembola diet was strongly interrelated and each species had to adjust its feeding behavior accordingly. *Prokelisia dolus*, a planthopper species, which when fed with 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. Based on these observations, it is clear that the feeding activities of animals are species specific. *Ampithoe longimana*, an amphipod also maintained its growth and fecundity by compensatory feeding of low quality diets in excess quantities (Cruz-Rivera

and Hay, 2000). This suggests that the role of food availability and quality in *F. candida* cannot be extrapolated to other Collembola species; hence, similar studies with other Collembolan species can provide a comprehensive understanding of their feeding behavior and diets.

The minor increases 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 $\delta^{13}\text{C}$ 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).

Presence of ^{15}N enriched ryegrass litter increased the $\delta^{15}\text{N}$ values of both soil and maize roots. However, maize roots had 10 fold higher $\delta^{15}\text{N}$ 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.

4.6 Conclusion

The importance of food quality in Collembola fungal diet has been identified and studied extensively (Booth and Anderson, 1979; Bengtsson et al., 1988; Lavy and Verhoef, 1996; Jørgensen et al., 2008), but the role of plant roots and their derived materials, in terms of both quality and quantity, in Collembola diets, has received much less attention (Krab et al., 2013). Endlweber et al. (2009) demonstrated a strong tendency for *P. fimata* to actively switch its diet from ryegrass litter to live maize roots in microcosms with an abundance of roots, attributing the switch largely to improved food quality. Results of this experiment have demonstrated that the $\text{C}_4\text{-C}$ 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. Quantity was an important factor in determining the food choice of *F. candida* but its occurrence across other Collembola species remains to be tested. These results also highlight the importance of considering food availability when designing experiments on diet-switching.

CHAPTER 5 SUMMARY

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

The study extended the existence of active diet-switching from plant litter to live plant roots to a second species of Collembola: *Folsomia candida*. When live maize roots were also present in the microcosms along with the ryegrass litter, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 *Folsomia candida* populations to almost twice that when feeding on ryegrass litter alone.

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

The study also revealed the positive influence of live root quality on the feeding behavior 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; survival and reproduction were unaffected. Presence of dry yeast masked the influence of maize roots as *F. candida* fed with yeast showed even higher body length growth on yeast 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 in a Collembola species such as *F. candida* was directly related to live root availability. *Folsomia candida* exposed to larger root biomass in microcosms showed a higher diet-switch towards maize roots when compared to smaller biomass. This is in line with the characteristic opportunistic feeding habits of Collembola which vary food depending on food availability. Collembola in natural soils experience various conditions of food availability, depending on the ecosystems they inhabit and must adapt their feeding behavior based on the available resources, an important factor for their success in various habitats.

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 proven 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 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. 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 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 that *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 this.

REFERENCES

- Aerts, R., 1997.** Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: A triangular relationship. *Oikos* 79, 439-449.
- Albers, D., Schaefer, M., Scheu, S., 2006.** Incorporation of plant carbon into the soil animal food web of an arable system. *Ecology* 87, 235-245.
- Anderson, J.M., Healey, I.N., 1972.** Seasonal and inter-specific variation in major components of the gut contents of some woodland Collembola. *Journal of Animal Ecology* 41, 359-368.
- Andren, O., Schnurer, J., 1985.** Barley straw decomposition with varied levels of microbial grazing by *Folsomia fimetaria* (L.) (Collembola, Isotomidae). *International Association for Ecology* 68, 57-62.
- Angers, D.A., Voroney, R.P., Cote, D., 1995.** Dynamics of soil organic matter and corn residues affected by tillage practices. *Soil Science Society of America Journal* 59, 1311-1315.
- Bakonyi, G., 1989.** Effects of *Folsomia candida* (Collembola) on the microbial biomass in a grassland soil. *Biology and Fertility of Soils* 7, 138-141.
- Balesdent, J., Mariotti, A., Boisgontier, D., 1990.** Effect of tillage on soil organic carbon mineralization estimated from ^{13}C abundance in maize fields. *Journal of Soil Science* 41, 587-596.
- Balesdent, J., Wagner, G.H., Mariotti, A., 1988.** Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. *Soil Science Society of America Journal* 52, 118-124.
- Balesdent, J., Mariotti, A., Guillet, B., 1987.** Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. *Soil Biology and Biochemistry* 19, 25-30.
- Baylis, J.P., Cherrett, J.M., Ford, J.B., 1986.** A survey of the invertebrates feeding on living clover roots (*Trifolium repens* L.) using ^{32}P as a radiotracer. *Pedobiologia* 29, 201-208.

- Beare, M., Crossley, D., Hendrix, P., Odum, E., 1995.** A hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. *Plant and Soil* 170, 5-22.
- Bengtsson, G., Erlandsson, A., Rundgren, S., 1988.** Fungal odour attracts soil Collembola. *Soil Biology and Biochemistry* 20, 25-30.
- Berg, B., McClaugherty, C., 2003.** *Plant Litter Decomposition, Humus Formation, Carbon Sequestration* Springer, Berlin, Germany.
- Berg, M.P., Stoffer, M., van den Heuvel, H.H., 2004.** Feeding guilds in Collembola based on digestive enzymes. *Pedobiologia* 48, 589-601.
- Bernays, E., Bright, K., Gonzalez, N., Angel, J., 1994.** Dietary mixing in a generalist herbivore: Tests of two hypotheses. *Ecology* 1997-2006.
- Boersma, M., Kreutzer, C., 2002.** Life at the edge: Is food quality really of minor importance at low quantities? *Ecology* 83, 2552-2561.
- Booth, R.G., Anderson, J.M., 1979.** The influence of fungal food quality on the growth and fecundity of *Folsomia candida* (Collembola: Isotomidae). *Oecologia* 38, 317-323.
- Briones, M.J.I., Ineson, P., Sleep, D., 1999.** Use of $\delta^{13}\text{C}$ to determine food selection in Collembolan species. *Soil Biology and Biochemistry* 31, 937-940.
- Brown, R., 1985.** Effects of some root-grazing arthropods on the growth of sugar-beet. Special publication of the British Ecological Society 285-295.
- Brüggemann, N., Gessler, A., Kayler, Z., Keel, S.G., Badeck, F., Barthel, M., Boeckx, P., Buchmann, N., Brugnoli, E., Esperschütz, J., Gavrichkova, O., Ghashghaie, J., Gomez-Casanovas, N., Keitel, C., Knohl, A., Kuptz, D., Palacio, S., Salmon, Y., Uchida, Y., Bahn, M., 2011.** Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: A review. *Biogeosciences* 8, 3457-3489.
- Bukovinszky T, Verschoor AM, Helmsing NR, Bezemer TM, Bakker ES, Vos M, de Senerpont Domis LN, 2012.** The good, the bad and the plenty: Interactive effects of food quality and quantity on the growth of different daphnia species. *PloS one* 7, 1-9.

- Chahartaghi, M., Langel, R., Scheu, S., Ruess, L., 2005.** Feeding guilds in Collembola based on nitrogen stable isotope ratios. *Soil Biology and Biochemistry* 37, 1718-1725.
- Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P., Evershed, R.P., 2006a.** Collembolan trophic preferences determined using fatty acid distributions and compound-specific stable carbon isotope values. *Soil Biology and Biochemistry* 38, 1275-1281.
- Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P., Evershed, R.P., 2006b.** The effect of diet on isotopic turnover in Collembola examined using the stable carbon isotopic compositions of lipids. *Soil Biology and Biochemistry* 38, 1146-1157.
- Chamberlain, P.M., McNamara, N.P., Chaplow, J., Stott, A.W., Black, H.I.J., 2006c.** Translocation of surface litter carbon into soil by Collembola. *Soil Biology and Biochemistry* 38, 2655-2664.
- Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P., Evershed, R.P., 2005.** Fatty acid composition and change in Collembola fed differing diets: Identification of trophic biomarkers. *Soil Biology and Biochemistry* 37, 1608-1624.
- Chamberlain, P.M., Bull, I.D., Black, H.I.J., Ineson, P., Evershed, R.P., 2004.** Lipid content and carbon assimilation in Collembola: Implications for the use of compound-specific carbon isotope analysis in animal dietary studies. *Oecologia* 139, 325-335.
- Charnov, E.L., 1976.** Optimal foraging, the marginal value theorem. *Theoretical Population Biology* 9, 129-136.
- Chen, B., 1995.** Food preference and effects of food type on the life history of some soil Collembola. *Pedobiologia* 39, 496.
- Churchland, C., Weatherall, A., Briones, M.J.I., Grayston, S.J., 2012.** Stable-isotope labeling and probing of recent photosynthates into respired CO₂, soil microbes and soil mesofauna using a xylem and phloem stem-injection technique on sitka spruce (*Picea sitchensis*). *Rapid Communications in Mass Spectrometry* 26, 2493-2501.
- Cole, L., Bardgett, R.D., 2005.** Animals: Microbial Interactions and Nutrient Cycling. In: Rattan, L. (Eds.), *Encyclopedia of Soil Science*, Second Edition (Print/online Version). Taylor & Francis, Columbus, pp. 113-116.

- Cortet, J., Joffre, R., Elmholt, S., Krogh, P.H., 2003.** Increasing species and trophic diversity of mesofauna affects fungal biomass, mesofauna community structure and organic matter decomposition processes. *Biology and Fertility of Soils* 37, 302-312.
- Cragg, R.G., Bardgett, R.D., 2001.** How changes in soil faunal diversity and composition within a trophic group influence decomposition processes. *Soil Biology and Biochemistry* 33, 2073-2081.
- Crouau, Y., Moïa, C., 2006.** The relative sensitivity of growth and reproduction in the springtail, *Folsomia candida*, exposed to xenobiotics in the laboratory: An indicator of soil toxicity. *Ecotoxicology and environmental safety* 64, 115-121.
- Crouau, Y., Cazes, L., 2003.** What causes variability in the *Folsomia candida* reproduction test? *Applied Soil Ecology* 22, 175-180.
- Cruz-Rivera, E., Hay, M.E., 2000.** Can quantity replace quality? food choice, compensatory feeding, and fitness of marine mesograzers. *Ecology* 81, 201-219.
- DeNiro, M.J., Epstein, S., 1981.** Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45, 341-351.
- DeNiro, M.J., Epstein, S., 1978.** Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta* 42, 495-506.
- Dombos, M., Stimmann, M.W., 2001.** Marking springtails (*Folsomia candida*) with rubidium. *Pedobiologia* 45, 551-560.
- Draheim, R., Larink, O., 1995.** Effects of differently cultured fungi as a diet of Collembola. *Acta Zoologica Fennica* 168-170.
- Drift, J.V.D., Jansen, E., 1977.** Grazing of springtails on hyphal mats and its influence on fungal growth and respiration. *Ecological Bulletins* 25, 203-209.
- Ehleringer, J.R., 1991.** $^{13}\text{C}/^{12}\text{C}$ Fractionation and its Utility in Terrestrial Plant Studies. In: Coleman, D.C., Fry, B. (Eds.), *Carbon Isotope Techniques*. Academic Press, Inc., San Diego, California, pp. 187-200.
- Eisenhauer, N., Sabais, A.C., Scheu, S., 2011.** Collembola species composition and diversity effects on ecosystem functioning vary with plant functional group identity. *Soil Biology and Biochemistry* 43, 1697-1704.

- Endlweber, K., Ruess, L., Scheu, S., 2009.** Collembola switch diet in presence of plant roots thereby functioning as herbivores. *Soil Biology and Biochemistry* 41, 1151-1154.
- Endlweber, K., Scheu, S., 2007.** Interactions between mycorrhizal fungi and collembola: Effects on root structure of competing plant species. *Biology and Fertility of Soils* 43, 741-749.
- Endlweber, K., Scheu, S., 2006.** Effects of Collembola on root properties of two competing ruderal plant species. *Soil Biology and Biochemistry* 38, 2025-2031.
- Environment Canada, 2007.** Biological test method: Test for measuring survival and reproduction of springtails expose to contaminants in soil - September 2007. EPS 1/RM/47.
- Filser, J., 2002.** The role of Collembola in carbon and nitrogen cycling in soil: Proceedings of the Xth international colloquium on apterygota, České Budejovice 2000: Apterygota at the beginning of the third millennium. *Pedobiologia* 46, 234-245.
- Fitter, A.H., Self, G.K., Brown, T.K., Bogie, D.S., Graves, J.D., Benham, D., Ineson, P., 1999.** Root production and turnover in an upland grassland subjected to artificial soil warming respond to radiation flux and nutrients, not temperature. *Oecologia* 120, pp. 575-581.
- Fitter, A., Garbaye, J., 1994.** Interactions between mycorrhizal fungi and other soil organisms. *Plant and Soil* 159, 123-132.
- Fountain, M.T., Hopkin, S.P., 2005.** *Folsomia candida* (Collembola): A “Standard” soil arthropod*. *Annual Review of Entomology* 50, 201-222.
- Fujii, S., Yamada, A., Fukushima, K., Saitoh, S., Kitayama, K., Takeda, H., 2013.** Relationships among distribution of fine roots, soil DOC concentration and Collembola. 7, 21-27.
- Garrett, C.J., Crossley, D., Coleman, D.C., Hendrix, P.F., Kisselle, K.W., Potter, R.L., 2001.** Impact of the rhizosphere on soil microarthropods in agroecosystems on the georgia piedmont. *Applied Soil Ecology* 16, 141-148.

- Gearing, J., M., 1991.** The Study of Diet and Trophic Relationships through Natural Abundance 13-C. In: Coleman, D.C., Fry, B. (Eds.), Carbon Isotope Techniques. Academic Press, Inc., San Diego, California, pp. 201-218.
- Green, C., 1964.** The effect of crowding upon the fecundity of *Folsomia candida* (Willem) var. *distincta* (Bagnall) (Collembola). *Entomologia Experimentalis et Applicata* 7, 62-70.
- Griffiths, B.S., Bardgett, R.D., 1997.** Interaction between Microbe-Feeding Invertebrates and Soil Microorganisms. In: van Elsas, J.D., Wellington, E., Trevors, J.T. (Eds.), *Modern Soil Microbiology*. Marcel Dekkar, New York, pp. 165-182.
- Gunn, A., Cherrett, J., 1993.** The exploitation of food resources by soil meso- and macro invertebrates. *Pedobiologia* 37, 303-320.
- Gutow, L., Leidenberger, S., Boos, K., Franke, H., 2007.** Differential life-history responses of two *Idotea* species (Crustacea: Isopoda) to food limitation. *Marine ecology-progress series* 344, 159-172.
- Hanlon, R.D.G., Anderson, J.M., 1979.** The effects of Collembola grazing on microbial activity in decomposing leaf litter. *Oecologia* 38, 93-99.
- Haubert, D., Langel, R., Scheu, S., Ruess, L., 2005.** Effects of food quality, starvation and life stage on stable isotope fractionation in Collembola. *Pedobiologia* 49, 229-237.
- Hopkin, S.P., 1997.** *Biology of the Springtails (Insecta: Collembola)*. Oxford University Press, Oxford.
- Huberty, A.F., Denno, R.F., 2006.** Consequences of nitrogen and phosphorus limitation for the performance of two planthoppers with divergent life-history strategies. *Oecologia* 149, 444-455.
- Hurej, M., Debek, J., Pomorski, R.J., 1992.** Investigations on damage to sugar-beet seedlings by the springtail *Onychiurus armatus* (Collembola, Onychiuridae) in lower Silesia (Poland). *Acta Entomologica Bohemoslovaca* 89, 403.
- ISO, 1999.** Soil Quality -- Inhibition of Reproduction of Collembola (*Folsomia candida*) by Soil Pollutants. International Standards Organization, Geneva, Switzerland, pp. 1-16.

- Johnson, D., Krsek, M., Wellington, E.M.H., Stott, A.W., Cole, L., Bardgett, R.D., Read, D.J., Leake, J.R., 2005.** Soil invertebrates disrupt carbon flow through fungal networks. *Science* 309, 1047.
- Jones, D., Nguyen, C., Finlay, R., 2009.** Carbon flow in the rhizosphere: Carbon trading at the soil–root interface. *Plant and Soil* 321, 5-33.
- Joosse, E.N.G., Testerink, G.J., 1977.** The role of food in the population dynamics of *Orchesella cincta* (Linné) (Collembola). *Oecologia* 29, 189-204.
- Jørgensen, H.B., Hedlund, K., Axelsen, J., 2008.** Life-history traits of soil collembolans in relation to food quality. *Applied Soil Ecology* 38, 146-151.
- Jørgensen, H.B., Johansson, T., Canbäck, B., Hedlund, K., Tunlid, A., 2005.** Selective foraging of fungi by Collembolans in soil. *Biology Letters* 1, 243-246.
- Jørgensen, H.B., Elmholt, S., Petersen, H., 2003.** Collembolan dietary specialisation on soil grown fungi. *Biology and Fertility of Soils* 39, 9-15.
- Kaneda, S., Kaneko, N., 2002.** Influence of soil quality on the growth of *Folsomia candida* (Willem) (Collembola). *Pedobiologia* 46, 428-439.
- Kaneko, N., McLean, M.A., Parkinson, D., 1995.** Grazing preference of *Onychiurus subtenuis* (Collembola) and *Oppeia nova* (Oribatei) for fungal species inoculated on pine needles. *Pedobiologia* 39, 538.
- Karowe, D.N., Martin, M.M., 1989.** The effects of quantity and quality of diet nitrogen on the growth, efficiency of food utilization, nitrogen budget, and metabolic rate of fifth-instar *Spodoptera eridania* larvae (Lepidoptera: Noctuidae). *Journal of insect physiology* 35, 699-708.
- Kato, M., 1995.** The aspidistra and the amphipod. *Nature* 377, 293.
- Kirk, K.L., 1997.** Life-history responses to variable environments: Starvation and reproduction in planktonic rotifers. *Ecology* 78, 434-441.
- Kreuzer, K., Bonkowski, M., Langel, R., Scheu, S., 2004.** Decomposer animals (Lumbricidae, Collembola) and organic matter distribution affect the performance of *Lolium perenne* (Poaceae) and *Trifolium repens* (Fabaceae). *Soil Biology and Biochemistry* 36, 2005-2011.

- Larsen, T., Gorissen, A., Krogh, P., Ventura, M., Magid, J., 2007.** Assimilation dynamics of soil carbon and nitrogen by wheat roots and Collembola. *Plant and Soil* 295, 253-264.
- Lavy, D., Verhoef, H., 1996.** Effects of food quality on growth and body composition of the Collembolan *Orchesella cincta*. *Physiological Entomology* 21, 64-70.
- Lee, Q., Widden, P., 1996.** *Folsomia candida*, a “fungivorous” Collembolan, feeds preferentially on nematodes rather than soil fungi. *Soil Biology and Biochemistry* 28, 689-690.
- Lynch, D.H., Voroney, R.P., Warman, P.R. 2006.** Use of ^{13}C and ^{15}N natural abundance techniques to characterize carbon and nitrogen dynamics in composting and in compost-amended soils. *Soil Biology and Biochemistry* 38, 103-114.
- McMillan, J.H., 1975.** Interspecific and seasonal analysis of gut contents of three Collembola (family: Onychiuridae). *Review of Ecology and Biology of Soil* 12, 449-457.
- Milcu, A., Partsch, S., Langel, R., Scheu, S., 2006.** The response of decomposers (earthworms, springtails and microorganisms) to variations in species and functional group diversity of plants. *Oikos* 112, 513-524.
- Mebes, K., Filser, J., 1998.** Does the species composition of collembola affect nitrogen turnover? *Applied Soil Ecology* 9, 241-247.
- Moore, J.C., Ingham, E.R., Coleman, D.C., 1987.** Inter- and intraspecific feeding selectivity of *Folsomia candida* (Willem) (Collembola, Isotomidae) on fungi. *Biology and Fertility of Soils* 5, 6-12.
- Nelson, K.L., Boiteau, G., Lynch, D.H., Peters, R.D., Fillmore, S., 2011.** Influence of agricultural soils on the growth and reproduction of the bio-indicator *Folsomia candida*. *Pedobiologia* 54, 79-86.
- OECD, 1984.** OECD Guidelines for Testing Chemicals/Section 2: Effects on Biotic Systems. Test no. 207: Earthworms, Cate Toxicity Test. OECD publishing, Paris, pp. 1.
- Ostle, N., Briones, M.J.I., Ineson, P., Cole, L., Staddon, P., Sleep, D., 2007.** Isotopic detection of recent photosynthate carbon flow into grassland rhizosphere fauna. *Soil Biology and Biochemistry* 39, 768-777.

- Paterson, E., Midwood, A.J., Millard, P., 2009.** Through the eye of the needle: A review of isotope approaches to quantify microbial processes mediating soil carbon balance. *New Phytologist* 184, 19-33.
- Pennings, S.C., Nadeau, M.T., Paul, V.J., 1993.** Selectivity and growth of the generalist herbivore *Dolabella auricularia* feeding upon complementary resources. *Ecology* 879-890.
- Persson, T., 1989.** Role of soil animals in C and N mineralisation. *Plant and Soil* 115, 241-245.
- Petersen, H., 1994.** The review of Collembolan ecology in ecosystem context. *Acta Zoologica fennica* 195, 111-118.
- Petersen, H., Luxton, M., 1982.** A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos* 39, 288-388.
- Peterson, B.J., Fry, B., 1987.** Stable isotopes in ecosystem studies. *Annual review of ecology and systematics*. Vol.18 18, 293-320.
- Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N., Weis, A.E., 1980.** Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics* 11, 41-65.
- Pollierer, M.M., Langel, R., Körner, C., Maraun, M., Scheu, S., 2007.** The underestimated importance of belowground carbon input for forest soil animal food webs. *Ecology Letters* 10, 729-736.
- Ponge, J.F., 2000.** Vertical distribution of Collembola (Hexapoda) and their food resources in organic horizons of beech forests. *Biology and Fertility of Soils* 32, 508-522.
- Ponge, J.F., 1991.** Food resources and diets of soil animals in a small area of scots pine litter. *Geoderma* 49, 33-62.
- Pyke, G.H., 1984.** Optimal foraging theory: A critical review. *Annual Review of Ecology and Systematics* 15, 523-575.

- Real, L., Caraco, T., 1986.** Risk and foraging in stochastic environments. *Annual Review of Ecology and Systematics* 17, 371-390.
- Reichle, D.E., 1977.** The role of soil invertebrates in nutrient cycling. *Ecological Bulletins* 25, 145-156.
- Römbke, J., Jänsch, S., Scroggins, R., 2006.** Identification of potential organisms of relevance to Canadian boreal forest and northern lands for testing of contaminated soils. *Environmental Reviews* 14, 137-167.
- Rusek, J., 1998.** Biodiversity of Collembola and their functional role in the ecosystem. *Biodiversity and Conservation* 7, 1207-1219.
- Sadaka-Laulan, N., Ponge, J., 2000.** Influence of holm oak leaf decomposition stage on the biology of *Onychiurus sinensis* Stach (Collembola: Onychiuridae). *European Journal of Soil Biology* 36, 97-105.
- Salamon, J., Schaefer, M., Alphei, J., Schmid, B., Scheu, S., 2004.** Effects of plant diversity on Collembola in an experimental grassland ecosystem. *Oikos* 106, 51-60.
- San Miguel, A., Raveton, M., Lempérière, G., Ravanel, P., 2008.** Phenylpyrazoles impact on *Folsomia candida* (Collembola). *Soil Biology and Biochemistry* 40, 2351-2357.
- Scheu, S., Folger, M., 2004.** Single and mixed diets in Collembola: Effects on reproduction and stable isotope fractionation. *Functional Ecology* 18, 94-102.
- Scheu, S., 2002.** The soil food web: Structure and perspectives. *European Journal of Soil Biology* 38, 11-20.
- Scheu, S., Ruess, L., Bonkowski, M., 2005.** Interactions between Microorganisms and Soil Micro- and Mesofauna. In: Varma, A., Buscot, F. (Eds.), *Microorganisms in Soils: Roles in Genesis and Functions*. Springer Berlin Heidelberg, pp. 253-275.
- Scheu, S., Simmerling, F., 2004.** Growth and reproduction of fungal feeding Collembola as affected by fungal species, melanin and mixed diets. *Oecologia* 139, 347-353.
- Schoener, T.W., 1971.** Theory of feeding strategies. *Annual Review of Ecology and Systematics* 2, 369-404.

- Schrader, S., Langmaack, M., Helming, K., 1997.** Impact of Collembola and enchytraeidae on soil surface roughness and properties. *Biology and Fertility of Soils* 25, 396-400.
- Seastedt, T.R., 1984.** The role of microarthropods in decomposition and mineralization processes. *Annual Review of Entomology* 29, 25-46.
- Seeber, J., Rief, A., Richter, A., Traugott, M., Bahn, M., 2012.** Drought-induced reduction in uptake of recently photosynthesized carbon by springtails and mites in alpine grassland. *Soil Biology and Biochemistry* 55, 37-39.
- Sih, A., Christensen, B., 2001.** Optimal diet theory: When does it work, and when and why does it fail? *Animal Behaviour* 61, 379-390.
- Slansky Jr, F., Stamp, N., Casey, T., 1993.** Nutritional ecology: The fundamental quest for nutrients. *Caterpillars: ecological and evolutionary constraints on foraging*. 29-91.
- Slansky, F., Scriber, J., 1985.** Food consumption and utilization. *Comprehensive Insect Physiology, Biochemistry and Pharmacology* 4, 87-163.
- Slansky, F., Rodriguez, J., 1987.** *Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates*. Wiley, New York.
- Smith, B.N., Epstein, S., 1971.** Two categories of $^{13}\text{C}/^{12}\text{C}$ ratios for higher plants. *Plant physiology* 47, 380-384.
- Staddon, P.L., 2004.** Carbon isotopes in functional soil ecology. *Trends in Ecology & Evolution* 19, 148-154.
- Stephens, D.W., 1986.** *Foraging Theory*. Princeton University Press, New Jersey.
- Stevenson, F.J., Cole, M.A., 1999.** *Cycles of Soil - Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients*. John Wiley and Sons, New York.
- Sterner, R., 1997.** Modelling interactions of food quality and quantity in homeostatic consumers. *Freshwater Biology* 38, 473-481.
- Sterner, R.W., Hessen, D.O., 1994.** Algal nutrient limitation and the nutrition of aquatic herbivores. *Annual Review of Ecology and Systematics* 1-29.

- Sterner, R.W., Robinson, J.L., 1994.** Thresholds for growth in daphnia magna with high and low phosphorus diets. *Limnology and Oceanography* 39, 1228-1232.
- Sticht, C., Schrader, S., Giesemann, A., Weigel, H., 2006.** Effects of elevated atmospheric CO₂ and N fertilization on abundance, diversity and C-isotopic signature of Collembolan communities in arable soil. *Applied Soil Ecology* 34, 219-229.
- Swift, M.J., Anderson, J.M., 1989.** Decomposition. In: Lieth, H., Werger, M.J.A. (Eds.), *Ecosystems of the World 14B. Tropical Rain Forest Ecosystems: Biogeographical and Ecological Studies*. Elsevier Scientific Pub. Co., Amsterdam, pp. 547-569.
- Swift, M.J., Heal, O.W., Anderson, J.M., 1979.** *Decomposition in Terrestrial Ecosystems*. Blackwell Scientific, Oxford.
- Takeda, H., Ichimura, T., 1983.** Feeding attributes of four species of Collembola in a pine forest soil. *Pedobiologia* 25, 373-381.
- Tenney, F.G., Waksman, S.A., 1929.** Composition of natural organic materials and their decomposition in the soil: IV. The nature and rapidity of decomposition of the various organic complexes in different plant materials, under aerobic conditions. *Soil Science* 28, 55.
- Testerink, G.J., 1981.** Starvation in a field population of litter-inhabiting Collembola. methods for determining food reserves in small arthropods. *Pedobiologia* 21, 427-433.
- Thimm, T., Hoffmann, A., Borkott, H., Munch, J.C., Tebbe, C.C., 1998.** The gut of the soil microarthropod *Folsomia candida* (Collembola) is a frequently changeable but selective habitat and a vector for microorganisms. *Applied and Environmental Microbiology* 64, 2660-2669.
- Tiunov, A., 2007.** Stable isotopes of carbon and nitrogen in soil ecological studies. *Biology Bulletin* 34, 395-407.
- Tugel, A.J., Lewandowski, A.M., Happe-vonArb, D. (Eds.), 2000.** *Soil Biology Primer*. Rev. Ed. Soil and Water Conservation Society, Ankeny, Iowa.

- Usher, M.B., Longstaff, B.C., Southall, D.R., 1971.** Studies on populations of *Folsomia candida* (Insecta: Collembola) - the productivity of populations in relation to food and exploitation. *Oecologia* 7, 68-79.
- van Amelsvoort, P.A.M., Usher, M.B., 1989a.** Egg production related to food quality in *Folsomia candida* (Collembola: Isotomidae): Effects on life history strategies. *Pedobiologia* 33, 61-66.
- van Amelsvoort, P.A.M., Usher, M.B., 1989b.** A method for assessing the palatability of senesced leaf litter using *Folsomia candida* (Collembola: Isotomidae). *Pedobiologia* 33, 193-198.
- Van Soest, P.J., 1994.** Nutritional Ecology of the Ruminant. Cornell University Press, New York.
- van Straalen, N.M., Verhoef, H.A., 1997.** The development of a bioindicator system for soil acidity based on arthropod pH preferences. *Journal of Applied Ecology* 34, 217-232.
- Verhoef, H.A., Brussaard, L., 1990.** Decomposition and nitrogen mineralization in natural and agro-ecosystems: The contribution of soil animals. *Biogeochemistry* 11, 175-211.
- Verhoef, H.A., Li, K.W., 1983.** Physiological adaptations to the effects of dry summer periods in Collembola. *International Colloquium of Soil Zoology*. 8, 345-356
- Visser, S., Whittaker, J., 1977.** Feeding preferences for certain litter fungi by *Onychiurus subtenuis* (Collembola). *Oikos* 320-325.
- Vitorello, V.A., 1989.** Organic matter and natural carbon-13 distribution in forested and cultivated oxisols. *Soil Science Society of America Journal* 53, 773-778.
- Wanniarachchi, S.D., Voroney, R.P., Vyn, T.J., Beyaert, R.P., MacKenzie, A.F., 1999.** Management for agricultural, forestry and urban uses - tillage effects on the dynamics of total and corn residue-derived soil organic matter in two southern Ontario soils. *Canadian Journal of Soil Science*. 79, 473-480.
- Wardle, D.A., 2013.** Communities and Ecosystems: Linking the Aboveground and Belowground Components (MPB-34). Princeton University Press, New Jersey.

Wolda, H., 1978. Seasonal fluctuations in rainfall, food and abundance of tropical insects. *The Journal of Animal Ecology* 47, 369-381.

Zak, D.R., Pregitzer, K.S., Curtis, P.S., Teeri, J.A., Fogel, R., Randlett, D.L., 1993. Elevated atmospheric CO₂ and feedback between carbon and nitrogen cycles. *Plant and Soil* 151, 105-117.

APPENDIX A: LABELLING YEAST WITH C₃ SIGNATURE

Collembola raised on commercial baker's yeast yield a C signature close to C₄ plants. In procedures designed to determine a potential diet-switch to C₄ food sources, such as described in Endlweber et al. (2009), the Collembola cannot possess 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 C₃ 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 C₃ 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 ¹³C value of -24.8‰ and the Collembola raised on the yeast had a signature of -24.2‰.

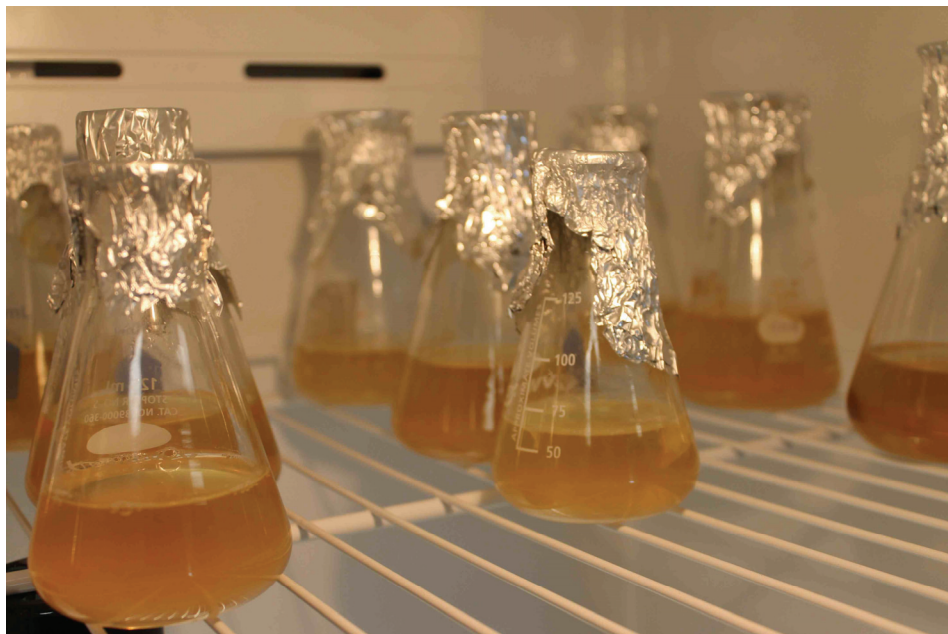


Fig. Conical flasks filled with Sabourad's media and inoculated with baker's yeast placed in incubator used in the production of C₃ yeast.

APPENDIX B: LABELLING RYE GRASS WITH ^{15}N FERTILIZER

Rye grass (C_3 plant) residue used in this experiment was labelled 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 rye grass is given below.

1. Rye grass 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 ($0.40\text{ mol. m}^{-3}\text{ KH}_2\text{PO}_4$, $0.15\text{ mol. m}^{-3}\text{ K}_2\text{HPO}_4$, $1.0\text{ mol. m}^{-3}\text{ K}_2\text{SO}_4$, $3.0\text{ mol. m}^{-3}\text{ CaCl}_2$, $0.50\text{ mol m}^{-3}\text{ MgSO}_4$, $0.10\text{ mol m}^{-3}\text{ NaCl}$, $0.025\text{ mol m}^{-3}\text{ H}_3\text{BO}_3$, $0.50310^{-3}\text{ mol. m}^{-3}\text{ CuSO}_4$, $0.002\text{ mol. m}^{-3}\text{ MnSO}_4$, $0.50310^{-3}\text{ mol. m}^{-3}\text{ Na}_2\text{MoO}_4$, $0.002\text{ mol. m}^{-3}\text{ ZnSO}_4$, and $0.02\text{ mol. m}^{-3}\text{ FeSO}_4$).
5. $5\text{mM } ^{15}\text{NH}_4^{15}\text{NO}_3$ solution was prepared by dissolving 4 g of the $^{15}\text{NH}_4^{15}\text{NO}_3$ (10% atom) in 100ml water and then making up the solution to 1 L. This is same as 2% solution.
6. Labelling procedure was done by a pulse labelling 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 rye grass seedling which were supplied with $^{15}\text{NH}_4^{15}\text{NO}_3$ solution and other nutrients to label ryegrass with ^{15}N .