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Complete List of Authors:	Eerpina, Ramesh; Vineland Research and Innovation Centre, Horticulture Boiteau, Gilles; AAFC, Potato Research Centre Lynch, Derek; Dalhousie University Faculty of Agriculture, Plant and Animal Sciences
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COLLEMBOLA DIET-SWITCHING IN THE PRESENCE OF MAIZE ROOTS VARIES WITH SPECIES

Ramesh Eerpina^a, Gilles Boiteau^b, Derek H. Lynch^c

^aVineland Research and Innovation Centre, 4890 Victoria Avenue North, Vineland Station
Ontario, L0R 2E0. email: Ramesh.eerpina@dal.ca

^bPotato Research Centre - Agriculture and Agri-Food Canada Research Centre,
Fredericton, New Brunswick, Canada. E3B 4Z7

^cFaculty of Agriculture, Dalhousie University, Truro, Nova Scotia, Canada. B2N 5E3

Abstract: Collembola are known to feed on a wide range of soil material, predominantly rhizosphere fungi and root derived substances. However, diet switching from these usual food sources to living roots (herbivory) was previously demonstrated for one species of collembola, *Protaphorura fimata*, an euedaphic species. The study objective was to determine if diet-switching can be applied to other collembola species *Folsomia candida* Willem. This hemi-edaphic species was given different combinations of maize plants ($-13.28\text{‰ } \delta^{13}\text{C}$, $3\text{‰ } \delta^{15}\text{N}$) and ^{15}N enriched rye grass litter ($-28.88\text{‰ } \delta^{13}\text{C}$, $17516.86\text{‰ } \delta^{15}\text{N}$) in a C_3 soil system ($-27.27\text{‰ } \delta^{13}\text{C}$, $5.27\text{‰ } \delta^{15}\text{N}$) under controlled conditions. After eight weeks, there was clear evidence of root feeding because the $\delta^{13}\text{C}$ signature in collembola tissue was -19.28‰ in the presence of maize plants alone and -18.29‰ with maize plants grown in soil mixed with ryegrass litter, whereas collembola in unplanted soil microcosms had a $\delta^{13}\text{C}$ signature of -23.66‰ . Data analysis with a two-source isotope mixing model indicates that up to 60% of the carbon requirements of *F. candida* were

derived from living maize roots. Whether collembola root feeding is due to grazing on roots directly or on mycorrhiza (root-fungus association) requires further investigation.

Keywords: Feeding behaviour, Collembola, Isotope signature, *Folsomia candida*, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$

Collembola are small, wingless hexapods that form the largest subset of soil arthropods. Their polyphagous nature and large numbers enables them to play an important role in the decomposer (soil) food webs. Collembola commonly known graze on fungi (Moore et al. 1985) and preference towards saprophytic fungi (Jørgensen et al. 2003; Klironomos and Kendrick, 1996) had been documented. Two species, *F. candida* and *Protaphorura armata* had increased growth and reproduction when exposed to different combinations of seven saprophytic fungi (Scheu and Summerling 2004). Similarly, another collembola species, *Heteromurus nitidus*, benefited by feeding on a mixed diet of ectomycorrhizal fungi and algae (high quality and low quality food respectively) with increased reproduction compared to single diets (Scheu and Folger, 2004). Other studies showed *P. fimata*, *H. nitidus* and *F. candida* preferentially feed on non-ectomycorrhizal fungi over mycorrhizal fungi (Gange 2000; Tiunov and Scheu, 2005). Visual examinations of collembola guts also showed the presence of cellulase enzyme, responsible for digesting plant tissue (Berg et al. 2004; Chahartaghi et al. 2005). Tracer ($\delta^{15}\text{N}$) techniques indicated that collembola trophic guilds can range from primary decomposers to herbivores (feeding on living plant tissues), implying a wide resource base (Chahartaghi et al. 2005).

Roots contain a wide variety of constituents and products such as carbohydrates, organic acids, amino acids and other secondary metabolites (Johansson 1992) which also

can form an abundant source of food for soil arthropods. Collembola also feed root on derived products (Ostle et al. 2007) and are closely linked to the rhizosphere carbon (C) flow. Root grazing by collembola has been reported by Brown (1985) and many recent studies showed soil food webs rely on plants roots for their C requirements (Albers et al. 2006; Larsen et al. 2007; Ostle et al. 2007; Pollierer et al. 2007). Collembola feeding promoted root growth with longer, thinner and more root tips, suggesting collembola form nutrient rich patches in soil, thereby affecting root growth (Bonkowski et al. 2009).

Endlweber et al. (2009) used isotopic techniques to demonstrate an active diet-switch from plant residues to live roots in the collembola species *P. fimata*. Such herbivory could be one of the strategies used by collembola to adjust their feeding according to the availability and quality of diet (Scheu and Folger 2004; Jørgensen et al. 2008; Jørgensen and Hedlund 2013; Larsen et al. 2007). Collembola prefer food resources that are in their immediate proximity (Ponge 2000) and have the ability to switch diets depending on availability (Chahartaghi et al. 2005). This suggests that switching to resources that are more palatable may be a strategy in their feeding behavior to ingest higher quality food material or to create resource partitioning when more than one species is present (Cortet et al. 2003; Eisenhauer et al. 2011). Despite the understanding of collembola habits in the presence of fungal or algal materials, little is known about their feeding preferences in the presence of live plants (roots). The majority of studies reporting collembola root feeding are largely based on direct observations or gut content analysis. Studies using isotopic analyses can provide more conclusive evidence that living roots and root exudates (sugars, phosphatases, organic and amino acids) play an important role for supplying nutrients for collembola by tracing C flows between food resources and

collembola. Since the nitrogen (N) cycle is closely linked to the C cycle, analyses of N along with C has provided additional understanding of C changes (Nelson et al. 2011; Wardle 2013).

Folsomia candida, a model organism used in many toxicological studies, is a non-pigmented, soil dwelling organism and is a common springtail species in most soils. Scanning electron microscopic analysis of *F. candida* gut contents revealed they ingest bacterial and fungal components (Thimm et al. 1998). Like many other springtail species *F. candida* shows strong preferences for certain fungi over others. They prefer *Cladosporium cladosporioides* to *Aspegillus niger* and *Pencillium sp.* to increase their growth and reproduction (Scheu and Summerling 2004). Briones et al. (2009) showed *F. candida* preferred C₄ maize litter over C₃ soil when given a choice between both.

The objective of the current study is to investigate whether the diet-switch reported by Endlweber et al. (2009) is species specific or if it can be extended to other collembola species such as *F. candida*. Based on the observations of Endlweber et al. (2009), it was hypothesized that *F. candida* satisfies both its C and N requirements from litter residues but switches to living plant roots when they are present. Given the objective and to compare the results, the present study used the same protocol as Endlweber et al. (2009) with microcosms containing C₄ plants (Maize – *Zea mays L.*) in a C₃ soil mixed with ¹⁵N enriched C₃ ryegrass (*Lolium perenne L.*) litter. For logistical reasons, our study was replicated over time.

Materials and methods

An initial colony of *F. candida* was obtained from the 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). These collembola raised on commercial baker’s yeast had a C signature similar to C₄ plants (-11 ‰ $\delta^{13}\text{C}$). To obtain *F. candida* with C signatures closer to C₃ plant values and that of *P. fimata* in Endlweber et al. (2009), a fresh colony of *F. candida* was raised on yeast cultured on a modified Sabourad’s media consisting of a mixture of beet sugar and soya peptone (C₃ plants) (Chamberlain et al. 2004). The resultant *F. candida* had a C and N isotope signature of -24.22‰ $\delta^{13}\text{C}$ and 9.74‰ $\delta^{15}\text{N}$.

Enriched ryegrass litter was added to half of the treatments. To obtain enriched residue, rye grass was grown in a silica sand and perlite media. All essential nutrients were supplied in the form of inorganic nutrient solution except for N which was applied thrice in the form 10 % ammonium nitrate over a 45 day period. After two months, plants were harvested by cutting the stems just above soil line and the leaves were dried and powdered.

To obtain collembola that were age synchronized for the experiment, groups of 200 – 300 adults were transferred into new plaster boxes to induce egg laying. Once the eggs had hardened, generally a week after laying, they were transferred into new 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 nymphs emerged egg cards were removed. Nymphs left in the rearing boxes were thus age synchronized within 2 days of emergence and were used when they attained the required age (15 days) for the experiment.

Collembola were grown in soil microcosms in a randomized block factorial experiment with four treatments formed by the presence and absence of maize and ryegrass litter: (1) maize plants + rye grass litter, (2) maize plants only, (3) rye grass litter only and (4) control treatment of soil alone with no maize plants or ryegrass litter. Due to logistical reasons blocks were established on January 21st, February 19th, March 11th and March 18th, 2013. Since the blocks were separated in time, 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 air-dried soil (C/N ratio 12.5, % C = 2.0 $\pm$ 0.4, pH 6.8) collected from the surface 15 cm of an organic cropping system field trial (Truro, Canada) with no prior history of corn or other C₄ crop production. Soil texture was loamy sand to sandy loam and the soil is classified as an ortho-humic ferric podzol (Truro soil association). Soil was sieved through a 2 mm mesh screen using a sieve shaker (Ro-Tap, Mantor, USA) and defaunated at -80° C for 24 h (Mebes and Filser 1998) to kill or reduce the native populations of collembola. 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.

Soil (1 kg) was filled to a height approximately 15 cm with the remaining 10 cm to the top of the PVC microcosm acting as a barrier to *F. candida* emigration. Prior to

placing soil into microcosms, two grams of finely ground ryegrass material (<0.5 mm, 47.7 ± 2.5 % C, 2.5 ± 0.1 % N) enriched with ^{15}N ($\delta^{15}\text{N}$ 17516.5 ± 113 ‰), was uniformly mixed into the soil in half of the microcosms (ryegrass litter containing treatments). Maize treatments received two seeds thinned after emergence to retain one healthy seedling per microcosm (roots, 41.2 ± 1.8 % C, 0.8 ± 0.1 % N). As the defaunation process kills all or most native microorganisms, soil in microcosms was re-inoculated by adding 30 mL supernatant of soil suspension prepared by mixing 200 g fresh soil in 2 L water and allowing it to settle for 3 h, after mixing ryegrass and sowing maize seed. Seven days after sowing, 100 individuals of *F. candida* (15 days old) were added to each microcosm which were randomly placed in growth chambers (Conviron, Controlled Environments Ltd., Winnipeg, Canada), 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 was maintained at field capacity by adding water every alternate day until the initial total weight (adjusted for plant growth) was achieved. Weed seedlings were removed from the microcosms upon emergence, as required.

Eight weeks after their transfer into microcosms, maize plants were removed from the microcosms by cutting the stem at the soil level and then the roots are excavated. Plant roots, along with the complete soil column were pulled out of the microcosms and were transferred along with the loose soil into large plastic containers. Approximately 25 g of soil was collected from several locations and depths in the microcosms and composited, then dried at 65° C until a constant weight was obtained. Microcosms were also rinsed into the plastic containers to transfer the adhering soil. Small quantities, approximately 100 g of soil in water suspension, were transferred into 1 L Mason jars with 500 mL water and stirred so the collembola would float to the surface due to their hydrophobic integument.

Collembola thus floating were immediately skimmed off repeatedly and transferred into 2 mL glass vials. Soil was stirred continuously to dislodge any collembola attached to the soil particles and stirring was repeated to make sure all the collembola are extracted. The extraction process was repeated until all the soil from the microcosms was extracted. Vials were freeze-dried in a freeze drier (Dura stop, Kinetic thermal systems, Stone Ridge, USA). After washing, maize roots were examined for any remaining *F. candida* and separated from shoots and dried at 65° C for 24 h.

Dried root and soil samples were powdered in a ball mill (Retsch, Haan, Germany) and samples of freeze-dried collembola (1.0 ± 0.1 mg), ground soil (5.0 ± 0.4 mg) and root (2.0 ± 0.2 mg) were packed into tin capsules for isotope analysis. Stable isotope ratios of the samples were analyzed in an elemental analyzer (Costech ECS4010, Costech Analytical, Valencia, USA) coupled to an isotope ratio mass spectrophotometer (IRMS) (Delta V mass spectrophotometer, Thermo scientific, Bremen, Germany) at the Stable Isotope laboratory, University of Saskatchewan, Saskatoon, Canada. The IRMS provided direct measurement of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of samples using V-PDB limestone and atmospheric N as standards, respectively. For internal calibration purposes, International standards IAEA-N1, IAEA-N2, IAEA-CH6 and USGS-24 were used. The fraction of C incorporated into collembola from maize and ryegrass litter sources were calculated using the two source mixing model (Eq. 1) described by (Gearing 1991).

$$F_x = (\delta R_m - \delta R_y) / (\delta R_x - \delta R_y) \times 100 \quad (1)$$

Here F_x is the fraction (%) of C from source X (maize roots), $\delta^{13}\text{R}_m$ is the isotopic ratio of the collembola exposed to mixture, $\delta^{13}\text{R}_x$ is the isotopic ratio of the source X, and

$\delta^{13}\text{R}_y$ is the isotopic ratio of the source Y (ryegrass litter). R is the respective isotope signature of C. Gearing (1991) suggested replacing the signature of the food source itself by the use of δR_x and δR_y for the same animal species feeding on only one source of food at a time (corrected C source values) to increase the accuracy of the estimated C fractionation between food resources. These corrected estimates are uncommon in the literature because the necessary data are usually not available.

Statistical analysis

Two-factor Analysis of variance (ANOVA) was conducted for collembola tissue C and N concentrations and isotope signatures using Proc GLM procedures with maize and ryegrass litter as two factors each with two levels (either present or absent) with replications and time as a blocking factor in SAS 9.3 (SAS Institute, Cary, USA). 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 the statistical assumptions of normality and constant variance were met.

Results

Maize plant response to treatments

Addition of ^{15}N enriched ryegrass litter, as expected, increased the root $\delta^{15}\text{N}$ signature significantly ($F_{1,19} = 137.6$, $P < 0.0001$) but had no significant effect on maize shoot and root biomass, root C and N concentrations, or root $\delta^{13}\text{C}$ signature (Table 1).

Folsomia candida

There was growth in the population of *F. candida* in all treatments, but number of collembola recovered from individual microcosm differed significantly ($F_{3,41} = 8.56$, $P < 0.0001$) among the treatments. The highest number was recovered from microcosms with maize plants and ryegrass litter (211.83 ± 122.66), followed by microcosms with maize plants alone (199.75 ± 115.63), ryegrass litter only (127.50 ± 79.06) and soil only control (94.00 ± 67.22). However, *F. candida* tissue C and N content was not significantly influenced by the presence of maize plant and/or ryegrass litter (Table 2).

Presence of maize plants alone had a significant influence on the $\delta^{13}\text{C}$ ratio of *F. candida* tissue ($F_{3,39} = 25.96$, $P < 0.0001$, Fig. 1). The $\delta^{13}\text{C}$ signature of *F. candida* exposed to maize and ryegrass litter combined ($-19.28 \pm 3.12\text{‰}$) or maize plants without ryegrass ($-18.29 \pm 3.41\text{‰}$) did not differ and were between those for maize plant tissue and ryegrass litter $\delta^{13}\text{C}$ signatures. In treatments without maize, $\delta^{13}\text{C}$ did not differ significantly from the control treatment; the signature for ryegrass litter in the soil was $-28.88 \pm 0.02\text{‰}$ and for soil alone was $-27.17 \pm 0.14\text{‰}$. Applying the $\delta^{13}\text{C}$ signature of *F. candida* exposed to maize plants and ryegrass litter (δR_m) and that for maize plants alone (δR_x) and litter treatment alone (δR_y) to the two source mixing model indicated that $59.7 \pm 19.4\%$ of this species C was derived from live maize roots.

The $\delta^{15}\text{N}$ signatures for *F. candida* were lowest for the control ($9.98 \pm 3.04\text{‰}$) and maize plants only ($12.91 \pm 2.41\text{‰}$) treatments and these were not significantly different from each other. However, addition of ^{15}N enriched ryegrass litter significantly ($F_{3,39} =$

91.04, $P < 0.0001$) increased the $\delta^{15}\text{N}$ of *F. candida* for the treatment with maize plants and without maize plants (Fig 2). However, these latter two treatments were not statistically different from each other. The amount of N derived by *F. candida* from maize roots/ryegrass litter calculated using the two source mixing model indicated that this collembola species derived its N almost exclusively from maize roots ($97.17 \pm 0.88\%$).

Discussion

It was hypothesized that *F. candida* obtains both C and N from ryegrass litter but switches to maize roots when they are present. However, in contrast to our hypothesis and compared to *P. fimata*, when given a choice, *F. candida* did not completely switch their diet from ryegrass litter to maize for both C and N but only partially for C. Although the amount of collembola C and N did not change, the fraction of C and N derived from maize and ryegrass litter varied greatly. This shows that although maize roots and ryegrass litter has varying amounts of C and N and contributed different proportions of tissue C and N, their composition remained stable in collembola, indicating consumers follow stoichiometric balance of the nutrients (Moe et al. 2005). Without maize roots, litter resources satisfied 80% of *F. candida* C requirement, but when ryegrass litter was present in combination with maize it accounted for approximately 40% of *F. candida* tissue C and collembola essentially derived the same proportion of its C from maize irrespective of the presence or absence of ryegrass litter. This indicates that *F. candida* rely on soil microbes or soil itself (Briones et al. 2009) to satisfy their C requirement at least to some extent. Nonetheless, we did not investigate this microbial feeding pathway. In contrast to the continuous availability of maize root C, the majority of ryegrass litter C and N might have been mineralized during the eight-weeks incubation period and consequently not available

for *F. candida*. The rates of C incorporation from roots found by Larsen et al. (2007) for three collembola species, *P. minuta* (54 %), *P. armata* (77 %) and *F. fimerata* (59 %) in microcosms containing wheat plants and enriched ryegrass litter is on par with the current study. The potential for diet-switch from plant roots to litter was not investigated by these authors. In contrast, *P. fimata* foraged almost 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). A thorough understanding of the distinction between diet switching (shift in isotopic ratios) and a shift to more omnivorous or mixed diet will require more research. Applying the corrected C values to our study provided a slightly higher (72 %) estimated fraction of *F. candida*'s C originating from maize roots. Both *P. fimata* and *F. candida* are euedaphic species, nevertheless, only the former species switched exclusively to maize for its nutritional requirements. The total diet-switch to maize observed with the former species suggests that the plant may have provided more of the nutritional requirements for that species than for *F. candida*. It is not clear why collembola tend to derive more C from live roots than leaf litter, perhaps this can be due to nutritional imbalances in the two food sources.

It is interesting to note that there were twice as many *F. candida* in the presence of maize plants than in ryegrass litter only treatments, suggesting that partial diet-switching did not only benefit nutrition, but also survival and/or population growth. In fact, Usher et al. (1971) suggested that amount of food supply plays an important role in regulating the population of collembola and *F. candida* population growth benefited when food is not a limiting factor. *Protaphorura fimata* density was shown to be benefited by the presence of *Trifolium repens* and/or *Lolium perenneae* plants, however the density was higher in the

presence of grasses than legumes (Endlweber and Scheu 2007). Kaneda and Kaneko (2002) and Nelson et al. (2011) also demonstrated that life history traits of *F. candida* are also dependent on the quality of the soil. Results from the present study support existing evidence of the importance of food, its quality and availability in regulating the growth and reproduction of soil organisms.

As observed by Kreuzer et al. (2004) in *T. repens*, an increase in *F. candida* numbers did not cause any significant changes in C and N uptake of roots and shoots and plant biomass. Feeding activities of collembola results in nutrient rich microsites in soil which is available to maize roots, hence their presence might cause changes in nutrient content of roots. This possibility was not investigated in the study and must be considered for future experiments. The complete maize root herbivory by *P. fimata* demonstrated by Endlweber et al (2009) might have increased fine root hair growth which could have in turn improved the N-uptake and subsequently shoot biomass.

Changes in the $\delta^{15}\text{N}$ signature from lower to higher trophic levels has been generally attributed to changes in trophic positions of the consumers. The $\delta^{15}\text{N}$ of a consumer typically exceeds that of its diet (Chahartaghi et al. 2005) by a mean trophic fractionation of $3.0 \pm 2.6 \text{ ‰}$ (DeNiro and Epstein 1978; Post 2002). The $\delta^{15}\text{N}$ signature of *F. candida* in the presence of maize plants alone was 9.9 δ units higher than the signature of maize roots, suggesting a shift of two trophic levels (Haubert et al. 2005) and implying that *F. candida* could have obtained their N from fungi, but not from feeding on plant roots directly. Feeding by collembola decreased mycorrhizal hyphae and subsequently colonization of the roots of *T. repens* (Endlweber and Scheu 2007). When maize roots

were absent, the $\delta^{15}\text{N}$ signature of *F. candida* increased by 4.7 δ units suggesting a shift of only one trophic level. Quantifying the amount of fungal (and other microbial) presence and *F. candida* feeding on this microbial biomass could have significantly improved our understanding of the feeding behaviour. Collembola dependency on maize roots can be through a mycorrhizal pathway and investigating this pathway can be beneficial.

Dietary specialization of collembola to avoid niche overlap has been reported and our study adds to the increasing evidence that they can feed on different types of food materials (Scheu and Summerling 2004). Although collembola were classified as decomposers predominately, new evidence suggests that they can feed on live plant roots. This feeding is synergistic and benefit both collembola, by providing C resources and plants by mineralizing nutrients. Unlike many other collembola species that are shown to be omnivorous in nature, *F. candida* was able to make the switch from litter residue to forage on maize root resources, when available. This opportunistic feeding by collembola (Petersen and Luxton 1982) could be an important reason behind their success in various environments and in performing their role as important drivers of the decomposition process.

Conclusion

Although soil collembola have been traditionally associated with the detrital food webs feeding mostly on decomposing matter, our results add to the increasing number of species shown to use the root-rhizosphere channel as an equally important source of C and sometimes N (Larsen et al. 2007; Endlweber et al. 2009). The partial diet-switch of *F. candida* shown in our study and the complete diet-switch of *P. fimata* (Endlweber et al.

2009) towards maize roots from ryegrass litter are just two examples of feeding strategies available to collembola. In this, we were not able to quantify the microbial pathway of C assimilation by collembola and also the nutritional imbalances between different food sources. Future studies will have to focus in depth on these aspects to improve our understanding of their feeding ecology. If these results can be confirmed for other soil biota, our understanding of the functioning of underground systems will change. This will have major implications for the study of C fluxes and functioning of decomposer systems. Different combinations of root availability and root quality could have affected the level of diet-switching observed here with *F. candida*, an aspect that has to be investigated in the future studies.

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TABLES AND FIGURES

Table 1 Maize shoot and root biomass, root C and N concentrations, and root $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope signatures when grown in soil microcosms containing *Folsomia candida*, with and without ryegrass litter. The P values for each column represent pairwise t-test comparisons of the mean ($\pm$ S.D, n=6) values.

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 $\pm$ 1.9	1.4 $\pm$ 0.6	39.7 $\pm$ 1.1	0.9 $\pm$ 0.1	-13.2 $\pm$ 0.2	1036 $\pm$ 369
<i>F. candida</i> only	4.6 $\pm$ 1.6	1.4 $\pm$ 0.5	39.9 $\pm$ 1.3	0.9 $\pm$ 0.1	-13.2 $\pm$ 0.4	12.2 $\pm$ 1.7
<i>P</i> -value	0.54	0.89	0.54	0.11	0.49	0.0001***

Table 2 Changes in tissue C and N concentration (mean $\pm$ S.D, n=12) of *Folsomia candida* in response to maize plus ryegrass litter, ryegrass litter alone, maize alone and soil alone over an eight weeks in soil microcosms

Treatment	<i>F. candida</i> C (%)	<i>F. candida</i> N (%)
Maize + ryegrass litter	46.7 $\pm$ 3.2	10.3 $\pm$ 0.8
Ryegrass litter only	55.2 $\pm$ 9.4	9.8 $\pm$ 4.4
Maize only	47.1 $\pm$ 3.4	11.0 $\pm$ 0.7
Control	44.9 $\pm$ 3.4	11.2 $\pm$ 0.9
ANOVA <i>P</i> -value	0.48	0.7

Figure 1. The $\delta^{13}\text{C}$ values of experimental substrates soil, maize, ryegrass litter (white bars) and *Folsomia candida* (F.c) from soil microcosms exposed to maize plus ryegrass litter, ryegrass litter alone, maize alone and soil alone (black bars). Values are the mean $\pm$ standard deviation (n=12). Means were compared with a Tukey's HSD test at $P < 0.05$ and bars with the same letter are not significantly different.

Figure 2. The $\delta^{15}\text{N}$ values of experimental substrates soil, maize, ryegrass litter (white bars) and *Folsomia candida* (F.c) from soil microcosms exposed to maize plus ryegrass litter, ryegrass litter alone, maize alone and soil alone (black bars). Values are the mean $\pm$ standard deviation (n=12). Means were compared with a Tukey's HSD test at $P < 0.05$ and bars with the same letter are not significantly different.