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Short communication

# Use of $\delta^{13}C$ to determine food selection in collembolan species

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We have investigated the feeding preferences of two collembolan species, *Folsomia candida* Will. and *Proisotoma minuta* Tullberg, on two different naturally-labelled diets ($C_3$ soil and $C_4$ litter) using carbon stable isotope analysis. The results showed a strong preference for litter material, by both species, with very little isotopic fractionation. Additionally, isotopic composition showed that *P. minuta* did not switch diet in the presence of *F. candida*. This study shows the feasibility of applying isotopic techniques to smaller components of the soil system.

Collembola are one of the most abundant and widespread groups of soil arthropods, yet investigation of their natural diet is problematic due to their small size and their opaque environment. Culture experiments indicate that they can consume fungal hyphae, spores, bacteria and decaying plant material (Hopkin, 1997). Collembola are known to exhibit specific grazing preferences for certain soil fungi (e.g. Thimm and Larink, 1995) although recent evidence points to preferential feeding on nematodes (Lee and Widden, 1996). These studies rely on direct observations using video cameras (Lee and Widden, 1996), comparisons of productivity (van Amelsvoort and Usher, 1989), colour-marking techniques (Thiele and Larink, 1990), as well as multiple-choice experiments (e.g. Klironomos and Kendrick, 1996), mouthpart structure (Chen et al., 1996), gut content analyses (Leonard and Anderson, 1991; Bardgett et al., 1993; Hasegawa and Takeda, 1995) and radiotracer approaches (Baylis et al., 1986).

The isotopic composition of the whole body in small animals gives an accurate estimate of the $\delta^{13}C$ value of their diet due to the short time period for dietary carbon to become incorporated in tissues (DeNiro and Epstein, 1978a). The $\delta^{13}C$ value of an animal's diet must approximate to that of the carbon assimilated, with fractionation effects occurring through assimilation (Fry et al., 1978; Martin et al., 1992a) and through losses in respiration (DeNiro and Epstein, 1978a) and excretion. This leads to the observed discrepancy between the isotopic values of the animal and the feed, resulting in a slight enrichment in $\delta^{13}C$ of the animal by about 1‰ in relation to the diet (DeNiro and Epstein, 1978a) and represents one of the major problems in using stable isotope techniques.

Studies into the dietary preferences of other animals have used C and N stable isotopes (e.g. hyrax species, DeNiro and Epstein, 1978b; grasshoppers, Fry et al., 1978; African herbivores, Tieszen et al., 1979; termites, Spain and Reddell, 1996; earthworms, Martin et al., 1992a,b; Schmidt et al., 1997). Here we describe the preferential feeding activity of *F. candida* and *P. minuta* on two different naturally-labelled diets ($C_3$ soil and $C_4$ litter) using carbon stable isotope analysis. These two collembolan species were specifically chosen because of their divergent responses to elevated $CO_2$ (CPB, 1996), suggesting that strong population interactions may occur. Coexistence of different species in the same habitat suggests resource partitioning, but very little is known of the changes in feeding habits between soil animals with similar ecological niches.

Collembolans were raised on a diet of dried baker's yeast of constant carbon isotopic composition and kept in plastic boxes containing a substrate of plaster of Paris and activated charcoal (Goto, 1960). Microcosms were established using maize litter (*Zea mays* L.; $C_4$) and '$C_3$ soil' (*sensu* Ineson et al., 1996) collected from Cartmel Fell, Cumbria, UK (Nat. Grid Ref. SD 375785). The litter and soils were air-dried at room temperature, with the soil being coarsely sieved (2 mm mesh) and the litter sorted to remove unwanted debris and petioles.

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The soil, 40 g, and the litter, 2.0 g, were placed into 80 microcosm inner containers (Anderson and Ineson, 1982) resulting in experimental units containing litter (L), soil (S) and soil plus litter (M). Each microcosm was re-hydrated by the addition of 200 ml of deionized distilled water, allowed to soak for 24 h, drained and the leachate discarded. The microcosms were incubated at room temperature for 1 week to allow microbial growth prior to inoculation with animals.

Animals were randomly selected from the cultures using a fine paintbrush and added to the microcosms as individual species (designated F for *F. candida* and P for *P. minuta*) and as species mixtures (FP), with 15 *F. candida* and 20 *P. minuta* per microcosm. This number of animals was calculated from the animal dry weights, to provide approximately the same biomass for each species. Each of the three treatments FM, PM and FPM was replicated to enable destructive sampling of four replicates of each treatment at 2, 4, 6 and 8 weeks incubation. Additionally, 8 replicates of the FL, FS, PL and PS treatments were established to permit destructive analyses in weeks 6 and 8. Replicate samples of the initial (week 0) litter, soil, yeast and animals grown on yeast were also analysed for $^{13}C$ isotope composition. The microcosms were incubated in a water-cooled cabinet at 15°C for 8 weeks with a randomised block design with one replicate of each treatment being placed on each of the cabinet's shelves.

In every destructive sampling the soil and the litter from each microcosm were placed in glass dishes and flooded with deionized distilled water, with gentle stirring. The collembolans readily floated to the surface and were easily picked off with a paintbrush and transferred to a vial containing deionized distilled water in order to remove any superficial debris.

Individuals from each separated species and from every single microcosm were bulked and frozen to −10°C for 1 h. After thawing, each animal sample was oven dried at 105°C for 1 h. Isotopic analyses were performed using an ANCA-MS system (Europa Scientific, Crewe, Cheshire, UK); this analytical system is fully described by Barrie and Prosser (1996). Beet sucrose was used as a reference material, determined to be −25.96‰, relative to PDB. Analytical precision over 10 beet sucrose replicates was better than 0.2‰.

One-way ANOVA followed by Fisher's LSD comparison of means was used to compare mean isotopic values of the animals, in single and mixed species cultures, when feeding on soil plus litter at the different sampling dates. Similarly, ANOVA was also used to compare isotopic composition of the animals in both single and mixed cultures and their feeds at week 6.

Changes in diet from the initial yeast culture ($C_3$) to $C_4$ were reflected in the $\delta^{13}C$ value of collembolans when grown in a mixture of soil and litter (M), indicating a clear preference for maize litter as the food resource base. Initially an increase in $\delta^{13}C$ value, close to that for the $C_4$ material, was observed for both species either in single and mixed species cultures, but still significantly different to the maize value, except

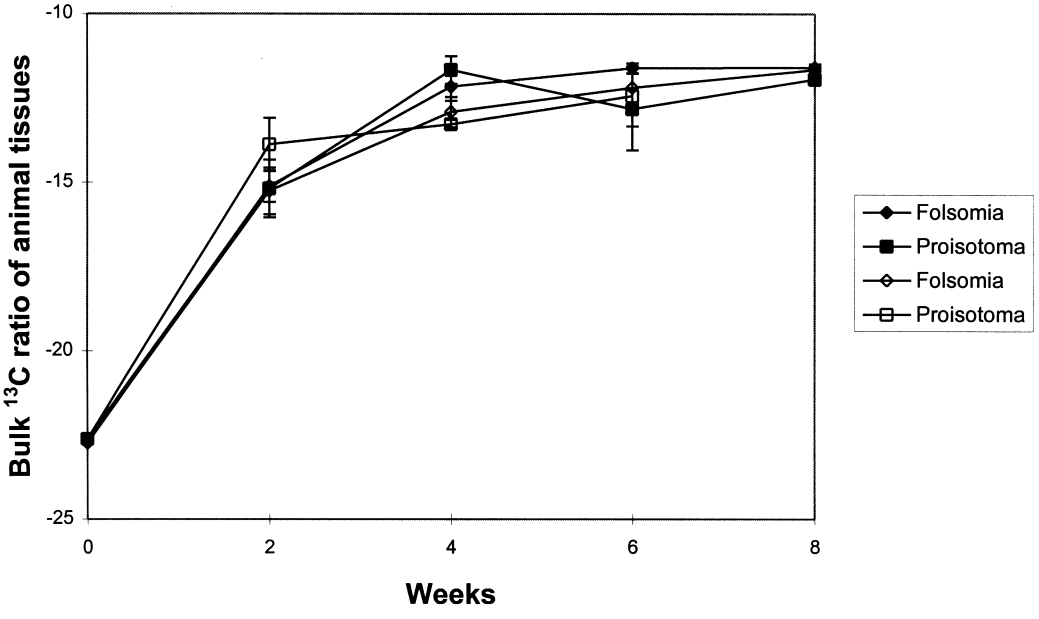



Fig. 1. Changes in the $\delta^{13}C$ values of the two collembolan species in single (solid symbols) and mixed species cultures (open symbols) growing in soil plus litter (M) during the experimental period (8 weeks). Values are means with standard errors, $n = 4$.

Table 1
Results from the analysis of variance (ANOVA) followed by Fisher's LSD test comparison of means for $\delta^{13}C$ values of *Folsomia candida* and *Proisotoma minuta* from single species cultures (F, P) and mixed species cultures (FP) when growing in units containing soil plus litter (M) at the different sampling dates (2, 4, 6 and 8 weeks)

| Treatments | 2 | 4 | 6 | 8 |
|---|---|---|---|---|
| FM | a | ab | a | a |
| PM | a | a | a | a |
| *Folsomia*–FPM | a | ab | a | a |
| *Proisotoma*–FPM | a | b | a | ND |

Significant differences between treatments are expressed as different letters, $P < 0.05$.ND = no determination.

for *Proisotoma* in mixed cultures. Once the equilibrium of $\delta^{13}C$ values (week 4) was attained this response remained so for the entire experimental period making it a good indicator of dietary changes (Fig. 1). The population size of *Proisotoma* in mixed cultures (FPM) decreased abruptly through the experiment and on week 8 there was insufficient material for isotopic measurements. Significant differences between treatments are shown in Table 1.

Fig. 2 summarises the isotopic composition of the animals, in single and mixed cultures and the resource type, at week 6. *Folsomia* growing on yeast had a $\delta^{13}C$ signal ca. 1.5‰ higher than the yeast on which it was feeding ($P < 0.05$), with *Proisotoma* being 1.7‰ higher ($P < 0.05$). When the food source was soil (S), *Folsomia* was about 4‰ higher than the bulk soil signal ($P < 0.05$), yet was only 1.2‰ higher ($P > 0.05$) when this species was feeding in maize (L). For *Proisotoma*, not enough material was obtained when feeding in soil (S) but in maize litter (L) there was a difference of 2.5‰ higher than the substrate ($P < 0.05$). There was also a significant difference ($P < 0.05$) in the $\delta^{13}C$ between the two species in single species cultures on maize litter, with *Proisotoma* having the more positive signal. No significant differences in relation to the maize value were detected for mixed cultures and single species cultures of *Proisotoma* when feeding in soil plus litter (M), however the isotopic composition of *Folsomia* from the mixed cultures and single species cultures was still significantly different to the litter value.

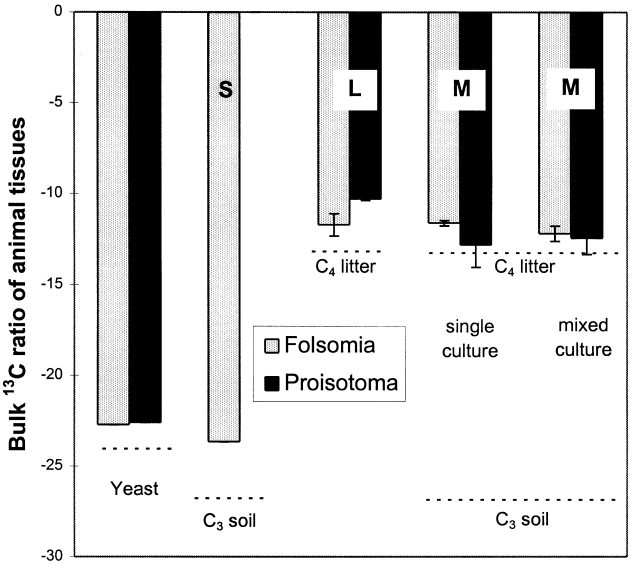



Fig. 2. $\delta^{13}C$ composition of the animals (*Folsomia* and *Proisotoma*) feeding on yeast, soil (S), maize (L) and in soil plus litter (M) and their feeds (yeast, $C_3$ soil and $C_4$ litter) at week 6 of the experiment. Values are means with standard errors, $n = 4$.

In summary, we have shown that the two species show a clear preference for the (new) organic matter derived from the maize litter (or from the microorganisms growing on it) in both single and mixed species cultures and that the response was convergent with time (Anderson, 1978). Additionally, it is possible to conclude that there was no evidence of resource partitioning since the presence of both species in the mixed culture did not result in any significant shift to (old) $C_3$ soil derived material by either species.

The two collembolan species employed here are known to show preferences for different species of fungi, as a result of different protein content (Usher et al., 1982) or avoidance of inhibitory substances in some hyphae (Visser and Whittaker, 1977). Many soil processes are affected by the activities of soil fauna and the grazing preferences shown by collembolans can play an important role during the early stages of decomposition when there is fairly rapid succession of different fungi species (Hopkin, 1997). Alterations in collembolan community structure may therefore have implications for nutrient cycling. The analysis of tissue carbon isotope composition is considerably less time consuming that traditional observational and gut content analyses. This study shows, for the first time, evidence for the feasibility of using stable isotope techniques to investigate the feeding ecology of small soil animals, such as collembola.

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