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Nutrient allocations and metabolism in two collembolans with contrasting reproduction and growth strategies

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Summary

1. Physiological mechanisms such as allocation and release of nutrients are keys to understanding an animal's adaptation to a particular habitat. This study investigated how two detrivores with contrasting life-history traits allocated carbon (C) and nitrogen (N) to growth, reproduction and metabolism. As model organisms we used the collembolans, *Proisotoma minuta* (Tullberg 1871) and *Protaphorura fimata* (Gisin 1952).

2. To estimate allocations of C and N in tissue, we changed the isotopic composition of the animal's yeast diets when they became sexually mature and followed isotope turnover in tissue, growth and reproduction for 28 days. In addition, we measured the composition of C, N and phosphorus (P) to gain complementary information on the stoichiometry underlying life-history traits and nutrient allocation.

3. For *P. minuta*, the smallest and most fecund of the two species, the tissue turnover of C and N were 13% and 11% day^{-1}, respectively. For *P. fimata*, the equivalent rates were 5% and 4% d^{-1}, respectively. *Protaphorura fimata* had the lowest metabolic rate relative to total body mass but the highest metabolic rates relative to reproductive investment. Adult *P. fimata* retained approximately 17% of the nutrient reserves acquired while a juvenile and adult *P. minuta* about 11%. N and P contents of total tissue were significantly higher in *P. minuta* than in *P. fimata*, suggesting that tissue turnover was correlated with high protein-N and RNA-P.

4. Our results suggest that the lower metabolism and nutritional requirements by *P. fimata* than *P. minuta* is an adaptation to the generally low availability and quality of food in its natural habitat.

5. The methodological approach we implemented tracking mass balance, isotope turnover and elemental composition is promising for linking nutrient budgets and life-history traits in small invertebrates such as Collembola.

Key-words: Collembola, carbon, ecological stoichiometry, invertebrate, metabolism, nitrogen, stable isotope, tissue turnover

Introduction

Physiological mechanisms such as allocation and release of nutrients are keys to understanding an animal's adaptation to a particular habitat. Detrivores are, in spite of their enormous distribution and vast importance for decomposition and cycling of nutrients, among the least known group of invertebrates in terms of linking their nutrient budgets and life-history strategies (Chown & Nicolson 2004; Bardgett *et al.* 2005). Linking these physiological parameters is important for understanding how detrivores function in an environment that is considered extremely nutrient limited and very patchy in terms of food resources.

Collembola are among the most abundant of all soil-dwelling arthropods. They are considered to feed mainly on decaying vegetation and soil fungi although recent findings suggest

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that root exudates are an important food source as well (Pollierer *et al.* 2007; Larsen *et al.* 2007a). Collembola belong to a very heterogenous group with a wide range of life-history traits. These particular traits affect the nutritional requirements of the animals (Jørgensen, Hedlund & Axelsen 2008). Martinson *et al.* (2008) speculated that soil detrivores adapted to higher quality food resources might have higher nitrogen (N) and phosphorus (P) content than those adapted to lower quality food resources. Similarly, Collembola adapted to living in nutrient-poor habitats often have a lower fecundity than those living in nutrient rich habitats (Larsen *et al.* 2008). For animals living in a nutrient poor habitat one might also expect adaptations to overcome periods of food shortages, such as relying on nutrient reserves. However, the physiological mechanisms behind dietary requirements and resource partitioning to reproduction, nutrient reserves and other pools are not well understood because no studies have implemented a methodological approach that could link these parameters.

Only a limited number of studies have estimated nutrient allocation in invertebrates. O'Brien, Schrag & del Rio (2000) successfully documented resource allocation patterns in Lepidoptera by isotopically labelling diet and subsequently keeping track of isotope change in tissue. This isotope change approach was based on studies that emerged almost two decades earlier (Fry & Arnold 1982; Tieszen *et al.* 1983), but to our knowledge no studies have conducted a complete invertebrate nutrient budget encompassing growth, reproduction and metabolic turnover. Changing the isotope composition of an animal's diet provides a marker for tracking the rates of growth and tissue turnover. In fecund invertebrates the nutrient pools are allocated to reproduction, metabolism and moulting.

The aim of our study was to investigate how different physiological traits in Collembola relate to nutrient allocations during growth, reproduction and metabolism. To address this question, we estimated C and N allocations in two collembolans with different physiological traits, *Proisotoma minuta* (Tullberg 1871) and *Protaphorura fimata* (Gisin 1952). *Proisotoma minuta* lives in the soil–litter interface (hemiepedaphic) and is pigmented (greyish or bluish) with fully developed compound eyes and furca (Fjellberg 2007) (Fig. 1). *Protaphorura fimata* lives below the litter-surface layer (euedaphic) and has adapted traits typical for its habitat: it lacks pigmentation (white) and has reduced compound eyes and furcas (salutatory organ) (Fjellberg 1998) (Fig. 1). *Proisotoma minuta* is small (1·1 mm in length) and has a faster reproductive cycle than the larger *P. fimata* (2·2 mm in length) (Larsen *et al.* 2007b). While *P. fimata* predominantly lives in forest soils, *P. minuta* is a cosmopolitan species that occasionally can be found in very large number in habitats with nutritious organic matter (Wiggins & Curl 1979; Hågvar & Kjøndal 1981; Fjellberg 1998, 2007).

To ensure that the diet quality would not effect nutrient allocations adversely (Frost *et al.* 2005), we fed the animals a high quality diet, dried baker's yeast (*Saccharomyces cerevisiae*), which is considered to balance the nutritional requirements of the animals (Haubert *et al.* 2005; Larsen *et al.* 2008). We

Fig. 1. The two Collembola species in our study, *Proisotoma minuta* (greyish, < 1·1 mm in length) and *Protaphorura fimata* (white, < 2·2 mm in length).

changed the composition of the stable isotopes, ^{13}C and ^{15}N, in diet when the animals entered sexual maturity to estimate two parameters: (i) how much of egg C is derived from juvenile vs. adult diets (O'Brien *et al.* 2000); and (ii) how much does egg manufacturing contribute to turnover of ^{13}C and ^{15}N in tissue. In addition, we investigated the composition of C, N and P in the animals relative to their diet to gain complementary information on the regulatory processes underlying life-history traits and nutrient allocation (Ventura 2006). We hypothesized that the smallest and most fecund of the two species, *P. minuta* would have a higher tissue turnover rate than *P. fimata* as these traits are likely to be metabolically expensive (West, Woodruff & Brown 2002; Gratton & Forbes 2006).

Meterials and methods

STUDY ORGANISMS AND DIETS

The stock of *P. fimata* and *P. minuta* were obtained from laboratory cultures that lived for many generations on commercial freeze-dried bakers yeast (*S. cerevisiae*, De Danske Spritfabrikker A/S). *Protaphorura fimata* is in this study identified in the narrow sense (*s.s.*) but was in a previous study (Larsen *et al.* 2007a) identified under the species complex name *P. armata* (Tullberg 1869) in the less strict sense (*s.l.*). The mode of reproduction of *P. fimata* and *P. minuta* is not well known, but assumed to be sexual as males are found in natural populations. The prevalent mode of reproduction of our laboratory cultures is also believed to be sexual. In laboratory cultures *P. fimata* eats moulted exuvia (skin), whereas *P. minuta* does not eat its own exuvia.

The control treatment fed the commercial freeze-dried bakers yeast had the following elemental composition: C, 42·3 ± 0·1; N, 6·7 ± 0·0; and P, 0·88 ± 0·02% (average dry mass ± SE, *n* = 3). The diet change treatment was fed ^{13}C and ^{15}N labelled yeast and this had been grown in an aqueous medium at 28 °C over 2 days. Homogenous labelling was obtained by growing *S. cerevisiae* in an amino acid free medium. The medium was enriched with ^{13}C and ^{15}N to approximately twice the natural abundance to ensure that the isotopic values of the two diets were distinct thus diminishing errors associated with isotope fractionation. The medium contained:

45·2 mg L^{-1} D-Glucose 99% U-^{13}C6 (Cambridge Isotope Laboratories), 4·0 g L^{-1} D(+)-glucose (Sigma), 0·99 mg L^{-1} 99 atom% ^{15}N-$(NH_4)_2SO_4$ (Cambridge Isotope Laboratories), 264·0 mg L^{-1} $(NH_4)_2SO_4$ (Sigma), 3·4 g L^{-1} 'Yeast Nitrogen Base without amino acids and $(NH_4)_2SO_4$' (Fluka). Yeast was extracted from the medium by centrifugation, then freeze dried and homogenized by grinding with mortar and pestle. The elemental composition of labelled yeast was: C, 39·0 ± 0·1; N, 7·0 ± 0·1; and P, 2·9 ± 0·1% ($n = 3$). A preliminary growth experiment showed that the mass of the collembolans was not significantly different between the unlabelled and labelled diet treatments ($n = 3$, $P < 0·05$, *P. fimata* approximately 28 days old and *P. minuta* approximately 35 days old).

EXPERIMENTAL DESIGN AND SAMPLING

Animals were incubated at 20 °C in Petri dishes with plaster of Paris $(CaSO_4)$ substrates and were fed twice a week *ad libitum*. Each generation of animals was hatched from eggs within 3 days, resulting in an age varying between 0 and 3 days. Each replicate was initiated by transferring 40–60 eggs to new substrates. To differentiate between juvenile and adult nutrient pools, diet was changed from unlabelled to labelled diet when the animals entered sexual maturity in the 'Diet change Parent' (DP) treatment (Fig. 2). To have reference isotope values of animals in equilibrium with their diets, we had a control treatment called 'Control Parent' (CP, $n = 4$ for each of the two sampling occasions) where animals were fed the non-labelled diet during their entire life cycle. The labelled diet treatment, called 'Labelled Gen. 1' (LG, $n = 4$ for the only sampling occasion), were hatched from eggs that were laid by animals fed labelled diet (Labelled Parent – LP, $n = 4$) (Fig. 2). In the DP treatment, hatchlings ($n = 4$ for each of the three sampling occasions) were raised on unlabelled control yeast until sexual maturity (*P. minuta* 21–23 days, *P. fimata* 28–30 days). All animals sampled after sexual maturity were transferred to new substrates without mixing animals between replicates. The substrates were subsequently replaced with new substrates once a week until sampling to avoid inhibitory effects of info-chemicals on fecundity (Verhoef 1984). Animals from the CP

treatment were collected for analysis at sexual maturity and 28 days after sexual maturity and eggs after 7, 14 and 28 days (Fig. 2). In the DP treatment, animals and eggs were collected 7, 14 and 28 days after sexual maturity. The analysis included counting the number of animals, determining batch fresh (FM) and dry mass (DM) and carrying out elemental (C, N and P) and isotopic (^{13}C and ^{15}N) analyses. To reduce stress on the animals due to handling, the FM of each replicate was determined only twice; at the designated sampling day and at the sampling day proceeding it. Eggs were dried and weighed before elemental and isotopic analysis. To obtain sufficient exuvial biomass for elemental and isotopic analysis, exuvia collected from *P. minuta* was pooled between maturity and termination for each treatment. DW of all treatments was determined after drying at 50 °C for 24 h in pre-weighed tin capsules.

Collembolans follow a sigmoid growth model where juvenile growth can be described according to an intrinsic exponential growth model and adult growth to an asymptotic exponential model (Folker-Hansen, Krogh & Holmstrup 1996). Therefore we used an asymptotic exponential model to characterize adult growth:

$$W(t) = W_n - W_d \times e^{-k_a t} \qquad \text{eqn 1}$$

where t is time after reaching maturity, k_a is the asymptotic growth rate, $W(t)$ is the body mass at sampling, W_n is the asymptotic mass and W_d is the difference between W_n and $W(t_0)$ (mass at sexual maturity). The three parameters W_n, W_d and k_a were estimated by nonlinear least squares minimization. Fecundity was calculated as number of eggs laid per individual per day (eggs ind^{-1} d^{-1}), and reproductive investment was expressed as the dry mass of the reproductive output per day relative to the dry mass of the parents (% d^{-1}).

CHEMICAL ANALYSES

Each dried sample of animals was divided into two subsamples of at least 300 µg and 20–30 animals. A Sartorius MC210 microbalance was used for weighing. Elemental P analysis was carried out in pre-weighed Teflon capsules and determined by acid-persulphate
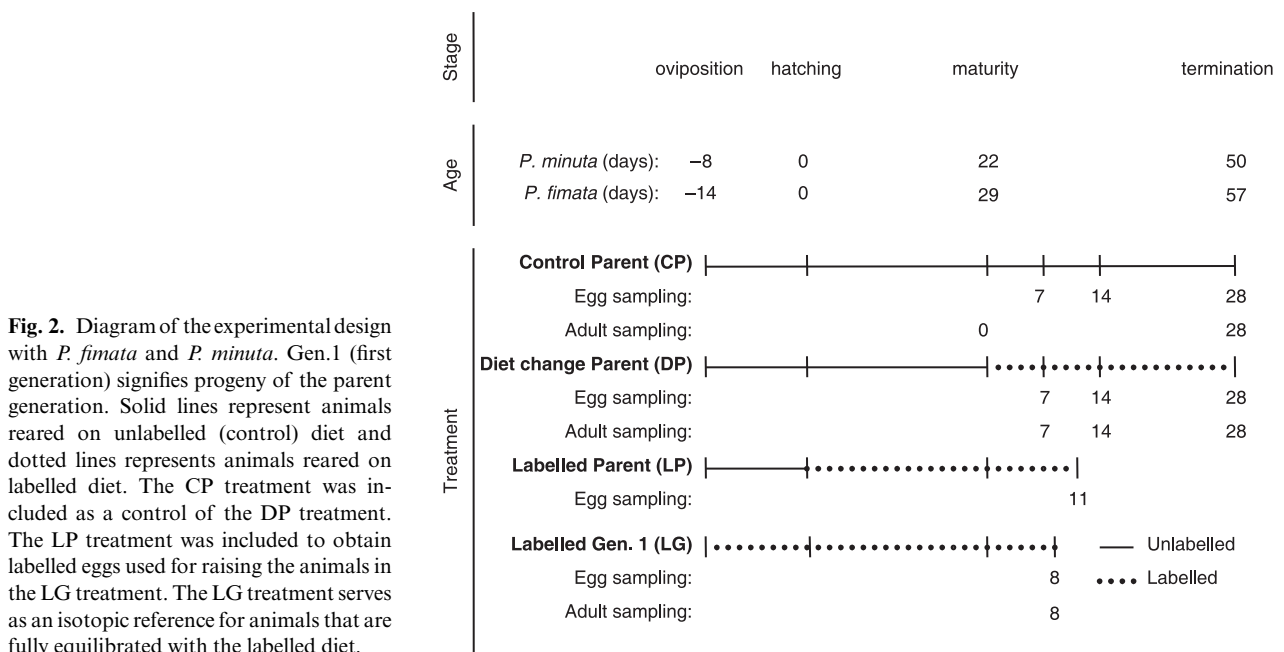
Fig. 2. Diagram of the experimental design with *P. fimata* and *P. minuta*. Gen.1 (first generation) signifies progeny of the parent generation. Solid lines represent animals reared on unlabelled (control) diet and dotted lines represents animals reared on labelled diet. The CP treatment was included as a control of the DP treatment. The LP treatment was included to obtain labelled eggs used for raising the animals in the LG treatment. The LG treatment serves as an isotopic reference for animals that are fully equilibrated with the labelled diet.

digestion followed by phosphate analysis using the ammonium molibdate method (Grasshoff *et al.* 1983). ^{13}C and ^{15}N isotope ratios and concentrations were determined at the UC Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The working standard for N was purified $(NH_4)_2SO_4$ with a $\delta^{15}N$ value of +1·33‰, calibrated against IAEA N1 and IAEA N2. The working standard for C was beet (*Beta vulgaris* L.) sucrose with a $\delta^{13}C$ value of –23·83‰, calibrated against NIST SRM 8539 and NIST SRM 8542 standards. The isotopic ratios are reported with units of per mil (‰) difference according to the equations in Appendix S1 in Supporting Information.

CARBON (C) AND NITROGEN (N) TURNOVER

To assess turnover of collembolan ^{13}C and ^{15}N, animals were switched from unlabelled to labelled diet when entering sexual maturity. Subsequently, isotopic ratios in total body mass and eggs were tracked for 28 days. Isotopic turnover followed an asymptotic exponential model akin to that used in previous diet change studies (e.g. Tieszen *et al.* 1983; Hesslein, Hallard & Ramlal 1993):

$$\delta_a^t = \delta_a^n - \delta_a^d \times e^{-\lambda t} \qquad \text{eqn 2}$$

where δ^t is the isotopic ratio of animals at the time *t*, subscript *a* refers to adult DP animals, δ^n is the asymptotic isotope ratio of the curve, δ^d is the difference between δ_a^n and the intercept value at $t = 0$. λ is the turnover rate per day, which also can be presented as the half-life: $t_{1/2} = \ln(2)/\lambda$. The turnover rate is a first order constant and applies to the mixing fraction (β_a) (defined as the fraction of tissue that changes isotopically after diet switch). β_a is obtained from:

$$\beta_a = \frac{\delta_a^n - \delta_{CP}^e}{\delta_{LG}^e - \delta_{CP}^e} \qquad \text{eqn 3}$$

where δ_{CP}^e is the is the isotope ratio of animals in equilibrium with the unlabelled diet (CP) and δ_{LG}^e is the isotope ratio of animals in equilibrium with the labelled diet (LG). The remaining fraction, the non-mixing fraction $(1 - \beta_a)$, is built during juvenile growth and not replaced after sexual maturity (Fig. 3). To find the isotope change rate relative to the total body mass (Λ_a), we multiplied the turnover rate (λ) by the mixing fraction (β_a). While the term 'change rate' encompasses the contribution of growth, metabolism and reproduction to isotopic change, 'turnover' encompasses the contribution of metabolism and reproduction only.

The processes contributing to isotopic change in adult Collembola are growth and tissue turnover (Fig. 3). We calculated isotope change due to growth (δ_g^t) as:

$$\delta_g^t = \frac{W(t) - W(t_0)}{W(t)(\delta_{LG}^e - \delta_{CP}^e)} \qquad \text{eqn 4}$$

Collembolan growth follows an asymptotic exponential model (Eqn 1). For this reason, isotope change due to growth (δ_g^t) also follows an asymptotic exponential model. Because both δ_g^t and δ_a^t (isotope ratio of total body mass) follow asymptotic exponential curves, the rate of tissue turnover (δ_r^t) is found by subtracting δ_g^t from δ_a^t. The mixing fraction values for δ_g^t and δ_r^t were found by substituting δ_a^n in Eqn 2 with either δ_g^n or δ_r^n. To find the change rates due to growth (Λ_g) and tissue turnover (Λ_r) we multiplied the fraction change rates of δ_g^t and δ_r^t by their respective mixing fraction values.

NUTRIENT ALLOCATIONS TO EGGS

Previous studies have shown that invertebrates allocate nutrients for egg production from two sources: directly from the diet and indirectly through tissue reserves (O'Brien *et al.* 2000). To investigate whether nutrients used for egg production in Collembola were supplied directly from the diet or indirectly through body reserves (the mixing fraction) we used a simple two-compartment model of nutrient flow that took into account the time it takes to produce an egg. Like a growing animal, the production of an egg follows a particular growth pattern. However, as no data exist in the literature on the growth patterns of eggs we assumed the simplest possible model, which is linear growth. The dietary isotope values are expressed as a function of time, $f(t)$, where one diet source represents before change and the other after change:

$$f(t) = \begin{cases} \delta^c + \varepsilon & t < 0 \\ \delta^l + \varepsilon & t \geq 0 \end{cases} \qquad \text{eqn 5}$$

where δ^c is the isotope value of the control diet (before diet change), δ^l is value of the labelled diet, *t* is time of diet change and ε is the isotopic fractionation associated with manufacturing eggs, that is, the isotopic difference between adults and their eggs. The next function $v(t)$ expresses the isotope values of whole body as a function of time:

$$v(t) = \begin{cases} \delta^a + \varepsilon & t < 0 \\ \delta^n - \delta^d \times e^{-\lambda t} + \varepsilon & t \geq 0 \end{cases} \qquad \text{eqn 6}$$

where δ^a is the isotope value of juveniles (before diet change), and δ^n, δ^d and λ are the parameters from Eqn 2 describing isotopic change

□ The non-mixing fraction $(1 - \beta)$ ▨ The mixing fraction (β)   ⬚ Tissue fraction
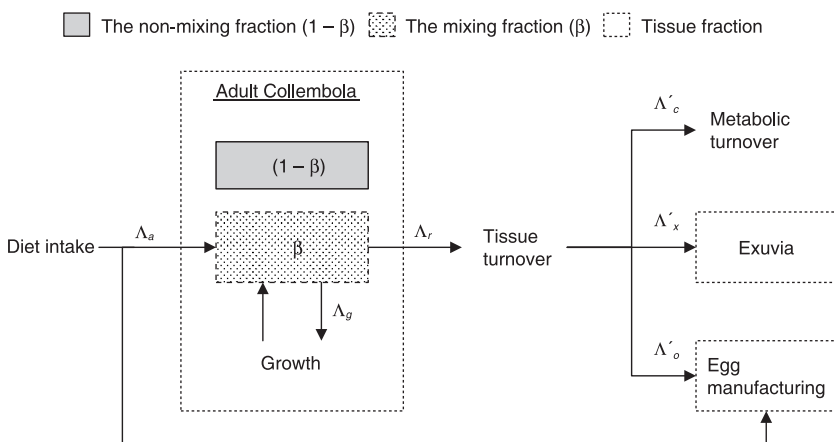

Fig. 3. The total rate of isotopic incorporation (Λ_a) into collembolan tissue depends on the rates of growth (Λ_g) and tissue turnover (Λ_r). In this model we make an operational distinction between the mixing and a non-mixing fraction in an adult Collembola. The mixing fraction (β) is somatic tissue that is renewed through diet intake (Λ_a) and replaced through tissue turnover (Λ_r). The fractions responsible for tissue turnover are egg manufacturing (Λ_o'), metabolic turnover (Λ_c') and moulting of exuvia (Λ_x'). The non-mixing fraction $(1 - \beta)$ is incorporated during the juvenile stage before sexual maturity and not replaced in adults.

of adults. The next function $h(x)$ describes the fraction that is directly allocated from the diet (γ) and the remaining fraction provisioned from the tissue $(1 - \gamma)$:

$$h(x) = \gamma \times f(t) + (1 - \gamma) * v(t) \qquad \text{eqn 7}$$

Finally, the last function $g(t)$, estimates T, the time it takes to develop or grow an egg from initiation (init) to oviposition (init + T):

$$g(t) = \int_{\text{init}}^{\text{init}+T} \frac{dV_{\text{egg}}}{dt} h(t) dt \qquad \text{eqn 8}$$

The residual variance is assumed to be normally distributed and is estimated by the sum of squares of the residuals divided by the sample size (Seber & Wild 1989). The joint Bayesian posterior distribution of the parameters in the model was sampled using the Metropolis–Hastings algorithm with a multinomial candidate distribution (100 000 iterations with a burn-in period of 1000), assuming uniform prior distributions of the parameters with the constraints that γ should be between 0 and 1, and T between 0 and 10 (Carlin & Louis 1998). The sampling procedure is checked by visual inspections of the sampling chains.

TISSUE TURNOVER FRACTIONS

Beside egg production, the fractions responsible for tissue turnover are metabolic turnover and shedding of exuvia (Fig. 3). The contribution of exuvia (Λ'_x) to tissue turnover can readily be found by multiplying the rate of shedded exuvia by its elemental content. To calculate the contribution of egg production (Λ'_o) to tissue turnover, the proportion allocated directly from the mixing fraction to eggs, $(1 - \gamma)$ was multiplied by reproductive investment (% d^{-1}). Metabolic tissue turnover (Λ'_c) is the fraction of tissue turnover that remains after subtracting egg production (Λ'_o) and exuvia (Λ'_x) from tissue turnover (Λ_r) (Fig. 3).

STATISTICAL ANALYSES

All statistical analyses and modelling were performed with R, version 2·7·1 (R Development Core Team 2008). All treatments were tested for variance homogeneity before applying ANOVA or Student's t-test. Nonlinear functions were fitted by nonlinear least squares minimization and were compared to one another with the significance test described by Motulsky and Ransnas (1987). To test lack-of-fit, we compared the nonlinear functions with general ANOVA models using a likelihood ratio test. Prior to comparing the curves for δ^{13}C and δ^{15}N we normalized their values by accounting for their different isotope equilibrium values. The Satterthwaite approximation was used to derive standard errors of pooled samples. Deviations are given as standard errors.

Results

LIFE HISTORIES

After sexual maturity, the growth of *P. minuta* and *P. fimata* followed an exponential asymptotic growth curve (Fig. 4). The two growth curves of the two species in the DP treatment were, after normalization of the initial mass, tested to be significantly higher for *P. fimata* than *P. minuta* ($F_{3,29} = 6\cdot6$, $P =$
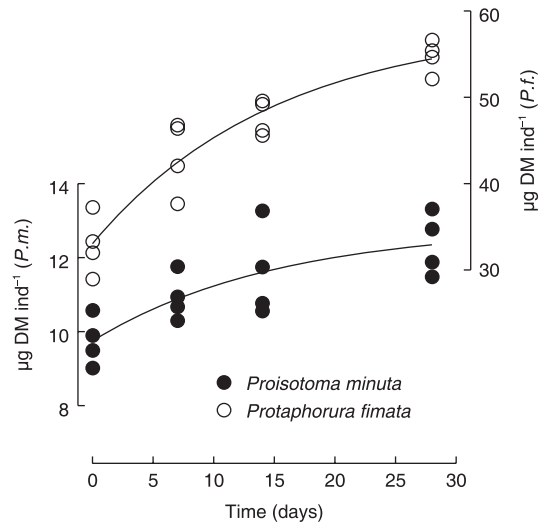

Fig. 4. Individual mass (W) of *P. minuta* (*P.m.*, left axis) and *P. fimata* (*P.f.*, right axis) after sexual maturity in the diet change (DP) treatment ($n = 4$) fitted with an exponential asymptotic growth curve ($n = 16$): *P. minuta*; $W(t) = 12\cdot8 - 3\cdot1e^{0\cdot0655}$, *Pr. fimata*; $W(t) = 58\cdot5 - 25\cdot4e^{0\cdot0660}$.

0·0016). Body growth for an average adult was 0·096 μg day^{-1} for *P. minuta* and 0·58 μg day^{-1} for *P. fimata*. For total tissue production, which encompasses egg manufacturing, growth and shedded exuvia, was 0·69 μg day^{-1} for *P. minuta* and 1·0 μg day^{-1} for *P. fimata*. These values demonstrate that *P. fimata* allocated dietary resources equally between growth and reproduction, while *P. minuta* allocated more resources to reproduction than growth. The different strategies on reproduction can also be seen from the significantly higher fecundity and reproductive investment of *P. minuta* than *P. fimata* (Table 1, $P < 0\cdot05$). The reproductive investment for *P. minuta* was 5·8% day^{-1}, which is five times higher than for *P. fimata*. Fecundity was 1·2 eggs ind^{-1} day^{-1} for *P. minuta*, and 0·39 eggs ind^{-1} d^{-1} for *P. fimata* (Table 1). The growth and fecundity parameters of the control and diet change treatments were not significantly different for each species (Table 1, $P > 0\cdot05$).

ELEMENTAL CONTENT AND IMBALANCES

The elemental compositions of labelled *P. minuta* and *P. fimata* adults and eggs (Fig. 5) were significantly different (ANOVA, $P < 0\cdot05$). The differences in elemental composition between the two species were more pronounced for eggs than adults. *Proisotoma minuta* adults and eggs contained significantly less C but more N and P than *P. fimata* (ANOVA, $P < 0\cdot05$). C : N and C : P ratios (by atoms) for adults and eggs were thus significantly lower for *P. minuta* than *P. armata* (ANOVA, $P < 0\cdot05$). The N : P ratios were similar for adults but significantly lower for *P. minuta* than *P. fimata* eggs (ANOVA, $P < 0\cdot05$). The labelled diet was balanced to the requirements of the collembolans except for a negative elemental imbalance

Table 1. Life-history parameters for the Diet change Parent (DP) and Control Parent (CP) (means ± SD, $n = 4$). Different letters denote significant differences and apply to rows (ANOVA, $P < 0.05$)

		Proisotoma minuta		*Protaphorura fimata*	
		DP	CP	DP	CP
$W_{hatchlings}$	µg DM ind⁻¹	0·55	0·55	1·40	1·40
$W_{maturity}$		9·7 ± 0·7[a]	10·1 ± 0·2[a]	32·8 ± 3·4[b]	35·2 ± 1·4[b]
W_{final}		12·4 ± 0·8[a]	12·6 ± 0·3[a]	54·7 ± 1·9[b]	54·1 ± 1·8[b]
Fecundity	Eggs ind⁻¹ d⁻¹	1·16 ± 0·05[a]	1·50 ± 0·34[a]	0·386 ± 0·064[b]	0·446 ± 0·035[b]
Reproduction	% d⁻¹	5·68 ± 0·11[a]	7·34 ± 0·84[a]	1·21 ± 0·10[b]	1·40 ± 0·06[b]
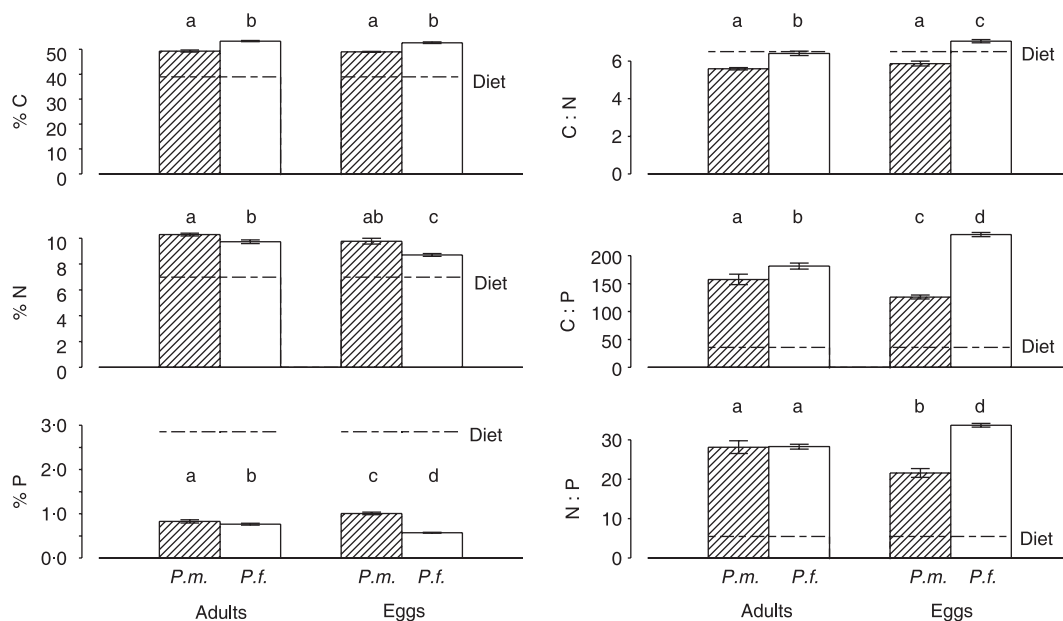
Fig. 5. Elemental composition and ratios (by atoms) from the diet change treatment with *P. minuta* (*P.m.*) and *P. fimata* (*P.f.*), their eggs and diet ($n = 4$, error bars display standard errors). Horizontal broken line is the elemental content of the labelled yeast diet. Different letters signify significant differences (ANOVA, $P < 0.05$).

of C : N relative to adult *P. minuta* and *P. fimata* ($P < 0.05$). Exuvia collected from *P. minuta* contained 7·4% C; 1·3% N; and 0·032% P (C : N : P = 597 : 90 : 1). The elemental compositions of C, N and P of the adult animals was not significantly different between the diet switch and control treatments (ANOVA, $P > 0.05$).

CARBON (C) AND NITROGEN (N) TURNOVER

The isotope ratios of the animals and their diets can be found in Table S1 in Supporting Information. Isotopic change rates were higher for *P. minuta* than *P. fimata*. The asymptotic exponential curves for isotope turnover differed significantly between the two species (Fig. 6A, $\delta^{13}C$: $F_{3,26} = 36$, $P < 0.0001$; Fig. 6B, $\delta^{15}N$: $F_{3,26} = 68$, $P < 0.0001$), with half-lives ranging between 4 and 5 days for *P. minuta*, and between 6 and 7 days for *P. fimata* (Table 2). The curve fits for $\delta^{13}C$ and $\delta^{15}N$ for were similar for both species (*P. minuta*: $F_{3,26} = 0.39$, $P = 0.76$; *P. fimata*: $F_{3,26} = 0.49$, $P = 0.69$). The mixing fraction (β_a) was

89% for *P. minuta* and significantly larger than the 82% for *P. fimata* ($P < 0.001$, Table 2). Hence, *Pr. fimata* utilizes compared to *P. minuta* a larger nutrient pool built during the juvenile stage that is not replaced after sexual maturity as indicated by the larger non-mixing fraction $(1 - \beta_a)$. Finally, we estimated Λ_a, the isotopic change rate relative to the entire body: Λ_a rates were 14·3% C d⁻¹ and 12·8% N d⁻¹ for *P. minuta*, and 9·4% C d⁻¹ and 8·7% N d⁻¹ for *P. fimata* (Table 2).

After estimating mixing fractions and tissue change rates for the entire body, we modelled the contribution of growth and tissue turnover to isotopic change in the animals. The mixing fraction values for growth (β_g) for *P. minuta* were 22% and 23%, and for *P. fimata* 40% and 41% (Table 2), showing that *P. fimata* invested almost twice as much in growth than *P. minuta*. *Proisotoma minuta* allocated relatively more resources to tissue turnover than *P. fimata* with β_r approximately 68% for *P. minuta* and 41% for *P. fimata* for both C and N (Table 2). The change rates for growth (Λ_g) differed between 1·7% and 1·9% d⁻¹, and 4·2% and 4·8% d⁻¹ for *P. minuta* and
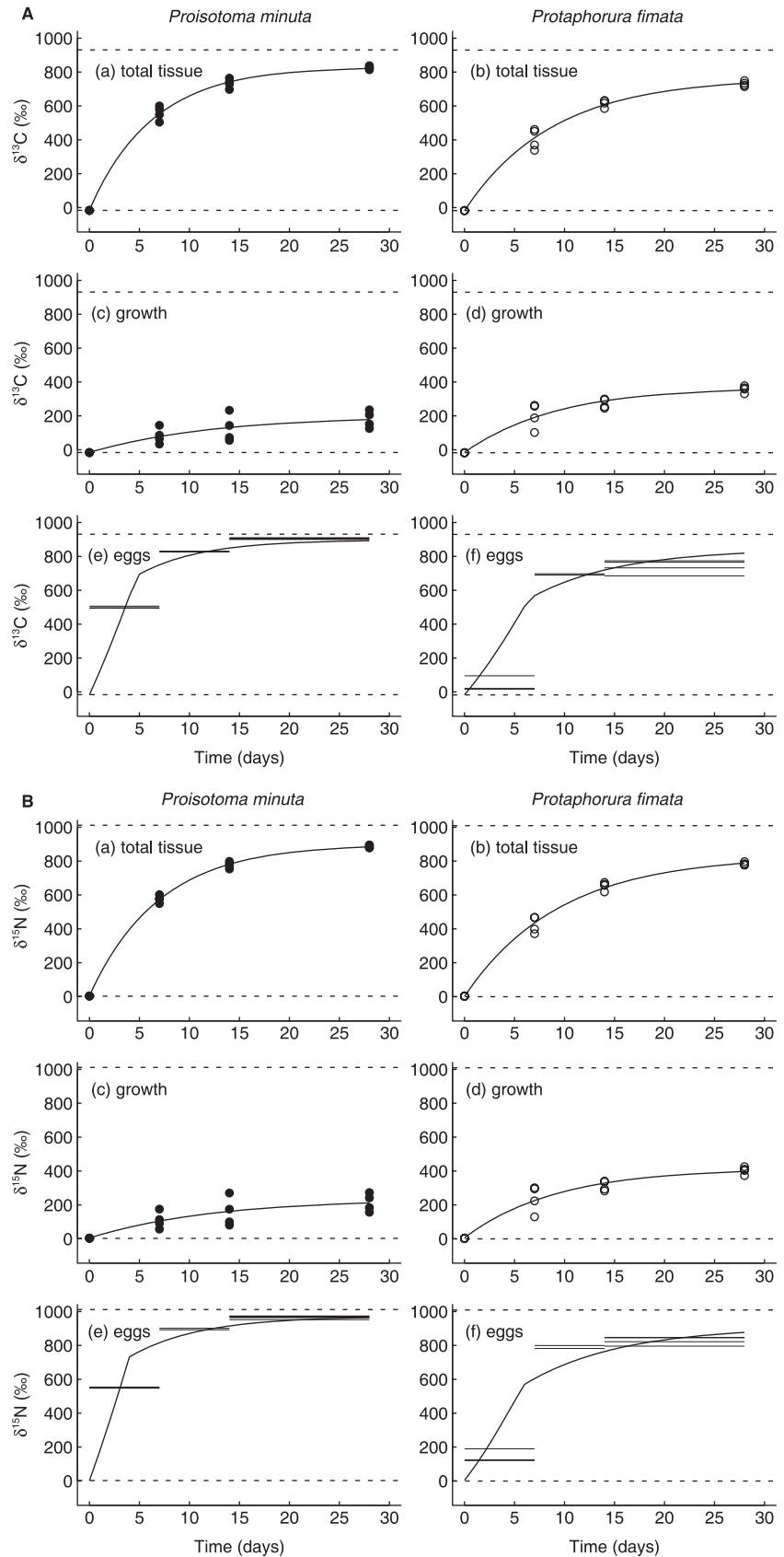
Fig. 6. Changes in δ¹³C (A) and δ¹⁵N (B) in adult *P. minuta* and *P. fimata* 0–28 days after diet change; curves were fitted by a nonlinear regression (a–d). Dashed lines represent animals in equilibrium with un-labelled (control parent) or labelled diets (labelled gen.1). Changes in isotopes due to growth were estimated from changes in biomass. For eggs, the horizontal lines (e–f) represent δ¹³C values of the oviposition periods (0–7, 7–14, 14–28 days). The curves for egg δ¹³C were fitted using Bayesian modelling (see Fig. S1).

Table 2. Model parameters for isotope turnover (means ± SD, $n = 4$). The tissue change rate (Λ) signifies isotopic change relative to total body mass. Growth (g) and Tissue turnover (r) are sub-fractions of Total (a), and Metabolism (c), Eggs (o) and Exuvia (x) are sub-fractions of Tissue turnover (r) (Fig. 2). Different superscript letters indicate significant differences ($P < 0.05$)

Species		Asymptote (δ^n)	Delta (δ^d)	Turnover (λ)	Half-life ($t_{1/2}$)	Mixing fraction (β)	Tissue change rate (Λ, % d^{-1})
Total (a)							
P. minuta	$\delta^{13}C$	832 ± 13	848 ± 18	0·160 ± 0·009[a]	4·34	0·893 ± 0·01[a]	14·3 ± 0·8
P. fimata		767 ± 23	786 ± 26	0·114 ± 0·009[b]	6·10	0·825 ± 0·03[b]	9·4 ± 0·6
P. minuta	$\delta^{15}N$	901 ± 9	896 ± 11	0·144 ± 0·004[a]	4·82	0·890 ± 0·01[a]	12·8 ± 0·4
P. fimata		831 ± 21	830 ± 24	0·106 ± 0·007[b]	6·56	0·824 ± 0·02[b]	8·7 ± 0·6
Growth (g)							
P. minuta	$\delta^{13}C$	201 ± 55	217 ± 55	0·081 ± 0·048	8·58	0·216 ± 0·102	1·7 ± 1·0
P. fimata		370 ± 30	386 ± 34	0·110 ± 0·024	6·31	0·398 ± 0·032	4·4 ± 1·0
P. minuta	$\delta^{15}N$	236 ± 58	232 ± 59	0·081 ± 0·048	8·58	0·233 ± 0·101	1·9 ± 1·1
P. fimata		416 ± 32	411 ± 36	0·110 ± 0·024	6·31	0·413 ± 0·032	4·5 ± 1·0
Tissue turnover (r)							
P. minuta	$\delta^{13}C$	634 ± 25	651 ± 35	0·189 ± 0·029	3·66	0·681 ± 0·030	12·9 ± 2·0
P. fimata		380 ± 24	401 ± 28	0·117 ± 0·020	5·94	0·408 ± 0·024	4·8 ± 0·8
P. minuta	$\delta^{15}N$	685 ± 22	682 ± 29	0·167 ± 0·019	4·16	0·677 ± 0·028	11·2 ± 1·3
P. fimata		417 ± 30	419 ± 33	0·102 ± 0·020	6·78	0·413 ± 0·030	4·2 ± 0·8
Metabolism* (c)							
P. minuta	$\delta^{13}C$						10·7 [7·6–12·7]
P. fimata							4·2 [3·6–4·7]
P. minuta	$\delta^{15}N$						8·9 [5·7–10·9]
P. fimata							3·6 [3·0–4·2]
Eggs[(i)] (o)							
P. minuta	$\delta^{13}C$						2·1 [0·1–5·2]
P. fimata							0·54 [0·03–1·16]
P. minuta	$\delta^{15}N$						2·3 [0·3–5·5]
P. fimata							0·67 [0·27–1·17]
Exuvia (x)							
P. minuta	$\delta^{13}C$						0·092
P. fimata							NA
P. minuta	$\delta^{15}N$						0·081
P. fimata							NA

*Tissue turnover for metabolism and eggs are given as medium values and 95% confidence intervals.

P. fimata, respectively (Table 2). The tissue turnover rates (Λ_r) were 12·9% C d^{-1} and 11·2% N d^{-1} for *P. minuta*, and 4·8% C d^{-1} and 4·2% N d^{-1} for *P. fimata*.

EGG ALLOCATION

The mean $\delta^{13}C$ and $\delta^{15}N$ values of eggs laid by *P. minuta* after diet change were equal or more enriched than adult animals indicating that the nutrients used for egg production were a mixture of diet and body reserves (Fig. 6A and 6B). In contrast, the $\delta^{13}C$ and $\delta^{15}N$ values of *P. fimata* eggs laid during the first week were depleted relative to the adults indicating that nutrients for egg production were provisioned from tissue reserves created before the diet change (Fig. 6A and 6B). However, *P. fimata* eggs harvested at 14 and 28 days were similar or more enriched than the adults indicating that nutrients for egg production were provisioned directly from diet.

To investigate the dynamics of nutrient provisioning to eggs, we estimated γ (Eqn 7 – the proportion of egg C or N provisioned directly from diet) and T (Eqn 8 – the average time it took to build an egg). Median values of T ranged were 3·8–4·3 days for *P. minuta* and 6·3–6·6 days for *Pr. fimata* (see Fig. S1 in Supporting Information). The development time of eggs was longest for *P. fimata*, concurrent with the larger egg biomass of *P. fimata* than *P. minuta*. Median values of γ ranged 0·59–0·64 for *P. minuta* and 0·45–0·56 for *P. fimata* (see Fig. S1), indicating that the direct provisioning from diet to eggs was slightly higher for *P. minuta* than for *P. fimata*. To evaluate how well the modelled parameters fit the actual values of isotope change of eggs, median values of γ and T were integrated over time and plotted in Fig. 6A and 6B. The modelled parameters fitted the isotopic change of *P. minuta* eggs well, although the enrichments of ^{13}C and ^{15}N were underestimated. Contrary to *P. minuta*, the fit for *P. fimata* eggs overestimated enrichments of ^{13}C and ^{15}N, particularly during the first week.

TISSUE TURNOVER FRACTIONS

The fractions responsible for tissue turnover (Λ_r) are metabolic turnover (Λ_c'), egg production (Λ_o') and for *P. minuta* shedding

of exuvia (Λ'_x) (Fig. 3). For both species, the metabolism was the largest contributor to tissue turnover. For *P. minuta* we estimated median values for metabolic rates (Λ'_c) for C and N as 10·7% and 8·9% d^{-1}, respectively, and for *P. fimata* 4·2% and 3·6% d^{-1}, respectively (Table 2). The rates of the 95% confidence intervals do not overlap between the two species, suggesting that *P. minuta* had the highest metabolic rate. The median C and N values for egg production (Λ'_o) were for *P. minuta* 0·54% and 0·67% d^{-1}, respectively, and for *P. fimata* 2·1% and 2·3% d^{-1}, respectively (Table 2). The rate of moulting exuvia (Λ'_x) in *P. minuta* was very small (< 0·01% d^{-1}) compared to total tissue turnover (11·2–12·9% d^{-1}) (Table 2).

Discussion

Our data support that isotope change in Collembola tissue is primarily attributed to metabolism (Λ'_c) and, to a lesser degree, growth (Λ_g). This contrasts with findings from similar diet change studies with poikilotherms such as whitefish (Hesslein *et al.* 1993), fish larvae (Herzka & Holt 2000), young postlarval shrimp (Fry & Arnold 1982) and crustaceans (Ventura & Catalan 2008), where most of the changes in either C or N were attributed to growth. However, these studies included animals growing at low temperatures, likely resulting in low metabolic rates (Clarke & Johnston 1999). In contrast, our experiment was performed at 20 °C, and our results are more in line with observations from homeotherms (Ponsard & Averbuch 1999). We did find strong differences between the two collembolan species, which suggests that in addition to the direct effects of temperature on metabolism, physiological traits are also an important factor in explaining tissue turnover.

We confirmed our hypothesis that *P. minuta* has a higher metabolic rate than *P. fimata*. The two most important physiological traits contributing to the relatively high metabolism in *P. minuta* are probably its smaller size and higher fecundity compared to *P. fimata*. Petersen (1981) found the allometric scaling exponent (*b*) relating metabolic rate to body mass (metabolic rate = $a \times$ mass^b) to range 0·67–0·83 for eu- and hemiedaphic Collembola (*b* = 0·78 for *Onychiurus armatus* s.l., no species resembling *P. minuta* were included). When we modelled allometric scaling between the two species in our study, the 95% confidence values for *b* were 0·25–0·48 (see Fig. S2). The relatively small value of the allometric scaling exponent in our study indicates that *P. minuta* had a proportionally higher metabolic rate than what can be explained by allometric scaling, that is, the mass differences between the two species. Therefore, it is likely that the much higher rate of reproductive investment (egg manufacturing, Λ'_o) in *P. minuta* than *P. fimata* also contributed to its high metabolic rate. Gratton and Forbes (2006) conducted a feeding experiment on beetles and compartmentalized turnover of ^{13}C in different organs in beetles. They found that the isotopic signature in body fat and reproductive organs changed more rapidly than the more metabolically inert tissues, such as muscles and cuticle. In terms of optimizing reproductive investment relative to metabolic rates, our data indicate that *P. minuta* is more efficient than *P. fimata* as the ratios of metabolism to reproductive

investment were 3–4 times higher for *P. fimata* than for *P. minuta*. A possible trade-off for the high reproductive investment of *P. minuta* compared to *P. fimata* could be a higher somatic damage associated with replacing tissue cells. This interpretation is supported by a longevity study with fruit flies (O'Brien *et al.* 2008) where females with the greatest ratio of nutrient investment to somatic tissue vs. reproduction were the longest living.

The C metabolic rates for the two collembolans were in the same range as previously found with classic allometric approaches (direct measurements of respiration) (Petersen 1981). The animals in Petersen's study were measured at a lower temperature than the present study. At 10 °C, the respiratory rate by *O. armatus* s.l. was 1·2 mL O_2 g^{-1} h^{-1}, and using a temperature coefficient (Q_{10}) of 3·2 (c.f. Petersen 1981) the respiratory rate would be 4·0 mL O_2 g^{-1} h^{-1} at 20 °C. The comparable metabolic rate for *P. fimata* in the present study was 3·3 mL CO_2 g^{-1} h^{-1} (assuming that all metabolic C was catabolized to CO_2). For *P. minuta*, we estimated the metabolic rate to 8·3 mL CO_2 g^{-1} h^{-1}. The metabolic rates of both studies are likely to underestimate actual rates. In Petersen's study, the animals were subject to resting conditions during a 1–4 h period and in our study we only estimated carbon catabolized from tissue, thus not taking into account what was catabolized directly from diet. In terms of N metabolic rates, the values reported here are much higher than previously reported with direct measurement of excreted ammonium (Sjursen & Holmstrup 2004; Larsen *et al.* 2007b). The ammonium excretion rates of *P. minuta* and *P. fimata* were < 40% of the N metabolic rates estimated in this present study (Larsen *et al.* 2007b). The lower N metabolic rates previously measured could either be because the animals were under resting conditions or that collembolans excrete nitrogenous waste in other forms than ammonium-N, such as uric acid (Verhoef *et al.* 1983).

We distinguished between the mixing and non-mixing fractions, the latter belonging to the nutrient fraction that was built during the juvenile stage and not replaced after sexual maturity. The size of the non-mixing fraction after subtracting the contribution of growth was larger for *P. fimata* than *P. minuta*. This difference demonstrates that the species with the lowest tissue turnover, *P. fimata*, retained more of its juvenile-acquired nutrient reserves as an adult. A contributing factor for the higher non-mixing fraction values of *Pr. fimata* than *P. minuta* could be that *P. fimata* re-ingested their exuvia. However, the losses of C and N through shedding of exuvia were very small for *P. minuta* relative to total tissue turnover. The significance of adult animals having large juvenile reserves could be that they more easily can cope with starvation or nutritional stress and still maintain a normal reproduction rate.

Growth and reproduction requires N- and P-rich materials such as amino acids, phospholipids and ribosomes, while catabolism and storage of energy needs C-rich materials like lipids and carbohydrates. We linked stoichiometry with tissue turnover to evaluate the requirements of two different Collembola species. We found that *P. minuta* had a significantly lower C : N ratio and higher tissue turnover of both C and N than *P. fimata*, suggesting that *P. minuta* has a higher synthesis

rate of proteins than *P. fimata*. This finding also underlines the importance of looking at both stoichiometry and tissue turnover when evaluating nutrient requirements. The demand for P was also highest for *P. minuta* because adults and eggs contained significantly more P than *P. fimata*. In animal tissue, P predominantly is found in phospholipids, nucleotides and nucleic acids (Sterner & Elser 2002; Ventura 2006) and is involved in processes inside the cell that govern growth and reproduction (Elser *et al.* 2003). Adult *P. minuta* had the highest P content and tissue turnover. This supports the growth rate hypothesis, which states that differences in organismal C : N : P ratios are caused by differential allocations to RNA necessary to meet protein synthesis demands of growth and reproduction (Sterner & Elser 2002). Proteins contain about 16% N and 52% C and approximately half of the body dry mass of animals is made of proteins or free amino acids (Ventura 2006). As the asymptotic curves for ^{13}C and ^{15}N were similar for both species it indicates that proteins were the primary drivers of tissue turnover.

Both species possess traits that are typical for their respective habitats (Fjellberg 1998, 2007) except that euedaphic living species tend to be smaller than hemi-edaphic species. It is suggested that species living in the mineral layer of the soil are adapted to a less nutritious diet than species living in relatively fresh litter (Faber 1991; Berg & Verhoef 1998; Berg & Bengtsson 2007). A number of parameters in our study indicate that *P. fimata* is adapted to lower food quality and availability than *P. minuta* by having large nutritional reserves, low fecundity, low metabolic rate and low protein synthesis rate. Presently, we are cautious to relate these parameters to vertical stratification as this would require a much larger assemblage of species than included in this study. However, the methodological approach we implemented, combining mass balance, isotope turnover and stoichiometry, is promising for linking nutrient budgets and life-history traits.

In conclusion, we found that ^{13}C and ^{15}N changes in Collembola were primarily attributed to metabolism and not growth, contrasting previous allometric studies on poikilotherms, such as fish and crustaceans. The euedaphic *P. fimata* had a significantly lower metabolic rate and reproductive output than the hemi-edaphic *P. minuta*. The two most important parameters explaining the higher metabolic rate of *P. minuta* than *P. fimata* were most likely its small body size and high reproductive investment. Our stoichiometric data indicate that *P. minuta* may have higher nutritional requirements for reproduction as the N : P ratio of its eggs was significantly higher than that for *Pr. fimata*. The relatively low metabolism and nutritional requirements by *P. fimata* might be an adaptation to the generally low food availability and quality in the euedaphic habitat. Our approach of tracking isotope turnover and mass balance after sexual maturity allowed us to estimate nutritional reserves, reproductive investments and metabolic turnover.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Calculations of stable isotope ratios.

Table S1. Isotope ratios for *Proisotoma minuta* and *Protaphorura fimata*

Fig. S1. Collembolan allocation of carbon and nitrogen to egg manufacturing.

Fig. S2. The allometric scaling coefficient between *Proisotoma minuta* and *Protaphorura fimata*.

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