Unravelling collembolan life belowground: Stoichiometry, metabolism and release of carbon and nitrogen

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Data sheet

Abstract:	This thesis investigated carbon and nitrogen dynamics of soil dwelling Collembola by using direct measurements and stable isotope additions. In an isotope change experiment, collembolans exchanged between 6 and 10 % of carbon and nitrogen in their body tissue per day to metabolism and between 0.5 and 2 % to reproduction. When collembolans on low and high protein diets were compared, animals on the low protein quality depleted their tissue ^{15}N values relative to those on high quality diet indicating that the nitrogen turnover decreased on the low protein quality diet. In a wheat microcosm investigating source contributions from soil, roots and isotope labelled green manure the mixing model analysis indicated that photosynthate (root derived C) was the main carbon source for collembolans (54 – 79 % of total C) indicating that the rhizosphere channel is very important for collembolans in addition to the detritus based channel.

Contents

Stille

Du Slægt, der som en storm i høst
henover Jorden haster,
men aldrig i dit eget bryst
til bunden loddet kaster;
du, som vil granske livet ud,
men glemmer livets kilde,
søg dog engang dig selv og Gud -
vær stille!

Hør op med denne vilde larm,
den hvileløse trængsel,
læg øret til din egen barm,
hvor sjælen bor i fængsel;
dæm op for dine lysters elv,
lad strømmen sig skille,
søg på hjertes bund dig selv,
men stille.

Hør op at færdes uden ro
imellem livets døre;
hvor kan i slig en larm du tro,
at du Guds røst kan høre?
Han drager ej i hjertet ind
som tordenstorm i skoven,
han kommer som en sagte vind
fra oven.

Du slægt, som fuld af harm og had
igennem verden jager,
mens bævende liig espens blad
bestandig tungen klager!
Hvad er dit mål, hvad er dit med,
hvad søger du i vrimlen?
Se, blomsten vokser tavs i fred
mod himlen.

Hør, overalt i mark og lund
en bøn om stilhed lyder,
selv middagssolens gyldne mund
i skovens stilhed byder;
hør, stjernerne langs himlens kyst
på sølverharper spille,
og bede dig med bønlig røst:
Vær stille!

Oh bøj dig til disse stemmers klang,
du travle slægt, dit øre!
Der kommer dog den tid engang,
da du får lov at høre:
Når gravens dybe klokke slår,
når dag og nat sig skille,
og døden råber kold og hård:
Vær stille!
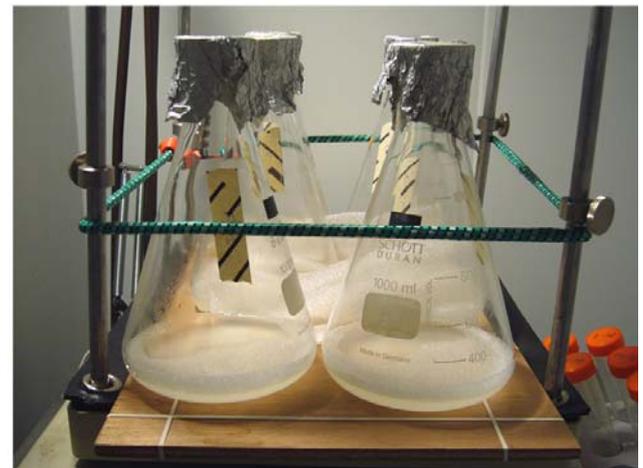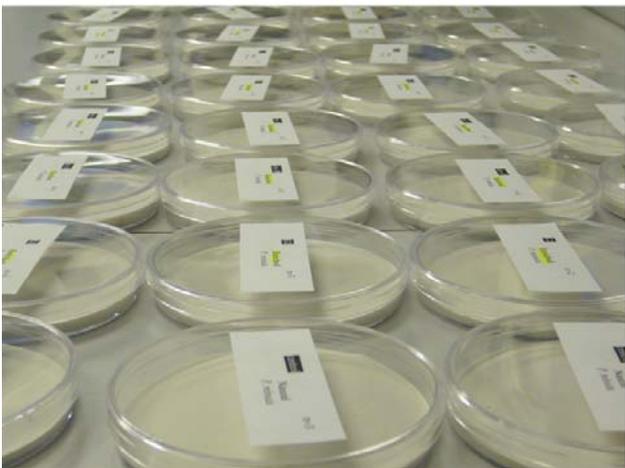
Left-top: The collembolans *Protaphorura armata* (large white) and *Proisotoma minuta* (small grey)
Right-top: The wheat microcosm study (Paper. II)
Left-bottom: Petri-dishes with collembolans (Paper III)
Right-bottom: Culturing isotope labelled yeast (Paper III)

Acknowlegdement

I would like to thank my principal supervisor Jakob Magid for fostering the idea of this thesis: to integrate the methodologies of two laboratories with each their different approaches. While the "Plant Nutrition and Soil Fertility Laboratory" traditionally has researched in mass fluxes in soil and plant nutrition, the "Department of Terrestrial Ecology" has carried out environmental research using test organism to study ecosystem processes. Jakob Magid proposed that the understanding of the role that soil organisms play for nutrient release could be improved by implementing the isotope labelling techniques of agronomic research. In turn, these organisms could be used to investigate parameters influencing isotope fractionation. I am grateful to my project supervisor, Paul Henning Krogh, for generously sharing his enthusiasm and vast knowledge on collembolans with me, and for his support during the last three years. During the thesis, I also integrated traditions from a third research discipline, freshwater ecology, thanks to Marc Ventura. I am deeply indebted to Marc Ventura who during our many discussions made me aware of the potentials of ecological stoichiometry. This brought me to a deeper understanding of how individual and larger scale processes are interlinked with each other

Summary

Soil Collembola are important for decomposition and cycling of carbon and nitrogen but only few studies have quantified collembolan release of these elements. Quantifying the release rates and understanding the mechanisms mediating them might be a key for modelling how collembolans alter the biochemical cycling of nutrients in soil. The objectives of the thesis were to: i) investigate turnover of carbon and nitrogen by collembolans in relation to their physiology and dietary requirements; and ii) use collembolans as a tool to assess biological mediated fluxes of carbon and nitrogen in soil. The soil food web is a nitrogen limited environment which also limits the availability of the nitrogen rich amino acids. No studies have yet addressed how amino acid imbalances in the diet affect collembolan productivity and physiology. An insight into physiological responses can be obtained by measuring tissue composition of carbon, nitrogen and phosphorus that are key elements in biochemical compounds. Information on internal physiological processes can also be obtained by analyzing ^{15}N because nitrogen stress is likely to lead to an increased recycling of nitrogen and consequently retention of ^{15}N in the tissue. This thesis is composed of four separate studies that progressively address specific aspects of collembolan physiology and uptake/ release of carbon and nitrogen. To link the studies with each other, two species of Collembola, the small hemiedaphic *Proisotoma minuta* and the large euedaphic *Protaphorura armata*, were included in all the studies allowing us to compare different aspects of collembolan physiology. Release of carbon and nitrogen by Collembola has been estimated by mass balance models and by direct measurements but these methods have in their current forms some disadvantages. Instead we implemented an isotope mass-balance approach that would allow us to follow how two collembolans with contrasting life history strategies, *P. minuta* and *P. armata*, allocated carbon and nitrogen to growth, reproduction and metabolism. We also examined how two diets with different amino acid imbalances affected elemental turnover and isotope discrimination. Finally, two plant microcosm studies were included to i) investigate how collembolans (and soil mesofaun) affected plant growth and nutrient release, and to ii) determine the sources of carbon and nitrogen in collembolans using a linear mixing model. The results showed that life history strategies of collembolans were key factors in explaining carbon and nitrogen allocations. We estimated that metabolism contributed to replacement rates of tissue of ~10 % day^{-1} for *P. minuta*, and ~6 % day^{-1} for *P. armata* (for carbon and nitrogen). In comparison, reproductive tissue replacement rates were ~2 % day^{-1} for P. minuta and ~0.5 % day^{-1} for *P. armata*. The relative expenditures to metabolism relative to reproduction were thus lower for *P. minuta* than *P. armata*. The study on amino acids imbalances revealed that low protein quality increased C:N and C:P ratios significantly ($p<0.05$) indicating that carbon rich storage compound (lipids and fatty acids) increased relative to nitrogen-phosphorus rich compounds (RNA and proteins). However, *P. armata* had a much weaker response compared to the two other species. We also measured isotope compositions and found significantly lower ^{15}N values ($p<0.05$) in the tissue of collembolans raised on the low than high protein quality diet. This contrasts the hypothesis proposing that amino acid deficiency increases retention of ^{15}N in tissue. A possible explanation for this result might be that collembolans preferentially digested proteins with amino acids depleted in ^{15}N. In the microcosm studies, we did not find a significant increase in plant growth in treatments with Collembola. Analysis of source contributions indicated that photosynthate (root derived C) was the main carbon source for collembolans (54 – 79 % of total C) supporting recent results that the rhizosphere channel is very important for collembolans in addition to the detritus based channel.

Resumé på dansk

Springhaler spiller en vigtig rolle for jordbundens nedbrydningsprocesser og cirkulering af kulstof (C) og kvælstof (N), men kun ganske få forsøg har undersøgt deres direkte frigivelse af C and N. Bestemmelse af springhalers frigivelse af C og N og undersøgelse af de mekanismer der fordrer frigivelsen er centralt for at kunne modellere hvordan dyrene påvirker stofstrømmene i deres omgivelser. Afhandlings formål var at i) bestemme springhalers turnover af C og N i relation til deres fysiologi og ernæringsbehov og ii) at anvende springhaler som et redskab til at vurdere biologiske stofstrømme i jordbunden. Jordbundens fødekæde er N-begrænset hvilket også begrænser tilgængeligheden af de N-holdige aminosyrer. Det er endnu ikke blevet undersøgt hvordan aminosyre-ubalancer i føden påvirker springhalers produktivitet og fysiologi. Springhalers fysiologi kan undersøges ved at måle på deres sammensætning af C, N og fosfor (P) som er essentielle for opbygningen af biokemiske stoffer. Den fysiologiske tilstand kan også vurderes vha. ^{15}N analyser fordi mangel på N fører til øget recirkulering af N med deraf følgende berigelse af ^{15}N i vævet. Denne afhandling er sammensat af fire separate forsøg som hver undersøger forskellige aspekter af springhalers fysiologi og optag / frigivelse af C og N. For at opnå en rød tråd mellem forsøgene, blev to arter, den lille hemi-edafiske *Proisotoma minuta* og den store eu-edafiske *Protaphorura armata*, anvendt i alle fire forsøg så forskellige aspekter af deres fysiologi kunne sammenlignes. Frigivelse af C og N er hos springhaler blevet undersøgt ved hhv. masse-balance modeller og direkte målinger, men der er ulemper ved begge fremgangsmåder i deres nuværende udformning. Vi implementerede i stedet for en isotop masse-balance fremgangsmåde hvorved vi kunne følge allokeringer af C og N fra føden til hhv. vækst, reproduktion og stofskifte hos *P. minuta* og *P. armata* som har forskellige livshistorie strategier. Vi undersøgte også hvordan føde med hhv. lille og store aminosyre-ubalancer påvirkede forskellige fysiologiske parametre. Endelig inddrog vi også to plante-mikrokosmos forsøg for 1) at undersøge om springhalerne (og mesofaunaen) påvirkede plantevækst og næringsstoffrigivelse og for 2) at bestemme vha. en 'linear mixing model' hvorfra springhalers får deres C og N. Vores resultater viste at springhalers livshistorie strategier var afgørende for deres allokeringer af C og N. Udskiftningen af kropsvæv som følge af stofskifte var ~10 % dag^{-1} hos *P. minuta* og ~6 % day^{-1} hos *P. armata* (både C og N). Til sammenligning, var udskiftningen af kropsvæv som følge af reproduktion ~2 % dag^{-1} hos *P. minuta* og ~0.5 % day^{-1} hos *P. armata*. Den relative stofskifte-omkostning forbundet med reproduktion var således mindre hos *P. minuta* end *P armata*. Aminosyre-forsøget viste at der var en signifikant (p<0.05) stigning i C:N og C:P på lav-kvalitetsføden (stor aminosyre-ubalance), hvilket indikerer at C-rige indholdsstoffer (lipider og fedtsyrer) steg i forhold til N- og P-rige indholdsstoffer (protein og RNA). Dog var denne effekt langt mindre udtalt hos *P. armata* end hos de to andre arter i forsøget. Vi målte også på isotop-sammensætningen, og fandt at ^{15}N indholdet var signifikant lavere (p<0.05) hos dyr der spiste lav-kvalitetsføden end hos dyr der spiste høj-kvalitetsføden. Disse resultater var i modstrid med antagelsen om at ^{15}N indholdet stiger i kropsvævet når dyr spiser lav-kvalitetsføde. En mulig forklaring kan være at dyrene fortrinsvis fordøjede proteiner med ^{15}N-fattige aminosyrer. I de to mikrokosmosforsøg påvirkede springhalerne ikke plantevæksten. Derimod målte vi at rhizosfære (rod) C var en meget vigtig C-kilde for springhalerne (54 – 79 % af total C) hvilket stemmer overens med andre forsøg af nyere dato der støtter at den rhizosfære-baserede fluks er meget vigtig (udover den nedbrydningsbaserede fluks).

1

Introduction

Ever since I first became acquainted with Collembola I have been particularly fascinated by their ancient history. Collembola and their early ancestors were among the animals pioneering land about 400 million years ago, between the late Silurian and early Devonian periods, when bacteria, algae and plants began to colonize land (Hopkin 1997). As land plants evolved, collembolans established themselves in almost all terrestrial ecosystems from tropical rain forests to polar regions, and it is today one of the most abundant arthropod species on the planet. In temperate regions, the majority of collembolans are small, between 0.5 and 5 mm in size, and live in narrow confines in soil where they feed on fungi, detritus, plant roots and possibly microfauna. Because collembolans so successfully have co-existed with plants and microbes for millions of years, knowledge on how these animals interact with their environment is a key to understanding terrestrial ecosystem processes.

Interactions among soil fauna and microbes are central to many processes in soil ranging from decomposition to the functioning of the rhizosphere (Lussenhop 1992). When an organism interacts with its environment, it exchanges nutrients that in turn affect other organisms in that system. To understand the environment in which collembolans live we need to investigate the interactions among these organisms and the mechanism underlying resource use. A number of studies have investigated the effects of collembolans on nutrient release in microcosm studies, experimental systems that are representative of larger ecosystems, by exclusion and inclusion of an assemblage of species (e.g. Cragg and Bardgett 2001; Milcu et al. 2006). Differences between the treatments in plant growth or microbial parameters can then be attributed to the presence of the animals. Other studies have used molecular tools to unravel their dietary preferences and physiological responses to environmental stress (e.g. Chamberlain et al. 2006; Kærsgaard et al. 2004; Ruess et al. 2004). Fewer studies have, however, investigated these animals' direct release of carbon and nitrogen and the mechanisms mediating them (Petersen 1980; Sjursen and Holmstrup 2004; Verhoef et al. 1988). Because Collembolans belong to a group of animals that are highly diverse and specialized it is important to take their individual physiological traits into consideration when assessing their role in nutrient cycling. For example, Petersen (1980) and Sjursen and Holmstrup (2004) found that the release of carbon and nitrogen differed between different species. Likewise, the resources quality available for the collembolans might influence their productivity and hence release of carbon and nitrogen (Chen et al. 1995). The importance of parameters have not yet been investigated in detail and filling this gap of knowledge might be fundamental to model how collembolans alter the biochemical cycling of carbon and nitrogen in the soil food web. To explain why relatively few studies have coupled collembolan physiology with release of carbon and nitrogen the answer might be found in the methodological problems associated in handling these tiny animals. Another reason might be that the advanced techniques that have been commonly used in other research disciplines for decades only recently have become standard techniques within terrestrial ecology. Hence, in this thesis I have attempted to build upon the traditions of collembolan research and implemented some of the more advanced techniques such as analysis of biochemical compounds and use of stable isotope additions to investigate collembolans' release of nutrients and to understand some of the physiological mechanisms determining their release rates.

The cycling of nutrients is critical for ecosystems' sustenance. While microbes (bacteria and fungi) are the major agents of nutrient release, animals are important for modifying the activity of microbes and the translocation of organic matter (Coleman et al. 2002). Animals often require the three essential elements, carbon, nitrogen and phosphorus, in different ratios than are provided by

their food. For simplicity, I will refer to all three elements as nutrients unless otherwise noted. The availability and composition of carbon, nitrogen and phosphorus varies greatly because the distribution of resources is often patchy and the elemental ratios change over the course of decomposition (Farley and Fitter 1999; Hobbie 2000). This patchiness particularly affects collembolans living in the soil environment because they are constrained to live in the soil pore system. They are thus more limited in the physical space available to them for scavenging new food resources than are their aboveground counterparts, the herbivores. In herbivory-driven food webs, the diet contains on average 10-20 times less nitrogen and phosphorus than the typical consumer (Fagan and Denno 2004). Detritus is more depleted in nitrogen and phosphorus than live –plants, which suggests that nutritional imbalances could be even more important for detrital food webs (Moe et al. 2005). Therefore, it is essential to understand detritus-based food webs and their living components, because they contribute substantially to flows between trophic levels and element cycling in ecosystems.

The amount of nutrients ingested and released by an animal must follow the principles of mass balance. Nutrients that are ingested but not assimilated through an animal's gut wall are released as faeces. Faecal nutrients are not usually directly available to primary producers, which require nutrients in dissolved form. However, faecal nutrients may subsequently become available to primary producers via decomposition and remineralization by microbes. Assimilated nutrients have two fates: they can be bound in animal tissues via growth and reproductive tissue, or they can be released as metabolic waste products such as urine via the labial nephridia, in insoluble forms via the midgut epithelium or as gaseous losses via respiratory processes (Hopkin 1997). A large part of the nitrogenous and phosphorous waste generated by Collembola is probably released as ammonia (Sjursen and Holmstrup 2004), uric acid (Verhoef et al. 1983) and phosphate. Although collembolans primarily influence their environment through their grazing activities and spreading of propagules, the excretion of waste products also play an important role in forming soil microstructures and in providing nutrients for primary producers and heterotrophic microbes (Rusek 1998).

Metabolic excretion in Collembola has been estimated by mass balance models or by direct measurements. While excretion rate by mass balance is estimated as nutrients ingested minus nutrients allocated to egestion, growth and reproduction, direct measurements is done by keeping animals in sealed enclosures in which their waste products can be quantified. Relatively few studies have attempted to quantify waste products in Collembola, and both approaches have had their advantages and disadvantages. Verhoef et al. (1988) studied the nitrogen budgets of the epigeic collembolan *Tomocerus minor* and estimated with the mass balance approach that on a high quality diet 63 % of their diet was excreted as urine. The approach used by Verhoef et al. (1988) allowed them to incubate the animals, with food, for a long time, but it may not be methodologically feasible to carry out a similar study on soil-dwelling species since they are much smaller than *T. minor* that despite its name, reaches a length of 4.5 mm. Direct measurements have the advantage that even very small species can be handled, but as far as Collembola studies go, the animals were without food. Sjursen and Holmstrup (2004) collected ammonium from five Collembola species during two days of starvation. Based on their measurements, the authors hypothesized that ammonium excretion is the most important pathway in the nitrogen-metabolism of Collembola. Collembolan metabolism was also measured by Petersen (1980) but as oxygen consumption. The animals were put into ampoules with a NaOH buffer, without food. It might, however, lead to a large error keeping the animals without food because it lowers their metabolism. Thus, the current implementations of mass balance models and direct measurement for Collembola needs to be modified to obtain realistic values for soil-dwelling species.

Body size and diet quality are among the factors that mediate metabolic release of carbon and nitrogen. Because of allometric constraints on metabolism, mass-specific nutrient excretion rates of animals usually decline with increasing body mass. This is a fundamental causal constraint limiting

life history options because small species are able to maintain a large mass-specific production, i.e. growth and reproduction (Brown and Sibly 2006). Collembolans generally possess traits that are characteristic for *r*-selected animals by their relative small size, short generation time and high fecundity. While collembolans can be categorized as *r*-selected animals in contrast to larger K-selected animals in the soil decomposer system such as earthworms, the large variations in their morphology (Berg et al. 2004), growth and reproduction (Chamberlain et al. 2005) and dietary preferences (Jørgensen et al. 2005) suggest that collembolans have quite contrasting life history strategies. Moreover, collembolans appear to adjust their strategies according to the availability and quality of diet (Booth and Anderson 1979; Chen et al. 1995; van Amelsvoort and Usher 1989). How collembolan metabolism is affected by life history strategies has not yet been quantified and a more detailed knowledge on this matter might improve our understanding of their role in the soil decomposer system.

The size of animals is likely to affect their nutritional requirements. Small sized animals are advantaged by a large mass-specific production but might be disadvantaged when it comes to exploiting low quality diets because it requires a large intestinal system (Brown and Sibly 2006). Collembolan studies dating back more than four decades have established a correlation between functional morphology and ecological niche (Christiansen 1964; Haarløv 1960). However, early studies on food selection by gut analyses revealed no species-specific differences (Bödvarsson 1970), although later studies have observed distinctly different feeding behaviours and preferences by Collembola (e.g. Hutson 1980; McMillan 1976; Thimm and Larink 1995), and have found dissimilar concentrations of digestive enzymes in the gut (Berg et al. 2004; Borkott and Insam 1990; Urbasek and Rusek 1994). Low availability of preferred food in the field might, however, lead the animals to generalized feeding behaviours (Shaw 1988) that in turn are likely to affect their production and release.

Isotopic analyses of soil fauna have proven to be a valuable tool for investigating carbon dynamics in soil. Evidence has been accumulating that root exudates play an important role for supplying carbon to microorganisms and soil fauna (Coleman et al. 2002; Garrett et al. 2001; Ostle et al. 2007). These authors pulse labelled the air surrounding the plants with $^{13}CO_2$ or $^{14}CO_2$ so the labelled carbon could be tracked to soil organisms. The extent that soil animals assimilated root derived carbon differed widely between species and functional groups. For example, Coleman et al. (2002) found in their study of agricultural soil food webs that nematodes had contrasting assimilation patterns of the ^{14}C label than microarthropods, and speculated whether this pattern was due to nematodes preferentially assimilating bacteria and microarthropods assimilating fungi. Some animals might also feed directly on roots. Therefore, accurate knowledge on feeding preferences (or requirements) by the various soil species can provide a deeper biological understanding of carbon fluxes in soil. Analyses of additional elements such as nitrogen can also improve the understanding of carbon fluxes because the cycling of carbon is intrinsically linked to that of nitrogen (Wardle 2002). Carbon as well nitrogen were tracked in a field study by Albers et al. (2006) where maize, a C4 plant (enriched in ^{13}C), was grown in a C3 soil (depleted in ^{13}C). By analyzing ^{13}C and ^{15}N in different meso- and macrofauna taxon (grouped into genera) during two seasons, the authors found that carbon derived from the maize plants was incorporated quickly into the animals during the growing season and that incorporation of carbon and nitrogen varied within the taxonomic grouping of collembolans supporting the notion that detailed knowledge on species level is important for interpreting carbon and nitrogen fluxes correctly.

Understanding the fate of nutrients in individual animals is central to predicting how food webs alter nutrient cycling. Because nutrient excretion is constrained by mass balance, rates must be affected by the nutrient composition of the animal's body and food (Sterner and Elser 2002). Ecological stoichiometry theory proposes that individual animal species maintain relatively constant proportions of nutrients in their bodies. To maintain overall homeostasis, animals should release elements in food in excess to requirement, while maintaining most of the limiting element

(Anderson et al. 2005). Nutrient limitations appear to be a common phenomenon for collembolans living in the soil decomposer system due to seasonal variations in resource availability and fierce competition for nutrients among soil organisms. Most terrestrial animal studies including those with Collembola have focused on nitrogen (Fagan and Denno 2004; Leonard and Anderson 1991; Verhoef et al. 1988) and less on phosphorus (Kay et al. 2006; Markow et al. 1999) and carbon (Hessen et al. 2004) limitations. Carbon is the major elemental constituent in the body, but is normally not the limiting nutrient because it is supplied to the decomposer system in excess amounts by primary producers. However, these resources provided by primary producers might not be available to collembolans before they have been processed by microbes and transformed into high quality substrates such as fatty acids (Chamberlain et al. 2004; Ruess et al. 2005). Transformation of nitrogen and phosphorus into high quality substrates is likewise important. While it has been documented that production by collembolans can be limited by nitrogen in soil (Booth and Anderson 1979), it is still unclear to what extent phosphorus is a limiting nutrient (Jensen et al. 2006). In tissue, phosphorus predominantly is found in phospholipids, nucleotides and nucleic acids (Ventura 2006) so it is involved in processes inside the cell that govern growth and reproduction (Elser et al. 2003). Nitrogen, being an important amino acid/enzyme constituent, also plays a key role by catalyzing biochemical reactions in cells and in the creation of connective tissue. The composition of amino acids has long been a widely studied subject in many research disciplines, (e.g. Fantle et al. 1999; Lindan and Work 1951; O'Brien et al. 2002) but not in soil animal research, which is surprising given that a number of amino acids are indispensable and usually imbalanced to dietary requirements. Moreover, about half of the body dry weight in animals is made of proteins or free amino acids (reviewed by Ventura 2006). So there is substantial evidence to suggest that better knowledge of amino acid requirements in Collembola is necessary for understanding these animals' metabolism and excretion of nutrients.

Nutritional imbalances affect chemical processes in animal tissue. During these chemical processes bonds are made or broken between atoms all the time. Atoms with a low mass, the light isotopes, will react fast than atoms with a high mass. As a consequence, the abundance of stable isotopes of an element will vary among the reactants and products of a chemical reaction. The variation between reactants and products is called fractionation. During catabolism, preferentially light isotopes are released in CO_2 or NH_4^+ form leaving the heavy isotopes behind. If the remaining substrate containing the heavy isotopes is retained in tissue, it follows that tissue is enriched in the heavy isotope. While the distance or the discrimination factor between $\delta^{13}C$ of diet and bulk animal tissue normally is very small (<1 ‰) (De Niro and Epstein 1978), it is quite large for $\delta^{15}N$ (~3.4 ‰) (De Niro and Epstein 1981). Several studies have found that discrimination of $\delta^{15}N$ is variable and normally increases if the diet is N-limited (reviewed by Ben-David et al. 1999). Not only the quantity but also the quality of N is likely to affect discrimination because deficiency of indispensable amino acids will lead to greater protein requirements and ingestion and hence to higher catabolism of the amino acids not needed for protein synthesis (Martínez del Rio and Wolf 2005).

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Objectives

The objectives of the thesis are to: i) investigate turnover of carbon and nitrogen by collembolans in relation to their physiology and dietary requirements; and ii) use collembolans as a tool to assess biological mediated fluxes of carbon and nitrogen in soil. I will pursue these objectives through four hypotheses. The first hypothesis is related to collembolan physiology and is based on the allometric theory stating that small, *r*-selected species can maintain a larger mass-specific production than large, K-selected species (Brown and Sibly 2006). Thus, I hypothesize that i) *r*-selected collembolans have higher metabolic rates of carbon and nitrogen than K-selected collembolans. The next hypothesis is the one proposed by Sjursen and Holmstrup (2004) saying that ii) collembolans primarily excrete nitrogen in NH_4^+-N form. The third hypothesis is related to how dietary N imbalances affect physiological processes in collembolans. I hypothesize that iii) that $\delta^{15}N$ in tissue will increase relative to that of the diet as the protein quality decreases. The fourth and last hypothesis is related to carbon dynamics in a soil-crop system where I propose that iv) during the growing season root derived carbon is the largest source of carbon for collembolans.

3

Summary of Papers

The thesis is based on four separate studies that each addresses specific aspects of carbon and nitrogen dynamics in relation to Collembola. To interlink the studies, two species of Collembola, the small and hemiedaphic *Proisotoma minuta* and the large and euedaphic *Protaphorura armata,* were included in all the studies allowing us to compare different aspects of collembolan physiology. In our first study, "*Properties of anaerobically digested and composted municipal solid waste assessed by linking soil mesofauna dynamics and nitrogen modelling*" (Paper I), we investigated how a mesofauna assemblage consisting of one enchytraeid species, five collembolan species and one predatory mite affected plant growth and release of nutrients. In addition, we measured the direct release of NH_4^+-N by collembolans. Our next study, "*Differential assimilation of carbon and nitrogen by wheat roots and soil-dwelling Collembola*" (Paper II), was designed to estimate the significance of the wheat root uptake of low molecular weight (LMW) compounds derived from green manure. Quantifying root uptake of exogenously applied LMW compounds is important for understanding the cycling carbon and nitrogen in the soil. Our primary reason for including collembolans was not to assess their role in nutrient cycling but to use them as sampling devices for the microbes and to evaluate functional difference between three Collembola species. We used a green manure enriched with ^{13}C and ^{15}N to track the fate of these elements to roots and collembolans. Our objective in the third study, "*The implications of contrasting life histories for nutrient allocations and elemental composition in Collembola*" (Paper III), was to investigate carbon and nitrogen allocations in Collembola and relate their physiological responses to their life history traits. To overcome the issues related with the current methodologies of estimating carbon and nitrogen release in Collembola we implemented an isotope mass-balance approach that has been used previously for other animal groups such as large insects (O'Brien et al. 2000), mammals (MacAvoy et al. 2005) and fish (Hesslein et al. 1993). This approach would allow us to follow how different Collembola species allocated carbon and nitrogen to growth, reproduction and metabolism. In addition, we investigated the composition of carbon, nitrogen and phosphorus in the animals in relation to their diet in order to gain complementary information on the regulatory processes underlying life history traits and nutrient allocation. In our last study, "*Contrasting effects of amino acid imbalances on elemental turnover and isotope discrimination in three species of Collembola*" (Paper IV), we raised the animals on diets with low and high protein quality to investigate physiological responses to amino acid imbalances by Collembola. We measured isotope discrimination because N-imbalances normally affect isotope partitioning. As in the previous study, we also measured carbon, nitrogen and phosphorus in collembolans and their diets.

The manuscripts in Paper I-IV can be acquired from Thomas Larsen (thl@natursyn.dk) once they are in print.

3.1
Summary of Paper I

Properties of anaerobically digested and composted municipal solid waste assessed by linking soil mesofauna dynamics and nitrogen modelling

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We investigated how anaerobically digested (ADMSW) and composted (CMSW) municipal solid waste affected population in detrital food web, plant nitrogen uptake and nutrient dynamics. Anaerobically digested waste often releases much larger amounts of readily available compounds than are released by composted waste. These compounds fuel the soil microbes, and plant available nutrients are immobilized. Then, when the easily available energy sources are exhausted, the nutrients are released back into the soil. In contrast to anaerobically digested waste, composts are normally left to mature in order to avoid the release of excessive amounts of organic acids that can harm plant roots and cause the immobilization of plant nutrients (Bernal et al. 1998; Lampkin 1990). Thus, the quality of organic materials affects decomposition processes and the functioning of the soil food web. We therefore hypothesized that ADMSW would immobilize nitrogen immediately after addition to a soil sample, in contrast to the addition of CMSW, which was hypothesized to have no immobilizing effect on nitrogen.

It is well established that microbial grazing and ingestion of labile organic matter by the soil mesofauna can affect the cycling of nitrogen and other plant nutrients (Coleman and Crossley 1996; Didden 1993). The effects of mesofauna are mostly thought to be indirect, through grazing on the primary decomposers. However, little work has been done to measure their release of plant available N except for Sjursen and Holmstrup (2004) and Verhoef et al. (1988). In this study, we quantified the release of NH_4^+-N and urea-N, because both N forms can be readily taken up by plants. While release of NH_4^+-N has been documented for mesofauna, no studies have quantified whether or not they release urea-N (Edwards and Lofty 1977; Sjursen and Holmstrup 2004). In relation to soil fauna, we hypothesized that the mesofauna consisting of enchytraeids and microarthropods, would stimulate N release from the decomposer community. Lastly, we hypothesized that only enchytraeids would release urea.

The results showed that both anaerobically digested waste (ADMSW) and compost (CMSW) initially immobilized N, indicating that little N was available for barley uptake (Paper I, Fig. 1a and

1b). Our hypothesis that CMSW was mature and would not immobilize N was therefore not supported. Probably as a result of N limitations, barley growth and N content were low, between 0.6 and 0.8 % N, in all treatments. That the systems were N deficient was also indicated by the fact that plant uptake of N greatly exceeded the estimated N-mineralization at 57 days (Paper I, Table 3 and Fig. 1a and 1b). However, both amendments greatly increased fungal biomass and mesofauna abundance compared to the Control without amendments. Mesofauna abundance was not correlated with fungal biomass, an important food source for mesofauna, in the ADMSW and CMSW treated soils. The enchytraeids' abundance was highest in the ADMSW treated soil, and this was probably because enchytraeids could feed directly on decomposed ADMSW materials. In spite of a high mesofauna abundance reaching $> 100,000$ microarthropods m^{-2} and $> 500,000$ enchytraeids m^{-2}, plant growth and plant N content were similar between treatments with and without mesofauna. Nevertheless, NH_4^+-N released by the mesofauna was estimated to be substantial and was about 20 % relative to the amount of N taken up by barley in the ADMSW treated soil. An explanation for the discrepancy between the apparently high release of NH_4^+-N by the mesofauna and the lack of effects on plant parameters might be that other members of the detrivore and microbivore community carried out the functions of the mesofauna in the treatments without mesofauna. None of the mesofauna species released urea-N, and our hypothesis that enchytraeids would release urea-N was not confirmed. The reason for this might be that enchytraeids mostly release urea under stress, as has been suggested for earthworms (Edwards and Lofty 1977).

3.2
Summary of Paper II

Differential assimilation of carbon and nitrogen by wheat roots and soil-dwelling Collembola

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It has been demonstrated that plant roots can take up small amounts of low molecular weight (LMW) compounds from the surrounding soil (Kuzyakov and Jones 2006). Root uptake of LMW compounds have been previously investigated by pulse injection of isotopically labelled sugars or amino acids. To our knowledge, no studies have used the approach of using labelled organic matter to investigate root uptake of LMW compounds. Application of labelled organic matter may hold some advantages over pulse injection for monitoring root uptake of MSW compounds. 1) The compounds are released continuously and gradually over a longer period and 2) they are released in local hot spots where the density of roots might be larger than in the surrounding, less nutrient rich soil. In contrast, direct pulse injection might favour microbial capture because the compounds are spread uniformly throughout the soil with the possibility that the significance of root carbon uptake is underestimated (Kuzyakov and Jones 2006). In this study, we tested whether wheat roots took up LMW compounds released from dual-labelled (^{13}C and ^{15}N) green manure by analyzing for excess ^{13}C in roots. In order to estimate the fraction of green manure carbon that was potentially available for root uptake, the amount of excess ^{13}C and ^{15}N of the primary decomposers was estimated by analyzing soil-dwelling Collembola. Collembolans were used as 'sampling devices' of the metabolically active pool in soil because they primarily feed on fungi or microfauna.

The experimental setup consisted of defaunated soil microcosms with dual-labelled green manure additions (216±2 δ^{13}C and 827±5 δ^{15}N). Soil was defaunated because we intended to follow three Collembola species that we added from laboratory cultures: *P. minuta*, *P. armata* and *Folsomia fimetaria* that presumably have different dietary preferences. Wheat was sown at the same time that the green manures were added. Growth, plant N and recoveries of ^{13}C and ^{15}N in soil, roots, shoots and Collembola were measured at 27, 56 and 84 days. We included ^{15}N in addition to ^{13}C labelling to distinguish between the three sources from which the collembolans could derive their carbon and nitrogen: roots (photosynthate), soil resource and green manure.

We found a small but significant amount of excess ^{13}C in roots at 27 days but not at 56 and 84 days. The amount found at the first sampling was equivalent to that ~0.4 % of total root carbon derived from the labelled green manure. About 50 % of green manure carbon was not recovered from the soil-plant system at 27 days and an additional 8 % was not recovered at 84 days. Up to 23% of *P. minuta* carbon derived from the green manure at 56 days (The data from the 27-day sampling were lost). In comparison, the similar number for *P. armata*, which is capable of feeding on roots, was 7 %. Using a linear mixing model, we estimated that photosynthate was the main carbon source for collembolans (54 – 79 %). Roots and shoots assimilated differential amounts of green manure nitrogen. Roots contained 5 % less nitrogen from the green manure than shoots.

Because of the very small or total lack of recovery of excess ^{13}C in wheat roots, we do not find that there is solid support for claiming that roots assimilated green manure derived carbon. During the incubation, the pool of green manure derived carbon available for root uptake decreased due to decomposition. However, the isotopic composition in Collembola indicated that there was a considerable fraction of green manure derived carbon in the decomposer system at 56 days. This supports the premise that LMW compounds containing carbon from the green manure was released throughout the incubation. Our results are thus is in line with findings from injection studies reporting that uptake of exogenously LMW compounds is small or insignificant, indicating that roots are poor competitors for LMW compounds (Boddy et al. 2007; Despland and Noseworthy 2006; Kuzyakov and Jones 2006; Owen and Jones 2001). Rather, roots or root effluxes appeared to be the main supplier of energy in the soil food web, which agrees with previous findings (Garrett et al. 2001; Pelz et al. 2005; Ruf et al. 2006). However, the large loss of carbon at the beginning of the experiment indicates that the green manure derived carbon was very important for fuelling the microbial mediated release of mineral nitrogen and subsequent plant growth. We found that roots took up less nitrogen from labelled green manure than shoots, and speculate that mycorrhizal associations could have supplied mineral nitrogen to roots that was depleted in ^{15}N (Michelsen et al. 1998). In conclusion, root uptake of LMW compounds was not supported using the dual-labelled green manure. The methodological approach might be improved using higher ^{13}C enrichment than was used in this study and a slower decomposable green manure. However, the methodology of using ^{13}C and ^{15}N labelled green manure in a plant-soil system, combined with isotopic analysis of soil fauna, provided valuable information on carbon and nitrogen dynamics in soil and the significance of plant derived carbon for the soil food web.

3.3
Summary of Paper III

The implications of contrasting life histories for nutrient allocations and elemental composition in Collembola

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The understanding of the physiological processes underlying resource use in animals is a key to building a mechanistic link from individual to ecosystem processes (Anderson et al. 2005). Limitation of productivity by nutrient elements appears to be common in almost all ecosystems and especially for herbivores and detrivores. The means for optimizing use of resources vary between animals according to their life history strategies. We investigated correlations between life history traits and nutrient allocations in two detrivores with contrasting life history strategies. We hypothesized that the smallest and the most reproductive of the two collembolans, *P. minuta*, would have larger rates of tissue replacement of carbon and nitrogen than *P. armata*. Tissue is replaced due to metabolism, reproduction and moulting, and we expected that allocations from tissue to metabolism as well as reproduction would be higher for *P. minuta* than *P. armata*

To estimate nutrient allocations, we changed the isotopic composition (^{13}C and ^{15}N) of the animal diets and followed growth, reproduction and isotope change in tissue and eggs during 28 days. The principle behind this approach is that isotope composition of a tissue is the result of the integration of isotopic inputs over time and by shifting the isotopic composition of diets and by quantifying growth and reproductive output, the relative contributions of assimilated nutrients can be tracked to different pools (Hesslein et al. 1993; Tieszen et al. 1983).We used a yeast diet with a high nutritional value for collembolans. In addition, we also measured the composition of the elements carbon, nitrogen and phosphorus to gain complementary information on the regulatory processes underlying life history traits and nutrient allocation.

Our results confirmed that *P. armata* primarily allocated its dietary resources to growth and that *P. minuta* allocated its resources to reproduction. The total production, i.e. reproduction and growth, was largest for *P. minuta*. We found support for our hypothesis that *P. minuta* had the largest tissue

replacement rates for both carbon and nitrogen. To partition between reproductive and metabolic tissue replacement, we used a two-compartment model that predicted how adults allocated nutrients to eggs. We estimated metabolic tissue replacement rates for both elements to ~10 % day^{-1} for *P. minuta* and ~6 % day^{-1} for *P. armata*. In comparison, reproductive tissue replacement rates were ~2 % day^{-1} for *P. minuta* and ~0.5 % day^{-1} for *P. armata*. The relative expenditures of metabolism relative to reproduction were thus lower for *P. minuta* than *P. armata*. The content of nitrogen and phosphorus were significantly higher in *P. minuta* than *P. armata* indicating that high production rates were correlated with high protein-N and RNA-P content in tissue.

In conclusion, we showed that *P. minuta* with high production rates and short generation times, has a competitive advantages compared to *P. armata*. However, this *r*-selected strategy also requires high quality diets; this can be critical because nitrogen and phosphorus are often limited nutrients in the soil decomposer system. The different nutritional requirements of Collembola can in part explain different dietary preferences observed by e.g. Chamberlain et al. (2006) and Jørgensen et al. (2005). While some of the dietary preferences might be linked to the collembolan's digestive systems and morphological features, we successfully unravelled some of the physiological mechanisms underlying nutritional requirements and allocations in Collembola.

3.4
Summary of Paper IV

Contrasting effects of amino acid imbalances on elemental turnover and isotope discrimination in three species of Collembola

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The quality of dietary resources is a key aspect of the function and performance of animals living in the detrital food web. Especially nitrogen is a limiting nutrient in the decomposer system, which is why it is essential that we understand how the function and performance of the system's inhabitants are regulated by nitrogen constraints. Several studies have examined the performance of decomposer animals under varying nitrogen levels (Booth and Anderson 1979; Haubert et al. 2005; Jensen et al. 2006; Lavy and Verhoef 1996). To our knowledge, however, no studies have yet investigated the influence of protein quality on the performance of soil decomposer animals. The qualitative approach of evaluating nitrogen constraints in soil is very important because several amino acids are indispensable for animals and can only be obtained through dietary sources.

In this study, we examined how three epedaphic collembolans, *Folsomia candida*, *P. armata* and *P. minuta*, all members of the decomposers community in the soil, were affected by two fungal diets with amino acid compositions that were balanced and imbalanced, respectively, to their requirements. We hypothesized that 1) the collembolans would decrease their performance (i.e. productivity and metabolism) on the imbalanced diet and that 2) the decreased performance would lead to an enrichment of ^{15}N in their tissue due to an increased recycling of amines. A higher recycling rate would increase the excretion of ^{14}N relative to ^{15}N due to isotope fractionation. We followed the performance of the collembolans by changing the isotope levels of ^{13}C and ^{15}N in their diets and subsequently tracked isotopic change in their tissue during a 28 day period. Change in isotopic composition would be due to growth, reproduction and metabolic tissue replacement (Paper III). We also tracked the elemental composition of carbon, nitrogen and phosphorus because it

could indicate whether the collembolans changed their biochemical constituents in response to the two diets.

Contrary to our hypothesis, animals were depleted in ^{15}N on the imbalanced diet in spite of a significant decrease in performance of *F. candida* and *P. minuta*. In contrast, *P. armata* did not decrease its performance on the imbalanced diet, and its tissue was depleted in ^{15}N on both diets. The decrease in performance by *F. candida* and *P. minuta* on the imbalanced diet was correlated with a significant increase in C content and decrease in N and P content ($p<0.05$). N:P ratios remained constant for both diets. These elemental changes indicate that the content of lipids or fatty acids increased while proteins, nucleic acids and nucleotides decreased. *P. armata* did not change its elemental content except for a significant increase in the C:P ratio on the imbalanced diet ($p<0.05$). However, this increase was small compared to the two other species.

We cannot provide an explanation for the depleted ^{15}N values on the imbalanced diet, but speculate that more isotopically light amino acids were readily available during assimilation than heavy amino acids. The range of ^{15}N values in individual amino acids has been found to be quite large and can vary from -9 to 16 ‰ (Petzke et al. 2005). Depleted ^{15}N values in collembolans have been reported a few times in the literature, mostly on low-N diets (Larsen et al. Manuscript; Ruess et al. 2004), and we suggest that isotope analysis of the individual amino acids is necessary to resolve this issue. *P. armata*, which, unlike the other two species, is a generalist feeder, maintained a stable performance on both diets and appeared to be the most robust of the three collembolans. Similar characteristics have been observed for generalist herbivores, but the nature of the mechanisms used to overcome nutritional imbalances remains unknown. Lavy and Verhoef (1996) observed compensatory feeding for the collembolan *Orchesella cincta* and proposed that surplus N was metabolized for energy. Collembolans might also contain endosymbioents releasing metabolites and enzymes, but only few investigations have been carried out on this subject (Borkott and Insam 1990). The correlation between decreases in isotopic change, i.e. performance, and higher carbon and lower nitrogen and phosphorous compared to the balanced diet was very strong. This suggests that the animals changed their biochemical composition in response to the imbalanced diet. A better understanding of the physiological responses and mechanisms employed to overcome nutrient-poor conditions is a key to understanding belowground species interactions, as well as to addressing the question of why the biodiversity of belowground systems is very high.

4

Discussion

4.1
Carbon and nitrogen dynamics in Collembola

How collembolans allocate nutritional resources to growth, reproduction and metabolism is important for understanding their role and physiology. In the nutrient allocation study (Paper III), the small and highly reproductive *P. minuta* had a higher replacement of tissue to metabolism and reproduction than the large and slow reproducing *P. armata* (Paper III). The results from the nutrient allocation study thus confirmed the first hypothesis that *r*-selected collembolans have higher metabolic rates of carbon and nitrogen than K-selected collembolans. While it was expected from metabolic theory that smaller organisms have more resources relative to body mass to allocate to reproduction (Brown and Sibly 2006) we also estimated that *P. minuta* had the highest reproductive efficiency relative to metabolic investments. Thus, the higher productivity of *P. minuta* not only compensated for a presumably shorter lifespan it also gained a competitive advantage over *P. armata* relative to invested resources. The underlying assumption is that the nutritional value of the diet was the same for both species. This was however not completely supported by our protein quality study because *P. armata* was limited by a higher number of indispensable amino acids in the diet than *P. minuta* (Paper IV). The influence of these dietary imbalances on nutrient allocations might, however, have been insignificant as the relative imbalance of the first limiting amino acid, methionine, was the same for both species. We only used one diet in the nutrient allocation study (Paper III) but the approach of combining mass balance and tracking isotope changes might be a promising tool for understanding the complexity of the processes involved in meeting nutritional requirements of soil dwelling collembolans.

We used direct and indirect methods to estimate collembolan release of N. The indirect method employed in the nutrient allocation study (Paper III), showed that *P. minuta* had the highest metabolic release of ~9 % N day^{-1} and *P. armata* had the lowest of ~5 % day^{-1}. These rates were surprisingly high compared to those determined by direct measurements of NH_4^+-N. Sjursen and Holmstrup (2004) reported release rates <0.8 % NH_4^+-N day^{-1} (assuming that 10 % of tissue is N) at 20ºC and our own similar measurements were 1.1 and 3.4 % NH_4^+-N day^{-1} for *P. armata* and *P. minuta*, respectively (Paper I). The excretion rates of NH_4^+-N were 22 and 38 %, respectively, compared to the metabolic rates from the nutrient allocation study thus rejecting the second hypothesis that collembolans primarily excrete metabolic nitrogen in NH_4^+-N form. This comparison of nitrogen rates might, however, be incorrect because of differences in incubations conditions between direct and indirect methods. In the direct measurement study, we incubated the animals for 6 hours (without food) whereas Sjursen and Holmstrup (2004) incubated their animals for 48 hours, and that probably explains why our values were higher and stresses the importance of carrying out physiological studies where the animals are active. We do not know whether metabolic nitrogen rates (Paper III) where higher than NH_4^+-N rates because of 6 hours of starvation, but the animals did not empty their guts completely during this period (as observed during the experiment) indicating that they continued to assimilate nutrients. Another explanation for the discrepancy might be that collembolans excrete N in other forms than NH_4^+-N (Verhoef et al. 1983). The influence on collembolan metabolism by resting conditions is also illustrated by comparing our estimates for carbon release (Paper III) with the measurements of O_2 consumption by Petersen (1980). Petersen's quantification of O_2 consumptions was equivalent to 0.74 and 0.86 kJ g^{-1} DW d^{-1} (thermal equivalent 20 kJ O_2 l^{-1}) for *Onychiurus furcifer* and *Folsomia nana,* respectively at 15ºC. *O. furcifer* and *F. nana* are the species in Petersen's study that resemble *P. armata* and *P. minuta* the most.

Assuming that all metabolically released carbon was catabolised to CO_2, our estimates yielded 1.3 and 2.6 kJ g^{-1} DW d^{-1} (thermal equivalent 24 kJ CO_2 l^{-1}) for *P. armata* and *P. minuta*, respectively. *P. armata* and *P. minuta* were reared at a slightly higher temperature (20ºC) than the collembolans in Petersen's study probably causing a small increase in metabolism. Our rates (Paper III) might, however, have been underestimated because we only estimated carbon catabolised from tissue and not directly from the diet. This discrepancy in carbon or energy release rates between animals with and without food leaves the question on how to apply laboratory data to the field where animals might be less active than the laboratory animals we reared. Field conditions can be mimicked in future nutrient allocation studies by restraining animal living conditions, for example by offering diets with lower nutritional value than *S. cerevisiae*.

4.2
Protein quality affects isotope discrimination

The discrimination factor between diet and consumer can contain valuable information about the nutritional value of the diet (Martínez del Rio and Wolf 2005). Several authors have hypothesized that animals in negative nitrogen balance become progressively enriched in ^{15}N (reviewed by Ben-David et al. 1999). Amino acid imbalances between diet and the consumer can contribute to negative N imbalances, why I hypothesized that $\delta^{15}N$ in tissue would increase relative to that of the diet as the protein quality decreased. This hypothesis was not supported because we measured depleted ^{15}N values in the tissue of collembolans raised on the low quality diet. In addition, *P. armata* was depleted in ^{15}N when raised on high quality diet in two studies (Paper III and Paper IV). This led us to speculate in the protein quality study (Paper IV), whether the depleted ^{15}N values by *P. armata* were caused by bacteriocyte associated-endosymbionts in the gut. If there were endosymbionts synthesizing amino acids in the gut this would alter the assimilatory pathways of nutrients and possibly tissue-diet discrimination of ^{15}N. While this is highly speculative, it could explain why the performance of *P. armata* was similar on both the balanced and imbalanced diets. Nutritional ecology studies have shown that to meet micronutrient requirements animals such as ruminants (Tan and Murphy 2004) and aphids (Baumann 2005) harbour endosymbionts. Endosymbionts releasing digestive enzymes has previously been found in Collembola (Borkott and Insam 1990). Whether collembolans have also generated a physiological dependency for micronutrients provided by symbiontic gut bacteria deserves further attention because it might explain how nutritional deficiencies are overcome in a nutrient limited environment.

Synthesis of amino acids by endosymbionts is a very unlikely explanation for the depleted ^{15}N values of *P. minuta* and *F. candida* because they performed poorly on the nutritionally imbalanced *F. culmorum* diet. An alternative explanation might be that the fungal diets assimilated nitrogen from organic and inorganic natural abundance sources with possibly slight differences in ^{15}N values. However, it fails to explain why *P. armata* was depleted in ^{15}N when raised on the homogenously labelled yeast diet (Paper III). Rather, the explanation might be that bulk ^{15}N values of the diets not were fully representative of what the animals actually assimilated. Several processes affect discrimination of isotopes and some of these occur as fractionation at the atomic level and others as mixing at the organismal or system level (Fry 2006). Isotope mixing on the system level is a general principle because most organisms live in open systems where resources constantly are replenished by multiple sources. At the organism level, the nutrients and the elements in them can undergo varying degrees of mixing through various metabolic pathways. A visual representation of this mixing can be found by looking at Fig. 3a and 3b in the protein quality study (Paper IV). When collembolans were fed the enriched, but heterogeneously labelled diets their isotope compositions were distinctly apart indicating that they assimilated nutrients and elements in them differently. That collembolans have different assimilatory pathways is also supported in the studies by Berg

(2004) and Urbasek and Rusek (1994) where the authors found significantly difference in specific enzymatic activities among collembolans.

Obtaining information on the mechanisms underlying isotope composition is often difficult when relying on bulk analysis of isotopes and micronutrients. Whether mixing was responsible for the depletion in ^{15}N might be solved by tracking biosynthetic sources. Biosynthetic pathways specific to different organisms has been used to determine trophic transfer of carbon to collembolans by analyzing the isotope composition of fatty acids (Evans et al. 2003; Haubert et al. 2006). Similarly, ^{15}N values of individual amino acids have been used to disentangle dietary sources of humans (Petzke et al. 2005) but the biosynthetic source of amino acid ^{15}N cannot be traced with the same precision as fatty acid ^{13}C. While the carbon-skeleton of a particular fatty acid remain intact until it is catabolized, the amines of most amino acids are readily transaminated during synthesis of proteins. The amino acids that do not undergo transamination are lysine and threonine (Preston 2006) and both amino acids are relatively abundant in collembolans (Paper IV). No collembolan studies have yet analyzed ^{15}N of these two amino acids in collembolans but the method might hold a great potential to trace two important N-sources because the organisms synthesizing amino acids, plants, bacteria and fungi, all have their particular ways of assimilating nitrogen possibly leading to different ^{15}N signatures.

4.3
Ecological stoichiometry

Ecological stoichiometry describes the role of stoichiometry (typified by C:N:P) in controlling trophic processes such as nutrient cycling, population dynamics and life history strategies. We investigated how diet quality and life histories affected the elemental composition in tissue (Paper III). Animals raised on the low quality diets increased their C:P and C:N ratios compared to high quality diet and maintained a constant N:P ratio indicating that carbon-rich storage compounds increased relative to nitrogen- and phosphorus-rich compounds such as proteins, nucleic acids and nucleotides (Ventura 2006). A similar compensation has been observed for insects (Sterner and Elser 2002) and Collembola feeding on low nitrogen diets (Haubert et al. 2004; Lavy and Verhoef 1996; Ruess et al. 2004). The animals compensate for low levels of dietary nitrogen by consuming more food to maintain a relatively constant amount of proteins and as a consequence accumulate more fat. It is also possible that the amount of proteins, nucleic acids and nucleotides decreased in addition to the increase in fat. The protein quality study (Paper IV) did not support the growth rate hypothesis that is based on the assumption that the phosphorus rich RNA is the most likely biochemical contributor to varying C:P and N:P in tissue (Sterner and Elser 2002). To meet protein synthesis demands of rapid production the organism would then raise its relative RNA content. The higher performance by the collembolans on the high quality diet compared to the low quality diet was not accompanied by lower N:P ratios, i.e. higher phosphorus relative to nitrogen. We did, however, find support for the growth rate hypothesis in the nutrient allocation study (Paper III). *P. minuta* eggs that are small and only need a short incubation period contained significantly more phosphorus relative to nitrogen (p<0.05, C:N ~20) than *P. armata* eggs (C:N ~32) (Paper III, Fig. 3, Table 1). Although we did find overwhelming support for the growth rate hypothesis, the availability of phosphorus in the diet probably plays an important role for collembolans. High phosphorus (and nitrogen) content in tissue was positively correlated with a high performance or productivity.

Soil dwelling Collembola have mostly been linked to the detritus based food web because they typically feed on decomposer organisms (Rusek 1998). In our wheat microcosm study (Paper II) we investigated whether collembolans obtained their carbon and nitrogen from soil, rhizosphere channel (photosynthates) or the added green manure. Applying a linear mixing model on the isotope data we estimated that the rhizosphere channel was the main carbon source for collembolans, ~60 %

of total carbon for *P. minuta* and *F. fimetaria* and ~78 % for *P. armata* (Paper II, Table 5). That rhizosphere carbon is important for fueling the soil food web is line with previous studies (Coleman et al. 2002; Garrett et al. 2001; Ostle et al. 2007) and supportive of the last hypothesis that root derived carbon would be the largest source of carbon for collembolans. The reason why *P. armata* derived more carbon from the rhizosphere channel than the two other species could be due to its capability to digest roots (Ulber 1983). Sources of carbon and nitrogen were quite similar for *P. minuta* and *F. fimetaria* in spite of their ecomorphological differences. That collembolans are closely linked to the rhizosphere channel was also demonstrated by Ostle et al. (2007) in their ^{13}C pulse-labelling study of rhizosphere fauna in grasslands. Collembolans accounted for ~45 % of the tracer ^{13}C measured within the fauna biomass (earthworms, enchytraeids and microarthropods) within a 20 days period after pulse labelling of plants. Collembola was the group of animals that incorporated most photosynthate carbon although they only represent 0.7 % of total fauna biomass. This led the authors to suggest that collembolans also are closely linked to the rhizosphere channel either directly by grazing on hyphae or root exudates or indirectly as a result of predation on soil microbes and microfauna.

Understanding nutrient flow is a central goal of both organismal biology and ecosystem ecology but only few models link the flows of carbon and nitrogen through groups of organisms (de Ruiter et al. 2005; Hungate et al. 1997). We modelled the direct release of NH_4^+-N by collembolans in the barley (Paper I) and wheat (Paper II) microcosm studies and concluded that their direct release of readily available plant nutrients was very small (<0.5 % of total plant nitrogen). However, the excretion by enchytraeids were a magnitude higher (~20 % of total plant nitrogen) than that of the collembolans but we did not detect a significantly greater plant growth in treatments with mesofauna compared to missing mesofauna treatments. A number of studies have found, however, that collembolans (and mesofauna) in spite of their small biomass can influence microbial metabolism and plant growth (Bardgett and Chan 1999; Cole et al. 2004; Partsch et al. 2006), which suggest that the functional characteristics of some species, rather than their biomass *per se*, is important for their role in maintaining ecosystem processes. For most soil animals, only scarce knowledge exists on diets, preferences and physiology. To integrate organismal biology into ecosystem functioning some of the techniques presented in this thesis might hold lot of potential (Scheu 2002). In food web experiments, it is essential to have a good knowledge of turnover rates of organismal nutrient pools because animals incorporate nutrients at different rates. This knowledge is also important when tracing specific sources in the soil food web. For example, in the aforementioned study by Ostle et al. (2007) it would be useful to integrate rates of metabolism and productivity with the isotope values of soil animals to elucidate whether tissue replacement rates or feeding preferences lead to the much higher incorporation of photosynthate carbon by collembolans than the other soil animals. In stoichiometric studies where elemental requirements by animals normally are obtained by quantifying elemental content of tissue knowledge of turnover or replacement rates is likewise important. Elements might be replaced at different rates thus affecting their requirements as we documented in the nutrient allocation study where replacements rates for carbon was ~10 % higher than for nitrogen.

4.4
Isotope additions

In complex biological systems, isotope values are net values of multiple processes, reflecting balances between gains and losses. At natural abundance, these processes can lead to large errors when tracking dietary sources because the difference between isotope values are small and often <10 ‰. To overcome the problems associated with fractionation and unknown mixing, an isotope label can be added to a source and its importance can readily be quantified. We used isotope additions for several purposes from investigating root uptake of low molecular weight compounds

to estimating nutrient allocations in Collembola. While a small amount of a label can give a strong signal, a lot of supplementary information is needed for an accurate interpretation of the isotope data. For example, our modelling of nutrient allocations to reproduction (Paper III) could be improved if we had a more knowledge on how eggs are developed in Collembola. However, some of the shortcomings of our model could be overcome by increasing the number of sampling days and number of replicates, which would allow us to constrain some of the parameters and increase the degrees of freedom. A disadvantage of isotope additions is that it is methodologically difficult and costly to label organic sources homogenously. In the wheat microcosm study (Paper II), we used homogenously labelled ryegrass as a green manure and mixed it with natural abundance ryegrass because of the great costs of labelled material. In the nutrient allocation study (Paper III), we obtained a homogenously labelled diet by growing a special strain of yeast capable of auto-synthesizing all the required amino acids. Growing the yeast diet was relatively inexpensive and the costs of labelled plant materials might get more inexpensive in the future as the demand increases. But it would be almost impossible to label municipal organic waste (Paper I) consisting of multiple sources homogenously. Even in a laboratory flask with just two nutrient sources, one organic and the other inorganic, the isotope values in the final product of different nutrients and their elements appeared to be very diverse (Paper III). These different isotope values did not appear because of fractionation, but because of mixing. To unravel the pathways of the individual compounds, it might hold a lot of potential to include compound specific isotope techniques in isotope addition studies because the variations in signals of the different compounds will be very well-defined. Integrating isotope additions with natural abundance surveys can also be useful to dismantle the mixing and fractionation puzzle. For example, in the natural abundance treatment with wheat (Paper II), we estimated root ^{15}N discrimination relative to the actual uptake of two sources rather than just bulk soil by integrating mixing data from the isotope addition treatment. Similarly, this approach could be used to estimate isotope discrimination by collembolans in studies where their diet is composed of multiple sources, and as a supplement to compound specific analysis. To separate between multiple food sources it would be necessary to add stable isotopes such as ^{18}O, ^{34}S and ^{2}H in addition to ^{13}C and ^{15}N. Another benefit of including analysis of multiple isotopes is that each element can address specific questions in relation to source mixing and partitioning of ecosystem processes. Most mass spectrometers are limited to analyzing one or two elements at a time but development of new techniques have already made it possible to analyze C, N, and S simultaneously, and in few years it might be possible to analyze all five elements from one sample.

4.5
Conclusions

This thesis investigated carbon and nitrogen dynamics of soil dwelling Collembola. To investigate these dynamics, we primarily used stable isotope additions in various experimental settings. Two collembolans with contrasting life history strategies, the small *P. minuta* and the large *P. armata*, were included in all four studies to disentangle different aspects of their biology and physiology. Direct measurement in the first study (Paper I) revealed that the excretion rate of NH_4^+-N were three times higher for *P.minuta* than *P. armata* indicating that *P. minuta* is the most metabolically active of the two species. In the next study (Paper II), collembolans were used as sampling devices for microbial uptake of labelled green manure that was added to wheat microcosms. *P. armata* derived more of its carbon from roots than *P. minuta* suggesting that the animals had different feeding preferences. The third study (Paper III) investigated nutrient allocations in Collembola by feeding them homogenously labelled yeast and subsequently monitoring production and change of isotope values. We confirmed our result from the first study (Paper I) that *P. minuta* is more metabolically active than *P. armata*. In spite of the higher metabolic tissue replacement rate of *P. minuta* than *P. armata*, *P. minuta* had the highest reproductive efficiency relative to metabolic

investment. The metabolic nitrogen rates determined were considerably higher than the NH_4^+-N rates determined in the first study (Paper I). This suggests that collembolans excrete metabolic nitrogen in other forms than NH_4^+-N. Another possibility is that removal of diet decreases collembolan metabolism considerably, thus leading to errors when estimating their release of nutrients under field conditions where the animals supposedly are active. The last study (Paper IV) investigated how protein quality affected the performance (i.e. productivity and metabolism) and ^{15}N values of collembolans. One species, *P. armata*, maintained the same performance on either diets contrary to *P. minuta* and *F. candida*. The physiological response to the low quality diet was a significantly increase in C:N and C:P ratios indicating that carbon-rich storage compounds increased relative to nitrogen- and phosphorus-rich compounds such as proteins and RNA. The changes in elemental composition were correlated with their performance. We found depleted ^{15}N values in the tissue of collembolans raised on the low quality diet. This is not supportive of the hypothesis stating that nutritional imbalances lead to retention of the heavy isotope. We suggest that mixing rather than inverse fractionation contributed to these results. In summary, this thesis investigated some aspects of collembolan physiology such as excretion of NH_4^+-N, carbon and nitrogen allocations and physiological response to protein quality. These findings contributed to a more accurate understanding of the implications of collembolan life history strategies. The results might also be applied to field labelling studies because knowledge of tissue replacement is important to estimate how fast animals incorporate and release carbon and nitrogen in the decomposer system.

5

Outlook

Human activities affect cycling of elements as well as ecosystem processes. Increased CO_2 levels and nitrogen depositions affect primary production with a subsequent cascading effect through the belowground food web because it is driven by the quantity and quality of plant-derived organic materials. The biotic interactions belowground are important in governing decomposition and mineralization processes. While most decomposition is directly carried out by bacteria and fungi, those animals that interact with microbes usually promote greater rates of microbial turnover and consequently more rapid rates of decomposition of organic materials and nutrient mineralization. The quantitative role that Collembola play in promoting decomposition might be relatively small compared other soil fauna members such as nematodes, enchytraeids and earthworms. However, this and previous studies have shown that collembolans are useful for understanding the biological mechanisms underlying carbon and nitrogen dynamics: Collembola respond quickly to changes in the soil food web because of their high metabolism, they are closely associated to rhizodeposition and their different feeding preferences make them useful as sampling devices of various biotic components in soil. So an accurate knowledge of their attributes can help us to extract information that can be applied at a larger scale. At the scale of the whole ecosystem, the entire producer and entire decomposer subsystems are obligately dependent on each other. Plants deposit organic compounds in their immediate rooting zone that are taken up by microorganisms. These microorganisms might in turn stimulate root development by releasing growth stimulating plant hormones. Further, by fueling microbes plant roots stimulate decomposition and eventually release of plant available nitrogen. How collembolans interact with this plant-microbial loop is not clear but they probably stimulate this loop at intermediate levels of grazing. The nature of these loops involving plant, microbes and soil fauna are likely to be affected by human-induced changes of elemental cycling. The increased CO_2 levels and nitrogen depositions favour plants adapted to fertile conditions. As a result, plant species that grow more rapidly generally produce higher-quality foliage and lower concentrations of lignin and structural carbohydrates; this result in a higher quality of those organic materials entering the soil food web in turn affecting soil biota composition and carbon and nitrogen dynamics (reviewed by Wardle 2002). These changes might eventually affect ecosystem stability and induce regime shifts. Such shifts are mostly documented in aquatic food webs (Scheffer et al. 2001) but terrestrial food webs might be equally affected as indicated by the succession of plant communities in for example meadows and heath lands. A major factor contributing to this succession is input of inorganic nitrogen that normally exists in low concentrations in natural ecosystems (van Breemen 2002). So the nitrogen cycle is not only affected quantitatively but also the qualitatively, and regime shift are as likely to occur aboveground as belowground. The different nutritional requirements by Collembola strongly suggest that nitrogen depositions will affect species composition. Likewise, increased rhizodepositions of readily available carbon compounds might also shift species composition. Not only species composition but also system processes are affected. To investigate changes in system processes stable isotope analyses are increasingly employed because anthropogenic sources are depleted in ^{13}C and ^{15}N compared to natural background levels (Fry 2006; Norra et al. 2005). A closer integration of natural abundance surveys and isotope additions might be a useful approach to partition between sources and especially if supplemented with compound specific isotope analysis and bulk analysis of multiple isotopes such as ^{13}C, ^{15}N and ^{34}S.

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This thesis investigated carbon and nitrogen dynamics of soil dwelling Collembola by using direct measurements and stable isotope additions. In an isotope change experiment, collembolans exchanged between 6 and 10 % of carbon and nitrogen in their body tissue per day to metabolism and between 0.5 and 2 % to reproduction. When collembolans on low and high protein diets were compared, animals on the low protein quality depleted their tissue ^{15}N values relative to those on high quality diet indicating that the nitrogen turnover decreased on the low protein quality diet. In a wheat microcosm investigating source contributions from soil, roots and isotope labelled green manure the mixing model analysis indicated that photosynthate (root derived C) was the main carbon source for collembolans (54 – 79 % of total C) indicating that the rhizosphere channel is very important for collembolans in addition to the detritus based channel.

Unravelling collembolan life belowground:
Stoichiometry, metabolism and release of carbon and nitrogen