Trophic interactions as indicators of ecosystem regeneration in disturbed grassland - A stable isotope approach

Dissertation

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"You are what you eat … plus a few per mil"

Michael J. DeNiro & Samuel Epstein (1981)

Influence of diet on the distribution of nitrogen isotopes in animals.
Geochimica et Cosmochimica Acta **45**, p. 348

Dedicated to Tobias, Selma and Eva

Curriculum vitae

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Selbständigkeitserklärung

Die geltende Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena ist mir bekannt.

Die vorliegende kumulative Dissertation habe ich selbständig angefertigt und keine anderen als die von mir angegebenen Quellen, persönlichen Mitteilungen und Hilfsmittel benutzt.

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Die vorgelegte Dissertation wurde weder als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung noch als Dissertation an der Friedrich-Schiller-Universität Jena beziehungsweise an einer anderen Hochschule eingereicht.

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Overview of the thesis and the included manuscripts

This thesis comprises an introductory chapter, four scientific manuscripts, and a final discussion. Being the first author of manuscripts I – IV I largely completed the following tasks myself: (1) organized and performed the laboratory experiments and the field work, the selection and preparation of samples and their analysis, (2) researched the respective references, (3) composed the manuscripts, incorporating the comments of the co-author, and (4) revised the manuscripts until final acceptance by the editors.

The introduction outlines the relation between ecosystem recovery, nutrient cycling and food web research. Ecosystem development following disturbance is controlled by regeneration processes which return the system into a "healthy" state. This requires the re-establishment of ecosystem structure and functions. The (re-)assembly of communities and the involved changes of the nutrient cycles strongly influence food web structure and, therefore, become reflected by the development of the trophic network. One way to characterize trophic relationships is by means of stable isotopes. The potential of stable carbon and nitrogen isotopes for studying food web regeneration is illustrated.

Manuscripts I – III provide experimental proof that the stable isotope technique represents a powerful tool to study trophic interactions. Model food chain experiments have been conducted using terrestrial isopods (woodlice). This group of macro-decomposers very likely played a major role in rebalancing decomposition processes in a disturbed grassland that has been investigated by the graduate research group "Analysis of the Functioning & Regeneration of Degraded Ecosystems". The experiments focussed on factors affecting the animal performance and the changes of the elemental and isotopic composition of their biomass. In addition, the chemical and isotopic alteration of organic matter accomplished by woodlice and the role of coprophagy in this context had been investigated. Manuscript IV contains the results obtained by applying stable nitrogen isotopes to analyze structure and functioning of regenerating food webs under field conditions. Spatial differences and temporal dynamics of animal ^{15}N signals are used to assess the developmental state of ecosystems differently impacted by pollution.

The discussion integrates the overall results of the manuscripts with respect to:

– the suitability of the used methods,

– the potential role of macro-decomposers in regeneration processes,

– the significance of coprophagy for the transformation of organic matter, and

– the use of stable isotope techniques as a tool in ecological restoration.

Manuscript I

Rothe, J., Gleixner, G. (2000) **Do stable isotopes reflect the food web development in regenerating ecosystems?** - *Isotopes in Environmental & Health Studies* 36, 285-301.

We evaluated the magnitude of isotopic changes occurring in woodlice feeding on artificial diets of different quality. It seems accepted that feeding on a certain food should lead to an isotopic enrichment of the consumer over its food source in the range of 3 – 4‰ in ^{15}N and 0 – 2‰ in ^{13}C. However, a number of factors such as food quality, animal age and sex influence trophic isotopic enrichments.

We have shown that low quality diet caused large shifts in woodlouse $\delta^{15}N$ and $\delta^{13}C$ values. The isotopic difference between consumer and diet even exceeded the reported trophic level shifts (TLS). We suggested that burning of proteins and storage fats to overcome nutritional stress were the main reasons. In one treatment the uptake of faeces (coprophagy) by woodlice partly balanced the shortage of nutrients. This, however, caused even higher animal ^{15}N enrichment because the recycled faeces had higher $\delta^{15}N$ values than the supplied food. Modelling the contribution of recycled faeces to the diet (assuming a constant TLS of 3‰) indicated that coprophagy is an important factor influencing the isotopic signals of animals.

Manuscript II

Rothe, J., Gleixner, G. (submitted) **The effect of coprophagy by woodlice (*Porcellio dilatatus*) on C and N balances and on the isotopic signatures of animals and faeces in experimental microcosms**. - *Soil Biology & Biochemistry*.

With regard to the previous experiment we investigated the importance of coprophagy for the nutrition of woodlice. We also studied the influence of coprophagy on the isotopic composition of woodlice and faeces. Woodlice recycling faeces performed better with regard to carbon and nitrogen acquisition. This was probably an effect of faster adaptation of the digestive apparatus to the experimental diet and of the supply with essential compounds, enzymes and pre-digested matter derived from the microorganisms in the faeces. Recycled faeces initially accounted for ca. 20% of the diet. Its contribution reduced to ~10% indicating the special importance of coprophagy in the first phase after a food change. While $\delta^{15}N$ and $\delta^{13}C$ values of the animals remained nearly constant, the δ values of the faeces significantly increased due to coprophagy. Extrapolation of the trophic shifts ($\Delta^{15}N$ and $\Delta^{13}C$) demonstrated that the trophic isotopic enrichment approached the expected levels faster if woodlice utilized faeces.

Manuscript III

Rothe, J., Gleixner, G. (submitted) **Elemental and isotopic changes during the transformation of organic matter by terrestrial isopods (Crustacea, Oniscidea) and the significance of coprophagy**. – *Applied Soil Ecology*.

To elucidate the role of macro-decomposers for the alteration of organic matter (OM) and to identify the contribution of coprophagy we fed adult woodlice artificial diet labelled with ^{13}C by adding C3 and C4 cellulose. Faeces derived from that diet were exchanged between both groups. The feeding experiment ran for 8 weeks.

Despite significant changes in the C and N contents as well as in the δ values of animals, the isotopic shift between woodlice and diet generally remained too small to account for the trophic level enrichment in ^{13}C and ^{15}N, respectively. We suggested that high diet quality and low level of internal C and N turnover in full-grown animals were responsible for the lack of isotopic equilibration. Relative to the diet the faeces produced were enriched in ^{13}C (up to 4‰), in ^{15}N (~ 2.5‰) and in the proportion of cellulose causing higher C:N ratios. Apart from the transformation of the ingested diet into faeces during the gut passage, significant elemental and isotopic changes by woodlice were shown also for the non-ingested part of the supplied OM.

Manuscript IV

Rothe, J., Gleixner, G. (accepted) **Application of stable nitrogen isotopes to investigate food web development in regenerating ecosystems**. - In: Temperton V, Nuttle T, Hobbs R, Halle S (eds.) *Assembly Rules and Restoration Ecology – Bridging the Gap Between Theory and Practice*. Island Press, California.

In a study on the unassisted recovery of disturbed grassland we collected plant, animal and soil samples at four locations along the pollution gradient at various times in the regeneration process. Biological samples were selected to represent different taxonomic units, functional groups as well as trophic levels to obtain a general picture of the food web structure by arranging the species according to their $\delta^{15}N$ values.

Woodlice, herbivorous beetles and herbivorous bugs represented the level of primary consumers. The woodlouse species could be differentiated into primary and secondary decomposers. Carnivorous beetles and bugs were enriched in ^{15}N relative to decomposers and herbivores. However, the average enrichment accounting for all supposable trophic interaction varied among the four sites and was only partly related to the level of impact. In addition, differences in the temporal dynamics of animal and soil $\delta^{15}N$ values suggested, that both least impacted systems already recovered from disturbance.

Contents

1. Abstract

Using stable isotope techniques to identify regeneration patterns in the trophic structure of the reassembling communities, this thesis aims to describe the development of a disturbed grassland ecosystem (Steudnitz) that had been subjected to anthropogenic pollution for more than 30 years. The study should contribute to the understanding of processes and factors governing ecosystem development which provides the scientific basis for the ecological restoration of landscapes degraded by man, e.g. by mining activities or industrial pollution.

Prior to the application of this method to field samples from the Steudnitz ecosystem, an essential step was to test the suitability of stable carbon and nitrogen isotopes to characterize trophic interactions and to check the validity of assumptions required to interpret stable isotope data such as the '3.4‰-rule'. Three feeding experiments were conducted in order to study the isotopic relations between consumer and diet and to identify additional factors that influence the isotopic signature of organisms. The isotopic shift between an animal and its food source(s) is most important for evaluating the significance of trophic links and for defining the animal's food web position. In the experiments, woodlice (*Porcellio dilatatus*) were used as they represent model organisms which probably take up a vital role in ecosystem regeneration.

In the first experiment woodlice were reared on two different artificial diets for seven weeks. The trophic isotopic enrichments in both, ^{15}N and ^{13}C, varied among the two groups of woodlice depending on the quality of their diet (C:N ratios of 54 vs. 200). In the case of the slightly better diet, the ^{15}N shift of 5.7‰ exceeded the expected magnitude – the so-called trophic level shift (TLS), reported with 3.4 ± 1.0‰ – by more than 2‰. For this unexpectedly strong enrichment coprophagy by woodlice was addressed. While the uptake of ^{15}N-enriched faeces in addition to the diet in order to overcome nutritional shortage explained trophic shifts above the normal level, the differences in the amount of recycled faeces and in the nitrogen content of the ingested diet caused the different ^{15}N shifts in both groups of woodlice.

Furthermore, physiological stress due to low diet quality was reflected also by the $\delta^{13}C$ values of the animals. Burning of storage fat which tends to be depleted in ^{13}C relative to the bulk animal biomass resulted in ^{13}C shifts of 1.6‰ and 1.0‰, respectively, whereby the higher value reflecting a lower diet quality. The results obtained by creating nutritional stress to the consumers ('starvation effect') show that the effect of diet quality on animal isotopic signatures may superimpose the regular trophic isotopic enrichment. Just as low diet quality, also fasting causes isotopic enrichment in the animal. Coprophagy was demonstrated to be a third factor additionally influencing trophic isotope fractiona-

tion. The investigation of trophic interaction by means of stable isotopes has to account for these factors. Moreover, modelling of the proportion of faeces in the overall diet suggested a considerable contribution of coprophagy to woodlouse nutrition.

The second experiment revealed the significance of coprophagy for the nutrition of woodlice. In order to demonstrate the effect of coprophagy on the elemental and isotopic composition of the organisms and of the produced faeces, one group of woodlice was prevented from coprophagy by daily faeces collection. Coprophagy clearly affected C and N balances and the isotopic signatures of animals and faeces as it led to increased food consumption and mass loss from the system but reduced the amount of collectable (i.e. non-eaten) faeces and the loss of woodlouse biomass. The stronger increase of $\delta^{15}N$ and $\delta^{13}C$ values in the faeces derived from food and faeces (0.62 and 0.52‰ $week^{-1}$, respectively) compared to faeces derived from pure food (0.27 and -0.02‰ $week^{-1}$, respectively) provided evidence that the metabolic turnover in woodlice was positively influenced by coprophagy.

The proportion of faeces in the diet was inversely related to the level the digestive system had adapted to the new diet. While accounting for more than 20% in the first week, this proportion declined suggesting that woodlice were gradually adjusting to the supplied high quality food. In contrast to other studies, it was concluded that woodlice generally benefit from coprophagy and that the availability of faeces is a premise for the optimal performance of these macro-decomposers. The results point to the ecological role of coprophagy for organic matter decomposition and to the potential interference with the natural ^{15}N and ^{13}C labelling of food web components.

Clarifying the effect of changing food sources on animal biomass, the role of woodlice in organic matter transformation as well as the significance of coprophagy for the alteration of organic matter and the isotopic adaptation of woodlice, the third experiment contributed to the understanding of organic matter cycling in terrestrial ecosystems. For tracing the flow of matter, adult woodlice were fed two types of ^{13}C-labelled food and recycled either self-produced faeces or supplied faeces, the latter being differently labelled than the food. During the conversion from food into faeces organic matter ingested by woodlice became enriched in ^{13}C (up to 4‰) and ^{15}N (~ 2.5‰). By grazing the microorganisms growing on the surface of food items, woodlice altered even the non-ingested part of the supplied faeces. Enabled by the application of isotopically labelled food and faeces, the influence of coprophagy on the elemental and isotopic composition of woodlice and faeces was demonstrated.

During the adaptation of woodlice to the new food the contribution of faeces to nutrition decreased from 18% to 6%, whereas the efficiency of faeces assimilation gradually increased from ~50% to more than 80%. This shows that coprophagy accelerates the

physiological adaptation to changing food sources and improves food utilization by decomposing animals. However, the isotopic adaptation of adult woodlice to a new diet was very slow. In addition, woodlouse $\delta^{13}C$ values surprisingly decreased irrespective of the ^{13}C label of the diet. This reflected that some component of the mixed diet became preferentially assimilated. After eight weeks the isotopic shift between woodlice and diet was still too small to account for the reported trophic level shift for ^{13}C and ^{15}N. The high quality of the supplied diet combined with a low internal C and N turnover in full-grown animals were addressed.

Based on the experimental experience that indicated the suitability of stable isotopes to investigate trophic relationships and, within this, to reliably trace the path of organic matter, ^{15}N signatures of plants, animals and soil were analyzed in order to describe food web development in a regenerating grassland ecosystem (Steudnitz) and to get insight into the mechanisms and principles governing the structural and functional reorganisation of communities after disturbance.

The mean value of the frequency distribution of $\delta^{15}N$ differences between consumers and their diet indicated the adjustment of food web components to their food sources with trophic level enrichments between 3 and 4‰ (‘3.4‰-rule’) at the most recovered sites which were the least disturbed sites on the upper slope. Changes of the animal ^{15}N signatures with time reflected the development of the food web at the four differently impacted sites. Similar dynamics, i.e., minimal variations between species representing different trophic levels, imply functional stability of the trophic relationships.

The difference between soil and animal $\delta^{15}N$ values mirrored the level of functional integration between soil food web and aboveground food web. Calibration against reference systems should allow evaluating the developmental state and to assess regeneration progress. In addition, parallel development of the $\delta^{15}N$ values of epigeic species relative to those of the soil indicated the attainment of stable relations between aboveground and belowground processes enforcing ecosystem stability. The study highlights that – due to its ‘isotopic memory’ – substantial information to understand the functioning of terrestrial ecosystem may be found in the soil.

2. Zusammenfassung

Ziel der vorliegenden Arbeit ist es, die Entwicklung eines Rasenökosystems zu beschreiben, das für mehr als 30 Jahre starken anthropogenen Belastungen ausgesetzt war (Steudnitz, Thüringen). Dafür wurden die in der Struktur des Nahrungsnetzes verankerten raum-zeitlichen Regenerationsmuster entlang eines Belastungsgradienten mit Hilfe von stabilen Isotopen charakterisiert. Die Arbeit soll damit zum tieferen Verständnis der für die Ökosystementwicklung maßgeblichen Prozesse und Faktoren beitragen, deren Kenntnis die wissenschaftliche Voraussetzung für eine *ökologische* Restaurierung vom Menschen zerstörter Landschaften darstellt.

Vor Anwendung der analytischen Methode auf Pflanzen-, Tier- und Bodenproben des Ökosystems Steudnitz, wurden die Eignung stabiler Kohlen- und Stickstoffisotope, trophische Interaktionen zu charakterisieren, und die Gültigkeit von Grundannahmen, die für die Interpretation von Isotopendaten erforderlich sind (zum Beispiel der „3,4‰-Regel"), kritisch überprüft. In drei Laborversuchen wurden die Beziehungen zwischen den Isotopengehalten von Konsument und Nahrung im Detail untersucht. Zudem wurde nachgewiesen, welche Faktoren zusätzlich zur Nahrung Einfluss auf die Isotopensignatur von Organismen ausüben.

Schlüsselparameter, um die ökologische Wertigkeit trophischer Verknüpfungen abzuschätzen und die Position der Organismen im Nahrungsnetz zu definieren, ist die Differenz der Isotopengehalte (δ-Werte) von Konsument und Nahrung, die auch als ‚trophische Verschiebung' oder ‚Trophiestufeneffekt' bezeichnet wird. Als Modellorganismen dienten Asseln, eine Makrodestruenten-Gruppe, die für die Regeneration des analysierten Ökosystems wahrscheinlich eine wichtige Rolle spielte.

Im ersten Experiment variierte die trophische ^{15}N- und ^{13}C-Isotopenanreicherung in den Tieren in Abhängigkeit von der Nahrungsqualität (C:N-Ratio: 54 bzw. 200). Nach 7 Wochen überstieg die mit der besseren Nahrung verbundene ^{15}N-Verschiebung (5,7‰) den empirischen Richtwert für den isotopischen Abstand zwischen zwei benachbarten Trophiestufen von 3,4±1,0‰ um mehr als 2‰. Die unerwartet starke Anreicherung wird damit begründet, dass die Tiere zusätzlich zur Nahrung ihre eigenen, ^{15}N-angereichten Faeces nutzten (Koprophagie), um den Nährstoffmangel auszugleichen. Die ungleiche ^{15}N-Anreichung in beiden Asselgruppen folgte den Unterschieden im Stickstoffgehalt des Futters und in der Menge genutzter Faeces.

Der aus der geringen Futterqualität resultierende physiologische Stress spiegelte sich auch in den $\delta^{13}C$-Werten der Tiere wider. Die Metabolisierung körpereigenen Fetts, welches im Vergleich zum Gesamtbiomasse an ^{13}C verarmt ist, führte zu trophischen ^{13}C-Verschiebungen von 1,6 bzw. 1,0‰. Dabei war die Größe der Verschiebung proportional zur C:N-Ratio des Futters. Die durch Ernährungsdefizite hervorgerufenen Veränderungen der Isotopensignatur der Konsumenten beweisen, dass Futtereffekte die normale trophische Anreicherung überlagern können. So führt z.B. Hungern ebenfalls zur ^{15}N- und ^{13}C-Anreicherung der Organismen. Auch Koprophagie gehört zu den Faktoren,

die bei der Untersuchung trophischer Interaktionen mittels stabiler Isotope berücksichtigt werden müssen, da sie die trophische Isotopenfraktionierung beeinflussen. Modellrechnungen zeigen außerdem, dass Koprophagie signifikant zur Ernährung von Asseln beitragen kann.

Um den Koprophagie-Effekt auf die Element- und Isotopenzusammensetzung der Konsumenten und der Faeces zu demonstrieren, wurde im zweiten Experiment in einem der beiden Ansätze durch tägliches Absammeln der Faeces Koprophagie durch Asseln stark eingeschränkt. Es zeigte sich, dass sowohl die C- und N-Bilanzen als auch der Isotopengehalt der Tiere und der Faeces durch Koprophagie deutlich beeinflusst waren. Faecesnutzung steigerte Konsumption und Atmungsverluste, verringerte dagegen die Biomasseverluste der Asseln. Höhere Anstiege der $\delta^{15}N$- und $\delta^{13}C$-Werte bei aus Futter *und* Faeces gebildeten Faeces (0,62 bzw. 0,52 ‰/Woche) im Vergleich zu rein aus Futter gebildeten Faeces (0.27 bzw. -0.02 ‰/Woche) deuten an, dass Koprophagie den Stoffwechsel der Asseln positiv beeinflusst.

Der Faecesanteil in der Gesamtnahrung korrelierte negativ mit dem Anpassungsgrad des Assel-Verdauungssystems an das neue Futter. In der ersten Woche machten Faeces ca. 20% der Nahrung aus. Nach 8 Wochen hatte sich ihr Anteil auf unter 10% verringert, was verdeutlicht, dass die Tiere aufgrund der immer besseren Ausnutzung der Nahrung Faeces zur Deckung ihres Energiebedarfs nicht mehr benötigten. Die Grundfunktion von Koprophagie – der Erwerb essentieller Substanzen – blieb jedoch erhalten. Deshalb wird, im Gegensatz zu anderen Arbeiten, betont, dass Koprophagie für Asseln von effektivem Nutzen ist und längerfristig eine Voraussetzung für optimales Wachstum und optimale Ernährung darstellt. Daraus lässt sich die ökosystemare Bedeutung dieser Ernährungsstrategie für den Abbau organischen Materials und den Nährstoffkreislauf ableiten. Außerdem wird der potentielle Einfluss von Koprophagie auf die natürliche ^{15}N- und ^{13}C-Markierung der Organismen im Nahrungsgefüge hervorgehoben.

Um Stoffkreisläufe in terrestrischen Ökosystemen besser zu verstehen, befasste sich das dritte Experiment detailliert mit den Wechselbeziehungen zwischen benachbarten Trophiestufen. Es untersuchte den Einfluss des Futterwechsels auf die Konsumenten, die Rolle der Asseln für die Umwandlung organischer Substanz sowie die Auswirkungen von Koprophagie auf den Umwandlungsprozess und die isotopische Anpassung der Asseln an das neue Futter. Mit dem Ziel, den Stofffluss, besonders das Rezyklieren der Faeces, nachvollziehen zu können, wurden adulte Asseln über 8 Wochen mit zwei verschieden ^{13}C-markierten Futtermischungen und gleichartig bzw. invers zum Futter markierten Faeces ernährt. Die Umwandlung des Futters in Faeces im Darmtrakt der Asseln führte zur ^{13}C- (bis 4‰) und ^{15}N-Anreicherung (~2,5‰) des organischen Materials. Am Beispiel der Faeces wurde nachgewiesen, dass die Tiere durch Abweiden der die Futteroberflächen besiedelnden Mikroorganismen auch die Nahrungsreste verändern. Die ^{13}C-Markierung ermöglichte, den Koprophagie-Effekt auf die Element- und Isotopenzusammensetzung der Asseln und der Faeces zu studieren, ohne die Faecesaufnahme an sich zu beschränken.

Im Zuge der Anpassung der Asseln an das neue Futter verringerte sich der Anteil der Faeces an der Nahrung von 18% auf 6%. Gleichzeitig stieg in 8 Wochen die Assimilationseffizienz der aufgenommenen Faeces graduell von ca. 50% auf über 80% an. Diese Ergebnisse verdeutlichen, dass Koprophagie die physiologische Anpassung der Tiere an sich ändernde Nahrungsquellen beschleunigt und die Futterverwertung erheblich verbessert. Die isotopische Anpassung der adulten Asseln an die Nahrung verlief dagegen sehr langsam. Zudem verringerten sich in allen Fällen die Assel-δ^{13}C-Werte unabhängig von der Markierung des Futters, ein Hinweis darauf, dass bestimmte Komponenten des Mischfutters bevorzugt assimiliert wurden. Nach 8 Wochen lag die trophische Anreicherung immer noch weit unter dem erwarteten Trophiestufeneffekt. Als Ursachen dafür kommen zum einen die hohe Qualität des Futters, zum anderen der reduzierte C- und N-Stoffwechsel in den Geweben ausgewachsener Tiere in Betracht.

Gestützt auf die experimentellen Erfahrungen, die bestätigen, dass sich stabile Isotope für die Untersuchung trophischer Beziehungen und die Kennzeichnung von Stoffflüssen eignen, wurden die ^{15}N-Signaturen der im Rasenökosystems Steudnitz entnommenen Proben ausgewählter Pflanzen- und Tierarten und des Bodens analysiert. Mit der Beschreibung der Regeneration des Nahrungsnetzes soll der Einblick in die Mechanismen und Grundregeln, die der Reorganisation der biozönotischen Strukturen und Funktionen nach Ökosystemstörung zu Grunde liegen, vertieft werden.

Der Mittelwert der Häufigkeitsverteilung der gemessenen ^{15}N-Verschiebungen zwischen Konsumenten und deren Nahrung ist ein quantitatives Maß für die Anpassung der Nahrungsnetzkomponenten an ihre Nahrungsquellen. Die in der Regeneration am weitesten fortgeschrittenen Flächen am Oberhang wiesen eine mittlere trophische Anreicherung von 3-4‰ auf, d.h., die 3,4‰-Regel bestätigte sich. Die zeitlichen Veränderungen der δ^{15}N-Werte der Tiere spiegeln wider, dass sich die vier unterschiedlich geschädigten Flächen tatsächlich weiterentwickelt haben. Eine ähnliche Dynamik der δ^{15}N-Werte, d.h., eine minimale Varianz zwischen Arten verschiedener Trophiestufen impliziert dabei eine entsprechende funktionelle Stabilität in den trophischen Wechselbeziehungen.

Die Differenz zwischen den δ^{15}N-Werten der Tiere und des Bodens diente als Zeigerwert für den Grad der funktionellen Verflechtung von Boden- und oberirdischem Nahrungsnetz. Basierend auf dem Vergleich dieser Differenzwerte mit Werten aus Referenzökosystemen, sollten die Abschätzung des Entwicklungsstandes des Systems und die Bewertung des Regenerationsfortschritts möglich sein. Ein weiterer diagnostischer Parameter ist der Grad der Konformität (d.h. der Parallelität) in der zeitlichen Entwicklung der δ^{15}N-Werte der untersuchten Tierarten in Relation zum Boden. Er gibt an, ob stabile Beziehungen zwischen ober- und unterirdischen Prozessen vorherrschen, welche wiederum eine Basis für Ökosystemstabilität darstellen. Die Arbeit unterstreicht, dass der Boden aufgrund seines ‚Isotopen-Gedächtnisses' wesentliche Informationen für das Verständnis der Funktion terrestrischer Ökosysteme speichert.

3. Introduction

This thesis is embedded in the scope of the graduate research group "Analysis of the Functioning & Regeneration of Degraded Ecosystems", hosted by the Friedrich-Schiller-University Jena. Since 1996 this group (Graduiertenkolleg) has been investigating the natural unassisted recovery of a severely degraded grassland ecosystem near the village Steudnitz 10 km north of Jena. The study site and its degradation history are comprehensively described by Heinrich (1984). In short, the grassland had been impacted for more than 30 years by the emission of alkaline dusts and gases containing high amounts of phosphorus, sodium, calcium as well as potentially toxic elements such as cadmium, lead, fluorine and many others (Langer and Günther, 2001). The pollutants originated from an adjacent phosphate fertilizer plant and became distributed by wind across the exposed slope according to the size of dust particles (Fig. 1). The impact was most dramatic on the lower slope. There it resulted in the total loss of plant species except for one salt tolerant grass. The impact gradually reduced with increasing distance from the emission source (Fig. 1).

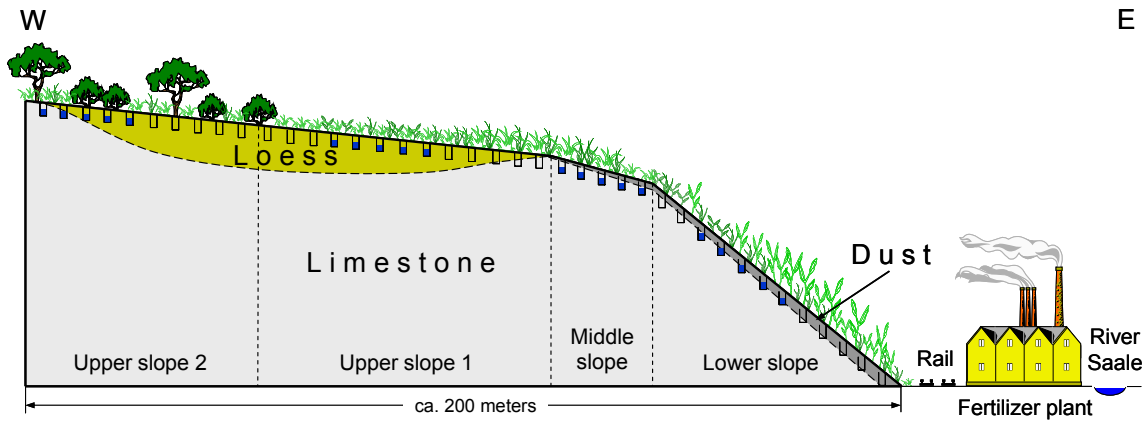

Figure 1 Steudnitz research area: Profile view along the pitfall trap transect, divided into four sections. Within each section animal material from 5 pitfall traps (indicated as half-filled) and the adjacent sweep net catches was used for stable isotope analysis

The regeneration of the grassland system started in 1990 after the shut down of the fertilizer plant. While the recovery was well reflected by the vegetation succession at different locations along the pollution gradient (Heinrich et al., 2001), there was only limited understanding of the underlying processes and controlling factors. Therefore, a graduate research group was established aiming to identify which basic ecosystem functions had been destroyed and – starting from this degraded state – to extract the

general processes and guiding principles which drive regeneration. The results should be integrated in a conceptual regeneration model applicable to a variety of ecosystems.

In order to successfully manipulate ecosystems practitioners need to be provided with the know-how in terms of concepts, models, methodologies and tools (SER, 2002). Since acquiring fundamental knowledge on how structure and function re-establish and how communities re-assemble is a rather complex task, the graduate research group favoured a complex interdisciplinary approach to ecosystem analysis. Co-workers from the fields of ecology, biology, chemistry, geography and soil science contributed to get insight into the interactions between ecosystem components acting in different compartments (soil, aboveground) and to describe the involved processes as summarized in a preliminary structural model (Fig. 2).

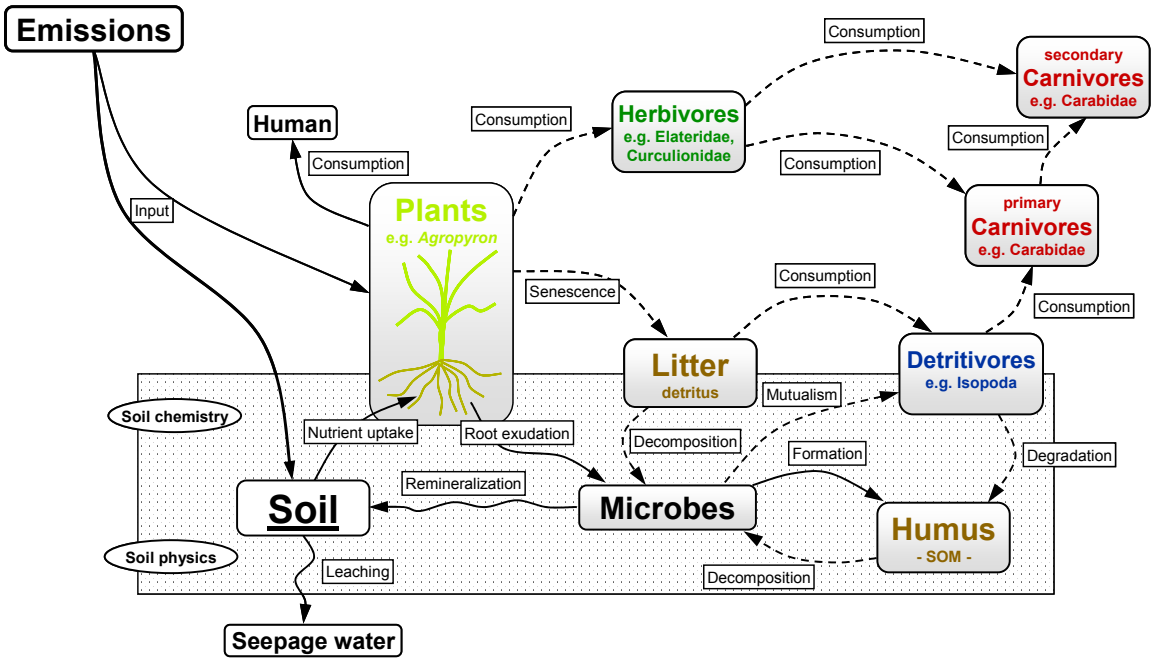

Figure 2 Structural model of the components and processes investigated in the Steudnitz grassland ecosystem by the graduate research group "Analysis of the Functioning & Regeneration of Degraded Ecosystems" in its first (1996-1999) and second phase (1999-2002). Components (shaded boxes) and interactions (dashed arrows) related to the experiments and to the field work included in this thesis are highlighted.

Based on the analysis of stable isotopes of the elements carbon and nitrogen, the main goal of the thesis is to describe ecosystem development following disturbance by reconstructing the general food web structure of the community at differentially degraded sites and at different times of regeneration. Using stable isotope data, it tries to assess regeneration progress and suggests important factors that may have controlled the

development of the Steudnitz system from a degraded state towards recovery. One factor is the cycling of elements, especially of carbon and nitrogen, which demands the functioning of the decomposer system consisting of decomposing animals (detritivores) and microorganisms, mainly acting in the soil and on the soil surface. Upon trophic interactions organic matter is transferred from the base of the food web to the top-consumer level. These interactions involve changes of the elemental and isotopic composition of the transferred organic matter and of the respective consumers; i.e., paths of carbon and nitrogen through an ecosystem that define food web structure can be traced. For this purpose, stable isotope techniques have been revised in the lab and were finally applied to the investigated grassland system. The marked ecosystem components (shaded boxes) and processes (dashed arrows) refer to the investigations covered by this thesis (Fig. 2). Three feeding experiments using woodlice, an important group of macro-decomposer species in the Steudnitz system, dealt with changes of the elemental and isotopic composition of the animals themselves as well as of the consumed and unconsumed organic matter (Manuscripts I – III). The trophic interactions between organisms representing various food web levels were the main focus of the field study on ecosystem regeneration (Manuscript IV).

3.1. Ecosystem regeneration

Ecosystem regeneration following disturbance comprises the re-establishment of abiotic and biotic system components, of their relationships and of essential ecosystem functions. In this process the system leaves the degraded state and moves on a certain trajectory towards its recovery (Hobbs and Norton, 1996). The regenerated ecosystem will not necessarily represent the pre-disturbance state since contemporary constraints and conditions may cause it to develop along an altered trajectory (SER, 2002). While ecological restoration attempts to return an ecosystem to its historic trajectory, this trajectory of a heavily impacted system may be difficult or impossible to determine. The latter holds true also in the case of unassisted recovery by natural succession. For that reason, we do not know exactly how the Steudnitz grassland system would look like without the long-term impact by the fertilizer plant. This implies that we are constricted in assessing whether after ten years of regeneration the present system can be regarded as fully recovered or not.

Nevertheless, there is a general understanding as to which conditions have to be assured in order to make ecosystems function (Bradshaw, 1987; Begon et al., 1996). One of them is the flow of energy which is partly combined with the cycling of nutrients (Odum, 1973). While nutrient availability in the broad sense is clearly one of the key factors controlling ecosystem development, the thesis concentrates on the two most important organic elements: carbon (C) as the dominant energy source of heterotrophic organisms and nitrogen (N).

3.2. Carbon and nitrogen cycling

In the biosphere the cycles of C and N are tightly connected because both elements are combined to organic molecules. In terrestrial ecosystems organic matter is primarily produced by plants. Primary production comprises the fixation of atmospheric CO_2, the uptake of N mainly from the soil and the conversion of inorganic forms of C and N into organic forms. After its formation the major part of the organic matter finally becomes converted back into inorganic compounds, comprising the processes of respiration (formation of CO_2 from organic C sources), excretion and remineralization. The latter process is mainly achieved by microorganisms but involves the action of a great number of animals, together referred to as the decomposer system. Primary production, decomposition and remineralization are vital ecosystem functions which need to be in balance in order to maintain the nutrient cycles and the energy flow through the system connecting all biota (Odum, 1973).

Beside other factors such as salt stress, water stress and toxicity, the imbalance between primary production and decomposition in particular could have been a major obstacle for development in the initial phase of regeneration at the most impacted sites of the Steudnitz ecosystem. It has been reported that the soil microflora suffered from the pollution (Langer and Günther, 2001) and that decomposing animals suitable to prevent accumulation of plant litter were lacking (Peter, 1984). From 1990 onwards macro-decomposers such as woodlice re-colonized the site (Eggers, 1997) in parallel with the amelioration of the soil conditions (Metzner et al., 1997). Until 1994, in the initial phase of regeneration, the individual numbers of the two most abundant woodlouse species increased only slowly. During that time abundances were inversely correlated to the level of impact showing highest values at the least disturbed site (Fig. 3).

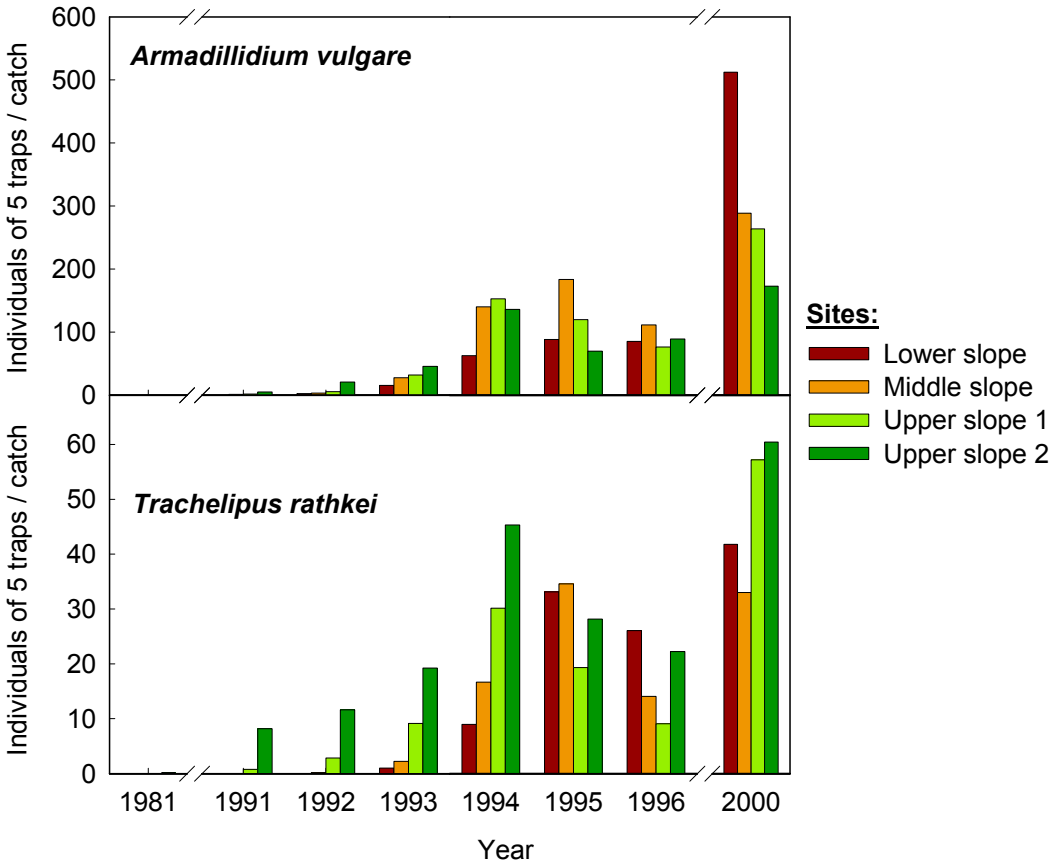

Figure 3 Temporal development of the individual numbers of the two most abundant woodlouse species in the Steudnitz grassland ecosystem between 1981 and 2000 at 4 differently impacted sites (T. Eggers and J. Rothe, unpublished data). The trapping area of 5 pitfall traps covered ~ 150 m^2. The time period of one catch was 14 days. Sites are labelled according to Manuscript IV, Fig. 1. Note the breaks in the time scale.

After 1994 this order disappeared. In 2000 the abundance of *Trachelipus rathkei* was highest at the uppermost slope site whereas *Armadillidium vulgare* was most abundant on the lower slope which had been the most degraded site. Additionally, by 2000 woodlice had become the most important group of the epigeic arthropod fauna in terms of individual numbers and biomass. Moreover, during succession the population size of the investigated woodlouse species correlated with the plant species number on the lower slope, but not on the uppermost slope. This suggests that in the case of severe degradation the re-establishment of balanced conditions between primary production and decomposition is highly important for ecosystem development (Brussaard, 1998; Setälä, 2002).

The special role of the decomposer system for the cycling of C and N and for the functioning of ecosystems has been shown repeatedly (Zheng et al., 1997; Mikola and Setälä, 1998; Scheu and Schaefer, 1998; Setälä et al., 1998; Laakso et al., 2000). However, little is known about the importance of macro-decomposers such as woodlice

for the transformation of organic matter (Jambu et al., 1988). The same applies to coprophagy, i.e. the uptake of faeces in addition to the food. Even though this nutritional strategy is practiced by a number of detritivores, its ecological significance is still uncertain (Kautz et al., 2002). Coprophagy is expected to affect the performance of woodlice as well as the quality and quantity of the faeces which represent a valuable food source for following decomposers. Therefore, the degradation of organic matter by woodlice was studied in more detail (Manuscript III).

3.3. Food webs

The paths organic C and N take through an ecosystem are defined by the trophic interactions between the organisms forming the biocoenosis (Odum, 1973). Once fixed by plants, C flows in chains from a primary consumer to the secondary consumer and so forth, always the latter representing a higher level in the trophic hierarchy. Typically food chains are cross-linked to form food webs in which each species has a certain trophic position. In terrestrial systems food chains usually comprise 3 – 4 steps from the primary producer to the top consumer (Begon et al., 1996; Post, 2002).

Despite the fact that the number of trophic levels is limited, food webs can be very complex. Food web complexity is defined by the number of trophic levels, the number of elements (species) at each level and the number of links between these elements. Each single trophic link is characterized by the amount of carbon (quantity) and the type of organic compounds (quality) transferred to the consumer. Only a few paths within the complex network of trophic interactions may be responsible for the main carbon turnover while others could be neglected (Ponsard and Arditi, 2000).

Overlaps in the food requirements and in the spectrum of potential predators create redundancy within the food web; i.e., some species have the same function either as food source or as consumer. Consequently, food webs are stabilized against small-scale disturbances and tolerate a limited loss of species. However, severe and prolonged disturbance such as reported from the Steudnitz system (Heinrich, 1984) may drastically reduce the number of species and essentially lower the complexity and functionality of the food web. This does, however, not imply that food-chain length is shorter in more highly disturbed extant food webs (Post, 2002).

In contrast to disturbance, ecosystem regeneration is assumed to go along with a stepwise increase of the food web complexity and functional stability (Manuscript IV).

This relationship should apply to any ecosystem irrespective of type, history and trajectory it presently moves on. If the assumption was valid, the correlation between food web stability/complexity and regeneration progress would offer a means to assess the present state of an ecosystem regenerating from disturbance or being subjected to restoration measures. This, however, requires the analysis of the food web structure and trophic interactions and to reliably conclude on the complexity and – more importantly – on the functional stability reached by the food web. To approach this requirement stable isotope analysis was applied.

3.4. The stable isotope approach

Over the last decades stable isotopes have developed to a widely used tool in all fields of ecology and environmental science (Lajtha and Michener, 1994; Unkovich et al., 2001). The majority of chemical elements have more than one stable isotope offering a multitude of applications. Most ecological research is done on isotopes of the five bioelements carbon, hydrogen, oxygen nitrogen and sulphur (C, H, O, N and S) using the range of their natural abundance (Wada et al., 1995). H, C and N have two, O has three and S even four stable isotopes. Always the light(est) isotope is by far the most abundant (Tab. I).

Table I: Mean abundance of the stable isotopes of the 5 bioelements at global scale

Element	Isotope	Abundance (%)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.890
	^{13}C	1.110
Nitrogen	^{14}N	99.630
	^{15}N	0.370
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Sulphur	^{32}S	95.006
	^{33}S	0.760
	^{34}S	4.220
	^{36}S	0.014

Concentrating on the elements C and N, three aspects should be mentioned which define the utility of stable isotopes: Firstly, in contrast to radio isotopes, stable isotopes cannot disappear during any process; i.e., at global scale the mean relative abundances of the heavier (^{13}C and ^{15}N) and the lighter isotope (^{12}C and ^{14}N) are constant. Secondly, during equilibration processes and (bio)chemical reactions the heavier and the lighter isotope underlie different reaction kinetics. Typically, isotopomers get preferentially processed by enzymes if a certain position of the molecules is occupied by either the lighter or the heavier isotope. This isotope discrimination leads to deviations from the global abundances of stable isotopes at smaller scales (Hoefs, 1997). Kinetic isotope effects result in isotope fractionation only as long as the reactions are not quantitative, i.e. the yield is lower than 100% (Hayes, 2002). Thirdly, stable isotope ratios create a characteristic label that allows concluding on the origin and the fate of the studied organic matter (Wada et al., 1995). The ratio of the heavier to the lighter isotope ($^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively) is commonly given as so-called delta (δ) value in parts per thousand (‰) in relation to international standard material (Coplen et al., 1992; Coplen, 1996).

An example for isotopic labelling caused by isotope discrimination is the fixation of atmospheric CO_2 and its conversion into organic matter by plants. Mainly due to the difference in the initial carboxylating enzyme which is either Ribulose bisphosphate carboxylase-oxygenase (RUBISCO) or Phosphoenolpyruvate (PEP) carboxylase, C_3 plants are much more depleted in the heavier carbon isotope ^{13}C than C_4 plants. Hence, $\delta^{13}C$ values of C_3 plants are typically around –27‰, those of C_4 plants around –14‰, while the $\delta^{13}C$ value of atmospheric CO_2 is –8‰ (O´Leary, 1981). This demonstrates that isotope fractionation may create organic matter with different isotopic composition thus representing distinctly labelled food sources.

The relative abundance of stable isotopes in living organisms depends on the isotopic composition of their food sources and on their internal fractionation. Often N is a limiting resource for nutrition that undergoes transformations different from C. Therefore, isotope patterns of N and C allow different insights into biological processes ranging from the molecular (Schmidt and Kexel, 1998; Gleixner et al., 1999; Gleixner et al., 2002) up to the ecosystem level (Wada et al., 1995; Buchmann et al., 1998; Ehleringer et al., 1998; Schulze, 2000).

The utility of stable isotopes for studying trophic interactions and the structure of food webs is based on the fact that internal isotope fractionation generally leads to an

enrichment of the heavier isotope in the consumer (e.g. a predator) relative to its diet (e.g. the prey of this predator). This trophic isotopic enrichment phenomenon is mainly due to the discrimination of the enzymes involved in respiration and N excretion against the heavier isotope (i.e. ^{13}C and ^{15}N, respectively) in favour of the lighter, leading to a measurable fractionation (DeNiro and Epstein, 1978; DeNiro and Epstein, 1981). The resulting characteristic isotopic shift between consumer and diet is referred to as trophic level shift (TLS). According to empirical data derived from laboratory experiments as well as from field observations the TLS accounts for 3 – 4‰ in ^{15}N (Minagawa and Wada, 1984; Peterson and Fry, 1987) and 0 – 2‰ in ^{13}C (DeNiro and Epstein, 1978; Fantle et al., 1999). Most recently, Post (2002) presented isotopic shift values that average over multiple trophic pathways (i.e. entire food webs) and summarize most of the published and a large number of own laboratory and field data. Those values, 3.4 ± 1.0‰ for ^{15}N and 0.4 ± 1.3‰ for ^{13}C, almost exactly match the TLS values reported earlier (Gearing et al., 1984 as cited in Ponsard and Arditi, 2000; Minagawa and Wada, 1984). The rather small, sometimes insignificant trophic isotopic shift in C makes $\delta^{13}C$ values a reliable tool for tracing diets (Sealy et al., 1987; Koch et al., 1994; Michener and Schell, 1994). Because isotope fractionation is more pronounced in N, in investigating the trophic structure of communities $\delta^{15}N$ values are used to assign consumer species to certain trophic levels (Vander Zanden et al., 1999; Scheu and Falca, 2000).

The structure of food webs can be visualized by plotting $\delta^{15}N$ vs. $\delta^{13}C$ values (e.g. Fantle et al., 1999; Stapp et al., 1999; chapter 5.5. Fig. 4 at p. 121). In general, plants reveal lower $\delta^{15}N$ and $\delta^{13}C$ values compared to the consumer species because for primary producers the trophic enrichment practically does not apply. Plants and plant derived material such as plant litter form the trophic ground level and plot in the lower left corner of the graph. With increasing trophic level, the organisms shift towards the upper right corner. The representatives of the highest trophic level, i.e. top carnivores, are most enriched in ^{15}N and ^{13}C. This clear food web structure results from the increasing isotope enrichment along the food chains. From the difference between the $\delta^{15}N$ value of a top-consumer (e.g. 7.8‰) and that of the plant biomass (e.g. –2.5‰; arbitrary values) the mean food chain length can be calculated. Dividing the difference (10.3‰) by 3.4‰, i.e. the reported TLS for ^{15}N (Post, 2002), the resulting value incremented by 1 (~ 4) is the number of trophic levels covered by the food web example. Since plant biomass is the starting point for all grazer and decomposer food chains, its isotopic composition defines the ‘isotopic baseline’ of the food web. This baseline depends on the isotopic

composition of the C and N sources for plant growth and on the present environmental conditions. Consequently, its position differs among ecosystems; i.e., the isotopic baseline is largely system-specific and provides a reference value to compare the trophic structure of different ecosystems (Ponsard et al., 2000; Post, 2002).

Knowledge on the overall isotope fractionation associated with the transfer of C and N upon trophic interaction is a prerequisite for using stable isotopes in food web research. However, because isotope fractionation depends on many factors, the isotopic difference between consumer and diet can vary; i.e., the 'rule of a constant TLS' is valid only under certain circumstances (Gannes et al., 1997; Focken, 2001). Numerous studies exist illustrating the isotopic changes that occur in animals due to feeding on a specific diet under defined conditions (DeNiro and Epstein, 1978; DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Sick et al., 1997; Focken and Becker, 1998; Webb et al., 1998). After a diet switch, changes in the isotopic composition of the different tissues or body components of an animal depend on how fast these constituents are turned over (Gannes et al., 1998). Hence, apart from diet quality that might cause nutritional stress, also the life stage of an organism and its overall physiology will affect the isotopic equilibration between whole animal and diet and may, therefore, interfere with food tracing and food web reconstruction. As environmental conditions are variable and often the factors influencing animal isotopic signatures are unknown or immeasurable, the problem arises that in certain cases stable isotopes might neither reflect the food sources nor the current trophic position of an organism. Nevertheless, despite a large variation in the trophic isotopic enrichment in the case of single trophic relationships, e.g. 1.3 to 5.3‰ in ^{15}N (Minagawa and Wada, 1984), the average over entire food webs should be ideally in the order of 3.4 ± 1.0‰ (Post, 2002), i.e. the empirical TLS value for ^{15}N. Deviations from that value could be related to ecosystem disturbance and be based on imbalanced trophic interactions. Investigating the relationship between the current state of an ecosystem and the isotope patterns of its food web seems to be a promising approach to describe ecosystem development and to conclude on the progress in ecosystem regeneration from stable isotope data (Manuscript IV). The implementation of the suggested relationship in the analysis of ecosystems, however, demands the validity of the mean TLS values adopted for ^{13}C and ^{15}N be carefully checked (Gannes et al., 1997). This had been a major issue of the three laboratory experiments (Manuscripts I – III).

Summarizing the introductory section, the goal of this thesis is to address the following questions:

- What is the influence of food quality, nutritional stress, changing food sources and coprophagy on the isotopic shift between consumer and diet?
- Which role do macro-decomposers play in the alteration of organic matter during decomposition processes? Does coprophagy matter?
- Can ecosystem development following disturbance be described using stable isotopes; i.e., are stable isotopes able to identify structural patterns in food webs that relate to regeneration?
- What is the present state of the investigated ecosystem with respect to the recovery from a degraded state?

Answering these questions is important to food web research and the advance in ecosystem analysis and restoration ecology for the following reasons:

- The application of stable isotopes to field studies requires detailed information on the factors influencing animal isotopic signals. Current knowledge needs to be verified.
- The contribution of macro-decomposers to carbon and nitrogen cycling is supposed to be important for the functioning of ecosystems. The significance of coprophagy within this is not yet understood.
- The suitability of stable isotopes to uncover temporal and spatial patterns in ecosystem development has not been proven so far. Assessing regeneration progress from isotope data offers a new tool for ecosystem analysts and for practitioners in ecological restoration.
- The knowledge about the dynamics, governing factors and the time-scale of regeneration processes in relation to the level of ecosystem degradation is limited. In order to fill this gap appropriate case studies are needed.

The posed questions were addressed by carrying out the following:

- a laboratory study to quantify the effect of diet quality on the isotopic composition of woodlice (Manuscript I),
- a laboratory study to test the role of coprophagy for the nutritional performance of woodlice and their isotopic enrichment (Manuscript II),

- a laboratory study to define changes in the elemental and isotopic composition of organic matter and woodlice caused by feeding activities including coprophagy (Manuscript III), and,
- a field study using stable nitrogen isotopes to describe the general trophic structure of a disturbed grassland ecosystem and its temporal dynamics in relation to the former degree of impact (Manuscript IV).

4. Manuscripts

4.1. Manuscript I

Do stable isotopes reflect the food web development in regenerating ecosystems?

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DO STABLE ISOTOPES REFLECT THE FOOD WEB DEVELOPMENT IN REGENERATING ECOSYSTEMS?

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We evaluated the use of δ^{15}N- and δ^{13}C-values to monitor the development of food web complexity and biodiversity in a regenerating ecosystem. Therefore a model food chain was established feeding cultivated woodlice (*Porcellio dilatatus*) on a cellulolytic fungus (*Chaetomium globosum*) grown on cellulose paper. Two diets of different quality (C:N ratios of 54 vs. 200) with different δ^{15}N- (1.3‰ vs. 3.1‰) but identical δ^{13}C-values caused low and high dietary stress in animals of treatment **A** and **B**, respectively. After an incubation time of 7 weeks amount, elemental and isotopic composition of collected faeces and exuviae as well as woodlice and remaining food were determined.

The increase of δ^{15}N-values of woodlice relative to the diet was 5.7‰ and 2.5‰ in treatments **A** and **B**, respectively, whereas δ^{13}C-shifts were 1.0‰ and 1.6‰, showing a reverse relationship. Modelling of elemental and isotopic mass balances indicated that faeces recycling explains the unexpected high ^{15}N-enrichments. Moreover, ^{13}C-enrichments were positively correlated to the degree of starvation. Considering the effects of starvation and recycling of faeces, stable isotopes represent a useful tool to elucidate trophic interactions in regenerating food webs.

Keywords: Carbon 13; *Chaetomium globosum*; Diet; Faeces; Mass balances; Natural variations; Nitrogen 15; *Porcellio dilatatus*; Recycling; Woodlice

INTRODUCTION

Long-term studies investigating the effect of a phosphorus fertilizer plant on a calcareous grassland ecosystem in Thuringia, Germany,

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indicated that dust pollution affected species composition, biodiversity and ecosystem functions [1–3]. Since dust emissions stopped in 1990 the ecosystem has regenerated continuously, *i.e.*, soil development took place [4] and organisms recolonized this site [5]. These regeneration processes are likely to have increased biodiversity, ecosystem functions and hence, ecosystem complexity [6–10].

Soil biodiversity has a key role in the regeneration processes by enforcing biogeochemical cycling [11] and primary production [12]. Increasing biodiversity also allows more trophic interactions and longer food chains [6]. Therefore, ecosystem regeneration will lead to an increase in food web complexity [8].

Stable isotopes have shown to be useful to investigate the structure of trophic webs [13–16]. Organisms reflect the stable isotope ratios of their diet, although generally being slightly enriched in the heavier isotope [17, 18]. The trophic level shift (TLS) is stronger for δ^{15}N than for δ^{13}C. The generally accepted trophic level shift for nitrogen is about 3‰ [19], for carbon it ranges from 0–2‰ [14]. While concurrent measurements of δ^{13}C-values provide additional information, δ^{15}N-values are preferred to study food webs because of the characteristic difference of 3.4‰ between two trophic levels [20]. However, for particular organisms often quite different values are reported [13, 16]. Hence, it is important to investigate isotopic shifts for single species under controlled conditions [14].

Woodlice became increasingly abundant in the regeneration process of the studied grassland ecosystem (T. Eggers, pers. comm.). Accordingly, a model food chain was conceptualized using woodlice as important representatives of the soil food web [21]. Woodlice are saprophagous crustaceans which regularly shed their chitinous skin [22]. They play a major role in the decomposition of plant leaf litter in deciduous forests and grassland ecosystems [16, 21, 23]. Woodlice shred wood, litter and carcass and inoculate their diet with microorganisms [22, 24]. Microorganisms digest the food within the intestine, but also colonize the excreted faecal pellets [25–28]. Recycling of faeces (coprophagy) is supposed to be an essential step in the nutrition of woodlice in environments with low food quality [24, 28]. These recycling processes keep nutrients for a longer period at the same food web position and may result in additional isotope fractionations, leading to TLS for ^{15}N higher than 3‰.

Moreover, woodlice starve voluntary for several days during the molt cycle, commonly ingest their skins (exuviae) and practice cannibalism [14, 22], all factors which can influence their isotopic signature irrespective of the initial diet. Consequently, higher values of TLS would be measured and the exact trophic state of a species misinterpreted. This complicates the direct use of stable isotope data for food web analysis.

Therefore, main objectives of this work are:

(1) to determine factors which control the carbon and nitrogen isotope signature of woodlice,
(2) to develop a simple conceptual model to estimate the importance of nutrient recycling processes,
(3) to evaluate if the use stable carbon and nitrogen isotopes in food web studies can provide new information on the importance of biodiversity in regenerating ecosystems.

MATERIALS AND METHODS

Experimental Setup

We established an experimental food chain consisting of three elements: (1) cellulose paper as substitute for primary production (*e.g.* leaf litter), (2) the cellulolytic fungus *Chaetomium globosum* Kunze ex Steud. 1824 (Ascomycotina) grown on this paper as representative of the soil microflora and (3) cultivated woodlice (*Porcellio dilatatus* BRANDT, 1833) as a saprophagous consumer feeding on paper plus fungus.

The fungus was grown for 8 weeks at 24.5°C in a breeding chamber in two different ways producing two types of diet (see below). In one series (**A**) the cellulose paper was placed on 15ml of nutrient solution containing inert agar as solidifying agent while in the other series (**B**) 2ml of nutrient solution were applied directly onto the paper. Hence, in series **A** the fungus had 7.5 times more nitrogen (as sodium nitrate in the nutrient solution) for growth compared to series **B**. It was observed, that *Chaetomium globosum* produced more biomass in series **A** than in series **B**. Finally, in both series paper plus fungus were harvested, lyophilized, ground and the powder finally rewet for feeding purposes.

Prior to experiments *Porcellio dilatatus* was held at densities of 200–300 individuals in translucent plastic boxes (L ∗ W ∗ H = 40 ∗ 30 ∗ 20 cm), those bottoms contained a water reservoir of foam rubber, covered with plaster (gypsum, $CaSO_4 \cdot 2H_2O$) to a depth of 3 cm from the base of the box. On top a 2–3 cm layer of wet sand provided refuges for woodlice individuals. The boxes were closed by a lid, but had perforations at one side to allow gas exchange. Relative humidity was kept close to 100% at constant temperature of 22°C. Subdued light was applied for 10 hours per day. Normal diet of the woodlice consisted of fresh sliced carrots and fresh lettuce at surplus amounts, served on glass dishes and changed twice a week. The δ^{15}N- and δ^{13}C-values for a mix of 2 parts of carrots and 1 part of lettuce (based on dry weights) were between 1.7 and 2.0‰ and between –30.9 and –31.1‰, respectively. Selective food choice of different woodlice caused intra-specific variations in the isotope signature. In a preincubation phase of 3 weeks woodlice were fed on food similar to the experimental diet.

In the experiment both treatments **A** and **B** consisted of 3 Petri dishes (150 ∗ 25 mm) each containing 5 adult males. The Petri dishes were placed in a growth chamber at controlled light and temperature regime (8 hours light at 20°C, 16 hours dark at 15°C) with relative humidity close to 100%. Both treatments of woodlice (**A** and **B**) were supplied with chemically similar diet of identical δ^{13}C- (= –24.5±0.1‰) but different δ^{15}N-values, consisting of cellulose paper and cellulolytic fungus, grown either with (**A**) or without agar (**B**). Initially, the food of treatment **A** contained 0.7% nitrogen with a δ^{15}N-value of 1.3±0.2‰. The food of treatment **B** contained only 0.2% nitrogen, but was isotopically enriched (δ^{15}N = 3.1±0.5‰). The supplied food had a very unfavourable C:N ratio of 54 in treatment **A** and 200 in treatment **B**, which should simulate low and extremely low nutrient quality, respectively [14].

Fresh and dry weights of input and output components were recorded, except the gases. Woodlice are ammoniotelic (M. Zimmer, pers. comm.), *i.e.*, they do not produce urine as such but excrete nitrogen as ammonia, which is transported by the water piping to the maxillary glands in the head region and from there released as gas [29]. This volatile N-component was not trapped. Therefore, it is unknown, how much isotopically light ammonia (Fig. 4) was produced by the woodlice. However, the total mass balance for nitrogen as well as the amount of respired carbon dioxide were calculated.

Faeces and exuviae were collected weekly. After 7 weeks the remaining food was harvested and the woodlice were frozen in liquid nitrogen. Finally, all material was lyophilized, ground and stored in a vacuum desiccator.

Isotope Analysis

Measurements of δ^{15}N- and δ^{13}C-values and nitrogen and carbon contents were performed using an Elemental Analyser-Isotope Ratio Mass Spectrometer system. Amounts of 50–200µg N or 30–200µg C per sample were weighed into tin capsules and measured using a Thermoquest Finnigan Delta Plus XL (analytical precision ± 0.2‰ for ^{15}N and ^{13}C). Working standards were acetanilide (δ^{15}N = 1.78‰, δ^{13}C = –33.94‰) and caffeine (δ^{15}N = –1.16‰, δ^{13}C = –51.80‰), calibrated against international standards IAEA-N2 and NBS-22. Isotopic ratios are expressed in conventional delta (δ) notation in parts per thousand: δX (‰) = ($R_{sample}/R_{standard} - 1$) ∗ 1000‰, where X = ^{15}N or ^{13}C and R = ^{15}N/^{14}N or ^{13}C/^{12}C, respectively. Standard is air for ^{15}N and V-PDB for ^{13}C.

Statistics and Calculations

Unifactorial analysis of variance (SPSS for Windows 9.01) was used to evaluate the significance of differences between measured parameters, followed by post hoc Student-Newman-Keuls test to identify homogeneous subgroups (normally N = 3, level of significance: $p < 0.05$).

The relative amount of respired food per consumed food (R_{rel}) was calculated from the mass balance

$$R_{rel} = \frac{R_{food}}{\Delta_{food}} = \frac{\Delta_{food} - \text{Faeces} - \text{Exuviae} - \Delta_{WL}}{\Delta_{food}}$$

where R_{food} is the amount of respired food [mg], Δ_{food} is the amount of consumed food (*i.e.*, initial minus final amount of food [mg]), Faeces is the amount of collectable faeces [mg], Exuviae is the amount of shed exuviae [mg] and Δ_{WL} is the biomass change of woodlice (*i.e.*, initial minus final biomass of woodlice [mg]).

RESULTS

Feeding Experiment

The total mass balance for nitrogen over the whole experiment was closed in both treatments even though any possible excretion of gaseous ammonia by woodlice was disregarded, indicating no effective loss of nitrogen from the system. For carbon 29.6 ± 3.3% was respired in treatment **A** *vs.* 23.0±1.0% in treatment **B** (data not shown). The relative contents of N and C in diet and woodlice also changed over the experiment (Fig. 1). While the percentage of nitrogen slightly

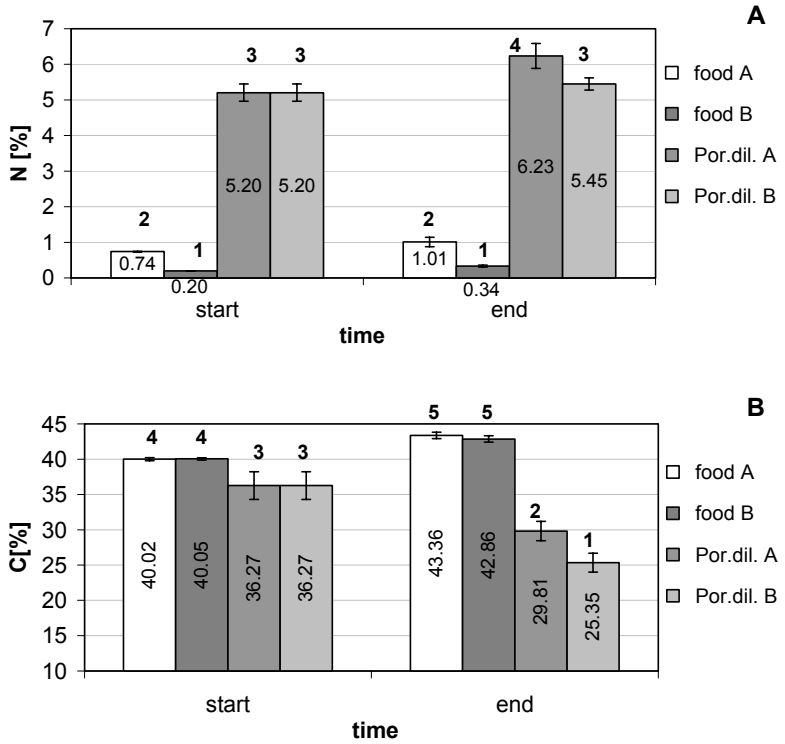

FIGURE 1 Differences of mean initial (start) and final values (end) of (A) nitrogen and (B) carbon contents of food and woodlice (Por.dil.) of treatments **A** and **B** (N = 3). Homogeneous subgroups are indicated by numbers above columns, *i.e.*, same numbers refer to samples belonging to the same statistical entity, while different numbers refer to significant differences between samples (unifactorial ANOVA followed by post hoc Student-Newman-Keuls test, $p < 0.05$). Error bars indicate 1 standard deviation of the mean.

increased for food and organisms in both treatments (Fig. 1A), the loss of woodlice carbon was striking (Fig. 1B).

This carbon loss explained the relative increase of nitrogen in food and organisms. Therefore, the elemental mass balance was applied to demonstrate the appropriate N and C relations (Fig. 2). In average, woodlice of treatment **A** had a nitrogen accretion of 8.4 ± 3.2% but lost 25.6 ± 4.0% of their carbon, whereas in treatment **B** the amount of N and C decreased by 8.8 ± 3.8% and 39.1 ± 4.2%, respectively, as indication of the higher dietary stress (*i.e.*, degree of starvation) in treatment **B**.

However, in both treatments animals consumed the same amount of food, *i.e.*, the mass ratio of used food per woodlouse biomass was about 2.3 (data not shown). The collectable amount of faeces per unit consumed food was only 38.3 ± 8.9% in treatment **A** vs. 49.2 ± 11.1% in **B**, while shed exuviae accounted for 4.1 ± 1.5% and 1.0 ± 0.8%, respectively (Tab. 1). The weight loss of woodlice as a measure of the degree of starvation was 9.5 ± 3.4% in treatment **A** *vs.* 13.0 ± 2.3% in **B** (data not shown; differences not significant). Accordingly, the proportion of respired food per consumed food was 61.6 ± 8.1% in treatment **A** vs. 55.4 ± 9.9% in **B** (Tab. I).

Our results confirmed that δ^{15}N- and δ^{13}C-values of the remaining woodlice biomass increased relative to the diet (Fig. 3). Due to the switch from breeding to experimental diet, the δ^{13}C-values of woodlice

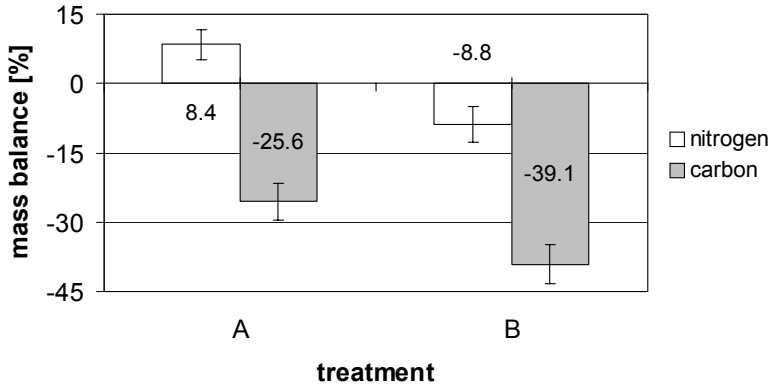

FIGURE 2 Mass balances of nitrogen and carbon in woodlouse biomass of treatments **A** and **B** (means of $N = 3$). Negative values refer to effective losses. Error bars indicate 1 standard deviation of the mean.

TABLE 1 Net percentage of consumed food transferred to different compartments of treatments **A** and **B** (calculated from dry weights; values given as mean ± 1 SD of the mean, *N* = 3). The differences between treatments are not significant. Mass losses of woodlice relative to consumed food were included and accounted for a proportional increase in metabolic respiration. These negative values correspond to the weight losses of the animals (9% *vs.* 13%) given in the text

	Treatment	
Compartment	*A*	*B*
Faeces	38.3 ± 8.9%	49.2 ± 11.1%
Exuviae	4.1 ± 1.5%	1.0 ± 0.8%
Woodlice	-4.0 ± 1.4%	-5.6 ± 1.0%
Metabolic respiration	61.6 ± 8.1%	55.4 ± 9.9%
Total	100%	100%

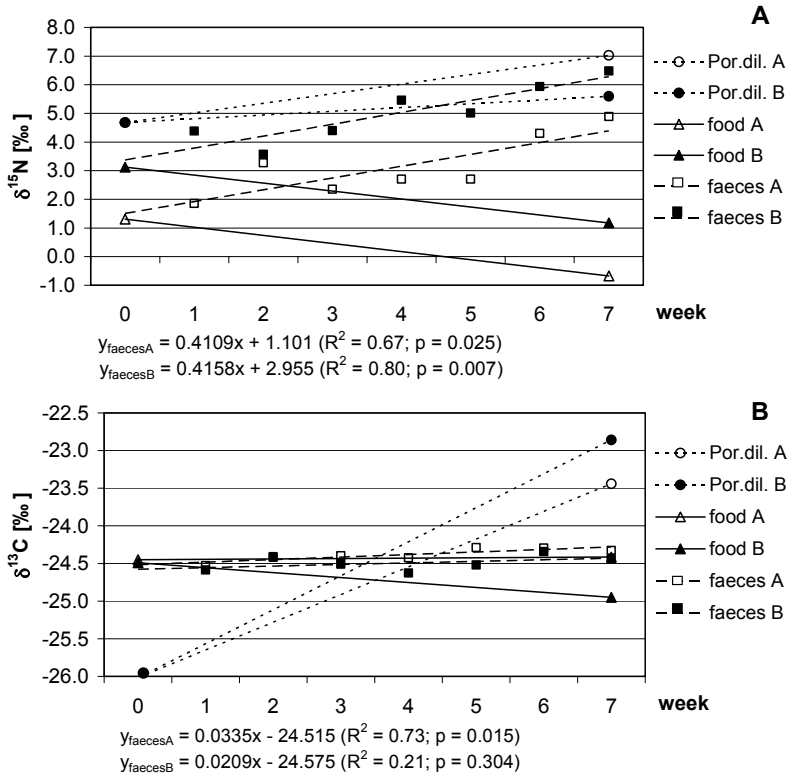

FIGURE 3 Temporal shifts of means of A) δ^{15}N- and B) δ^{13}C-values of woodlice (Por.dil.), food and faeces of treatments **A** and **B** (*N* = 3). Initial and final values of woodlice and food are linked by straight lines. For faeces regression lines of linear regression are plotted, regression equations given below with (R^2 and *p* values of ANOVA of linear regression in brackets). In average, one standard deviation of the mean of δ^{15}N- and δ^{13}C-values, respectively, are 0.5 and 0.3‰ in woodlice, 0.6 and 0.1‰ in food, and 0.4 and 0.1‰ in faeces.

shifted within 7 weeks from –26.0 ± 0.2‰ (reference organisms from culture boxes) over more than 2.5 units to values of –23.4 ± 0.6‰ and –22.9 ± 0.3‰ in treatments **A** and **B**, respectively.

However, the observed trophic level shifts for ^{15}N were different, *i.e.*, 5.7 ± 0.8‰ in treatment **A** and 2.5 ± 0.8‰ in treatment **B**. In consequence, organisms fed on food richer in nitrogen became isotopically heavier than organisms fed on nitrogen poor food (Fig. 3A). Although not significant, the $\delta^{13}C$-shifts were different for animals of treatment **A** (1.0 ± 0.6‰) and treatment **B** (1.6 ± 0.3‰) as well (Fig. 3B), but showed the reverse relationship: Organisms fed on diet with slightly more nitrogen became less enriched in ^{13}C compared to organisms fed on diet with extremely low nitrogen.

The $\delta^{13}C$-values of faeces and diets stayed rather constant, only the food of treatment **B** decreased significantly by 0.5‰ (Fig. 3B). In contrast, faeces of both treatments displayed a distinct and parallel enrichment in ^{15}N with time, while the reverse is true for the diet (Fig. 3A). The diet was expected to stay isotopically unchanged over the whole experiment, but its final decrease in the $\delta^{15}N$-value (possibly an effect of microbial infection) even enlarged the trophic level shift ($\Delta^{15}N$ = 7.7‰ and 4.4‰ in treatments **A** and **B**, respectively).

Modelling the Importance of Faeces Recycling

In order to explain a $\delta^{15}N$-shift of woodlice higher than the expected trophic level shift (TLS) of about 3‰, a simple model (illustrated in Fig. 4) was used to calculate the appropriate proportion of faeces (x_{faeces}) in the total diet which gives for a defined TLS the observed $\delta^{15}N$-shift of woodlice at each time step.

For both supplied food and faeces the same TLS of 3‰ was assumed, however, other factors like starvation or cannibalism cannot yet be included. From the mass balance (1) and the isotope mass balance (2) the proportion of faeces x_{faeces} can be calculated (3).

$$1 = x_{food} + x_{faeces} \tag{1}$$

$$\delta_{WL} = \frac{(\delta_{food} + TLS) \cdot x_{food} \cdot N_{food} + (\delta_{faeces} + TLS) \cdot x_{faeces} \cdot N_{faeces}}{x_{food} \cdot N_{food} + x_{faeces} \cdot N_{faeces}} \tag{2}$$

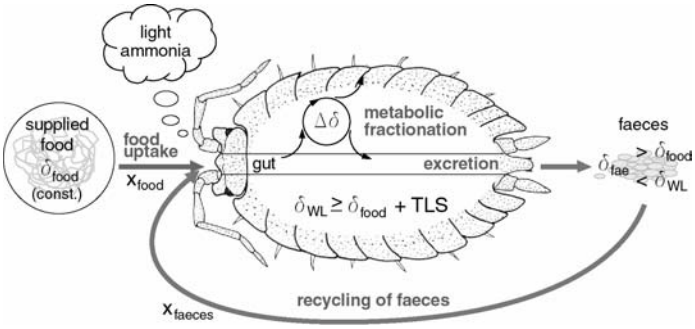

FIGURE 4 Diagram illustrating components and processes involved in the change of δ^{15}N-values of a feeding woodlouse. Food uptake is followed by metabolism of N containing compounds. Isotopically light ammonia is released as enzymes involved in the nitrogen excretion discriminate against the heavier isotope (^{15}N). Woodlice and excreted faeces become enriched in ^{15}N, but the enrichment of faeces is always smaller relative to the woodlice. Metabolic fractionation and subsequent excretion of N containing products cause the described trophic level shift. Recycling of faeces enhances ^{15}N gain of woodlouse, leading to a δ^{15}N-value of the animal higher than δ^{15}N of supplied food plus normal trophic level shift. $\delta = \delta^{15}$N, TLS = trophic level shift, WL = woodlouse, x_{faeces} = proportion of faeces in the total diet, x_{food} = proportion of supplied food in the total diet.

$$x_{faeces} = \frac{N_{food} \cdot (\delta_{WL} - \delta_{food} - TLS)}{N_{food} \cdot (\delta_{WL} - \delta_{food} - TLS) + N_{faeces} \cdot (\delta_{faeces} - TLS - \delta_{WL})} \quad (3)$$

where x_{faeces} is the proportion of faeces in the total diet, x_{food} is the proportion of food in the total diet, TLS is the assumed trophic level shift, δ refers to δ^{15}N-values and N to nitrogen percentages of woodlice ($_{WL}$), supplied food ($_{food}$) and faeces ($_{faeces}$).

In treatment **A** after one week a x_{faeces} of 57% was calculated. It increased to 89% within 7 weeks. The increment of x_{faeces} between time steps was always positive, but reduced continuously (Fig. 5), *i.e.*, the rate of faeces recycling enforced during the whole experiment and became finally saturated.

The initially negative values of x_{faeces} in treatment **B** mathematically refer to a substantial loss of ^{15}N by producing faeces without uptake as the supplied food is sufficiently enriched to cause the observed enrichment of the woodlice. After 4 weeks effective faeces uptakes took place and x_{faeces}, which finally reached 20%, was still increasing

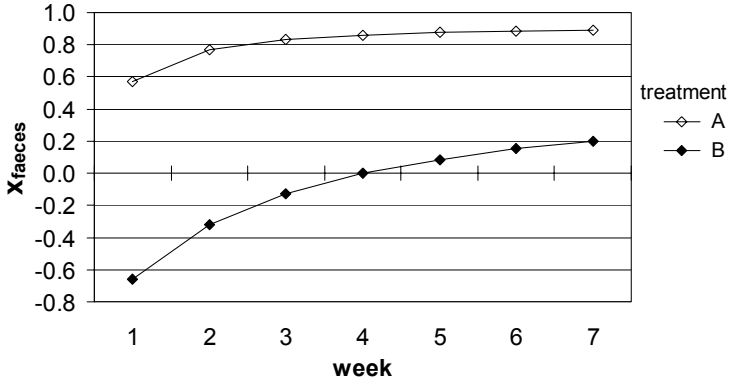

FIGURE 5 Calculated temporal shift of the proportion of faeces in the total diet (x_{faeces}) of treatments **A** and **B**.

at the end of the experiment. In general, the curve indicates a lower importance of coprophagy in comparison to treatment **A**.

DISCUSSION

Small differences in low quality food, represented by C:N ratios, led to unexpected isotopic differences in cultivated woodlice. The physiological responses to starvation which are accompanied by positive isotope fractionations of endogenous amino acids recycling through the keto acid pool [30] and catabolism of own biomass [14] should be addressed.

However, the observed ^{15}N-shift of woodlice relative to their diet was neither identical in both treatments nor were animals with the higher degree of starvation stronger enriched in ^{15}N. Woodlice of treatment **A** which consumed better food and had less dietary stress (*i.e.*, lost less weight, less carbon and even assimilated some nitrogen, Fig. 2) were more enriched in ^{15}N than woodlice of treatment **B**. This suggests that additional factors are needed to interpret the observed isotopic shifts.

The mass balance demonstrated a higher total carbon loss and a higher relative metabolic respiration rate in treatment **A** (Tab. I), indicating an elevated metabolic activity of treatment **A.** Surprisingly, in treatment **A** less faeces were collected suggesting that from the pool

of excreted faeces a greater proportion was recycled immediately. This also explains the higher ^{15}N-enrichment in treatment **A**. As faeces became heavier in ^{15}N with time (Fig. 3A), uptake of excrements in addition to the ^{15}N-depleted experimental diet should cause a higher animal δ^{15}N-shift than feeding solely supplied food. This proposed relationship is reflected by different Δ^{15}N-values in treatment **A** (5.7‰) and **B** (2.5‰) which correspond inversely to the proportion of collectable faeces (38% and 49%, respectively). Thus, a higher rate of faeces recycling is supposed in treatment **A**.

Faeces recycling can be seen as mechanism to overcome partly the physiological effects of starvation caused by low nutrient quality. The higher N contents in woodlice of treatment **A** is in accordance to the isotopic results. It is presumed, that intense recycling of faeces (which contained about 50% more N than the supplied food) led to an effective accretion of nitrogen (Fig. 2).

Laboratory studies indicated, that food quality influences the isotopic difference between food source and organisms [14, 30, 31]. Feeding on protein-poor diets can force animals to catabolize their own lipids and proteins for energy requirements in response to starvation, leaving the remaining biomass enriched in ^{15}N [14]. However, fasting earthworms displayed no change of the δ^{15}N-value after 56 days [32]. It was assumed that the lighter products of amino acid recycling were not excreted from the body, hence, no fractionation was realized when measuring total biomass.

Mass balances can prove if starvation is accompanied by substantial loss or gain of nitrogen. In our experiments woodlice of treatment **B** lost 9% of their nitrogen, but became less enriched in ^{15}N than woodlice of treatment **A** with better food and an nitrogen gain of 8%. Obviously, endogenous N recycling mechanisms can fractionate only on small amounts of the internal nitrogen [30]. Therefore, it is likely that the continuous uptake of isotopically enriched N by faeces recycling mainly increases the whole animal δ^{15}N-value. Moreover, in our study the excretory loss of gaseous, isotopically light ammonia from woodlice bodies cannot serve as mechanism to explain the measured ^{15}N-shifts, as no nitrogen was lost effectively from the system. Degree of starvation and isotopic enrichment were positively correlated in the case of δ^{13}C-values. Individuals of treatment **B** showed a higher degree of starvation and lost significantly more

carbon than those of treatment **A** (Figs. 1B and 2). These changes in carbon contents were caused by extremely low diet quality which did not meet the nutritional requirements of woodlice [22–24]. Because of the very low N contents of diet **B**, these animals had to consume 4 times as much food as animals in treatment **A** to get the same amount of nitrogen. In turn, this would require a very high metabolic turnover which did not occur (Tab. I). Instead, woodlice seemed to metabolize their own tissues as suggested by [14], thus losing N and C (Fig. 2).

Metabolic processes which cause carbon losses can also lead to isotopic shifts of whole animal biomass. Consumption of storage fats which are depleted in ^{13}C relative to bulk [30, 33] would elevate the $\delta^{13}C$-value of woodlice. Furthermore, an increasing proportion of the exoskeleton could be assumed since the organisms declined more in carbon than in weight. Analyses of exuviae showed a carbon percentage lower than 18% (nitrogen below 2%), thus proposing a high proportion of ash which might partly balance carbon losses. In addition, exoskeletal carbon was found to be highly enriched in ^{13}C ($\delta^{13}C$-values above –20.6‰). This is in accordance to investigations by Gleixner *et al.* (1993) who suggested that chitin is isotopically heavier compared to primary metabolic products [34]. Therefore, the higher $\delta^{13}C$-shift of woodlice of treatment **B** (Fig. 3B) is interpreted as the effect of starvation such as a reduction of body lipids which can be seen directly from a higher weight loss (13%) in relation to treatment **A** (9%).

Modelling the importance of faeces recycling reflects a different importance of coprophagy in both treatments (Fig. 5). A higher increment of x_{faeces} until week 4 mirrors the switch from preexperimental to experimental diet which causes disequilibrium between animals and new food source. In the second half of the experiment the slope of both curves decreases, finally stabilizing in treatment **A** as indication of equilibrium, whereas woodlice of treatment **B** adapted less rapidly to their food.

The calculated values of x_{faeces} seem too high in treatment **A**, because the total diet of the woodlice had to consist of about 60% of faeces already at the beginning and even of 90% at the end of the experiment. This is unlikely, although not entirely impossible, as own observations have shown, that during normal cultivation of woodlice accumulation of produced faeces was of little account, only.

In contrast, in treatment **B** the calculated values of x_{faeces} are too low, as practically negative values should not occur. Thus, at least one additional process (e.g.starvation) is missing to model observed δ^{15}N-shifts satisfactorily. As the dietary stress was different in both treatments and the degree of starvation can also vary in the course of the experiment, the implementation of this dynamic factor into the model should lead to a better fit of x_{faeces} for a given TLS.

The implication of coprophagy helps to understand observed isotopic spacing between diet and consumers, but does not explain the changes of δ^{15}N-values as such. In fact, a certain TLS (3‰ according to the literature) was necessary to obtain appropriate values. Assuming a TLS of 2‰, for treatment **A** the model would give nonsensical values of x_{faeces} above 100%, while a TLS of 4‰ causes always negative values for treatment **B**. Because TLS is presumed to be the same in both treatments, only a shift around 3‰ leads to reasonable x_{faeces}-values. But since both, recycling effects and TLS may be under- or overestimated, results of the calculations are exemplary and show the need for a reliable definition of TLS.

Our study revealed that nutrient recycling by uptake of excrements and starvation cause uncorrelated changes in $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios. As these changes are superimposed on the trophic level shift normally expected and complicate the interpretation of isotope patterns in natural food webs, both, δ^{13}C- and δ^{15}N-measurements are needed to disentangle trophic interactions, effects of starvation and nutrient recycling processes.

The actual role of faeces recycling in natural soil environments is unknown, but seems to be underestimated. Ullrich (1991) has shown that woodlice fed on faecal pellets can survive more than 100 days [28]. Microorganisms convert food initially indigestible to woodlice into easily accessible C and N containing compounds like proteins, oligo and monosaccharids [21, 23, 25, 35]. Thus, “matured” faeces may become more attractive than low quality leaf litter, as woodlice are able to find suitable food sources by responding to the odour of cellulolytical active microorganisms colonizing the potential diet [36]. Faeces feeding was also observed in collembola [37], one of the most abundant and widespread groups of decomposing soil arthropods [16, 38]. As regenerating ecosystems are subjected to changes in food quality and quantity the consequences of physiological and

behavioural responses as starvation and coprophagy must be assessed to extract trophic relationships from isotopic information, thus elucidating the path of food web development.

CONCLUSIONS

Feeding experiments revealed much higher ^{15}N-enrichments in woodlice than expected and disproved the thesis that the δ^{15}N-shift substantially reflects solely isotopic differences of the supplied food. In fact, the measured changes in stable isotope ratios cannot be explained by simple feeding, but involve processes which preferentially occur in disturbed ecosystems. These processes include starvation due to dietary constraints [14] or belong to normal or stress-induced behaviour of animals like coprophagy [28] and cannibalism [13].

While higher ^{15}N-enrichments referred to faeces recycling processes, a higher ^{13}C-enrichment was correlated to the degree of starvation. Therefore, the use of both stable isotope ratios may reflect the impact of different ecosystem processes in relation to trophic interactions. Especially in regenerating ecosystems the estimation of biodiversity, food chain length and food web complexity by interpreting δ-values has to consider nutrient recycling and starvation.

A simple laboratory food chain demonstrated, that even small differences in the diet can lead to unexpected changes in the final isotopic pattern. Modelling the relative role of nutrient recycling by faeces explained changes of δ^{15}N-values higher than the generally accepted trophic level shift of 3‰.

Further research focussing on the determination of faeces recycling rate will give a better estimate of its real importance and allow us to calculate more precisely real trophic level shifts.

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4.2. Manuscript II

The effect of coprophagy by woodlice (*Porcellio dilatatus*) on C and N balances and on the isotopic signatures of animals and faeces in experimental microcosms

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Abstract

We investigated the importance of coprophagy for *Porcellio dilatatus* and its influence on the elemental (C, N) and isotopic composition (^{13}C, ^{15}N) of woodlouse biomass and faeces in a food chain experiment. For eight weeks, woodlice either freely **r**ecycled their own faeces derived from an artificial diet of carrots and lettuce (treatment **R**) or were prevented from coprophagy by daily faeces **c**ollection (treatment **C**).

Coprophagy led to increased food consumption and mass loss from the system. At the same time it reduced the amount of collectable faeces and the loss of woodlouse biomass. Moreover, coprophagy induced a higher molt frequency indicating better conditions for growth. The rather stable C:N ratios of faeces relative to those of food and animals suggested a faster adaptation to the experimental diet of woodlice recycling faeces. The stronger increase of faeces $\delta^{15}N$ and $\delta^{13}C$ values with time in treatment **R** (0.62 and 0.52‰ $week^{-1}$, respectively) compared to treatment **C** (0.27 and -0.02‰ $week^{-1}$, respectively) provided evidence that the metabolic turnover in woodlice was positively affected by coprophagy. The importance of faeces uptake was reflected by the proportion of faeces in the diet, which in the first week accounted for >20% in treatment **R**. Thereafter, this proportion declined suggesting that woodlice were adjusting to the high quality food. Including the proportion of recycled faeces we modeled the isotopic shift between woodlice and their diet and found different dynamics in treatments **C** and **R** with regard to ^{15}N and ^{13}C. Extrapolations – even though on a weak basis – suggest that due to coprophagy the isotopic shift would reach faster the generally accepted values for one step in the trophic cascade, i.e. ~3.4‰ for ^{15}N and 0-1‰ for ^{13}C. We conclude that woodlice benefit from coprophagy. The availability of faeces is a premise for the optimal performance of these macro-decomposers. Our results emphasize the ecological significance of coprophagy for organic matter decomposition and illustrate the effect on natural ^{15}N and ^{13}C labelling of food web components that needs to be considered when studying element cycling in trophic networks.

Key words: ^{15}N, ^{13}C, stable isotopes, isotopic shift, Porcellio dilatatus, food web, nutritional state, decomposition

1. Introduction

The decomposer system is an essential part of ecological systems (Wardle et al., 1995; Zheng et al., 1997; Brussaard, 1998). Its members accomplish the closure of the carbon cycle by the degradation and subsequent remineralization of organic matter. Woodlice (Isopoda, Crustacea) are involved in the breakdown of plant litter and wood (Gruner, 1966). This primary degradation is a prerequisite for decomposition eventually leading to complete mineralization into inorganic products or (temporary) stabilization as soil organic matter (Bernoux et al., 1998; Wolters, 2000; Gleixner et al., 2002). Woodlice are widespread and form a dominant component of the soil arthropod macro-decomposer community in many temperate habitats. They commonly inhabit the transition zone of aboveground and belowground compartments of woodland and grassland ecosystems. By their activity woodlice mediate between aboveground food web (AFW) and soil food web (SFW), i.e. they are key regulators of the ecosystem functions of decomposition and nutrient recycling (Paoletti and Hassall, 1999).

Nutritional behaviour and efficiency of woodlice do not only define their food web position but also their role in the transformation of organic matter. It was shown that in the presence of woodlice the disappearance of leaf litter from the ground increased drastically (Kautz and Topp, 2000). The level of food uptake by woodlice determines largely how much CO_2 will be respired and how much faeces can be produced. While the respired CO_2 is the final product of remineralization, faeces represent an important source of organic matter for following decomposers including microbes (Lee, 1997; Zimmer and Topp, 2002). Woodlice themselves also take up faeces supplementary to their primary food sources (coprophagy). Even though there is still debate on that issue (Kautz et al., 2002) and strong experimental prove for the nutritive benefits of coprophagy is missing (Szlávecz and Maiorana, 1998) this behaviour is supposed to be advantageous for woodlice and to play a role in the completion of their ecological function (Ullrich et al., 1991).

In general, coprophagy should have positive effects on the nutritional state of macro-decomposers such as woodlice (Kukor and Martin, 1986) but also freshwater pulmonate snails (Brendelberger, 1997) because of the supply with vitamins, amino acids and enzymes (e.g. for degrading cellulose, chitin, and phenolics). This is especially true if the available food has a low nutritive value and consists of compounds the degradation of which hardly provides the amount of energy required to maintain basic metabolic functions (Rothe and Gleixner, 2000). Therefore, uptake of processed faeces might even be a vital step in woodlouse nutrition (Ullrich et al., 1991; Zimmer and Topp, 1998).

Coprophagy recycles (matured) faeces back to its producers, introducing additional loops to the food web. To trace the actual path of organic matter, i.e., to identify trophic interactions and to describe the overall food web structure, analysis of stable carbon and nitrogen isotope ratios has become a widely used tool. According to studies by DeNiro and Epstein (1978; 1981), the isotope pattern of any organism closely resembles that of its diet. Normally, the consumer becomes slightly enriched in ^{15}N and ^{13}C compared to the diet, because the lighter isotope (^{14}N and ^{12}C) is preferred in the metabolism and therefore faster excreted. The isotopic shift between representatives of consecutive trophic levels, often referred to as trophic level shift (TLS), was found to be 3.4±1.1‰ for ^{15}N (Minagawa and Wada, 1984; Post, 2002) and 0-2‰ for ^{13}C (e.g. Michener and Schell, 1994, and references therein; Fantle et al., 1999). The stepwise enrichment in ^{15}N along the trophic chain can be used to assign organisms to trophic levels assuming that the 'trophic level shift' is a constant value. However, this assumption might not always be valid (Gannes et al., 1997). The isotopic difference between an animal and its diet is influenced not only by the isotopic composition of the diet but also by its quality and the ingested quantities (Sick et al., 1997; Focken, 2001). Other interfering factors are species, age, sex and nutritional state of an animal. Considering these factors, in any trophic relationship the isotopic shift should show temporal dynamics. We hypothesize that coprophagy as an additional factor may affect this dynamics and expect changes in the isotopic composition of woodlice and faeces in accordance with the degree of coprophagy.

Depending on their relative abundance, the control woodlice exert on the carbon turnover in terrestrial ecosystems may reach ecological importance. Primary degradation of plant-derived matter as well as recycling of faeces by woodlice causes physical and chemical alterations of the original material (Rothe and Gleixner, submitted). Quantity and quality of the produced faeces directly affect the transfer of organic matter to subsequent consumers (Jambu et al., 1988). The resulting isotope patterns could help to characterize processes involved in nutrient cycling in food webs. Therefore, the present study aimed to

1) investigate the effect of coprophagy by woodlice on food uptake, respiration and animal biomass;
2) demonstrate the influence of coprophagy on the isotopic signature of the woodlice and of faeces derived from coprophagy;
3) identify the significance of coprophagy in the nutrition of woodlice;
4) estimate the impact of coprophagy on the isotopic shift between consumer and diet and on the isotopic equilibration of the animal body with a new food source.

2. Materials and methods

2.1. Experimental setup

For the experiment running eight weeks, we divided 100 woodlice (*Porcellio dilatatus* BRANDT, 1833) of similar size and weight from a permanent standard culture (Rothe and Gleixner, 2000) into two groups to perform two treatments, one with and one without coprophagy. Both groups were subdivided into ten replicates of five male adults kept in a Petri dish. The Petri dishes were incubated at ca. 100% relative humidity under controlled light and temperature conditions (Rothe and Gleixner, 2000). In both treatments we supplied surplus amounts of autoclaved high quality food (C:N ratio of 15) twice a week. This food consisted of a mixture of freeze-dried carrot (2.7 parts) and lettuce (1 part, w/w) ground to particles <1 mm in size.

In both treatments we recorded changes of the dry weight of woodlice, the amount of ingested food and the amount of collectable faeces on a weekly basis in all present replicates, i.e., ten in the first week, nine in the second and so forth and three in the eighth week. In treatment "**C**" we daily **c**ollected the faeces, thus minimizing coprophagy. Per replicate the collected faeces of one week were pooled, and the pooled sample was subjected to further analysis. In treatment "**R**" **r**ecycling of faeces was permitted, i.e., faeces were sampled only in the replicate that was sampled by the end of every week. For the elemental and isotope analysis of faeces and woodlice, one replicate per treatment was sampled every week except for the last sampling date, after eight weeks, when the remaining three replicates were sampled. Woodlice and faeces were frozen immediately. The material was lyophylized, ground and stored at -18°C. Note that for stable isotope and C and N analyses in the time series from week 1 to week 8 the number of replicates per treatment *N* is 1 per week and that therefore the significance of changes could not be tested.

To determine the elemental and isotopic composition of woodlice, supplied food and collected faeces, we weighed samples according to 150 µg N or 50 µg C into tin capsules (separately for ^{15}N and ^{13}C). Samples were combusted in an EA 1110 Elemental Analyzer (ThermoQuest, 20090 Rodano, Italy) and analyzed for ^{15}N and ^{13}C content in a DeltaPlusXL isotope ratio mass spectrometer (Finnigan MAT, 28127 Bremen, Germany). The analytical precision was ±0.2‰ for ^{15}N and ^{13}C. Working standards were acetanilide ($\delta^{15}N$=1.78‰, $\delta^{13}C$= -33.94‰) and caffeine ($\delta^{15}N$= -1.16‰, $\delta^{13}C$=

-51.80‰), calibrated against international standards IAEA-N2 and NBS-22. Accuracy and repeatability of measurements were assured according to Werner and Brand (2001). Isotope ratios are expressed in conventional delta (δ) notation in parts per thousand: $\delta X(‰)=(R_{sample}/R_{standard} - 1) * 1000‰$, where $X = {}^{15}N$ or ${}^{13}C$ and $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$, respectively. Standard was AIR for ${}^{15}N$ and V-PDB for ${}^{13}C$.

2.2. Mass balance parameters

Weekly recorded *input* components were food utilized by the woodlice (*food*) and woodlouse biomass at the beginning of the week (wl_{start}) (Eqn. 1); *output* components at the end of the week were collected faeces (*faeces*), woodlouse biomass (wl_{end}), shed woodlouse skins (exuviae, *ex*) and biomass of dead woodlice (*dead*) (Eqn. 2). The difference between *input* and *output* was named '*respiration*' (Eqn. 3). This calculated term includes mass loss by respiration, gaseous excretion (e.g. ammonia) and escape of volatile organic compounds (e.g. pheromones).

$$input = food + wl_{start} \quad (1)$$

$$output = faeces + wl_{end} + ex + dead \quad (2)$$

$$\text{'}respiration\text{'} = input - output \quad (3)$$

The change of woodlouse biomass (Δwl) was calculated according to Eqn. 4:

$$\Delta wl = (wl_{end} + ex + dead) - wl_{start} \quad (4)$$

From Eqns. 1-4 follows that *food* can be split into 3 different fractions (Eqn. 5).

$$food = faeces + \text{'}respiration\text{'} + \Delta wl \quad (5)$$

The mass balance parameters of Eqn. 5 were related to the number of woodlouse individuals at the time of sampling. Since the large variance in the described parameters among the replicates made the present trends nearly unrecognizable, we decided to compare both treatments using sums over all present replicates of one week instead of mean values. The weekly sums were added up to obtain a cumulative sum for every week which was then related to the respective cumulative sum of woodlouse individuals. For example, regarding food consumption, in week 1 the amount of food consumed in the ten replicates of each treatment was summed and divided by the sum of animals present in these replicates at the end of week 1. This gave the amount of consumed food per

individual and week after the first week. In week 2 the sums of consumed food of week 1 and 2 were summed and divided by the sum of individuals present in week 1 and 2. This gave the amount of consumed food per individual and week averaged over the elapsed time period (two weeks). The calculation was done for all weeks and resulted in week 8 in a weekly per capita consumption rate, which represented an average over the whole time. This data reduction procedure provided per week only one value per treatment and no information on the variance of the respective mass balance parameter, however, these values allowed comparing general trends in time among the treatments.

The described procedure was applied to the amount of collected faeces, respired material (calculated according to Eqn. 3) and woodlouse biomass change (negative values!) giving for all eight weeks the per capita faeces collection rate, 'respiration' rate and rate of woodlouse biomass loss. In the same way we calculated the elemental balance, i.e. the loss per woodlouse individual of carbon and nitrogen on a weekly basis. C:N ratios were computed from the cumulative amounts of carbon and nitrogen in input (*food*) and output components (*faeces*, '*respiration*' and wl_{end}).

To test the significance of differences in the investigated parameters between treatments **C** and **R** non-parametric statistical tests for related samples (Wilcoxon Test, samples paired by week; SPSS for Windows 11.0) were performed.

2.3. Coprophagy and the trophic isotopic shift

To assess the importance of coprophagy in both treatments we estimated the *P*roportion of *R*ecycled *F*aeces (P_{RF}) in the woodlouse diet. For this, we calculated the amount of recycled faeces (*rf*) as the difference between the amounts of produced (*pf*) and collectable faeces (*cf*). These calculations were based on the assumption that under surplus supply of identical food the amount of produced faeces per woodlouse biomass (*wb*) should be statistically identical in both treatments. Also, we considered that in treatment **C** the collection of produced faeces was not complete since faeces were removed only once per day. This gave the woodlice some opportunity to feed on their faeces. However, the fraction of faeces taken up within 24 hours after their deposition is supposed to be small, because in general woodlice leave freshly dropped faeces untouched for at least some days (Hassall and Rushton, 1985; Zimmer and Topp, 1998). According to our observations on faeces consumption in-between the daily sampling events we estimated a constant collection coefficient *F* of 0.9 in treatment **C**; i.e. of the produced faeces 90% were collected.

Using F, we calculated for every week the mean amount of produced faeces per woodlouse biomass from the mean amount of collectable faeces per mean woodlouse biomass (Eqn. 6).

$$pf / wb = (cf / wb)\, F^{-1} \quad (6)$$

For every week, the mean amount of recycled faeces per woodlouse biomass was calculated from the cf / wb ratio using the pf / wb ratios obtained for treatment **C** also in treatment **R** (Eqn. 7).

$$rf / wb = pf / wb - cf / wb \quad (7)$$

The rf / wb ratio was used to calculate for every week for all present replicates the absolute amount of recycled faeces rf (Eqn. 8) and finally the proportion of recycled faeces in the diet P_{RF} (Eqn. 9).

$$rf = (rf / wb)\, wb \quad (8)$$

$$P_{RF} = rf / (rf + food) \quad (9)$$

The mean P_{RF} values were related to the according week number (Fig. 5). Natural log-regression gave the best and most likely fit for the curves of both treatments. The P_{RF} values derived from the regression equations were used to determine weekly δ values of the total diet (δ_{diet}) consisting of the supplied food and the recycled faeces (Eqn. 10).

$$\Delta X = \delta_{wl} - \delta_{diet} = \delta_{wl} - \frac{\delta_{faeces} * P_{faeces} * P_{RF} + \delta_{food} * P_{food} * (1 - P_{RF})}{P_{faeces} * P_{RF} + P_{food} * (1 - P_{RF})} \quad (10)$$

For every week the actual isotopic shift between woodlice and their diet ΔX ($X = {}^{15}N$ or ${}^{13}C$) was calculated (Eqn. 10) from the $\delta^{15}N$ or $\delta^{13}C$ values of woodlice, supplied food and collected faeces (δ_{wl}, δ_{food} and δ_{faeces}) and the nitrogen or carbon content of food and faeces (P_{food} and P_{faeces}) after linear regression of the original isotope and elemental data in order to overcome the partly large variation in these data.

To test the significance of differences in the obtained P_{RF} values between treatments **C** and **R**, non-parametric statistical tests for independent samples (Mann-Whitney Test; SPSS for Windows 11.0) were performed.

3. Results

3.1. Mass balance parameters

Within eight weeks, twice as much faeces were collected in treatment **C** (314 mg) compared to treatment **R** (157 mg). The weekly rate of collected faeces per individual averaged over the elapsed time period was significantly higher in treatment **C** (Wilcoxon Test: n=8; p=0.012). It decreased from 2.0 mg ind^{-1} week^{-1} after the first week to 1.3 mg ind^{-1} week^{-1} averaged over eight weeks. In treatment **R**, it decreased from 1.0 to 0.9 mg ind^{-1} week^{-1} (Fig. 1).

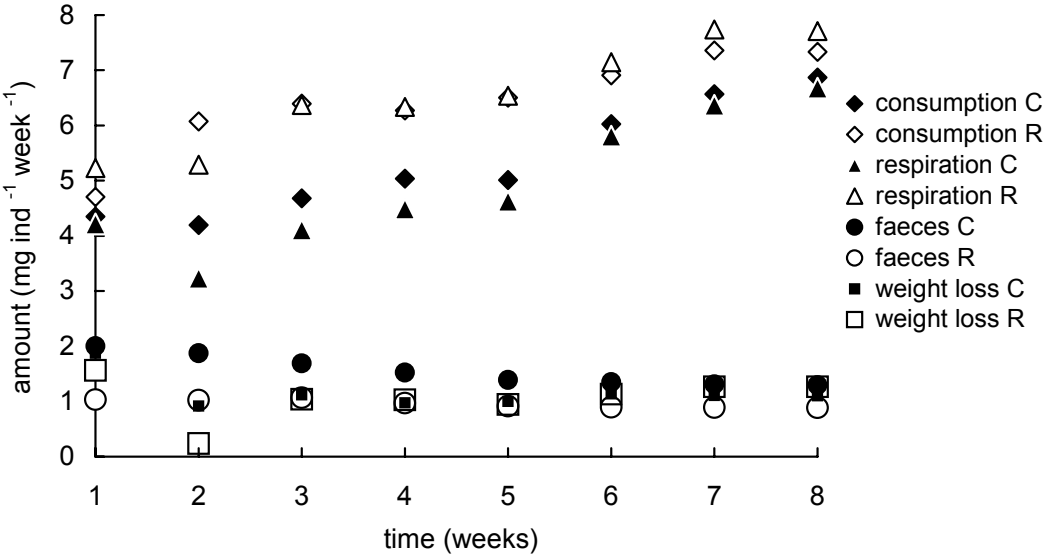

Fig. 1. Amounts of consumed food (consumption), respired matter (respiration), collected faeces (faeces) and lost woodlouse biomass (weight loss) per individual and week, averaged over the elapsed time period. In treatment **C** (C) faeces produced by *Porcellio dilatatus* were collected daily, in treatment **R** (R) faeces were left for recycling.

In contrast, weekly per capita consumption of experimental diet and 'respiration' rates were significantly higher in treatment **R** (Wilcoxon Test: n=8; p=0.012), both increasing with time (Fig. 1). In treatment **C**, the amount of consumed food increased from 4.3 mg ind^{-1} week^{-1} during the first week to 6.9 mg ind^{-1} week^{-1} averaged over eight weeks; in treatment **R**, from 4.7 to 7.3 mg ind^{-1} week^{-1}. While in treatment **C** 'respiration' was always lower than consumption, we found the opposite in treatment **R** (except for week 2) which is a consequence of a higher woodlouse biomass loss in comparison to the faeces collection rate (see Eqn. 5).

Compared to the initial weight (ca. 40 mg ind^{-1}), within eight weeks woodlouse biomass decreased by 13.1% in treatment **C** and by 10.9% in treatment **R**. There was no significant difference in the rate of biomass loss between treatments **C** and **R**. Compared to the amount of collected faeces, the loss of woodlouse biomass was lower in treatment **C** and higher in treatment **R** (except for week 2).

The loss of carbon and nitrogen through respiration and excretion from the experimental systems was significantly higher in treatment **R** compared to treatment **C** (Wilcoxon Test: n=8; for C: p=0.012, for N: p=0.049) (Fig. 2). Averaged over the elapsed time period the amount of lost carbon (nitrogen) increased from 1.6 (0.09) and 1.9 (0.10) mg ind^{-1} $week^{-1}$ to 2.6 (0.18) and 2.9 (0.21) mg ind^{-1} $week^{-1}$ in treatments **C** and **R**, respectively.

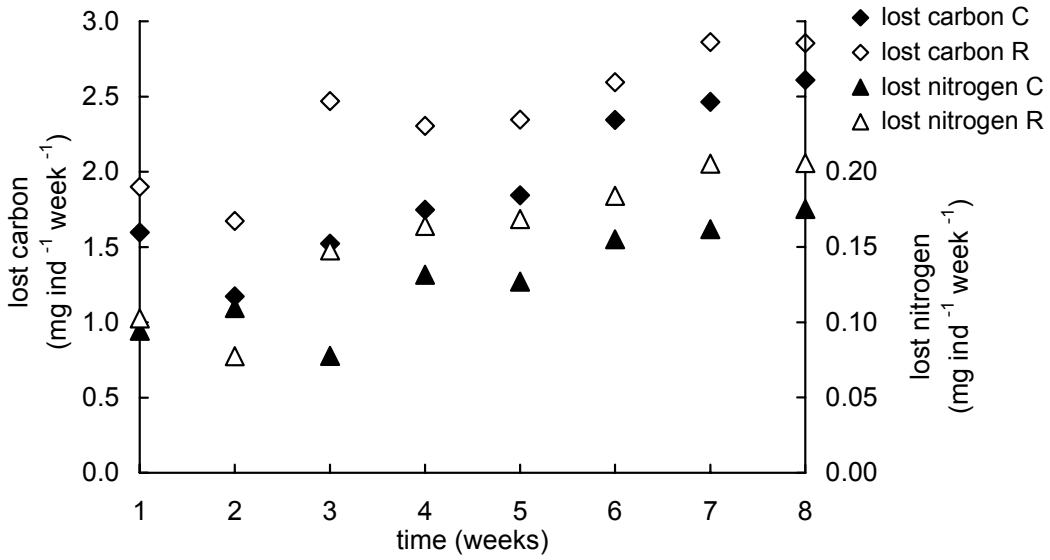

Fig. 2. Amounts of carbon and nitrogen per individual and week lost from the experimental system through respiration and gaseous excretion, averaged over the elapsed time period, in treatments **C** (C) and **R** (R) with *Porcellio dilatatus* as consumer.

The calculated weekly C:N ratio of the 'respired' material (which is a theoretical figure since the respired material cannot be analyzed for C and N) stabilized after initial fluctuations from week 5 onwards to become similar to the C:N ratio of the supplied food (~ 15) in treatment **C** and slightly lower (~ 14) in treatment **R** (Fig. 3). The C:N ratios of woodlice were almost identical in both treatments (Fig. 3). They remained relatively stable with time (6.3±0.1) but became slightly higher in treatment **R** after week 5 (differences statistically not significant; Wilcoxon Test: p>0.05). Within eight weeks, starting from 14.6 and 8.6, respectively, the C:N ratio of faeces continuously adjusted to a constant value of 11.6 in treatment **C** and 8.2 in treatment **R** (Fig. 3). The faeces of

treatment **C** started with a C:N ratio of 14.6 close to the used food (15.1) and remained – even though it was shifting to lower values – always closer related to the food. In contrast, in treatment **R** the faeces C:N ratio in week 1 was only 8.6 and decreased by 0.4, only.

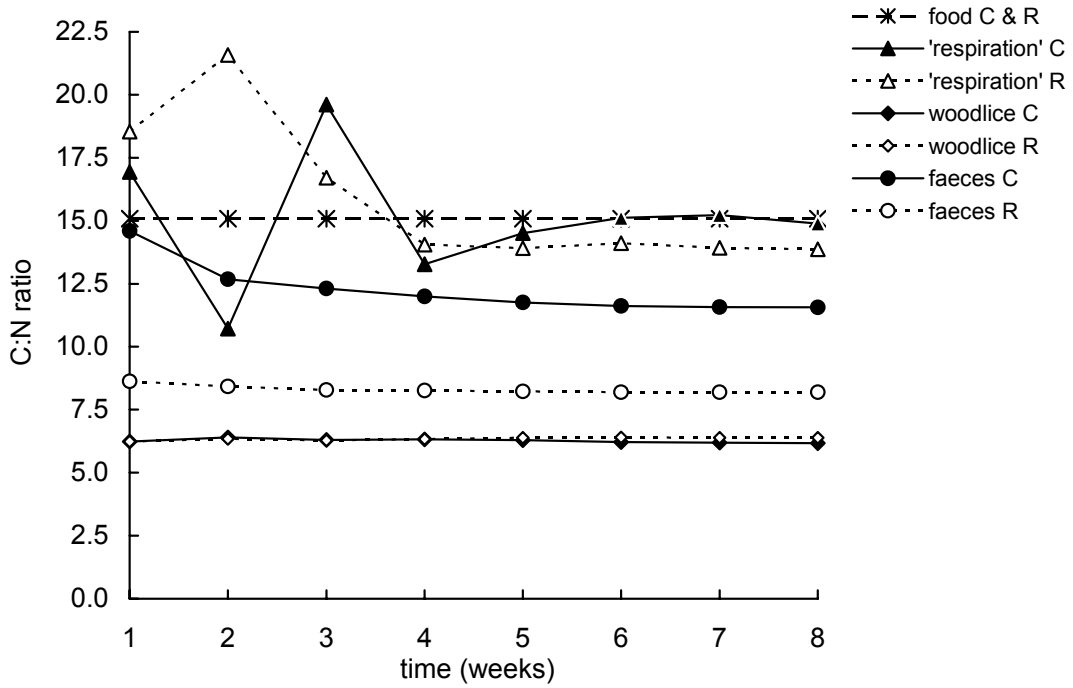

Fig. 3. Weekly C:N ratios of food, respired matter (respiration), woodlice and faeces, averaged over the elapsed time period, in the experimental treatments **C** and **R** with (C) and without (R) daily collection of faeces produced by *Porcellio dilatatus*.

3.2. Isotope patterns

Within eight weeks, animal $\delta^{15}N$ values in tendency slightly increased in both treatments (Fig. 4A). Woodlice started with a $\delta^{15}N$ value of 2.7‰ which was 0.7‰ higher than the supplied food (2.0‰). The final isotopic difference between woodlice and food was less than 1.3‰. The $\delta^{15}N$ value of the collected faeces – even though rather fluctuating – showed a small increase of averaged 0.27‰ $week^{-1}$ in treatment **C**, but a larger increase of averaged 0.62‰ $week^{-1}$ in treatment **R** (Fig. 4A).

While the $\delta^{13}C$ value of the supplied food remained constant (~ -31‰), woodlouse $\delta^{13}C$ values decreased from -25.9‰ to -26.8 and -27.1‰ in treatments **C** and **R**, respectively (Fig. 4B). This led to a final isotopic shift between woodlice and food of about 4‰. Although not significant, the small difference between treatments **C** and **R** in the ^{13}C-depletion of woodlice was influencing the calculation of the ^{13}C shift between woodlice and diet.

In treatment **C**, the faeces $\delta^{13}C$ value fluctuated strongly between the weeks (up to 5‰); however, the linear trend showed a decrease of averaged -0.02‰ week^{-1} (Fig. 4B). In contrast, in treatment **R** the $\delta^{13}C$ value of faeces generally increased by averaged 0.52‰ week^{-1}.

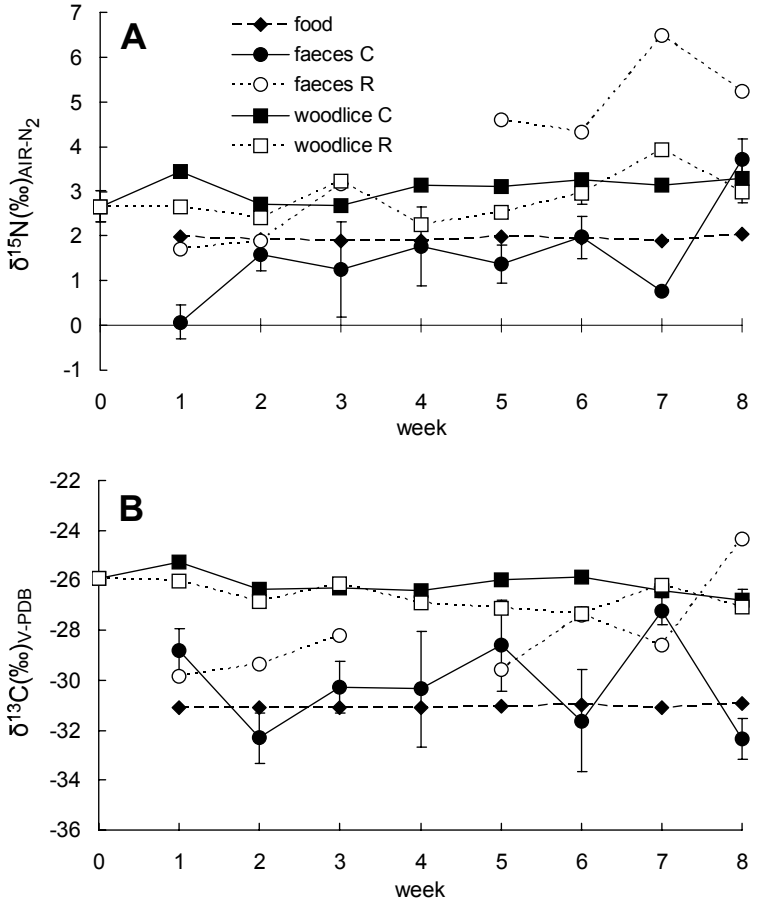

Fig. 4. Changes over time of $\delta^{15}N$ (A) and $\delta^{13}C$ values (B) of supplied food, collected faeces and harvested woodlice (*Porcellio dilatatus*) in treatments **C** and **R** with (C) and without (R) daily faeces collection. If material of more than one replicate was analysed data points represent means (±1 SD).

3.3. Coprophagy and the trophic isotopic shift

Compared to treatment **C**, the calculated P_{RF} values were significantly higher (Mann-Whitney Test) in treatment **R** in week 1 (n=10; p<0.001), 2 (n=9; p=0.001), 3 (n=8; p=0.015), 4 (n=7; p=0.004) and 6 (n=5; p=0.032). In week 5, no significant differences were found, in weeks 7 and 8 the sample size was too small (Fig. 5). The mean P_{RF} values showed a distinct decrease with time in both treatments. This indicated gradually reduced importance of coprophagy. In treatment **R** the proportion of recycled faeces in the diet ranged from 22% in week 1 to only 1% in week 7. The fluctuations of P_{RF} were rather high in comparison to treatment **C** where recycled faeces accounted for only 5% (weeks 1 and 2) to less than 1% (weeks 6 and 7) of the diet.

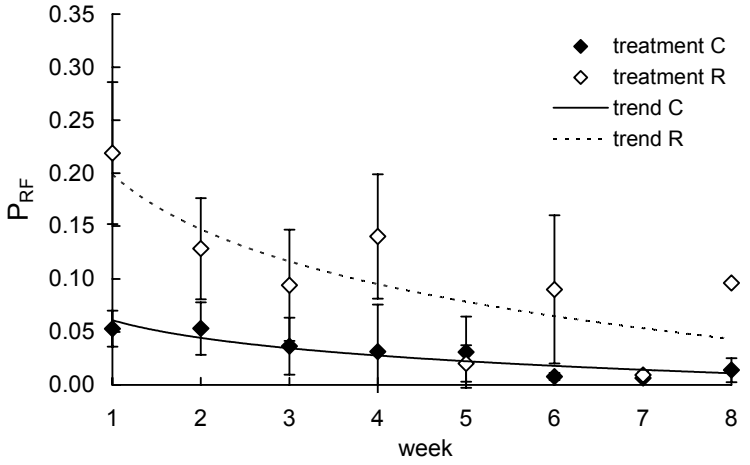

Fig. 5. Changes of the proportion of recycled faeces in the diet (P_{RF}) with time in treatments **C** (filled symbols) and **R** (open symbols). The trend lines for treatments **C** (solid) and **R** (dashed) derived from natural log-regression with R^2-values of 0.62 and 0.83, respectively. If applicable data points represent means (±1 SD).

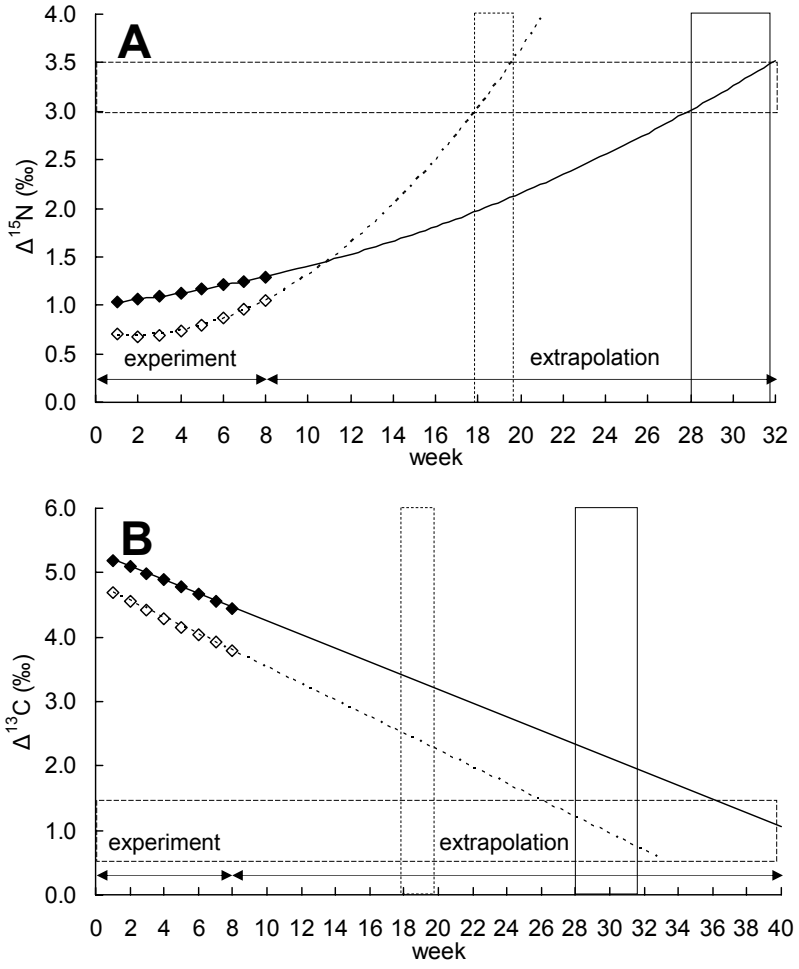

Fig. 6. Temporal dynamics of the isotopic differences (Δ) between woodlice (*Porcellio dilatatus*) and their diet (supplied food plus recycled faeces) for ^{15}N (A) and ^{13}C (B) in treatments **C** (filled symbols) and **R** (open symbols). The $\Delta^{15}N$ and $\Delta^{13}C$ values base on linear regressions of the $\delta^{15}N$ and $\delta^{13}C$ values of food, faeces and woodlice, respectively, and on natural log-regression of calculated P_{RF} values. The trend lines obtained for the eight weeks of experiment were extrapolated until they reached the range of the isotopic trophic level shift TLS (indicated by the long-dashed horizontal boxes). In Figure 6A, the vertical dashed box marks the time interval when the $\Delta^{15}N$ value of treatment **R** (dashed line) would pass through the predefined ^{15}N-TLS range, and the same for the solid box and the $\Delta^{15}N$ value of treatment **C** (solid line). In Figure 6B, the marked time intervals correspond to those in 6A (for more explanation see text).

During the experimental time, the calculated ^{15}N shift between woodlice and diet ($\Delta^{15}N$) was always higher in treatment **C**, increasing from 1.04‰ in week 1 to 1.29‰ in week 8; however, the slope of the curve increased more slowly compared to treatment **R** (Fig. 6A). In treatment **R** $\Delta^{15}N$ increased from 0.70‰ in week 1 to 1.05‰ in week 8. The initially lower isotopic shift in treatment **R** was due to the dynamics of coprophagy (Fig. 5) with a higher uptake of faeces in the first weeks. As faeces were enriched in ^{15}N compared to the supplied food (Fig. 4A) this caused higher ^{15}N content in the total diet. Later, increasing faeces $\delta^{15}N$ values were balanced by decreasing P_{RF} values. Eventually, the continuous rise of woodlouse $\delta^{15}N$ values led to the increasing slope of the $\Delta^{15}N$ curve in treatment **R** (Fig. 6A).

The ^{13}C shift between woodlice and diet ($\Delta^{13}C$) showed a linear decline (Fig. 6B), i.e. the woodlouse $\delta^{13}C$ values tended to approach those of the total diet. Again a higher isotopic shift was found in treatment **C**. The $\Delta^{13}C$ values decreased from 5.2‰ in week 1 to 4.5‰ in week 8, but the slope of the curve was less steep than in treatment **R**, the latter with a respective decrease from 4.7‰ to 3.8‰.

Being aware of the difficulty of the following extrapolation, just for a thought experiment we assumed that the treatment-specific dynamics of the isotopic shift would have continued if the experiment run longer than eight weeks. This was to estimate the time the $\Delta^{15}N$ and $\Delta^{13}C$ values would require to reach the range indicative for consumers that have isotopically adjusted to their diet (i.e. 3.0-3.5‰ for ^{15}N; 0.5-1.5‰ for ^{13}C), approximating the reported isotopic trophic level shifts (TLS) for ^{15}N and ^{13}C. Second order polynomials were fitted to the curves obtained from the calculated $\Delta^{15}N$ values over time (R^2 >0.999 and >0.995 for treatments **C** and **R**, respectively; Fig. 6A). Linear fits suited best in the case of the $\Delta^{13}C$ values (R^2 >0.999 for both treatment; Fig. 6B). We then extrapolated the trend lines until they crossed the predetermined range (marked by the horizontal long-dashed boxes) and read the elapsed time (Figs. 6A&B). In the case of ^{15}N the trend line of treatment **R** crossed that of treatment **C** three weeks after the experiment was finished (week 11) and reached the 3‰-level in week 18 (Fig. 6A). Because of the flatter slope in treatment **C** an isotopic shift of 3‰ was achieved only ten weeks later in week 28. The (theoretical) adjustment of the $\Delta^{13}C$ value to the defined range needed even longer. The 1.5‰-level was reached in week 26 by treatment **R** and in week 36 by treatment **C**; the 1‰-level approximately in weeks 30 and 40, respectively (Fig. 6B).

4. Discussion

4.1. Mass balance parameters

In treatment **R**, where woodlice had free access to the produced faeces, the amount of collected faeces was lower compared to treatment **C**, although the amount of consumed food was higher (Fig. 1). Since from the consumed food always a certain proportion becomes transferred into excrements, the amount of produced faeces should increase with the amount of consumed food. The high consumption rate in conjunction with a low faeces collection rate in treatment **R** clearly indicated that woodlice indeed utilized faeces. In contrast to our assumption that an increased food uptake resulted from a shortage of faeces, in treatment **R** a higher degree of coprophagy obviously induced higher per capita consumption and 'respiration' rates. Consequently, the transformation of the ingested organic matter into inorganic compounds was higher (by 15% on average) in treatment **R** compared to treatment **C**. The contradiction between the hypothesized benefits of coprophagy and the increase in food uptake and respiration in woodlice utilizing faeces should be discussed.

In *Porcellio scaber* reared on artificial diet, after one gut passage 40-70% of the cellulose present in the food was digested and the faeces did not contain any free glucose (Zimmer and Topp, 1998). This indicates a low energetic value of freshly deposited excrements compared to the supplied matter. However, after the proliferation of microorganisms, faeces represent organic material enriched in nitrogen and microbe-derived compounds. In our case, additional nitrogen as such might have been less important since the N content of the primary food was high compared to normal plant leaf litter. Although still in debate (Kautz et al., 2002), the advantage of coprophagy most likely lies in the opportunity to ingest organic matter which is easier degradable (e.g. microbial biomass) and contains compounds (e.g. enzymes, vitamins, precursor molecules) which would be unavailable to woodlice otherwise (Kukor and Martin, 1986). By this means, coprophagy in addition to the consumption of plant litter may facilitate the digestion of material present in the gut. Also, we have to consider a positive feedback of coprophagy on food consumption due to an appetizing effect connected with the utilization of faeces (M. Zimmer, pers. comm.) and the guarantee of efficient (re-) inoculation of the gut content with suitable microbes (Ullrich et al., 1991). The latter was of special importance in our experiment where the uptake of microbes with the primary food was impeded as it was autoclaved. The reduced availability of matured faeces due to daily collection in treatment **C** may have either reduced the abundance of

microorganisms in the intestine (dilution effect) or extended the gut passage to allow sufficient proliferation of microbes in the gut.

Taking into account these factors, we assume that woodlice in treatment **R** were able or even stimulated to take up more food compared to treatment **C**. Faster gut passage combined with more effective assimilation increased the flow of matter through the digestive system, thereby forcing woodlouse nitrogen and carbon pools to turn over faster and more complete than in woodlice of treatment **C**. This explanation is supported by the ^{15}N and ^{13}C dynamics of both treatments indicating a faster adaptation of the woodlouse isotope pattern to the experimental food in treatment **R** (Fig. 6).

From the consumed food the major proportion was just respired (Fig. 1). Unexpectedly, in treatment **R** the 'respiration' rate even exceeded the consumption rate. Note that 'respiration is a calculated term which accounts for respiration and gaseous excretion together, i.e. those output components that have not been recorded. Mathematically, the 'respiration' term exceeds food consumption if the loss of woodlouse biomass exceeds the amount of collected faeces (Eqn. 5) which has been the case in treatment **R**. For the additional mass loss from the experimental microcosms via respiratory CO_2 and nitrogenous excretions we suggest two treatment-specific reasons: Firstly, microbial activity on the produced faeces is an additional path for the production of respiratory CO_2 that may escape from the system. As the CO_2 released during the respiration process tends to be depleted in ^{13}C in comparison to the organism (DeNiro and Epstein, 1978), the remaining faeces substrate including the attached microbes will enrich in ^{13}C with time. Thus, the loss of respiratory CO_2 was mirrored by the increasing faeces $\delta^{13}C$ values. Secondly, woodlice presumably experienced enhanced stress due to the transition from breeding to experimental conditions (see below).

We did not find significant differences in the loss of woodlouse biomass per individual between treatments **C** and **R**; i.e., our hypothesis that coprophagy represented a positive factor for growth was not supported. However, despite the fact that woodlice were supplied with surplus amounts of high quality food, in both treatments they metabolized their own biomass. Additional feeding trials revealed that if food quality exceeds a specific threshold this can even have negative effects on woodlice (data not shown). Because the digestive system of woodlice is normally adapted to low quality food, e.g. plant litter, prolonged utilization of artificial diet with low C:N ratio and low content of fibre and refractory compounds as the only food source may cause digestive disorders which finally result in the loss of animal body mass. This shows that food quality can also be too high for ensuring optimum nutrition.

Beside the fact that food quality was probably above the optimum range, for the mass loss from the experimental system we also address the stress situation under experimental conditions. Already the switch to autoclaved high quality food demands the adaptation of the gut microflora and may temporarily reduce assimilation efficiency. Here, faeces uptake may ensure the effective inoculation of the ingested matter with microbes accelerating the adaptation process. In fact, in the first two weeks of the experiment the loss of woodlouse biomass was considerably lower in treatment **R** (Fig. 1). After three weeks, however, the positive effect of coprophagy on growth disappeared since both treatments became differently impacted by cannibalism during molt events. Woodlice molted much more frequently in treatment **R**. At the end of the experiment, 21 of initially 50 individuals (42%) had died in treatment **R**, but only 8 (16%) in treatment **C**. This high mortality in treatment **R** appears to be related to coprophagy as the one factor in a chain of causation which applied to treatment **R**. The switch to high quality food in combination with the availability of matured faeces changed internal conditions of the woodlice what triggered the signal to molt. As the Petri dishes did not provide refuges to protect molting animals which are highly vulnerable almost all molting woodlice were attacked by their associates resulting in 16 victims in treatment **R** after week 4. Even though we tried to remove cannibalized individuals as soon as possible there certainly remained a contribution of cannibalism to the higher respiratory losses (see above). Rather than a proof that coprophagy was detrimental, the high mortality in treatment **R** was due to the specific experimental conditions and could have been prevented by keeping each individual in a separate vessel. While food consumption and 'respiration' clearly were positively affected by coprophagy, for woodlouse biomass we can only assume from the results of the first two weeks that the uptake of faeces in addition to the primary food is of advantage for regular growth, especially in the long term and especially in the case of low food quality (Kautz et al., 2002).

In both treatments, the losses of carbon and nitrogen were tightly linked. The higher 'respiration' rate in woodlice recycling faeces (Fig. 1) was not simply caused by the release of respiratory CO_2 but accompanied by a proportional loss of N-containing compounds (Fig. 2). In treatment **R**, the nitrogen loss was more pronounced and led to a lower theoretical C:N ratio of the 'respired' matter compared to treatment **C** (Fig. 3). Concordantly, averaged over the experiment, the C:N ratio of woodlice was slightly higher in treatment **R**.

The elemental losses from woodlouse bodies also influenced the C:N ratios of the collected faeces (Fig. 3). Since in treatment **C** faeces were collected every day and immediately frozen, the time for microbes to act on this organic matter was almost negligible. Thus, the continuous shift from a C:N ratio nearly as high as the supplied

food to distinctly lower values cannot be explained by external microbial faeces processing. Instead, we address the increasing influence of material derived from woodlouse nitrogen and carbon pools on the production of faeces and the successive adaptation of the gut microflora to the experimental diet.

In treatment **R**, however, faeces were left in the Petri dishes for at least seven days up to five weeks to stimulate coprophagy. Here, the C:N ratio of faeces was very low (8.6) in the first week, already (Fig. 3). Both, a pronounced contribution of woodlouse-derived nitrogen and a quick proliferation of microbiota in the gut (supported by a fast inoculation of the autoclaved food due to the uptake of faeces) would allow for a high nitrogen concentration in the material present in the posterior hindgut and may have caused low C:N ratios in the produced faeces. In subsequent external processing steps, microbes further reduced the carbon content of the faeces by the release of respiratory CO_2 while N-containing compounds escaped to a smaller extent. This was mirrored by the average contents of C and N: In treatment **R**, faeces were strongly depleted in C (23±0.8%) but enriched in N (2.9±0.1%) compared to faeces in treatment **C** (28±1.7% and 2.5±0.1%, respectively) and the supplied food (38.2±0.1% and 2.5±0.1%, respectively). Like in treatment **C**, the slight decrease of the faeces C:N ratio in treatment **R** after week 1 suggests that, especially during the first phase of the experiment after the switch to the artificial diet, the gut microbiota of the woodlice were adapting to the new food source.

Similar to the relative accumulation of nitrogen in woodlouse faeces, Mangrove Sesarmine Crabs (*Sesarma messa*) seemed to remove primarily carbon rather than nitrogen from the mangrove litter, as reflected by the decrease in C but slight increase in N content of the crab faeces over mangrove litter (Lee, 1997). Accordingly, the C:N ratio of the faecal material was significantly lower than that of the mangrove litter from which it was generated. It decreased further in the next decomposition step which was mediated by a detritivorous amphipod using the crab faeces as food source (Lee, 1997). This corroborates our findings that coprophagy contributes to the enrichment of N relative to C in the recycled organic material.

4.2. Isotope patterns

In general, the stable isotope ratios of nitrogen and carbon were in accordance with the mass and elemental balance data. They provided strong evidence for a positive treatment effect as mirrored by the larger ^{15}N and ^{13}C shifts in woodlice recycling faeces compared to woodlice prevented from coprophagy and by the enrichment of ^{15}N and ^{13}C isotopes in the faeces of coprophagous woodlice (treatment **R**).

In a previous study on the effect of food quality on the consumer's isotopic signatures, within seven weeks $\delta^{15}N$ values of woodlice increased by 0.9‰ (C:N 200) and 2.3‰ (C:N 50) depending on the food quality. The isotopic shift for ^{15}N between woodlice and the supplied food reached 2.5 and 5.7‰, respectively (Rothe and Gleixner, 2000). In this study, woodlice feeding on high quality food (C:N 15) for eight weeks became enriched in ^{15}N by less than 0.7‰. The final isotopic difference between woodlice and food, $\Delta^{15}N$, of less than 1.3‰ was much lower than the recorded average ^{15}N- trophic level shift of 3.4‰ (Minagawa and Wada, 1984) which was confirmed by a number of field studies (Peterson and Fry, 1987; Ponsard and Arditi, 2000; Scheu and Falca, 2000; Post, 2002).

Contrasting levels of nutritional stress could be the most important factor determining the different extent of ^{15}N change in animals found in both studies. Stress can be caused by the uptake of inadequate quantities of nutriments or by an insufficient quality. In rats, for example, the isotopic difference between plasma protein and diet was clearly increasing when protein ingestion went below and above the protein requirements (Sick et al., 1997). It also increased with decreasing protein quality. Adams and Sterner (2000) found a significant positive linear relationship between the $\delta^{15}N$ value of daphnids and the C:N ratio of the green algae the daphnids were reared on. Juvenile blue crabs feeding on protein-poor diet fractionated nitrogen isotopes to a much greater extent than crabs on diet nutritious enough to meet the metabolic requirements (Fantle et al., 1999). Both, too little utilizable protein (as in our former study) or the oversaturation of the digestive system, which causes high deamination rates and excretion of high amounts of ^{15}N depleted urea or ammonia, may increase animal $\delta^{15}N$ values (Focken, 2001). Most likely, in the present study the offered diet matched the metabolic requirements with respect to the nitrogen supply leading to the observed little change of the woodlouse ^{15}N signatures.

In our former study, woodlouse $\delta^{13}C$ values increased by ~3‰ due to utilization of storage fat by the starving animals as a clear indication for dietary deficits (Rothe and Gleixner, 2000). This was not the case in the present study, although in both treatments woodlice lost weight. The $\delta^{13}C$ values of woodlice decreased by ca. 1‰ towards the $\delta^{13}C$ value of the supplied food. Even though woodlice were slowly adapting to the food, the isotopic difference between woodlice and food, $\Delta^{13}C$, did not reach the typical range of the ^{13}C- trophic level shift (0.5-1.5‰) by the end of the experiment. Like in the case of ^{15}N, also in ^{13}C a period of eight weeks was too short for *P. dilatatus* to isotopically equilibrate with the experimental food.

In treatment **C**, faeces derived from primary food during the first gut passage; i.e., the isotope pattern of faeces was influenced by woodlouse internal processes, only. Therefore, in treatment **C** the collected faeces changed isotopically only to a minor extent. In treatment **R**, however, the initial material could pass the gut several times due

to coprophagy. Thus, the former food material aged either within the gut or (between gut passages) as faeces outside the woodlice. Like in many crustaceans, the waste product of nitrogen metabolism in woodlice is ammonia which is strongly depleted in ^{15}N (Fantle et al., 1999). This ammonia is excreted by maxillary glands in the head region of the animals and may escape from the experimental system. Thus, the ^{15}N signature of the dropped faeces should not be influenced by 'light' ammonia, but mainly by fractionation on nutrient assimilation from the gut into the woodlouse metabolism and by microbial activity inside (internal processing) as well as outside the woodlouse body (external processing). The specific microbiological situation in treatment **R** forced the nitrogen enrichment in the faeces (i.e. lower C:N ratios compared to treatment **C**; see above), and led to increased faeces $\delta^{15}N$ values (Fig. 4).

The mechanism underlying this ^{15}N enrichment is not fully understood yet. The excretion of gaseous ^{15}N-depleted metabolic waste products of the microbes involved in faeces production is probably the most important aspect. However, also the repeated uptake of faeces which contain microbe-derived free substances (e.g. exoenzymes) and products of the enzymatic activity (e.g. free amino acids) plays a role. Such compounds are supposed to be depleted in ^{15}N relative to intact microbial cells and the source material microbes act on. Upon ingestion, woodlice can easily assimilate those 'lighter' compounds while other parts of the ingested matter are more refractory. By this means, the excrements (a mixture of the old and of young faeces) become enriched in ^{15}N. The cycle might repeat as long as the faeces support microbial growth. Further isotope fractionation during the external processing of faeces could be expected, but was not observed in the absence of woodlice, yet (Rothe and Gleixner, submitted).

The ^{13}C development of faeces in treatment **R** is in accordance with the explanation given for ^{15}N. Increasing faeces $\delta^{13}C$ values indicate the loss of ^{13}C depleted respiratory CO_2 due to internal and external microbial activity (Fig. 4B).

4.3. Coprophagy and the trophic isotopic shift

Mass balance parameters as well as isotopic signatures were influenced by the extent of coprophagy. In both treatments the proportion of recycled faeces in the diet P_{RF} decreased with time to finally less than 5%, i.e., faeces represented an important dietary component during the first days after the change to the experimental diet, however, their significance became reduced as the digestive system of the animals adjusted to the artificial diet (Fig. 5). Consequently, coprophagy facilitated the adaptation of the digestive system of woodlice to changing food sources. Moreover, the temporal reduction

of the importance of faeces uptake supported the assumption that in this experiment for energetic requirements woodlice were not depending on coprophagy.

The latter result does not imply that coprophagy could generally be neglected when studying nutrient cycling and trophic interactions in the field. Just from the evolutionary point of view it can be supposed that in coprophagous animals such as woodlice the uptake of faeces in addition to the primary food is a premise for their optimal performance, especially in the long term. Regarding this, coprophagy may gain significance even at the ecosystem level because of its involvement in the functioning of the entire decomposer system. The main ecological function of woodlice, for example, is the mechanical disintegration and microbial inoculation of organic matter. We have shown that coprophagy by woodlice increased food consumption and 'respiration' leading to an increased turnover and transformation of organic matter. The produced faeces were enriched in nitrogen compared to the primary food and represent an attractive source of nitrogen to other organisms. Because of the commonly lower food quality in the field compared to the experiment, macro-decomposers such as woodlice need 'microbial auxiliaries' to reduce nutritional stress (Ullrich et al., 1991) and coprophagy is supposed to be an important environmental factor (Kautz et al., 2002).

For assessing the trophic state of (coprophagous) animals in food webs using isotope data a reliable value for the isotopic shift between two adjacent trophic levels (trophic level shift, TLS) has to be adopted. It is still a matter of investigation if there is a more or less sharp value which can be regarded as 'equilibrium-TLS' after a consumer has physiologically and whereby isotopically adjusted to its diet (Focken, 2001; Post, 2002). Our results show clearly that, depending on the food quality, the isotopic difference between a consumer and its diet might need a long time until a predefined TLS is reached (e.g. 3.4‰ for ^{15}N as reported by Minagawa and Wada, 1984), even in small animals such as woodlice. As a thought experiment, we assumed constancy in the dynamics of the isotopic changes in food, faeces and woodlice. The extrapolation of the experimental results revealed that adult individuals of *Porcellio dilatatus* theoretically needed at least 18 weeks to reach a $\delta^{15}N$ value 3‰ higher than the diet (Fig. 6A). Coprophagy reduced the time required to achieve the pre-defined TLS value markedly that again suggests an enhanced internal carbon and nitrogen turnover in woodlice utilizing faeces.

The influence of coprophagy on the isotope patterns in trophic relationships does neither affect the studied organisms nor any ecosystem processes, however, it is important to consider these factors that influence trophic isotopic shifts when studying nutrient cycling and food web structure in the field. In addition, in nature we would not expect such differences originating from the shortage of faeces as there is no artificial restriction of coprophagy. Instead, it might be even difficult to find (matured) faeces in

the field as they become eaten by other decomposers and degrade rapidly (Hopkin and Martin, 1984). Nevertheless, ^{15}N-enriched faeces resulting from more or less intense coprophagy may contribute to the isotopic enrichment of the soil organic matter and to the shift of the decomposer system to higher $\delta^{15}N$ values.

Within eight weeks, the $\Delta^{13}C$ values changed more rapidly (by -0.7 and -0.9‰ in treatments **C** and **R**, respectively,) than the $\Delta^{15}N$ values (0.25 and 0.35‰, only). Nevertheless, woodlice needed longer (at least 26 weeks) to reach a $\delta^{13}C$ value 1.5‰ higher than the diet. One reason was the large initial difference between woodlice and diet of ~5‰. Like in the case of ^{15}N, the extrapolation demonstrated that due to a higher uptake ^{13}C-enriched faeces and a higher woodlouse internal carbon turnover coprophagy can accelerate the isotopic equilibration of the woodlouse body with a new diet (Fig. 6B).

While confirming and explaining nicely the mass balance results, the isotope data also question under which circumstances the application of a fixed TLS value is suitable to define trophic relationships from stable isotope measurements. The same issue was brought up recently by a study on the effect of ration size on the TLS (Focken, 2001), specifying numerous parameters which influence the isotopic signature of whole organisms and single fractions (quality and quantity of the diet, species, age, physiological state, metabolic rates etc.) and suggesting well-designed laboratory experiments to achieve better understanding of the different processes involved. We demonstrated that after the switch to a higher food quality isotope ratios in adult woodlice shifted rather slowly. Other studies have shown that in juvenile animals with higher metabolic rates (Fantle et al., 1999) and under low food quality (Rothe and Gleixner, 2000) isotopes may become fractionated to a much greater extent. The question remains if in natural food webs there is an effective stabilization of the trophic relationships which causes the isotopic shift between consumer and diet to level off, and at what TLS value this stabilization would occur under varying environmental conditions. Therefore, in addition to laboratory experiments, field studies should be increasingly conducted to find more evidence to either accept or reject the use of predefined TLS values to draw trophic webs from ^{15}N and ^{13}C data.

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4.3. Manuscript III

Elemental and isotopic changes during the transformation of organic matter by terrestrial isopods (Crustacea, Oniscidea) and the significance of coprophagy

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Elemental and isotopic changes during the transformation of organic matter by terrestrial isopods (Crustacea, Oniscidea) and the significance of coprophagy

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Abstract

We investigated changes of the elemental and isotopic composition of woodlice and their diet after feeding on isotopically different food and recycling labelled and unlabelled faeces. Elucidating the effect of changing food sources on animal biomass, the role of woodlice in the transformation of organic matter (OM), and the significance of coprophagy for OM alteration and isotopic adaptation of woodlice, this study should improve the understanding of OM cycling in terrestrial ecosystems in order to describe ecosystem regeneration by reconstructing trophic interactions.

Over eight weeks adult woodlice were fed two types of food labelled with ^{13}C using C3- and C4-cellulose. Additionally, animals recycled either self-produced faeces or supplied faeces differently labelled with ^{13}C than their food. Weekly harvested food, faeces and woodlouse samples were analyzed for C and N contents and for isotopic composition.

Feeding on the experimental diet increased $\delta^{15}N$ values, but decreased C:N ratios and $\delta^{13}C$ values of woodlice irrespective of the ^{13}C label. In turn, relative to the diet faeces were enriched in ^{13}C (up to 4‰) and ^{15}N (~ 2.5‰) and revealed higher C:N ratios. Unexpected alteration of non-ingested OM was shown for the supplied faeces. Caused by the action of both, microorganisms and woodlice, in the remaining faeces $\delta^{13}C$ value and C content increased, whereas $\delta^{15}N$ value and N content decreased. Isotopically, adult woodlice only slowly adapted to the new diet. After eight weeks the isotopic shifts between woodlice and diet were still too small to account for the trophic level enrichment in ^{13}C and ^{15}N. Introducing coprophagy and including only the assimilated part of the diet in the trophic shift modelling did not improve the result. The high diet quality and a low level of internal C and N turnover in full-grown animals were addressed.

Coprophagy clearly affected the elemental and isotopic composition of woodlice and faeces. While the contribution of recycled faeces to nutrition decreased from 18% to 6% during the adaptation of woodlice to the new food, the efficiency of faeces assimilation increased to more than 80%. Our study highlights that coprophagy accelerates the adaptation to changing food sources and improves food utilization by decomposing animals.

Key words: ^{13}C label; adult woodlice; decomposition; isotopic adaptation; isotopic shift; *Porcellio dilatatus*; trophic interaction; trophic level

1. Introduction

Woodlice (isopods) play an important role in the transformation of organic matter (OM) in terrestrial ecosystems (Hassall et al., 1987). They are involved in the primary degradation and microbial inoculation of mainly plant-derived OM. In the litter layer of woodlands and grasslands they shred considerable amounts of litter and wood into small fragments and deposit rapidly decomposable faecal pellets (Gruner, 1966). These faecal pellets are an important source of OM for other decomposers and for the whole food web (Jambu et al., 1988; Lee, 1997; Zimmer, 2002). In addition, woodlice graze microbial biomass growing on surfaces (Gunnarson, 1987) and also recycle their own faeces (Hopkin, 1991). So far, little is known about the changes in the chemical and isotopic characteristics of OM during the conversion of woodlouse food into faeces and about the significance of coprophagy (faeces recycling) in this process (Zimmer and Topp, 1998; Zimmer and Topp, 2002).

Woodlice are as macro-decomposers integral part of the food web. To determine their position within the food web, in general the ratios of stable carbon and nitrogen isotopes, expressed as $\delta^{13}C$ and $\delta^{15}N$ values in per mill (‰), are used. It is known that the trophic level shift (TLS), i.e. the isotopic difference between organisms from two adjacent trophic levels, is in the range of 3.4 ± 1.1‰ for ^{15}N (Minagawa and Wada, 1984) and 0 – 2‰ for ^{13}C (DeNiro and Epstein, 1978; Fantle et al., 1999). Therefore, commonly $\delta^{15}N$ values are used to assign species to trophic levels and to reconstruct food webs (Stapp et al., 1999; Scheu and Falca, 2000). The $\delta^{13}C$ values in combination with naturally labelled OM, e.g. C3 and C4 plant biomass, allow tracing food sources (Schoeninger, 1982; Michener and Schell, 1994; Heaton, 1999).

According to the theory, when utilizing a constant diet in the longer run, the animal body shall isotopically adapt to the ^{13}C and ^{15}N signature of the diet until equilibration (DeNiro and Epstein, 1978; DeNiro and Epstein, 1981; Ponsard and Averbuch, 1999). At this time the actual isotopic shift between animal and diet (Δ) should be in the order of TLS. However, due to their effect on the animal isotopic composition many factors influence Δ and mask the real trophic position. These factors comprise nutritional stress caused by insufficient diet quantity and/or quality, age and species (Sick et al., 1997; Rothe and Gleixner, 2000; Focken, 2001). Also, isotopic equilibration requires that a consumer is able to exploit the same food source(s) for an adequate period of time; i.e., the relative stability of the trophic interactions is a prerequisite for Δ to approach TLS. In

consequence, to apply stable isotope techniques to food web research in highly dynamic ecosystems stability in particular is most important.

A number of field studies from intact ecosystems implying well-tuned trophic interactions largely confirmed the reported TLS for ^{15}N (Peterson and Fry, 1987; Hobson et al., 1994; Kwak and Zedler, 1997; Ponsard and Arditi, 2000). However, in developing ecosystems such as systems that regenerate from disturbance species turnover and fluctuating abundances of plants and animals may cause rapid changes in the food sources available to a consumer (Rothe and Gleixner, accepted). If the time required to isotopically adjust to a new food source exceeds the time of source availability, the isotopic signature of a consumer will not reflect that of the recently exploited food source. For that reason it is essential to have information on how fast a consumer does isotopically adapt to new food sources.

Because they can be taxonomically identified more easily than juvenile stages, in field studies often adult individuals are preferred for stable isotope analysis. Therefore, it is especially interesting how adult organisms adapt to changing food sources. Moreover, there is indication that coprophagy may accelerate the nutritional and also the isotopic adaptation to a new food source (Ullrich et al., 1991; Rothe and Gleixner, submitted).

Consequently, we conducted a feeding experiment with ^{13}C labelled food and faeces to investigate changes of the C and N contents and the $\delta^{13}C$ and $\delta^{15}N$ values of OM and woodlouse biomass over time. We hypothesized that depending on the level of faeces uptake coprophagy will influence the process of isotopic adaptation to a new food source and the alteration of OM by woodlice. Because woodlice were full-grown we also expected the isotopic adaptation of animal biomass to occur at reduced speed. Our questions to the experimental system are:

1. How and how fast is the elemental and isotopic composition of adult woodlice changed by new food?
2. What is the elemental and isotopic relation between consumed food and produced faeces?
3. Do woodlice influence the elemental and isotopic composition of the remaining food?
4. How is coprophagy feeding back on the elemental and isotopic composition of produced faeces and woodlice?

2. Materials and methods

2.1. Experimental setup

In a feeding experiment woodlice took up food and faeces either equally or differently labelled in ^{13}C (Fig. 1). We used male adults of *Porcellio dilatatus* (BRANDT, 1833) of similar size and weight from a permanent standard culture (Rothe and Gleixner, 2000) to set up four treatments and two faeces production lines each with ten replicates (in faeces exchange treatments five replicates). Each replicate consisted of seven individuals kept in one Petri dish. The Petri dishes were incubated at 90 – 100% relative humidity under controlled light and temperature conditions for eight weeks. In addition, for reference (**R**) five times eight woodlice were collected from the permanent culture at time zero.

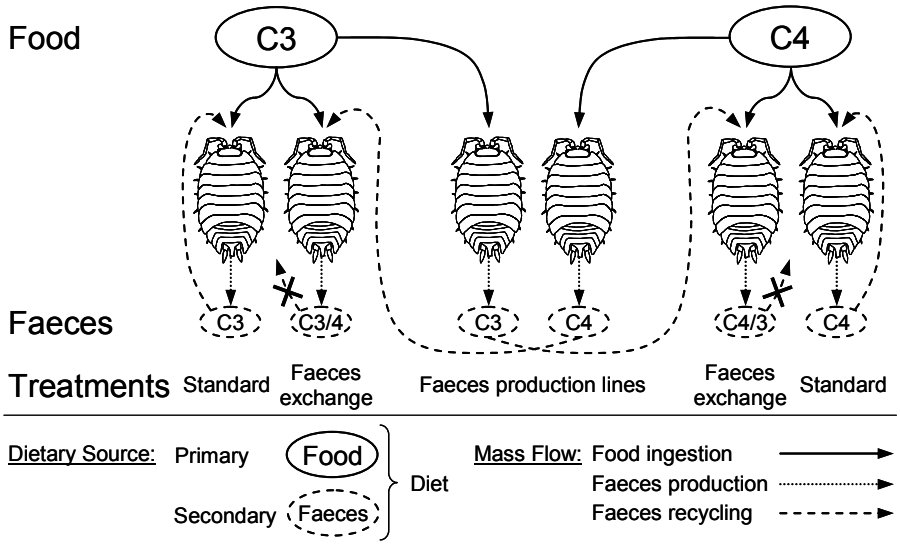

Figure 1. Scheme of the experimental setup. In standard treatments woodlice recycled their self-produced faeces showing the same ^{13}C label as the consumed food. In faeces exchange treatment woodlice recycled faeces differently labelled than the supplied food and produced faeces with a mixed isotopic signal. The exchanged faeces originated from respective faeces production lines. The flow of organic matter during food ingestion, faeces production and faeces recycling is indicated by arrows.

In the standard treatments **C3** and **C4** woodlice were supplied with C3- and C4-food and were able to recycle their own faeces produced from C3-food (C3-faeces) and C4-food (C4-faeces), respectively (Fig. 1). In the faeces exchange treatments **C3/4** and **C4/3** woodlice were also supplied with C3- and C4-food, respectively, but were hindered to take up their own faeces by a stainless steel grid on the bottom of the Petri dish which was passed by faeces pellets. To allow for recycling of faeces differently labelled than

the food, C3-fed woodlice were supplied with C4-faeces and C4-fed woodlice with C3-faeces originating from the respective productions lines (Fig. 1). Accordingly, the faeces produced in faeces exchange treatments contained both, C3 and C4 labelled material, and were named C3/4- and C4/3-faeces, respectively (Fig. 1). The sum of ingested matter, i.e. the food as primary and the faeces as secondary dietary source together, was referred to as the diet.

The food (C: N ratio = 35) consisted of freeze-dried carrot-lettuce mix (one part) as nitrogen supplying high-quality component with a $\delta^{13}C$ value of -31.2‰ and of cellulose to introduce the ^{13}C label and to increase the fibre content (one part; w/w). The cellulose originated either from spruce, a C3 plant, or from maize, a C4 plant, with $\delta^{13}C$ values of -23.5‰ and -11.9‰, respectively. C3 labelled cellulose was purchased (Sigma); C4 labelled cellulose was extracted from maize straw by sodium chlorite oxidation (Loader et al., 1997). In C3- and C4-food both components were homogenized and ground to powder. The difference of the $\delta^{13}C$ values between C3- and C4-food accounted for -7‰.

From the C3- or C4-food we provided surplus amounts (~ 100 mg dry weight) to each replicate twice a week. In faeces exchange treatments faeces (10 – 30 mg dry weight) was supplied once per week. The supply started in the second week. Exchange faeces were obtained from pooling the last-week faeces harvested in the production lines. Faeces were homogenized by adding a small volume of sterile water and stirring. The resulting faeces mash was divided into portions, one for analysis and five for the replicates of the faeces exchange treatments. In addition, we used homogenized and portioned spare faeces from the faeces production lines to incubate faeces for eight weeks under the same conditions like in the treatments, however, in the absence of woodlice (nine replicates).

Every week, for each replicate we recorded woodlouse biomass, amount of consumed food and amount of collected faeces. Additionally, in faeces exchange treatments the amount of consumed faeces was recorded. In all replicates we weekly harvested the produced faeces; in faeces exchange treatments also the remnants of the supplied faeces. Moreover, in the standard treatments we harvested one replicate per week from week 1 to 7. In week 8, the five replicates of both faeces exchange treatments and the residual three replicates of both standard treatments were harvested at once. Woodlice, produced faeces and remnants of supplied food and faeces were frozen immediately. The material was lyophylized, ground and stored at -18°C.

To determine the elemental and isotopic composition of food, faeces and woodlice we weighed separate samples for ^{15}N and ^{13}C according to 150 µg N or 50 µg C into tin capsules. Samples were combusted in an EA 1110 Elemental Analyzer (ThermoQuest, 20090 Rodano, Italy) and analyzed for ^{15}N and ^{13}C contents in a DeltaPlusXL isotope ratio mass spectrometer (Finnigan MAT, 28127 Bremen, Germany). The analytical precision was ± 0.2‰ for ^{15}N and ^{13}C. Working standards were acetanilide ($\delta^{15}N$ = 1.78‰, $\delta^{13}C$ = -33.94‰) and caffeine ($\delta^{15}N$ = -1.16‰, $\delta^{13}C$ = -51.80‰), calibrated against the international standards IAEA-N2 and NBS-22. Accuracy and repeatability of the measurements were assured according to Werner and Brand (2001). Stable isotope ratios were expressed in conventional delta (δ) notation in parts per thousand: δ (‰) = ($R_{sample}/R_{standard}$ - 1) · 1000‰, where δ = $\delta^{15}N$ or $\delta^{13}C$ and R = $^{15}N/^{14}N$ or $^{13}C/^{12}C$, respectively. Standard was AIR for ^{15}N and V-PDB for ^{13}C.

2.2. Calculations

2.2.1. Proportion of recycled faeces in the diet ($P_{RF\text{-}diet}$)

The proportion of recycled faeces in the diet ($P_{RF\text{-}diet}$) values was derived from separate mass balances for treatments **C3/4** and **C4/3** (Eqn. 1). The difference of supplied and returned amounts of food and faeces gave the amount of consumed food (A_{food}) and the amount of recycled faeces (A_{RF}), respectively. The proportion of recycled faeces in the diet was determined for each replicate; weekly means were calculated thereafter.

$$P_{RF\text{-}diet} = \frac{A_{RF}}{A_{food} + A_{RF}} \quad (1)$$

The $P_{RF\text{-}diet}$ values obtained for the two faeces exchange treatments were also applied to the standard treatments according to the supplied food (C3 or C4) assuming that the ingested food was the main determinant of the faeces composition. This assumption was supported by our results on food consumption and faeces production (data not shown).

2.2.2. Proportion of recycled faeces in the produced faeces ($P_{RF\text{-}faeces}$)

We also calculated the proportion of recycled faeces in the produced faeces ($P_{RF\text{-}faeces}$) in the faeces exchange treatments on a weekly basis. It was assumed, that – except for the

^{13}C label of the food – the other factors like the amount of faeces woodlice recycle and the isotope fractionation during the processes that influence faeces production (e.g. food assimilation, respiration, excretion) were statistically identical in all treatments.

The calculation of $P_{RF\text{-}faeces}$ based on weekly means of the $\delta^{13}C$ values (δ) and the C contents (C) of the supplied C3- or C4-food (*food3* and *food4*) and of the supplied C4- or C3-faeces from the production lines (*faeces4* and *faeces3*). Since both dietary sources, food and recycled faeces, formed the base material of faeces production, their individual proportions in the produced faeces $P_{FOOD\text{-}faeces}$ and $P_{RF\text{-}faeces}$ add up to 1 (Eqn. 2).

$$1 = P_{FOOD\text{-}faeces} + P_{RF\text{-}faeces} \tag{2}$$

The $\delta^{13}C$ value of the produced faeces (*faeces3/4* and *faeces4/3*) was determined by $P_{FOOD\text{-}faeces}$, by $P_{RF\text{-}faeces}$ and by the isotope fractionation (F) occurring in-between ingestion and faeces disposal (Eqns. 3 and 4)

$$\delta_{faeces3/4} = \frac{\delta_{food3} \cdot C_{food3} \cdot P_{FOOD\text{-}faeces} + \delta_{faeces4} \cdot C_{faeces4} \cdot P_{RF\text{-}faeces}}{C_{food3} \cdot P_{FOOD\text{-}faeces} + C_{faeces4} \cdot P_{RF\text{-}faeces}} + F \tag{3}$$

$$\delta_{faeces4/3} = \frac{\delta_{food4} \cdot C_{food4} \cdot P_{FOOD\text{-}faeces} + \delta_{faeces3} \cdot C_{faeces3} \cdot P_{RF\text{-}faeces}}{C_{food4} \cdot P_{FOOD\text{-}faeces} + C_{faeces3} \cdot P_{RF\text{-}faeces}} + F \tag{4}$$

According to our assumption that $P_{RF\text{-}faeces}$ and F are equal in treatments **C3/4** and **C4/3** we eliminated the unknown variable F yielding in a quadratic equation to extract $P_{RF\text{-}faeces}$ (Eqn. 5). One solution was negative; the other met the range between 0 and 1.

$$P_{RF\text{-}faeces\ 1,2} = -\frac{Y}{2X} \pm \sqrt{\left(\frac{Y}{2X}\right)^2 - \frac{Z}{X}} \tag{5}$$

In Eqn. 5 X, Y and Z represent

$X = C_{food3} \cdot C_{food4} \cdot (\delta_{food3} - \delta_{food4} - \delta_{faeces3/4} + \delta_{faeces4/3}) - C_{food3} \cdot C_{faeces3} \cdot (\delta_{food3} - \delta_{faeces3} - \delta_{faeces3/4} + \delta_{faeces4/3}) - C_{food4} \cdot C_{faeces4} \cdot (\delta_{faeces4} - \delta_{food4} - \delta_{faeces3/4} + \delta_{faeces4/3}) + C_{faeces3} \cdot C_{faeces4} \cdot (\delta_{faeces4} - \delta_{faeces3} - \delta_{faeces3/4} + \delta_{faeces4/3})$;

$Y = C_{food3} \cdot C_{faeces3} \cdot (\delta_{food3} - \delta_{faeces3} - \delta_{faeces3/4} + \delta_{faeces4/3}) + C_{food4} \cdot C_{faeces4} \cdot (\delta_{faeces4} - \delta_{food4} - \delta_{faeces3/4} + \delta_{faeces4/3}) - 2 \cdot C_{faeces3} \cdot C_{faeces4} \cdot (\delta_{faeces4} - \delta_{faeces3} - \delta_{faeces3/4} + \delta_{faeces4/3})$;

and $Z = C_{faeces3} \cdot C_{faeces4} \cdot (\delta_{faeces4} - \delta_{faeces3} - \delta_{faeces3/4} + \delta_{faeces4/3})$.

2.2.3. Proportions of carrot-lettuce mix and cellulose in the faeces ($P_{MIX\text{-}faeces}$, $P_{CELL\text{-}faeces}$)

The weight proportion of 1:1 of the two food components might not necessarily describe their actual contribution to faeces production. Therefore, we split the proportion of food in the produced faeces $P_{FOOD\text{-}faeces}$ and modeled the proportions of carrot-lettuce mix ($P_{MIX\text{-}faeces}$) and cellulose ($P_{CELL\text{-}faeces}$) in the produced faeces on a weekly basis. Calculations were done separately for each of the faeces exchange and standard treatments using the $P_{RF\text{-}faeces}$ values derived from Eqn. 5 (see above). The models describe the simple mixing of three components excluding additional isotope fractionation and base on weekly means of $\delta^{13}C$ values (δ) and C contents (C) of carrot-lettuce mix (*mix*), cellulose (*cell*), recycled faeces (*RF*) and produced faeces (*PF*).

The proportions of carrot-lettuce mix, cellulose and recycled faeces in the produced faeces add up to 1. The calculation of $P_{MIX\text{-}faeces}$ follows Eqn. 6.

$$P_{MIX\text{-}faeces} = 1 - (P_{CELL\text{-}faeces} + P_{RF\text{-}faeces}) \quad (6)$$

In the isotopic mass balance for the $\delta^{13}C$ value of the produced faeces δ_{PF} (Eqn. 7) we substituted $P_{MIX\text{-}faeces}$ according to Eqn. 6 and rearranged to extract $P_{CELL\text{-}faeces}$ (Eqn. 8).

$$\delta_{PF} = \frac{\delta_{mix} \cdot C_{mix} \cdot P_{MIX\text{-}faeces} + \delta_{cell} \cdot C_{cell} \cdot P_{CELL\text{-}faeces} + \delta_{RF} \cdot C_{RF} \cdot P_{RF\text{-}faeces}}{C_{mix} \cdot P_{MIX\text{-}faeces} + C_{cell} \cdot P_{CELL\text{-}faeces} + C_{RF} \cdot P_{RF\text{-}faeces}} \quad (7)$$

$$P_{CELL\text{-}faeces} = \frac{C_{mix} \cdot (\delta_{mix} - \delta_{PF}) \cdot (1 - P_{RF\text{-}faeces}) + C_{RF} \cdot (\delta_{RF} - \delta_{PF}) \cdot P_{RF\text{-}faeces}}{C_{mix} \cdot (\delta_{mix} - \delta_{PF}) + C_{cell} \cdot (\delta_{PF} - \delta_{cell})} \quad (8)$$

Applying $P_{CELL\text{-}faeces}$ and $P_{RF\text{-}faeces}$ to Eqn. 6 we finally calculated $P_{MIX\text{-}faeces}$.

2.2.4. Trophic shifts for diets without (Δ_2) and with faeces recycling (Δ_3)

The trophic shift, i.e. the isotopic difference between woodlice and their diet, was calculated on a weekly basis from week 2 to week 8 for both, ^{13}C and ^{15}N. The calculation of the $\Delta^{13}C$ and $\Delta^{15}N$ values (Δ) based on weekly means of $\delta^{13}C$ and $\delta^{15}N$ values (δ) and of C and N contents (C), respectively, of woodlice (*wl*), supplied food (*food*) and recycled faeces (*RF*). We distinguished between a two component diet represented by the supplied food composed of carrot-lettuce mix and cellulose (Eqn. 9)

$$\Delta_2 = \delta_{wl} - \delta_{food} \quad (9)$$

and a three component diet consisting of the supplied food and faeces. The proportions of food ($P_{FOOD\text{-}diet}$) and recycled faeces ($P_{RF\text{-}diet}$) in the diet add up to 1 (Eqn. 10).

$$1 = P_{FOOD\text{-}diet} + P_{RF\text{-}diet} \quad (10)$$

In the isotope mass balance (Eqn. 11) $P_{FOOD\text{-}diet}$ was substituted by $(1 - P_{RF\text{-}diet})$. $P_{RF\text{-}diet}$ was known from Eqn. 1.

$$\Delta_3 = \delta_{wl} - \frac{\delta_{food} \cdot C_{food} \cdot P_{FOOD\text{-}diet} + \delta_{RF} \cdot C_{RF} \cdot P_{RF\text{-}diet}}{C_{food} \cdot P_{FOOD\text{-}diet} + C_{RF} \cdot P_{RF\text{-}diet}} \quad (11)$$

2.3. Statistics

In the comparison of $\delta^{15}N$ and $\delta^{13}C$ values and of the N and C contents of woodlice (Figs. 2 and 3) the significance of the difference between mean values was tested by Oneway-ANOVA and following post hoc Student-Newman-Keuls test ($p<0.05$). The number of replicates *n* in faeces exchange treatments and in the reference amounted to five, in standard treatments to three.

In the temporal development of $\delta^{13}C$ and $\delta^{15}N$ values as well as C and N contents of faeces and food (see Figs. 4 to 6) the pairwise comparison of weekly means by Independent Sample T-Tests (in faeces exchange treatments $n = 5$; in standard treatments *n* ranged from 10 in week 1 to 3 in week 8) revealed that if the standard deviations represented by the error bars did not overlap, the respective means differed significantly at the $p < 0.05$ level. On that basis we assessed the relevance of differences between the compared data series. All statistical analyses were performed using SPSS for Windows 11.0 (SPSS Inc. 2001).

3. Results

3.1. Changes of the elemental and isotopic composition of woodlice induced by new food

Over a period of eight weeks the woodlouse C content significantly increased in both faeces exchange treatments (> 36.5%) compared to the standard treatments and the initial reference, with ~ 35% (Fig. 2). Woodlouse $\delta^{13}C$ values generally decreased compared to the reference value. The decrease was significant in the faeces exchange treatments **C3/4** and **C4/3**. C4-fed woodlice of faeces exchange and standard treatments were slightly enriched in ^{13}C compared to C3-fed woodlice. The difference in the woodlouse $\delta^{13}C$ values was significant between treatments **C3/4** and **C4/3** (Fig. 2).

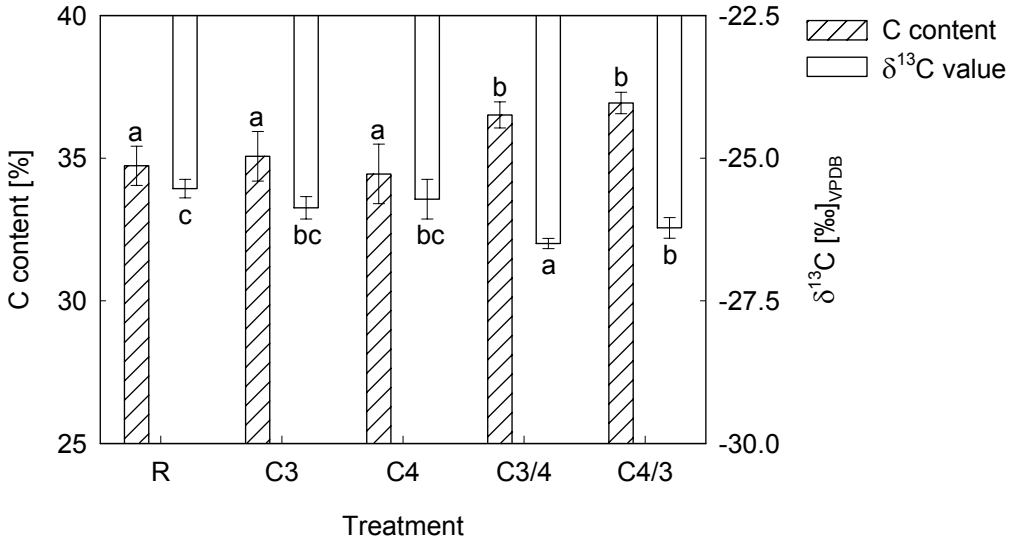

Figure 2. Mean values of woodlouse C content (hatched columns) and $\delta^{13}C$ values (unfilled columns) at the beginning of the experiment (**R** = reference) and after eight weeks in standard (**C3** and **C4**) and faeces exchange treatments (**C3/4** and **C4/3**). Values sharing the same letter are not significantly different at the $p < 0.05$ level (Oneway-ANOVA followed by post hoc Student-Newman-Keuls Test). Error bars indicate one standard deviation of the mean.

After eight weeks the woodlouse N content was significantly higher and the C:N ratio significantly lower in all treatments compared to the initial value (**R**) except for treatment **C4** (Fig. 3). In all treatments woodlouse $\delta^{15}N$ values had increased significantly by 0.5‰ (treatments **C3/4** and **C4/3**) up to 1.3‰ (treatments **C3** and **C4**) compared to the reference. The differences between faeces exchange and standard treatments were significant, too.

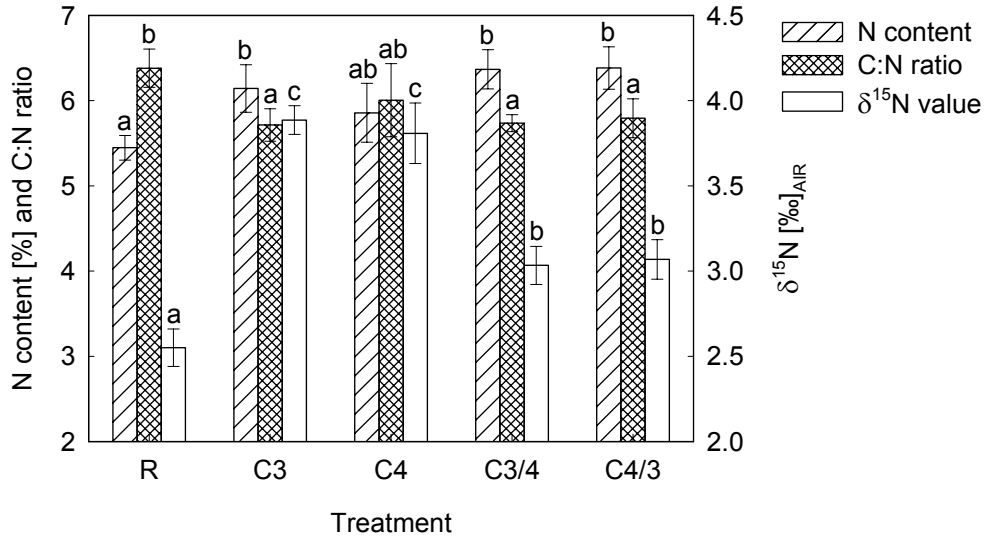

Figure 3. Mean values of woodlouse N content (hatched columns), C:N ratios (cross-hatched columns) and $\delta^{15}N$ values (unfilled columns) at the beginning of the experiment (**R** = reference) and after eight weeks in standard (**C3** and **C4**) and faeces exchange treatments (**C3/4** and **C4/3**). Values sharing the same letter are not significantly different at the $p < 0.05$ level (Oneway-ANOVA followed by post hoc Student-Newman-Keuls Test). Error bars indicate one standard deviation of the mean.

3.2. Development of the isotopic differences between woodlice and food (Δ_2) over the experiment

The differences between the $\delta^{13}C$ and $\delta^{15}N$ values of woodlice and supplied food were monitored over the experiment. These differences, referred to as $\Delta^{13}C$ and $\Delta^{15}N$ values (see Eqn. 9), reflect the actual trophic shift. The trophic shift for ^{13}C and ^{15}N after week 1 and after week 8, respectively, as well as the differences between the trophic shifts after week 8 and week 1 were calculated for all treatments without accounting for the recycled faeces (Tab. 1).

After the first week the $\Delta^{13}C$ values of ~ 1.5‰ in treatments with C3-food fell into the expected range of 0 – 2 ‰. At this initial stage the woodlouse $\delta^{13}C$ values were still very similar in all treatments. The $\delta^{13}C$ values of the C3- and C4-food, however, differed by 7‰ so that the $\Delta^{13}C$ values in treatments with C4-food were strongly negative (ca. -5.5‰). Over the experiment the $\Delta^{13}C$ values decreased significantly in all treatments except for treatment **C4**. After week 8 this decrease was more pronounced in faeces exchange treatments (-0.6 to -0.8‰) compared to standard treatments (-0.1 to -0.4‰). In the treatments with C3-food (**C3** and **C3/4**) the trophic shift for ^{13}C still matched the expected range. In treatments with C4-food the negative $\Delta^{13}C$ values did not shift towards the expected positive range but further decreased to values lower than -6‰ in treatment **C4/3** (Tab. 1).

Table 1. Mean trophic shift for ^{13}C ($\Delta^{13}C$) and ^{15}N ($\Delta^{15}N$) between woodlice and supplied food after one week and after eight weeks in faeces exchange (**C3/4** and **C4/3**) and standard treatments (**C3** and **C4**). The significance of the differences between the trophic shifts after week 8 and after week 1 was proven by Independent Sample T-Tests.

		Isotopic shift between woodlice and food		
		after week 1	after week 8	
Parameter	Treatment	Mean ± s.d. [‰]	Mean ± s.d. [‰]	Difference [‰]
$\Delta^{13}C$	C3/4	1.43 ± 0.15	0.59 ± 0.12	-0.84 ***
	C4/3	-5.52 ± 0.17	-6.12 ± 0.15	-0.60 **
	C3	1.45 ± 0.15	1.04 ± 0.19	-0.41 *
	C4	-5.41 ± 0.11	-5.55 ± 0.28	-0.15 n.s.
$\Delta^{15}N$	C3/4	0.17 ± 0.10	0.59 ± 0.10	0.42 ***
	C4/3	-0.02 ± 0.09	0.43 ± 0.11	0.45 ***
	C3	0.29 ± 0.12	1.30 ± 0.13	1.02 ***
	C4	-0.01 ± 0.11	1.04 ± 0.17	1.05 ***

After the first week $\Delta^{15}N$ values were ~ 0‰ in treatments with C4-food and slightly higher (0.2 – 0.3‰) in treatments with C3-food; i.e., the trophic shifts for ^{15}N were below the expected range of 3.4 ± 1.1‰. Over the experiment $\Delta^{15}N$ values increased significantly in all treatments. After eight weeks the increase was more pronounced in treatments **C3** and **C4** (~ 1‰) compared to treatments **C3/4** and **C4/3** (~ 0.4‰). Finally, the $\Delta^{15}N$ values remained below 1.4‰ in standard and below 0.6‰ in faeces exchange treatments; i.e., within eight weeks the ^{15}N shifts did not reach the range reported for one trophic level (TLS). However, the development of animal $\delta^{13}C$ and $\delta^{15}N$ values over the experiment suggested a further decrease of the $\Delta^{13}C$ values and a further increase of the $\Delta^{15}N$ values in all treatments if the experiment was continued (data not shown).

3.3. Isotopic differences between woodlice and food considering coprophagy (Δ_3)

Woodlice did not exclusively feed on the supplied food, consisting of the two components carrot-lettuce mix and cellulose, but also on the faeces available for recycling. Therefore, we included faeces as the third dietary component in the calculation of $\Delta^{13}C$ and $\Delta^{15}N$ values (see Eqn. 11). Because the faeces were generally enriched in ^{13}C and ^{15}N relative to the food (see below), accounting for coprophagy necessarily resulted in higher $\delta^{13}C$ and $\delta^{15}N$ values of the whole diet in comparison to pure food. Consequently, the trophic shift was smaller when coprophagy was considered (Tab. 2). One exception was treatment **C4/3** where the recycled faeces were strongly depleted in

^{13}C relative to the food. In this treatment the trophic shift was larger for the 3-component diet (Tab. 2) compared to the 2-component diet (Tab. 1).

Table 2. Mean trophic shift for ^{13}C ($\Delta^{13}C$) and ^{15}N ($\Delta^{15}N$) between woodlice and their diet after one week and after eight weeks in faeces exchange (**C3/4** and **C4/3**) and standard treatments (**C3** and **C4**). The diet comprised both, food and faeces. The significance of the difference between the trophic shift after week 8 and after week 1 was proven by Independent Sample T-Tests.

		Isotopic shift between woodlice and diet		
		after week 1	after week 8	
Parameter	Treatment	Mean ± s.d. [‰]	Mean ± s.d. [‰]	Difference [‰]
$\Delta^{13}C$	C3/4	-0.46 ± 0.12	0.15 ± 0.09	0.61 ***
	C4/3	-4.72 ± 0.16	-5.82 ± 0.17	-1.09 ***
	C3	1.14 ± 0.16	0.93 ± 0.20	-0.21 n.s.
	C4	-6.11 ± 0.13	-5.78 ± 0.31	0.32 n.s.
$\Delta^{15}N$	C3/4	-0.24 ± 0.11	0.40 ± 0.08	0.64 ***
	C4/3	-0.40 ± 0.09	0.27 ± 0.10	0.67 ***
	C3	-0.07 ± 0.13	1.16 ± 0.11	1.23 ***
	C4	-0.46 ± 0.14	0.94 ± 0.09	1.40 ***

Except for treatment **C3** with 1.1‰, after the first week the $\Delta^{13}C$ values were below the expected range (0 – 2‰), especially in treatments with C4-food (< -4.7‰). Over the experiment the trophic shift for ^{13}C increased by 0.3 to 0.6‰ if woodlice were using C4-faeces (significant in treatment **C3/4**) and decreased by 0.2 to 1.1‰ if woodlice were using C3-faeces (significant in treatment **C4/3**). After week 8 in both treatments with C3-food the trophic shift was in the range of 0 – 2‰. In treatment **C4** the $\Delta^{13}C$ value developed into the 'right' direction towards more positive values (Tab. 2), however, it finally remained with -5.8‰ still below that derived for woodlice feeding on pure food (-5.5‰; Tab. 1).

The $\Delta^{15}N$ values after week 1 were below 0‰ in all treatments. Over the experiment they increased significantly to reach 0.3 to 0.4‰ in faeces exchange and 0.9 to 1.2‰ in standard treatments; i.e., the increase was more pronounced in the standard treatments. Although the difference of the isotopic shifts after week 8 and after week 1 exceeded in all treatments that obtained for the 2-component diet (Tab. 1), the final $\Delta^{15}N$ values were lower than the respective values calculated without considering coprophagy (Tab. 2).

The difference between trophic shifts calculated either with or without faeces was depending on the proportion of faeces in the diet ($P_{RF\text{-}diet}$) and became reduced as the faeces uptake by woodlice decreased (see below).

3.4. Elemental and isotopic changes during the conversion of food into faeces

From the food consumed by the woodlice 40 to 50% became converted into faeces. As weight changes in woodlice were negligible, mass balances indicate that 50 to 60% of the ingested organic matter (OM) had been transformed to gaseous compounds, mainly respiratory CO_2 and excreted ammonia. The conversion of food into faecal pellet during the gut passage was combined with significant changes of the elemental and isotopic composition of the original OM. We evaluated the role of woodlice for this alteration process from the temporal development of C and N contents and of $\delta^{13}C$ and $\delta^{15}N$ values of faeces produced in the standard treatments relative to the supplied food (Fig. 4).

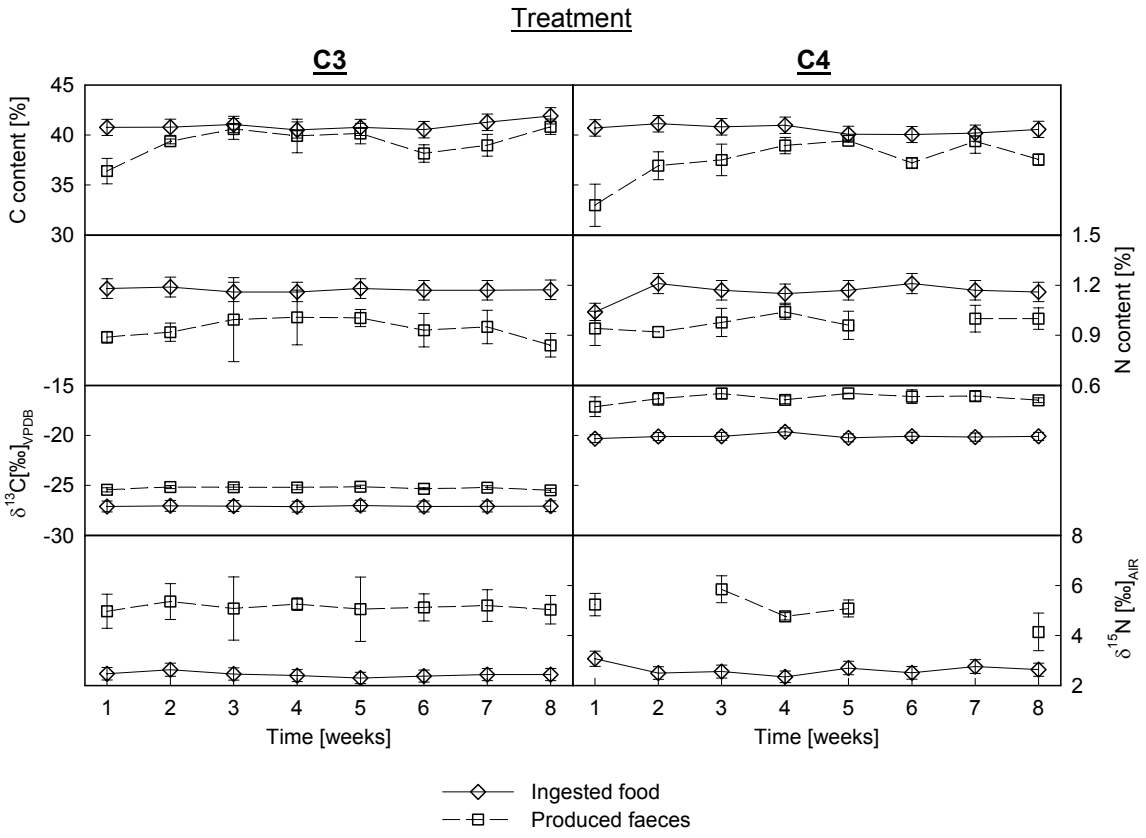

Figure 4. Elemental (C; N) and isotopic ($\delta^{13}C$; $\delta^{15}N$) differences between supplied food and faeces produced by woodlice in standard treatments **C3** and **C4**. Error bars indicate one standard deviation of the weekly mean.

Compared to the food the C content of the produced faeces was slightly (1.3 ± 0.8%) and significantly lower (2.4 ± 1.3%) in treatments **C3** and **C4**, respectively. In both treatments also the N content of the produced faeces was significantly lower (0.2 ± 0.06%) than that of the food. The produced faeces were significantly enriched relative to the food by 1.8 ± 0.1‰ and 3.8 ± 0.5‰ in ^{13}C and by 2.7 ± 0.1‰ and 2.4 ± 0.6‰ in ^{15}N in treatments **C3** and **C4**, respectively (mean differences over eight weeks ± s.d.).

3.5. Elemental and isotopic alteration of non-ingested faeces

In the faeces exchange treatments **C3/4** and **C4/3** we monitored the differences between the supplied faeces and the non-ingested remnants after one week to illustrate the effect of woodlice on the alteration of OM without gut passage (Fig. 5).

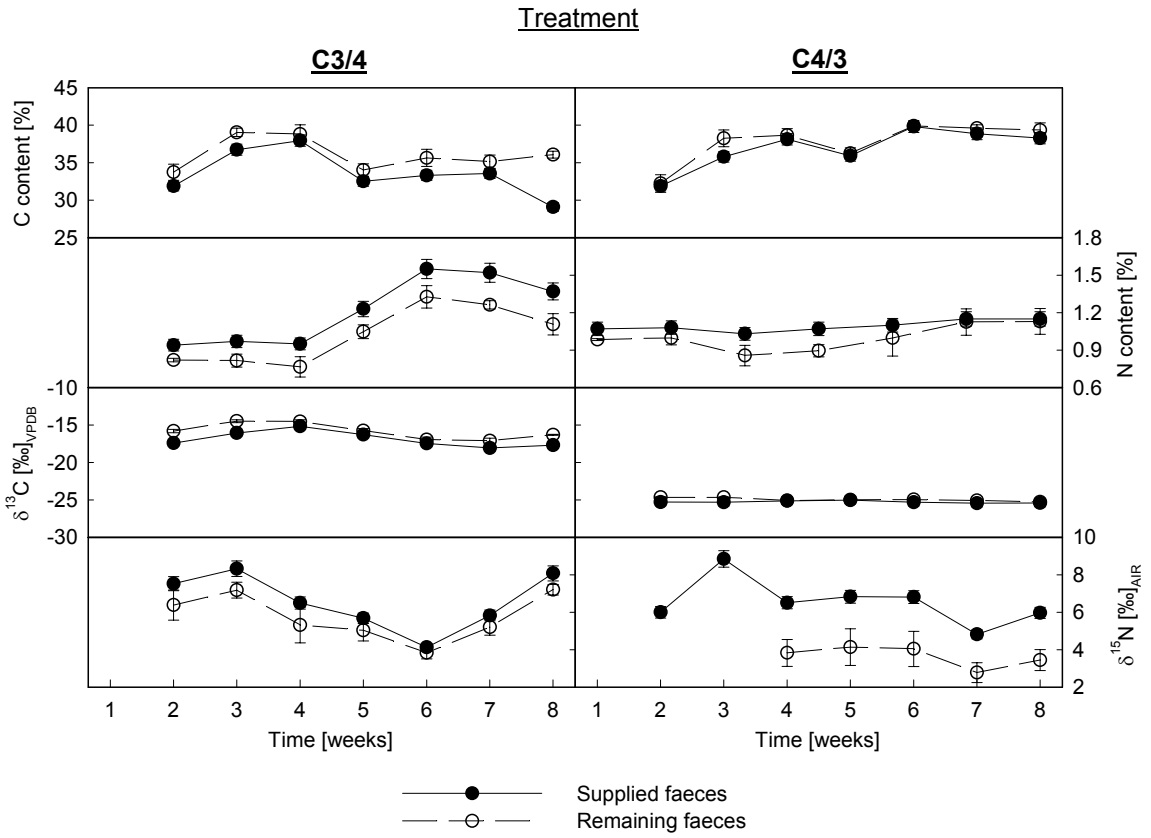

Figure 5. Elemental (C; N) and isotopic ($\delta^{13}C$; $\delta^{15}N$) differences between the faeces supplied for nutrition and the non-ingested remnants of the supplied faeces in faeces exchange treatments **C3/4** and **C4/3**. Error bars indicate one standard deviation of the weekly mean.

In both treatments the elemental and isotopic composition of the supplied faeces and that of its remnants were strongly correlated in time. In treatment **C3/4**, we found significant differences between the C content and $\delta^{13}C$ values of supplied and remaining faeces (Independent Sample T-Tests; always df = 8 and $p < 0.05$). Comparing mean values over seven weeks (± 1 s.d.), the C content of the remaining faeces exceeded that of the supplied faeces by 1.74 ± 0.55% and 0.79 ± 0.80% in treatments **C3/4** and **C4/3**, respectively (Fig. 5). Furthermore, residual faeces were enriched in ^{13}C relative to the input by 1.03 ± 0.48‰ and 0.33 ± 0.26‰ in treatments **C3/4** and **C4/3**, respectively.

In contrast, both, N content and $\delta^{15}N$ values were significantly lower in the remaining compared to the supplied faeces (Independent Sample T-Tests; always df = 8 and $p < 0.05$). The mean differences over seven weeks between output and input values accounted for -0.20 ± 0.05% and -0.10 ± 0.06% in the N content and for -0.84 ± 0.34‰ and -2.54 ± 0.29‰ in the $\delta^{15}N$ values in treatments **C3/4** and **C4/3**, respectively.

3.6. Influence of woodlice on the alteration of non-ingested faeces

Because the described changes in the non-ingested part of supplied faeces were unexpected, we tested if woodlice were directly involved in this alteration process. We incubated C3- and C4-faeces in the absence of woodlice and compared the weekly changes in the C and N contents and in the $\delta^{13}C$ and $\delta^{15}N$ values to changes in the presence of woodlice (Tab. 3).

Table 3. Weekly changes in the C and N contents and in the $\delta^{13}C$ and $\delta^{15}N$ values of faeces incubated in the absence and the presence of woodlice (means of 9 to 14 replicates ± 1 s.d.). The significance of the changes was evaluated by testing the mean differences between the remaining and the supplied faeces against zero (One Sample T-Tests, Test value = 0). The differences in the changes of the faecal organic matter in the absence and presence of woodlice were tested for significance by Independent Sample T-Tests.

		Changes of the faeces within one week	One-Sample Test [1]			T-Test for Equality of Means		
Woodlice	Parameter	Mean ± s.d.	df	t	p	df	t	p
Absent	N [%]	0.04 ± 0.08	8	1.48	n.s.	21	5.55	< 0.001
Present		-0.15 ± 0.08	13	-7.11	<0.001			
Absent	$\delta^{15}N$ [‰]	-0.05 ± 0.36	8	-0.45	n.s.	15	5.13	< 0.001
Present		-1.55 ± 0.93	11	-5.80	<0.001			
Absent	C [%]	0.24 ± 3.15	8	0.23	n.s.	8.8	-0.92	n.s.
Present		1.23 ± 0.83	12	5.34	<0.001			
Absent	$\delta^{13}C$ [‰]	0.07 ± 0.26	8	0.78	n.s.	20	-3.75	< 0.01
Present		0.68 ± 0.52	13	4.88	<0.001			

[1] Test value = 0

In the absence of woodlice the weekly changes in the stable isotopes and the C and N contents of the incubated faeces were not significant. By contrast, in the presence of woodlice C content and $\delta^{13}C$ values of the faeces significantly increased and the N content and $\delta^{15}N$ values significantly decreased (Tab. 3, One Sample T-Test).

The direct comparison of these weekly changes in the absence or presence of woodlice revealed that the differences between the changes were significant for the N content, the $\delta^{13}C$ and the $\delta^{15}N$ values (Tab. 3, Independent Sample T-Test). Alterations of the C content of the faeces were statistically similar; however, this was mainly the effect of the large scatter of this parameter when woodlice were absent. The data strongly suggest that in the studied system significant alteration of organic matter at a time scale of one week required the presence of woodlice.

3.7. Elemental and isotopic differences between faeces produced in faeces exchange and in standard treatments

To demonstrate the influence of coprophagy on the elemental and isotopic composition of the produced faeces we monitored the differences between the faeces produced in standard and in faeces exchange treatments (Fig. 6).

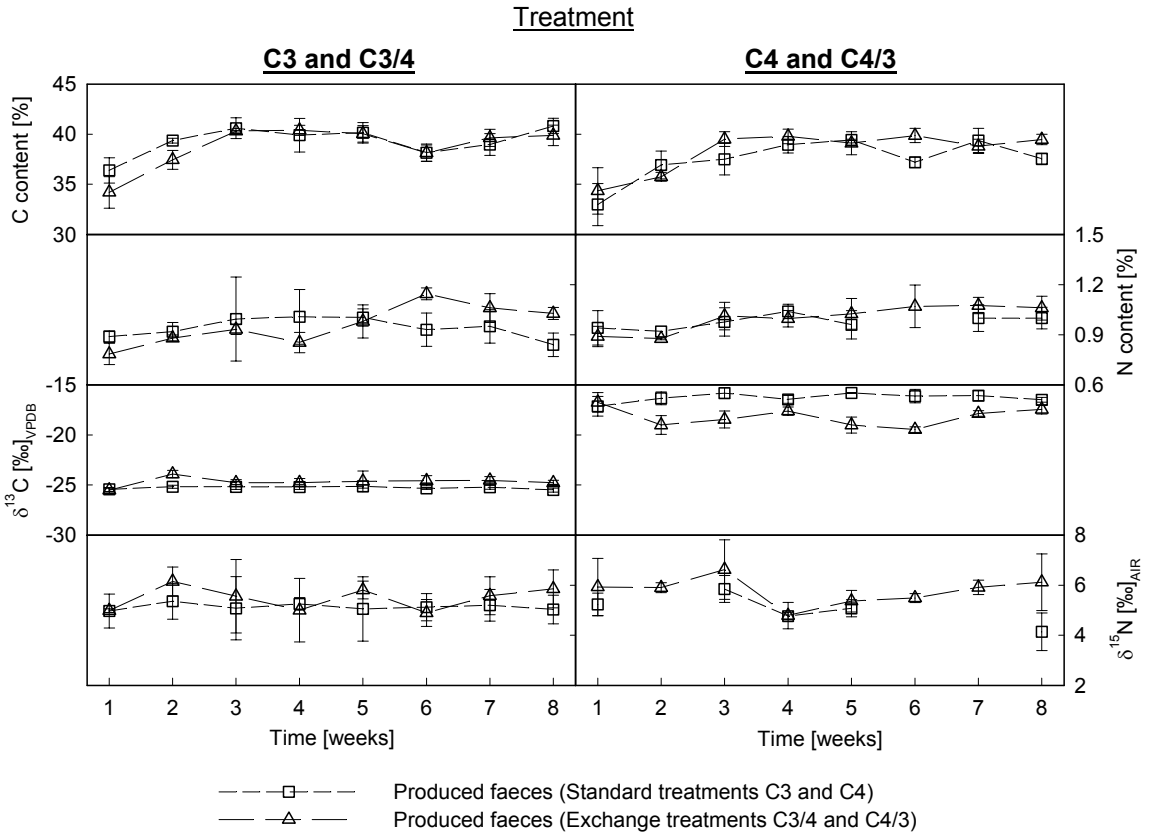

Figure 6. Elemental (C; N) and isotopic ($\delta^{13}C$; $\delta^{15}N$) differences between the produced faeces in standard and faeces exchange treatments. In standard treatments **C3** and **C4** faeces were produced from the food (see Fig. 4) and the recycled faeces (identical to the produced faeces). In faeces exchange treatments **C3/4** and **C4/3** faeces were produced from the food, however, for recycling faeces were supplied with a ^{13}C label different from that of the food. Error bars indicate one standard deviation of the weekly mean.

In the standard treatments **C3** and **C4** the elemental composition of the produced faeces (Fig. 6) was clearly related to that of the food (Fig. 4). However, in the faeces exchange treatments **C3/4** and **C4/3** the C and N contents of the produced faeces (Fig. 6) mostly mirrored that of the supplied faeces (Fig. 5). Therefore, the C and N contents of faeces produced in faeces exchange treatments did not show a fixed relation to the C and N contents of faeces produced in standard treatments. For example, in accordance with the changing N content of the supplied faeces, the N content of faeces produced in treatment **C3/4** was lower until week 4, while it was higher from week 6 onwards in comparison to the faeces produced in treatment **C3** (Fig. 6).

In the standard treatments **C3** and **C4** which had the same type of ^{13}C label in food and recycled faeces, respectively, also the isotopic composition of the produced faeces (Fig. 6) was related to that of the food (Fig. 4). In treatment **C3/4**, the uptake of faeces which were about 10‰ enriched in ^{13}C relative to the food was reflected by on average 0.7‰ higher $\delta^{13}C$ values of the produced faeces compared to faeces produced in treatment **C3** (Fig. 6). In contrast, in treatment **C4/3** the uptake of faeces about 5‰ depleted in ^{13}C relative to the food was reflected by on average ~ 2.3‰ lower $\delta^{13}C$ values of the produced faeces compared to faeces produced in treatment **C4**. Depending on the ^{15}N content of faeces consumed in treatments **C3/4** and **C4/3** (Fig. 5), which was often higher than that of faeces consumed in treatments **C3** and **C4** (Fig. 4), the $\delta^{15}N$ values of the faeces produced in faeces exchange treatments **C3/4** and **C4/3** were on average 0.4 and 0.8‰ higher than those produced in standard treatments **C3** and **C4**, respectively (Figs. 6).

3.8. Composition of the woodlouse diet and of the produced faeces

Focussing on the importance of faeces for woodlouse nutrition and faeces production, we weekly calculated the mean proportion of recycled faeces in the woodlouse diet $P_{RF\text{-}diet}$ (see Eqn. 1) and in the produced faeces $P_{RF\text{-}faeces}$ (see Eqn. 5) in treatments **C3/4** and **C4/3** (Fig. 7). $P_{RF\text{-}diet}$ decreased from 0.18 in week 2 to 0.06 in week 8. $P_{RF\text{-}faeces}$ decreased from 0.17 in week 2 to 0.02 in week 8 and reached lower values than $P_{RF\text{-}diet}$. The regression lines show the assumed temporal trend of both parameters (logarithmic decrease).

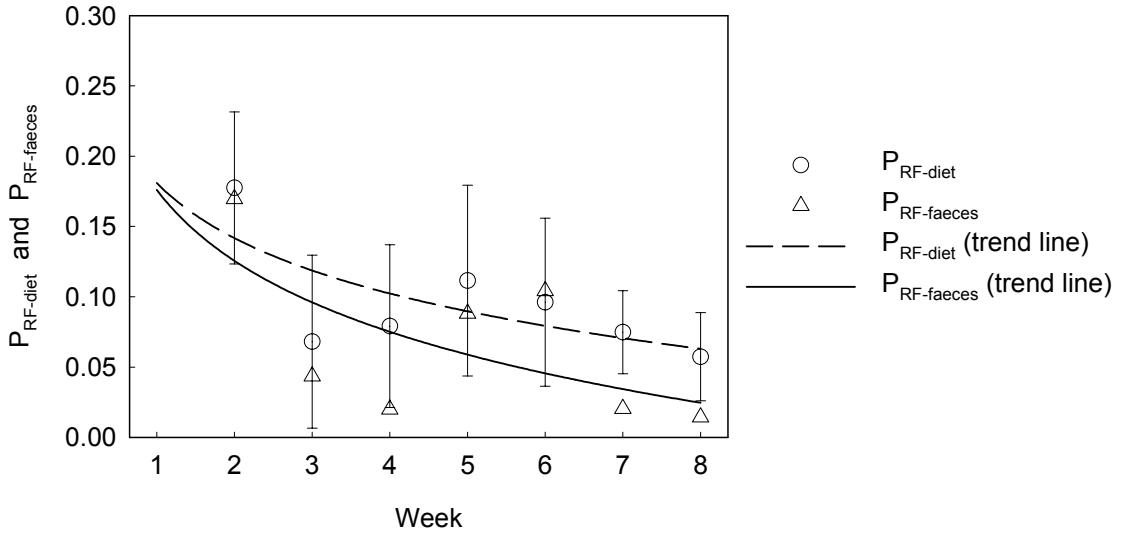

Figure 7. Weekly means of the proportion of recycled faeces in the ingested matter ($P_{RF\text{-}diet}$) and in the produced faeces ($P_{RF\text{-}faeces}$) in faeces exchange treatments **C3/4** and **C4/3**. Error bars indicate one standard deviation of the mean. The trend lines derived from natural logarithmic regression with $R^2 = 0.48$ and 0.38 for $P_{RF\text{-}diet}$ and $P_{RF\text{-}faeces}$, respectively.

To assess the contribution of carrot-lettuce mix and cellulose to the production of faeces we calculated on a weekly basis the proportions of both food components in the produced faeces $P_{MIX\text{-}faeces}$ and $P_{CELL\text{-}faeces}$ (see Eqns. 6 to 8). In the calculations we applied the obtained $P_{RF\text{-}faeces}$ values (Fig. 7) to both, faeces exchange and standard treatments (Fig. 8).

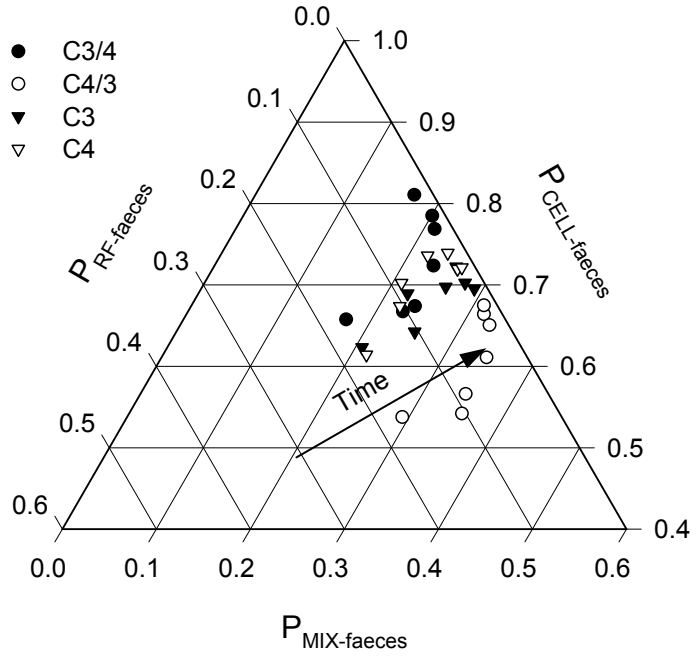

Figure 8. Ternary plot of the individual contribution of the three dietary components cellulose, carrot-lettuce mix and recycled faeces to the production of woodlouse faeces in faeces exchange (**C3/4** and **C4/3**) and in standard (**C3** and **C4**) treatments from week 2 to week 8. The progress in time is indicated by the arrow. Note the different scaling of the three axes.

Faeces were mainly formed of the cellulose contained in the food. In all treatments $P_{CELL\text{-}faeces}$ increased with time ranging from 0.54 to 0.81. In treatments with C3-food (**C3** and **C3/4**) the produced faeces showed higher proportions of cellulose compared to treatments with C4-food (**C4** and **C4/3**). While the proportion of recycled faeces $P_{RF\text{-}faeces}$ was decreasing (see also Fig. 7), the proportion of carrot-lettuce mix $P_{MIX\text{-}faeces}$ was increasing (except for treatment **C3/4**) and covered values from 0.17 to 0.35 (Fig. 8). Thus, ranking the three source materials, clearly the fibre component cellulose was the most important constituent of the produced faeces followed by the high-quality carrot-lettuce component. Recycled faeces were of major importance in the first two weeks of the experiment.

In addition, from the ingested amounts we calculated the assimilated proportions of carrot-lettuce mix, cellulose and recycled faeces and – integrating over the three components – of the whole diet (Fig. 9).

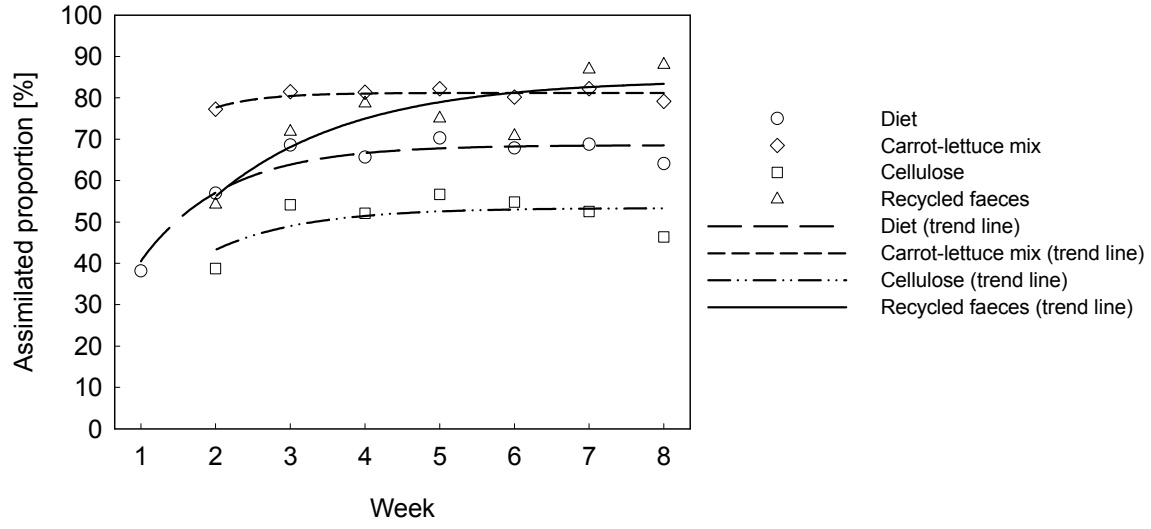

Figure 9. Assimilated proportions of the diet and of the three individual dietary components carrot-lettuce mix, cellulose and faeces. The assimilated proportions were calculated from the weekly ingested amounts. The symbols represent averages over all treatments. The trend lines derived from exponential rise to maximum regressions with R^2 values of 0.95, 0.56, 0.50 and 0.75 for diet, carrot-lettuce mix, cellulose and recycled faeces, respectively.

The assimilated proportion of all dietary components was increasing with time. Accordingly, the assimilation efficiency of the whole diet increased from less than 40% in week 1 to more than 68% in week 8. With respect to the regression lines, the high-quality component carrot-lettuce mix was finally assimilated to 80%, the fibre component cellulose only to 53%. The most pronounced increase from 56% in week 2 to 83% in week 8 occurred in the recycled faeces with a still increasing tendency after week 8 (Fig. 9).

3.9. Isotopic differences between woodlice and the assimilated part of the diet

Finally, we calculated the trophic shifts between woodlice and the assimilated part of the diet by applying the proportions of the three dietary components in the assimilated diet to Eqn. 11. The resulting $\Delta^{13}C$ and $\Delta^{15}N$ values (Tab. 4) differed clearly from those obtained in the calculations based on the ingested diet (Tab. 2).

The $\Delta^{13}C$ values after week 1 followed a regular order according to the ^{13}C label of food and faeces. With 3.2‰ the highest ^{13}C trophic shift was found in the purely C3-fed treatment **C3**; the values decreased via treatments **C3/4** (0.9‰) and **C4/3** (-2.1‰) to the purely C4-fed treatment **C4** with -4.7‰. Except for treatment **C3/4** with a significant increase, after eight weeks the $\Delta^{13}C$ values had decreased in all treatments. The decrease was significant in treatment **C3**. While in treatments **C3/4** (2.0‰) and **C3** (1.6‰) the final trophic shifts were in the expected range of 0 – 2‰, in treatments **C4/3** (-2.9‰) and **C4** (-5.1‰) the strongly negative values contradicted the principle of trophic enrichment.

Table 4. Mean trophic shift for ^{13}C ($\Delta^{13}C$) and ^{15}N ($\Delta^{15}N$) between woodlice and the assimilated part of the ingested diet after one week and after eight weeks in faeces exchange (**C3/4** and **C4/3**) and standard treatments (**C3** and **C4**). The diet comprised both, food and faeces. The significance of the difference between the trophic shift after week 8 and after week 1 was proven by Independent Sample T-Tests.

		Isotopic shift between woodlice and diet		
		after week 1	after week 8	
Parameter	Treatment	Mean ± s.d. [‰]	Mean ± s.d. [‰]	Difference [‰]
$\Delta^{13}C$	C3/4	0.94 ± 0.66	1.97 ± 0.45	1.03 **
	C4/3	-2.14 ± 0.73	-2.90 ± 0.85	-0.77 n.s.
	C3	3.21 ± 0.39	1.57 ± 0.36	-1.64 ***
	C4	-4.71 ± 0.46	-5.08 ± 0.16	-0.36 n.s.
$\Delta^{15}N$	C3/4	-0.11 ± 0.09	0.48 ± 0.09	0.59 ***
	C4/3	-0.32 ± 0.15	0.24 ± 0.10	0.56 ***
	C3	0.20 ± 0.11	1.23 ± 0.13	1.03 ***
	C4	-0.49 ± 0.09	0.92 ± 0.11	1.41 ***

Starting with slightly negative to slightly positive (treatment **C3**) values the ^{15}N trophic shift significantly increased in all treatments. The increase was > 1‰ in the standard treatments **C3** and **C4** and < 0.6‰ in faeces exchange treatments **C3/4** and **C4/3**. Despite this increase, the $\Delta^{15}N$ values (0.5 and 0.2‰ in treatments **C3/4** and **C4/3**; 1.2 and 0.9‰ in treatments **C3** and **C4**) finally did not reach the expected range of 3.4 ± 1.1‰.

4. Discussion

4.1. Elemental and isotopic changes in adult woodlice due to new food

Differences between the initial and the final elemental and isotopic composition of *Porcellio dilatatus* reflected significant changes in adult woodlice induced by the experimental food. The general decrease of the animal C:N ratio indicated that woodlice were able to adequately use the new food for nutrition. The adaptation of the woodlouse digestive capabilities to the experimental food was clearly demonstrated by the increasing efficiency of assimilation of the ingested matter. In the case of the carrot-lettuce mix and the cellulose the assimilation efficiency reached constancy by week 4; i.e., food changes initiated the adjustment and the optimization of the digestive system. During this process woodlice revealed decreasing $\delta^{13}C$ and increasing $\delta^{15}N$ values. However, while woodlice had physiologically adapted to the new food after four weeks already, their isotopic adaptation was still ongoing by the end of the experiment.

Depending on the composition of the dietary sources the elemental and isotopic changes in the woodlice varied among the treatments. The differences between woodlice of faeces exchange and standard treatments, e.g. in the animal $\delta^{15}N$ values, were related to the fact that in faeces exchange treatments at the beginning of every week faeces from the previous week were supplied whereas in standard treatments woodlice had to produce fresh faeces first. This result indicates that woodlice respond very sensitive to diet quality.

4.2. Isotopic adaptation of adult woodlice to new food as reflected by the trophic isotopic enrichment

How fast woodlice adapted to new food sources was evaluated from the isotopic difference between woodlice and diet ($\Delta^{13}C$ and $\Delta^{15}N$) as a measure of the trophic shift. In all treatments $\Delta^{15}N$ changed into the presumed direction from lower to higher values, whereas the development of the $\Delta^{13}C$ values did not show a clear pattern. Except for the ^{13}C shift in treatments with C3-food that matched the range of 0 – 2‰ reported for one trophic level, the isotopic differences between woodlice and diet did not show the expected numbers even after eight weeks of constant food supply. We suggest two reasons:

Firstly, the experimental time was inadequate for adult woodlice to complete isotopic adaptation. As hypothesized the isotopic adaptation of adult animals may require a long time. In the case of $\Delta^{15}N$ values, which shifted into the expected direction, eight weeks were too short, even though the experiment ensured stable conditions for the isotopic equilibration between woodlice and diet. This period was rather long compared to periods of source availability under field conditions. In other studies on the influence of the diet on animal δ values animals had been grown on a specific diet either as juveniles and only for up to four weeks (Minagawa and Wada, 1984; Focken and Becker, 1998; Adams and Sterner, 2000), rarely longer (e.g. Fellerhoff, 2002), or for a number of generations (DeNiro and Epstein, 1978). This implied that animal biomass was formed either entirely or to a major proportion from the diet ensuring a respective isotopic shift. In our study on full-grown animals, however, the ^{15}N shift between woodlice and food ($\leq$ 1.3‰) remained far below the reported trophic level shift of 3.4‰ (Minagawa and Wada, 1984). The main reason is probably the low incorporation rate of N from the new food source into the animal body. It has been suggested that growing individuals should reflect a new diet faster than non-growing individuals in which the new diet is only incorporated via tissue maintenance turnover (Ponsard and Averbuch, 1999).

Secondly, in addition to the lack of adaptation time, the strongly negative ^{13}C trophic shifts in treatments with C4-food indicated that woodlice were not isotopically adjusting to the diet as a whole. In the same context, the only small differences between the $\delta^{13}C$ values of C3- and C4-fed woodlice were as surprising as decreasing $\Delta^{13}C$ values irrespective of the ^{13}C label of the diet. Relating woodlouse $\delta^{13}C$ values to either food only, i.e. neglecting coprophagy, or to food and recycled faeces together gave diverse results concerning the development of the ^{13}C shifts. This is because coprophagy alters the composition of the diet and also affects the isotopic enrichment in the consumer (Rothe and Gleixner, submitted). Both aspects need to be considered when assessing the food web position of animals (Fantle et al., 1999; Scheu and Falca, 2000) and tracing diets (Koch et al., 1994; Phillips and Koch, 2002) from isotope data.

In fact, accounting for coprophagy allowed defining exactly the isotopic composition of the ingested diet. However, it did not solve the problem of the strongly negative and further decreasing ^{13}C trophic shifts. Even using only the assimilated part of the diet to calculate the ^{13}C trophic shift was not sufficient to approach the typical range of 0 – 2‰ (DeNiro and Epstein, 1978; Fantle et al., 1999). It might therefore be important to further distinguish between what of the assimilated C becomes finally incorporated into woodlouse biomass and what becomes released by respiration and excretion. We have shown that woodlice mainly assimilated the high-quality component of the food (carrot-lettuce mix) with a $\delta^{13}C$ value of -31.2‰ and that the contribution of the ^{13}C labelled food component (cellulose) to the biomass of adult woodlice was small. We propose that the C which entered the structural C pool of woodlice originated mainly from the carrot-lettuce mix, while the C contained in the cellulose was almost entirely respired without noteworthy affecting the woodlouse isotopic composition. This explains why woodlouse $\delta^{13}C$ values were decreasing in all treatments. Sufficient time for isotopic adaptation provided would allow to analyze the δ values of woodlice and diet after isotopic equilibration and to calculate the individual contribution of the dietary components to woodlouse biomass and to respiration.

Contrary to the incomplete isotopic adaptation in the experiment, in the standard culture *Porcellio dilatatus* had $\delta^{15}N$ and $\delta^{13}C$ values which were 2.5 – 3.5‰ and 1.1 – 1.4‰ higher, respectively, than the food on which the species was reared for several months until full-grown; i.e., cultured individuals revealed the expected trophic level enrichments. This clearly shows, that irrespective of their ability to quickly adapt their digestive system and to utilize changing food sources for nutrition (Hassall and Rushton, 1985; Wolters, 2000; Zimmer, 2002), the isotopic equilibration of adult woodlice may

need an extended period of time depending on the level of effective C and N uptake relative to the amounts of internal C and N and on the turnover between metabolic and structural pool. Therefore, we strongly suggest differentiating between growing and adult organisms when investigating the isotopic adaptation of a consumer to a new diet.

4.3. Alteration of organic matter by woodlice during the conversion of food into faeces

The differences between the supplied food and the faeces produced from that food mirrored the impact of woodlice on the chemical and isotopic composition of organic matter (OM).

The C:N ratio of the produced faeces (38 – 42) exceeded that of the food (35). This was surprising because faeces are considered as valuable source of N-containing compounds offering relative excess of N compared to the food (Torrallardona et al., 1996; Brendelberger, 1997; Zimmer and Topp, 2002). In a previous study using carrot-lettuce mix as food (C:N ~ 15), within eight weeks the C:N ratio of faeces decreased from 11 to 8 (Rothe and Gleixner, submitted). Likewise, in other studies the C:N ratios of animal faeces had always been lower than that of the OM source they derived from (Lee, 1997; Kautz et al., 2002). One aspect explaining our result is that the faeces were given only one week to mature. During maturation the faeces C:N ratio increases because of the release of microbial respiratory CO_2 from the substrate. Probably more important, however, was the high proportion to which cellulose supplied with the food became excreted. The fact that woodlice recycled faeces despite their higher C:N ratio compared to the food approved that the main function of coprophagy is not the acquisition of N or C as such, but the gain of compounds which woodlice cannot produce themselves (Kukor and Martin, 1986; Ullrich et al., 1991).

The ^{13}C enrichment in woodlouse faeces resulted from the mixing of the three dietary components during faeces production and was dominated by the $\delta^{13}C$ values of C3- and C4-cellulose in treatments with C3- and C4-food, respectively. Moreover, relative to the food produced faeces became enriched in ^{15}N by 2 – 3‰ what is close to the trophic level shift of 3.4 ± 1.1‰ (Minagawa and Wada, 1984). Consequently, woodlouse faeces which amounted to 40 – 50% of the consumed food in this study, but can make up even 80 – 90% (Jambu et al., 1988; Kautz et al., 2002), provide a source of OM enriched in ^{15}N as well as in microbial biomass compared to the original food. With respect to its quantity and quality this source may considerably influence nutrient transfer to higher trophic levels and other ecosystem processes (Mikola and Setälä, 1998; Laakso et al., 2000).

4.4. Alteration of non-ingested organic matter by woodlice due to microbial grazing

The similarity of the temporal dynamics of the C and N contents and the $\delta^{13}C$ and $\delta^{15}N$ values between supplied faeces and their one week old remnants indicated that changes in the elemental and isotopic composition of OM caused by woodlice did not occur randomly.

The higher C:N ratio of the non-ingested (~ 38) compared to the supplied faeces (~ 31) was due to a stronger loss of N relative to C. This loss of N required the presence of woodlice. Without woodlice the C:N ratio of supplied faeces remained more or less constant indicating the role of animals as a controlling factor of OM alteration. While microbial activity was responsible for the chemical and isotopic changes of the supplied faeces, woodlice exerted influence on the performance of microorganisms thereby affecting the overall chemical and isotopic composition of the faeces. For the ^{15}N depletion in supplied faeces under woodlouse control we suggest the following mechanism:

In the faeces mash obtained from homogenization of the faeces harvested in the production lines microbial growth takes place predominantly on the surface. Within a few hours a high amount of microbial biomass is formed the C and N of which almost entirely originates from the faeces substrate. Due to branches in the metabolic pathways which direct molecules into different pools (e.g. for protein synthesis) isotope fractionation takes place leading to a more or less intense ^{15}N enrichment of the microbial biomass relative to the substrate (Macko et al., 1986; Macko et al., 1987; Werner and Schmidt, 2002). In contrast, the excreted N-metabolites are depleted due to the discrimination against the ^{15}N isotope in nitrogen excretion (DeNiro and Epstein, 1981; Minagawa and Wada, 1984). These compounds become excreted into the substrate; i.e., while the heavier isotope (^{15}N) accumulates in the microbial biomass, the lighter isotope (^{14}N) is transferred back into the faeces mash, thus reducing its overall ^{15}N content. Since woodlice access the faeces mash from the surface, they predominantly scrape and ingest the uppermost layer containing a high proportion of ^{15}N-enriched microbial biomass whereas the remainder contains most of the excreted ^{15}N-depleted metabolites. This separation process reduces both the N content and the $\delta^{15}N$ value of the residual substrate. The proliferation of microbes causing ^{15}N fractionation and the grazing by woodlice of the microbial ‘lawn’ on the substrate surface may repeat several times resulting in a measurable difference between supplied and remaining faeces. Without the grazing by woodlice isotope fractionation between microbial biomass and residual substrate would be not detectable as both fractions are harvested and analyzed together.

Moreover, the grazing by woodlice might keep the microbial population in a phase of intense growth leading to a high throughput of OM at high rates of respiration and excretion and, consequently, amplifying isotope fractionation (Henn et al., 2002). Our results obtained from faeces incubation in the presence and absence of grazing animals support this view. While in the presence of woodlice the release of ^{13}C-depleted microbial respiratory CO_2 caused a general increase of the $\delta^{13}C$ values of the remaining faeces, in the absence of animals the ^{13}C changes were negligible; i.e., the alteration of organic matter was achieved by the concerted action of microorganisms and woodlice. Thus, even the simple case of a 'one diet – one consumer' model food chain represents a complex system in which different interactions and feed-back mechanisms between food, microbial community and decomposers need to be considered.

4.5. The impact of coprophagy on the elemental and isotopic composition of the faeces

Recycled faeces accounted for up to a fifth of the ingested matter in our experiment (Fig. 7). In the field exact numbers on the significance of coprophagy are hard to obtain (Carefoot, 1993; Zimmer and Topp, 1998; Kautz et al., 2002), but it is assumed to meet the range indicated by our experiment (Hassall and Rushton, 1982; Hopkin and Martin, 1985; Zimmer, 2002) in which the exchange of ^{13}C-labelled faeces visualized the involvement of coprophagy in the alteration of OM.

Ingested faeces become mixed with the primary food during the gut passage. Thus, the released faeces differ from faeces excreted in the case of pure food ingestion. This has already been shown directly by constraining faeces uptake by woodlice (Rothe and Gleixner, submitted). The faeces exchange experiment, however, offered the advantage to assess the involvement of coprophagy in OM alteration by comparing two groups of woodlice both recycling faeces. Depending on the type of faeces available for uptake, the faeces woodlice produced differed significantly in their elemental and isotopic composition. This indicates that coprophagy may determine the quality of the produced faeces even though the contribution of recycled faeces (10 – 20%) to faeces production was much lower than that of the food (80 – 90%).

Similar to a previous study (Rothe and Gleixner, submitted), the proportion of faeces in the diet decreased due to the adaptation of the digestive system to the new food source. While in the first phase woodlice required the help of 'microbial auxiliaries' to satisfy their demands for energy and essential substances (Ullrich et al., 1991), coprophagy be-

came scaled down during the adjustment of intestinal enzymes and gut microflora to the new conditions. Although faeces partly lost their significance as secondary source of C and N they were still vital part of the diet facilitating the uptake of essential substances and the microbial inoculation of the primary food (Rothe and Gleixner, submitted). This was mirrored by the continuously increasing assimilation efficiency of recycled faeces, which balanced the decreasing faeces uptake. The increase in faeces assimilation also demonstrates the importance of coprophagy for food utilization and, hence, for efficient OM degradation by woodlice. Due to the ongoing optimization of internal and external digestion processes, in the second gut passage woodlice assimilated almost all of the OM formerly supplied as food (Fig. 9).

4.6. The impact of coprophagy on the elemental and isotopic composition of woodlice

The elemental and isotopic differences between woodlice in faeces exchange and standard treatments were caused by utilizing faeces of different quality. In the exchange treatments one week old faeces (C:N ~ 31) were supplied, in standard treatments woodlice recycled freshly produced faeces (C:N 38 – 42). The higher C and N contents of woodlice in faeces exchange treatments suggest the availability of matured faeces to be an advantage. Consuming processed faeces in addition to the food enabled woodlice to access microbial C- and N-sources and compounds derived from microbial activity (e.g. sugars, amino acids, vitamins) which are partly essential (Ullrich et al., 1991; Kautz et al., 2002). Because cellulose – the main C source of the supplied food – cannot be digested by woodlice directly but requires microorganisms acting in the gut as well as in the excreted faeces (Kukor and Martin, 1986; Zimmer and Topp, 1998; Zimmer et al., 2002), recycling of matured faeces also provided additional C for nutrition.

The smaller ^{15}N enrichment in woodlice of faeces exchange treatments was related to the constant supply with faeces of higher quality, too. The effect of diet quality on consumer $\delta^{15}N$ values has been shown for rats (Sick et al., 1997), locusts (Webb et al., 1998), daphnids (Adams and Sterner, 2000) and woodlice (Rothe and Gleixner, 2000; Rothe and Gleixner, submitted). These studies consistently indicate that higher food quality results in a smaller isotopic shift, i.e. in a decelerated isotopic adaptation of the consumer to the food source.

5. Conclusions

Changing a consumer's food source may result in significant changes of its elemental and isotopic composition. While the adaptation of the digestive system is accomplished within days the isotopic equilibration between adults and their new diet may require weeks to months. Therefore, in rapidly changing environments the $\delta^{15}N$ and $\delta^{13}C$ values of full-grown organisms do not necessarily reflect their current food sources and their trophic position. Although our experiment allowed to define the contribution of coprophagy to the trophic shift and to even discriminate between assimilated and non-assimilated diet, this has not been sufficient to verify the reported trophic level shifts for ^{13}C and ^{15}N (DeNiro and Epstein, 1978; DeNiro and Epstein, 1981; Minagawa and Wada, 1984). Hence, in food web research, especially in field applications, it is recommended to distinguish between juveniles and adults.

Food ingested by woodlice becomes significantly altered on its conversion into excrements during the gut passage. The released faeces represent a source of ^{15}N and ^{13}C enriched organic matter that may considerably contribute to the nutrient transfer to higher trophic levels. Also, the remaining part of the food that does not enter the digestive system becomes altered by woodlice due to the grazing of microbial biomass growing on the food surface. In the case of the supplied faeces this process separated heavier and lighter isotopes of N and C fractionated by microorganisms and led to ^{15}N-depleted and ^{13}C-enriched remnants.

Coprophagy influences the elemental and isotopic composition of both, woodlice and faeces. Woodlice respond very sensitive to faeces quality showing significantly lower $\delta^{15}N$ values when utilizing matured in comparison to fresh faeces. Feeding back on the digestive capabilities of woodlice, coprophagy accelerates the nutritional adaptation to a new food source and optimizes food assimilation. Variations in the ^{13}C signature of the recycled faeces are maintained during faeces production. Therefore, the application of isotopically labelled dietary components in feeding experiments is an appropriate method to identify the role of coprophagy for woodlouse nutrition.

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4.4. Manuscript IV

Application of stable nitrogen isotopes to investigate food web development in regenerating ecosystems

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Application of stable nitrogen isotopes to investigate food web development in regenerating ecosystems

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Introduction

Stable isotope analysis has become an essential tool in ecology and environmental science to investigate the cycling of carbon and nitrogen at various spatial and temporal scales in order to understand organic matter dynamics (Lajtha and Michener 1994). The path organic matter takes through an ecosystem is defined by the ecosystem structure. Food webs can be regarded as 'biotic backbone' of the system as they connect all organisms by trophic relationships. The food web structure reflects the energy flow, which is a major driving force of ecosystem development. Consequently, structural changes in food webs (e.g. due to species loss or species turnover) influence the pattern of organic matter and energy transfer whereby also affecting the isotopic composition of single food web constituents and the general isotopic patterns within the food web.

Recently Vander Zanden et al. (1999) and Stapp et al. (1999) demonstrated that natural and anthropogenic disturbances cause deviations from system specific isotope patterns. As such, improvement of ecosystem health during the recovery from disturbances should also be mirrored by the isotopic situation. However, this has so far not been systematically investigated in regenerating ecosystems.

Ecosystem regeneration after disturbance comprises the re-establishment of abiotic and biotic system components (structural recovery) and of their interactions (functional recovery). At each stage in the transition from a disturbed to an intact 'healthy' state (Hobbs and Norton 1996) the rules underlying community assembly ensure that the present ensemble of system components is able to maintain vital ecosystem functions, such as primary production, decomposition and remineralization of organic matter. The order in which species assemble is governed by the species traits and by the multi-factorial complex of environmental conditions acting as a dynamic filter (Chapter 6, Fattorini and Halle). The stepwise assembly requires

dynamic adaptation of the food web in terms of structure and functioning and leads to increasing complexity and stability of the trophic network. For each trophic link both, its actual interaction strength and its relative stability contribute to the isotope signal built up in the consumer. Therefore, in stabilized trophic relationships, the isotopic difference between consecutive trophic levels is supposed to be rather constant. However, the involved species as such might reveal an isotope shift according to isotopic changes of their food sources during ecosystem regeneration.

In this study we used stable nitrogen isotopes to describe the progress in ecosystem development after disturbance, which is a key issue of restoration ecology (Hobbs and Norton 1996). Based on a small number of macro-invertebrate species we monitored temporal changes of the food web structure at 4 differently degraded grassland sites. Also functional aspects concerning food web complexity and stability were considered. Because the investigated systems are by far to complex to uncover the causalities between changing isotope patterns and regeneration processes from a few analyses, our study is a first attempt to find descriptive parameters which promise to be useful in the context of community assembly and ecological restoration. Community ecologists as well as restoration ecologists need tools appropriate to find patterns, which are relevant to understand the factors and processes involved in community development and to detect rules which govern the assembly of the system (Chapter 1, Fattorini et al.). In this chapter, therefore, we address the following questions: Can stable isotopes:

- distinguish developmental states reached by differently disturbed ecosystems?
- reflect changes in the structure and dynamics of food webs of regenerating ecosystems?
- provide information on food web and ecosystem complexity, functionality and stability?

Stable isotope method

The element nitrogen (N), which is a major component of organic matter, has two stable isotopes: ^{15}N and ^{14}N. Due to isotope discrimination in the attainment of equilibrium (thermodynamic isotope effects) as well as in (bio)chemical reactions (kinetic isotope effects) the isotopic composition of organic matter depends on 1) the isotopic composition of the reactants, 2) the involved biochemical pathways, 3) the reaction kinetics and 4) the physical and chemical conditions during its (bio)synthesis, transformation and degradation (Wada et al. 1995; Gleixner et al. 2001). The combination of all 4 factors lead to a dynamic stable isotope fingerprint in every biogenic material. Isotope ratios are expressed in conventional delta (δ) notation in parts per thousand relative to international standard materials (Eq. 1.1).

$$\delta^{15}N\ (‰) = (R_{sample}/R_{standard} - 1) * 1000\ ‰ \quad (1.1)$$

R stands for the ratio between the heavier and the lighter isotope ($^{15}N/^{14}N$) of a sample or a standard. International standards, e.g. AIR for N, are provided by the International Atomic Energy Agency (Coplen et al. 1992). Commonly isotope ratios are determined using Isotope Ratio Mass Spectrometers (IRMS) in combination with on-line and off-line coupled sample preparation systems (Brand 1996).

Stable isotopes in food webs

The relative abundance of stable isotopes in living organisms depends on the isotopic composition of their food sources and their internal fractionation. Generally, the latter leads to an enrichment of the heavier isotope in consumers relative to their diet. For ^{15}N the enrichment is caused by preferential excretion of ^{15}N-depleted metabolites like ammonia. Regardless of the habitat, Minagawa and Wada (1984) reported an average ^{15}N enrichment of 3.4±1.1‰ (s.d.) per trophic level. This difference in $\delta^{15}N$ values is also referred to as Trophic Level Shift (TLS). As empirical parameter, TLS is used to assign organisms to distinct trophic levels (Ponsard and Arditi 2000; Rothe and Gleixner 2000; Scheu and Falca 2000).

Generally, the ^{15}N shift of 3.4‰ reflects conditions in which the consumers were able to isotopically equilibrate with their diet according to the thermodynamic and kinetic constraints governing isotope fractionation. In fact, the actual isotopic difference between consumer and diet often deviates from the 3.4‰ target value in either direction depending on factors such as food quality, ration size, age and sex (Focken 2001), but also on the time of adaptation to the food source. Nevertheless, previous work has confirmed that stable N isotopes provide a time-integrated measure of food web relationships as they indicate the trophic position of a consumer at a given time (Fantle et al. 1999; Stapp et al. 1999; Vander Zanden et al. 1999). The comparison of trophic positions of certain species among ecosystems requires, however, taking into account the system-specific isotope background determined by the isotopic composition of the organic matter input at the base of the food web (Ponsard and Arditi 2000). Moreover, food web studies in terrestrial ecosystems need to include the key compartment soil, even if the main focus is on the aboveground food web (AFW). While changes in soil properties during regeneration will have equivalent impact on the entire biocoenosis (Scheu et al. 1999), changes in biotic ecosystem components feed back to the soil (Scheu 1997). Thus, soil isotopic signatures may reflect the history of structural and functional ecosystem development (Bardgett et al. 1998; Bengtsson et al. 1998; Mikola et al. 2001).

Materials and methods

Study site

We studied the regeneration of a grassland ecosystem (Fig. 1; for general site characteristics see Heinrich 1984 and Chapter 12, Wagner) which had been disturbed for 30 years by dust and gaseous emissions originating from an adjacent phosphate fertilizer plant (Metzner et al. 1997). Major parts of the lower slope were almost completely degraded (Heinrich et al. 2001). Restrictions in the cycling of organic matter caused by dissimilarities between primary production and decomposition hampered the re-establishment of ecosystem structure and functioning in the first years of regeneration.

Sampling and sample preparation

In 1990 the Institute of Ecology (University of Jena) installed a permanent transect of 40 pitfall traps over 200 meters to monitor faunal development after disturbance along the former pollution gradient. For the present study, we combined 5 adjacent sampling points at 4 segments of the transect to define 4 distinct sites, which were differently impacted (Fig. 1): (1) the most disturbed steep lower slope next to the polluter (LS), (2) the flatter middle slope (MS) just below the edge of the valley shoulder, (3) the lower part of the slightly inclined upper slope (US1) and (4) the least impacted upper part of the upper slope already invaded by woody plants (US2).

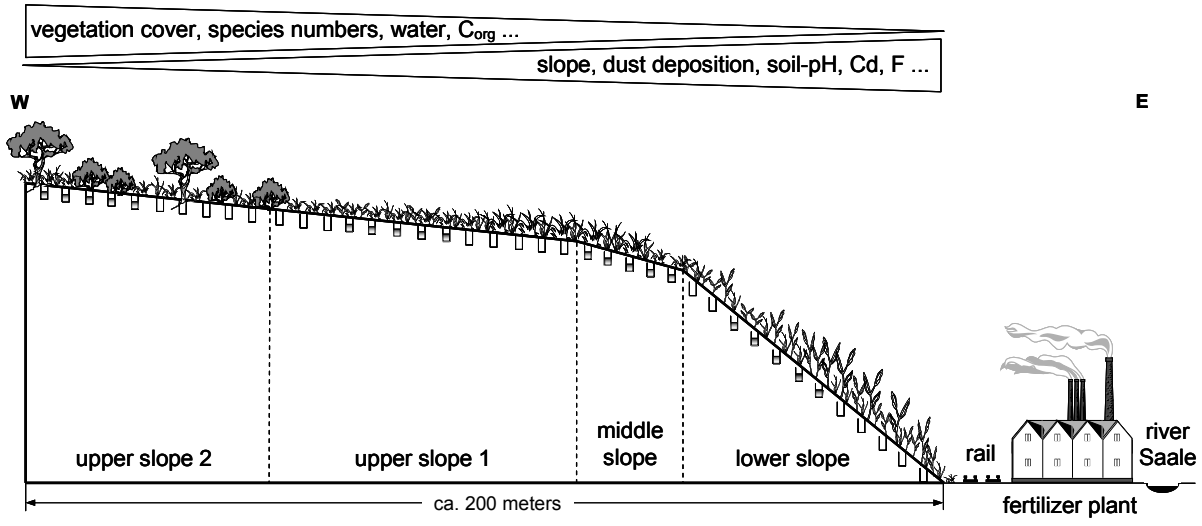

Figure 1: Steudnitz research area: Profile view along the pitfall trap transect, divided into 4 sections. Within each section animal material from 5 pitfall traps (indicated as half-filled) was used, representing distinct sites (see text). Main environmental gradients as present at the beginning of ecosystem regeneration are indicated.

From 1991 to 1996 and in 2000 pitfall traps were emptied every 14 days to sample epigeic arthropods. In addition, at LS, US1 and US2 the fauna in the higher parts of the vegetation was sampled by sweep net catching. Animals were preserved in 70% ethyl alcohol. After taxonomic identification animals caught at 3 successive sampling dates were pooled per species and site to obtain sufficient amounts of animal dry mass. Each pooled sample contained 20-50 individuals.

For isotope analysis, from the most dominant species we selected 14 to represent different taxonomic categories, trophic levels, and functional groups. We chose woodlice, which are important macro-decomposers sensitive to potentially harmful elements such as zinc, cadmium, copper and lead (Beyer and Anderson 1985; Hopkin 1990; Jones and Hopkin 1998). Their recolonization is thought to have a pronounced effect on quantity and quality of soil organic matter and thus on ecosystem development in general. Several phyto- and zoophagous beetle and bug species, respectively, were selected to include the trophic levels of herbivores and carnivores. The final selection comprised samples of spring, summer (in 2000 only) and autumn catches, respectively, of 2 woodlouse (1991-96 and 2000), 7 beetle (1991, 1996 and 2000) and 5 bug species (1992, 1994-1996 and 2000; at US1 and US2, only). The nomenclature of the investigated species followed Gruner (1966) for woodlice, Köhler and Klausnitzer (1998) for beetles, and Wagner (1967) for bugs.

In 1994, 1998 and 2000 around each pitfall trap we took 10 soil cores from the A-horizon (0-10 cm) for a pooled sample. Soil was air dried, sieved to <2 mm, cleared from plant residuals and stored in paper bags. The 5 samples from each of the 4 sites were analyzed separately and the data were pooled. Also, in 2000 separate samples of the most abundant plant species and pooled litter samples were taken at the 4 sites. Plants were divided in shoots and roots, dried at 35°C and stored in paper bags.

Isotope analysis

Prior to isotope analysis ethyl alcohol was removed from the animal material. Plant, animal and soil samples were freeze dried, ground and stored in air tight glass bottles at -10°C. Samples equivalent to 150 µg N were weighed into tin capsules and combusted in an EA 1110 Elemental Analyzer (ThermoQuest, 20090 Rodano, Italy). The resulting N_2 gas was analyzed for ^{15}N content in a DeltaPlusXL isotope ratio mass spectrometer (Finnigan MAT, 28127 Bremen, Germany). The analytical precision was ±0.2‰. Working standards were acetanilide and caffeine, calibrated against international standard IAEA-N1. Accuracy and

repeatability of measurements were assured according to Werner and Brand (2001). Isotope ratios were expressed as $\delta^{15}N$ values (Eq.1.1).

Calculations and Statistics

In 2000 the differences of $\delta^{15}N$ values between the analyzed animal species and their likely food sources were calculated separately for all supposable combinations at each site considering trophic links between: carnivores – herbivores/detritivores, herbivores – plant shoots, detritivores – plant shoots and detritivores – litter. Intratrophic-level predation was excluded. The resulting data was divided into $\delta^{15}N$ classes of 1‰ width.

We used the mean value of the frequency distribution of classified $\delta^{15}N$ differences to evaluate the developmental state of the food web after 10 years of regeneration. This is based on the idea that stability and functionality of food webs are accomplished by the whole array of trophic links. Since the isotopic equilibration of a consumer with its diet is reflected by a shift in the $\delta^{15}N$ value of 3.4±1.1‰ (Minagawa and Wada 1984), we assume, that developmentally advanced food webs with a high proportion of stable trophic links should show distribution mean values between 3 and 4‰. The distribution raw data were subjected to Bootstrap analysis (1000 replications; S-PLUS 6.0, Insightful Corp. 2001) to test the reliability of the observed mean. The bias between observed and empirical means was below 0.002‰. In addition, we tested if the distribution mean values observed in 2000 resulted from developmental processes, i.e. we calculated the distribution mean values in 1991, 1996 and 2000 for the trophic interactions of the predators. Linear regression was applied to analyze temporal trends (SPSS for Windows 11.0, SPSS Inc. 2001).

As regards the temporal ^{15}N dynamics from 1991 to 2000, the isotopic difference between soil and species representing the AFW was used to characterize changes in the coupling between aboveground and belowground parts of the ecosystem with ongoing regeneration. This difference should decrease the more AFW and soil food web (SFW) become structurally and functionally connected. Depending on the degree of functional integration the isotopic signatures of the soil and of the aboveground components should show similar dynamics. Temporal trends and differences in the dynamics (i.e. the degree of parallelism) of the measured $\delta^{15}N$ values (annual means of 2-3 seasonal records) were tested by generalized linear models (GLM) based on univariate analysis of variance (ANOVA). The interaction between species and duration of regeneration was included (SPSS for Windows 11.0, SPSS Inc. 2001). Data sets of 4 beetle and 3 bug species were omitted because of incompleteness.

Results

Developmental state of the food web

In 2000, after 10 years of regeneration, the frequency distribution of $\delta^{15}N$ differences between consumer species and their likely diet varied clearly among the investigated sites (Fig. 2).

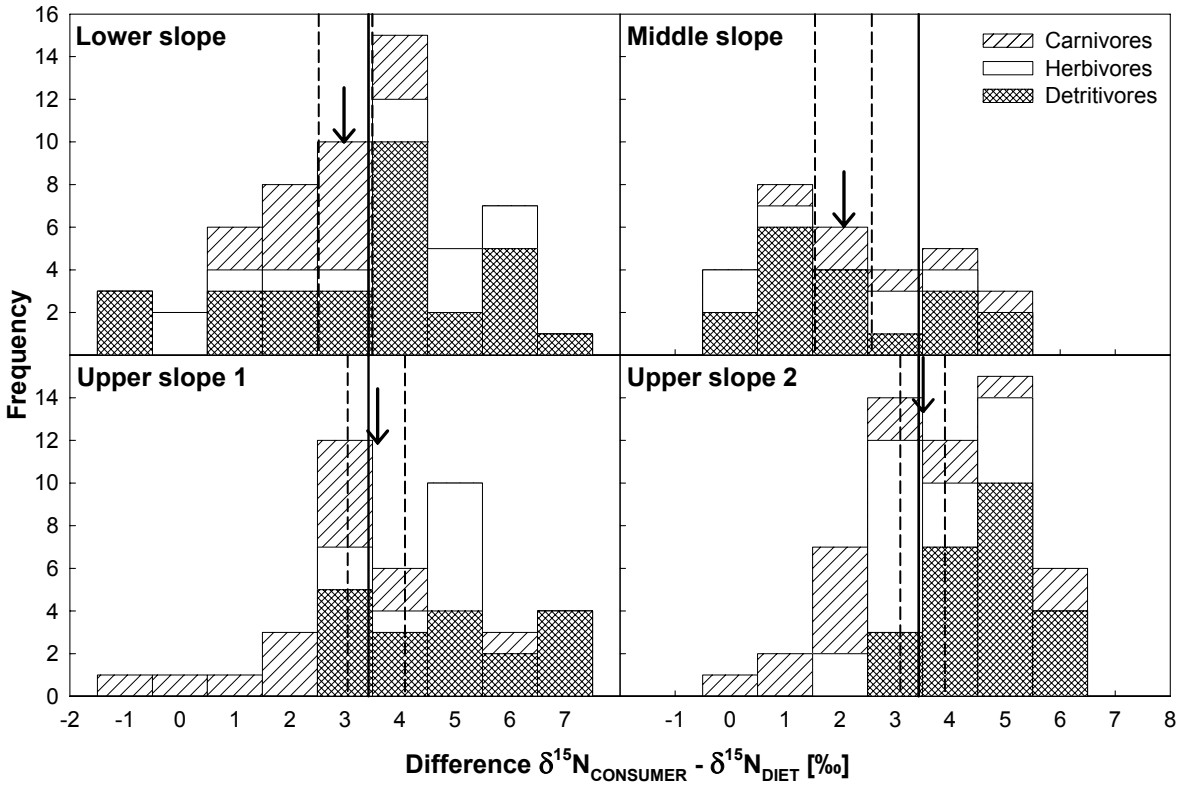

Figure 2: Frequency distribution (class width: 1‰) of the isotopic difference (^{15}N) between food web components and their putative food source at the 4 sites of the Steudnitz study area in 2000. The distribution mean values are indicated by arrows, the 95% confidence interval of the means by vertical dashed lines. The filling patterns illustrate the contribution of the 3 different types of consumers.

Distribution mean values (given as Bootstrap mean with lower and upper bound of the 95% confidence interval) were highest at the least disturbed sites US1 (3.65‰; 3.11-4.18; N=41) and US2 (3.57‰; 3.22-3.93; N=57), intermediate at the most disturbed site LS (3.01‰; 2.53-3.46; N=68) and lowest at MS (2.13‰; 1.58-2.69; N=30). The overall mean was 3.18‰ (N=185). While means were statistically identical at US1 and US2, the means found at LS and MS did not fall into the 95% confidence interval at the other sites.

From 1991 to 1996 the mean ^{15}N shift between predators and their possible prey strongly increased at all 4 sites (Fig. 3); thereafter it remained constant. The linear trend was significant in the case of LS (p=0.01) and US2 (p=0.02). The increase in the isotopic shift

between adjacent trophic levels agreed with our hypothesis that advance in regeneration should be mirrored by the stable isotope pattern of the assembling community.

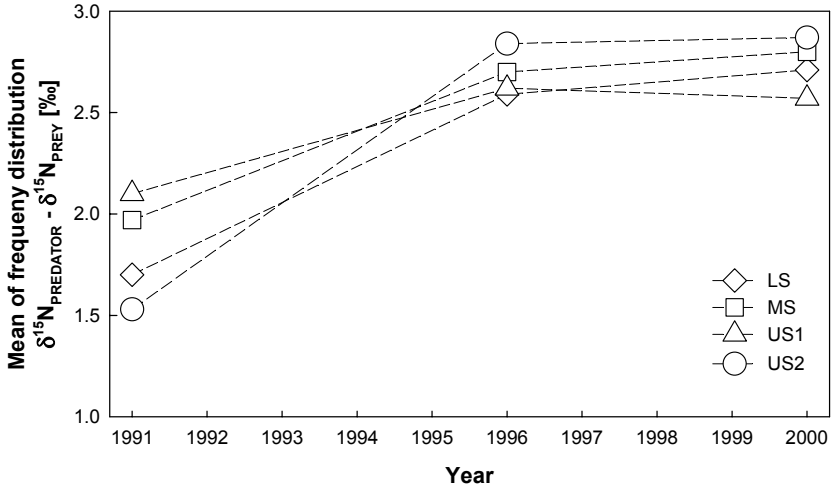

Figure 3: Temporal variation of the mean of $\delta^{15}N$ differences between predators and potential prey, spanning over 10 years of ecosystem regeneration after disturbance. Calculations based on samples from 7 beetle, 5 bug and 2 woodlouse species at 4 differently impacted sites (LS = lower slope, MS = middle slope, US1 = lower part of upper slope, US2 = upper part of upper slope).

Food web structure

Food web dynamics over 10 years was reflected by the development of animal $\delta^{15}N$ values (Fig. 4). At all 4 sites the general trophic order of the selected species was identical throughout the time. The woodlouse *Armadillidium vulgare* and the phytophagous beetle *Otiorhynchus raucus* were consumers at the base of the food web. The woodlouse *Trachelipus rathkei* was always about 2‰ enriched in ^{15}N compared with *A. vulgare* and represented a distinctly higher level within the trophic hierarchy. The next level was composed by predatory organisms (i.e. the beetle *Calathus melanocephalus* and the bug *Nabis brevis*), but also included one beetle species assumed to be predominately phytophagous (*Agrypnus murina*). The plant sap sucking bug *Notostira elongata* was found at US2 at the level of herbivores, at US1, however, at the level of carnivores.

Dynamics of the entire food web

Based on the ^{15}N data of animals and the soil univariate analysis of variance (GLM) confirmed that at LS ($p=0.013$), US1 ($p=0.001$) and US2 ($p=0.007$) the entire system shifted towards lower $\delta^{15}N$ values with time. At MS statistically no relationship between isotope pattern and time of regeneration was detectable (GLM, $p=0.269$). However, the hump-shaped

curves (e.g. in *T. rathkei* and *A. murina*) show that after a general shift to higher $\delta^{15}N$ values the whole system returned to a ^{15}N level similar to the initial situation in 1991.

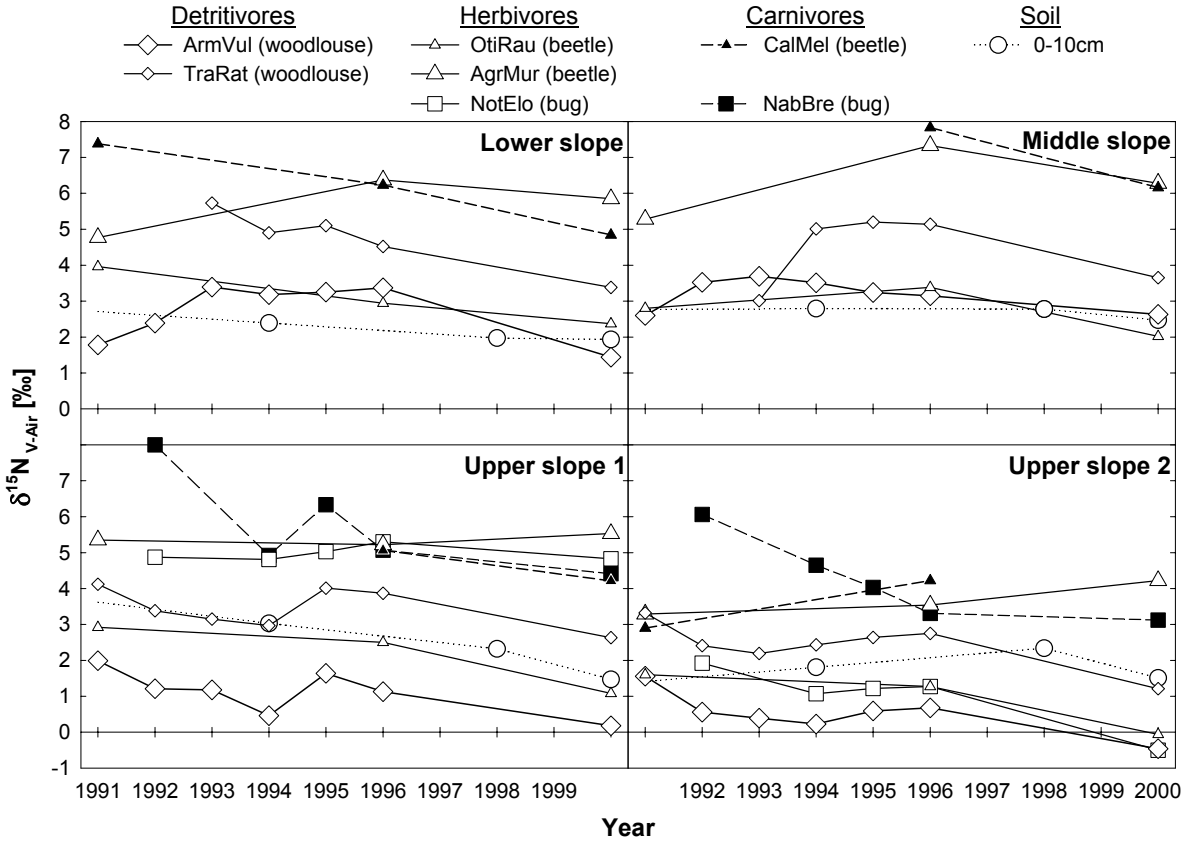

Figure 4: $\delta^{15}N$ values of the main consumer species and soil $\delta^{15}N$ values (extrapolated from 3 data points) at the 4 sites of the Steudnitz study area between 1991 and 2000. If seasonal data of animal species were available the arithmetic mean of 2-3 values was used (standard deviations always <0.8‰). Data points for $\delta^{15}N$ values of soil are represented by circles, those of animals by angular symbols (open symbols for detritivorous and phytophagous species, filled symbols for zoophagous species). Dotted lines connect data points of soil measurements, solid lines data points of detritivorous and phytophagous species, dashed lines data points of zoophagous species. ArmVul – *Armadillidium vulgare*, TraRat – *Trachelipus rathkei*, AgrMur – *Agrypnus murina*, OtiRau – *Otiorhynchus raucus*, CalMel – *Calathus melanocephalus*, NotElo – *Notostira elongata*, NabBre – *Nabis brevis*.

Stability of trophic relationships

Concerning the similarity in the ^{15}N dynamics of species of various trophic levels, at MS ($p=0.555$) and US1 ($p=0.137$) the model rejected that the isotope signals of the analyzed species behaved differently in time. Instead, the lack of significant differences in the interactions between species ^{15}N signals and year of sampling suggests a high level of stability in the processes and factors governing the isotope dynamics at both sites. At LS the species-time interaction was almost significant ($p=0.074$), i.e. the similarity in the ^{15}N patterns of the analyzed species was lower at LS compared with MS and US1. At US2 the species-time interaction was significant ($p=0.002$). However, when omitting the beetle species

C. melanocephalus and *A. murina* from the analysis (both showing positive ^{15}N trends, Fig. 4) it becomes evident that the $\delta^{15}N$ values of the 4 species at the base of the food web shifted almost in parallel with time (GLM, p=0.163).

Development of the soil relative to the aboveground food web

Along the pollution gradient from LS to US2 the soil ^{15}N content shifted consistently towards that of higher trophic levels of the AFW (Fig. 4). At LS the $\delta^{15}N$ values of the soil were clearly (~1‰), at MS slightly below the $\delta^{15}N$ values of *A. vulgare* and *O. raucus*. At US1 and US2 the soil $\delta^{15}N$ values were >1‰ above *A. vulgare* and slightly to well above *O. raucus*, respectively.

Over 10 years of regeneration at all 4 sites the ^{15}N dynamics of the soil was similar to that of the AFW species (GLM, p>0.05). However, between 1998 and 2000 at LS and MS animal $\delta^{15}N$ values further decreased while the soil remained rather constant. This could reflect the ongoing integration of AFW and SFW at both formerly most impacted sites. In comparison, the ^{15}N patterns at US1 and US2 suggest that the coupling of aboveground and soil processes at the least impacted sites stabilized between 1991 and 1996, already.

Discussion

Developmental state of the food web

The mean of the frequency distribution of classified $\delta^{15}N$ differences between consumer species and their likely diet (Fig. 2) served as descriptive variable for evaluating the developmental state of food webs. This parameter differentiates between sites along a pollution gradient representing differently advanced stages in ecosystem regeneration. The site-specific ecosystem complexity estimable from vegetation and macro-invertebrate data (Heinrich et al. 2001; Perner and Voigt unpublished) corresponds with the calculated distribution mean values. At the least disturbed sites US1 and US2 the means (3.65 and 3.57‰, respectively) were closest to the reported 3.4‰ target value (Minagawa and Wada 1984). In their study on litter macro-invertebrates of undisturbed woodlands, based on a total of almost 300 samples of detritivorous and predatory species taken in 3 seasons at 3 sites, Ponsard and Arditi (2000) found an average ^{15}N shift between detritivores and the analyzed litter (L-horizon) of 3.5±1.2‰. Between predators and detritivores the ^{15}N shift (3.6±1.0‰) significantly differed from the 3.4‰ target value only in 1 of the 9 cases. This corroborates our hypothesis that the

distribution mean value should be close to 3.4‰ at the sites most advanced with respect to food web regeneration.

At LS the number of grass and herb species increased from 2 in 1991 to 50 in 2000 (Heinrich et al. 2001). This strong increase in plant diversity corresponds with a distribution mean value of 3.0‰ close to the 3.4‰ target value; i.e. the particular community dynamics at LS led to a faster stabilization of trophic relationships compared with MS. At MS biodiversity was lowest just like the distribution mean value (2.1‰). The vegetation was strongly dominated by the grass *Agropyron repens*. Due to its low palatability for macro-decomposers the litter of *A. repens* was only partly degraded and accumulated to a thick layer on the soil surface (Heinrich 1984). The barrier of undecayed litter presumably caused a decoupling between soil and aboveground processes, which, in turn, reduced the diversity of trophic interactions and the speed of regeneration. In consequence, the developmental state was less advanced at MS compared with LS and – even more pronounced – with US1 and US2.

Considering this in conjunction with the evidence that the mean trophic shifts for ^{15}N found in 2000 resulted from developmental processes (Fig. 3), the system on the upper slope in Steudnitz (sites US1 and US2) can be regarded as recovered from disturbance. We hypothesize that community assembly has reached an 'optimum state' in so far as the addition of new species would not necessarily improve functionality and stability of the food web but would only increase complexity by introducing further functional redundancy.

Food web structure

Our ^{15}N data allowed detecting differences in the food web structure of 4 grassland sites within 200 meters along a pollution gradient (Fig. 4). Although stable isotope analysis is incapable of providing a complete picture of the trophic links between individual species in food webs, this technique allows a general and reliable evaluation of the trophic structure of a community (Ponsard and Arditi 2000; Scheu 2002). Moreover, ^{15}N signatures gave insight into the nutritional behaviour of the analyzed species. For example, the $\delta^{15}N$ values of woodlice indicated a clear niche differentiation in the food sources at all 4 sites, which was stable in time. Over 10 years the ^{15}N signals of *Armadillidium vulgare* and *Trachelipus rathkei* differed consistently by 2‰, suggesting that *A. vulgare* fed mainly on fresh litter and plant biomass while *T. rathkei* used materials more enriched in ^{15}N such as degraded litter, faeces and carcasses, i.e. detritus including the associated microbes. This is in accordance with field studies, which have shown that differences in the feeding mode of taxonomically related species are traceable by $^{15}N/^{14}N$ ratios (Schmidt et al. 1997; Scheu and Falca 2000).

Scheu and Falca (2000) suggested distinguishing primary and secondary decomposers to describe the continuum of decomposer species according to the preferential food sources.

The high $\delta^{15}N$ values at the level of carnivores frequently found in the beetle *Agrypnus murina* were in contrast to the a priory classification as phytophagous. We assume that apart from plant material this polyphagous, large-sized elaterid beetle extensively used ^{15}N enriched food sources. Beside a possible switch to a saprophagous feeding mode (including dead animal biomass) zoophagy should be considered as an alternative nutritional strategy in *A. murina*, a fact, that has rarely been reported, yet (J. Perner, pers. comm.). Corroborated by a C:N ratio (4.4±0.2) only slightly above that of obligate carnivorous beetles such as *Calathus melanocephalus*, *Anisodactylus binotatus* and *Poecilus cupreus* (4.3±0.2), we propose that in the Steudnitz system adults of *A. murina* – contrary to the common classification – were predominantly acting as predators and therefore showed higher $\delta^{15}N$ values than detritivores and herbivores (Fig. 4). In addition, depending on the time of sampling relative to the date of emergence, the isotopic signature of an imago can show a respective influence of the larval diet. Consequently, if holometabolic species are selected for food web reconstruction, the use of species such as *Otiorhynchus raucus* (herbivorous generalist) and *C. melanocephalus* (carnivorous generalist) with a common functional type of nutrition in larva and imago is advantageous.

The differing ^{15}N signals of the plant sap sucking bug *Notostira elongata* at US1 and US2 (Fig. 4) relate to variations in the isotopic composition of the accessible diet. In our case, *N. elongata* prefers immature seeds of *Agropyron repens* (W. Voigt, pers. comm.), which we found to be up to 4‰ enriched in ^{15}N compared with other abundant grasses in Steudnitz. While *A. repens* was present at US1, it was lacking at US2. Thus, at US1 the presence of *A. repens* allowed the apparently anomalous ^{15}N enrichment observed in *N. elongata* resulting in a carnivore-like ^{15}N signal, while at US2 this bug exploited food sources with lower ^{15}N content similar to those of the phytophagous beetle *O. raucus*. The example shows that feeding preferences can be useful to trace food sources but may also provoke misleading conclusions concerning trophic level membership. Within functional groups, therefore, generalists, which integrate over a number of food sources, should be selected to obtain an unbiased picture of the trophic structure of the studied community.

Dynamics of the entire food web

The $\delta^{15}N$ values of the investigated species reflected that all trophic levels were affected by regeneration processes; i.e. the entire food web showed an 'isotopic' response to changing environmental conditions (Fig. 4). We argue that the system ^{15}N shifts at the Steudnitz sites were controlled by two factors: the performance of N_2-fixing plants and the efficiency of organic matter decomposition.

At US1 and US2 the $\delta^{15}N$ values of the basal food web components decreased while the abundance of two species of the genus *Vicia* (Fabaceae) was increasing or permanently high (Heinrich et al. 2001). *Vicia* is able to fix atmospheric N_2 with $\delta^{15}N$ values close to 0‰ (Nadelhoffer and Fry 1994). The continuous input of fresh N with $\delta^{15}N$ values below the mean ecosystem value caused the observed decrease of animal and soil $\delta^{15}N$ values at US1 and US2. The high and almost constant soil $\delta^{15}N$ values at LS and MS are due to the absence of legumes (Fig. 4). As the availability of N influences plant species composition, biomass production, and also the performance of soil microbes, it affects community assembly with respect to diversity and strength of trophic interactions. Our study demonstrates that food web regeneration may benefit from the presence of N_2-fixing plants. In fact, we found the ecosystems at US1 and US2 to be most advanced in regeneration because of the effective supply with N which accelerated developmental processes. The role of N and N_2-fixing plants for ecological restoration has been intensively discussed by Bradshaw (Chapter 15), who came to a similar conclusion that single plant species may become key components in community assembly.

Second factor governing the ^{15}N dynamics was the presence of macro-decomposers. Due to their sensitivity to heavy metals and high salt concentrations (Beyer and Anderson 1985; Hopkin et al. 1986; Paoletti and Hassall 1999), woodlice were almost absent from Steudnitz before 1990. Heinrich (1984) observed that primary production exceeded the decomposition rate leading to litter accumulation on the soil surface. The investigated woodlouse species (*A. vulgare* and *T. rathkei*) obtained significant abundances not until 1994, but since then they facilitated the decomposition of accumulated litter and freshly produced plant material. Likewise the improvement of soil conditions supported the recovery of the soil microflora (Langer and Günther 2001). Both aspects ensured the input of new ^{15}N-depleted compounds into the soil, which was also recognizable at LS and MS (Fig. 4). Because the lack of (macro-) decomposers may considerably hamper developmental progress due to restrictions in the cycling of matter, site preparation in restoration projects needs to consider this aspect.

Stability of trophic relationships

From 1991 to 2000 at each of the 4 Steudnitz sites the trophic hierarchy was rather constant (Fig. 4), i.e. the principle food web structure was already established in the initial phase of regeneration. We suggest that the attainment of functional stability in trophic relationships can be identified from analyzing the ^{15}N dynamics of species representing different trophic levels (Fig. 4). For example, at US1 and US2 the $\delta^{15}N$ values of most AFW components went parallel during the last years. This reflects that in less than 10 years in both systems on the upper slope regeneration processes have compensated the negative effects of disturbance with respect to the relative stability of the food web and its functioning.

Development of the soil relative to the aboveground food web

The relative shift of soil $\delta^{15}N$ values towards those of AFW species increased along the pollution gradient in Steudnitz. At the least impacted site US2 the soil $\delta^{15}N$ values of the Ah-horizon were in-between those of primary consumers (i.e. primary decomposers and herbivores) and higher trophic levels (secondary decomposers and predators). Similarly, in intact forest ecosystems the ^{15}N content of the Ah-horizon was found to be higher (1.5‰ on average) compared with detritivores, but lower (1.9‰ on average) compared with predators, both present in the litter layer (Ponsard and Arditi 2000). From this we hypothesize that the $\delta^{15}N$ difference of AFW components and soil reflects the level of functional integration between AFW and SFW which should be most advanced in undisturbed conditions.

The level of integration influences functionality, efficiency and stability of the entire food web. While stable interactions between SFW and AFW are prerequisite for essential ecosystem functions such as nutrient cycling including decomposition and remineralization, disturbance may lead to severe interruptions in the cycling of elements whereby – at least in part – decoupling ecosystem components from each other (Asner et al. 1997). The ^{15}N data of Steudnitz imply an increasing level of integration of the trophic network with a decreasing degree of disturbance. If our hypothesis was valid, the developmental state of an ecosystem on its trajectory from disturbed towards recovered could be estimated in relation to reference ecosystems.

The temporal development of the soil $\delta^{15}N$ values relative to those of detritivores and herbivores describes the interdependence of SFW and AFW based on structural and functional links, which comprise bottom-up control of the aboveground community by soil animals as well as top-down forces by generalist predators benefiting from belowground energy supply (Scheu 2001). Stable relations between aboveground and belowground

processes should cause the ^{15}N signals of AFW species and the soil to run parallel with time as demonstrated for the most advanced Steudnitz sites US1 and US2.

So far, our interpretation of stable isotope data is a first attempt to scale up from species-specific measurements to ecosystem properties. More evidence is needed to develop this approach into a universal tool in ecological restoration.

Stable isotopes in ecosystem research

The potential of stable isotopes to assess developmental progress is applicable to describe natural succession as well as to evaluate the success of restoration measures. Since the rules creating isotope patterns are universal, also unknown systems can be approached. Previous knowledge about the attributes and the history of the system under focus is an asset but no imperative to apply isotope techniques successfully. Various types of ecosystems with differing species compositions can be compared. A small sample size already allows one to get an impression of the structure and functional performance of a community. Considering the amount of work needed to gather the data necessary to study community structure and its variation in time, the isotope methods are faster and easier to use than the classical ones (Ponsard and Arditi 2000). However, to compare the system under restoration with a reference system the 'isotopic baseline' defined by the organic matter input at the base of the trophic network needs to be included. In addition, isotope analysis of the soil is required to get information on the functional coupling between above- and belowground compartments, which is assured by trophic links (Scheu 2001).

In our study the good correspondence of spatial and temporal isotope patterns with the abiotic and community development provided arguments to 1) conclude from isotope data to ecosystem properties such as stability, functionality and complexity and to 2) translate these parameters into 'developmental states'. Both steps demand high levels of abstraction bearing the risk of an over-simplistic interpretation of the complex reality. To account for this, comprehensive studies of various ecosystems (e.g. Ponsard and Arditi 2000; Scheu and Falca 2000) should provide statistical evidence for a causal relationship between ecosystem state and descriptive variables derived from isotope data, e.g. the mean value of the frequency distribution of $\delta^{15}N$ differences between consumer and diet as suggested above.

Empirical findings such as the '3.4‰-rule' encourage one to use the information offered by isotope patterns. These patterns are produced by the same forces driving the piece by piece assembly of species to combine to a harmonic entity named 'community' – despite (or because of?) the presence of thresholds and environmental filters (section 2 on environmental

filters and assembly). The driving forces are the thermodynamics and the reaction kinetics of biogeochemical processes (Schmidt et al. 1995; Wada et al. 1995; Gleixner and Schmidt 1997) acting in the framework set by the environmental conditions. They control the flow of energy and matter and introduce structure into the systems, whereby creating the 'dynamic stable isotope fingerprint' in biogenic material, which can be used in ecological studies. Since the studied patterns and processes span over several spatial and temporal scales (Chapter 6, Fattorini and Halle) the search for assembly rules has to cover various levels of complexity. Different resolution and, hence, different insight can be extracted from raw data by suitable data reduction methods and statistical procedures. Using such tools to screen our isotope data for hidden patterns and synoptic variables allowed us to focus on community attributes such as stability and complexity, which could not be easily assessed otherwise. In this context, our view on the development of regenerating ecosystems from the perspective of stable isotopes may serve as encouraging example.

Conclusions

With regard to assembly rules in the context of restoration ecology stable nitrogen isotopes offer great potential to describe food web development in regenerating ecosystems and to get insight into the mechanisms and principles governing structural and functional reorganisation of communities after disturbance. Due to its 'isotopic memory' substantial information to understand the functioning of terrestrial ecosystem can be found in the soil.

The mean value of the frequency distribution of $\delta^{15}N$ differences of consumers and their diet indicates the adjustment of food web components to their food sources with trophic level enrichments between 3 and 4‰ at the most recovered sites ('3.4‰-rule').

Temporal variations of animal ^{15}N signatures describe the development of the food web of disturbed ecosystems. Similar dynamics imply functional stability of the trophic relationships between species representing different trophic levels.

The difference of soil and animal $\delta^{15}N$ values reflects the level of functional integration between soil food web (SFW) and aboveground food web (AFW). Calibration against reference systems should allow to estimate regeneration progress.

The parallel development of $\delta^{15}N$ values of AFW species relative to the soil indicates the attainment of stable relations between aboveground and belowground processes enforcing ecosystem stability.

Attributes such as complexity, stability and functionality, which are related to the developmental state of food webs and ecosystems can be assessed from species-specific and soil ^{15}N data. Further validation of this method is required prior to the use in community and restoration ecology.

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5. Discussion

Analysis of stable isotope ratios of carbon and nitrogen has been proven very powerful to characterize trophic interactions. The method was applied in a sequence of laboratory experiments that used woodlice feeding on different artificial diets as model organisms

- to demonstrate if the isotopic composition of a consumer truly reflects its diet (DeNiro and Epstein, 1978; DeNiro and Epstein, 1981) and to identify the influencing factors (Gannes et al., 1997; Focken, 2001),
- to revise empirical knowledge on the magnitude of the trophic isotopic enrichment (Minagawa and Wada, 1984; Gannes et al., 1998; Ponsard and Averbuch, 1999; Post, 2002),
- to study the role of macro-decomposers for organic matter alteration during the decomposition process (Hassall et al., 1987; Zimmer and Topp, 1999; Zimmer, 2002; Zimmer and Topp, 2002), and,
- to illustrate the significance of coprophagy for the nutrition of woodlice in this context (Hassall and Rushton, 1982; Kautz et al., 2002).

Secondly, stable nitrogen isotopes provided a quantitative tool to reconstruct the trophic structure of the animal communities (Stapp et al., 1999; Vander Zanden et al., 1999; Vander Zanden and Rasmussen, 1999; Scheu and Falca, 2000) at differentially disturbed sites of a regenerating grassland ecosystem and to describe their temporal dynamics. Food web structure and dynamics on the one hand and mean ^{15}N isotopic shifts averaged over multiple trophic interactions (sensu Post, 2002) on the other allowed concluding on the ecosystem developmental state with respect to regeneration.

Although stable isotopes are widely used by ecologists (Lajtha and Michener, 1994; Wada et al., 1995) and their advantages and limitations are well-documented (Peterson and Fry, 1987; Gannes et al., 1997; Peterson, 1999; Post, 2002), some methodological aspects of the applied techniques related to this thesis should be discussed, followed by a synthesis on the trophic level shift issue.

5.1. The stable isotope approach to investigate trophic interactions

There exist various techniques to investigate the trophic structure of communities and to identify the paths energy and matter take through food webs (Begon et al., 1996). The classic taxonomic approach is certainly the best way to obtain a view on how organisms

are trophically connected at the species level and allows assessing the complexity of food webs in the field. However, this method to construct so-called connectance (Post, 2002) or connectivity webs (Scheu, 2002) is very labor-extensive, time-consuming and requires special taxonomic knowledge to identify plant and animal samples at the species level. Moreover, if no direct observation were made, it can only be assumed from the current understanding of the nutritional biology of a certain species, which food source(s) could have been used by that species and, from the relative abundance of the other species or food source, what the importance of the suspected trophic links could have been. Especially in studying ecosystem regeneration it seems more important to define the major paths of energy flow and the magnitude of the effect of one species on another species, i.e. to construct energy flow webs and interaction strength webs, respectively (Post, 2002; Scheu, 2002).

The stable isotope approach will mostly fail to depict single trophic links between species in the field. However, the isotopic composition of an organism directly indicates the mean number of trophic transfers between basal species and this organism, weighted with respect to the flow of organic matter (Ponsard and Arditi, 2000). In searching for patterns that reflect community assembly during ecosystem development from a disturbed state towards re-establishment of ecological structure and function, stable nitrogen and carbon isotopes offer some advantages in comparison to a purely taxonomic approach as they are

- a time-integrated measure of food web relationships based on energy flow; i.e., they take into account not the ingested food but only the effectively assimilated proportion,
- a quantitative measure of the trophic position of a consumer at a given time,
- indicative for the food source of different organisms, and,
- system-specific, capable to reveal differences in the dynamics of different ecosystems (Hobson and Wassenaar, 1999; Stapp et al., 1999; Post, 2002).

Before stable isotope were applied to analyze trophic interactions, sampling techniques, sample storage and sample preparation required to be re-evaluated with respect to their influence on the isotopic composition of biological material, especially in the case of animal samples. In order to assure the reliability of the animal isotope data, it was tested if, firstly, short-term preservation (at maximum 14 days) of the catches in 3% formaldehyde solution used in pitfall trapping of the epigeic fauna and, secondly, long-term storage of the animal samples in 70% ethyl alcohol caused changes in the original $\delta^{13}C$ and $\delta^{15}N$ values. Two test series on two different woodlouse species, *Porcellio*

dilatatus and *Porcellio scaber*, revealed that neither sampling in formaldehyde nor ethanol storage for up to six month affected the animal $\delta^{15}N$ values and the N content. However, a general depletion in ^{13}C of ~ 1‰ was observed which was accompanied by an increase of the C content of 3 – 4% (unpublished data). The ^{13}C depletion is in agreement with other studies on preservation effects (Ponsard and Amlou, 1999; Arrington and Winemiller, 2002; Sarakinos et al., 2002). Surprisingly, ^{13}C enrichment was found in the original Steudnitz samples preserved since 1991. Regardless of the species, the animal $\delta^{13}C$ values were the higher the longer the sample had been stored. A possible explanation is that certain biochemical fractions dissolvable in ethanol leached out over a storage time of up to ten years, presumably the lipids which are usually depleted in ^{13}C relative to proteins and sugars (Ponsard and Amlou, 1999). Because of the resulting temporal bias towards lower animal $\delta^{13}C$ values only the $\delta^{15}N$ values were used to reconstruct the trophic relationships at the differently degraded sites of the Steudnitz grassland ecosystem.

Another factor which could have influenced the results of the Steudnitz field study was the selection of the species from the pool of stored samples for analysis. Aiming to demonstrate that by using stable isotopes the analysis of a few species is sufficient to get a general impression of the current food web structure and, simultaneously, saves time and costs (Ponsard and Arditi, 2000), a rather limited number of species (Appendix, Tabs. I and II) was chosen to represent entire food webs. In order to comply with concerns about the influence of the selected species (Gannes et al., 1997) on the isotope patterns finally used to illustrate trophic networks, species which occurred at all four Steudnitz sites and throughout the studied years were preferably selected. This created a major constraint due to variations among the trapped species in the amount of available biomass according to varying abundances in the course of regeneration at the different sites.

In consequence, only those species which had been most abundant over ten years of sampling were chosen for analysis. This bears the criticism that, even though the selected species belonged to the most dominant ones, there is no guarantee that they are really ‘representatives’ or ‘key species’ with respect to regeneration. In fact, while the food web patterns derived from the isotope measurements nicely fit with the observed or hypothesized regeneration patterns along the pollution gradient, this plausibility does not provide the direct proof for the causality between stable isotope patterns and ecosystem state. To advance on this the overall picture provided by the results on vegetation, faunal and soil development (e.g. Metzner et al., 1997; Heinrich et al., 2001; Langer and

Günther, 2001) needs to be considered in order to discover if the obtained food web patterns truly reflect regeneration. If the correlations between observations and isotope measurements were statistically verified it should be possible to describe ecosystem development after disturbance and to assess the present ecosystem state from isotope data directly. Consequently, this technique would be a valuable tool for ecosystem analysts and restoration ecologists in both, theory and practice (Temperton et al., in press).

While the results on the Steudnitz food webs indeed suggest that measuring isotopic signals in a small number of samples is sufficient to extract information on the ecosystem development, the selection of species should be improved in forthcoming field studies. Sampling techniques (e.g. collection by hand vs. pitfall traps) and frequencies should be adjusted to obtain adequate biomass of the target species. Moreover, there is no demand to select always the same species provided that representatives of different functional groups and trophic levels are sampled. As shown by a number of studies (e.g. Stapp et al., 1999; Ponsard and Arditi, 2000; Scheu and Falca, 2000), comparison of ecosystems which lack common species is viable. Because sample selection can be adapted to the changing pool of species, there are no species constraints in studying food web dynamics.

The direct comparison of different systems requires, however, considering the isotopic baseline of each system (Post, 2002). This baseline depends on the isotopic signature of the plant-derived organic matter input at the base of the food web. In most ecosystems primary production is achieved by a great number of plant species all of which exhibit temporal variation of their abundance as well as of their isotopic composition (Handley and Scrimgeour, 1997). Therefore, the precise definition of the isotopic baseline is not a straightforward task. As suggested for aquatic systems, one way to capture the spatial and temporal variation at the base of food webs would be to analyze long-lived primary consumers – serving as integrators of the isotopic variations – instead of plant samples (Post, 2002). The results of the feeding experiments presented in this thesis indicate that woodlice are well-suited to serve as integrators in terrestrial environments. But, in order to calculate isotopic baselines from such data still the trophic isotopic shift between plant material and consumers needs to be defined.

Neither plant nor litter samples had been archived in the first years of regeneration; i.e. it was impossible to generate isotopic baselines for the different Steudnitz sites. Instead, relative parameters such as ^{15}N differences between trophic levels were compared. To evaluate the trophic position of species from different ecosystems directly and to obtain a more complete picture of the trophic structure that includes also the trophic ground level,

sampling of plant and plant litter as practiced in 2000 (see Fig. 4, p. 121) is indispensable. There is some indication, that due to the permanent input of plant-derived material the isotopic composition of the soil is related to that of the trophic ground level (unpublished data). If this relation was verified, the isotopic record contained in the soil could potentially serve as a reference for baseline definition.

As already mentioned, the most critical aspect in applying stable isotopes to food web research is the classification of animals into trophic levels. This step implies the validity of the empirical trophic level shifts, e.g. 3.4‰ in the case of ^{15}N (Minagawa and Wada, 1984; Post, 2002). The actual trophic isotopic enrichment of a consumer relative to its diet has been shown to vary depending on a number of factors such as diet quality and quantity, species, age and sex (Gannes et al., 1997; Webb et al., 1998; Focken, 2001). Therefore, the verification of the isotopic shifts between consecutive trophic levels was given special attention by conducting several feeding experiments using the woodlouse species *Porcellio dilatatus* as model organism.

5.2. The magnitude of the trophic isotopic enrichment – a key factor in food web research

Feeding experiments on woodlice revealed that low diet quality may strongly influence the isotopic composition of the consumer (Manuscript I). The resulting isotopic shifts in ^{13}C and ^{15}N even exceeded the reported values of the trophic level shift (TLS). In contrast, in woodlice fed a diet of sufficient quality only small changes of the animal $\delta^{13}C$ and $\delta^{15}N$ values were observed which did not account for the TLS, either. A similar relationship between diet quality and the magnitude of the trophic isotopic enrichment was found for a variety of organisms (Sick et al., 1997; Webb et al., 1998; Adams and Sterner, 2000; Focken, 2001) indicating that the isotopic composition of the food is possibly the major, however, not the only determinant of the isotopic composition of a consumer. Corresponding with the life stage, also age was shown to affect the ability of the animal body to isotopically adapt to the diet after a diet switch. In fact, the adaptation was decelerated in adults compared to juveniles (Manuscript III). While the speed of this equilibration process is a function of the turnover rate of the different animal tissues (Gannes et al., 1998; Ponsard and Averbuch, 1999), the final isotopic difference between consumer and diet results from isotope fractionation during the biochemical reactions involved in animal nutrition (Schoeller, 1999). In addition, the speed of isotopic

adaptation seems to depend on the species, as well. For example, in the case of adult woodlice eight weeks were too short to isotopically equilibrate with the food (Manuscript III), whereas 28 days old adult rats reached the equilibrium isotope composition in less than two weeks (Ponsard and Averbuch, 1999). The main reason for these differences is probably the much higher tissue maintenance turnover in small mammals compared to arthropods. Once full-grown, in arthropods the internal turnover which adds new C and N to the existing structural pool is estimated to be very small, particularly in holometabolic insects, but also in hemimetabolic insects and terrestrial isopods (woodlice) with several molt cycles. Therefore, the isotopic signal of a new food source will become incorporated into an adult's body only slowly. This needs to be considered when using stable isotopes to characterize the trophic structure of arthropod communities, especially if the external conditions change faster than organisms are able to adapt (Manuscript III).

For the reasons described above (another reason will be discussed below) the reported ^{15}N- and ^{13}C-TLS values were not confirmed by any of the three food chain experiments (Manuscripts I – III) and should, therefore, be used cautiously. For example, the first experiment (Manuscript I) indicated, that 'careless' implementation of empirical TLS values to model the uptake of faeces in addition to the supplied food may yield in unrealistic results. Based on a fixed ^{15}N shift of 3‰, in two different treatments the proportion of faeces in the diet was calculated either much too high (up to 90%) or even negative indicating that the real ^{15}N shifts strongly differed from 3‰. Moreover, to adopt a constant trophic shift to model all phases of the experiment seems unrealistic as the consumers need time to adapt to the new food (Manuscript III).

The deviation of the experimental results from the empirical TLS values does not mean, however, that these values are invalid. In fact, in woodlice from the standard culture as well as in the animal samples taken from the Steudnitz ecosystem, the trophic shifts met the reported range. It can be concluded that under suitable environmental conditions, especially if the organisms grow up with their food sources, the 'rule' of a 3.4‰ trophic shift in ^{15}N should apply more often than under unfavourable conditions, e.g. instabilities in the availability of food sources. Since real ecosystems are dynamic systems, the variations of the $\delta^{15}N$ values of animals at the same trophic level can be large in relation to the expected isotopic shift between trophic levels. Confirming this, trophic shifts of several delta units below and above the 'target value' of 3.4‰ have been reported (e.g. DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Ponsard and Arditi, 2000; Fellerhoff, 2002).

In this context, one additional aspect that might partially explain the observed variation of the isotopic shift between consumers and their (putative) food sources is the effect of coprophagy on animal δ values. Coprophagy is wide-spread among the species of the decomposer system and is involved in decomposition and nutrient cycling (Hassall et al., 1987). Hence, coprophagy is likely to influence trophic interactions especially in soil food webs the structure of which can be studied using stable isotopes (Scheu and Falca, 2000; Scheu, 2002).

5.3. Coprophagy – just a nutritional strategy?

It seems accepted that the uptake of faeces in addition to the food (coprophagy) by decomposing animals such as woodlice is beneficial (Hassall and Rushton, 1982; Hassall and Rushton, 1985). As coprophagy has evolved in organisms which mainly utilize low quality food, i.e. organic matter rich in refractory substances like lignin and cellulose and poor in N-containing compounds (e.g. wood debris and plant litter), it can be postulated that recycling of faeces is essential – at least in certain stages of the life cycle of those species or under certain environmental conditions (Ullrich et al., 1991). However, the ecological significance of coprophagy is not yet understood (Kautz et al., 2002). Feeding experiments using woodlice as representatives of coprophagous macro-decomposers revealed that coprophagy is an important physiological factor that influences in several ways the decomposition of organic matter as well as the isotopic shift between consumer and diet, thus interfering with the analysis of food webs (Manuscripts I – III).

The effect of coprophagy on the isotopic difference between consumer and diet is firstly due to the change of the isotopic composition of the overall diet that results from the mixing of different C and N sources upon the uptake of faeces in addition to the food. Faeces are normally enriched in ^{15}N compared to the food they derived from (Manuscript I). Thus, a mixed diet consisting of food and faeces will – depending on the proportion of faeces – reveal a higher $\delta^{15}N$ value than pure food (Manuscript II). Secondly, coprophagy also affects the animal isotopic composition. This is partly caused by the isotope content of the mixed diet. In addition, coprophagy exerts indirect control on the internal isotope fractionation of woodlice by enhancing their nutritional performance (Hopkin, 1991; Ullrich et al., 1991). Namely by its effect on digestion and assimilation coprophagy modifies the internal turnover of C and N (Manuscript III). This essentially feeds back on the woodlouse isotopic composition (see chapter 5.2.).

Moreover, woodlouse $\delta^{15}N$ values depend on the quality of the recycled faeces and on the importance of coprophagy in relation to normal food consumption (Manuscript III, but see also Kautz et al., 2002). As described above, nutritional conditions close to the optimum result in minor isotopic changes regardless if an individual utilizes faeces or not (Manuscript II). In contrast, very low food quality may force woodlice to recycle faeces to overcome nutritional shortage and may initiate drastic isotopic changes (Manuscript I). Also in the field coprophagy is assumed to be most important for animals encountering low food quality (Kautz et al., 2002; Zimmer, 2002); i.e., the ecological significance of coprophagy should be highest in environments that generally provide low food quality. This assumption could be proven by measuring animal isotope signals.

The decomposition of organic matter is influenced by coprophagy in at least two ways. On the one hand, the positive effect of coprophagy on digestion and food assimilation was found to significantly increase the rate of organic matter turnover by woodlice due to higher rates of food uptake, respiration and gaseous excretion (Manuscript II). So, a higher amount of organic matter per unit of time becomes converted into inorganic compounds. In real ecosystems, this reduces the accumulation of organic C and N in the litter layer, forces the cycling of these elements and facilitates the coupling between aboveground and belowground ecosystem compartments. These aspects gain special importance in ecosystem regeneration as suggested by Manuscript IV.

On the other hand, the excrements produced from a mixed diet of food and faeces may notably differ in their elemental and isotopic composition from excrements produced from pure food (Manuscripts II and III). Thus, the contribution of recycled faeces to faeces production provides a higher food quality to organisms which rely on faeces as nutritional source. Like for other food sources, amount and quality of the produced faeces will affect consumers at higher trophic levels of the decomposer system and, therefore, the process of organic matter decomposition in general (Lee, 1997).

Acquiring detailed knowledge on the effect of coprophagy on animal stable isotope signals is first of all of scientific interest. For instance, isotopic variations which are caused by physiological changes upon the uptake of faeces could be used to improve the understanding of the nutritional biology of coprophagous organisms. Therefore, eco-physiologists are encouraged to make use of the advantages of stable isotope tools, e.g. non-invasiveness, more frequently (Gannes et al., 1998). In food web research and ecosystem analysis the interpretation of isotope data derived from field samples needs to consider factors which influence animal δ values such as coprophagy. For example, the

distinct and consistent difference in the ^{15}N signals of two sympatric woodlouse species collected in Steudnitz (Manuscript IV) could be explained partly by coprophagy. The higher $\delta^{15}N$ values in *Trachelipus rathkei* compared to *Armadillidium vulgare* indicate that the former species utilized organic matter enriched in ^{15}N compared to fresh plant biomass and recent plant litter. The potential spectrum of food sources of *T. rathkei* includes plant litter processed by microbes, carcass and, most likely, matured faeces. The hypothesis, that coprophagy played a bigger role in *T. rathkei* than in *A. vulgare* awaits to be tested, however. In any case, this example demonstrates that niche differentiation corresponding to the specific foraging strategies and digestive capabilities of sympatric species (Zimmer and Topp, 2000) can be detected by stable isotope analysis.

While variations of the stable isotope ratios provide a diagnostic tool, the effect of coprophagy on decomposition is of ecological importance. Experimental results suggest that the ability to exploit faeces for nutrition is advantageous in the colonization of new habitats because coprophagy facilitates the adaptation to new food sources (Manuscripts II-III) and enables woodlice to more efficiently acquire nutrients contained in indigestive plant tissues such as grass litter and wood. As also shown by other studies, coprophagy presents an appropriate strategy for the further degradation and utilization of refractory organic material for which one gut passage is too short or too inefficient (Brendelberger, 1997). This strategy is thought to be a major cause for the great success of woodlice in the reassembling communities at the degraded sites of the Steudnitz grassland ecosystem. In addition, the successful performance of woodlice presumably was an important step in the regeneration of the polluted grassland (Manuscript IV).

5.4. The transformation of organic matter by woodlice and its ecological significance

The importance of woodlice for ecosystem development originates from their main ecological function: the first mechanical breakdown of plant litter and its inoculation with microbes (Gruner, 1966; Hassall et al., 1987; Hopkin, 1991). Without the action of macro-decomposers the litter may accumulate on the soil surface and impede the transfer of energy and nutrients between belowground and aboveground sphere. This has most likely been the case in the Steudnitz ecosystem during the period of fertilizer production from the 1950ies until 1990 (Heinrich et al., 2001) because macro-decomposers went nearly extinct from the system (Peter, 1984) and, also the soil microbiota suffered from

unfavourable environmental conditions (Langer and Günther, 2001). The constraints to C and N cycling, that resulted from imbalanced relations between primary production and decomposition, and the loss of functional links between soil food web and aboveground food web gradually reduced after the shut-down of the fertilizer plant in 1990. From 1990 onwards the edaphic conditions improved (Metzner et al., 1997). The decomposer system restructured from the primary degradation being solely based on soil mesofauna, particularly springtails (Fritzlar et al., 1986; unpublished data), towards being mainly based on macro-decomposers, especially woodlice (Eggers, 1997; unpublished data; see Fig. 3).

In addressing the achievements of woodlice that define their ecological significance feeding experiments uncovered the magnitude of elemental and isotopic changes during the conversion of ingested organic matter into faeces (Manuscript III). Generally, the faeces released by woodlice are easier to decompose than the original plant material (Hopkin, 1991), have a higher N content (Kautz et al., 2002) and contain microbial substances; i.e., woodlouse faeces offer a valuable source of organic matter that allows species to exist which are unable to utilize unprocessed plant litter. The dependency of higher trophic levels of the decomposer system on this food source is corroborated by the fact that woodlouse faeces can be hardly found in the litter layer of woodlands and grasslands (Hopkin and Martin, 1984).

Organic matter transformation by woodlice is influenced by the feedback coprophagy exerts on consumption, assimilation, respiration and excretion (see chapter 5.3.). Both, the transformation rate (quantity) and the elemental and isotopic composition of the transformation products (quality) depend on the proportion coprophagy contributes to nutrition (Manuscripts II and III). It should be mentioned that the significance and the interdependency of the processes involved in the conversion of organic matter from food into faeces could not be disentangled without the help of stable isotopes.

5.5. Food web development from the stable isotope perspective

Extensive studies on the food web of terrestrial ecosystems that take advantage of stable isotope techniques are scarce (Ponsard and Arditi, 2000; Scheu and Falca, 2000). Addressing primarily structural aspects, these studies enable to consider also functional aspects of the investigated communities because the reported isotope patterns result from trophic interactions and do reflect energy flow as well as interaction strength. This may demonstrate that stable isotope analysis is capable of contributing notably to illuminate

the functioning of ecosystems by constructing energy flow webs and interaction strength webs, respectively (Post, 2002; Scheu, 2002). Using the great potential of stable isotopes in providing information on structure and functioning of communities to uncover patterns of ecosystem regeneration, it should also be possible to conclude – by further generalizing in the interpretation of isotope data – on the current position of an ecosystem as it moves from a degraded state towards recovery (Manuscript IV).

Stable isotopes have proven to represent a precise tool to visualize and to compare the food web structure at four differently impacted sites of the Steudnitz grassland ecosystem (Fig. 4). Based on ^{15}N and ^{13}C signals of plants and animals sampled in 2000, after ten years of regeneration (Appendix Tabs. I and II), the food web images differentiate clearly between the trophic levels of producers, herbivores, carnivores and decomposers. The decomposers can be even further divided into primary and secondary decomposers. Data points representing the same trophic level are encircled by colored envelopes (Fig. 4).

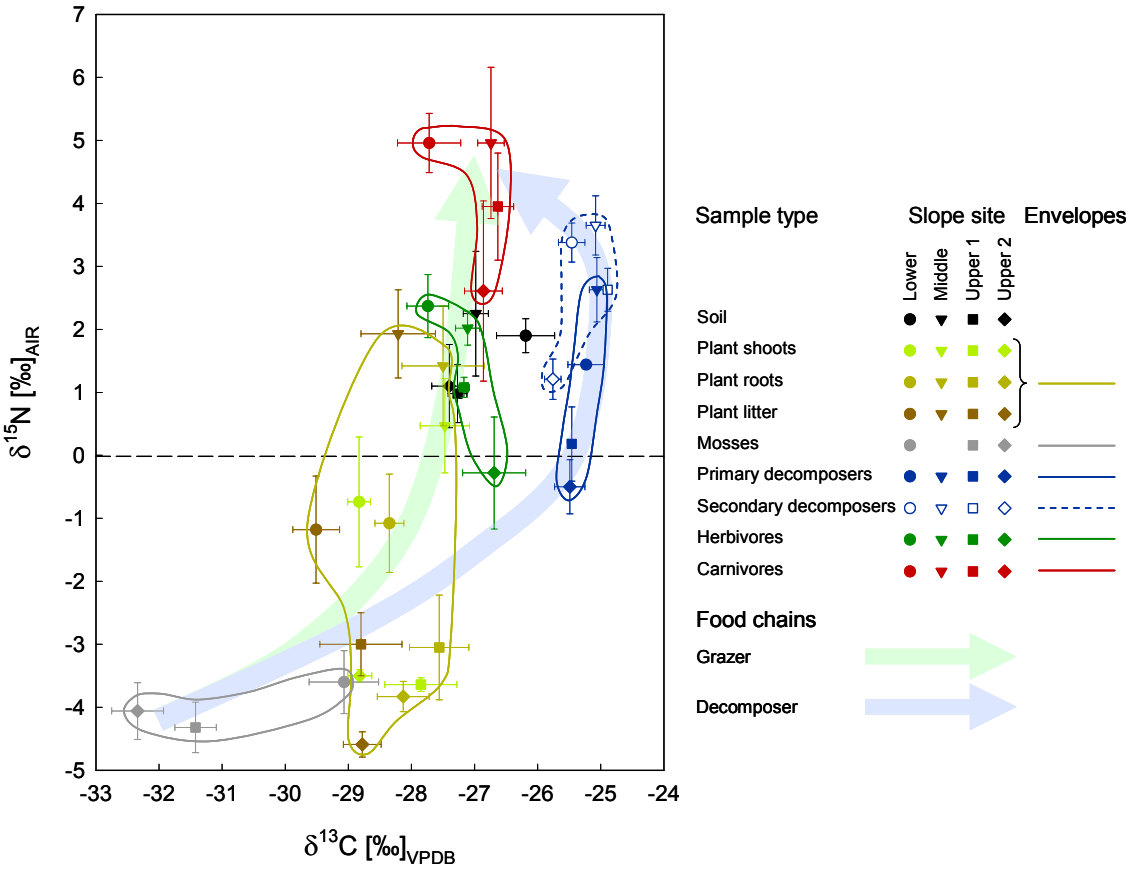

Figure 4 Reconstruction of the food web at four sites of the Steudnitz study area in 2000 based on mean $\delta^{15}N$ and $\delta^{13}C$ values of soil, plant (shoots and roots), litter, moss, and animal samples (Appendix Tabs. I and II). Error bars indicate 1 standard deviation of the mean. The slope site the samples originated from is indicated by different symbols. Sample types can be distinguished by color. Data points representing the same trophic level are highlighted by a common envelope (mosses separately from higher plants). Food chains are symbolized by colored arrows.

At all four sites, the food webs – indicated by different symbols – generally mirror the expected pattern (see chapter 3.4.) with the flow of matter from the lower left to the upper right part of the plot, also symbolized by a green and a blue arrow representing the grazer and the decomposer food chain, respectively. Along the $\delta^{15}N$ axis, at each site the position of the organisms above the trophic ground level (plants) mostly corresponds with the position of the respective plant samples and reflects the reported ^{15}N trophic level shift (TLS; i.e. 3 – 4‰). For example, in relation to the other sites, at the upper slope site 2 plant shoots, plant roots and plant litter reveal lowest $\delta^{15}N$ values. Accordingly, herbivores, decomposers and carnivores reveal lowest $\delta^{15}N$ values, too (Fig. 4). This demonstrates that the absolute position along the $\delta^{15}N$ axis of the data points belonging to one and the same food web is site-specific confirming that the comparison between ecosystems requires the definition of isotopic baselines (Post, 2002). Furthermore, the isotopic relations among the trophically linked organisms within the Steudnitz food webs generally account for the trophic enrichment phenomenon (Ponsard and Averbuch, 1999) and corroborate the empirical TLS for ^{15}N of 3.4 ± 1.0‰ (Minagawa and Wada, 1984; Post, 2002).

In contrast to the general picture, at the middle slope site the plant material reveals exceptionally high $\delta^{15}N$ values in relation to the $\delta^{15}N$ values of the consumers (Fig. 4). Such deviations from the 'normal' pattern certainly challenge the validity of the 3.4‰-'rule'. With regard to the issue of ecosystem regeneration from disturbance, however, significant deviations from the 'target' value could be interpreted simply by assuming that the (environmental) conditions required to reach trophic enrichment in the range of TLS were not provided.

While it is undoubted, that the uncertainties about isotope fractionation create a limit for the interpretation of isotope data (Gannes et al., 1997; Focken, 2001), comprehensive food web studies which found mean ^{15}N trophic shifts in the range of 3 – 4‰ (Ponsard and Arditi, 2000; Scheu and Falca, 2000) may serve as an example that under natural conditions the requirements for approaching the reported TLS can be fulfilled. In fact, trophic enrichment in the range of TLS requires a respective level of stability of the 'environment' in which isotope fractionation takes place as well as a sufficient period of time for isotopic equilibration between the consumer and its food to occur (Ponsard and Averbuch, 1999). Consequently, isotopic shifts are likely to account for the reported TLS only if the trophic interactions have stabilized (Manuscript IV) considering that stable isotopes provide a time-integrated measure of food web relationships (Stapp et al., 1999).

Note, that the empirical TLS for ^{15}N of 3.4‰ does not necessarily apply to each single trophic interaction (mostly ranging between ~2‰ and 5‰) but is a valid approximation of trophic fractionation only when averaged over multiple trophic pathways (Post, 2002). Accordingly, the food webs at the four Steudnitz sites were compared based on mean isotopic shifts accounting for the variation among the trophic relations (Manuscript IV).

As the food web images of the year 2000 (Fig. 4) also contained information on the trophic ground level, the data sets were best suited for statistical analysis allowing to compare the different sites and to evaluate ecosystem state. However, although most comprehensive, these data provided only a static view on the situation after ten years of regeneration. By using additional ^{15}N data of preserved animal samples ranging back until 1990, the starting point of ecosystem regeneration, temporal changes in the trophic interactions between decomposers, herbivores and carnivores could be observed at the four sites along the pollution gradient (Manuscript IV). These changes are suggested to reflect the adaptation of the food web to changing environmental conditions and the regeneration progress of the ecosystem in general. At the time temporal variations become minimal, trophic interactions can be regarded as balanced. This implies that the factors governing community structure such as competition or top-down and bottom-up forces (Scheu, 2001) reached balanced levels, as well. Balanced trophic interactions enable permanent flow of matter through a stabilized food web structure. Regarding the function of the trophic network in mediating the distribution of energy and matter across the entire community, balanced trophic interactions indicate that the food web has reached functional stability. A functionally stable food web is a major characteristic of intact ecosystems.

This argumentation has been applied to compare the four Steudnitz sites (Manuscript IV). Considering both, the situation in 2000 and the temporal dynamics from 1990 until 2000, according to the stable isotope data the food webs at both upper slope sites have fully recovered from disturbance; i.e., the trophic structure of the communities became equivalent to that reported from intact ecosystems (Ponsard and Arditi, 2000; Scheu and Falca, 2000). In contrast, the systems at the lower and the middle slope site are less advanced concerning the developmental state. Generally, the re-establishment of macro-decomposers and the presence of N_2-fixing plants at high abundances represented important factors controlling and accelerating the regeneration process (Manuscript IV).

5.6. Concluding remarks

Stable isotopes are capable to uncover the dynamics of trophic interactions in regenerating ecosystems. Synthesizing the current understanding on the trophic isotopic shift and the influencing factors, this thesis tries for the first time to conclude from stable isotope patterns on the stability of trophic interactions and, furthermore, on the developmental state of ecosystems. The isotopic shifts between consumers and food approach the empirical 'target' value (3.4‰-'rule') only if the trophic interactions are balanced and sufficient time of stability to isotopically adjust to the food is provided. Therefore, isotopic shifts averaged over entire food webs offer a useful measure to conclude on ecosystem attributes such as the functional stability of the food web especially if reference or time series data for comparison are available.

Mass balances and – in combination with stable isotope data – isotope mass balances allow to evaluate the efficiency of woodlice in converting food into faeces and to define the quantitative and the qualitative relations between food and faeces. By using stable isotope techniques it is possible to differentiate between those parts of the ingested food and of the recycled faeces that became assimilated, excreted as faeces or released from the body in an inorganic form (respiration and gaseous excretion), respectively. Moreover, stable isotopes allow defining the involvement of coprophagy in the nutrition of woodlice and in organic matter transformation. This exemplary demonstrates the suitability of stable isotopes in characterizing trophic interactions.

Like the definition of reliable values of the trophic isotopic enrichment and the identification of factors controlling the isotopic composition of biological samples, the present experimental results on the significance of woodlice for the degradation of organic matter belong to the archive of information which is required

- to conclude on the role of organisms with respect to ecosystem development,
- to identify factors and processes involved in ecosystem regeneration,
- to interpret isotope patterns in the food web of ecosystems,
- to develop tools to assess the ecosystem state e.g. in ecological restoration, and,
- to model ecosystem development properly.

Prospectively, it is important to further validate the stable isotope method with special focus on the application of trophic isotopic shifts in food web research. This urges to conduct feeding experiments with juvenile animals, favourably using species which exploit similar food sources from the postnatal to the adult stage, for instance

hemimetabolic insects with several larval stages such as bugs (Heteroptera) and locusts (Saltatoria) or woodlice which continuously develop with regular molt even as adults. In addition, gas exchange measurements including the analysis of stable isotopes in the gas phase are essential to obtain complete isotope mass balances.

Based on that, the methodology used in this thesis to evaluate the ecosystem state from isotope data by calculating mean trophic shifts may be improved and applied to other grasslands with different species and a different history than the Steudnitz ecosystem. Subsequently, food webs of grasslands and woodlands should be analyzed in order to prove the assumption that different types of ecosystems can be compared and evaluated with respect to their developmental state. This requires checking the results against reference ecosystems. The expected increase in the understanding of ecosystem processes as well as the growing amount of experimental and field data should allow modelling ecosystem regeneration that combines classical approaches to ecosystem analysis with stable isotope techniques.

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7. Appendix

Table I: Animal species analyzed from the Steudnitz research area, their taxonomic relationship, main ecological function, mode of nutrition and locations of sampling, sampled between 1991 and 1996 and in 2000.

animal group	(order, class)			
species	(family)	main function[3]	nutrition[3]	slope site[2]
woodlice	(Isopoda, Crustacea)			
Armadillidium vulgare	(Armadillidiidae)	decomposer	saprophagous[1]	1-4
Trachelipus rathkei	(Trachelipidae)	decomposer	saprophagous[1]	1-4
beetles	(Coleoptera, Insects)			
Agriotes murina	(Elateridae)	leaf chewer	phyto-/saprophagous	1-4
Otiorhynchus raucus	(Curculionidae)	leaf chewer	phytophagous	1-4
Amara aulica	(Carabidae)	predator	zoo-/phytophagous	1-4
Anisodactylus binotatus	(Carabidae)	predator	zoophagous	1-4
Calathus melanocephalus	(Carabidae)	predator	zoophagous	1-4
Harpalus affinis	(Carabidae)	predator	zoo-/phytophagous	1
Poecilus cupreus	(Carabidae)	predator	zoophagous	1-4
bugs	(Heteroptera, Insects)			
Amblytylus nasutus	Miridae	plant-sap sucker	phytophagous	1
Myrmus miriformis	Coreoidae	plant-sap sucker	phytophagous	1, 3, 4
Notostira elongata	Miridae	plant-sap sucker	phytophagous	1, 3, 4
Trigonotylus coelestialium	Miridae	plant-sap sucker	phytophagous	1
Nabis cf. *brevis*	Nabidae	predator	zoophagous	1, 3, 4

[1)] including necrophagy and coprophagy, i.e. all types of dead organic material

[2)] 1 = lower slope (LS), 2 = middle slope (MS), 3 = lower part of the upper slope (US1), 4 = upper part of the upper slope (US2)

[3)] of the imago (in the case of beetles)

Table II: Dominant vascular plant species from the Steudnitz research area, family, plant type, and location of sampling, sampled in 2000.

plant species	family	type	slope site[1]
Artemisia vulgaris	Asteraceae	perennial herb	ls
Lactuca serriola	Asteraceae	perennial herb	ls
Picris hieracioides	Asteraceae	perennial herb	ls
Atriplex nitens	Chenopodiaceae	annual herb	ls
Agropyron repens	Poaceae	perennial grass	ms
Inula conyza	Asteraceae	perennial herb	ms
Arrhenatherum elatius	Poaceae	perennial grass	us1
grass mixture[2]	Poaceae	perennial grass	us1
Bromus erectus	Poaceae	perennial grass	us2
Festuca rubra	Poaceae	perennial grass	us2
Brachypodium pinnatum	Poaceae	perennial grass	us2
Ceratodon purpureus	Ditrichaceae	acrocarpic moss	ls
Brachythecium rutabulum	Brachytheciaceae	pleurocarpic moss	ls, us2
Eurhynchium hians	Brachytheciaceae	pleurocarpic moss	us1

[1)] ls = lower slope, ms = middle slope, us1 = lower part of upper slope, us2 = upper part of upper slope

[2)] mixture of *Poa angustifolia* and *Festuca rubra*

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