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Titel der Diplomarbeit

Stable isotope ^{15}N and ^{13}C labelling of different functional groups of earthworms and their casts: implications for studying below-aboveground interactions

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Verfasserin / Verfasser:	Barbara Heiner
Matrikel-Nummer:	0002199
Studienrichtung /Studienzweig (lt. Studienblatt):	Biologie/ Ökologie
Betreuerin / Betreuer:	Dr. Johann G. Zaller Uni.-Prof. Mag. Dr. Thomas Frank Uni.-Prof. Mag. Dr. Wolfgang Waitzbauer

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1. ABSTRACT

1. The activity of earthworms (Oligochaeta: Lumbricidae) has substantial effects on the structure and fertility of soils with consequences for the diversity of plant communities and associated ecosystem functions. However, we still lack a clear understanding of the functional role earthworms play in terrestrial ecosystems, partly because easy-to-use methods to track the activities of earthworms are missing.

2. Here we tested whether earthworms and their casts can be dual-labelled with ^{15}N and ^{13}C by cultivating them in soil substrate added with ^{15}N ammonium nitrate and $^{13}C_6$ -glucose stable isotopes.

3. Additionally, we also wanted to know whether (i) earthworms and casts from different functional groups (soil-feeders vs. litter-feeders) would differ in their incorporation of stable isotopes, (ii) the labelling signal would differ if the same amount is applied once or staggered, and (iii) the duration of the isotopic enrichment in casts changes if they are stored under constant conditions or were inserted in potted field soil.

4. Our findings show the feasibility of dual-labelling both for the tissue and casts of both litter-feeding (*L. terrestris*) and soil-feeding (*A. caliginosa*) earthworms using the same method. The advantage of this method is that it labels earthworms and their casts under realistic conditions where earthworms need to be cultivated for only four days in soil that received a one-time addition of commercially available stable isotopes. In earthworms, the isotopic enrichment remained at a stable level for at least 21 days; labelled casts can be stored for at least 105 days without a significant decrease in their isotopic signals.

5. This simple and efficient method opens new avenues for tracing the activities of these important ecosystem engineers, exploring the functional relationships between earthworms and other organisms or studying ecosystem nutrient cycling.

Keywords: carbon isotope, nitrogen isotope, soil invertebrates, tracer study, labelling method

2. INTRODUCTION

In most temperate terrestrial ecosystems, earthworms (Oligochaeta: Lumbricidae) represent the dominant fraction of the soil faunal biomass, often serving as ecosystem engineers (Jones et al. 1994) with substantial effects on the structure and fertility of soils. Most earthworm communities consist of different functional groups of earthworms comprising litter-dwellers (epigeics), soil-dwellers (endogeics) and vertical-burrowers (anecics) (Bouché 1977). In temperate grasslands up to 1000 earthworms m^{-2} have been reported (Edwards et al. 1995) and by producing up to 42 tons ha^{-1} a^{-1} of nutrient rich casts (Edwards and Bohlen 1996) they are a key component of nutrient cycling in soil (Lavelle 1988). Earthworm casts contain more plant nutrients than bulk soil (Sharpley and Syers 1976; McKenzie and Dexter 1987; Tomati et al. 1994; Schrader and Zhang 1997; Chaoui et al. 2003) and are also hot spots of microbal (Blair et al. 1995; Brown et al. 2000) and macroinvertebrate activity (Decaens et al. 1999). In calcareous grasslands it could be shown that an earthworm cast production rate of 16 tons ha^{-1} a^{-1} (Zaller and Arnone 1997) together with a species-specific relationships between casts and plants are also suggested to have helped defining the structure of highly diverse grassland communities and vice versa (Zaller and Arnone 1999). Moreover, seed herbivory and seed transport are important mechanisms by which earthworms selectively alter the diversity of grassland ecosystems (Willems and Huijsmans 1994; Zaller and Saxler 2007).

Despite the earthworms' paramount importance, we still lack a mechanistic understanding of interactions between earthworms and other ecosystem components in many aspects (Curry and Schmidt 2007). In order to examine functional relationships, stable isotopes have widely been used on a wide range of organisms (Hood-Nowotny and Knols 2007). Several researchers have successfully studied the feeding habits of earthworms by means of stable isotope natural abundances (Spain et al. 1990; Martin et al. 1992; Schmidt et al. 1997; Spain and Feuvre 1997; Scheu and Falca 2000; Ruess et al. 2004; Schmidt et al. 2004; Elfstrand et al. 2008; Seeber et al. 2009). However, while stable isotope natural abundances are important tools for

investigating patterns in food-webs, they provide only limited information on functional relationships between involved organisms or trophic groups. These functional relationships can elegantly be studied by the use of isotopic tracers, however so far only two studies used stable isotope labelling of earthworms (Whalen and Janzen 2002); (Dyckmans et al. 2005). While Whalen and Janzen (2002) where able to label the tissue and mucus of one epigeic species after a four-week labelling period, Dyckmans et al. (2005) successfully labelled one endogeic species after a four-day cultivation of earthworms in isotopically enriched soil. In the current study, we basically employed the method developed by Dyckmans et al. (2005), however expanded this method in several important aspects that have never been addressed before: (i) we aim to label the tissue of one anecic (*Lumbricus terrestris*) and one endogeic (*Aporrectodea caliginosa* (Savigny 1826)) species using the same method, (ii) we will also examine isotopic signals in earthworm casts of these species, and (iii) we will investigate the effect of storage time on labelled casts over a longer period of time. Successful labelling of earthworms and casts would provide a great tool for soil and ecosystem ecologists to specifically examine the role of these important soil organisms.

In a set of laboratory experiments, we specifically tested the following key questions: (i) Is it possible to label earthworms and their casts of different functional groups in a non-invasive, natural way by feeding ^{15}N and ^{13}C enriched soil directly to the earthworms? (ii) How do the isotopic enrichments in earthworm tissue and casts develop in the short term after a transfer of earthworms to unlabelled soil? (iii) For how long will the ^{15}N and ^{13}C signal in *L. terrestris* casts remain stable?

3. MATERIAL AND METHODS

3.1 Preparation of ^{13}C and ^{15}N enriched cultivation substrate for earthworms

The idea of the current study was, that earthworms cultivated in soil enriched with stable isotopes will incorporate the isotopes in their tissue and defecate isotopically enriched casts. Therefore, soil (Haplic Chernozem, silty loam, pH = 7.6, Corg = 2.2%) was collected from an arable field at the research farm of the University of Natural Resources and Applied Life Sciences Vienna near Groß-Enzersdorf bordering to Vienna, sieved (2 mm) and sterilized (12h at 120°C). Following Dyckmans et al. (2005) we used $^{13}C_6H_{12}O_6$ and $^{15}NH_4NO_3$ in order to dual-label earthworm species and casts with several modifications. First, we either incubated or not incubated (Fig. 1) the soil containing $^{15}NH_4NO_3$ to improve the availability of nitrogen for earthworms through microbial metabolisation of ammonium nitrate. Second, we either applied 100 mg of $^{13}C_6H_{12}O_6$ and 100 mg of $^{15}NH_4NO_3$ at once or added 25 mg $^{13}C_6H_{12}O_6$ and 25 mg $^{15}NH_4NO_3$ on each of four consecutive days. This variant was chosen to determine the effect of ^{13}C loss by microbal respiration. Third, we either added or not added ground oat flakes as a food source for earthworms in order to improve the fitness of earthworms and thus facilitate the incorporation of stable isotopes. The later variant is also thought to more benefit the organic matter feeding *L. terrestris* than the geophagic *A. caliginosa* (Doube et al. 1997). These treatments were maintained in a full factorial design with three replicates; one unlabelled control was set up for each treatment.

Treatments including a seven day soil incubation were prepared by filling 200 g sieved and sterilized soil into polypropylene bags, adding 100 mg $^{15}NH_4NO_3$ and 400 mg unlabelled glucose dissolved in 4 ml deionized water (treatment “once+incub”), 100 mg $^{15}NH_4NO_3$ and 400 mg unlabelled glucose dissolved in 4 ml deionized water and 20 g ground oat flakes (treatment “once+incub+oats”) and 25 mg $^{15}NH_4NO_3$ and 400 mg unlabelled glucose dissolved in 4 ml deionized water (treatment “staggered+incub”) and stored these mixtures in the dark at 15°C for

7 days (Fig. 1). To ensure aerobic conditions and a homogeneous ^{15}N distribution, soil was stirred daily.

Treatments that did not include soil incubation were prepared seven days later. Here, soil was enriched with 100 mg $^{15}NH_4NO_3$ and 400 mg unlabelled glucose dissolved in 4 ml deionized water (treatment "once no incub") or 25 mg H_4NO_3 and 400 mg unlabelled glucose dissolved in 4 ml deionized water (treatment "staggered no incub") (Fig. 1).

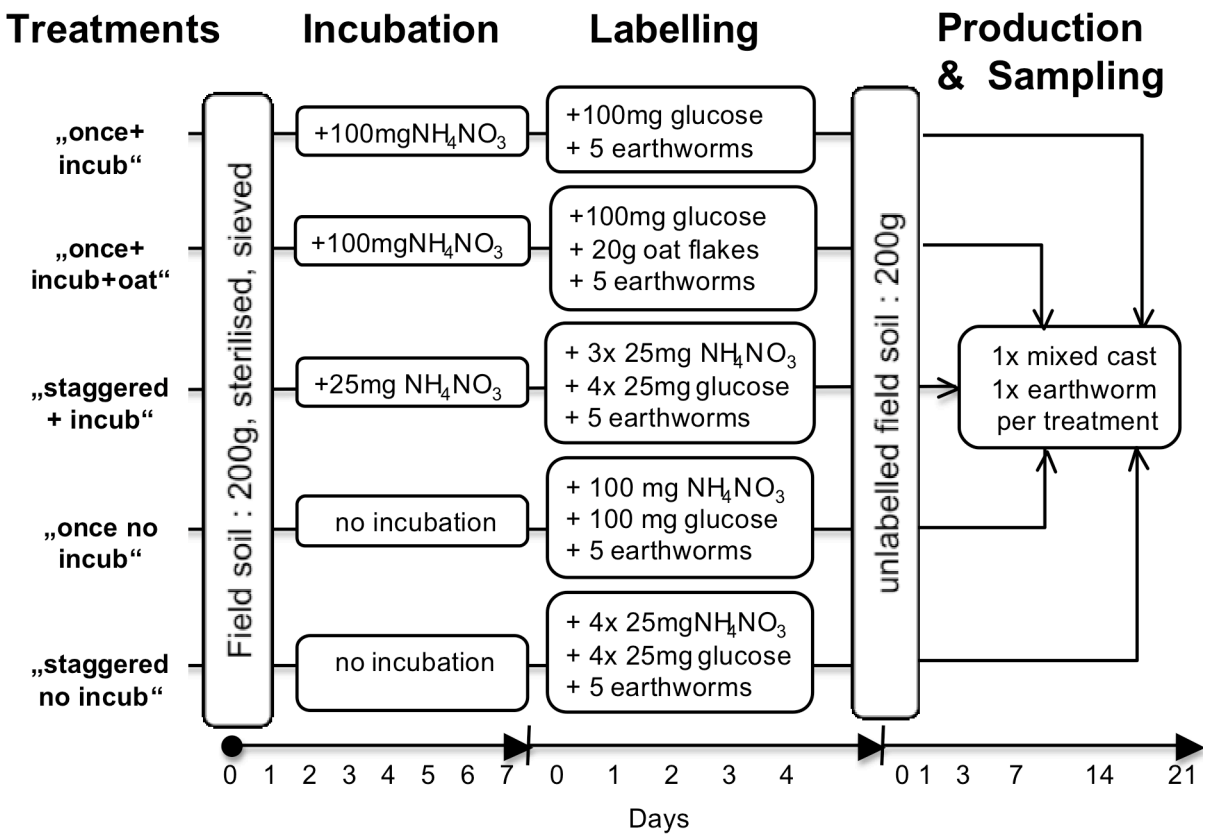

Fig. 1 Schematic diagram of the treatments used for $^{13}C/^{15}N$ labelling of *L. terrestris* and *A. caliginosa*. Controls are not shown in this diagram.

Afterwards, the labelled soil was transferred into polypropylene boxes (volume 500 ml) and 100 mg $^{13}C_6$ glucose dissolved in 25 ml deionised water were added to the treatments "once+incub", "once+incub+oats" and "once no incub". In treatments "staggered+incub" and "staggered no incub" 25 mg $^{13}C_6$-glucose dissolved in 25 ml deionised water were added (Fig. 1). On day 2, 3 and 4 of the labelling period (see next section) 25 mg $^{15}NH_4NO_3$, 400 mg unlabelled glucose and 25 mg $^{13}C_6$-glucose dissolved in 25 ml deionised water were added to treatment with staggered isotope labelling. Overall, all treatments received the same amount of either labelled or unlabelled ammonium nitrate or glucose before earthworms were added. For labelling we used 95 at. % $^{15}NH_4NO_3$ (Chemotrade, Leipzig) and 99 at. % $^{13}C_6$-glucose (Sigma-Aldrich, Austria).

3.2 Labelling of earthworms and casts

To label the earthworms, five individuals of each species (*L. terrestris* or *A. caliginosa* respectively) were separately held in boxes containing the soil labelled as described above. Boxes with earthworms were stored in the dark at constantly 15°C. We used adult individuals of *L. terrestris* obtained from a commercial supplier (Denu's Würmer Stuttgart, Germany) with a mean initial biomass of 3684 mg ± 365 mg. Adult and semi-adult individuals of *A. caliginosa* with a mean initial biomass of 705 mg ± 54 mg were collected by hand-sorting from an arable field (Bruck-Neudorf, Burgenland, Austria) in March 2008. After four days in the labelled soil, earthworms were transferred into new polypropylene boxes (volume 500 ml) containing 200 g unlabelled and sterilized moist soil. Boxes were again stored in the dark at 15°C and re-randomized daily.

On day 1, 3, 7, 14 and 21 after transferring the earthworms into unlabelled soil, a pooled sample of casts and one earthworm was collected from each replicate and analysed (see below).

3.3 Long-term storage of isotopically enriched earthworm casts

After day 21 of the sampling period, when the last worm was taken out of the soil boxes, labelled casts of *L. terrestris* were stored in two different ways in order to find out whether isotopic signature in the casts is affected by storage. First, three boxes containing the labelled casts from treatment "once+incub" were stored in the dark at 15°C in a conditioning cabinet. Second, six cast samples of each box were packed separately in plastic tissue capsules inserted at a depth of 30 cm in a pot filled with field soil (volume 40 l) in a greenhouse. A pooled cast sample of each box and a plastic tissue capsule corresponding to each box was taken every two weeks over a period of 105 days and prepared for the analyses.

3.4 Isotopic analysis, calculations and statistics

Earthworm casts and the earthworm tissue were analyzed for ^{13}C and ^{15}N by continuous flow isotope ratio mass spectrometry (CF-IRMS). For isotopic analyses, earthworm casts were dried at 60° for 24 h and homogenized with a ball mill.

The earthworms were individually rinsed in water, dried on tissue paper, weighed and deep-frozen. The earthworms were dissected and cleaned of internal organs including intestines by rinsing with a fine stream of distilled water. Only the anterior 15 segments of the frozen earthworms were used to avoid a contamination from the intestinal contents. Earthworm tissue was dried for 24 h at 60°C and manually pulverized using pestle and mortar. For calculations, isotopic enrichment is expressed in atom % excess (APE), where APE is the difference in atom % between the sample and the natural abundance level of ^{13}C and ^{15}N in the casts and worm tissue from the control treatments.

Since data on isotopic enrichments in tissue and casts of both earthworm species were not normally distributed we analysed them using a Mann-Whitney-U-test. Spearman correlations were calculated to test relations between isotopic enrichments in tissue and casts. For the

regression analysis, data were transformed if it did not show a normal distribution. For the comparison of the two treatments of the long-term storage experiment, a Mann-Whitney-U-test was used. Enrichment data of tissue and casts are given as the mean ± one standard deviation (SD).

4. RESULTS

Significant enrichments of ^{15}N and ^{13}C were found compared to the control treatments both in tissue and casts of both earthworm species for all enrichment treatments on each sampling date (Mann-Whitney-U-test, $P < 0.05$; Fig. 2).

Earthworm species differed in their tissue incorporation of both ^{15}N and ^{13}C to labelling treatments: while for *L. terrestris* a one-time labelling and prior soil incubation showed about a 5 times higher APE for ^{15}N and ^{13}C than the other treatments (Fig. 2 A+C), labelling treatments resulted in similar enrichments in *A. caliginosa* tissue (Fig.2 B+D).

Averaged across treatments, the values for the earthworm body tissue on day 1 were 0.20 ± 0.09 APE ^{15}N and 0.040 ± 0.026 APE ^{13}C for *L. terrestris* and 0.42 ± 0.21 APE ^{15}N and 0.064 ± 0.035 APE ^{13}C for *A. caliginosa* (Fig. 2). Generally, there was no significant decrease in the isotopic enrichment in tissue from day 1 to day 21 (^{15}N: 0.21 ± 0.29 APE for *L. terrestris* and 0.26 ± 0.14 APE for *A. caliginosa*; ^{13}C: 0.051 ± 0.067 APE for *L. terrestris* and 0.062 ± 0.051 APE for *A. caliginosa*, Fig. 2). Missing data points in Figure 2 are due to earthworm mortality.

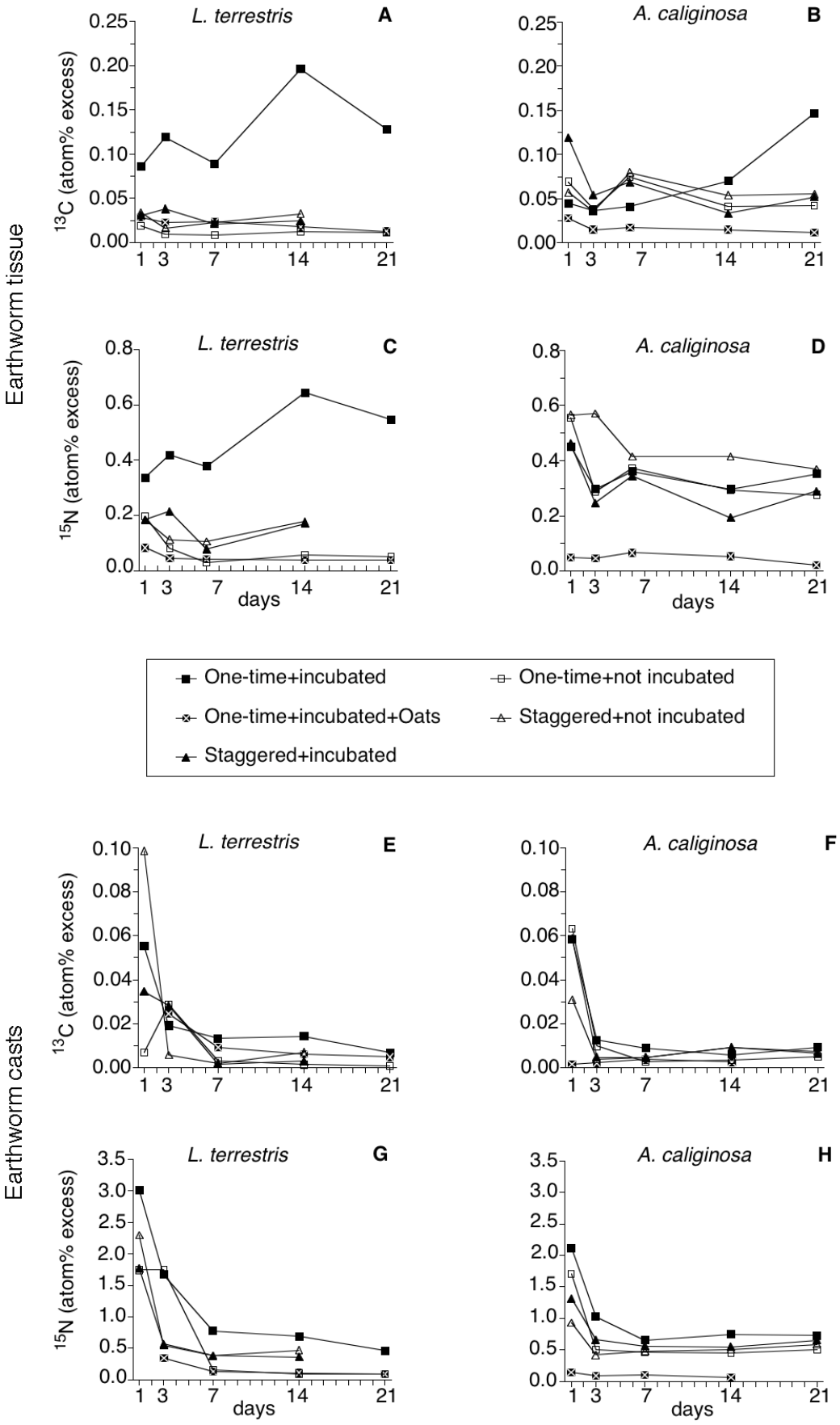

Fig. 2 Time course of ^{13}C and ^{15}N enrichment of earthworm tissue (A-D) and of earthworm casts (E-H) over 21 days after labelling (means, n=3).

Generally, isotopic enrichments in earthworm casts for both ^{15}N and ^{13}C did not significantly differ between the different labelling treatments (Fig. 2 E-H). For both, *L. terrestris* and *A. caliginosa*, the ^{15}N and ^{13}C enrichment of casts showed a similar exponential decline in all treatments. On day 1 following feeding on isotopically enriched soil for 4 days, casts of *L. terrestris* had a mean enrichment of 2.21 ± 0.60 APE for ^{15}N and 0.049 ± 0.038 APE for ^{13}C (Fig.2). The casts of *A. caliginosa* had a mean enrichment of 1.24 ± 0.76 APE for ^{15}N and 0.043 ± 0.026 APE for ^{13}C (Fig. 2). During the first three days, the tracer dropped down for ^{15}N to 0.74 ± 0.53 APE for *L. terrestris* and 0.54 ± 0.34 APE for *A. caliginosa* and for to 0.021 ± 0.009 ^{13}C APE for *L. terrestris* and 0.007 ± 0.004 ^{13}C APE for *A. caliginosa* (Fig. 2). From day 7 to day 21 the tracer signal stayed approximately on the same level (for ^{15}N: 0.32 ± 0.23 APE for *L. terrestris* and 0.50 ± 0.20 APE for *A. caliginosa*; for ^{13}C: 0.006 ± 0.004 APE for *L. terrestris* and 0.006 ± 0.003 APE for *A. caliginosa*, Fig.2).

Storage of labelled casts either in a climate chamber or a pot containing field soil had no significant influence on ^{15}N and ^{13}C enrichment (Fig. 3). After 105 days, casts stored under constant conditions had an enrichment of 0.45 ^{15}N APE and 0.008 ^{13}C APE, while casts stored in field soil had an enrichment of 0.26 ^{15}N APE and 0.011 ^{13}C APE (Fig.3; Mann-Whitney-U, P > 0.05).

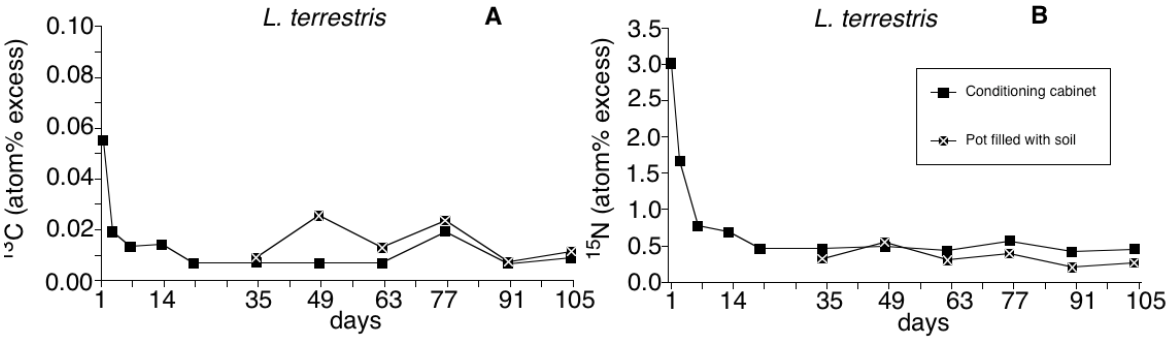

Fig. 3 Time course of ^{13}C (A) and of ^{15}N (B) enrichment of *L. terrestris* casts over 105 days after labelling (means, n=3).

Table 1

Spearman correlations between ^{15}N and ^{13}C APE for tissue and casts; spearman correlation between casts and tissue for ^{15}N and ^{13}C, respectively.

	L. terrestris		*A. caliginosa*	
	r_s	P	r_s	P
^{15}N APE vs. ^{13}C APE				
Tissue	0.782	< 0.001	0.643	< 0.001
Casts	0.703	< 0.001	0.648	< 0.001
Tissue vs. Casts				
^{15}N	0.757	<0.001	0.512	<0.001
^{13}C	0.462	0.001	0.424	0.001

The enrichments of both ^{15}N and ^{13}C in the tissue and casts were significantly positively correlated for both earthworm species (Table 1). For *L. terrestris* the ^{13}C enrichment of casts was positively correlated with the initial earthworm biomass (r^2 =0.827, P < 0.01, n=8) on day 7 of the labelling period; no such correlation was found for ^{15}N (r^2 =0.416, P = 0.084) or between *A. caliginosa* biomass and the isotopic enrichment in their casts.

5. DISCUSSION

This study demonstrates, that tissue and casts of the two adult earthworm species *A. caliginosa* and *L. terrestris* can be isotopically labelled in a simple and non-invasive way by cultivating earthworms in soil, enriched with ^{15}N and ^{13}C for only four days. From the different variants studied, a one-time addition of isotopes of previously incubated soil resulted in higher enrichments than a staggered addition of isotopes. For both species, a higher enrichment in tissue always correlated with a higher enrichment in casts. Our results also show that isotopically labelled casts can be stored over a period of at least 105 days without a significant decrease in the isotopic signal. It is noteworthy that the method works equally well for both earthworm species, although the soil-feeding *A. caliginosa* and the litter-feeding *L. terrestris* belong to different functional groups and differing in their feeding habits.

5.1 Isotopic enrichment in earthworm tissue

This is the first study attempting to isotopically label two different functional groups of earthworms using the same method. In contrast to our expectation, the isotopic enrichment in the tissue of both earthworm species was similar. This indicates that (i) the used method also works for worms which are not preferential soil-feeders, and (ii) it is not necessary to additionally feed *L. terrestris* with plant litter, like Dyckmans et al. (2005) proposed for litter-feeding earthworms. The anecic species, *L. terrestris*, one of the most active earthworm species in temperate soils has never been investigated before and our results show that cultivating this species for four days in isotopically enriched soil can result in a stable signature in its tissue for at least 21 days. The endogeic species, *A. caliginosa*, was also studied by Dyckmans et al. (2005), however although these authors added similar amounts of ^{15}N and ^{13}C to soil their isotopical signatures were about 20 % higher for ^{15}N and almost five times higher for ^{13}C than in the current study. Because the

average earthworm biomass in our study was similar compared to Dyckmans et al. (2005), we suspect that differences in the conditions of these earthworms might be responsible for the different isotopic signatures (Martinez del Rio et al. 2008). Wahlen et Janzen (2002) fed double-labelled wheat for up to 16 weeks to the surface-dwelling (epigeic) *A. tuberculata* and reached 0.055 APE ^{15}N and 0.380 APE ^{13}C after adult earthworms fed at labelled food for four weeks. At the moment we have data with different methods employed on isotopic labelling for only one species of earthworm per ecological group these data indicate that different functional groups of earthworms incorporate isotopes differently although isotopic incorporation is also known to vary considerably between individuals (Martinez del Rio et al. 2008). The latter explanation is also confirmed by the positive correlation between initial earthworm biomass and isotopic enrichment both in tissue and casts for both earthworm species. Also Whalen et Janzen (2002) and Dyckmans et al. (2005) reported that differences in biomass causes enrichment variability. Additionally, Martinez del Rio et al. (2008) also hypothesised that the signal of the tissue will also be influenced by growth and protein turnover of earthworms.

Contrary to our expectations, the addition of oat flakes to the labelled soil did not affect the incorporation of isotopic labels into earthworm tissue. Originally, we hypothesized that the signal in the litter-feeding *L. terrestris* would be more diluted than the signal in the soil-feeding *A. caliginosa.*

5.2 Isotopic enrichment in earthworm casts

This study is the first to test the feasibility of dual-labelling earthworm casts with ^{15}N and ^{13}C in a realistic way. The results show that even the simplest treatment, without incubation of the ammonium nitrate and with a one-time addition of glucose into the soil, labels the casts readily with stable isotopes. It is possible to store labelled casts over a period of 105 days without a significant loss of the labelling signal, which is very useful for planning and preparing an

experiment where labelled casts are needed. The used treatments also show that it is not necessary to additionally feed *L. terrestris* with plant litter, like Dyckmans et al. (2005) proposed for litter-feeding earthworms. The time course of isotopic signal in casts differed from that in earthworm tissue with an exponential decrease especially between day one and seven while in tissue the signal remained stable. This rapid loss during the first days indicates that perhaps earthworms still had labelled soil in their guts after the transfer to the unlabelled soil, which led to the high amount of label signal on day one. After day seven, the signal in the casts remained stable until day 21 although earthworms would feed on unlabelled soil and would thus dilute the isotopic signal. Mucus enrichment of *A. caliginosa* showed a similar pattern in the study of Dyckmans et al. (2005) and they suggested that two different pools of ^{15}N and ^{13}C with different turnover times might be responsible for this pattern.

5.3 Implications for ecological research

The method presented in this paper can help to better understand the functional relationships between earthworms and associated organisms in terrestrial ecosystems. For example, the labelled casts can be used as a tracer in a system equipped with soil, plants, mycorrhizae and earthworms, to examine the interactions between plants and earthworms concerning the nutrient use. This would help to disentangle trophic interactions where earthworms are involved ((Wurst, Dugassa-Gobena et al. 2004), (Wurst, Allema et al. 2008)) or could be used to trace back which organisms consume earthworms or live of their casts. The stable signal in casts would also enable longer-term experiments investigating the effect of soil microsites for nutrient distributions. Naturally, the method could also replace methods where radioactive ^{14}C was used to label other soil animals or their casts (e.g. springtails, Müller et Bieringer 1971).

In summary, the method presented in this study for producing isotopically labelled earthworm casts and tissue proved to be simple, effective and applicable both for soil-feeding and litter-feeding earthworms.

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8. APPENDIX

8.1 Zusammenfassung

1. Die Aktivität der Regenwürmer (Oligochaeta: Lumbricidae) hat einen substantiellen Effekt auf die Struktur und Fruchtbarkeit des Bodens, mit Konsequenzen für die Diversität der Pflanzengemeinschaft und deren Ökosytemfunktionen. Dennoch mangelt es an Erklärungen über die funktionelle Rolle die Regenwürmer in terrestrischen Ökosystemen spielen, was teilweise darauf zurückzuführen ist, dass die Methoden zur Untersuchung der Regenwurmaktivität mangelhaft erforscht sind.
2. In dieser Studie wurde untersucht, ob es möglich ist Regenwürmer und ihre Exkremente (Regenwurmhäufchen) gleichzeitig mit den stabilen Isotopen ^{15}N und ^{13}C zu markieren, indem Regenwürmer in mit ^{15}N-Ammoniumnitrat und $^{13}C6$-Glucose angereichertem Bodensubstrat kultiviert werden.
3. Zusätzlich wurde in dieser Studie untersucht, ob (i) Regenwürmer und deren Häufchen aus verschiedenen funktionellen Gruppen (epigäisch vs. anözisch), sich auch in der Anreicherung der stabilen Isotopen unterscheiden, (ii) sich die Signalstärke unterscheidet, wenn dieselbe Menge an stabilen Isotopen duch eine einmalige oder eine gestaffelte Zugabe eingesetzt wird und (iii) ob sich die Anreicherung an Isotopen in den Häufchen über einen längeren Zeitraumnachweisen lässt, wenn sie entweder unter Laborbedingungen oder eingegraben in mit Feldboden gefüllten Töpfen gelagert werden.
4. Die Ergebnisse dieser Untersuchung zeigen die Umsetzbarkeit einer zeitgleichen Markierung des Gewebes und der Häufchen, sowohl von anözisch (*L. terrestris*) als auch von epigäisch (*A. caliginosa*) lebenden Regenwurmarten, mit ein und derselben Methode auf. Der Vorteil dieser Methode ist, dass Regenwürmer und deren Häufchen innerhalb einer viertägigen Kultivierung in einmalig mit stabilen Isotopen angereichertem Bodensubstrat ausreicht, um eine Anreicherung unter realitätsnahen Bedingungen zu erzielen. Die isotopische Anreicherung in den Regenwürmern war auf einem stabilen Niveau für mindestens 21 Tagen

nachweisbar, die markierten Häufchen konnten für mindestens 105 Tage ohne einer signifikanten Abnahme des isotopischen Signals gelagert werden.

5. Diese einfache und effiziente Methode eröffnet neue Möglichkeiten der Untersuchung der (i) Aktivität der Regenwürmer als einer der wichtigsten Ökosystem-Ingenieure, (ii) funktionellen Beziehungen zwischen Regenwürmern und anderen Organismen und (iii) der Rolle der Regenwürmer im Nährstoffkreislauf von Ökosytemen.

8.2 Lebenslauf

Name	Barbara Heiner
Adresse	Kreuzgasse 87-89/ 9/ 14 1180 Wien

Persönliche Daten

geboren am	30. Juli. 1980	in Wien
Familienstand		ledig
Staatsbürgerschaft		Österreich

Ausbildungsdaten

Schulausbildung	1986 bis 1990	Volksschule in Wien
	1990 bis 1999	Gymnasium in Wien Abschluss: Matura
Studium	2000 bis 2006	Diplomstudium Biologie an der Universität Wien
	2006 bis 2010	Diplomstudium Ökologie an der Universität Wien

Berufspraxis

2002	Praktikum bei Echem Kompetenzzentrum für Elektrochemie
2007	Praktikum bei BioFoschung Austria
2008 bis 2009	Wissenschaftliche Mitarbeiterin auf der Universität für Bodenkultur
2009	Wissenschaftliche Mitarbeiterin bei FFG-Innovationsscheck (Vert. Nr. 822142)