

Isotopic labelling of enchytraeids under FACE conditions: A possible way to analyse the residue-enchytraeid-soil system considering elevated atmospheric CO₂ concentrations

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Abstract

A soil microcosm experiment was conducted in the laboratory with enchytraeids to assess the carbon (C) and nitrogen (N) turnover when feeding on barley straw. The straw originated from a field experiment where the crop was cultivated under elevated atmospheric CO₂ conditions (FACE = Free Air Carbon dioxide Enrichment). The CO₂ concentration amounted to 550 ppm compared to ambient air with 380 ppm. CO₂ applied to the crop in the enrichment plots was depleted in ¹³C; its δ¹³C value was -21‰. Additionally, one subplot of plants was labelled with ¹⁵N in both ambient air and elevated CO₂ treatments. The aim of our study was to evaluate if straw from plants grown under elevated CO₂ conditions and treated with labelled fertilizer can be used to trace carbon and nitrogen from the plants to the enchytraeids. Microcosms (n = 5) were filled with a previously defaunated silt loam soil, topped with ground barley straw and inoculated with a mixture of the two enchytraeid species *Enchytraeus crypticus* and *E. buchholzi* s.l.. One treatment without enchytraeids served as control. After 50 days of incubation at constant 20°C in darkness, the remaining straw was collected; the enchytraeids were extracted and counted. Samples of soil, straw and enchytraeids were analysed for C and N contents as well as ¹³C and ¹⁵N signatures. While the C/N ratio in the remaining straw material was significantly reduced, no change was observed in the enchytraeids. Under ambient air conditions, δ¹⁵N values of the enchytraeids were 27.6‰ in animals from plots with non-N-labelled straw, while those from labelled-N-treatments showed 52.2‰. In the FACE treatments δ¹⁵N-values of 15.7‰, and 29.3‰ were measured for animals from unlabelled and labelled treatments, respectively. The δ¹³C-values of the enchytraeids were as well significantly different reflecting isotope signatures of the consumed straw.

Keywords: soil mesofauna; ¹³C; ¹⁵N; microcosm experiment; carbon turnover; nitrogen turnover

1. Introduction

The analysis of the stable isotopes ¹³C and ¹⁵N provides a promising technique to trace consumed organic matter in food webs and to get insights into the structure of consumer communities (DeNiro & Epstein 1978, Robinson 2001). With respect to belowground communities, this technique has been successfully applied to various taxa of the makro- and mesofauna in forest soils (Scheu & Falca 2000) and arable soils (Briones et al. 2001, Schmidt et al. 2004). More recently, the abundances of ¹³C and ¹⁵N of soil fauna and their food sources in special environments like

vermicomposts were analysed (Sampedro & Dominguez 2008).

In the present study, ¹³C and ¹⁵N isotope analysis was used to trace the carbon (C) and nitrogen (N) translocation from crop residues to enchytraeids. Barley straw derived from a field experiment on effects of elevated CO₂ concentrations on the plant-soil-system (Free Air Carbon dioxide Enrichment = FACE) was used. Increasing atmospheric CO₂ concentrations are known to affect vegetation through enhanced photosynthetic rates and biomass production above- and below-ground, increase of plant water use efficiency (Ainsworth & Long 2005),

change in C/N ratios (e.g., Ehleringer et al. 2002), and modified rhizodeposition (Phillips et al. 2006). Direct influences of elevated atmospheric CO₂ concentrations on soil fauna can be excluded because of its adaptation to higher CO₂ concentrations in soil (Whalen & Sampedro 2010). However the soil food web may be indirectly affected by elevated atmospheric CO₂ concentrations through changes in litter quantity and quality like reduction of N concentrations (Cotrufo et al. 1998), as well as shifts in root turnover rates and nutrient exudation into the rhizosphere (Coûteaux & Bolger 2000). Previous studies showed changes in the stable ¹³C-signatures for collembolans (Sticht et al. 2008) and nematodes (Sticht et al. 2009), which were living in the rhizosphere and feeding on straw originating from crops cultivated under elevated CO₂ conditions in the same FACE treatment mentioned before. Stable isotope analysis of ¹³C and ¹⁵N offers the opportunity to test to which extent the isotopic signatures in soil fauna resemble those of their food (Schmidt et al. 2004, Sampedro & Dominguez 2008). The aim of our study was to analyse whether straw, which was produced under CO₂ enrichment conditions with a ¹³C label and which was additionally labelled with ¹⁵N, can be used for tracing both carbon and nitrogen from food to enchytraeids. In case of FACE straw as food, C/N relationships in food webs under elevated CO₂ conditions can be analysed, additionally. Our hypotheses were (1) Changes in C/N ratio of the food affect the C/N ratio of enchytraeids; (2) The ¹³C and ¹⁵N label of the straw affects the isotopic signature of enchytraeids.

2. Material and methods

2.1. Soil, litter and enchytraeids

Topsoil was sampled from an agricultural field located at the Johann Heinrich von Thünen-Institute (vTI) in Braunschweig, Lower Saxony, Germany (10°26' E 52°18' N, 79 m a.s.l.). It was a Luvisol derived from loess with a pH value of 7.3 and a mean organic matter content of 2.1%. The soil texture is characterised by 12% clay, 85% silt and 3% sand resulting in a silt loam. The soil was defaunated by freezing at -20°C for 24 h followed by thawing at room temperature for 24 h. This freezing-thawing cycle was repeated three times. This procedure is known to

significantly reduce the number of soil microarthropods and annelids (Wright et al. 1989). The soil was macroscopically cleared of organic plant residues like straw or roots and sieved (mesh size 2 mm). At the beginning of the experiment the soil moisture was 12%.

Straw from winter barley (*Hordeum vulgare* cv. Theresa) was obtained from the same field as the soil, where a FACE (Free Air Carbon dioxide Enrichment) experiment had been running for four years. The FACE equipment was constructed according to an arrangement developed by the Brookhaven National Laboratory in New York, USA: In circular plots, the standing crop was supplied with atmospheric air enriched in CO₂ up to 550 ppm (FACE treatment). Control plots under ambient air conditions revealed atmospheric CO₂ concentration of 375 ppm (ambient air treatment) (for details see Hendrey 1992, Weigel et al. 2006). The CO₂ used for the enrichment was depleted in ¹³C resulting in a δ¹³C of atmospheric CO₂ in the in the FACE treatment of -21.0‰ compared to a δ¹³C of -9.8‰ in the ambient air treatment (for details see Sticht et al. 2008). Furthermore, in either treatment subplots were fertilised with ¹⁵N labelled ammonium sulfate, ¹³C depletion and ¹⁵N labelling were used to trace C and N from litter in different compartments of the soil system. Straw from FACE and ambient air plots at natural ¹⁵N abundance as well as straw labelled with ¹⁵N from both treatments was collected, air-dried and ground. The initial C/N ratios of barley straw from FACE and ambient air plots were 69.8 and 58.0, respectively.

Two different enchytraeid species (*Enchytraeus crypticus* and *E. buchholzi* s.l.) were obtained from our own laboratory cultures. The enchytraeids were bred in petri dishes on solid agar at 20°C in darkness. Chopped oatmeal was offered for feeding. *E. crypticus* is common in fields and a standard test species; *E. buchholzi* s.l. is a widespread species complex (Schmelz & Collado 2010).

2.2. Soil, litter and enchytraeids

Perspex cylinders (6 cm in height and 4 cm in diameter) were used as microcosms, which were filled with moist soil up to a height of about 4 cm with a bulk density of 1.2 g cm⁻³. The soil of each microcosm was covered with 250 mg ground

barley straw and inoculated with both enchytraeid species in a mixed population (30 individuals). A total of 50 microcosms (25 with enchytraeids; 25 without enchytraeids as a control) were set up with 5 replicates of the following treatments: (1) FACE straw ^{15}N labelled; (2) FACE straw non-labelled; (3) Ambient air straw ^{15}N labelled; (4) Ambient air straw non-labelled; (5) No straw as control. All microcosms were covered with Parafilm and closed with nylon-gauze (20 μm mesh size) at the bottom. The microcosms were randomly placed on moist sand-baths to maintain soil moisture and kept in a climate chamber at 20°C in darkness.

After 50 days soil and remaining straw were sampled and dried. The enchytraeids were extracted according to Graefe (1984), collected in petri dishes with water for gut clearance, counted and stored in ethanol (96%). It was not distinguished between the two enchytraeid species initially inoculated. C and N contents of soil, straw and enchytraeids were measured by combustion in a TruSpec CN-Analyser (LECO). Furthermore, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of soil, straw and enchytraeids were measured with a mass spectrometer „Deltaplus“ (Finnigan MAT GmbH) coupled with an elemental analyzer FlashEA 1112 (ThermoQuest) via a „Continuous Flow Interface“ (ConFlo III, Thermo Finnigan MAT GmbH). The initial data for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ -values of soil, straw and enchytraeids in FACE and ambient air treatments are given in Tab. 1.

Tab. 1. Mean initial $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ -values of enchytraeids, straw and soil in FACE (elevated CO_2 concentration) and ambient air (control) treatments.

Treatment	Parameter	Enchytraeids	Straw	Soil
Ambient air	$\delta^{13}\text{C}$ [‰]	-23.38	-29.91	-27.09
FACE			-40.49	
Ambient air	$\delta^{15}\text{N}$ [‰]; non-labelled	20.69	4.46	6.90
FACE			5.29	
Ambient air	$\delta^{15}\text{N}$ [‰]; ^{15}N -labelled		70.99	
FACE			31.54	

2.3. Statistical analysis

All data were tested with the Kolmogoroff Smirnov-test for normal distribution. In case the data were not normally distributed the data were log-transformed to get an approximation to normal distribution. Normally distributed data were tested for significance with a Student-t test. Statistical analysis was done with the program SPSS for Windows.

3. Results

3.1. Enchytraeid abundance

At the end of the experiment, fewer individuals were extracted than inoculated before. Most enchytraeids per microcosm were found in the ambient air treatment (17.9 ± 28.1 individuals) followed by the control without straw (15.8 ± 4.0 individuals). In microcosms, where FACE straw was offered, 10.5 ± 13.0 individuals were found which significantly ($P < 0.01$) was less than found in the control without straw.

3.2. Carbon and nitrogen

The C/N ratio in remaining straw samples from the FACE treatments (with enchytraeids and control without enchytraeids) was significantly ($P < 0.001$) lower than in those of the ambient air treatments (Tabs. 2 and 3). In both, enchytraeids as well as the corresponding soil samples, differences were not significant. Furthermore, the C and N contents of enchytraeids, remaining straw and soil both in the enchytraeid and the non-enchytraeid treatment did not differ significantly between FACE and ambient air treatment samples (Tabs. 2 and 3). In enchytraeids, C contents increased with increasing N contents in the FACE ($y = 1.26x + 29.77$; $R^2 = 0.84^{**}$) as well as in the ambient air ($y = 1.17x + 31.58$; $R^2 = 0.84^{**}$) treatment.

Tab. 2. Means (\pm SD) of C and N contents of enchytraeids, remaining straw and soil samples as well as C/N-ratios in FACE (elevated CO_2 concentration) and ambient air (control) treatments with enchytraeids after 50 days experimental time.

Treatment	Parameter	Enchytraeids	Straw	Soil
Ambient air	C [mg g^{-1}]	527.77 ± 65.23	145.28 ± 53.42	11.04 ± 0.31
FACE		550.08 ± 82.12	103.65 ± 63.24	10.95 ± 0.28
Ambient air	N [mg g^{-1}]	181.14 ± 42.73	4.85 ± 1.81	1.17 ± 0.05
FACE		183.07 ± 34.47	4.59 ± 1.80	1.16 ± 0.03
Ambient air	C/N ratio	2.95 ± 0.45	31.84 ± 5.16	9.44 ± 0.31
FACE		2.89 ± 0.19	19.81 ± 4.37	9.45 ± 0.21

Tab. 3. Means (\pm SD) of C and N contents of remaining straw and soil samples as well as C/N-ratios in FACE (elevated CO_2 concentration) and ambient air (control) treatments without enchytraeids after 50 days experimental time.

Treatment	Parameter	Straw	Soil
Ambient air	C [mg g^{-1}]	118.64 ± 36.76	11.23 ± 0.46
FACE		96.10 ± 61.54	10.87 ± 0.37
Ambient air	N [mg g^{-1}]	3.26 ± 0.54	1.18 ± 0.02
FACE		3.93 ± 2.21	1.16 ± 0.02
Ambient air	C/N ratio	36.32 ± 9.07	9.55 ± 0.31
FACE		21.98 ± 7.49	9.35 ± 0.15

3.3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures

Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of enchytraeids, remaining straw and soil collected at the end of the experiment are presented in Fig. 1. Samples from the FACE straw treatment were significantly depleted in $\delta^{13}\text{C}$ values ($P < 0.05$) compared to those from ambient air straw treatment. Furthermore, enchytraeids and soil from both straw treatments showed $\delta^{13}\text{C}$ values significantly ($P < 0.05$) more negative compared to the control without straw. Samples of remaining straw and soil harvested from

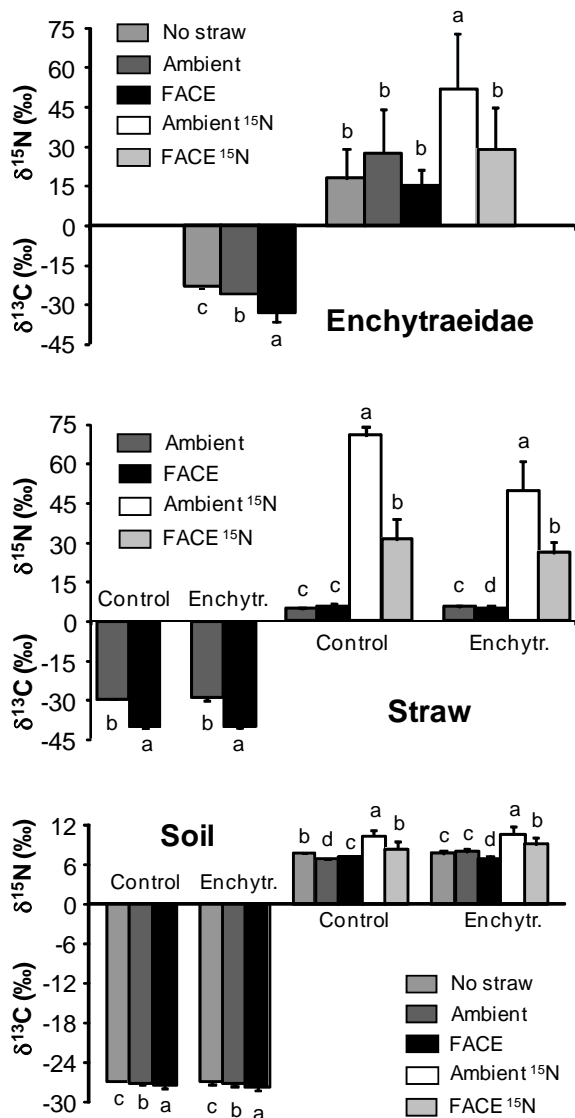


Fig. 1. Mean δ -values (\pm SD) of remaining barley straw, enchytraeids and soil samples from treatments with enchytraeids (Enchytr.) and the non-enchytraeid control after 50 days of incubation. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are presented for treatments applied with straw grown under ambient air and FACE conditions, additionally labelled with ^{15}N and a non-straw control treatment. Different letters within the same group of columns indicate significant differences ($p < 0.05$).

microcosms inoculated with enchytraeids did not differ in $\delta^{13}\text{C}$ values compared to those from non-enchytraeid control microcosms.

After 50 days of experimental time, ^{15}N label was present in all compartments, which were in contact with ^{15}N (Fig. 1). Hence, the $\delta^{15}\text{N}$ values here were significantly higher ($P < 0.05$) compared to unlabelled treatments. The highest $\delta^{15}\text{N}$ values were found for enchytraeids, remaining straw and soil in the ambient air treatment where ^{15}N labelled straw had been applied (Fig. 1). The $\delta^{15}\text{N}$ -value of enchytraeids amounted to 52.2‰ for labelled and 27.6‰ for non-labelled straw from ambient air treatment. $\delta^{15}\text{N}$ -values of enchytraeids in the FACE treatments were less enriched, they read 29.3‰ for labelled and 15.7‰ for non-labelled straw.

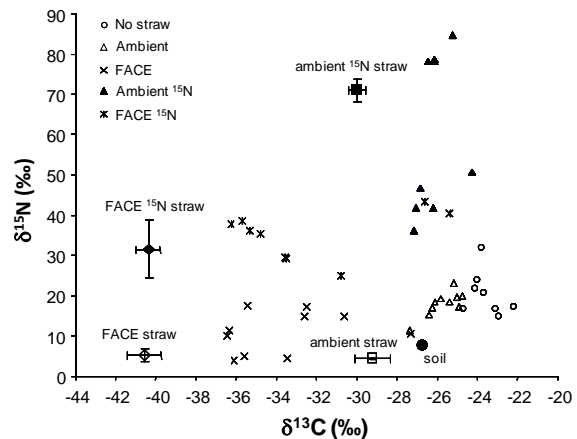


Fig. 2. Dual plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ patterns of enchytraeids depending on their food source (barley straw) after 50 days of incubation. Additionally, mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of soil as well as non-labelled and ^{15}N -labelled barley straw produced under ambient air and FACE conditions are given.

The C and N isotopic composition of the enchytraeids from all treatments gave insight into the translocation of C and N from straw, which had been initially offered for feeding. Enchytraeids, which were kept in soil without straw, showed $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values slightly enriched compared to soil (Fig.2). Those animals, which fed on straw produced under ambient air conditions, gave slightly more negative $\delta^{13}\text{C}$ values than before, indicating the uptake of C from the straw. The $\delta^{15}\text{N}$ values remained more or less the same. The C isotopic composition of straw cultivated under FACE conditions significantly differed from straw under ambient air conditions. So did the enchytraeids: their $\delta^{13}\text{C}$ values significantly shifted towards the

isotopic composition of their food source to more negative values (Fig. 2). $\delta^{15}\text{N}$ again remained in the range of all other individuals so far. With respect to the ^{15}N labelled straw, a clear shift in $\delta^{15}\text{N}$ was measured in enchytraeids - again towards the label of their food source. Since straw produced with ^{15}N label under ambient air condition contained much more ^{15}N than did straw produced under FACE conditions, the N isotopic signature in enchytraeids grown on ^{15}N straw from ambient air showed the most profound change in $\delta^{15}\text{N}$. The ^{13}C values, however, were always in the range of the respective straw. These results indicate enchytraeids as consumers of the straw as food source within the experimental time of 50 days.

4. Discussion

The initial individual density of enchytraeids was reduced in all treatments at the end of the experiment. Although refaunation of defaunated soils can be restricted in terms of recolonization (Wright et al. 1989), the laboratory conditions of the present study were chosen according to recommendations of Römcke et al. (2005) for the use of enchytraeids in standardized tests and hence should have been appropriate. It might be possible that a lack of adaptation to soil conditions after breeding the enchytraeids in solid agar led to a general decline of individual numbers. Nevertheless, a treatment effect was found with lowest enchytraeid numbers in microcosms containing FACE straw. Recently, Maraldo & Holmstrup (2010) summarized results on indirect effects of elevated CO_2 concentrations on enchytraeids. According to this review, increased CO_2 might affect the reproduction of enchytraeids. Furthermore, it is discussed that the composition of their food source (e.g. the balance between fungi and bacteria) might alter (Maraldo & Holmstrup 2010).

Concerning the N content of remaining straw, Cotrufo et al. (1998) reported a mean N reduction of 11% in leaf litter of C3 plants grown under elevated atmospheric CO_2 concentrations. In our study, we also observed a less profound reduction in N content of 5.4% (enchytraeid treatments). The C/N ratio in the straw material originating from cultivation under FACE conditions was also altered. Although a reduction of C/N was visible in the straw, the C/N ration in

the enchytraeids was not affected. Thus, our first hypothesis, that changes in C/N ratio of the food affect the C/N ratio of enchytraeids, cannot be confirmed.

According to Didden (1993) enchytraeids mainly feed on microorganisms which colonize dead organic material and utilize simple organic compounds. This indicates an indirect role of enchytraeids in decomposition processes. According to an analysis of the trophic structure of soil fauna communities in beech forests enchytraeids are classified as secondary decomposers (Scheu & Falca 2000). Our result presented in this study, however, revealed that enchytraeids incorporated part of the labelled organic material. The results of our ^{13}C and ^{15}N isotopic analyses confirm our second hypothesis, that ^{13}C and ^{15}N label of the straw affects the isotopic signature of enchytraeids. This indicates crop residues (besides soil microorganisms) being an important food source for enchytraeids. Hence, enchytraeids are directly involved in decomposition processes feeding on fragmented litter. However, so far this conclusion is restricted to the two species *E. buchholzi* s.l. and *E. crypticus*, which were used in the present study. Further studies on food selection and feeding behaviour of enchytraeids on the species level is needed.

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