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Natural ^{13}C abundance reveals age of dietary carbon sources in nematode trophic groups

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ABSTRACT

We determined the $^{13}C/^{12}C$ ratio (expressed as $\delta^{13}C$ ‰) of microbial biomass and nematode trophic groups in a small-plot field experiment with soil converted from C3- to C4-crop (silage maize) 20 years ago. During this period, the plots were subjected to three different organic input treatments: 1) maize stubbles and roots left after harvest (MS), 2) MS plus annual addition of aboveground maize biomass (MS + B), and 3) MS plus annual addition of faeces from sheep fed exclusively with maize (MS + F). The different $\delta^{13}C$ value of C3- and C4-crops allowed us to distinguish between old (> 20 years old) C3-derived C and recent (< 20 years old) C4-derived C incorporated into microbial biomass and nematodes.

The $\delta^{13}C$ value of phytophagous nematodes closely matched that of the maize. Bacterivorous nematodes had higher $\delta^{13}C$ values than fungivorous nematodes and microbial biomass indicating that the C sources of bacterivorous nematodes are more recent than those of fungivorous nematodes and microbial biomass. At low abundance of fungivorous nematodes (MS and MS + F), the microbial biomass had higher $\delta^{13}C$ values than the fungivorous nematodes, whereas their $\delta^{13}C$ values were comparable at higher densities of fungivorous nematodes (MS + B). The higher C4-derived input in MS + F and MS + B treatments increased the $\delta^{13}C$ values of bacterivorous nematodes and microbial biomass.

In MS and MS + B treatments, recent C4-derived C accounted for 50 and 70% of microbial biomass-C, respectively. Corresponding values for fungivorous and bacterivorous nematodes were 30 and 75%, and 65 and 85%, respectively.

We conclude that fungal-based decomposition pathways contribute more to the turnover of old soil C than bacterial-based decomposition. A substantial fraction of the microbial biomass and fungivorous nematode C in the MS treatment (50 and 70%, respectively) was C deposited in the soil more than 20 years ago, confirming that decade-old SOC remains biologically active.

1. Introduction

Decomposition of organic matter (OM) in soil is governed by the activity of and interactions between soil microorganisms and decomposer fauna that constitute the decomposer food web. The classical notion is that bacteria are primarily involved in decomposition of labile OM of recent origin, whereas fungi, primarily due to their ligninolytic activity, are considered to govern decomposition of recalcitrant and older OM (de Boer et al., 2005; Paterson et al., 2008; Koranda et al., 2014). However, bacterial ligninolytic activity may be more common and the bacterial contribution to recalcitrant OM decomposition more important than previously assumed (Strickland and Rousk, 2010). *Vice versa*, fungal use of labile OM may be much more significant than hitherto perceived (de Vries and Caruso, 2016). Recent evidence also suggests that the perception of old soil OM as biologically inactive is too simplistic as microorganisms continuously recycle carbon (C) retained in “old” soil OM pools (Gleixner et al., 2002; Basler et al., 2015a, 2015b; Dippold and Kuzyakov, 2016). The current perception of

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pathways through which C in old and recent soil OM pools pass during decomposition, not least the role of soil fauna, thus remains incomplete.

Soil living nematodes play key roles in the soil decomposer food web; nematode taxa are positioned at several different trophic levels, i.e. most taxa are either phytophagous, fungivorous, bacterivorous, predatory or omnivorous (Yeates et al., 1993). Nematodes are prey to microarthropods (Heidemann et al., 2014) and perhaps also contribute to the diet of earthworms (Curry and Schmidt, 2007). Except for phytophagous nematodes, the abundance of individual nematode trophic groups often mirror growth responses of their respective food sources (Ferris, 2010; Christensen et al., 2012). Together with protozoa, microbivorous nematodes are the main grazers of microorganisms and regulate the size, composition and activity of microbial communities as well as rates of C and nitrogen turnover (Rønn et al., 2012).

Application of isotope labelled (^{13}C and ^{14}C) substrates is a powerful approach for tracing the shorter-term fate of C derived from specific compounds or tissues through decomposer food webs and its incorporation into soil OM pools (Andresen et al., 2011; Paterson et al., 2011; Zieger et al., 2017). However, this approach cannot reveal the bioavailability of old soil OM because isotope labelled compounds do not represent the chemical and physical complexity of old OM stabilized *in situ* in the soil matrix (Christensen, 2001). A considerable fraction of the old soil OM has been biologically processed over decades to centuries and has become physically protected by incorporation into differently sized organo-mineral complexes (Christensen, 2001; von Lützow et al., 2006). Due to different isotopic fractionation in the photosynthetic pathways of C3- and C4-plants, long-term conversion from C3 vegetation to C4 vegetation provides an efficient *in situ* labelling of the soil OM pools. The higher $^{13}C/^{12}C$ ratio of C4-plants than C3-plants permits determination of the fate of post-conversion C4-derived C and pre-conversion C3-derived C in different compartments of the soil system (Staddon, 2004; Scheunemann et al., 2010). In the present study we employed an experiment, where C4-plants (silage maize, *Zea mays*) have been grown continuously for 20 years in soil with a pre-conversion history of C3-plants only (Thomsen and Christensen, 2010). The experiment includes three different treatments with annual addition of 1) maize roots and stubbles only (MS), 2) MS plus aboveground biomass (MS + B), and 3) MS plus maize-derived sheep faeces (MS + F). Soil kept under C3-crops served as reference treatment.

With recent developments of micro-sample elemental analysis-isotopic ratio mass spectrometry (μEA-IRMS) it is now possible to analyse stable C and N isotopic ratios of microgram samples (Langel and Dyckmans, 2014). This progress makes it feasible to isolate sufficient nematode biomass for $^{13}C/^{12}C$ ratio assessment of individual nematode taxa or trophic groups (Melody et al., 2016). Conventional IRMS requires the isolation of considerably more nematode biomass (Kudrin et al., 2015).

To determine to which extent individual nematode trophic groups are involved in the decomposition of recent soil OM and soil OM older than 20 years, we compared the $\delta^{13}C$ of bacterivorous and fungivorous nematodes in the long-term C3 to C4 conversion experiment with the $\delta^{13}C$ of recent plant-derived organic input and C deposited into the soil OM pool at least 20 years ago. For reference, we assessed $\delta^{13}C$ of phytophagous nematodes and soil microbial biomass in the converted soils and in soil maintained under C3 crops.

We hypothesized that C in phytophagous nematodes exclusively consists of recently photosynthetically fixed C, that C in bacteria, and thus in bacterivorous nematodes, is more enriched in recent C than the total soil microbial biomass, and that old C contributes more to the diet of fungivorous than to bacterivorous nematodes.

2. Materials and methods

2.1. The C3 to C4 conversion experiment

In 1996 bulk soil (Inceptisol, Orchrept; 109 mg clay g^{-1} soil) from the 0–25 cm layer was collected from an arable field at Askov Experimental Station, Denmark (55° 28'N, 09° 07'E). The soil had been exclusively under C3 plants and had a $\delta^{13}C$ of −27.5‰. The soil was sieved (4 mm), mixed and placed outside in open-ended cylinders (diameter 0.986 m, depth 0.5 m) that were inserted 45 cm into the ground. The cylinders were placed on top of the undisturbed clayey subsoil and filled with soil adjusted to a bulk density of 1.5 g cm^{-3}.

Except for one reference cylinder maintained under C3-crops, cylinders have been cropped exclusively with maize every year since spring 1997 and subjected to three different organic input treatments. Three replicate cylinders receive only maize roots and stubbles left after harvest of the maize (treatment MS), another three cylinders receive an additional annual input of coarsely chopped aboveground maize biomass at a dose of 0.8 kg dry matter (DM) m^{-2} (treatment MS + B), and finally three cylinders receive a similar quantity of maize-derived sheep faeces (treatment MS + F). All cylinders are fertilized annually with mineral fertilizers corresponding to 170–200 kg N ha^{-1}, 36–40 kg P ha^{-1} and 190 kg K ha^{-1}. Soil pH is kept in the range 5.5–6.5 by addition of $Ca(OH)_2$. Every year, the silage maize is whole-crop harvested by mid-October, leaving behind c. 4 cm stubbles. The annual maize yields for the three input treatments are shown in Table 1.

The maize-derived faeces was produced by feeding adult sheep with silage maize for 39 days. During the first 9 days, the sheep were fed with increasing amounts of silage, and for the remaining 30 days they were fed with maize silage exclusively. The faeces was collected daily from day 29–39 and frozen. The sheep were kept with no bedding during the 39 days. The C concentrations in maize biomass and faeces are 444 mg C g^{-1} DM. The $\delta^{13}C$ of maize biomass and sheep faeces is −12.0 and −13.7‰, respectively (Thomsen and Christensen, 2010).

2.2. Soil sampling and analyses

We sampled three soil cores (diameter 5 cm) to 10 cm depth in each cylinder in April 2016 prior to crop establishment and pooled the three subsamples into one composite sample for each cylinder. The soil

Table 1
Mean annual maize crop yield in plots subjected to different maize-derived input (stubbles and roots (MS), stubbles, roots and sheep faeces (MS + F) or stubbles, roots and aboveground maize biomass (MS + B) followed by SE.

Year	Mean maize crop yield (t ha^{-1})					
	MS	SE	MS + F	SE	MS + B	SE
1997	33.21	1.73	39.45	2.42	33.95	3.57
1998	22.14	0.64	26.39	1.58	20.75	1.84
1999	22.69	0.19	28.94	0.73	21.20	2.93
2000	21.16	0.36	26.03	1.30	20.50	1.44
2001	21.40	1.03	27.01	1.76	23.95	1.36
2002	27.34	1.25	36.00	0.90	30.91	0.61
2003	29.24	1.08	38.01	2.38	29.35	1.50
2004	20.56	1.37	27.34	0.62	23.37	0.57
2005	25.33	0.67	30.68	0.20	28.96	0.34
2006	27.92	0.19	33.24	0.87	29.62	0.19
2007	21.72	1.86	25.88	0.18	24.53	0.14
2008	29.06	1.07	34.29	0.44	29.96	0.32
2009	26.78	1.42	34.93	1.30	30.00	0.40
2010	25.15	0.27	30.07	0.66	26.52	0.28
2011	22.03	1.48	29.84	0.53	24.34	0.45
2012	23.21	0.85	30.61	0.97	26.23	1.51
2013	29.22	1.64	31.11	1.90	28.89	5.13
2014	25.79	0.24	31.75	1.13	28.41	0.23
2015	21.93	0.29	31.06	1.71	26.48	1.38

ANOVA $P_{treatment} < 0.001$; MS < MS + B < MS + F (Tukey $P < 0.05$).

samples were kept at 4 °C until they were processed the following day. The soil was sieved (2 mm), and 10 g fresh weight (fw) was dried at 103 °C for soil moisture determination.

We determined microbial biomass C content using the chloroform-fumigation method (Vance et al., 1987). We vacuum-incubated 10 g fw soil with ethanol-free chloroform ($CHCl_3$) for 24 h, after which the soil was extracted for 1 h at 200 rpm in 50 mL 0.05 M K_2SO_4. Similarly, we extracted another 10 g fw soil, which was not exposed to chloroform-fumigation. Extracts, including those of blank controls, were filtered through Whatman GF-D filters (Whatman Ltd., Maidstone, UK) and frozen immediately. Total soil organic C (TOC) in extracts was determined with a Shimadzu TOC-5000A. Chloroform-labile microbial biomass C was estimated as the difference between fumigated and unfumigated samples using an extractability factor of 0.45 (Joergensen, 1996).

For $^{13}C/^{12}C$ isotope ratio determination of the microbial biomass C, we freeze-dried aliquots of the extracts and transferred 20 and 12 mg of the dried K_2SO_4 salt and dried extract of unfumigated and fumigated samples, respectively, to tin capsules (5 mm × 9 mm). For $^{13}C/^{12}C$ isotope ratio of the soil organic C (SOC) we transferred 10 mg of dried and ground soil to tin capsules.

2.3. Nematode extraction and analyses

Nematodes were extracted for 72 h using a combination of the Baermann pan and the Whitehead tray (Whitehead and Hemming, 1965) methods. For each soil sample, we extracted one subsample of 40 g fw to assess the abundance of individual nematode feeding groups. Another eight 40 g subsamples were extracted for isolation of the trophic groups and subsequent $^{13}C/^{12}C$ isotope ratio determination.

We counted the number of individual nematode trophic groups based on morphological characteristics of mainly the buccal cavity and oesophagus (see Yeates et al., 1993) of live organisms using an inverted microscope at 200–400 × magnification.

For isotope measurements, we identified specimens of phytophagous, bacterivorous and fungivorous nematodes using a stereomicroscope at 115 × magnification and transferred a minimum of 100 individual specimens, where available, with an eyelash with handle (Ted Pella, Inc., Redding, CA) into pre-weighed silver capsules (8 mm × 5 mm) (CE Instruments, Milan, Italy). In some samples, the low abundance of fungivorous nematodes prevented the retrieval of 100 individuals. For the individual samples, we isolated between 1.8 and 14 µg C for bacterivorous nematodes, between 0.48 and 1.6 µg C for fungivorous nematodes and between 1.7 and 2.7 µg C for phytophagous nematodes. Before use, the silver capsules were heated at 500 °C for 4 h to minimize any background C contamination. The capsules with nematodes were dried at 40 °C overnight.

2.4. $^{13}C/^{12}C$ isotope ratio measurements

µEA-IRMS measurements of isotope ratios were made by high-temperature dry combustion using a modified elemental analyzer (Eurovector, Milano, Italy) coupled to an isotope ratio mass spectrometer (Thermo Delta Vplus; Thermo Scientific, Bremen, Germany) as described by Langel and Dyckmans (2014). Results were corrected for blank values, and we used acetanilide ($\delta^{13}C = -29.6‰$) and wild boar liver ($\delta^{13}C = -17.3‰$) for blank corrections. The results were used to determine the blank amount and isotopic composition for C in a Keeling-plot type graph (Langel and Dyckmans, 2014). The blank was 0.2 µg C with an isotopic value of −28‰.

2.5. Data analyses

We calculated the fraction of C4-derived C in microbial biomass and nematodes as the difference between δ^{13} values in the individual organismal group in C4- and C3-planted soil in relation to the difference between δ^{13} values of the plant input in the C4- and C3-planted soil:

$$Fraction\ of\ C\ derived\ from\ C4\ source = \frac{\delta^{13}C_{C4\ sample} - \delta^{13}C_{C3\ sample}}{(\delta^{13}C_{C4\ source} - \delta^{13}C_{C3\ source})}$$

where $\delta^{13}C_{C4\ sample}$ and $\delta^{13}C_{C3\ sample}$ denote the $\delta^{13}C$ value of the sample, i.e. the individual organismal group, in C4 and C3 planted soil, respectively, $\delta^{13}C_{C4\ source}$ denotes the $\delta^{13}C$ value of the maize (−12.0‰), and $\delta^{13}C_{C3\ source}$ denotes the $\delta^{13}C$ value of the C3 plant input (−27.5‰). In the MS + F treatment, stubbles and roots left after harvest and sheep faeces had different $\delta^{13}C$ signatures and the fraction of C derived from each of these two sources was not calculated.

We tested the effect of input treatment on microbial biomass and the abundance of individual nematode trophic groups with one-way ANOVAs followed by Tukey tests for pair-wise treatment comparisons. Phytophagous nematode abundances were log-transformed prior to statistical analysis to obtain homoscedasticity.

We compared the $\delta^{13}C$ values of nematode trophic groups and microbial biomass across the three input treatments with a two-way ANOVA, where factors were treatment and organismal group, i.e. nematode trophic group and microbial biomass, followed by Tukey tests for pair-wise comparisons. Statistical analyses were performed in SigmaPlot 14.0.

3. Results

3.1. Microbial biomass and nematode abundance

The mean microbial biomass ranged from 538 to 726 µg C g^{-1} soil, but did not differ significantly between the three input treatments (Table 2). The abundance of phytophagous nematodes tended to be higher in MS (12 individuals g^{-1} soil) than in the MS + B (4 individuals g^{-1} soil) treatment (ANOVA $P = 0.065$) (Table 2). In MS + F

Table 2
Mean soil $\delta^{13}C$ signature, C content, microbial biomass C and nematode trophic group abundances in soils exclusively subjected to maize (C4 plant) cropping and to different maize-derived input (stubbles and roots (MS), stubbles, roots and sheep faeces (MS + F) or stubbles, roots and aboveground maize biomass (MS + B)) for 20 years followed by SE (in parentheses), ANOVA P values for effects of input. Values followed by different letters are significantly different (Tukey $P < 0.05$). $n = 3$. Data are also reported for a reference soil exclusively subjected to C3 vegetation.

	MS		MS + F		MS + B		C3 reference soil	P
$\delta^{13}C$ soil (‰)	−22.33a	(0.32)	−19.07b	(0.18)	−19.51b	(0.17)	−27.47	< 0.001
C content (%)	1.27a	(0.07)	2.11b	(0.10)	1.97b	(0.15)	1.38	0.004
Microbial biomass C (µg g^{-1} soil)	538.32	(92.49)	725.93	(58.80)	620.41	(51.48)	416.36	0.243
Phytophagous nematodes (ind. g^{-1} soil)	11.97	(1.24)	7.32	(2.56)	4.02	(0.59)	6.34	0.065
Bacterivorous nematodes (ind. g^{-1} soil)	9.60a	(2.32)	12.24 ab	(2.83)	22.91b	(3.19)	9.35	0.033
Fungivorous nematodes (ind. g^{-1} soil)	0.24a	(0.08)	1.06a	(0.46)	3.00b	(0.52)	0.47	0.007
Predatory nematodes (ind. g^{-1} soil)	0.16	(0.06)	0.13	(0.09)	0.04	(0.02)	0.00	0.449
Omnivorous nematodes (ind. g^{-1} soil	0.13	(0.06)	0.30	(0.16)	0.25	(0.15)	0.27	0.665

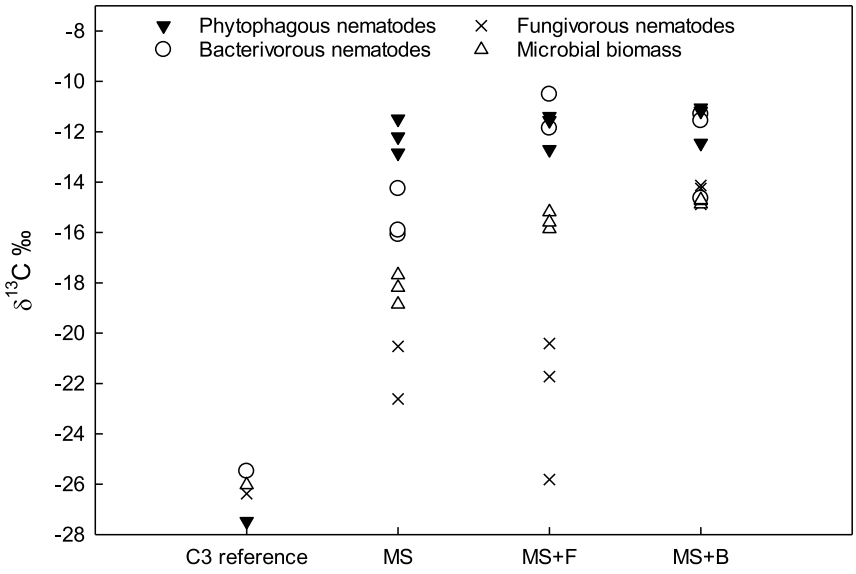

Fig. 1. $\delta^{13}C$ values (‰) of chloroform-fumigated microbial biomass C and three nematode trophic groups in soils cropped exclusively with maize (C4-plant) for 20 years and a reference soil cropped exclusively with C3 plants (C3-reference). C4-planted soils were subjected to three different annual input treatments: MS, only maize roots and stubbles were incorporated; MS + F, addition of 0.8 kg DM m^{-2} maize-derived sheep faeces; MS + B, addition of 0.8 kg DM m^{-2} chopped aboveground maize biomass.

there were 7 phytophagous nematodes g^{-1} soil. The mean abundance of bacterivorous nematodes in MS + B was 23 individuals g^{-1} soil, which was significantly higher than the 10 individuals g^{-1} soil found in MS (ANOVA $P = 0.033$, Tukey $P = 0.035$). The mean abundance was 12 g^{-1} soil in MS + F, which was not significantly different from the two other treatments (Tukey $P > 0.05$). The abundance of fungivorous nematodes was very low in MS and MS + F with 0.2 and 1.1 individuals g^{-1} soil. Annual inputs of aboveground maize biomass significantly increased the abundance of fungivorous nematodes to 3 individuals g^{-1} soil (ANOVA $P = 0.007$, Tukey $P < 0.05$).

With less than 0.3 individuals g^{-1} soil, the abundances of omnivorous and predatory nematodes were negligible, and they did not differ between treatments (Table 2).

3.2. Isotope abundances

The $\delta^{13}C$ value of phytophagous nematodes in the C3 reference soil (Fig. 1) was very close to that of C in the C3-crops (- 27.5‰) (Table 2). Moreover, the $\delta^{13}C$ values of microbial biomass and of bacterivorous and fungivorous nematodes were only slightly higher than that of the C3-crop and phytophagous nematodes (Fig. 1).

Across the three treatments with maize-derived inputs, $\delta^{13}C$ values varied between organismal groups (two-way ANOVA $P_{organism} < 0.001$). The $\delta^{13}C$ of phytophagous nematodes (mean = −11.9‰) was similar to that of the maize plant (- 12.0‰) and did not vary between treatments (Fig. 1). For the microbial biomass and fungivorous and bacterivorous nematodes, the $\delta^{13}C$ values depended on input treatment (two-way ANOVA $P_{input} < 0.001$, $P_{organism \times input} < 0.001$). The microbial biomass $\delta^{13}C$ values were lower than those of phytophagous nematodes in all input treatments (Tukey $P \leq 0.018$) and lower than those of bacterivorous nematodes in MS and MS + F (Tukey $P \leq 0.043$) (Fig. 1). In MS, the $\delta^{13}C$ of bacterivorous nematodes was −15.4‰, which was significantly lower than the $\delta^{13}C$ of phytophagous nematodes (Tukey $P = 0.017$). The $\delta^{13}C$ values of bacterivorous nematodes were higher in MS + F and MS + B than in MS (Tukey $P \leq 0.020$) and reached a level similar to that of phytophagous nematodes. Likewise, microbial biomass $\delta^{13}C$ increased from −18.2‰ in the MS to −15.5‰ and −14.8‰ in MS + F and MS + B, respectively (Tukey $P \leq 0.032$).

In MS and MS + F, the abundances of fungivorous nematodes were low (Table 2), and we were not able to isolate the targeted 100 individuals for μEA-IRMS analysis. Although the isotopic ratio measurements in these two input treatments was based on samples ranging between 0.48 and 1.15 μg C, this amount of C is considerably higher than the 0.2 μg C determined in our blanks. The mean $\delta^{13}C$ of fungivorous nematodes in the MS and MS + F treatments (−21.6‰ and −22.7‰, respectively) did not differ significantly but were lower than $\delta^{13}C$ in the microbial biomass and the two other nematode feeding groups (Tukey $P \leq 0.030$). The $\delta^{13}C$ of fungivorous nematodes increased to −14.4‰ in MS + B but remained lower than that of phytophagous nematodes (Tukey $P = 0.039$).

3.3. Proportion of C4-derived C in microbial biomass and nematode trophic groups

Across the MS and MS + B treatments, maize-derived C did not contribute equally to the C in the different organismal groups (two-way ANOVA $P_{organism} < 0.001$, $P_{organism \times input} < 0.001$). For both treatments, phytophagous nematode biomass C was almost exclusively of maize origin (95–106%; Fig. 2). Across the two treatments, phytophagous nematodes held more maize-derived C than all other organismal groups (Tukey $P < 0.001$), the bacterivorous nematodes held larger fractions of maize-derived C than did the fungivorous nematodes and the microbial biomass (Tukey $P \leq 0.013$) (Fig. 2). Overall, a larger fraction of organismal biomass C originated from the recent maize inputs in MS + B than in MS (two-way ANOVA $P_{input} < 0.001$).

In MS, 46–54% of the microbial biomass-C originated from maize. This was significantly less (Tukey $P < 0.001$) than in MS + B, where maize-derived C accounted for 72–73% of microbial biomass-C (Fig. 2). Maize contributed 61–73% of the C in bacterivorous nematodes in MS, which was less than in the phytophagous nematodes (Tukey $P < 0.001$). Compared to MS, the maize-derived C accounted for a significantly larger fraction (70–92%) of bacterivorous nematode C in the MS + B treatment (Tukey $P = 0.002$).

Recent maize-derived C contributed only 24–38% to the biomass of fungivorous nematodes isolated from the MS treatment. This was less than for all other organismal groups (Tukey $P \leq 0.020$). In contrast, the fraction of maize-derived C in fungivorous nematode biomass in MS + B (74–80%) was comparable to the fraction in the microbial biomass, but significantly lower than that in phytophagous nematodes (Tukey $P < 0.001$).

4. Discussion

This is the first direct assessment of the contribution of differently aged SOC pools to individual nematode trophic groups based on differences in $^{13}C/^{12}C$ ratios attained *in situ* by conversion from C3- to C4-planted soil. Previously, values for stable N and C isotopes have been

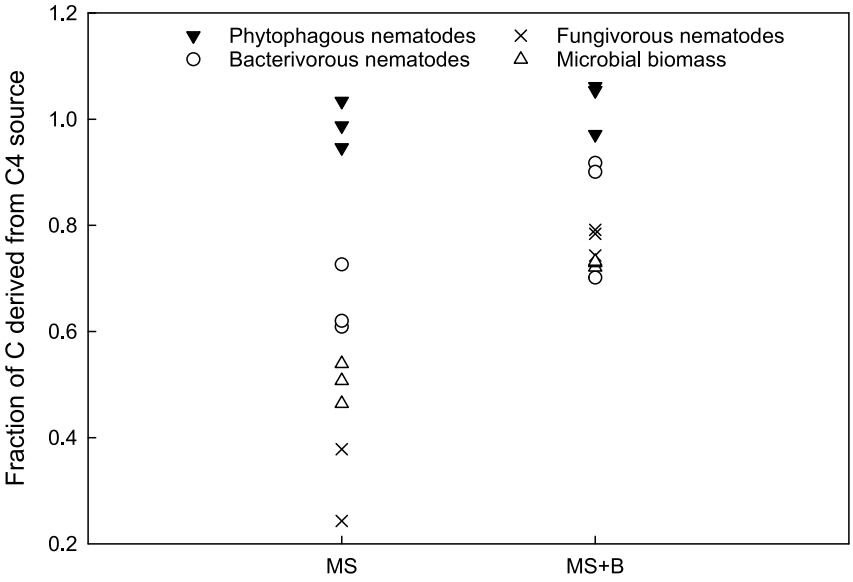

Fig. 2. Estimated fraction of C derived from C4 sources (maize-derived inputs) in chloroform-fumigated microbial biomass C and three nematode trophic groups in soils cropped exclusively with maize for 20 years. Soils were subjected to two different annual input treatments: MS, only maize roots and stubbles were incorporated; MS + B, addition of 0.8 kg DM m^{-2} chopped aboveground maize biomass.

reported for individual species (Neilson and Brown, 1999; Neilson et al., 2005) and taxa of mixed communities (Kudrin et al., 2015; Melody et al., 2016). Discrimination against the heavier ^{13}C isotope during microbial respiration may significantly increase the $^{13}C/^{12}C$ ratio of SOC when more decades are considered (Menichetti et al., 2015), but within the time-scale considered in the present study (20 years), the isotopic fractionation during soil C turnover can be considered as negligible (Christensen et al., 2011). Further, the magnitude of fractionation effect is about one order of magnitude smaller than the difference between C3 and C4 OM input and will thus not lead to substantial bias in our results.

For phytophagous nematodes isolated from the C4-soils and from the C3-reference soil, the $\delta^{13}C$ value closely resembled that of the food source plant (Fig. 1). Further, in the C3-reference soil, the $\delta^{13}C$ values of SOC, microbial biomass and microbivorous nematodes are almost identical and very close to that of the phytophagous nematodes (Table 2, Fig. 1). We are therefore confident that isotopic fractionation during soil C turnover and during nematode feeding (Potapov et al., 2018) did not compromise the validity of $\delta^{13}C$ values measured. However, to avoid the potential bias imposed by isotope fractionation we calculated the fraction of C4-derived C in microbial and nematode biomass as the difference between $\delta^{13}C$ values for the organism in C4 and C3 soils in relation to the difference in $\delta^{13}C$ values for the C4 and C3 plant inputs (see section 2.5). The $\delta^{13}C$ values for microbial biomass and nematodes in the C3 plot were not replicated, but they were very similar for the microbial biomass and microbivorous nematodes (varying between −26.4 and −25.5‰). Further, the $\delta^{13}C$ of the C3 soil remained constant at −27.5‰ during the 20 years (Thomsen and Christensen, 2010, Table 2). Hence, we are confident that the measured $\delta^{13}C$ values are good estimates of the C3 reference isotopic signature. If we assume that the −26.4 to −25.5‰ variation between decomposers in the C3 plot represent the variation within each group of organisms this would only infer a ± 0.05 range variation of the C4-derived fraction for the individual organismal groups. This limited variation would not affect our conclusions on patterns of recent and old SOC partitioning between the organisms.

With the current sensitivity of the μEA-IRMS methodology, $^{13}C/^{12}C$ ratio measurements of 0.48–14 μg nematode biomass C confirm the expected, i.e. that phytophagous nematodes rely on plant biomass exclusively. In the MS and MS + F treatments, the small numbers of fungivorous nematode gave samples with low C contents (0.48–1.15 μg C). Although this amount of C was higher than the 0.2 μg C in the blank samples used to correct measured $\delta^{13}C$ signals, we cannot exclude that background C contamination, although very small, may have influenced the isotopic signal measured for low-weight samples.

In general, our results show that microbivore nematodes feed on microbial biomass, which primarily originates from recent C pools; i.e. maize-derived C deposited within the past 20 years (Fig. 2). Hence, in the MS treatment with minimal incorporation of maize residues, maize-derived C still constituted c. 65% of the C in bacterivorous nematodes, and with the incorporation of chopped aboveground maize residues in the soil, averagely 84% of the C in this nematode group originated from recent maize input (Fig. 2). With c. 77% of their biomass C derived from maize residues, fungivorous nematodes in the MS + B treatment also relied primarily on OM deposited within the past 20 years. In the low-input MS treatment, the extremely low abundance of fungivorous nematodes (Table 2) and the lower contribution of C4-derived C in their biomass (26%) suggest that this group of nematodes starved and had to rely more on OM deposited more than two decades ago. The larger fraction of C4-derived C in bacterivorous nematodes than in microbial biomass (Fig. 2) agrees with our hypothesis that bacterial feeding nematodes feed mainly on microbial biomass engaged in the decomposition of more recently deposited SOM. Patches of fresh, labile OM residues support high microbial activity (Christensen et al., 1992) and are dominated by bacteria with high growth and respiration rates, which are likely to attract bacterivorous nematodes (Rasmann et al., 2012; Rønn et al., 2012; Liu et al., 2017). Further, a significant fraction of older SOM pools may reside in micro-aggregates within pores that are too small to allow nematode access to the associated bacteria (Jiang et al., 2018).

It is interesting that the $\delta^{13}C$ values of microbial biomass in MS and MS + F fall between the isotopic signatures of bacterivorous and fungivorous nematodes. This confirms that fungi as well as bacteria contribute to chloroform-labile microbial biomass. In MS + B where a higher abundance of fungivorous nematodes indicates enhanced fungal growth, the $\delta^{13}C$ of this group of nematodes closely matches that of the microbial biomass. This may reflect that fungi constitute the largest proportion of the chloroform-labile microbial biomass as previously suggested (Joergensen and Wichern, 2008). Whereas the bacterivorous nematodes attained 61–92% of their C from maize-derived sources (Fig. 2), C derived from OM deposited in the soil more than 20 years ago contributed more to microbial biomass and fungivorous nematodes. This supports our hypothesis that fungal-based decomposition is relatively more important for the turnover of old C than bacterial-based decomposition.

With mean densities of 0.2–3 individuals g^{-1} soil the densities of particularly fungivorous nematodes were low, and the highest total abundance of all nematode groups in MS + B reached 31 individuals g^{-1} soil, which is also considerably lower than the hundreds of nematodes g^{-1} recorded in microbial activity hotspots (Vestergård, 2004). Generally, nematode densities are low in temperate maize cropped systems (Glavatska et al., 2017; Čerevková et al., 2018). Further, we sampled in the spring six months after the last harvest and organic matter incorporation and before establishment of the next season's crop. The relatively low abundances thus reflect that the nematode communities were starved. The somewhat lower abundances of phytophagous nematodes in MS + F and MS + B than in MS probably reflect that enhanced plant productivity also increase plant capacity to resist phytophagous nematodes (Vestergård, 2004). As expected, the microbial biomass and populations of microbivorous nematodes were smallest in MS, where the annual input of fresh plant residues was restricted to stubbles and roots. Interestingly, although a similar quantity of additional organic input was added annually in the MS + F and MS + B treatments, only chopped aboveground maize residues significantly increased populations of bacterivorous and fungivorous nematodes, presumably reflecting a higher microbial production in MS + B than in MS + F. This agrees with earlier observations from this conversion experiment, which showed that a larger fraction of the C in maize biomass than in the faeces is readily mineralized (Thomsen and Christensen, 2010). It is expected that a large proportion of the labile substrate in the maize biomass is lost during passage through the sheep digestive tract. Thereby a smaller fraction of the OM added with sheep faeces is readily available for the soil decomposer food web and a larger fraction of the added C is retained in the more stable soil OM pool (Thomsen et al., 2013). Nevertheless, the notably higher $\delta^{13}C$ values recorded for the microbial biomass and bacterivorous nematodes in MS + F and MS + B than in MS (Fig. 2) shows that with high annual inputs of OM, the soil organisms base more of their biomass production on maize-derived OM than on OM added more than 20 years ago.

However, in the low-input MS treatment, c. 50% of the C in microbial biomass and c. 70% of the C in fungivorous nematodes originate from OM that was deposited in the soil more than 20 years ago (Fig. 2). This aligns with results obtained in recent studies, which show that old C is more dynamic than hitherto anticipated and that microbial recycling of old C continuously shuffles old C between recalcitrant SOM pools and the microbial biomass (Basler et al., 2015a, 2015b; Dippold and Kuzyakov, 2016). Likewise, a large proportion of earthworm and collembolan biomass originated form > 27 years old SOM in temperate arable soil (Scheunemann et al., 2010), which confirms that the soil food web organisms contribute to the turnover of old SOM (Eck et al., 2015). However, even exclusively soil feeding endogaeic earthworm biomass and termite biomass in temperate and tropical forest soils, respectively, originated from SOC with a soil residence time of less than a decade (Hyodo et al., 2006, 2012). Further research on C pool partitioning in soil food webs across ecosystem types and climatic gradients is thus necessary to reach firm conclusions on the significance of soil food webs on decomposition of old and recent SOC.

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