Natural ^{13}C abundance and carbon storage in Danish soils under continuous silage maize

S.M. Kristiansen^a, E.M. Hansen^a, L.S. Jensen^b, B.T. Christensen^{a,*}

^a Department of Agroecology, Danish Institute of Agricultural Sciences, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark

^b Department of Agricultural Sciences, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

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Abstract

Silage maize has replaced fodder beet as energy-rich winter forage for housed cattle. In Denmark silage maize now occupies more than 4% of the arable land, but little is known on the effect of continuous silage maize on soil carbon dynamics under NW European cropping conditions. Soil total-C and natural ^{13}C abundance were determined every 2–3 years in four Danish arable soils (0–20 cm) continuously cropped to maize for 14 years under identical climatic conditions. A set of soils was sampled after 11 years with maize and analysed for total-C and maize-derived C in >2 mm plant residues, in <2 mm soil, and in 250–2000 μm particulate organic matter (POM). Inputs of C in maize roots and stubbles induced an average annual increase in soil total-C of 90–470 kg C ha⁻¹. When soils were amended with an additional annual input of 8 t DM ha⁻¹ in chopped above-ground maize biomass, soil C storage increased 110–940 kg C ha⁻¹ per year. No changes in soil C/N ratio were observed. Carbon from maize roots and stubbles accumulated in the soil at an annual rate of 250–490 kg C ha⁻¹; with an additional annual input of 8 t DM ha⁻¹, the accumulation rate was 710–980 kg maize-C ha⁻¹. After 14 years with continuous silage maize, maize-derived C accounted for 7–18% of the total soil C; with additional maize biomass inputs, maize-C made up 18–31%. The retention in soil of the C added in above-ground maize biomass averaged 11–15%. The >2 mm maize residues accounted for 7–21% of all the maize-C retained in soil after 11 years of maize cropping. Between 17 and 41% of the maize-C found in <2 mm soil was associated with POM. The amount and composition of the POM reflected the intensity of maize residue inputs but POM did also include biologically recalcitrant organic matter derived from pre-maize vegetations. In Danish light-textured soils with moderate C concentrations and with a history of cereal dominated cropping, continuous silage maize was able to increase soil C storage. Changes in ^{13}C natural abundance showed that the accumulation of maize-C was substantial even in soil showing moderate changes in soil total-C.

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Keywords: Crop residues; $\delta^{13}\text{C}$; Particulate organic matter (POM); Sandy soil; Soil C sequestration

1. Introduction

The temperate, coastal climate in Denmark and other parts of NW Europe prevents maize (*Zea mays* L.) from reaching physiological maturity. Under these climatic conditions, maize is grown to provide

* Corresponding author. Tel.: +45-8999-1709; fax: +45-8999-1719.

E-mail address: bent.t.christensen@agrsci.dk (B.T. Christensen).

energy-rich silage that serves as winter forage for housed cattle. In this respect silage maize has now almost completely replaced fodder beet (*Beta vulgaris* L.). The area in Denmark with maize averaged 20,000 and 42,000 ha in the periods 1984–1993 and 1994–1999, respectively. By 2003, the area has increased to 118,000 ha (Pedersen, 2003) corresponding to 4.4% of the arable land, while that with beets has decreased from 130,000 ha in 1984 to 10,000 in 2002. This development has been driven mainly by an increased availability of maize cultivars better suited for whole crop harvest and silage production. Compared to beets, silage maize requires reduced inputs of pesticides and less management during crop growth. Further, maize crops carry more favourable economic subsidies.

The turnover of organic matter in soil is important in relation to soil quality and carbon sequestration (Christensen and Johnston, 1997). The level of soil carbon (C) is framed by site characteristics (climate and soil type) and management (crop sequence, residue disposal, inputs of fertilizers and manure, and tillage). The storage of C attained at a given site is determined by the balance between annual inputs of crop residues and other organic amendments and the mineralization rate of these inputs and of the organic matter already residing in the soil. Christensen (1990) reported annual losses of 320 and 362 kg C ha⁻¹ from the plough layer of an Askov sandy loam where fodder beet and silage maize, respectively, were grown in 4-year rotations with mineral fertilizers and removal of harvest residues. For crops relying on mineral fertilizers only, the retention in soil of crop residue C becomes crucial for the long-term maintenance of the soil C pool. However, little is known of the impact of silage maize on C storage and turnover in different soils subject to NW European cropping conditions.

The discrimination against the ¹³C isotope associated with photosynthesis results in depletion of ¹³C in plant biomass relative to atmospheric CO₂, the degree of depletion reflecting the type of photosynthetic pathway (Boutton, 1996). Plants with the Calvin cycle pathway (C3-plants) become distinctly lower in ¹³C than plants with the Hatch-Slack pathway (C4-plants). Traditional crops and native vegetation in cool temperate regions are all C3-plants, while warm-season grasses (e.g. maize) rely on the C4 photosynthetic pathway. Provided that isotopic fractionation during

C turnover is quantitatively insignificant, the soil organic matter derived from plant residues will carry a ¹³C signature characteristic of that residue type. The different ¹³C abundance in C3- and C4-plant residues thereby represents a valuable tool for studies on C turnover in soils where cropping with C3-plants has been replaced with C4-plants (Balesdent and Mariotti, 1996).

In this study, we examine total-C contents and ¹³C abundance in four soils continuously cropped with silage maize for 14 years. Soil was sampled from the 0–20 cm soil layer at the start of the experiment and subsequently every 2–3 years. The objective was to establish the direct contribution of maize residues to soil C storage in representative Danish arable soil types.

2. Materials and methods

2.1. Soils and climate

Bulk soil was collected in the autumn 1987 from the 0–25 cm layer of arable fields located at Lundgaard (LUN) and Askov (ASK) at 55°28'N, 09°07'E, Roskilde (ROS—55°54'N, 12°03'E), and Rønhave (RØN—55°28'N, 09°07'E) Experimental Stations. The four soils employed in this study represent the major arable soil types in Denmark. Their initial contents of soil C range from 10 to 25 mg C g⁻¹ dry soil and that of fine soil particles (<50 µm) range from 13 to 45% of soil dry weight. The pre-experiment cropping of the soils was dominated by cereals dressed with mineral fertilizers and crop residue removal upon harvest. While the LUN and ASK soils had never been subject to cropping with C4-plants, the ROS and RØN soils were sampled in fields which had been cropped with silage maize for 8 years before sampling. Due to lack of time-zero δ¹³C values, however, the amount of maize-derived C in the soil at sampling in 1987 could not be estimated reliably. Selected soil characteristics are shown in Table 1.

Upon sampling, the soils were transported to Askov Experimental Station, sieved to <4 cm, mixed, and placed outdoors in large open-ended cylinders (diameter 0.7 m; depth 0.5 m), that were inserted 45 cm into the ground. The soil was placed on top of the Askov clayey subsoil and during filling of the cylinders the soil was packed to regain its original density. The depth

Table 1

Selected soil characteristics of the Rønhave (RØN), Roskilde (ROS), Askov (ASK), and Lundgaard (LUN) soils, determined at the start of the experiments (1988)

Soil code	Texture class	Bulk density (g cm ⁻³)	Clay (<2 µm) (g kg ⁻¹)	Silt (2–50 µm) (g kg ⁻¹)	Fine sand (50–250 µm) (g kg ⁻¹)	Coarse sand (250–2000 µm) (g kg ⁻¹)	Soil total-C (g kg ⁻¹)	pH	C/N	δ ¹³ C (‰)
RØN	Sandy loam	1.30	176	269	386	168	14.8	6.6	10	–26.2 ^a
ROS	Sandy loam	1.27	137	263	368	231	14.6	7.4	11	–25.4 ^a
ASK	Sandy loam	1.12	141	212	306	340	24.7	6.4	13	–27.2
LUN	Sand	1.33	55	77	160	708	10.3	7.6	13	–27.2

Bulk density was determined in 1999. The pH value was measured in H₂O (1–2.5).

^a RØN and ROS soils had 8 years of maize cropping before sampling.

of the experimental soil layer (0–25 cm) was indicated in each cylinder by a coarse-meshed net. This ensured a fixed amount of soil into which added residues were incorporated (see later) and from which soil samples were drawn. Each soil type was represented by two or three cylinders.

Annual mean (1961–1990) temperature at Askov is 7.6 °C, the highest and lowest monthly means being 15.5 °C (July and August) and –0.2 °C (January and February). Annual mean precipitation is 862 mm and global radiation is 3384 MJ m⁻². During 15 April to 15 October (1971–1990 average) the Askov site accumulates 2400 Ontario Corn Heat Units (Brown and Bootsma, 1997), which is sufficient for silage maize to reach 30% dry matter before harvest in mid-October.

2.2. Agronomy

The first maize crop was planted in May 1988. The maize was sown every year when soil temperature had reached 10 °C. Following planting, the crop received mineral fertilizers, corresponding to 170–200 kg N ha⁻¹, 36–40 kg P ha⁻¹ and 190 kg K ha⁻¹. During the growth period, weeds were removed manually upon emergence. To avoid drought depression, water was added to the cylinders when prolonged dry periods were forecasted. The maize was harvested at growth stage BBCH 75 (Lancashire *et al.*, 1991), which was usually reached by mid-October. All above-ground plant biomass was removed (leaving 4 cm of stubbles), coarsely chopped, and subsamples were dried at 80 °C and stored. For each soil one cylinder was then amended with chopped fresh maize biomass (corresponding to 0.8 kg dry matter (DM) m⁻²), whereas the remaining cylinders received no additional inputs.

Three portions each of 10 l of soil were removed from every cylinder. Half of the maize biomass was then placed on the exposed soil surface and covered with the soil portion that contained the stubbles. The other half of the maize biomass was applied and covered with the remaining soil (treatment RSA). The remaining cylinders were subject to the same procedure except that maize biomass was not added (treatment RS). The cylinders were dug manually (to simulate ploughing) in early winter and then left undisturbed until seedbed preparation in late April. Over the course of the experiment, silage maize cultivars were changed whenever a variety was no longer available.

2.3. Sampling and sample preparations

Soil was sampled from the 0–20 cm layer in 1988, 1991, 1994, 1996, 1998, 2000 and 2002 in the spring before maize was planted. The soil samples were air-dried, sieved to <2 mm and stored until analyses.

In March 1999, a supplementary set of soil samples was retrieved with a stainless steel cylinder (diameter 30 cm, depth 20 cm). The soil was air-dried and sieved to <8 mm. Material left on the sieve (mainly maize residue fragments and gravel) was dried at 80 °C. The maize material was freed of soil particles and weighed. Soil passing the sieve was weighed, mixed and subsampled. One subsample was used for dry matter determination (105 °C for 24 h), the other was dried to constant weight and sieved to <2 mm. Material left on the 2 mm sieve was combined with the >8 mm maize residue to provide one >2 mm fraction for each treatment. Particulate organic matter (POM: 250–2000 µm) along with the corresponding sand fraction was isolated from <2 mm soil by wet sieving

(Gregorich and Ellert, 1993). Briefly, two portions of 37.5 g air-dry soil were each dispersed in 150 ml of a sodium hexametaphosphate solution (5 mg ml^{-1}) in 300 ml flasks. The suspension was shaken for 1 h at 40 rotations per minute, and then poured onto a $250 \mu\text{m}$ sieve. The POM and sand collected on the sieve was transferred to drying-pans, dried at 60°C to constant weight, and combined for the two soil portions.

2.4. Chemical analyses

All samples were analysed in 2000, except for the 2002 samples, which were analysed following sampling. The contents of total-C and total-N, and the $^{13}\text{C}/^{12}\text{C}$ isotopic ratio were determined by dry combustion of 1–30 mg dry and ball-milled subsamples. Samples from the period 1988–2000 were analysed by an ANCA-SL elemental analyser interfaced with a 20–20 isotope ratio mass spectrometer (Europa Scientific Ltd., Crewe, UK), while samples from 2002 were analysed by a Carlo-Erba 1108 interfaced with a Finnigan MAT Delta S mass spectrometer (Finnigan, Bremen, Germany). Chemical analyses were carried out in duplicate on each sample, and all results refer to 105°C oven-dry weight. The mean C content of the maize biomass amendments was 456 mg C g^{-1} dry matter (standard error of the mean: $\text{S.E.M.} = 1.8 \text{ mg C g}^{-1}$) and the C/N ratio 43. Accordingly, the annual addition of $0.8 \text{ kg biomass DM m}^{-2}$ corresponds to 365 g C m^{-2} .

Statistical analyses were mixed variance analysis on linear models of the four soils and the two residue treatments to allow test of temporal changes in the amount of soil total-C and maize-C. The statistical package SAS 8.2 (SAS Systems, Cary, NC, USA) and a 95% level of significance was adopted.

2.5. Calculations

By convention, the stable $^{13}\text{C}/^{12}\text{C}$ ratio is expressed in $\delta^{13}\text{C}$ (‰) units:

$$\delta^{13}\text{C}(\text{‰}) = \frac{R_{\text{samp.}} - R_{\text{stand}}}{R_{\text{stand}}} \times 1000$$

where $R_{\text{samp.}}$ is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and R_{stand} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the standard. The isotopic composition of the samples was calibrated against

the international PDB standard (Oztech Trading Co., USA). At a given site, the average $\delta^{13}\text{C}$ difference between C3 and C4 vegetation is approximately 15‰ (Balesdent and Mariotti, 1996).

When isotopic fractionation during C turnover is small compared to the difference in ^{13}C abundance in C3- and C4-plants, the fraction (X) of soil C derived from maize residues can be calculated by the equation (Balesdent and Mariotti, 1996):

$$X = \frac{\delta^{13}\text{C}_{\text{samp.}} - \delta^{13}\text{C}_{\text{soil}-0}}{\delta^{13}\text{C}_{\text{maize}} - \delta^{13}\text{C}_{\text{soil}-0}}$$

where $\delta^{13}\text{C}_{\text{samp.}}$ is the $\delta^{13}\text{C}$ of whole soil, POM ($250\text{--}2000 \mu\text{m}$) or maize residue ($>2 \text{ mm}$), $\delta^{13}\text{C}_{\text{soil}-0}$ is the $\delta^{13}\text{C}$ value of the soil sample from 1988 when the experiment was started, and $\delta^{13}\text{C}_{\text{maize}}$ is the $\delta^{13}\text{C}$ of maize biomass added to the cylinders. The average $\delta^{13}\text{C}$ value of the maize biomass added over the experimental period was -10.0‰ (range -9.3 to -10.7‰ ; $\text{S.E.M.} = 0.05\text{‰}$).

3. Results

Soil total-C levels increased during the 14 years with maize (Fig. 1). The exception was the ASK soil with the highest initial soil C content in which soil C remained almost constant. Applying a simple linear regression analysis ($y = ax + b$; where y = soil total-C and x = number of years), the mean annual increase in soil C ranged from 36 to 47 g C m^{-2} in the LUN, ROS and RØN soils without addition of above-ground biomass C (Table 2, treatment RS). When the soils received an additional input of above-ground maize biomass (treatment RSA), the LUN, ROS and RØN soils experienced an annual increase of 53 to 94 g C m^{-2} . The additional input of maize-C did not affect the soil C level in the ASK soil.

Initially the C/N ratio of the soil organic matter residing in the RØN, ROS, ASK and LUN soils was 10, 11, 13 and 13, respectively (Table 1), and these ratios did not change significantly over the course of the experiment, regardless of residue treatments (data not shown).

The initial (1988) $\delta^{13}\text{C}$ values were similar for ASK and LUN soils (-27.2‰) and typical for soils maintained under C3-crops for long periods (Table 1). The

Fig. 1. Total-C and soil maize-C in Rønhave (RØN), Roskilde (ROS), Askov (ASK), and Lundgaard (LUN) soils during 1988–2002. RS denotes treatments with incorporation of maize roots and stubbles only, while RSA denotes treatments with an additional annual input of 0.8 kg DM m^{-2} in chopped above-ground maize biomass. See Table 2 for data on linear models.

RØN and ROS soils had a somewhat higher ^{13}C abundance (-26.2 and -25.4% , respectively), reflecting the maize cropping that preceded the sampling in 1987 of these two soils.

Table 2

Mean annual change in soil total-C and maize-C in Rønhave (RØN), Roskilde (ROS), Askov (ASK), and Lundgaard (LUN) soils during the 14 years of maize growing with (RSA) and without (RS) addition of 0.8 kg DM m^{-2} per year in above-ground maize biomass

Soil code	Mean annual change (g C m^{-2} per year)			
	Soil total-C		Maize-C	
	RSA	RS	RSA	RS
RØN	94 aB	36 aB	98 aB	49 aB
ROS	53 aA	41 aA	76 aB	33 aB
ASK	11 bA	9 aA	78 aA	25 bA
LUN	74 aA	47 Aa	71 aA	33 aA

Changes were estimated by linear models ($y = ax + b$, where y is soil total-C or maize-C and x is numbers of years). Small letters (a, b) denotes significant ($P = 95\%$) differences between soil types within a treatment, and capital letters (A, B) denotes significant differences between treatments for a given soil type.

Fig. 2. The natural abundance of ^{13}C ($\delta^{13}\text{C}\%$) in RØN, ROS, ASK and LUN soils during 1988–2002. See Fig. 1 for further legend.

Silage maize cropping led to an increase in the ^{13}C abundance in all four soils (Fig. 2), the steepest increase being observed in treatments with additional maize biomass inputs (treatment RSA). The development in $\delta^{13}\text{C}$ values appeared to be little influenced by soil texture and previous cropping, although the ASK soil with the highest initial soil total-C content increased least in $\delta^{13}\text{C}$ in response to maize cropping. When considering the storage in the soils of maize-derived C (Maize-C, Fig. 1; Table 2), the average annual increase in maize-C was 25, 33, 33 and 49 g C m^{-2} in the ASK, LUN, ROS and RØN soils, respectively. When comparing the four soil types, only the ASK soil deviated significantly. The RØN soil retained more total-C than the three other soils. Compared with LUN and ASK soils, the ROS and RØN soil accumulated slightly more maize-C regardless of maize residue input levels. Thus during the 14 years of silage maize, the rhizodeposition in the growth period, the roots and the stubbles together left $350\text{--}686 \text{ g C m}^{-2}$ in the soil. With an extra annual input of 365 g C m^{-2} in above-ground maize biomass, the maize-derived C retained in soil after 14 years accounted for $994\text{--}1370 \text{ g C m}^{-2}$. In 2002, the maize-derived C in the soil accounted for 7–18%

Table 3

Content of total-C and maize-C in soil fractions isolated from the 1999 samples of Rønhave (RØN), Roskilde (ROS), Askov (ASK) and Lundgaard (LUN) soils

Soil code	Maize residues (a; >2000 μm)		POM (b; 250–2000 μm)		Soil (c; <2000 μm)		a + c	
	RSA	RS	RSA	RS	RSA	RS	RSA	RS
Soil total-C (mg C g ⁻¹ d.w.)								
RØN	181	141	9.6	7.8	17.7	16.1		
ROS	149	133	6.1	4.7	16.6	14.8		
ASK	133	121	8.7	6.2	30.3	26.7		
LUN	170	160	2.6	1.9	13.5	11.1		
C/N ratio								
RØN	52	46	21	22	10	10		
ROS	46	41	20	20	10	11		
ASK	37	37	19	18	14	14		
LUN	49	43	19	16	13	12		
$\delta^{13}\text{C}$ (‰)								
RØN	−13.0	−13.1	−15.6	−16.7	−21.7	−23.6		
ROS	−12.9	−13.2	−16.0	−18.0	−21.5	−23.6		
ASK	−14.1	−14.6	−17.9	−20.9	−24.1	−26.0		
LUN	−12.8	−13.1	−19.0	−22.1	−22.3	−24.2		
Soil total-C (g C m ⁻²)								
RØN	120	110	350	290	4600	4220	4720	4330
ROS	120	120	320	240	4110	3830	4230	4350
ASK	150	150	550	470	6410	6310	6560	6460
LUN	150	120	390	310	3490	2990	3640	3110
Maize-C (g C m ⁻²)								
RØN	90	90	230	170	1280	680	1370	770
ROS	80	90	190	110	1040	450	1120	540
ASK	110	110	300	170	1120	410	1230	520
LUN	120	100	190	90	990	520	1110	620
Maize-C (% fraction of total-C)								
RØN	81	81	65	59	28	16		
ROS	81	79	61	48	25	11		
ASK	76	73	54	36	18	7		
LUN	84	82	48	29	29	17		
Distribution of maize-C (%)								
RØN	7	12	17 (18) ^a	22 (25)	93	88	100	100
ROS	7	17	17 (18)	20 (24)	93	83	100	100
ASK	9	21	24 (27)	33 (41)	91	79	100	100
LUN	11	16	17 (19)	15 (17)	89	84	100	100
Mean	8	16	19 (21)	22 (27)	92	84		

POM denotes particulate organic matter (250–2000 μm). RS denotes treatments with inputs of roots and stubbles only while RSA denotes treatments with an additional annual input of 0.8 kg DM m⁻² in above-ground maize biomass. The <2000 μm (c) soil includes POM (b).

^a Numbers in parenthesis are the POM fraction in percentage of the <2000 μm soil ((b/c) $\times$ 100).

of the soil total-C (Fig. 3; treatment RS). When soils received an additional input of maize biomass (RSA), maize-C made up 18–31% of the soil total-C.

The cumulated input of C added in above-ground chopped maize biomass was related to its contribu-

tion to the soil C pool by subtracting the amount of maize-derived C in soil with inputs of root and stubble C only (RS) from treatments where additional maize biomass was added each year (RSA). A simple linear model ($y = ax$; y = soil C derived from inputs of

Fig. 3. The proportion of maize-derived C in RØN, ROS, ASK and LUN soils during 1988–2002. See Fig. 1 for further legend.

above-ground maize biomass, and x = amount of C added in the biomass) was fitted to the data (Fig. 4) and showed that the retention in soil of above-ground maize-C from this source ranged from 11% in the sand soil (LUN) to 14–15% in the sandy loams (ASK, ROS, RØN). The retention of maize was only slightly affected by initial soil total-C contents levels and cropping history. Assuming that a similar fraction of the C from maize roots and stubbles was retained in the soil, the annual C inputs from these sources to soil total-C were estimated to 180–322 g C m⁻², least in the ASK soil and most in the RØN soil.

The residues (>2 mm) isolated from the March 1999 soil samples showed a relatively high C/N ratio (37–52) and a $\delta^{13}\text{C}$ value of -12.8 to -14.6‰ (Table 3). It was calculated that 73–84% of the C in the >2 mm fraction could be ascribed to maize-derived C. The amount of maize-C residing in the fraction was 80–120 g C m⁻², corresponding to 7–11% of all maize-derived C retained in treatments where above-ground maize biomass had been added. In treatments without additional maize-C inputs, the >2 mm particles accounted for 12–21% of the maize-derived C. The POM fraction isolated from <2 mm soil collected in the spring 1999 showed intermediate C/N ratios and $\delta^{13}\text{C}$ values (Table 3). It was calcu-

Fig. 4. The retention in RØN, ROS, ASK and LUN soils of maize-C derived from above-ground maize biomass as a function of the cumulated input of C from this source. The linear model is $y = ax$, where a is the average retention of maize-C as a proportion of the input.

lated that 29–65% of the C in POM originated from maize.

4. Discussion

4.1. Evaluation of methodology

Studies addressing the effects of soil type on medium- to long-term retention of maize-derived C have been based primarily on analyses of soils sampled from different locations (e.g. Liang et al., 1998; Collins et al., 1999), whereby effects of soil and climate become confounded. The present experiment was designed (1) to allow the effect of soil type to be studied under identical weather conditions, eliminating any potential effects of soil-climate interactions on plant growth and C turnover in soil, and (2) to ensure that the various soils were under the same, well-defined management throughout the experimental period. This is advantageous compared to studies based on chronosequences of differently sited soils.

One distinct advantage of the present study is the availability of time-zero $\delta^{13}\text{C}$ values (1988) for each individual soil. When initial $\delta^{13}\text{C}$ values are not available, a reference site where the original C3-vegetation and the soil C level is assumed to have remained unchanged has to be located and sampled. However, significant spatial variations in soil C content and $\delta^{13}\text{C}$ may occur due to gradients in moisture, temperature and other parameters that regulate plant growth and litter decomposition (Van Kessel et al., 1994; Boutton, 1996). Thus Balesdent and Mariotti (1996) considered that the use of a non-representative reference soil may introduce a bias as high as 3‰ due to short-range site variations in $\delta^{13}\text{C}$.

Another key issue associated with the use of natural ^{13}C abundance to study the turnover of plant residue C in soil is the degree of isotopic fractionation during the decomposition processes. It is well documented that different plant components differ in their ^{13}C signature, notable features being the ^{13}C enrichments of easily-decomposable carbohydrates and the depletion in ^{13}C of the slowly-decomposing lignin fraction (e.g. Benner et al., 1987; Schweitzer et al., 1999). While the isotopic heterogeneity of plant residues and the isotopic fractionation during residue decomposition remain of importance in studies of short-term C turnover (Kristiansen et al., 2004), it is generally recognized that these sources of error are less important in studies concerned with longer-term C turnover in soil (Balesdent and Mariotti, 1996; Boutton, 1996; Ehleringer et al., 2000). Thus the duration of the experiment becomes decisive for the accuracy that can be obtained with the natural ^{13}C abundance methodology. In the present study we traced the changes in soil total-C and $\delta^{13}\text{C}$ from the time of change to maize cropping by eight sampling times over a 14-year period. This provides a time series of data, that balances the basically unreplicated nature of the present experimental facility.

4.2. Changes in soil total-C

Previous studies on the effect of continuous maize cropping show both positive and negative trends in soil total-C levels (e.g. Balesdent et al., 1990; Liang and MacKenzie, 1992; Angers et al., 1995; Liang et al., 1998; Clapp et al., 2000; Ludwig et al., 2003). Main factors influencing soil C levels appear to be the ini-

tial soil C concentration, and the quantity of maize biomass returned to the soil. For maize grown to physiological maturity in Indiana and Iowa, USA, Larson et al. (1972) and Barber (1979) found soil C contents to be linearly related to the quantity of residues returned after harvest. In contrast, Clapp et al. (2000) reported little effect of harvest residues in a grain maize experiment in Minnesota, USA, where residue removal and incorporation by ploughing were tested over a 13-year period. Although inputs of maize biomass are considered to be higher for grain maize than for silage maize (Bolinder et al., 1999), there appears to be no systematic differences between silage and grain maize in their effect on soil total-C. However, net changes in soil total-C storage provide little information on the retention of maize-derived C in soil, but merely reflect the overall balance between formation and decay of soil organic matter.

We found mean annual increases in soil total-C ranging from 90 kg C ha⁻¹ in the sandy loam soil with the highest initial soil C content (ASK) to 470 kg C ha⁻¹ in the coarse sand soil (LUN). With an additional annual input of 3650 kg C ha⁻¹ in above-ground maize biomass, annual increases in soil C storage ranged from 110 kg C ha⁻¹ in the ASK soil to 940 kg C ha⁻¹ in the most clayey sandy loam (RØN). It appears that maize cropping increases soil total-C storage except on soil with a high initial C content. In line with results of Clapp et al. (2000), the ASK and ROS soils showed little response in C storage when amended with additional maize biomass.

4.3. Maize-C in soil fractions

The >2 mm residue particles isolated by dry sieving were found to account for 7–21% (80–120 g C m⁻²) of the maize-C present in the soils sampled in March 1999. While the C/N ratio of this fraction reflected that of the fresh maize biomass incorporated in the previous autumn, the $\delta^{13}\text{C}$ and the total-C content were substantially reduced. This is ascribed to leaching and turnover of N-depleted, easily-decomposable and ^{13}C enriched carbohydrates (Boutton, 1996), and to the presence of soil particles depleted in ^{13}C and total-C. Even at this early stage of decomposition, the maize residues were already intimately associated with soil particles that could not be removed by the relatively gentle clean-up procedure applied in the present study.

It is recalled that soils at all other samplings were sieved to <2 mm before being subject to analyses. Consequently the >2 mm particles do not contribute to estimates of total-C and maize-C in soils sampled regularly at 2–3-year intervals.

In contrast, the 250–2000 μm POM is an integrated part of the <2 mm soils. The POM fraction constitutes a transitory pool between plant residues and mineral-associated organic matter (Christensen, 1992; Gregorich and Janzen, 1996). Its mean turnover rate is slower than that of recently incorporated plant biomass but faster than that of organic matter in silt and clay sized organomineral complexes (Balesdent, 1996). This is reflected in the intermediate C/N ratio and $\delta^{13}\text{C}$ value of POM (Table 3). Of the total-C residing in POM, between 29 and 65% was estimated to be derived from maize, suggesting that POM encompasses a significant quantity of C originating from previous C3-crops (Golchin et al., 1995; Cadish et al., 1996). The POM in the two soils which had been cropped to maize before the present experiment (RØN and ROS) contained a larger proportion of maize-C than the POM isolated from the ASK and LUN soils with no history of C4 crops. For each soil, the treatment taking additional inputs of maize biomass had more maize-POM than treatments receiving only roots and stubbles, regardless of pre-experiment cropping history. These results illustrate that the size and composition of soil POM may reflect the frequency and level of plant residue inputs.

In accordance with previous studies on continuous maize cropping and C turnover in soil (e.g. Angers et al., 1995; Gregorich et al., 1996), POM accounts for a substantial fraction of the maize-C retained in <2 mm soil. The POM fraction held 17–25% of the maize-C residing in the RØN, ROS and LUN soils after 11 years of maize cropping (Table 3). The ASK soil, which had a higher total-C content, retained more of its maize-C as POM (27–41%). Except for this soil, differences between soil types in the distribution of maize-C were small and inconsistent. However, estimates of maize-C in POM, based on ^{13}C abundance, should always be interpreted with some caution. The shorter-term C turnover of this transitory organic pool may well be significantly affected by the isotopic heterogeneity of substrates and by the isotopic fractionation associated with microbial activity. Generally, estimates of the proportion of maize-C in

POM are based on $\delta^{13}\text{C}$ values established for fresh maize biomass inputs because the exact $\delta^{13}\text{C}$ of the partly decomposed maize-POM remains unknown.

4.4. Retention of maize-C in soil

The proportion of soil total-C (0–20 cm) originating from maize roots and stubbles made up 7–18% after 14 years of continuous cropping, corresponding to 3.5–6.9 t C ha⁻¹. Similar results have been reported from other locations. In soil cropped to silage maize for 11–15 years (Quebec, Canada), maize-C made up 7% of the soil total-C equivalent to 3.6–4.5 t maize-C ha⁻¹ (Angers et al., 1995; Bolinder et al., 1999). Flessa et al. (2000) found that maize-C storage was 5.4 t C ha⁻¹ (14% of the soil total-C) after 37 years of continuous silage maize (Halle, Germany). It is generally found that maize-C retained in the plough layer (upper 20–30 cm) accounts for 75–90% of the whole profile maize-C (Balesdent et al., 1990; Gregorich et al., 1996; Bolinder et al., 1999; Flessa et al., 2000).

The input to the soil of maize plant residues is normally higher when maize is grown for grain harvest. Various harvest residues may provide an annual input of 5–15 t DM ha⁻¹ (Liang et al., 1998; Collins et al., 1999; Clapp et al., 2000). A treatment with an additional annual input equivalent to 8 t DM ha⁻¹ in fresh above-ground maize biomass was included in the present study. For this treatment we found 9.9–13.7 t maize-C ha⁻¹ in the 0–20 cm soil, corresponding to 18–31% of the soil total-C. We were only able to reveal minor, but significant, systematic differences between the four soil types in their ability to store maize-C. Our results agree with studies on maize-C storage in soils under grain maize with incorporation of harvest residues. Thus, Clapp et al. (2000) found 9.5–16.0 t maize-C ha⁻¹ after 13 years of cropping to grain maize and Balesdent et al. (1990) recorded 8–12 t maize-C ha⁻¹ after 17 years of cropping to grain maize.

The average retention in the soil of C added in above-ground maize residues ranged from 11 to 15% (Fig. 4). Similar retentions have been estimated for grain maize harvest residues in some experiments of comparable duration (Liang et al., 1998; Bolinder et al., 1999; Collins et al., 1999), while others (Angers et al., 1995; Clapp et al., 2000) reported that 21–37%

of the residue input was retained in soil after 11–13 years of continuous maize. However, the retention of residue C in soil reported in the literature is often flawed by crude estimates of the amount of C returned in harvest residues, roots and stubbles.

The main difference between silage maize and grain maize in terms of maize-C storage in soil is apparently related to differences in the amount of crop residues returned to soil. Any effects of differences in residue composition, in soil texture and soil C level, and in climatic conditions appear to be of secondary importance during the initial 10–15 years of maize cropping. In this phase, the rate of maize-C storage is almost linearly related to the number of years with maize (Fig. 1; Liang et al., 1998; Clapp et al., 2000; Ludwig et al., 2003). In the longer term, however, the rate of increase in maize-C storage in soil is expected to level off and the cropping system is assumed to approach a steady state in soil C turnover. In this later phase, soil type and climate are considered to determine the level of maize-C eventually reached under a given crop and soil management system. Apparently, relatively long periods of continuous maize cropping are required before an equilibrium in soil C storage is attained.

5. Conclusions

Continuous cropping to silage maize is able to increase soil total-C in light-textured soils with moderate C concentrations, irrespective of soil texture and cropping history. The main factors affecting soil total-C appear to be the amount of residue returned to the soil and initial soil C content. With an initial high soil C content, maize cropping does not cause a further increase in soil C regardless of residue input levels. Soil C/N ratio remained constant during maize cropping.

Particulate organic matter (POM, 250–2000 μm) accounted for a substantial proportion (17–41%) of the maize-C left in <2 mm soil. POM reflected the frequency and level of maize residue inputs, but POM most likely also encompasses a substantial fraction of biologically recalcitrant organic particles.

After 14 years of continuous cropping to silage maize, C derived from roots and stubbles accounted for 7–18% of the soil total-C found in the <2 mm plough layer soil (0–20 cm). There were no systematic differences between soils in their ability to store maize-C.

Retention of maize-C in soil appears to be similar for above-ground residue from silage and grain maize. After 14 years, 11–15% of the C that had been added in above-ground maize biomass remained in the soil.

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