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**Crop residue management and oxalate-extractable iron and aluminium explain long-term soil organic carbon sequestration and dynamics**

P. VAN DE VREKEN<sup>a,b</sup>, A. GOBIN<sup>b</sup>, S. BAKEN<sup>a</sup>, L. VAN HOLM<sup>a</sup>, A. VERHASSELT<sup>c</sup>, E. SMOLDERS<sup>a</sup> & R. MERCKX<sup>a</sup>

<sup>a</sup> KU Leuven - University of Leuven Department of Earth and Environmental Sciences, Kasteelpark Arenberg 20 box 2459, 3001 Leuven, Belgium, <sup>b</sup> Flemish Institute for Technological Research, Boeretang 200, 2400 Mol, Belgium, and <sup>c</sup> Interuniversity Institute for Biostatistics and statistical Bioinformatics, Universiteit Hasselt, Martelarenlaan 42, 3500 Hasselt, Belgium

Correspondence: P. Van De Vreken. E-mail: [philippe\\_van\\_de\\_vreken@hotmail.com](mailto:philippe_van_de_vreken@hotmail.com)

*Running title: Carbon sequestration in contrasting soil types*

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## 22 **Highlights**

23

24 • Different factors affect carbon stocks under two contrasting soils and four cropping systems

25 • Returning maize residues promotes substitution of native soil organic carbon by maize-derived  
26 carbon

27 • Organo-mineral interactions in the silt and clay fraction enhance carbon sequestration

28 • The legacy of historic plaggen manuring has a strong effect on native soil carbon stocks

29

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## Summary

The management of crop residues affects carbon (C)-sequestration. This study aimed to identify the interaction between residue management and soil properties on C-sequestration. The hypothesis was that larger silt and clay contents and larger residue inputs enhance C-sequestration. The soil was sampled in Belgium in long-term ( $\geq 15$  years) cropping systems with either grain maize, *Zea mays* L. (all stover (leaves and stalks) returned), silage maize (all stover removed) or permanent grass. The fields sampled were distributed over two adjacent regions; one with sandy soil (33% silt+clay) and one with silty loam soil (71% silt+clay). The  $^{13}\text{C}$  abundance of the soil organic carbon (SOC) revealed that topsoil (0–30 cm) under grain maize contained more maize-derived SOC than that under silage maize ( $14 \pm 1$  and  $9 \pm 1$  Mg C ha $^{-1}$ , respectively,  $P < 0.001$ , in sand, and  $17 \pm 1$  and  $14 \pm 1$  Mg C ha $^{-1}$ , respectively,  $P < 0.001$ , in silty loam). Total SOC stocks were unaffected by crop management, however, which suggests substitution of native SOC by fresh SOC derived from residues, especially in sandy soil where the silt and clay fraction is saturated with SOC. The maize-derived SOC stocks of the silty loam soil are 3.5 to 5.5 Mg C ha $^{-1}$  larger than those of the sandy soil, which confirms the larger potential of the former to sequester C. Surprisingly, the native C<sub>3</sub>-SOC stocks in the sandy soil were 25 to 30 Mg C ha $^{-1}$  larger than those of the silty loam soil. The concentration of SOC in the silt and clay fraction was on average three times larger for the sandy than the silty loam soil. The concentration of oxalate-extractable Al and Fe in the same fraction was on average 2.5 times larger for sandy than for silty loam soil. Silt and clay associated SOC correlated with the Fe+Al concentration across all regions and treatments ( $R^2=0.62$ ). This suggests that organo-mineral associations and the formation of organo-metal complexes promote C-sequestration in this temperate region.

## Introduction

Crop residues are an important source of soil organic matter in agro-ecosystems, but the increasing demand for biomass for renewable energy might reduce residue returns and therefore soil organic carbon (SOC) stocks. The cultivation of maize (*Zea mays* L.) for biogas can reduce fossil energy related CO<sub>2</sub> emissions considerably (Gerin *et al.*, 2008). However, the sustainability of residue removal is unknown in relation to maintaining SOC content. Larson *et al.* (1972) and Rasmussen *et al.* (1980) suggested that change in SOC is related linearly to the amounts of residues returned to a soil irrespective of their type. In contrast, Campbell *et al.* (1991) could not demonstrate that long-term straw removal reduced SOC compared to straw retention and incorporation. The available studies on maize are inconclusive. Reicosky *et al.* (2002) found no significant difference in SOC between the removal of stover (leaves and stems) or its return to the soil after 30 years of maize monoculture, whereas other studies (Robinson *et al.*, 1996; Dick *et al.*, 1998) found a significant increase in SOC when stover was returned to the soil.

Soil properties and management affect C-sequestration and might obscure the effect of crop residue management. Wilhelm *et al.* (2004) concluded that changes in SOC are proportional to the amount of crop residue returned, but that tillage affects the actual retention of SOC. Both silage and grain maize are often grown in long-term monoculture systems and will, therefore, affect the soil's capacity to sequester carbon. The decline in SOC was reported to be faster under continuous maize than under maize in rotation (Odell *et al.*, 1984; Huggins *et al.*, 1998). Robinson *et al.* (1996) attributed this to the smaller quantities of residue returned to

the soil and a greater tillage intensity under maize monoculture. The silt and clay contents might affect the role of crop residue input on C-sequestration because they affect the carbon saturation of soil (Six *et al.*, 2002). Another soil property that might determine the retention of C is the concentration of Al and Fe. Amorphous Al and Fe oxyhydroxides have been isolated from a variety of soil types, in particular from podzolic soil (Adams & Kassim, 1984; Evans & Wilson, 1985; Blaser *et al.*, 1997) for which positive correlations with SOC have been shown.

In this study we aimed to elucidate the effect of crop residue management and soil properties such as texture and metal concentration on C-sequestration; our hypothesis was that larger silt and clay contents facilitate greater C-sequestration when the annual return of residues is increased. We also hypothesized that a larger concentration of amorphous Al and Fe in the silt and clay fraction increases the soil's capacity to sequester C irrespective of soil type. Functional relations were sought, therefore, between soil organic carbon and either silt and clay content or the concentration of oxalate-extractable Al and Fe. The soil was sampled in fields that had been cropped for at least 15 years with either grain maize (all stover returned), silage maize (all stover removed) or permanent grass from adjacent regions with sandy soil and silty loam soil. We measured  $^{13}\text{C}$  natural abundance to identify the processes involved. This technique has been used repeatedly to establish the relative proportion of  $\text{C}_3$ - and  $\text{C}_4$ -crops to total SOC, and to gain insight into SOC turnover in long-term experimental fields after the introduction of maize monoculture (Balesdent *et al.*, 1987; Clapp *et al.*, 2000; Wilts *et al.*, 2004; Hooker *et al.*, 2005). Former studies were done on one or at best a few experimental sites, whereas we adopted a regional scale perspective by sampling 70 farmers' fields. Stable isotopes were measured both in the soil and in several SOC fractions.

Fractionation of soil samples enabled us to determine the extent to which both C<sub>4</sub>- and C<sub>3</sub>-SOC were protected against mineralization.

## **Materials and methods**

### *Selection of the fields*

Soil samples were obtained from 70 fields in Flanders (the northern part of Belgium), distributed over two adjacent regions: one with a sandy soil (40 fields) and the other with a silty loam soil (30 fields). The soil of each field was classified according to World Reference Base (WRB) by determining the reference soil groups (RSGs) for the geographic centroids of the fields (Dondeyne *et al.*, 2014; IUSS Working Group WRB, 2014). Classification names along with other major soil properties are provided in the supporting information (Table SI-2). The fields were selected from a crop geodatabase developed to reconstruct the cultivation history between 1997 and 2013 for about 500 000 agricultural fields in Flanders. The development of the crop geodatabase and the selection criteria for the fields are described in the supporting information, sections S1 and S2. The cropping systems were in place for at least 15 (sandy region) or 17 years (silty loam region). All maize fields have a level topography (confirmed by a digital elevation model) to minimize soil erosion. Four long-term cropping systems were investigated: (i) silage maize monoculture (SM) where all stover is harvested with short stubble only remaining, (ii) silage maize monoculture followed by grass, mainly Italian ryegrass (*Lolium multiflorum* Lam.), sown directly between the maize stubble by minimum or zero tillage and ploughed the next spring after a one-cut harvest (SMGRA), (iii) grain maize monoculture (GM) where all stover is returned because the grain only is harvested and (iv) permanent grass (pasture, PA) with a history of C<sub>3</sub>-vegetation only.

Forty fields (ten fields each for GM, SM, SMGRA and PA) were selected from the crop geodatabase in the northern part of Flanders. This region is characterized by a sandy to loamy sandy soil texture and was dominated by heathland until the end of the 18<sup>th</sup> century (de Ferraris, 2009). Because the region is dominated by intensive animal husbandry, the focus is on fodder production. Another 30 fields (ten fields each for GM, SM and PA; no SMGRA) were selected in the silty loam region in the southern part of Flanders. Agriculture in this region is characterized by mixed farming that combines arable cultivation with livestock farming. Within each region all fields were selected in a completely random way.

#### *Soil sampling*

All 40 fields in the sandy region were sampled between the end of November 2011 and mid-March 2012. The 30 fields in the silty loam region were sampled in February and March 2014. Each field was sampled following a slightly modified version of the ‘area-frame randomized soil sampling’ (AFRSS) protocol (Stolbovoy *et al.*, 2007). Briefly, the number of sampling sites per field depended on the area of the field and their location was determined by projecting a grid of random numbers over the field (detail is in the supporting information, section S3). For each sampling site nine subsamples were taken with an auger of 3.5-cm diameter that were mixed together to form a composite sample. The soil was sampled to a depth of 30 cm. The upper 30 cm of soil under PA was sampled in intervals of 10 cm (i.e. 0–10 cm, 10–20 cm and 20–30 cm). In contrast, for soil under maize a single sample was taken over the 0–30-cm layer because it is considered homogenous from the annual tillage. For every composite soil sample an undisturbed soil core was taken by a soil corer (with both a diameter and height of five cm) and a Kopecky sampler, after pre-digging with an Edelman auger. For the soil under maize the core was taken between 12.5 and 17.5 cm depth in the



profile. For the soil under permanent grass a separate core was taken for each of the three 10-cm layers sampled.

#### *Measurements on bulk soil samples*

Details of soil sample preparation are provided in the supporting information, section S4. Soil organic carbon and nitrogen contents were determined with an Elemental Analyzer (Thermo Scientific Flash HT EA, Waltham, MA, USA) with a thermal conductivity detector (furnace temperature of 1070 °C). The measurement error was determined from five repeated measurements of ten different samples (with both small and large percentages of SOC); it was 0.01%. The SOC contents (g C 100 g<sup>-1</sup> soil, or %C) were then converted to SOC stocks (Mg C ha<sup>-1</sup>).

The <sup>13</sup>C/<sup>12</sup>C ratio of SOC was determined with a Thermo Scientific, Delta V Advantage Isotope Ratio MS coupled with the EA through ConFlo IV (Thermo Scientific, Waltham, MA, USA). The carbon isotope ratio was converted to δ<sup>13</sup>C by

$$\delta^{13}\text{C} = \left( \frac{R_{\text{sam}}}{R_{\text{std}}} - 1 \right) \times 10^3, \quad (1)$$

where  $R_{\text{sam}}$  is the <sup>13</sup>C/<sup>12</sup>C ratio of the sample and  $R_{\text{std}}$  is the <sup>13</sup>C/<sup>12</sup>C ratio of the Vienna Pee Dee Belemnite standard. The fraction,  $f$ , of maize-derived carbon (C<sub>4</sub>-carbon) in a soil sample, expressed as a percentage of the total SOC, is given by

$$\delta_s = f \cdot \delta_m + (1 - f) \cdot \delta_g, \quad (2)$$

where  $\delta_s$  is the δ<sup>13</sup>C value determined on the soil sample,  $\delta_m$  is the δ<sup>13</sup>C value of the maize residue and  $\delta_g$  is the initial δ<sup>13</sup>C value of the soil before the introduction of maize. The  $\delta_m$  was

determined on 24 samples of maize residue which were taken in the field (more detail is given in the supporting information, section S5). The  $\delta_g$  was determined on the soil samples taken from the 10 to 20-cm soil layer of the fields under permanent grass (PA fields) that served as the ‘control’ treatment or isotopic reference. Data from a historical soil database suggest that these fields had never had maize grown there and have always been under continuous grass (see supporting information, section S2 for more detail). After calculating the fraction of SOC derived from maize residues by Equation (2) for the maize fields, the total SOC of these fields can be subdivided into C<sub>4</sub>-SOC and C<sub>3</sub>-SOC; the latter originated from the grassland vegetation before the transition to maize.

The particle-size distribution of all soil samples was determined by laser diffraction (Beckman Coulter LS13320 with Aqueous Liquid Module, Brea, CA, USA), see supporting information, section S4 for detail. One soil sample was selected from each field to measure pH (0.01 M CaCl<sub>2</sub>; soil:water ratio 1:5).

### *Fractionation of SOC*

For each of the 70 fields sampled, one composite topsoil sample was selected and fractionated by the method of Zimmermann *et al.* (2007). The method combines several physical and chemical fractionation techniques (Figure SI-3) to apportion the total SOC over five different fractions. Each fraction is assumed to be protected from microbial degradation to different extents. Two of the fractions are easily degraded (particulate organic matter (POM) and dissolved organic carbon (DOC)), two are degraded more slowly (carbon associated with silt and clay (s+c), and carbon stabilized in aggregates (S+A)), and one remains undegraded by microbial activity (oxidation-resistant carbon (rSOC)). The oxidation-resistant rSOC is a sub-fraction of the s+c fraction; the difference between s+c and rSOC fractions (to which we refer

hereafter as (s+c)-rSOC) is the oxidizable part of the s+c fraction. Details of the fractionation procedure are given in the supporting information, section S6. All solid fractions (POM, S+A, s+c and rSOC) were analysed for SOC, N and  $\delta^{13}\text{C}$  in an analogous way to analysis of the bulk soil. Prior to the analyses, the S+A, s+c and rSOC fractions were acidified with dilute HCl to remove any inorganic carbon (IC). The DOC concentration was measured with a TOC analyser (OI Analytical, Aurora 1030, College Station, TX, USA) with a nondispersive infrared (NDIR) detector by wet oxidation with sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) after the addition of phosphoric acid ( $\text{H}_3\text{PO}_4$ ) to remove any IC. The  $\delta^{13}\text{C}$  signature of the DOC was determined with the same mass spectrometer that was used for the solid fractions and the bulk soil samples. Maize-derived SOC in the fractions was calculated from the  $\delta^{13}\text{C}$  values with the fractionated PA soil, which served as the reference treatment (Equation (2)).

Oxalate-extractable Al and Fe were determined for the s+c fraction with a 0.2 M ammonium oxalate extraction solution at pH 3 (solid to liquid ratio of 40 mg:2 ml, end-over-end shaking for two hours in darkness; Schwertmann, 1964). Concentrations of Al and Fe were measured by ICP-OES (inductively coupled plasma optical emission spectroscopy) with an Optima 3300 DV (Perkin Elmer, Waltham, MA, USA).

### *Statistical analysis*

The statistical analysis was done in R, version 3.1.1 (R Core Team, 2015). The statistical analysis of the bulk soil properties differed slightly from that of the SOC fractions. For the latter, with only one fractionated composite soil sample per field we used a classical one-way analysis of variance (ANOVA) in which the regions were analysed separately and cropping history was the main effect, and a two-way ANOVA in which the regions were analysed

together with both region and cropping history as the main effects. However, to investigate the effect of cropping history on several response variables for the bulk soil (with 3, 4 or 5 observations per field, i.e. a nested sampling scheme with within-field replicates) we averaged the replicates before applying either of the ANOVAs. If the effect of cropping history on the response variable was significant at  $P = 0.05$ , treatment means were compared with Fisher's least significant difference (LSD) test (*LSD.test* in R-package *agricolae*). Means and standard errors of the mean were reported. The normality of the residuals was verified by Q–Q plots. If the residuals were not normally distributed, the data were transformed to natural logarithms. The same was done if heteroscedasticity was detected by Levene's test. When two soil variables were plotted against each other, we determined the functional relation between those variables. Functional relations were estimated with Equations (18) and (19) in Webster (1997).

## Results

### *Bulk soil*

Soil characteristics for the 70 fields sampled are summarized by region (Table SI-2). The median of soil pH is about 1 unit lower in the sandy soil than in the silty loam soil. The median of silt+clay content in the silty loam soil is twice that in the sandy soil. The sandy soil under permanent grass (PA) has significantly ( $P < 0.05$ ) larger SOC stocks in the 0–30-cm topsoil layer than does the arable soil under maize (SM, SMGRA and GM; Table 1). Crop residue management (removal or return of stover) or the sowing of a cover crop after harvest had no significant effect on total SOC stocks. The  $\delta^{13}\text{C}$  signatures show that sandy soil under continuous cultivation of grain maize has a larger proportion of maize-derived SOC than that

under silage maize (18% and 11%, respectively;  $P<0.001$ ). There was no difference in  $C_3$ -SOC between sandy soil with continuous cultivation of silage maize whether or not this was combined with a second annual  $C_3$ -grass crop (Italian ryegrass), and there was no difference in  $C_3$ -SOC between grain maize and silage maize.

The effects of crop management for the silty loam soil are similar to those observed for sandy soil. However, when the two soil types under maize are compared, total SOC and  $C_3$ -SOC are significantly smaller ( $P<0.001$ ) in the silty loam soil, whereas  $\delta^{13}C$ ,  $C_4$ -SOC and proportion of  $C_4$ -SOC are significantly larger. The soil under permanent grass, however, does not differ significantly in total SOC or in  $\delta^{13}C$ . The total SOC stock for soil under maize is about 20 to 25 Mg C ha<sup>-1</sup> less for the silty loam than for the sandy soil, which is associated with a 25 to 30 Mg C ha<sup>-1</sup> smaller  $C_3$ -SOC for the former. In contrast, the  $\delta^{13}C$  values of soil under SM and GM are about 2.5‰ larger in the silty loam soil than in the sandy soil, which results from the greater proportion of maize-derived SOC in the silty loam soil of about 15%. In absolute terms, the  $C_4$ -SOC stocks of the silty loam soil are up to 5.5 Mg C ha<sup>-1</sup> larger than in the sandy soil. The silty loam soil under GM contains ~3 Mg  $C_4$ -SOC ha<sup>-1</sup> more than that under SM, and the sandy soil under GM contains ~5 Mg  $C_4$ -SOC ha<sup>-1</sup> more than that under SM, but these differences are not significant. Some of the ANOVA-tables that are behind Table 1 are reported in Table 2.

### *The SOC fractions*

In both types of soil, about 60 to 70% of the SOC is present in the s+c fraction (Table 3). In the sandy soil, the second largest SOC fraction is attributed to sand and stable aggregates (S+A), followed by the POM fraction. In the silty loam soil, POM and S+A contribute equally

to SOC under maize, but under permanent grass S+A contributes significantly more ( $P<0.01$ ) to SOC than POM. For all SOC fractions in the sandy soil, no significant effects of cropping history were detected, except that the S+A fraction contains significantly more SOC ( $P<0.05$ ) in soil under continuous grass than continuous maize. In contrast, the silty loam soil under PA contains significantly more SOC ( $P<0.05$ ) than the soil under maize in all fractions except for POM; the largest difference is again for S+A. In spite of the larger SOC content of the s+c fraction in the sandy soil under maize, both types of soil have the same relative contribution of s+c to total SOC. Maize residue management has no significant effect either on the absolute amount of SOC per fraction or on the contribution of the fraction to total SOC.

The differences in  $\delta^{13}\text{C}$  between the cropping systems are consistent for all SOC fractions; soil under PA has significantly ( $P<0.001$ ) more negative  $\delta^{13}\text{C}$  values than all soil under maize, but soil under SM and SMGRA has significantly ( $P<0.05$ ) more negative  $\delta^{13}\text{C}$  than that under GM (Figure 1). There are no significant differences between  $\delta^{13}\text{C}$  of SM and that of SMGRA. These effects are similar to those for the bulk soil. The  $\delta^{13}\text{C}$  values under PA are similar for all fractions of the sandy soil and there are small differences only in the silty loam soil. In contrast, in maize-cropped soil the  $\delta^{13}\text{C}$  value is less negative, and the maize-derived SOC is larger in the labile (POM, DOC) than in the recalcitrant (rSOC) fractions. The  $\delta^{13}\text{C}$  of the fractions is less negative in the silty loam than in the sandy soil under maize, but not for POM and DOC. The POM fraction in the sandy soil has on average  $43 \pm 3\%$  of maize-derived SOC under GM and  $26 \pm 3\%$  under SM, whereas rSOC has only  $10 \pm 1\%$  C<sub>4</sub>-SOC under GM and  $5 \pm 1\%$  under SM. The GM-cropped soil has significantly more maize-derived SOC than does the SM-cropped soil in each fraction ( $P<0.001$ ), with on average 1.9 times more C<sub>4</sub>-SOC. For the silty loam soil the differences are smaller with on average only 40% more C<sub>4</sub>-SOC ( $P<0.05$ ) under GM. The S+A fraction of the silty loam soil has the largest proportion of C<sub>4</sub>-

SOC, and this is followed by the POM, (s+c)-rSOC, DOC and rSOC fractions. The latter is again the most stable fraction and consists mainly of old C<sub>3</sub>-SOC. When the two soil types are compared, the largest difference is for the S+A and (s+c)-rSOC fractions, which both have 20% more C<sub>4</sub>-SOC in the silty loam soil.

#### *The SOC and oxalate-extractable metals in the silt and clay fraction*

The amount of SOC associated with the s+c fraction is plotted against the silt+clay content of the soil (Figure 2). The data are plotted separately for both types of soil, but all cropping histories are combined because there is no difference in the functional relation between arable cropping and permanent grass (data not shown). An increase in silt and clay content increases the amount of SOC in the s+c fraction, especially in the sandy soil. The mean concentration of oxalate-extractable Al and Fe in the s+c fraction is about 2.5-fold larger in the sandy than in the silty loam soil, whereas the mean concentration of SOC in the s+c fraction differs by a factor of three (Figure 3). The concentration of oxalate-extractable Al and Fe of the s+c fraction explains the amount of SOC in this fraction ( $P<0.001$ ).

## **Discussion**

More than 15 years of stover incorporation in GM monoculture did not increase total SOC significantly, but it increased the fraction of maize-derived SOC. The absence of significant effects of residue management on total SOC could be attributed to the relatively short time scale of the maize cropping systems studied. In separate experiments, we recorded that the larger stover input in GM than for SM in this region is equivalent to an additional 3.54 Mg C ha<sup>-1</sup> year<sup>-1</sup>, or 53.05 Mg C ha<sup>-1</sup> over 15 years. With a retention coefficient of about 5 to 10%

of the fresh residue carbon (Allmaras *et al.*, 2004; Wilts *et al.*, 2004; Hooker *et al.*, 2005), this contribution is small relative to the total SOC stocks of 55–80 Mg C ha<sup>-1</sup>, and of the same order of magnitude as the standard error of the stocks. Likewise, Reicosky *et al.* (2002) found no effect of residue management on total SOC in the top 20 cm of the soil after 30 years of continuous maize cultivation. They attributed this to intensive mouldboard plough tillage (also used in Flanders), which stimulates decomposition and offsets any net gain in SOC from the larger residue additions under GM. Soil carbon saturation (Six *et al.*, 2002) might also explain why long-term stover return cannot increase total SOC stocks substantially more than long-term stover harvest. In the sandy soil, in particular, the SOC dynamics have probably reached a steady state, given the small silt and clay contents (Table SI-2), the relatively large total SOC stocks (Table 1) and the same SOC content in the (s+c)-rSOC and rSOC fractions for both permanent grass and maize monocropping (Table 3). The  $\delta^{13}\text{C}$  analysis revealed a clear effect of crop management on maize-derived SOC, but the additional C<sub>4</sub>-SOC might have been compensated for by an enhanced loss of old C<sub>3</sub>-SOC. The latter might be explained by a positive priming effect, which is defined as a strong short-term acceleration of the decomposition of native SOM after the addition of an easily decomposable substrate (Kuznyakov *et al.*, 2000).

The effects of maize residue management on the  $\delta^{13}\text{C}$  in soil are consistent with several long-term field trials. Balesdent *et al.* (1987) showed that  $\delta^{13}\text{C}$  was 1‰ less negative after 23 years of stover return under continuous maize cultivation than with stover removal. The maize-derived SOC amounted to 21% in the top 0–30 cm of the profile with stover returned and 13% with stover removed after 29 years of continuous maize cultivation (Wilts *et al.*, 2004). Clapp *et al.* (2000) found that C<sub>4</sub>-SOC content was 15% with stover returned, but only 5% with stover removed after 13 consecutive years of maize under no tillage. With mouldboard or



chisel ploughing, however, both stover treatments led to a maize-derived SOC proportion of about 10%.

The significantly larger C<sub>4</sub>-SOC stocks in the silty loam than in the sandy soil is because finer textured soil has a greater capacity to protect C<sub>4</sub>-SOC physically against microbial decomposition by the formation of soil aggregates, and to stabilize the C<sub>4</sub>-SOC chemically through association with silt and clay particles (Six *et al.*, 2002). The silty loam soil was also sampled two years later than the sandy soil and therefore it had more time to incorporate C<sub>4</sub>-SOC. However, this explains only one third of the observed difference in SOC stocks at most, when a retention coefficient of 10% is assumed for maize residue carbon and yearly stover inputs for GM and SM of 6.06 and 2.53 Mg C ha<sup>-1</sup> year<sup>-1</sup>, respectively (the latter were determined in a separate experiment).

Native C<sub>3</sub>-SOC and total SOC stocks are larger in the sandy than in the silty loam soil. This might result from their past land use and different soil chemical properties. At the end of the 18th century, the sandy region was dominated by heathland (de Ferraris, 2009), which was brought into cultivation during the following centuries. Peat sods from the heathland were transferred to livestock stables for use as animal bedding, which was later returned to the field as an organic amendment. This resulted in the development of Plaggic Anthrosols (IUSS Working Group WRB, 2014) that contain large concentrations of refractory organic matter (Springob & Kirchmann, 2002; Kalinina *et al.*, 2009). This recalcitrance can derive partially from the large content of soluble phenolic compounds present in *Calluna vulgaris* L. litter. The latter favours the formation of recalcitrant humic complexes (Kristensen & McCarty, 1999), i.e. native C<sub>3</sub>-SOC is biochemically stable. The fact that the concentration of SOC in the s+c fraction is about three times larger in the sandy than in the silty loam soil suggests

more chemical stabilization in the former. Other soil chemical factors might also be involved, in particular the markedly different oxalate-extractable Al and Fe content of the s+c fraction between both types of soil (Figure 3).

Six *et al.* (2002) showed that the regression lines between the silt and clay content of the soil and SOC associated with the silt+clay fraction have different slopes for 2:1 clays (dominant in temperate soil) and 1:1 clays (dominant in tropical soil), and for grassland and arable land. The two regions we sampled are only 70 km apart, therefore, the clay mineralogy is likely to be comparable and dominated by 2:1 clays because both soil types developed in a cool, humid climate (Driessen & Dudal, 1991). The lack of effect of cropping system on the functional relation for the sandy region (Figure 2) accords with the observations of Hassink (1997) that the amounts of SOC associated with the silt+clay fraction are no different for arable and grassland soil despite the smaller total SOC content of the former. Hassink (1997) concluded that the silt+clay fraction was saturated with SOC, and that the larger total SOC content of grassland soil could be explained by the greater accumulation of SOC than under arable cropping in the coarser sand-sized fractions. In our study, the sandy soil with either continuous grass or maize does not differ in the SOC content associated with the (s+c)-rSOC and rSOC fractions, whereas the fraction that represents sand and stable aggregates (S + A) contains significantly ( $P<0.01$ ) more SOC under permanent grass than under maize (Table 3). In contrast, the silty loam soil contains significantly more SOC in the (s+c)-rSOC and rSOC fractions under grass than under maize. This suggests that the s+c fraction is saturated with SOC in the sandy soil, but not in the silty loam soil.

The difference in carbon sequestration per unit of silt and clay between both soil types might be explained by the oxalate-extractable Al and Fe concentrations of the s+c fraction (Figure

3). The relation might be fortuitous because of the confounding effect of inputs from the previous heath vegetation of the sandy soil and different Al and Fe concentrations for both types of soil. The chemical interactions between organic matter and Al and Fe are well established, however. Acid ammonium oxalate extracts amorphous Al and Fe (Schwertmann, 1964) as well as organically bound Al and Fe. The former is involved in stabilization of SOC through organo-mineral associations, the latter through formation of organo-metal complexes. Adams & Kassim (1984) extracted Fe from the clay fractions ( $<2\ \mu\text{m}$ ) of several Podzol horizons and demonstrated a strong, positive linear relation between oxalate-extractable Fe in the clay fraction and SOC associated with it. Several incubation studies have shown that sorption on oxyhydroxides is a major mechanism by which Al (Schneider *et al.*, 2010) and Fe (Wagai & Mayer, 2007; Eusterhues *et al.*, 2014) can stabilize SOC. However, Wagai & Mayer (2007) observed that the OC:Fe ratio ( $\text{g g}^{-1}$ ) was larger than the maximum sorptive capacity of the iron oxyhydroxides for the spodic B-horizons of all Podzols investigated. Therefore, other stabilization mechanisms for SOC are important for these types of soil, in particular the complexation of  $\text{Fe}^{3+}$  ions with functional groups of SOC and subsequent precipitation of these complexes. Both low pH and large SOC content favour the formation of such complexes, not only with Fe (Karlsson *et al.*, 2008) but also with Al (Wagai & Mayer, 2007). This might explain why the sandy soil with its lower pH and often with podzolic properties (Table SI-2) can stabilize more SOC than the silty loam soil.

## Conclusions

The acid sandy soil studied is likely to stabilize more SOC in the silt+clay fraction than the silty loam soil by sorption on Al and Fe oxyhydroxides and through the formation of organo-metal complexes. The silt+clay fraction of the sandy soil is likely to be saturated with C,

unlike that of the silty loam soil which was suggested by the comparison of SOC fractions of adjacent permanent grassland and arable soil. The concept of carbon saturation also explains why recent C<sub>4</sub>-SOC is larger in the unsaturated silty loam soil than in the saturated sandy soil. Stover return under grain maize increases the C<sub>4</sub>-SOC in the medium term ( $\geq 15$  years) in both soil types, but the lack of effect on total SOC suggests C-substitution, especially in the C-saturated sandy soil. Increased stover return might have only small effects on soil carbon sequestration in the long term, whereas other factors such as soil mineralogy are more important.

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## **Supplementary material**

Supplementary material is available.

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548

549 Table 1 Mean SOC and  $\delta^{13}\text{C}$  in the top 30 cm of soil with selected cropping histories in the sandy and silty loam regions. The fraction of maize-  
550 derived SOC (Equation (2)),  $\text{C}_4\text{-SOC}$  and  $\text{C}_3\text{-SOC}$  were determined for cropping histories SM, SMGRA and GM. The standard error of the mean  
551 is also given.

Cropping History	SOC / g C 100 g <sup>-1</sup> soil	SOC / Mg C ha <sup>-1</sup>	$\delta^{13}\text{C}$ / ‰	Maize-derived SOC fraction / %	Maize-derived $\text{C}_4\text{-SOC}$		Native $\text{C}_3\text{-SOC}$	
					/ g C 100 g <sup>-1</sup> soil	/ Mg C ha <sup>-1</sup>	/ g C 100 g <sup>-1</sup> soil	/ Mg C ha <sup>-1</sup>
<b>Sand region</b>								
PA†	2.24 ± 0.12	91.19 ± 4.18	-28.40 ± 0.13					
SM	1.85 ± 0.10	78.76 ± 3.47	-26.63 ± 0.13	11.3 ± 0.8	0.21 ± 0.02	8.86 ± 0.74	1.64 ± 0.09	69.90 ± 3.17
SMGRA	1.65 ± 0.06	73.21 ± 2.41	-26.41 ± 0.18	12.7 ± 1.1	0.20 ± 0.01	9.07 ± 0.66	1.44 ± 0.06	64.15 ± 2.60
GM	1.76 ± 0.10	75.34 ± 3.45	-25.53 ± 0.18	18.3 ± 1.2	0.32 ± 0.02	13.74 ± 1.02	1.44 ± 0.09	61.60 ± 3.08
<b>Silt Loam Region</b>								
PA	2.44 ± 0.28	94.23 ± 8.99	-28.20 ± 0.22					
SM	1.21 ± 0.08	54.16 ± 2.96	-24.04 ± 0.20	26.9 ± 1.3	0.32 ± 0.02	14.32 ± 0.68	0.89 ± 0.08	39.84 ± 2.70
GM	1.20 ± 0.12	54.64 ± 4.89	-23.02 ± 0.38	33.5 ± 2.5	0.38 ± 0.01	17.30 ± 0.56	0.83 ± 0.11	37.33 ± 4.81

552 † PA = permanent grass (pasture), SM = monoculture of silage maize, SMGRA = monoculture of silage maize where the maize is followed by a second annual grass crop, GM = monoculture of  
553 grain maize

Table 2 (a) One-way ANOVA table for the dependent variable ‘maize-derived C<sub>4</sub>-SOC (Mg C ha<sup>-1</sup>)’ for the sandy region with averaged within-field replicates , (b) two-way ANOVA table for the dependent variable ‘maize-derived C<sub>4</sub>-SOC (Mg C ha<sup>-1</sup>)’ for the two regions combined with averaged within-field replicates, (c) one-way ANOVA table for the dependent variable ‘native C<sub>3</sub>-SOC (Mg C ha<sup>-1</sup>)’ for the sandy region with averaged within-field replicates and (d) two-way ANOVA table for the dependent variable ‘native C<sub>3</sub>-SOC (Mg C ha<sup>-1</sup>)’ for the two regions combined with averaged within-field replicates .

(a)

Source	Degrees of freedom	Sum of squares	Mean square	<i>F</i> ratio	<i>P</i>
Crop	2	154.9	77.44	11.21	< 0.001
Residuals	28	193.4	6.91		
Total	30	348.3	11.61		

(b)

Source	Degrees of freedom	Sum of squares	Mean square	<i>F</i> ratio	<i>P</i>
Region	1	342.7	342.7	59.67	< 0.001
Crop	2	190.2	95.1	16.56	< 0.001
Region × Crop	1	9.2	9.2	1.60	0.21
Residuals	46	264.2	5.7		
Total	50	806.3	16.1		

(c)

Source	Degrees of freedom	Sum of squares	Mean square	<i>F</i> ratio	<i>P</i>
Crop	2	382.3	191.17	2.08	0.14
Residuals	28	2570.8	91.81		
Total	30	2953.1	98.44		

(d)

Source	Degrees of freedom	Sum of squares	Mean square	<i>F</i> ratio	<i>P</i>
Region	1	8718	8718	75.55	< 0.001
Crop	2	328	164	1.42	0.25
Region × Crop	1	86	86	0.74	0.39
Residuals	46	5309	115		
Total	50	14 441	289		

Table 3 Mean concentrations of SOC in the different SOC fractions of soil with selected cropping histories in the sandy and silty loam regions. The standard error of the mean is also reported. The contribution of the SOC associated with a fraction relative to the total SOC of the bulk soil (percentage of total SOC) is reported in brackets.

Cropping History	POM*	DOC	S+A	(s+c)-rSOC	rSOC
/ g C kg <sup>-1</sup> soil					
<b>Sand region</b>					
PA <sup>\$</sup>	1.51 ± 0.39 (8)	0.45 ± 0.03 (2)	6.64 ± 0.55 (32)	10.20 ± 0.82 (49)	1.83 ± 0.35 (9)
SM	1.24 ± 0.18 (7)	0.40 ± 0.03 (2)	3.82 ± 0.45 (21)	12.05 ± 1.17 (63)	1.51 ± 0.13 (8)
SMGRA	2.04 ± 0.41 (11)	0.35 ± 0.02 (2)	4.22 ± 0.61 (24)	9.00 ± 0.50 (52)	1.79 ± 0.25 (11)
GM	1.30 ± 0.15 (7)	0.39 ± 0.02 (2)	3.88 ± 0.45 (22)	9.84 ± 0.82 (57)	2.10 ± 0.56 (11)
<b>Silt Loam Region</b>					
PA	1.97 ± 0.30 (10)	0.69 ± 0.07 (3)	6.14 ± 1.65 (27)	10.28 ± 1.43 (49)	2.37 ± 0.43 (11)
SM	2.35 ± 0.34 (19)	0.29 ± 0.02 (2)	1.44 ± 0.25 (13)	6.54 ± 0.71 (53)	1.53 ± 0.11 (13)
GM	1.83 ± 0.26 (16)	0.30 ± 0.02 (3)	1.42 ± 0.46 (12)	6.87 ± 0.99 (58)	1.38 ± 0.14 (12)

\$ PA, SM, SMGRA and GM: see Table 1

\* POM = particulate organic matter, DOC = dissolved organic carbon, S+A = SOC associated with the fraction containing sand and stable aggregates, (s+c)-rSOC = oxidizable part of the SOC associated with the silt and clay fraction, rSOC = non-oxidizable, chemically resistant part of the SOC associated with the silt and clay fraction

**Figure 1** The  $\delta^{13}\text{C}$  in the bulk soil (BS) and in the different SOC fractions of soil with selected cropping histories in (a) the sandy region and (b) the silty loam region, and the fraction of SOC derived from maize (%) determined by Equation (2) for the bulk soil and different SOC fractions in soils with selected cropping histories in (c) the sandy region and (d) the silty loam region. Abbreviations of cropping histories and SOC fractions are given in Tables 1 and 3.

**Figure 2** Functional relation between silt + clay content and the amount of SOC associated with the silt and clay fraction. All cropping histories are combined. The line for the sandy soil is the estimated functional relation (based on Equations (18) and (19) in Webster, 1997) of SOC associated with silt and clay as the dependent variable on silt + clay content. No such meaningful relation could be demonstrated for the silty loam soil.

**Figure 3** Functional relation between the concentrations of oxalate-extractable Al and Fe and the amount of SOC associated with the silt and clay fraction for the sandy and the silty loam soil. All cropping histories are combined. The line is the estimated functional relation (based on Equations (18) and (19) in Webster, 1997) of SOC amount associated with silt and clay as the dependent variable on oxalate-extractable Al and Fe for the sandy and silty loam soil combined.

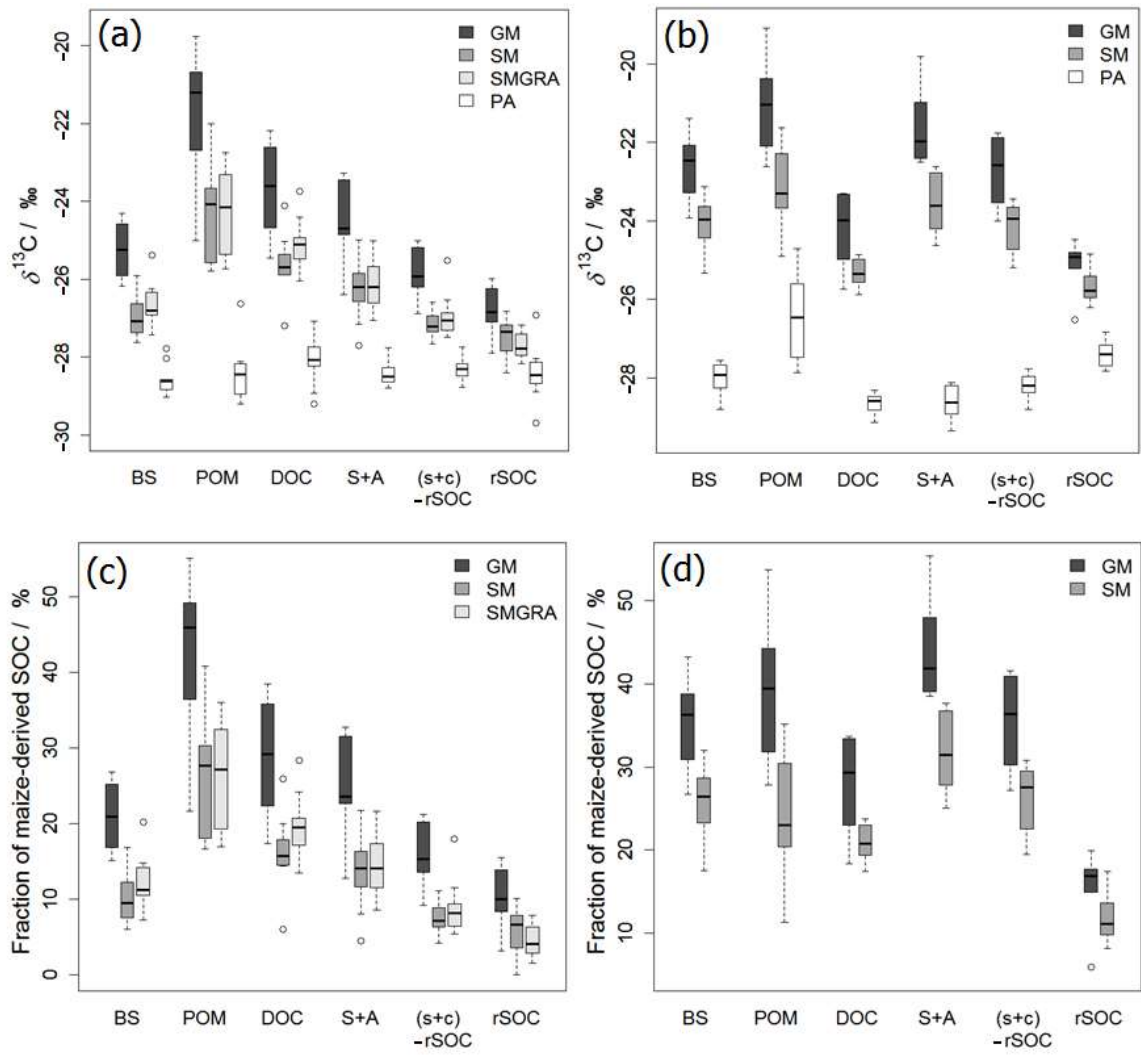


Figure 1

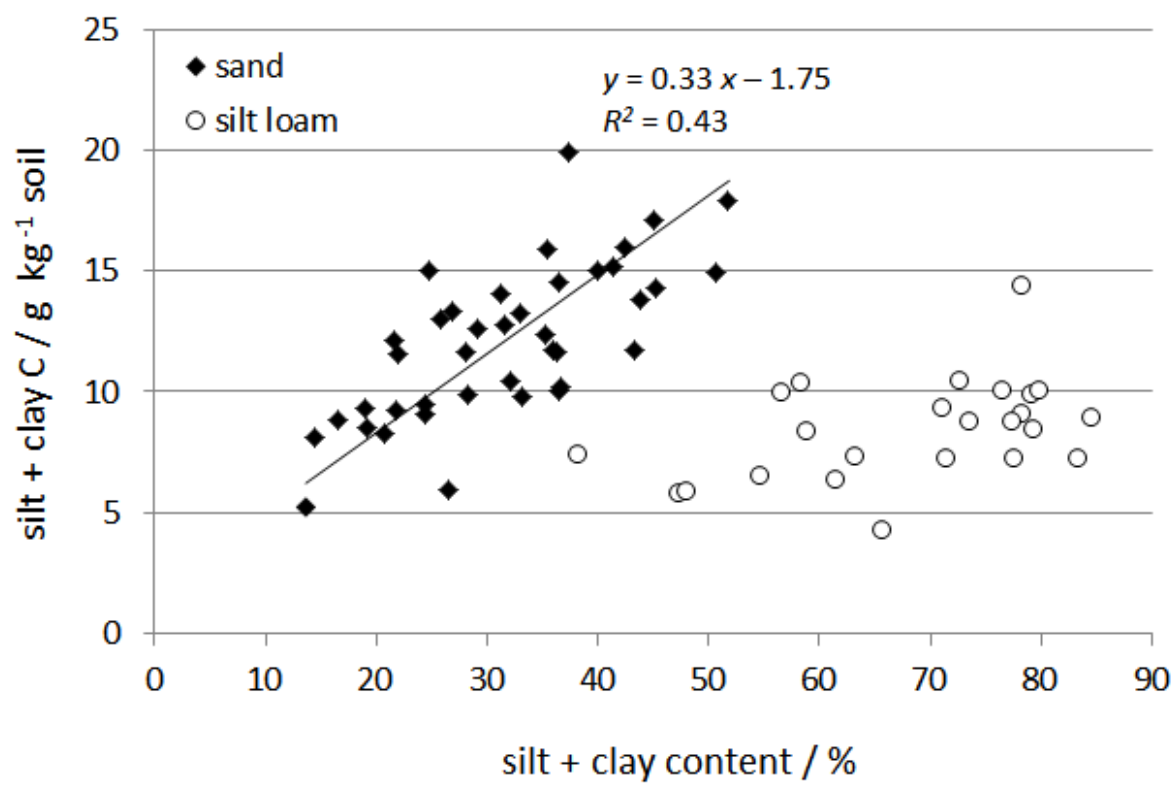


Figure 2



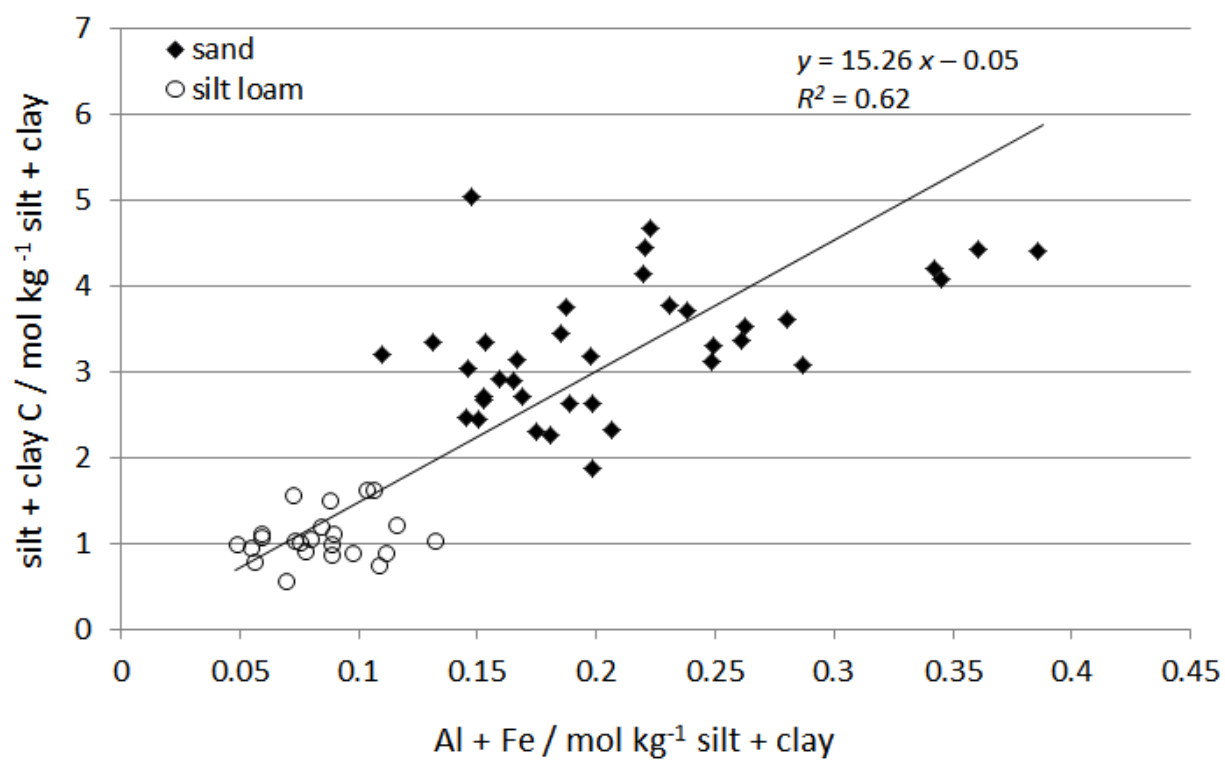


Figure 3