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An indicator for organic matter dynamics in temperate agricultural soils

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ABSTRACT

The heterogeneity of soil organic matter (SOM) and the small changes in soil organic carbon (SOC) compared to large total SOC stocks hinder a robust estimation of SOC turnover, in particular for more stable SOC. We developed a simple fractionation protocol for agricultural topsoils and tested it extensively on a range of soils in southern Belgium, including farmed soils, soils from long-term field trials, and paired sites after recent conversion to conservation farming. Our simple fractionation involves shaking the soil, wet sieving over 20 µm and analysing the SOC concentration in the soil as well as in the fine fraction (< 20 µm). Eight biological indicators measured in an earlier study across the same monitoring network for the 0–10 cm topsoil were analysed in a conditional inference forest model in order to investigate the factors influencing the SOC fractions. Soil microbial biomass N explained the largest proportion of variation in both fractions. The fine fraction was also associated with factors explaining the regional trend in SOC distribution such as farmyard manure input, precipitation, land use and flow length. The variation in SOC content between treatments both in long-term trials and in farmers' fields converted to conservation management was mainly attributed to changes within the coarse fraction. Thus, this fraction proves to be sensitive to management changes, although care should be taken to sample deep enough to represent the former plough layer inherited from the conventional tillage practice. Furthermore, the ratio between the coarse and the fine fraction showed a linear relationship ($r^2 = 0.66$) with the relative changes in SOC concentration over the last ten years. These fractions derived from a simple analytical approach are thus useful as an indicator for changes in SOC concentration. In analogy to biological indicators such as the soil microbial biomass C, the relationship between the fractions and relative changes in SOC concentration are likely to depend on climate conditions. Our methodology provides an indicator for use in routine analysis of agricultural topsoils, which is capable of predicting the effects of management practices on SOC concentrations in the short to mid-term (5–10 years).

1. Introduction

Agricultural soils have lost a portion of their organic matter through an imbalance between reduced C input, e.g. due to export of crops, and enhanced C output e.g. due to decomposition after disturbance by ploughing. Preserving and where possible enhancing SOM storage is one of the aims of conservation agriculture, resulting in improved soil functions such as enhanced water retention and nutrient cycling, in particular for soils with low SOM contents. Additionally, agricultural soils are considered to have the potential to mitigate climate change by C sequestration globally (Minasny et al., 2017) and thus compensate at least a part of the fossil fuel emissions and at the same time restore soil fertility (e.g. the '4 per mille' initiative launched by France at the COP 21 https://www.4p1000.org/).

Although fertilizers largely compensate the loss of soil fertility in temperate agricultural systems, less attention is paid to the compensation of SOM decreases, as these occur slowly and their effect on soil fertility, crop yields, and climate mitigation is not generally quantified

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(Kibblewhite et al., 2016). Conservation agriculture aims at reducing soil disturbance by ploughing, increasing input of organic amendments, diversifying the rotation and lengthening the period during which a crop is grown. These measures have a beneficial effect on the key indicators for soil health, be it biological properties (e.g. microbial biomass C, mean organic matter residence time, earthworms, soil enzymes, nematodes and pathogens, mycorrhizal fungi, soil respiration) or ecological functions (e.g. nutrient cycling, hydrological budget, energy budget, erosion, biodiversity, landscape processes; Lal, 2016).

Unfortunately, biological indicators and SOC content analysis are limited in their ability to detect changes at relevant temporal scales. Powlson et al. (1987) and Anderson and Domsch (1989) already demonstrated that microbial biomass C is sensitive to increases in straw incorporation in agricultural soils and proposed a threshold of 2.9% for the ratio of microbial C to SOC below which SOC decreases over time. However, the analysis of microbial C requires at least a day of fumigation, results are not directly related to mineralization rate (Kemmitt et al., 2008) and subject to seasonal variation (cv 29%; Krüger et al., 2018). Moreover, the heterogeneity of SOM is such that it is difficult to detect small changes in input against a large background of SOM that built over centuries (Powlson et al., 1987).

Routine soil analysis for fertilization recommendations is common practice for both croplands and grasslands and provides information on a limited number of soil parameters such as N, P, pH and SOM, with around 20,000 fields analysed each year in the Walloon Region (Genot et al., 2011). Recently, VisNIR spectroscopy is increasingly used in order to replace SOC analysis and estimate the clay content and CEC, while promising calibrations for soil fractions have been published by Jaconi et al. (2019). An additional indicator for the risk of SOM decline to be included in the soil fertility advice would enable farmers to engage in SOM management and eventually contribute to the challenges of e.g. the 4 per mille initiative. The constraints of such an indicator are obviously the sampling depth used for fertility advice (i.e. 0–20 cm in cropland and 0–10 cm in grassland), labour requirement and the insertion in a routine soil analysis chain. In this context techniques such as sonication and flotation in heavy liquids are rather complex and should be avoided (Poeplau and Don, 2014).

Different protocols were developed to separate fractions of SOC ranging from C stabilized by organo-mineral complexes to partly decomposed organic residues (von Lützow et al., 2007; Poeplau et al., 2018). Some of these fractions are in fact related to stable organic matter and reflect long-term (10–100 years) C storage. Liang et al. (2017) proposed a conceptual model reconciling decomposition and occlusion of OM and stressed the importance of stabilization with mineral surfaces in the fine fraction. The stabilization of organic matter by the fine silt and clay fraction (< 20 µm) has opened the debate on a maximum content of stable C for a given soil texture (Hassink, 1997; Six et al., 2002; Feng et al., 2013). Spatial models could be developed in order to estimate C sequestration potentials for this stable fraction (Angers et al., 2011; Wiesmeier et al., 2014). Chronosequences after land abandonment have shown that the changes in this stabilized C fraction over time are slow (> 60 years; Trigalet et al., 2016). Stewart et al (2008) demonstrated that three conceptual pools based on i) stabilization through chemical association with silt and clay particles, ii) physical protection within micro aggregates and iii) biological complexity of the organic compounds can be isolated through a three step fractionation using dispersion and wet sieving, flotation in heavy liquids and acid hydrolysis.

Our hypothesis is that through a wet sieving procedure after disaggregation we can derive two fractions corresponding to two of the three conceptual pools i.e. stabilization with silt and clay particles and physical protection within (micro) aggregates. Stewart et al (2008) stress that these fractions should be separated by dispersion and by wet sieving, as sonication will destroy the smaller (micro) aggregates. The fraction smaller than 20 µm contains free silt and clay as well as small micro aggregates. The organic matter in this fraction consists of stabilized organo-mineral complexes (Lal, 2016; Liang et al., 2017; Totsche et al., 2018) and the amount of C is limited by the sorption capacity of the soil, thus leading to C saturation (Hassink, 1997; Six et al., 2002; Feng et al., 2013). The fraction larger than 20 µm is more heterogeneous, consisting of micro aggregates, small macro aggregates (that withstand shaking), organic matter associated with coarse silt and sand, and particulate organic matter (POM). In agricultural soils, the POM fraction is rather limited at less than 10% of the total SOC (Wiesmeier et al., 2014). Given the hierarchical organization of aggregates (Tisdall and Oades, 1982), the macro aggregates also contain micro aggregates that reflect stabilized fraction (Six et al., 2002). The binding and storage capacity of coarse particles for C is limited, but the C stored in sand particles has been shown to be highly sensitive to land-use change (Leifeld and Kögel-Knabner, 2005)

We expected that a fraction > 20–50 µm would respond rather quickly to increases in return of crop residues to the soil and reduced soil disturbance. After all, these are the main aims of conservation agriculture. If such a management regime persists, an abundant coarse fraction that is decomposed rather quickly will feed the soil biota and gradually part of the C in the coarse fraction would be stabilized in the fine fraction. Thus, a sensitive indicator can be developed to inform farmers on the effects of management changes on SOM.

The aim of this paper was thus to test the capacity of a simple organic matter fractionation scheme as an indicator for the effects of management on the short to mid-term dynamics of organic matter (5–10 years) in agricultural soils. To do this we characterized SOC fractions and investigated the drivers (topography, soil, climate, land use, biological indicators) for variation in SOC fractions from sites within a soil monitoring network with additional sites from long-term trials and paired plots in farmers' fields. The biological indicators (respiration potential, microbial biomass carbon, microbial C/N ratio, net nitrogen mineralisation, metabolic potential of soil bacteria, earthworm abundance, microbial quotient, and metabolic quotient) were analysed on the same sampling points in order to develop a reference system (Krüger et al., 2018). We used a conditional inference forest analysis for determining the factors and ranking their importance as drivers for the SOC fractions (Hobley et al., 2015). Then we investigated the sensitivity of fractions to treatments in controlled conditions (long-term trials, paired plots), and finally selected the most effective indicator based on the fractions. Using this approach we were able to i) determine the drivers of and relations to soil functions for the derived C fractions, ii) demonstrate the sensitivity of the C fractions to the effects of management, and iii) propose an indicator for soil C status based on these C fractions.

2. Materials and methods

2.1. Study area

The data was collected in Wallonia, which is the southern part of Belgium and covers an area of c. 16,800 km^2. This region has been selected for a number of studies on the spatial distribution of SOC in agricultural soils, such as the evolution of SOC stocks over the last fifty years (Goidts and van Wesemael, 2007) and the mapping of SOC stocks (Chartin et al., 2017). Detailed information on the study area can be found in these papers. Briefly, the area has a maritime climate in the northwest (mean annual precipitation (P) = 700 mm and mean annual temperature = 10–11 °C) and a more continental climate in the southeast (P = 1060–1200 mm and mean annual temperature is 8–9 °C). The northern part comprises a plateau at around 100–120 m above sea level (asl) consisting of Quaternary niveo eolian loess sediment of silt to silt loam texture with a Haplic Luvisols (IUSS Working Group WRB., 2015). The soils are fertile and dominated by cropland systems in which wheat, barley and sugar beet are grown in rotation. The southeastern part consists of high plateau and valleys of the Ardennes (250–690 m asl) and has a substratum of Devonian rocks. Soils are thinner and often

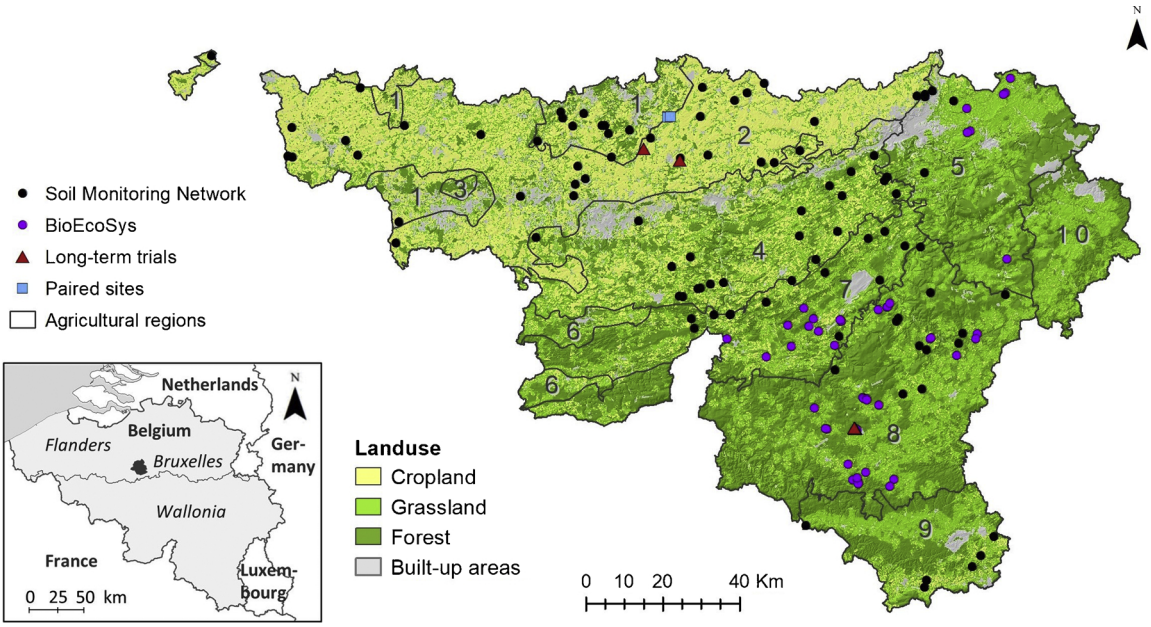

Fig. 1. Map of located sites and agricultural regions in Wallonia (1: Sandy-loam region, 2: Loam region, 3: Campine Hennuyère, 4: Condroz, 5: Herbagière de Liège, 6: Herbagère Fagnes, 7: Famenne, 8: Ardenne, 9: Jura and 10: Haute Ardenne.

stony, classified as Dystric Cambisols with Fluvisols in the valley bottoms. The land use in the southern part is dominated by extensive grassland systems for cattle breeding and forests. Within Wallonia ten agricultural regions are distinguished with, to a large extent, similar soils, topography and cropping practices (Fig. 1).

2.2. Soil monitoring network

Firstly, 434 profiles were sampled in two campaigns (August 2004 - August 2005 and October 2006 - May 2007) from 15 units with homogeneous land use, belonging to the same agricultural region and with similar soil texture, drainage and stoniness (referred to as LSU, or landscape unit; Goidts et al., 2009). The sampling was extended from March 2014 to June 2014, covering another 30 LSU's with 158 sampling sites (Chartin et al., 2017). These 592 sites were marked with an electromagnetic ball marker (3 M) buried at 1 m below surface in order to allow a precise relocation.

In April and May 2015, we re-sampled 30 sites in cropland and 30 sites in grassland selected among the 434 profiles originally sampled by Goidts et al. (2009). The sites were selected from ten LSU's using a latin hypercube for site selection (Minasny and McBratney, 2006), constrained by geographical coordinates and concentration of fine silt and clay from the maps produced by Chartin et al. (2017). Furthermore, we used 37 sites selected among the 158 additional sites mentioned above in order to increase the variability in environmental conditions.

2.3. Long-term trials

The long-term trials in Long Tours (50° 33′ N, 4° 43′ E) started in 1959 and consist of different treatments with organic amendments. The site is described in detail by Buysse et al. (2013) and Trigalet et al. (2014). The field is in a flat landscape position within the loam region and the soils are tilled using a mouldboard plough until 23–27 cm (Fig. 1). Additionally, the cereal stubbles are ploughed in with a spring tine cultivator (0–10 cm) and the seedbed is prepared with a disk harrow (5–8 cm). Initially, there was a four-year rotation of sugar beet, winter wheat, winter barley and horse bean, but it changed to a three-year rotation (sugar beet, winter cereal, winter cereal) in 1975. Two treatments were sampled: the control, where aboveground crop residues were exported and no organic fertilizer was used (RE treatment), and the residue return treatment (RR treatment), where residues were left in the field and a cover crop was grown in the winter preceding the sugar beet. The two treatments received the same amount of mineral fertilizer. Four composite samples in each treatment were collected in April 2016.

The long-term trial in Gentinnes (50°35′ N 4°35′ E) in the loam region dates from 2008 and encompasses three tillage treatments: i) conventional tillage with a mouldboard plough until 27 cm depth (CT), ii) deep de-compaction with a heavy tine cultivator until 30 cm (DT) every second year before the seeding of the sugar beet and a spring tine cultivator until 10 cm during the other year, and iii) reduced tillage with a spring tine cultivator until 10 cm depth (RT; Fig. 1). The seedbed for all treatments is prepared with a disk harrow to a depth of 5–8 cm. There is a two-year rotation with sugar beet and winter cereals. A cover crop is sown after tillage and left to decompose at the surface. The cereal straw is chopped and mixed in the topsoil (Jonard et al., 2013). Four composite samples in each treatment were collected in June, 2017.

The long-term trial in Libramont (49°55′N 5°21′E) is located in the Ardenne region (Fig. 1). The site was established in 1996 and converted to organic agriculture in 2010. The grassland consists of perennial rye grass and white clover and is grazed by cattle in rotation with on average five large livestock equivalents. Stocking density for each grazing was adapted according to available biomass and excess grass production in spring was mown for silage. There are four treatments: 1) composted farm yard manure (10 Mg $ha^{-1}y^{-1}$) until 2010 and no amendments or fertilizer afterwards, 2) mineral fertilizer until 2010 and no amendments or mineral fertilizer afterwards, 3) composted manure (10 Mg $ha^{-1}y^{-1}$) and 4) mineral fertilizer until 2010 and composted manure from 2010 onwards (10 Mg $ha^{-1}y^{-1}$). Each treatment consists of two replicates in each of which four composite samples were collected in March 2016.

2.4. Paired sites

Two paired sites were selected according to: i) availability of two comparable fields with one under conventional and the other under conservation agriculture, ii) the conventional site should be under winter wheat following a sugar beet crop. Four composite samples of each site were collected in June 2017. Furthermore, the fields under

Table 1
Management practices for the Bioecosys grasslands.

Management	Permanency	Type of management	Code	N[8] Ardenne	N[8] Famenne	N[8] Région herbagière	Management Intensity
Mown (and grazed)	(Temporary	Intensive conventional[1]	Mt1	3	-	-	intensive
		Intensive organic [2]	Mt2	3	-	-	
	Permanent	Intensive conventional[1]	Mp1	3	3	3	
		Intensive organic[2]	Mp5	3	-	-	
		Ist cut after 15 June[1]	Mp2	3	-	-	
		Natural grassland[3]	Mp3	3	3	-	
		Natural grassland with Late mowing[4]	Mp4	4	4	4	extensive
Grazed	Permanent	Continuous intensive[5]	Gp1	3	-	-	intensive
		Rotating[6]	Gp2	3	-	-	
		Natural grazing[7]	Gp3	4	-	-	extensive

[1]3-4 cuts with fertilizer and farmyard manure (FYM), suckler cows.
[2]3-4 cuts with FYM only, suckler and dairy cows.
[3]2-3 cuts, no intervention between 1 January and 15 June, a single spreading of FYM between 15 June and 31 July, suckler cows.
[4]1-2 cuts, no intervention between 1 January and 15 June, no spreading of FYM, suckler cows.
5Continuous grazing between April and October/November, suckler cows.
[6]Grazing for 8–15 years, return to the field in 5–7 weeks, suckler cows.
[7]no intervention between 1 January and 15 June, no spreading of FYM, suckler cows.
[8]number of fields sampled.

conservation agriculture and the two treatments in the Long Tours (see Section 2.3) were sampled in April, June (after flowering of the wheat) and August (after harvest) 2016 in order to investigate the seasonal variability in SOM fractions.

2.5. Bioecosys

The Bioecosys network consists of 49 grassland fields within three agricultural regions: 32 in the Ardenne, 10 in the Famenne and 7 in the Région herbagière de Liège (Fig. 1). These grasslands have been managed at least for the last five years according to the local practices and contain temporary and permanent grasslands that are grazed and/or mown. They are arranged along a gradient from intensive to extensive management (Table 1). One composite sample in each field was collected in November 2016.

2.6. Soil samples

For the soil monitoring network, composite samples consisted of five points sampled by auger and located within a 4 m radius circle around the center referenced by the buried ball marker (Chartin et al., 2017; Krüger et al., 2018). Samples of all long-term trials, paired sites and Bioecosys were taken on the corners of a 10 by 10 m square laid out using a handheld GPS. On each corner a composite sample was collected. All samples for SOC concentration and C fractions were taken from 0 to 20 cm in cropland and 0 to 10 cm in grassland. These depth intervals are in agreement with the sampling for routine soil fertility analysis. For biological indicators only the 0–10 cm topsoil was sampled in both croplands and grasslands (Krüger et al., 2018). The biological indicators and the SOM fractions were never used in the same statistical test. Instead, we used the biological indicators as co-variates together with the environmental co-variates (Section 2.9 and Table 2).

2.7. Fractionation of soil organic matter

All samples were air-dried, crushed using a pestle and mortar and sieved at 2 mm in agreement with the protocols for routine soil fertility analysis (Genot et al., 2011). For fractionation, 10 g of fine earth (< 2 mm) was mixed with 100 ml of de-ionized water in a plastic bottle and shaken horizontally for 15 min at 250 rpm. The fine earth water mixture was then quantitatively transferred to a 50 µm sieve and washed through in a beaker until the liquid that passed the sieve was clear. The material that passed through the 50 µm sieve was then poured onto a 20 µm sieve and washed through using a spray bottle filled with de-ionized water and a rubber spatula until the liquid that passed the sieve was clear. The first sieving step was included in order to prevent the clogging of the finest sieve. Care was taken to keep the total amount of water used for shaking and washing below 2 dm^3, in order to reduce losses during centrifugation and drying. The fraction remaining on the sieves was collected and dried in an oven at 60 °C. The liquid finer than 20 µm was centrifuged for 25 min at 3600 rpm and the clear supernatant discarded. The remainder was transferred into a beaker and dried at 60 °C for four days minimum. The mean recovery rate was 96%.

The fine earth (< 2 mm) as well as the fine fraction (< 20 µm) were

Table 2
Environmental co-variates.

Variable	Explanation	Depth (cm)	Reference
Topography			
eastness	Aspect, orientation towards East (Zar, 1999)	–	Derived from a 20 m resolution DEM provided by the NGI (Chartin et al., 2017)
northness	Aspect, orientation towards North (Zar, 1999)	–	
TPI 500m	Topographical position Index (Jenness, 2006)	–	
Flow length	Flow length according to RUSLE	–	
C factor	Crop factor according to RUSLE	–	
slope	Slope gradient (%)	–	
dem	Elevation (m)	–	
Climate		–	
Precipitation	Precipitation (mm)	–	Annual mean data (1971-2000) from meteo stations in Belgium and neighbouring countries, maps modelled using elevation from NASA SRTM DEM (Chartin et al., 2017)
Temp	Mean temperature (°C)	–	
Soil			
clay	Clay (%)	0-20	Maps of clay, silt and sand based on regression kriging of Aardewerk data (Chartin et al., 2017)
silt	Silt (%)	0-20	
sand	Sand (%)	0-20	
pH	pH KCl	0-10	This study see section 2.9
GWL min	Winter groundwater level (cm)	–	Based on Aardewerk database (Meersmans et al., 2008)
GWL max	Summer groundwater level (cm)	–	
Land use			
Land use	Grassland/cropland	–	Observations at sampling sites
FYM	Input of farmyard manure (and slurry) per municipality (Mg C ha^{-1} y^{-1})	–	Based on Dendoncker et al. (2004). Raster image with 40 m pixel
Biological indicators			
MBC	Microbial biomass C (mg C/kg)	0-10	This study see section 2.8
MBN	Microbial biomass N (mg N/kg	0-10	This study see section 2.8
PR	Potential respiration after 16 h pre-incubation at 15 °C and 55% field capacity (mg C-CO_2 kg^{-1} $hour^{-1}$)	0-10	
Nmin	Net N mineralization (mg N kg^{-1} day^{-1})	0-10	
Biolog	Functional diversity (%)	0-10	
qmic	Microbial quotient (MBC/SOC, unitless)	0-10	
qCO_2	Metabolic quotient (PR/MBC, mg C-CO_2 kg^{-1} C h^{-1})	0-10	

analyzed for their concentration of organic carbon. The C concentration of the coarse fraction (> 20 µm) was calculated from its mass and the mass and C concentrations of the fine earth and the fine fraction. All samples were tested for the presence of inorganic C (Ci) as calcium carbonate using a 5% HCl solution. Dolomite only occurs in Keuper limestones with a very limited outcrop on the border of the Jura and the Ardenne (Fig. 1; Boulvain and Pingot, 2013). As no samples originated from this area, a correction for dolomite was not required. If inorganic C was present, its content was determined by the calcimetric method using an electronic manometer (Sherrod et al., 2002). Carbon (Ci + o) was analyzed by dry combustion using a VarioMax CN Analyzer (Elementar GmbH, Germany). Finally, soil organic C concentration was calculated from the difference between total C and inorganic C (Eq. 1):

$$SOC = Ci + o - Ci \quad (1)$$

where all concentrations are expressed in g kg^{-1}.

Duplicates were fractionated for five samples, and the RMSE of the SOC content in the fine fraction was 0.45 g C kg^{-1} soil.

Twenty samples that were also treated by the shaking and sieving method described above were fractionated according to the scheme of Hassink (1997). The samples were selected in order to cover the range in SOC content of the soil monitoring network. Here, 20 g fine earth was dispersed in 150 ml deionized water and then sonified at 100 J ml^{-1}. The probe type sonicator (Branson 250) was calibrated to a power of 43.58 W over 344 s. The 500 ml beaker (86 x 181 mm) was cooled in a water bath with ice cubes in order to prevent heat build-up. We then sieved the soil-water mixture over 20 µm under pressure and rinsing with de-ionized water until the liquid was clear. The liquid finer than 20 µm was evaporated and freeze-dried. The mean recovery rate was 97%. The SOC content of the fine earth and the fraction finer than 20 µm was determined as explained above.

2.8. Biological indicators

Microbial C and N concentrations were measured after fumigation with chloroform (Vance et al., 1987). To do this, 20 g of non-fumigated soil and 20 g of fumigated soil (72 h with chloroform) were extracted with 100 ml K_2SO_4 (0.5 M) for one hour (180 rpm) and the supernatant was filtered (Whatman 42). Total N was measured colorimetrically with an auto-analyser equipped with a UV digester (Auto-Analyzer 3, Bran + Luebbe, Germany). Organic C was measured with an infrared analysis system (LABTOC, Pollution & Process Monitoring, UK). Biomass C- and N-pools were calculated as difference between organic C contents and total N contents of fumigated and non-fumigated soil extracts by the relationships (Eqs. 2 and 3):

$$MBC = E_c \text{ fumigated soil} - E_C \text{ non-fumigated soil}/k_{EC} \quad (2)$$

$$MBN = E_N \text{ fumigated soil} - E_N \text{ non-fumigated soil } /k_{EN} \quad (3)$$

Where MBC and MBN are the microbial biomass C and N (mg kg^{-1}), E_C and E_N the C and N extracted and k_{EC} and k_{EN} the conversion factors of 0.45 for C (Jenkinson et al., 2004) and 0.54 for N (Joergensen and Mueller, 1996).

Net nitrogen mineralization (Nmin) was calculated as the difference in mineral N concentration (nitrate, N-NO3- and ammonium, N-NH4) in a 40 ml KCl (1 M) extract before and after an incubation of 20 *g* fresh soil without roots during 29 days at 25 °C in the dark (Hart et al., 1994). The extracts were centrifuged for 10 min at 3000 rpm and the supernatant stored frozen until analysis. The N-NO3- and N-NH4 concentrations were measured using an auto-analyzer (Auto-Analyzer 3, Bran + Luebbe, Germany). N mineralization is expressed in mg N kg^{-1} day^{-1}.

The respiration potential (PR) was measured on fresh soil adjusted to 55% of field capacity after 16 h at 15 °C. The soil respiration was

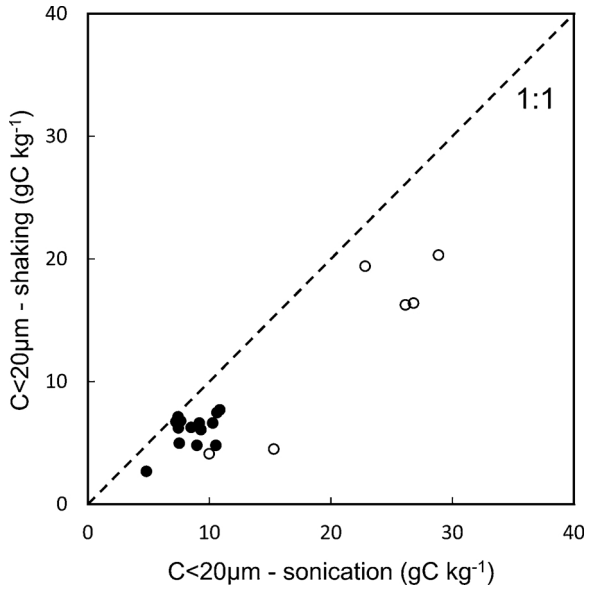

Fig. 2. Comparison of concentration of C < 20 µm between two fractionation techniques in a selection of croplands (closed symbols) and grasslands (open symbols): shaking (as applied in this study) and after sonication of 20 g soil in 150 ml water with an energy of 100 J ml^{-1} (energy level specified by Hassink, 1997).

determined by measuring the CO_2 accumulation produced by 20 g of soil in a 250 ml bottle. Gas samples of 4 ml were taken at 0, 120, 240 and 360 min incubation and were analyzed by infrared absorption (EGM-4, PPsystem, UK). The rate of CO_2 production was calculated by linear regression and expressed in mg $C-CO_2\,kg^{-1}\,h^{-1}$.

The functional or metabolic potential of the bacteria (Biolog) was measured using Biolog Ecoplates (BIOLOG™, California) containing 31 carbonated substrates. One gram of fresh soil was extracted with 9 ml of sodium chlorate (Rutgers et al., 2009). In order to evaluate the size of the microbial community, three dilutions (10-2, 10-3 and 10-4) were established in a 0.85% NaCl solution. A dilution corresponding to 1000–2000 CFU (colony forming units) was used for incubation. The Biolog plates were incubated for 72 h at 20 °C and the substrates with visible reactions were identified. The functional diversity was expressed as percentage of substrates used by the bacteria.

The microbial quotient (qmic) can be used as an index for the availability of carbon for micro-organisms (Bimüller et al., 2014). The quotient is calculated as the ratio MBC in g C kg^{-1} and the organic C in the bulk soil (Ctot in g C kg^{-1} ; Eq. 4) :

$$qmic = MBC/\ Ctot \quad (4)$$

The metabolic quotient (qCO_2) is an indicator for the energy required to maintain the microbial biomass (Anderson and Domsch, 1993). High values point to a stressed microbial community. The metabolic quotient is the ratio between the potential respiration (PR in mg $C-CO_2\,kg^{-1}\,h^{-1}$) and the MBC (g C kg-1) and is expressed in mg $C-CO_2\,kg^{-1}$ C h^{-1}(Eq.5):

$$qCO_2 = PR/MBC \quad (5)$$

2.9. Environmental co-variates

The co-variates are subdivided in the following categories: climate, soil, land use and biological indicators. Soil pH was measured in a suspension (1:2 w:w) of 1 M KCl using a pH meter (HI2550 Hanna Instruments). Apart from the pH, the climate and soil related co-variates were derived at the 89 sites from thematic maps (Table 2). The upstream flow length and crop cover (C) factor were used as in the RUSLE equation (Renard et al., 1991).

2.10. Data analysis

2.10.1. Conditional inference tree ensemble

In order to characterize the fractions and to understand their drivers we applied a conditional inference forest analysis with the C < 20 µm, C > 20 µm as dependent variables. Conditional inference trees are similar to a random forest and can be used to model non-linear interactions between the response variable (i.e. the C fractions) and predictor variables (see Table 2) without the requirements of normality and homoscedasticity (Hobley et al., 2016). In principle, all sites with a complete dataset were used. As some of the sites, in particular the long-term trials and paired sites, contained replicates of the samples, first an average from these replicates was calculated. All models were created using the party package in R (Strobl et al., 2007). The conditional inference forest was grown over 500 trees with the number of predictor variables randomly selected per split set to 2 and a significance relationship between predictor and response variable at $\alpha < 0.05$. Overall, the data set contained 89 observations and 24 predictor variables (Table 2). The overall performance of the models was evaluated on the RMSE and R^2 of the out-of-bag dataset. The relative variable importance (Hobley et al., 2015) was expressed as n = I/T*100, where I is the variable importance and T is the total variance explained by the model.

2.10.2. Differences between treatments and correlation matrix

For contrasts of long-term trials or paired plots with more than two treatments, the differences in Ctot, C < 20 µm and C > 20 µm between treatments were tested using permutation-based ANOVA (i.e., with treatment as between-subjects factor). A correlation matrix between the 23 environmental covariates (all variables in Table 2 except land use) and SOC fractions was calculated using the Pearson r. The significance level of all statistical tests was set at $\alpha = 0.05$.

3. Results and discussion

3.1. Factors controlling the concentration of the fractions

The comparison of both fractionation methods i.e. with and without sonication showed that the latter method yields ca 30% higher C < 20 µm concentrations (Fig. 2). This implies that a portion of fine micro-aggregates and primary particles (< 20 µm) remain encapsulated within large micro-aggregates (20–250 µm) after shaking. SOM fractionation protocols are sensitive to the disruptive power applied in order to separate the fractions (Poeplau and Don, 2014). We shook the sample for 15 min and thus cannot report the energy level. However, shaking is considered to be less disruptive than sonication, peptization or using a ball mill. Since the fractionation included neither sonication nor peptization, the protocol could be classified as an aggregate fractionation according to the classification of Poeplau et al. (2018). In contrast to the slaking procedure first suggested by Six et al. (2002) and adapted by Stewart et al. (2008), shaking for 15 min at 250 rpm probably destroys the macro aggregates (> 250 µm) but was not aggressive enough to destroy micro aggregates (Balesdent, 1996).

Within the agricultural regions the site selection was stratified according to landscape units with a similar soil texture, drainage and stoniness class (section 2.2; Fig. 3). Overall, with an increase in Ctot the contribution of the C > 20 µm in croplands increased from the NW to the SE at the expense of the C < 20 µm fraction. In grasslands, Ctot remained more or less constant with an increase in C < 20 µm resulting in a relative decrease of C > 20 µm. These general NW-SE trends correspond to increasing clay content, decreasing temperature and increasing precipitation (Meersmans et al., 2016; Chartin et al., 2017). The highest C < 20 µm contents in the Famenne grasslands are

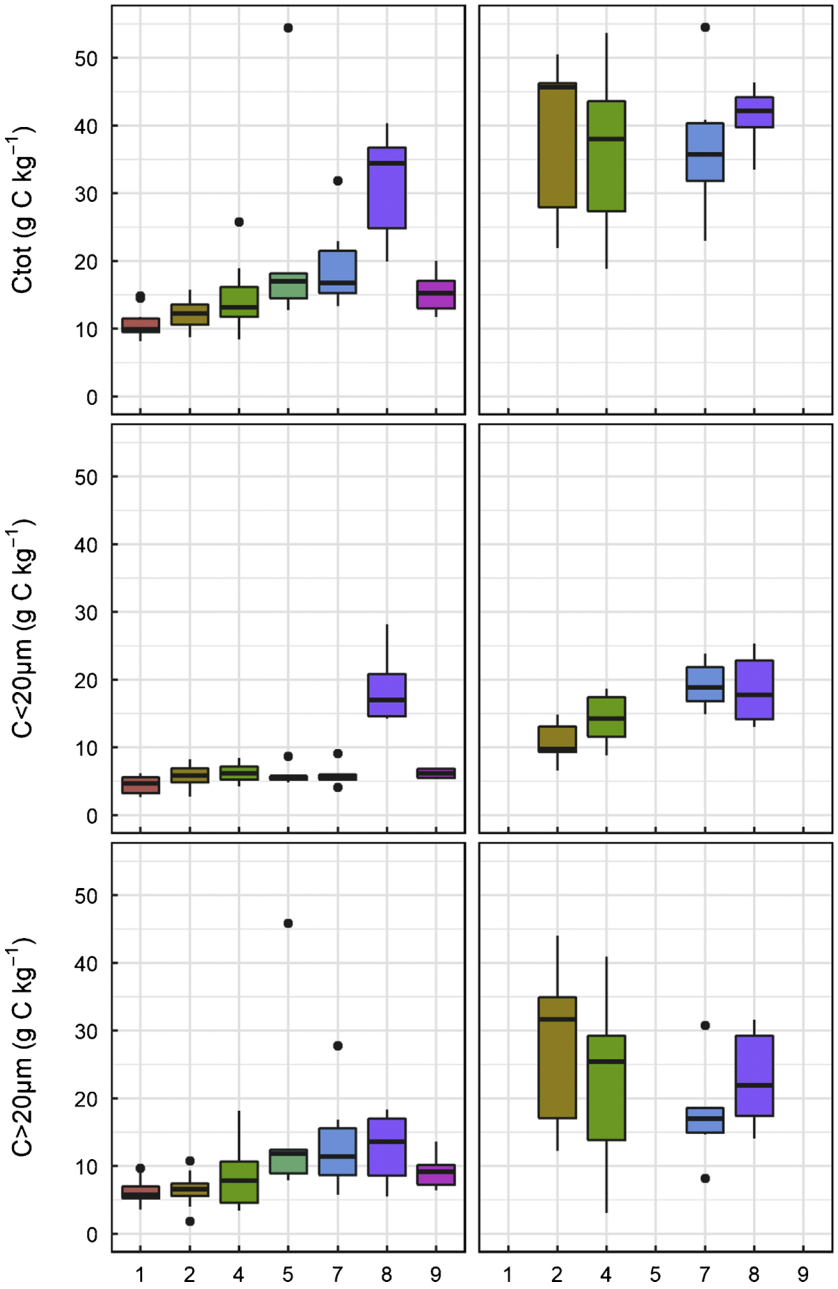

Fig. 3. Box plots of the Ctot (upper panels), C < 20 µm (middle panels) and C > 20 µm (lower panels) in croplands (0–20 cm; left hand) and grasslands (0–10 cm; right hand) of the soil monitoring network according to agricultural region: 1: sandy-loam region (cropland n = 12), 2: Loam region (cropland n = 20; grassland n = 6), 4: Condroz (cropland n = 12; grassland n = 12), 5: Herbagère de Liège (cropland n = 5), 7: Famenne (cropland n = 6; grassland n = 6), 8: Ardenne (cropland n = 6; grassland n = 6), 9: Jura(cropland n = 6). See Fig. 1 for a map of the agricultural regions.

consistent with the overall high clay content, low temperature and high precipitation in this region, whereas the lower contents in the loam region correspond to the lower clay content and lower precipitation at higher temperatures. These clear regional trends under grassland are in agreement with the theory of Hassink (1997) and Feng et al. (2013), who argued that the fine C fraction is associated with clay and fine silt. However, it should be noted that the clay content, precipitation and temperature all show similar gradients and there is a need for multi-factorial experiments to unravel the influence of each factor. The higher content of C < 20 µm in clay soils is therefore consistent with a higher storage capacity in the fine fraction due to their greater specific surface area combined with the greater relative abundance in heavy textured soils. In contrast, the total storage of C < 20 µm in light textured soils was limited due to the greater number of coarser particles in these soils (Hobley et al., 2013).

In contrast to the grasslands, there was no clear trend in C < 20 µm in the croplands, but concentrations were generally low (Fig. 3). This is consistent with lower C inputs into the croplands (1.93–2.15 Mg C ha^{-1}y^{-1}, van Wesemael et al., 2010) compared to the grasslands (2.9 Mg C ha^{-1}y^{-1}), and indicates that additional C storage capacity exists in the fine fraction of the croplands. However, the higher C > 20 µm in the grasslands than in the croplands indicates that this fraction is also depleted and has further C storage potential. This is consistent with C losses under croplands across both fractions, indicating that both fractions are sensitive to land-use (Hobley et al., 2016). Despite the lack of a clear regional trend in C < 20 µm, the C > 20 µm in croplands showed a regional-climatic trend corresponding to increase in precipitation and decrease in temperature from

Table 3
Correlation matrix. Bold numbers are significant ($p < 0.05$). See Table 2 for explanation of the co-variates.

	Ctot	C < 20 µm	C > 20 µm	pH	PR	MBC	MBN	biolog	N min	qmic	qCO2	clay	cfactor
Ctot	1	**0.78**	**0.93**	**−0.6**	**0.75**	**0.74**	**0.76**	**0.23**	**0.24**	0.07	0.06	**0.39**	**−0.75**
C < 20 µm		1	**0.5**	**−0.63**	**0.6**	**0.59**	**0.65**	0.12	**0.3**	0.01	0.11	**0.46**	**−0.65**
C > 20 µm			1	**−0.47**	**0.69**	**0.68**	**0.68**	**0.25**	0.17	0.09	0.02	**0.27**	**−0.66**
pH				1	**−0.53**	**−0.47**	**−0.53**	−0.09	−0.12	−0.07	−0.08	**−0.33**	**0.57**
PR					1	**0.88**	**0.87**	**0.44**	**0.38**	**0.3**	**0.52**	**0.51**	**−0.7**
MBC						1	**0.96**	**0.49**	**0.4**	−0.1	**0.67**	**0.51**	**−0.66**
MBN							1	**0.51**	**0.4**	−0.07	**0.58**	**0.48**	**−0.67**
biolog								1	0.16	0.02	**0.48**	0	−0.21
N min									1	0.12	**0.33**	**0.24**	**−0.38**
qmic										1	−0.19	−0.02	−0.14
qCO2											1	**0.35**	−0.19
clay												1	**−0.32**
cfactor													1
FYM													
dem													
GWL max													
GWL min													
eastness													
flow length													
silt													
northness													
precipitation													
sand													
slope													
temp													
TPI 500m													
C < 20 µm/Ctot													

	FYM	dem	GWL max	GWL min	eastn	flow lgth	silt	northn	prec.	sand	slope	temp	TPI 500m	C < 20 µm/Ctot
Ctot	**0.54**	**0.46**	−0.01	0.15	0.09	**0.44**	**−0.24**	0.06	**0.49**	0.01	**0.52**	**−0.48**	−0.16	**−0.48**
C < 20 µm	**0.72**	**0.65**	0	0.21	0.06	**0.61**	**−0.31**	0.13	**0.68**	0.05	**0.48**	**−0.66**	−0.06	0.08
C > 20 µm	**0.34**	**0.26**	−0.01	0.08	0.09	**0.26**	−0.16	0	**0.29**	−0.01	**0.45**	**−0.28**	−0.19	**−0.71**
pH	**−0.42**	**−0.42**	**0.25**	−0.16	0.03	**−0.47**	**0.26**	−0.17	**−0.44**	−0.11	**−0.5**	**0.44**	**0.31**	0.12
PR	**0.43**	**0.35**	−0.07	0.14	−0.08	**0.37**	**−0.3**	0.14	**0.41**	0	**0.48**	**−0.37**	**−0.28**	**−0.34**
MBC	**0.47**	**0.27**	−0.05	0.08	−0.02	**0.34**	**−0.26**	0.16	**0.34**	−0.04	**0.37**	**−0.29**	**−0.21**	**−0.32**
MBN	**0.5**	**0.38**	−0.04	0.09	−0.06	**0.39**	**−0.31**	0.18	**0.45**	0	**0.45**	**−0.4**	**−0.24**	**−0.27**
biolog	0.13	0.11	−0.04	0.08	−0.05	0.02	−0.07	0.15	0.14	−0.02	0.21	−0.11	−0.09	−0.21
N min	**0.33**	**0.28**	**0.27**	0.07	−0.06	0.17	−0.15	0.12	**0.32**	0	**0.24**	**−0.27**	0	0.03
qmic	−0.08	0.12	−0.09	0.19	−0.11	0.09	−0.09	−0.05	0.12	0.08	0.15	−0.13	−0.15	**−0.24**
qCO2	0.16	−0.04	−0.07	−0.06	−0.14	0.06	−0.12	0.17	0.03	−0.08	0	0.01	−0.1	0.08
clay	**0.37**	**0.2**	−0.04	−0.02	0.16	**0.36**	**−0.22**	0.04	**0.24**	**−0.27**	0.09	**−0.22**	0	0.03
cfactor	**−0.43**	**−0.38**	0.06	−0.04	0	**−0.39**	0.14	−0.09	**−0.39**	0	**−0.42**	**0.4**	0.19	**0.29**
FYM	1	**0.64**	0.14	0.14	0.15	**0.5**	**−0.31**	−0.12	**0.72**	0.04	**0.54**	**−0.65**	0.02	0.07
dem		1	0.06	**0.32**	−0.05	**0.72**	−0.17	0.01	**0.96**	0.06	**0.6**	**−1**	−0.01	0.08
GWL max			1	**0.42**	0.03	−0.13	−0.13	0.05	0.03	0.1	0.1	−0.05	0.1	0.08

(*continued on next page*)

Table 3 (*continued*)

	FYM	dem	GWL max	GWL min	eastn	flow lgth	silt	northn	prec.	sand	slope	temp	TPI 500m	C < 20 µm/Ctot
GWL min				1	−0.01	0.05	−0.11	0.05	**0.29**	0.14	**0.27**	**−0.31**	−0.01	−0.02
eastness					1	0.06	0.15	**−0.31**	−0.04	−0.15	−0.05	0.04	**0.3**	0.01
flow length						1	−0.05	0.03	**0.72**	−0.15	**0.39**	**−0.74**	−0.11	0.09
silt							1	−0.04	−0.21	**−0.67**	**−0.26**	0.16	0.01	0.07
northness								1	0.01	0.15	0.03	−0.02	**−0.33**	0.18
precipitation									1	0.07	**0.61**	**−0.97**	−0.1	0.06
sand										1	0.17	−0.05	−0.12	0.03
slope											1	−0.61	−0.2	−0.19
temp												1	0.07	−0.07
TPI 500m													1	0.16
C < 20 µm/Ctot														1

the NW to the SE, suggesting that this fraction is sensitive to climate-controlled inputs (C inputs from crop residues: sand loam region: 2.07 Mg C $ha^{-1}y^{-1}$, loam region: 1.93 Mg C $ha^{-1}y^{-1}$, Condroz: 2.06 Mg C $ha^{-1}y^{-1}$, Ardenne: 2.15 Mg C $ha^{-1}y^{-1}$; van Wesemael et al., 2010).

The conditional inference forest model performed better at explaining the C < 20 µm than the C > 20 µm, accounting for 63% of variance in the former, but only 44% of variance in the latter. Although the predictive performance may be improved by not constraining the minimum criterion for a split (Hobley and Wilson, 2016), our focus was on identifying predictors with statistically significant relationships with the fractions, which was ensured by this modelling approach. Note that due to the very strong correlation of temperature and elevation with precipitation (Table 3), we only included precipitation as a potential predictor in the models.

The microbial biomass N concentration proved to be the most important factor explaining C storage in both fractions (Fig. 4). This result suggests that C-storage is not only constrained by C-inputs (as indicated by the difference between grasslands: 2.9 Mg C $ha^{-1}y^{-1}$ and croplands: 1.93–2.15 Mg C $ha^{-1}y^{-1}$ van Wesemael et al., 2010) but that constraints on microbial activity, such as N limitation can also influence SOM storage (Allison et al., 2010; Craine et al., 2007; Fontaine et al., 2004; Kirkby et al., 2014). Although, the microbial biomass C and N were strongly correlated, the greater influences of MBN on C storage suggests an overall N limitation in the region. In particular, only five variables were identified as important to predicting C > 20 µm (Fig. 4), namely microbial N and C, land use, respiration and crop cover as approximated by the C factor of the RUSLE, of which the three most important variables were related with microbial activity. This indicates a close relationship between more labile C > 20 µm with potential respiration and land-use. The higher C > 20 µm in grasslands than croplands reflects the difference in C inputs in these systems (see data on C input in previous paragraph), whereas the microbial parameters indicate close association of less stable C > 20 µm with potential respiration. However, whether C storage controls microbial activity, vice-versa, or they are co-dependent could not be concluded from this analysis.

In contrast to the relatively simple controls on C > 20 µm, C < 20 µm was also associated with environmental and management co-variates such as carbon input from farmyard manure and slurry (FYM), precipitation at the regional scale, and upstream flow length as a proxy for fluxes of sediment and water at the catchment scale. Flow length has been associated with SOC spatial distribution in the region, as result of its effect on erosion and sedimentation, and relationship with elevation ($\rho = 0.29$) and therefore precipitation, which influences long-term productivity (Chartin et al., 2017). The importance of FYM and slurry to C < 20 µm suggests that the application of farm fertilizers leaded to their incorporation and retention into fine soil particles. Overall, these co-variates important to C < 20 µm (FYM, slurry, precipitation and flow length) are indicative of the balance of C input and decomposition over longer time scales and it is thus not surprising to find them as explanatory variables for a more stabilized fraction.

Overall, the conditional inference forest selected those variables with strongest relationships with the fractions, which is consistent with the constraint to significance imposed within the algorithm. The selected variables themselves were often correlated with many other variables, often indicating conditions favouring the input of biomass and retarding decomposition (Table 3). An example of such covariance between the variables selected by the conditional inference forests is the ratio of microbial biomass C to Ctot (qmic), which was positively correlated to potential respiration (PR), microbial biomass N (MBN), N mineralization (Nmin) and clay content. The negative correlation between the proportion of C < 20 µm and Ctot indicates that this fraction was important at low Ctot contents (Table 3; Hobley et al., 2013).

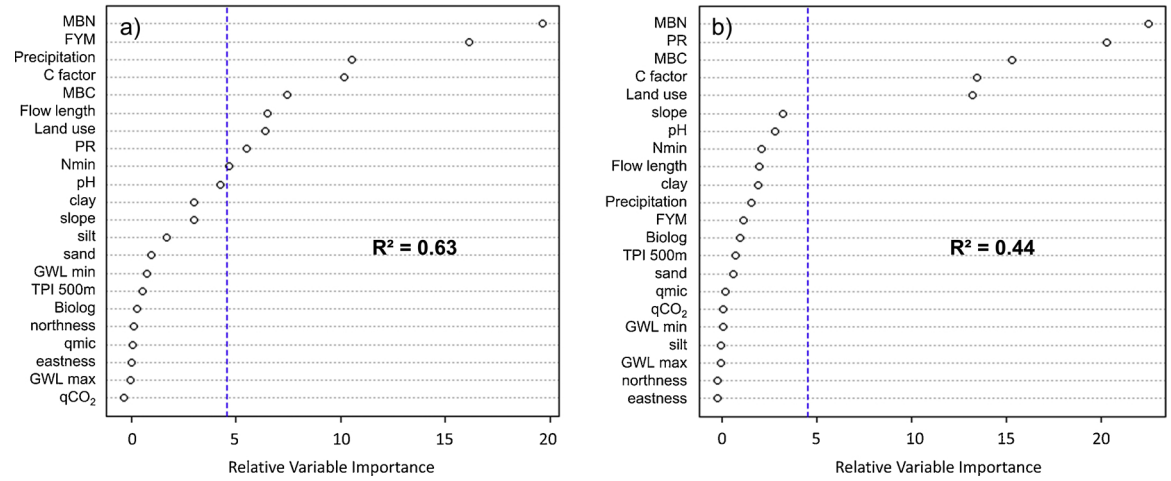

Fig. 4. Relative variable importance of co-variates (Table 2) identified using the conditional inference forest models for A) C < 20 µm, B) C > 20 µm. The vertical dashed lines indicates the average variable importance including all 22 covariates.

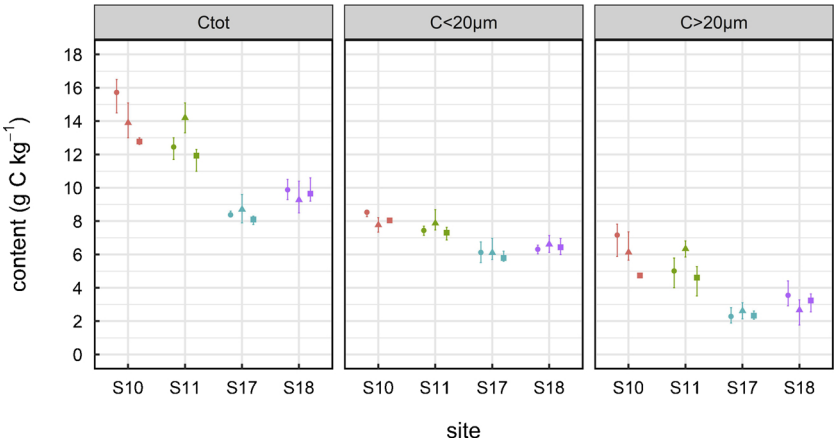

Fig. 5. Seasonal variability in Ctot and SOM fractions for sites with no-till (S10), shallow tillage (S11), long-term experiment (control S17 and cover crops S18). Sampling dates: April 2016 (diamonds), June 2016 (triangles), August 2016 (squares). The error bars represent the minimum and maximum values (n = 4 for each point).

3.2. Sensitivity of fractions to agricultural management

SOM fractions were analysed in April, June and August for two sites under conservation agriculture (see section 2.3) and a control as well as an organic amendment treatment of the long-term trial in Long Tours (see section 2.3; Fig. 5). Overall, the differences between the seasons were smallest for the C < 20 µm. For the C > 20 µm, there were no systematic differences between the seasons. The variability of Ctot reflected the trends in C > 20 µm, indicating that the coarse fraction was more sensitive to short-term influences on C dynamics, and this is reflected in Ctot concentration. Although sampling to a fixed depth of 20 cm did not account for any settling effects after tillage, there appeared to be no clear trends and thus no reason to restrict sampling to a specific season.

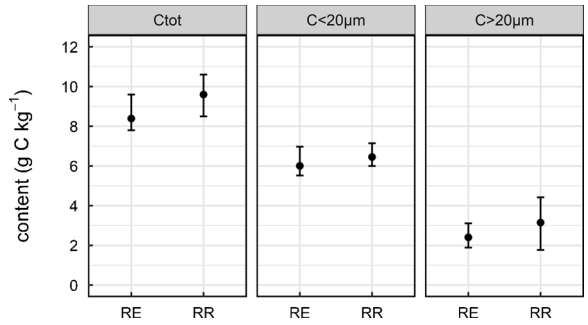

Fig. 6. Long-term trial on organic amendments set up in 1959; RE: Residue export without organic fertilizer, RR: Residue restitution and cover crop (see Section 2.3). The error bars represent the minimum and maximum values (n = 12 for each treatment).

The sampling throughout the seasons in the long-term trial of Long Tours provided a total number of 12 samples (i.e. 3 seasons and 4 replicates between the control (residue export) and the treatment with residue restitution and a cover crop once every three years in the rotation (Section 2.3; Fig. 6)). Despite high variability, particularly in C > 20 µm, ploughing in of residues increased C contents in the coarse and fine fractions, as well as Ctot (Fig. 6). Although both fractions responded positively to residue restitution, the effect was less pronounced in C < 20 µm than in C > 20 µm. This is consistent with a greater sensitivity to management in C > 20 µm than in C < 20 µm. This corresponds with the findings of Trigalet et al. (2014), who investigated the same treatments using samples from 1970 and 2012 and did not observe any differences between treatments in the fine fraction sampled in 1970. This confirms that increases in the fine fraction are relatively slow and require a sustained supply of C from the coarse fraction, where the differences between treatments are larger and occur more rapidly. Furthermore, this suggests that C < 20 µm is (at least partly) derived from a portion of C > 20 µm, namely the C which is not lost as CO_2 to the atmosphere but is biophysico-chemically altered and incorporated into the fine soil matrix.

The trial in Gentinnes consisted of three treatments including a cover crop and residue return to the soil, but with different tillage management (Section 2.3; Fig. 7). The effect of the tillage depth

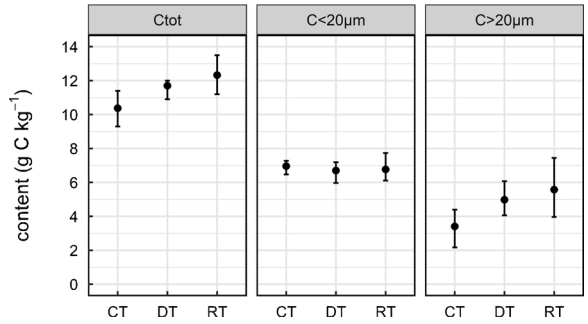

Fig. 7. Long-term trial (set up in 2005) with mouldboard tillage (CT), cultivator tine until 30 cm(DT) and spring tine cultivator until 10 cm (RT). The error bars represent the minimum and maximum values (n = 4 for each treatment).

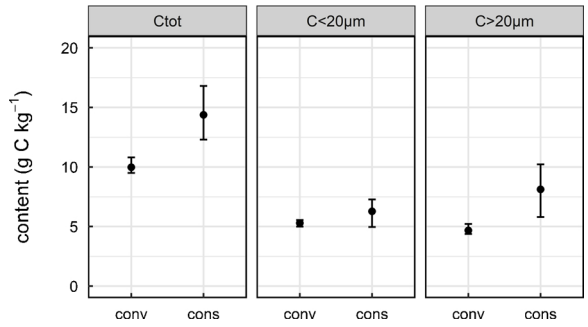

Fig. 8. Paired sites in farmers' fields with conventional (conv; 1 site) and conservation agriculture (cons; 2 sites) for at least 10 years (n = 12). The error bars represent the minimum and maximum values.

(mouldboard (CT) vs heavy tine cultivator until 30 cm (DT) and spring tine cultivator until 10 cm (RT)) can be seen from the higher C > 20 µm in the conservation treatments (i.e. DT and RT) (Fig. 7). This difference can be explained by the shallower tillage depth and the incomplete mixing of the plough layer by using the heavy tine cultivator (DT). However, in contrast to the trial in Long Tours that started in 1959, the length of the trial in Gentinnes (established in 2005) had not yet led to changes in C < 20 µm. Thus, the increase in C due to reduced tillage management occurred in C > 20 µm, consistent with its characterization as a short to mid-term management-sensitive indicator of C. It should be noted that a small bias is to be expected as the C concentration is no longer uniformly mixed throughout the 27 cm thick plough layer, but will be concentrated in the sampled 0–20 cm topsoil.

In contrast to the Gentinnes trial, the paired plot approach demonstrated that fields converted to conservation agriculture for at least 10 years had higher C < 20 µm concentrations (Fig. 8). This difference could be explained by the fact that farmers in general changed not only their tillage system (as in Gentinnes), but also increased the use of cover crops and crop residues (see Section 2.3). In fact, the difference in C > 20 µm between treatments for the paired plots (3.0 g C kg^{-1}) was somewhat higher than for the Gentinnes trial (2.0–2.5 g C kg^{-1}).

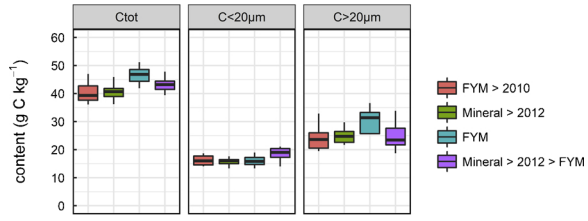

Fig. 9. Box plot of long-term trial on grassland fertilization set up in 1996. Organic farming started and mineral fertilization stopped in 2012 (n = 8 for each treatment).

Overall, differences in C < 20 µm between control and the treatments were observed over periods of 5–10 years only when the ratio C > 20 µm/Ctot was greater than 0.5. These results indicate that management can affect C < 20 µm over shorter time-periods, but that high inputs of coarse SOM are required to drive the production of fine SOM and its incorporation into the soil matrix in the longer term.

For the grasslands, the C < 20 µm (12–20 g C kg^{-1}) was rather high (Figs. 9 and 10). Moreover, the variability in C < 20 µm across the 49 sites of the Bioecosys network (Table 1) and the four treatments in Libramont was quite small (Figs. 9 and 10; Table 4). The proportion of C > 20 µm was also higher in the grasslands (0.57-0.79) compared to the croplands (0.45-0.59). This large and relatively constant C < 20 µm and high proportion of C > 20 µm for sites mainly in the Ardenne where the soil texture is relatively clayey suggests that these soils are well supplied by C input and may be close to their C saturation as first defined by Hassink (1997). Thus, changes in management will likely not further increase the C < 20 µm fraction, but be reflected in the C > 20 µm fraction. In fact, the treatment receiving composted manure clearly had the highest C > 20 µm in Libramont (Fig. 9). For the grasslands in the Ardenne, there was a general trend of increasing C > 20 µm with decreasing management intensity (Fig. 10). The differences between conventional (Mt1) and organic temporary grasslands (Mt2) were clear as well as the gradient in the grazed grasslands with increasing restrictions on cattle number and organic amendments (Gp1-Gp3; Fig. 10, Table 4). In summary, more intensively managed grasslands were generally associated with lower Ctot contents, with the reactive fraction being C > 20 µm, but the overall high inputs of C into grasslands systems indicate a saturation in C < 20 µm, so that it is not management sensitive. However, caution is required when comparing the results in the croplands and grasslands due to the differences in sampling depth, so that further research is required in order to substantiate the results.

3.3. SOC fractions as indicators for agricultural management

From the previous sections it appeared that the C < 20 µm represents a relatively stable fraction that is associated with the mineral complexes. The concentration of this fraction was highest in grassland soils, where at the same time the C > 20 µm concentrations were high (Fig. 3). This indicates that a large input of C is required to increase and maintain fine C storage. Long-term trials in cropland demonstrated that the coarse fraction (C > 20 µm) reacts to conservation tillage treatments started in 2008 (DT and RT; Fig. 7), but that the mineral associated fraction (C < 20 µm) had not yet reacted to these treatments. The relative change in Ctot over the last ~ 10 years (C_ER: enrichment ratio in Ctot) was assessed in 60 sites that were first sampled in 2005 and re-sampled in 2015 (see section 2.3). The ratio of C > 20 µm to C < 20 µm (qfrac) was linearly related to the relative change in Ctot (2005–2015; $r^2 = 0.66$, n = 60; Fig. 11). This demonstrates the importance of the C > 20 µm fraction not only as a sensitive indicator for the effect of changes in management practices, but that this ratio qfrac is a predictor of dynamics of topsoil SOC concentrations in these soils. Jaconi et al. (2019) demonstrated that a log ratio transformation of soil organic carbon fractions (in our case C > 20 µm/ C < 20 µm) gave the best results for their prediction by VisNIR spectroscopy. Therefore, qfrac is suitable for future use in standard soil fertility analyses, which are likely to include spectroscopy as a routine analysis (Genot et al., 2011).

Comparable to changes in qfrac, changes in microbial quotient (i.e. qmic) have already been suggested as useful and sensitive indicators to predict long-term trends in SOM and for monitoring changes in SOC e.g. due to changes in management (Powlson et al., 1987). The changes in qmic reflect organic matter inputs to soils, the efficiency of conversion to microbial C, losses of C from the soil and the stabilization of organic C by the soil mineral fractions (Anderson and Domsch, 1980, 1989; Wardle, 1992). Anderson and Domsch (1989) published an equilibrium

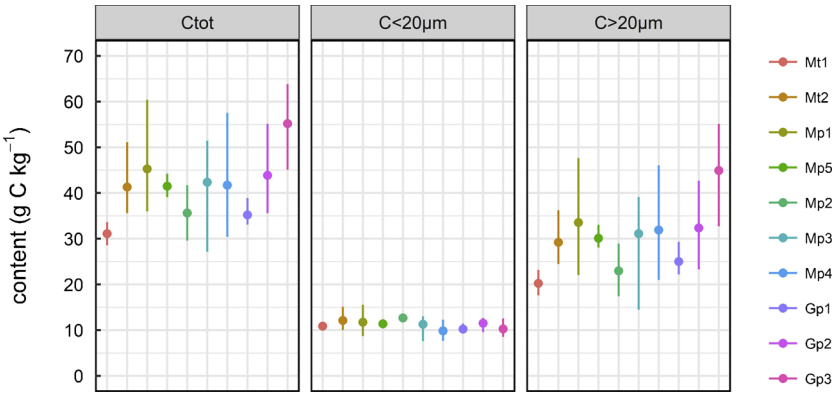

Fig. 10. Network of 49 grassland sites under agro-ecological schemes. Management intensity is decreasing from left to right along the X axis. See Table 1 for details on number of sites per treatment and management: M = mowing, G = grazing, p = permanent, t is temporary. The error bars represent the minimum and maximum values.

constant for qmic of 29 [mg MBC × g Corg^{-1}] for continuous crop rotations and of 23 [MBC × g Corg^{-1}] for monocultures. This equilibrium constant was valid for soils from the temperate climate zone of central Europe. Any deviation of the qmic from this equilibrium for any given soil would indicate that its C-content is increasing or decreasing. Insam et al. (1989) expanded this constant to an equilibrium function, which accounts for the influence of macroclimatic conditions by analyzing soils from 15 long-term sites from different climatic regions in the United States and Canada. The qmic showed a consistent trend depending on climate, as assessed by a precipitation/evaporation quotient (Insam et al., 1989). However, the relationship proposed by Insam et al. (1989), relating the qmic to a precipitation/evaporation quotient, generally underestimated the MBC contents of New Zealand soils, demonstrating that clay content and mineralogy also affect the qmic. It has to be tested if qfrac remains useful in soils from different climatic regions as an indicator for sites that restore/loose C.

4. Conclusion

Shaking a soil-water mixture and sieving at 20 µm enables separating organic matter in a fine (< 20 µm) and a coarse (> 20 µm) fraction. The C concentrations in these fractions are related to biological indicators, such as microbial biomass N and C and potential respiration, and management effects, such as C input from farmyard manure, and land use, as well as relationships with regional environmental covariates, such as, precipitation and – for the fine fraction - flow length. Long-term trials and paired plots revealed that the coarse fraction is more sensitive than the fine fraction to changes in management and that the former already responds within ˜5 years to changes in management. A different long-term behavior is observed between land-uses: i) in croplands, the fine fraction increases if the ratio of the coarse fraction to the Ctot > 0.5, ii) in grasslands, the fine fraction at the surface does not react to changes in intensity of grazing and mowing or use of fertilizer, but the coarse fraction reacts quickly. The relatively stable C < 20 µm content in grassland surface soils with high biomass C input is in agreement with the C saturation concept. After all, earlier

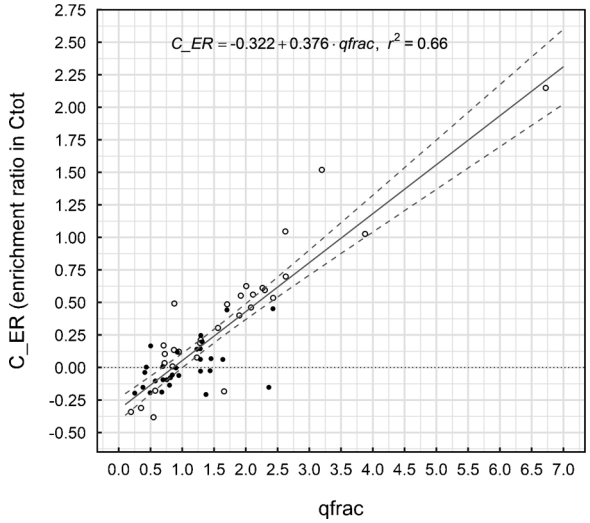

Fig. 11. The ratio C > 20 µm/C < 20 µm (qfrac) against enrichment ratio in Ctot (C_ER: ($Ctot_{2015}$-$Ctot_{2005}$)/$Ctot_{2005}$)) for cropland (0–20 cm; closed symbols) and grassland (0–10 cm; open symbols) sites in the Carbosol network sampled in 2005 and 2014-2016. The dashed lines are the 95% confidence limits of the regression.

studies used grassland soils as the C saturation potential at a given fine silt and clay content. The coarse fraction is crucial for maintenance/restoration of SOC because it: i) is the most reactive fraction from the biological point of view and in the longer term feeds the fine C fraction resulting in stable SOC stocks; and ii): reflects the integrated effect of agricultural management over ca. 10 years or more. For soils with reduced or no-till, care should be taken to sample deep enough to represent the former plough layer. Although the behavior of the indicators from the simple fractionation protocol is comparable to indicators based on soil microbial biomass, extrapolation to other climate and soil conditions as well as other sampling depths has not yet

Table 4
Results of ANOVA for differences between treatment in grassland sites. The SOC and the two fractions are analysed (C type). The treatments and the number of samples are explained in Sections 2.3 and 2.6.

differences	Sites/Trials	test	C type	p	df
Grassland fertilization	Libramont	ANOVA by permutation	Ctot	< 0.01	3
	4 treatments with 2 replicates	treatment as between-subjects factor	C < 20 µm	0.19	3
	4 samples per replicate		C > 20 µm	< 0.01	3
Grassland management	BioEcoSys	ANOVA by permutation	Ctot	< 0.01	9
	10 treatments in Ardenne	treatment as between-subjects factor	C < 20 µm	0.14	9
	3 (4)replicates		C > 20 µm	< 0.01	9

been tested. The same applies to the energy levels for the fractionation. Sonication has a greater capacity to break up aggregates and hence increases the fine fraction. As the sensitivity of the fractions to changes in management is of prime importance, the energy required to separate a fraction that responds to management changes probably varies according to environmental conditions, so that we recommend testing and validation of this protocol for other soils and management regimes.

Declaration of interests

None.

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