Interreg CENTRAL EUROPE

Co-funded by the European Union

Carbon Farming CE

CARBON SEQUESTRATION MONITORING METHODOLOGY

DELIVERABLE D.3.2.1

Version 1

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CONTENT

1. INTRODUCTION AND SCOPE OF THE TASK

In the Carbon Farming CE project, this Deliverable 3.2.1 is linked to Activity 3.2, which includes all activities for standardization of carbon sequestration monitoring.

The objective of Deliverable 3.2.1 is to provide a roadmap for monitoring soil C sequestration from carbon farming techniques.

Most C in agricultural soils is held in an organic form (soil organic carbon, SOC), and sequestering organic C in soils can have multiple benefits, including: i) offsetting of anthropogenic C emissions, ii) restoring soil function, iii) improving soil resilience (to erosion, pollution, disease and drought), iv) increasing agricultural productivity and sustainability, and v) improving food security (Lal et al., 2015). Because of these expected benefits, promoting SOC sequestration is of interest to both the farmers and policy makers.

There are various approaches to monitoring soil C, but here we present strategies aimed at assessing increases in: i) soil organic C, and ii) associated soil quality, which are expected to restore soil functionality and enhance the other multiple benefits promoted by SOC sequestration. Additionally, due to the interest of both farmers and policy makers, a multi-criteria approach for monitoring C sequestration due to different C farming techniques is presented: a simple and quick one for farmer self-assessment (visual soil assessment, VSA; FAO, 2008) and a more complex one based on C stock quantification and determination of soil enzyme activities (Bünemann et al., 2018; Giacometti et al., 2014; Gil-Sotres et al., 2005) and the related Soil Quality Index (SQI; Andrews et al., 2002; Askari and Holden, 2015; Mazzon et al., 2021) for independent audit. The roadmap also outlines the advantages and disadvantages of each C farming monitoring strategy, to support stakeholders in selecting the most appropriate one (Table 8).

As training material for farmers and end-users, this document is written in an easy-to-understand language, supported by explanatory boxes summarizing the three different monitoring methods (Boxes 1, 2 and 3). However, proper citations and references are provided to allow those interested to further explore the topic.

2. APPROACH FOR MONITORING SOIL CARBON SEQUESTRATION

2.1 Identifying goals of soil indicators for different monitoring strategy

The objectives of the proposed multi-criteria approach to soil organic C monitoring are: 1) quantifying the content of C stock of soils subjected to a specific carbon farming techniques; 2) evaluating the associated soil quality, which is expected to restore soil functionality and improve the other multiple benefits promoted by SOC sequestration; 3) defining several monitoring strategies characterized by a different level of complexity and capacity to monitor C in soils, but that can provide a simple and rapid methodology for farmers or a more complex and expensive assessment useful for agricultural advisors and policy makers.

2.2 Identifying standardized soil indicators

2.2.1 Visual Soil Assessment

The first soil index is the simplest, addressed to farmers and able to give them immediate feedback to them on soil quality through visual indicators of key physical, biological and chemical soil properties (Visual Soil Assessment; FAO, 2008).

Visual Soil Assessment is based on the visual assessment of key indicators of soil “status” and crop performance, which are presented on a scorecard (Figure 1). With the exception of soil texture, the soil indicators are dynamic indicators, i.e., they can change under different management regimes and land use pressures. Because they are sensitive to change, they are useful early warning indicators of changes in soil condition and as such provide an effective monitoring tool.

By following the pdf guide (https://www.fao.org/3/i0007e/i0007e.pdf) and observing the soil, you can fill in the form below. Each parameter is rated on a scale from 0 to 2 and a weight is assigned to each parameter observed. The sum of the values obtained makes it possible to obtain a soil quality index based on physical parameters, which is also directly correlated with the C content in the soil.

Landowner: Land use:
Site location: GPS ref:
Sample depth: Date:
Soil type: Soil classification:
Drainage class:

Textural group (upper 1 m):	☐ Sandy	☐ Loamy	☐ Silty	☐ Clayey	☐ Other
Moisture condition:	☐ Dry	☐ Slightly moist	☐ Moist	☐ Very moist	☐ Wet
Seasonal weather conditions:	☐ Dry	☐ Wet	☐ Cold	☐ Warm	☐ Average

Visual indicators of soil quality		Visual score (VS) 0 = Poor condition 1 = Moderate condition 2 = Good condition	Weighting	VS ranking
Soil texture	pg. 2		x 3	
Soil structure	pg. 4		x 3	
Soil porosity	pg. 6		x 3	
Soil colour	pg. 8		x 2	
Number and colour of soil mottles	pg. 10		x 2	
Earthworms (Number =) (Av. size =)	pg. 12		x 3	
Potential rooting depth (m)	pg. 14		x 3	
Surface ponding	pg. 18		x 1	
Surface crusting and surface cover	pg. 20		x 2	
Soil erosion (wind/water)	pg. 22		x 2	
SOIL QUALITY INDEX (sum of VS rankings)				

Soil Quality Assessment	Soil Quality Index
Poor	< 15
Moderate	15–30
Good	> 30

Figure 1. Scorecard provided by the FAO for the Visual Soil Assessment development. https://www.fao.org/3/i0007e/i0007e00.pdf

2.2.2 Development of pedotransfer functions (PTFs) for bulk density prediction

The second level will focus on the development of pedotransfer functions (PTFs) for predicting bulk density based on textural data and TOC (total organic C) content. Bulk density measurement is needed to quantify the soil C stocks (i.e., soil organic C on an area basis; Mg C ha^{-1}). However, the bulk density values are often missing from databases. The PTF approach will have the potential to help public officials or private companies to fill gaps in their database, allowing them to calculate soil C stocks.

A relationship between TOC and/or particle size content (in g kg^{-1}) and bulk density (in Mg m^{-3}) is defined, and the amount of C can be used to determine the bulk density values. Subsequently, the organic C stock in soils without skeleton (rock fragments >2 mm) is calculated by the following equation:

$$C\ stock\ [Mg\ ha^{-1}] = TOC\ [g\ kg^{-1}] \cdot bulk\ density\ [Mg\ m^{-3}] \cdot depth\ [m] \cdot\ 10\ m^2 ha^{-1} \qquad (1)$$

2.2.3. Determination of soil enzyme activities and related Soil Quality Index (SQI)

For the third level, a minimum dataset of soil chemical and biochemical indicators was selected according to the extensive literature application of soil biochemical indicators in the assessment of soil quality (Bünemann et al., 2018; Muñoz-Rojas, 2018; Gil-Sotres et al., 2005; Nannipieri et al., 2018, 2002; Sinsabaugh et al., 2008; Balota et al., 2004; Kwiatkowski et al., 2020; Wallenstein et al., 2012) and consists in the determination of:

CHEMICAL PARAMETERS

- Total nitrogen (TN) and total organic carbon (TOC) content (elemental analyser)
- C isotope composition ($\delta^{13}C$) (elemental analyser)

BIOCHEMICAL PARAMETERS

- Microbial biomass nitrogen (MBN) and carbon (MBC) content (Vance et al., 1987)
- Determination of seven extracellular hydrolytic enzyme activities (EEA) (Giacometti et al., 2014)
- C cycle: β-glucosidase (β-glu), α-glucosidase (α-glu), β-cellobiosidase (β-cel), β-xylosidase (β-xyl)
- N cycle: N-Acetyl-β-glucosaminidase (NAG)
- P cycle: Phosphomonoesterase (PME)
- S cycle: Arylsulfatase (AS)

Among the chemical parameters reported, C isotope composition ($\delta^{13}C$) is a measure that indicates the degree of C stabilization in the soil (Werth and Kuzyakov, 2010).

Chemical and biochemical parameters are used to determine **simple soil quality indicators** as the specific enzyme activities = EEA/MBC, and **complex soil quality indexes,** as the biochemical Soil Quality Index (SQI) were calculated. The **SQI** could be determined using the Soil Management Assessment Framework (SMAF) process (Figure 2), which included (i) indicator selection, (ii) indicator scoring, and (iii) integration of the scores into the index (Andrews et al., 2002; Andrews et al., 2004; Andrews and Carroll, 2001; Askari and Holden, 2015). Briefly, the minimum dataset (MDS) is selected from the principal component analysis (PCA), where the principal components (PC) with eigenvalues ≥1 and the properties with the highest loadings are assumed to best represent the system. Correlation is then used to reduce the redundancy between parameters, and only those parameters that did not correlate with each other are selected. Each selected parameter is standardized to a value between 0 and 1 using functions such as "more is better", "less is better", or "optimum" depending on the variable. Finally, the SQI is calculated using the "weighted additive" equation ($SQI = \Sigma WiSi$), which is the sum of the MDS scores (Si) multiplied by the amount of variation (Wi) in the corresponding PC. In general, higher SQI values correspond to higher soil quality (Mazzon et al., 2021).

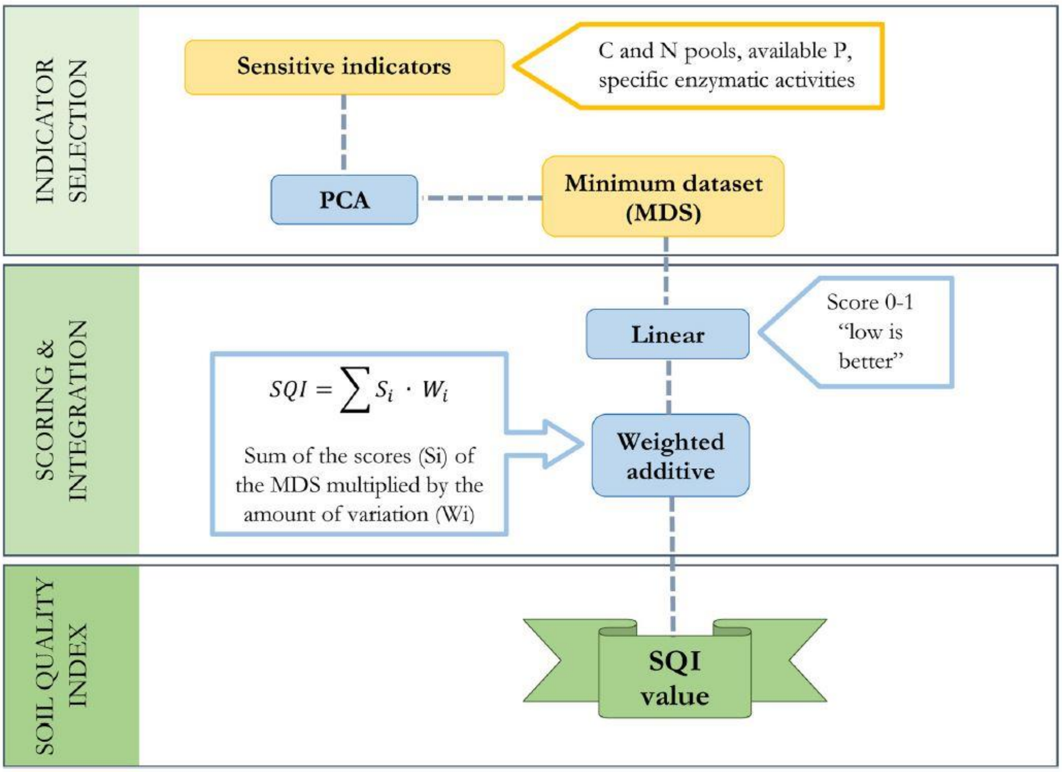

Figure 2. Schematic diagram with the steps for the determination of the SQI (Mazzon et al., 2021).

3. TEST AND IMPLEMENTATION OF CF STANDARIZED SOIL CARBON SEQUESTRATION MONITORING

3.1 Soil carbon sequestration monitoring test

The standard soil indicators selected are methods and parameters already used to assess soil quality. In the context of the CF Interreg project, these indicators outline three monitoring methods with three levels of complexity.

Two long-term field trials have therefore been selected for the monitoring test, where two of the CF techniques identified in D 1.1.1 are being tested.

Both trials are located at the experimental farm of the University of Bologna in Cadriano (about 10 km from Bologna), in the south-east of the Po Valley (Italy, 44°33' N, 11° 24' E; 23 m a.s.l.) (Figure 3).

The farm area is characterised by a fine, silty, mixed, mesic Udic Ustochrept soil (USDA Soil Taxonomy, 1999) and by a humid subtropical climate (Cfa, Köppen classification), with mean annual precipitation and temperature for the area of 747 mm and 14.2 °C, respectively.

Figure 3. Experimental trial location.

The two trials selected (Trial 29 and Trial 64) correspond to the CF techniques identified in the D 1.1.1 as:

A.1 – External organic fertilizer → Trial 29

B.2 – Crop rotation → Trial 64

3.1.1 External organic fertilizer techniques – Trial 29

The trial has been conducted since 1966 with a wheat-corn rotation characterized by the supply of manure, crop residues, and no organic supply (Control), with mineral N addition (at a dose of 200 kg_N ha^{-1} yr^{-1}) or without mineral N addition (Figure 4). The experimental design is completely randomized with three blocks and replicates.

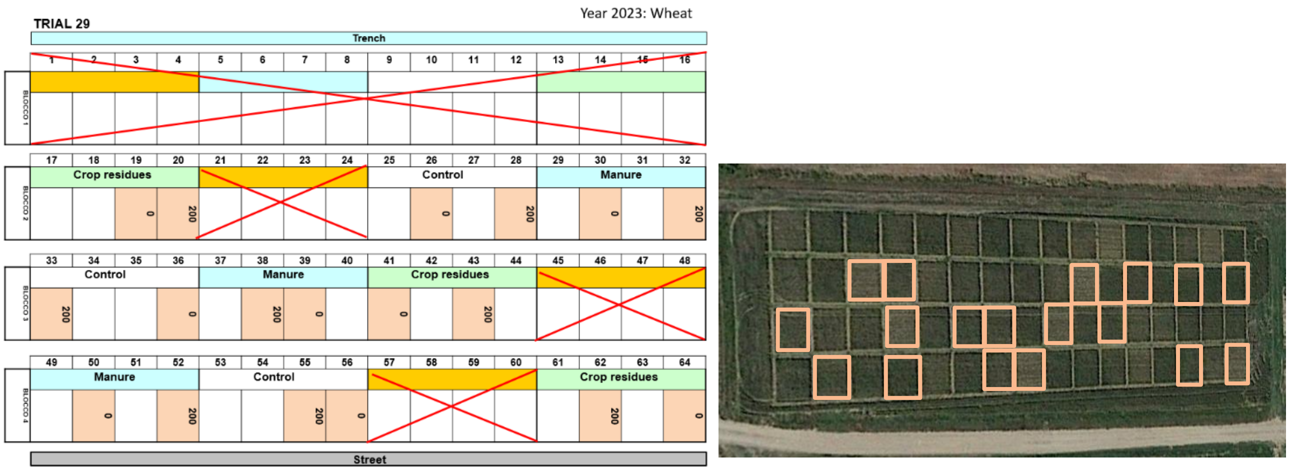

Figure 4. Experimental design of the Trial 29 – External organic fertilizer. From the plot highlighted soil was sampled and analyzed.

3.1.2 Crop rotation techniques – Trial 64

It consists of a long-term crop rotation trial with or without different combinations of organic and mineral (Figure 5). Considering the aim of the project and the intention to mainly observe the sole effect of crop rotation, we decided to consider those plots without any fertilization supply and named them "TEST" in the experimental design.

The crop rotation involved in the trial are:

- Continuous wheat
- Continuous corn
- Biennial rotation corn-wheat

- Two 9-year rotation both including: corn-wheat-corn-alfalfa-alfalfa-alfalfa-wheat-corn-wheat; the two 9-year rotation differ for the “starting” crop, thus resulting staggered over time.

Figure 5. Experimental design of the Trial 64 – Crop rotation. From the plot highlighted soil was sampled and analyzed.

3.1.3 Soil sampling

The soil was sampled and initially characterized on 7th of June 2023 in Trial 29 and on 14th of July 2023 in Trial 64. One soil sample was taken at a depth of 30 cm from each of the selected plots highlighted in Figures 4 and 5.

The first level of soil monitoring was carried out in the field (Figure 6) by following the Visual Soil Assessment procedure described in the following section (3.2). For the other two steps, a total of 28 soil samples (18 from Trial 29 and 10 from Trial 64) were taken to the Laboratory of Soil Chemistry and Pedology of the Department of Agricultural and Food Sciences of the University of Bologna, divided into two aliquots (one air-dried and one stored at 4°C) and analysed for the determination of the physical, chemical and biochemical soil indicators (section 3.3). In addition, undisturbed soil cores were taken at fixed depths (0-5, 5-10, 10-15 and 15-30 cm) for the measurement of bulk density (BD; section 3.3).

Figure 6. Photos from field of the holes and soil structure made during the VSA scorecard compilation.

3.2 Implementation of standarized soil carbon sequestration for farmers self-monitoring

As reported in section 2.2.1, the Visual Soil Assessment (VSA) method is based on the visual assessment of key soil condition and crop performance indicators of soil quality, presented as a scorecard (FAO, 2008). Figure 7 shows the scorecard used in the Interreg Carbon Farming project. As soil quality is known to be strictly related to soil C sequestration, the **VSA can be a simple, quick, cheap and indirect method to estimate the effect of C sequestration on soil quality that can be used by farmers themselves.** For each indicator, a visual score (VS) from 0 (poor) to 2 (good) is given based on field observations and comparison of soil samples with the photo gallery in the VSA manual (FAO, 2008). Each score is then multiplied by a weighting factor based on the relative importance of each indicator in assessing soil quality (Figure 7). The sum of the VS rankings gives the overall Soil Quality Index score for the sample being assessed. Compare this with the rating scale at the bottom of the scorecard to determine whether your soil is in good, moderate or poor condition.

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Visual Soil Assessment – annual crops

SOILSCORE CARD – VISUAL INDICATORS FOR ASSESSING SOIL QUALITY IN ANNUAL CROPS (FAO, 2008)

Site identification			
Landowner		Land use	
Site location		GPS coordinates	
Sample depth		Date	
Soil type		Soil classification	
Drainage class		Authors	

Textural group (upper 1 m)	◻ Sandy ◻ Loamy ◻ Silty ◻ Clayey ◻ Other
Moisture condition	◻ Dry ◻ Slightly moist ◻ Moist ◻ Very moist ◻ Wet
Seasonal weather condition	◻ Dry ◻ Wet ◻ Cold ◻ Warm ◻ Average

Visual indicators of soil quality	Visual score (VS) 0=poor condition 1=moderate condition 2=good condition	weighting	VS ranking
Soil texture		X 3	
Soil structure		X 3	
Soil porosity		X 3	
Soil colour		X 2	
Number and colour of soil mottles		X 2	
Earthworms (number ....) (average size ......)		X 3	
Potential rooting depth (........... m)		X 3	
Surface ponding		X 1	
Surface crusting and surface cover		X 2	
Soil erosion (wind/water)		X 2	
SOIL QUALITY INDEX (sum of VS ranking)			

Soil quality assessment	Soil Quality Index
Poor	< 15
Moderate	15-30
Good	> 30

Figure 7. Visual Soil Assessment –VSA- scorecard (modified from FAO, 2008).

The VSA was carried out in two long-term trials (29 and 64) at Cadriano (Bologna, Italy) (Table 1; see also section 3.1), comparing different carbon farming techniques. For external fertilizer techniques, each site had three replicates. For crop rotation techniques, each site had two replicates.

Table 1. List of carbon farming techniques tested and related ID of sampled sites.

CF techniques as codified in the deliverable D1.1.1	Trial	Short description	ID
A1, external fertilizer	29	No organic supply (Control)	**Control_0N**
	29	Control + mineral N (200 kg N ha^{-1} yr^{-1})	**Control_200N**
	29	Addition of manure	**Manure_0N**
	29	Addition of manure + mineral N (200 kg N ha^{-1} yr^{-1})	**Mannure_200N**
	29	Addition of crop residues	**Residues_0N**
	29	Addition of crop residues + mineral N (200 kg N ha^{-1} yr^{-1})	**Residues_200N**
B2, crop rotation	64	Continuous wheat	**Cont_wheat**
	64	Continuous corn	**Cont_corn**
	64	Biennial rotation corn-wheat	**Biennial**
	64	9-year rotation: corn-wheat-corn-alfalfa-alfalfa-alfalfa-wheat-corn-wheat (at sampling time = corn)	**9yrs_corn**
	64	9-year rotation: wheat-corn-wheat -alfalfa-alfalfa-alfalfa- corn-wheat-corn (at sampling time = alfalfa)	**9yrs_alfalfa**

Table 2 shows the VS for each indicator in each sampled site is reported. In Figure 8, the sum of the VS rankings gives the overall Soil Quality Index by VSA. All crop rotation techniques allowed to achieve high soil quality as assessed by VSA (VSA quality index >30; Figure 8). For external fertilizer techniques, the VSA quality index was >30 only for the addition of manure and mineral N. With the addition of crop residues, the VSA quality index assessed the soil as of moderate quality (VSA quality index between 15 and 30) and thus similar to the control.

A summary of the VSA approach is given in Box 1. For more details, the reader is referred to the FAO Field Guide (FAO, 2008).

Table 2. VS for each indicator (mean value, N = number of replicates [N = 3 in Trial 29; N = 2 in Trial 64], and st.dev. = standard deviation)

ID		texture	structure	porosity	colour	mottles	earthworm	roots depth	ponding	crusting	erosion
TRIAL 29											
Control_0N	mean	2.0	0.0	1.0	2.0	2.7	0.0	3.0	1.0	4.0	4.0
	st.dev.	*1.7*	*0.0*	*1.7*	*0.0*	*1.2*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Control_200N	mean	3.0	2.0	2.0	2.0	1.3	0.0	5.0	1.0	4.0	4.0
	st.dev.	*0.0*	*0.9*	*1.7*	*0.0*	*2.3*	*0.0*	*1.7*	*0.0*	*0.0*	*0.0*
Manure_0N	mean	3.0	1.0	2.5	3.3	4.0	0.0	4.0	1.0	4.0	4.0
	st.dev.	*0.0*	*1.7*	*0.9*	*1.2*	*0.0*	*0.0*	*1.7*	*0.0*	*0.0*	*0.0*
Mannure_200N	mean	3.0	3.0	5.0	4.0	4.0	1.0	3.0	1.7	4.0	4.0
	st.dev.	*0.0*	*0.0*	*1.7*	*0.0*	*0.0*	*1.7*	*0.0*	*0.6*	*0.0*	*0.0*
Residues_0N	mean	3.0	1.0	3.0	3.0	1.3	0.0	4.0	1.0	4.0	4.0
	st.dev.	*0.0*	*1.7*	*3.0*	*1.0*	*1.2*	*0.0*	*1.7*	*0.0*	*0.0*	*0.0*
Residues_200N	mean	3.0	0.0	2.0	2.7	2.7	0.0	3.0	1.0	4.0	4.0
	st.dev.	*0.0*	*0.0*	*1.7*	*0.6*	*1.2*	*0.0*	*3.0*	*0.0*	*0.0*	*0.0*
TRIAL 64											
Cont_wheat	mean	6.0	3.0	3.0	2.0	4.0	0.0	6.0	1.0	4.0	4.0
	st.dev.	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Cont_corn	mean	6.0	3.0	3.0	2.0	3.0	0.0	6.0	1.0	2.0	4.0
	st.dev.	*0.0*	*0.0*	*0.0*	*0.0*	*1.4*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
Biennial	mean	6.0	3.0	3.0	2.0	2.0	0.0	6.0	1.0	4.0	4.0
	st.dev.	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
9yrs_corn	mean	6.0	3.0	2.0	2.0	4.0	0.0	6.0	1.0	2.0	4.0
	st.dev.	*0.0*	*0.0*	*1.4*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*
9yrs_alfalfa	mean	6.0	3.0	3.0	2.0	4.0	0.0	6.0	1.0	4.0	4.0
	st.dev.	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*

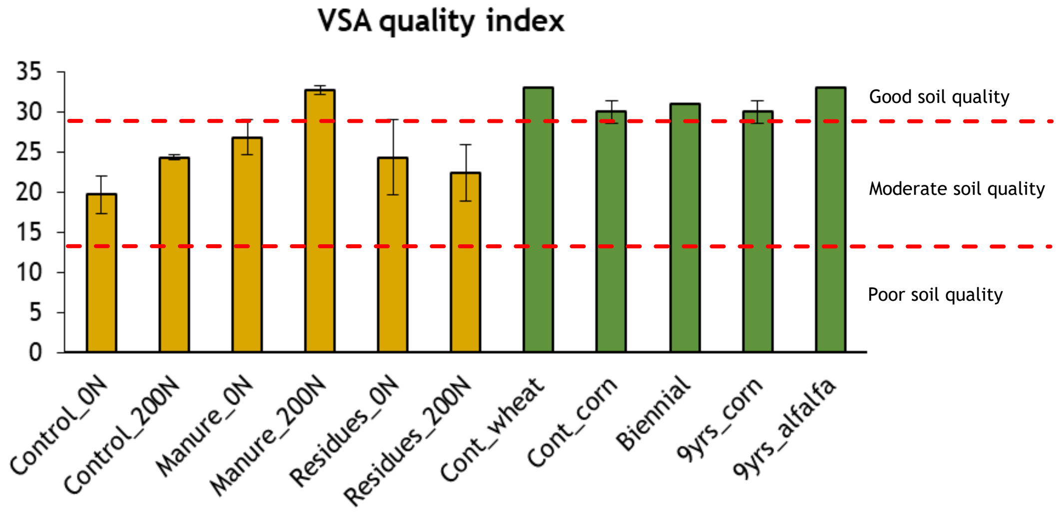


Figure 8. Visual soil assessment (VSA) quality index. Bars are standard deviation values.

Box 1 - HOW TO PROCEED FOR CF MONITORING BY VSA§
When?
• The test should be carried out when the soils are moist and suitable for cultivation.
Where?
• Select sites that are representative of the field avoiding heavily disturbed by traffic or at the field border
• For each field, at least 2 sites should be assessed. For field >1 ha, an adequate number of sites should be assessed (at least 2 sites over 1 ha)
• Record the position of the sites for future monitoring
• Dig a pit of 50x50 cm^2 until a depth of 30 cm with a spade
What?
• Work through the scorecard (Figure 7), assigning a VS to each indicator by comparing it with the photographs (or table) and description reported in the FAO Field Guide (FAO, 2008)

§Summary of how to proceed for VSA is reported (the complete description is available at https://www.fao.org/3/i0007e/i0007e.pdf)

3.3 Implementation of standarized soil carbon sequestration for external-farm reviews

3.3.1 Development of pedotransfer functions (PTFs) for bulk density prediction and quantification of soil C stocks

The measurement of bulk density is needed to quantify soil C stocks (i.e., soil organic C on an area basis referred to a specific depth; Mg C ha^{-1}). However, bulk density values are often missing from databases. Bulk density can be estimated using common pedotransfer functions (PTFs) for BD, but their applicability is often critical as significant overestimation or underestimation of SOC stocks can occur (e.g., Wiesmeier et al., 2012). To obtain consistent results, the best practice is to determine a local PTF and derive BD values from soil organic C content and soil texture (De Vos et al., 2005).
As reported in section 3.1, the determination of PTF and quantification of soil C stocks were carried out at the sites investigated for VSA (Table 1). The air-dried soil samples were sieved to 2 mm and an aliquot was finely ground. For each treatment, at least one sample was used for PTF determination (N=13 out of 28). For all samples, total organic C (TOC) was measured by Dumas combustion using an EA 1110 Thermo Fisher CHN elemental analyzer after dissolution of carbonates with 2 M HCl. Particle size distribution was measured using the pipette method (Gee and Bauder, 1986) on the fine soil samples of the sample group used for PTF determination. The BD of each site was quantified from the mass of oven dried (105°C) soil samples collected from 0 to 30 cm divided by the volume of the soil cores collected (Blake and Hartge, 1986). The SOC stock in the 0-30 cm soil depth was calculated according to equation (1). TOC and measured BD are reported in Table 3.

Table 3. Measured total organic C (TOC) and bulk density (BD) of the investigated sites (mean value, N = number of replicates [N = 3 in Trial 29; N = 2 in Trial 64], and st.dev. = standard deviation).

ID		TOC (g kg^{-1})	measured BD (g cm^{-3})	ID		TOC (g kg^{-1})	measured BD (g cm^{-3})
TRIAL 29				**TRIAL 64**			
Control_0N	Mean	6.91	1.55	**Cont_wheat**	mean	8.22	1.47
	st.dev.	*0.67*	*0.02*		*st.dev.*	*1.19*	*0.11*
Control_200N	Mean	7.89	1.46	**Cont_corn**	mean	6.09	1.53
	st.dev.	*0.62*	*0.14*		*st.dev.*	*0.96*	*0.03*
Manure_0N	Mean	14.32	1.44	**Biennial**	mean	5.73	1.62
	st.dev.	*4.36*	*0.07*		*st.dev.*	*0.35*	*0.01*
Mannure_200N	Mean	12.22	1.47	**9yrs_corn**	mean	7.65	1.50
	st.dev.	1.84	0.06		*st.dev.*	*0.07*	*0.02*
Residues_0N	Mean	8.08	1.49	**9yrs_alfalfa**	mean	9.28	1.36
	st.dev.	*0.74*	*0.05*		*st.dev.*	*1.34*	*0.01*
Residues_200N	Mean	8.14	1.47				
	st.dev.	*0.53*	*0.06*				

Figure 9 shows the SOC stocks at 0-30 cm depth using measured BD. SOC stocks varied from 24.5 to 79.6 Mg ha^{-1} and manure addition shows the highest SOC stocks, due to an additive effect. Among the other carbon farming practices, no significant differences were found with respect to the controls.

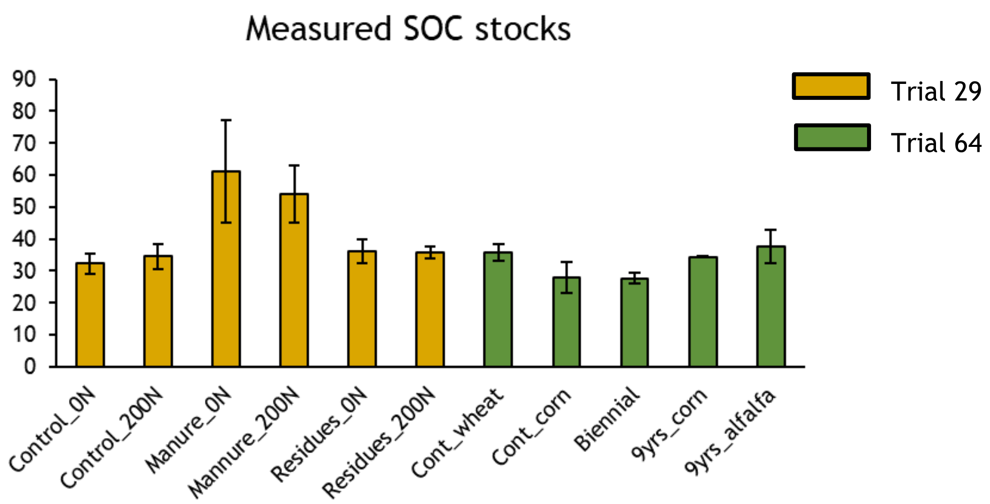


Figure 9. SOC stocks (Mg ha^{-1}) in 0-30 cm depth calculated by measured BD. Bars are standard deviation values.

In Table 4, the particle size distribution, TOC and BD of samples used for PTF determination is reported (N=13 out of 28).

Table 4. Particle size distribution, TOC and BD of samples used for PTF determination.

ID	Replicate	Clay (g kg^{-1})	Silt (g kg^{-1})	Sand (g kg^{-1})	TOC (g kg^{-1})	Measured BD (g cm^{-3})
TRIAL 29						
Control_0N	1	207	304	489	6.43	1.56
Control_0N	2	198	302	500	7.38	1.56
Control_200N	1	186	299	515	8.20	1.31
Manure_0N	3	165	327	508	11.95	1.51
Manure_200N	3	166	280	554	12.77	1.54
Residues_0N	1	173	308	519	7.22	1.48
Residues_200N	3	189	300	511	8.38	1.40
TRIAL 64						
Cont_wheat	1	274	418	308	7.38	1.54
Cont_wheat	2	312	423	265	9.06	1.39
Cont_corn	2	257	352	392	6.77	1.55
Biennial	1	189	350	461	5.97	1.62
9yrs_corn	1	196	357	447	7.60	1.51
9yrs_alfalfa	1	168	483	349	10.23	1.35

Considering that BD is correlated with TOC (rs=-0.613, p<0.05) and not with clay, silt and sand (Table 5), we calculated the following PTF for the estimation of BD from TOC (Figure 10):

$$BD = 3.21 - 0.38 \cdot TOC + 0.02 \cdot TOC^2 \qquad r^2=0.732,\ p>0.001 \qquad (2)$$

Table 5. Correlations between measured BD and clay, silt, sand and TOC for samples used for PTF determination (N=13 out of 28).

Clay		Silt		Sand		TOC	
r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
0.248	0.413	-0.116	0.706	-0.044	0.886	-0.613*	0.026

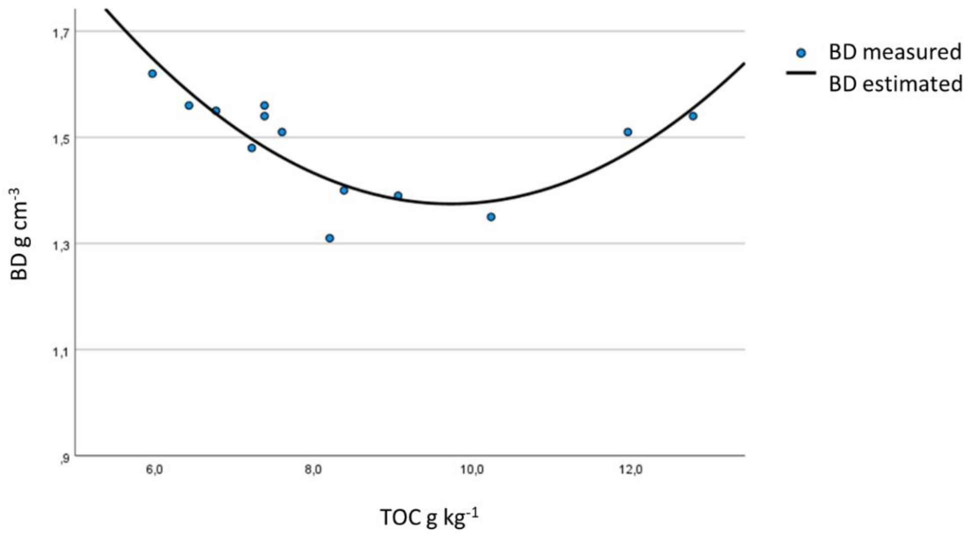


Figure 10. BD measured and BD predicted from TOC for the samples used for PTF determination (N=13 out of 28).

The accuracy of the PTF in predicting soil bulk density was then evaluated by calculating the root mean square error (RMSE, equation 3) and the mean error (ME, equation 4).

$$RMSE = \sqrt{\frac{\sum_1^N (BD_{mi} - BD_{pi})^2}{N}} \quad (3)$$

$$ME = \frac{\sum_1^N (BD_{mi} - BD_{pi})}{N} \quad (4)$$

where BD_{mi} and BD_{pi} were the measured and predicted BD (g cm^{-3}), respectively, for the *i*-th observation, and N was the total number of observations.

The RMSE value was 0.10 g cm^{-3}, demonstrating the good performance of the calculated PTF (De Vos et al., 2005), and the ME value allowed the evaluation of a negative bias of the PTF, indicating an average low tendency of underestimation of -0.03 g cm^{-3}.

Figure 11 shows the SOC stocks at 0-30 cm depth calculated from the predicted BD (Eq. 2). We exclude one sample from Manure_0N (the richest in C; TOC 19 g kg^{-1}), because the predicted BD was unrealistic (>3 g cm^{-3}) due to its C content. The calculated SOC stocks from the predicted BD varied from 28.2 to 72.5 Mg ha^{-1}, and again manure application shows the highest SOC stocks (Figure 11). Among the other carbon farming practices, it is confirmed that no significant differences were found with respect to the controls.

In Box 2 a summary of how to proceed for prediction of bulk density is reported.

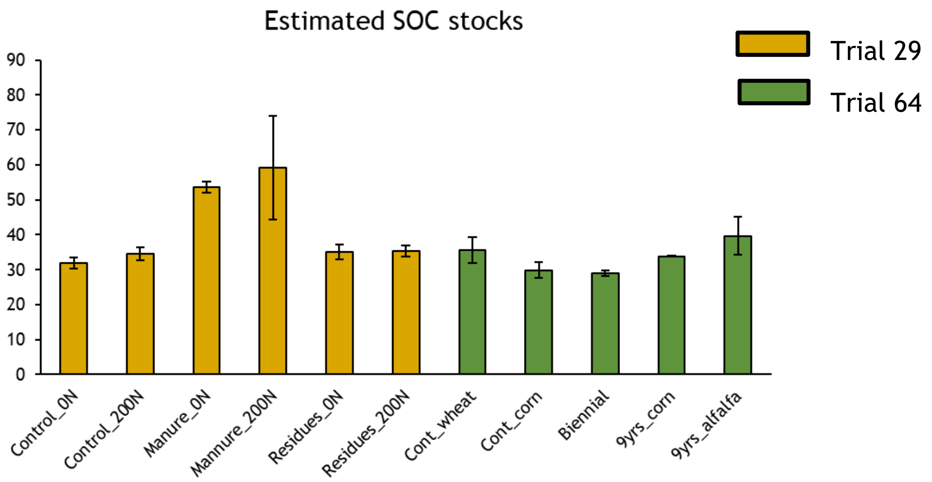


Figure 11. SOC stocks (Mg ha^{-1}) in 0-30 cm depth calculated by predicted BD. Bars are standard deviation values.

Box 2 - HOW TO PROCEED FOR CF MONITORING BY PTF FOR PREDICTION OF BULK DENSITY

When?

- The prediction of soil bulk density (BD) by pedotransfer function (PTF) should carried out when the BD density data is not available. BD can thus be estimated from other soil parameters, such as soil organic C and/or particle size distribution (clay, silt, sand).

Where?

- For each field, data on C organic content should be available
- For about 1/3 of fields, measured bulk density should be available
- Data on particle size distribution should be optional if the calculated PFT based on C organic content has a good accuracy

What?

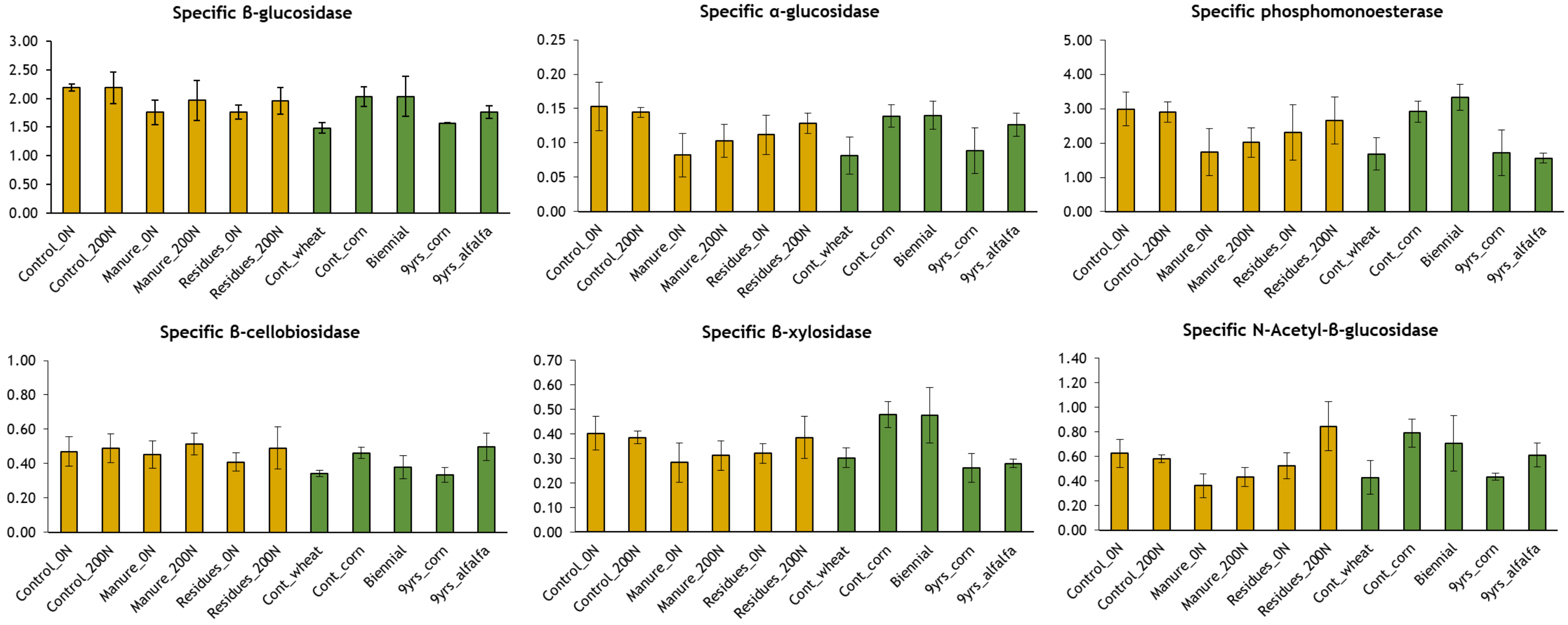
- By statistical interpolation the best significant model (higher r^2) relating organic C content and BD measured is evaluated, in order to predict bulk density
- A relationship between organic C (in g kg^{-1}) and bulk density (in Mg m^{-3}) is defined by statistical interpolation, and the PTF predicting the bulk density values from the amount of C is determine
- The accuracy of PTF in predicting soil bulk density is then evaluated by calculating the root mean square error (RMSE, Eq. (3)) and mean error (ME, Eq. (4)) on available data. A satisfying performance of the calculated PTF has RMSE value less than 0.26 g cm^{-3} (De Vos et al., 2005). If the accuracy of model is not satisfying, particle size distribution should be used in the model verifying then the accuracy of the new PTF.
- The C stock in each field can be calculated by Eq. (1) using predicted BD

3.3.2 SQI

The soil samples collected from the two experimental trials were characterized for their microbial biomass C (MBC) and N (MBN) content according to Vance et al. (1987) and for their total organic C (TOC) and N (TN) content, determined with a Flash 2000 elemental analyser (Thermo Fisher Scientific). The elemental analyser also allowed us to determine the value of the C stable isotope content ($\delta^{13}C$). The results of these analyses are given in Table 6.

Table 6. Carbon and nitrogen microbial biomass (MBC and MBN) content, total organic C and N (TOC and TN) content, TOC over TN ratio (CN), and C stable isotope content ($\delta^{13}C$) for the sites investigated (mean value, N = number of replicates [N = 3 in Trial 29; N = 2 in Trial 64], and st.dev. = standard deviation).

ID		MBC (mg kg_{ds}^{-1})	MBN (mg kg_{ds}^{-1})	TOC (g kg_{ds}^{-1})	TN (g kg_{ds}^{-1})	CN	$\delta^{13}C$ (‰)
TRIAL 29							
Control_0N	mean	43.64	5.79	6.91	0.86	9.31	-21.57
	st.dev.	*5.90*	*1.09*	*0.67*	*0.21*	*0.18*	*0.16*
Control_200N	mean	50.11	6.39	7.89	0.81	9.71	-22.81
	st.dev.	*8.62*	*1.94*	*0.62*	*0.06*	*0.63*	*0.60*
Manure_0N	mean	74.24	10.70	14.32	1.46	9.76	-24.98
	st.dev.	*18.99*	*2.67*	*4.36*	*0.35*	*0.65*	*1.17*
Manure_200N	mean	68.58	9.34	12.22	1.30	9.38	-24.40
	st.dev.	*6.69*	*1.13*	*1.84*	*0.21*	*0.14*	*0.22*
Residues_0N	mean	56.96	7.80	8.08	0.84	9.65	-22.69
	st.dev.	*11.64*	*2.06*	*0.74*	*0.09*	*0.22*	*0.53*
Residues_200N	mean	54.77	6.81	8.14	0.86	9.42	-22.06
	st.dev.	*15.57*	*2.65*	*0.53*	*0.06*	*0.31*	*0.32*
TRIAL 64							
Cont_wheat	mean	87.11	6.70	8.22	0.96	8.57	-25.27
	st.dev.	*10.51*	*0.33*	*1.19*	*0.16*	*0.20*	*0.43*
Cont_corn	mean	53.42	5.39	6.09	0.68	8.92	-19.89
	st.dev.	*8.24*	*0.51*	*0.96*	*0.12*	*0.12*	*0.49*
Biennial	mean	46.73	4.79	5.73	0.67	8.57	-22.46
	st.dev.	*5.61*	*0.75*	*0.35*	*0.07*	*0.39*	*0.35*
9yrs_corn	mean	93.14	7.50	7.65	0.86	8.89	-24.33
	st.dev.	*17.15*	*3.00*	*0.07*	*0.03*	*0.27*	*0.42*
9yrs_alfalfa	mean	145.93	15.48	9.28	1.00	9.27	-24.87
	st.dev.	*16.22*	*2.44*	*1.34*	*0.13*	*0.19*	*0.77*



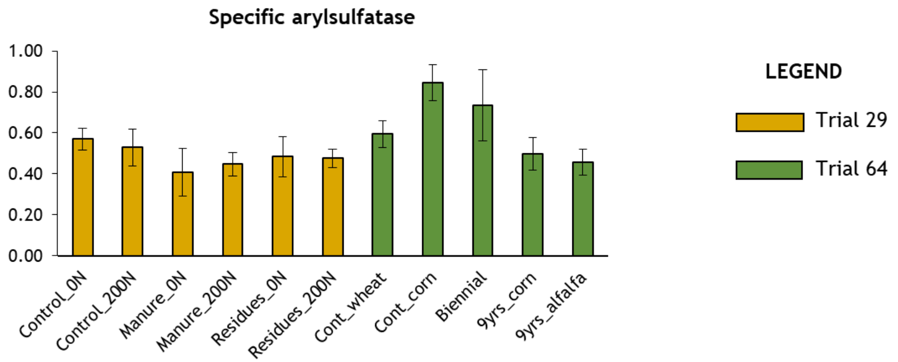


Figure 12. Soil extracellular specific hydrolytic enzymatic activities (expressed as $\mu mol_{MUF}\ mg_{MBC}^{-1}\ h^{-1}$) for the sites investigated. Bars are standard deviation values.

Both the CF techniques had a significant effect on the TOC content, with higher values corresponding to manure application and 9yrs_alfalfa rotation, respectively (Table 6). The C stable isotope content was also influenced by the CF techniques: more negative values were measured in Trial 29 with manure application and in Trial 64, where the C signature was indicative of the continuous corn rotation. Concerning the other parameters, microbial biomass (both MBC and MBN) showed significant differences only in Trial 64 with higher values corresponding to the 9yrs_alfalfa rotation, indicating its positive effects on both soil TOC sequestration and microbial biomass growth.

Specific enzymatic activities were determined as simple indicators of soil quality (Figure 12). There were not many differences between the CF techniques. The long-term application of organic fertilizers affected the specific α-glu (higher in the Controls) and the specific NAG (higher in the Residues_200 and lower with manure supply); on the contrary, the crop rotation, especially the two 9-year rotations, significantly reduced the specific PME and β-xyl activities.

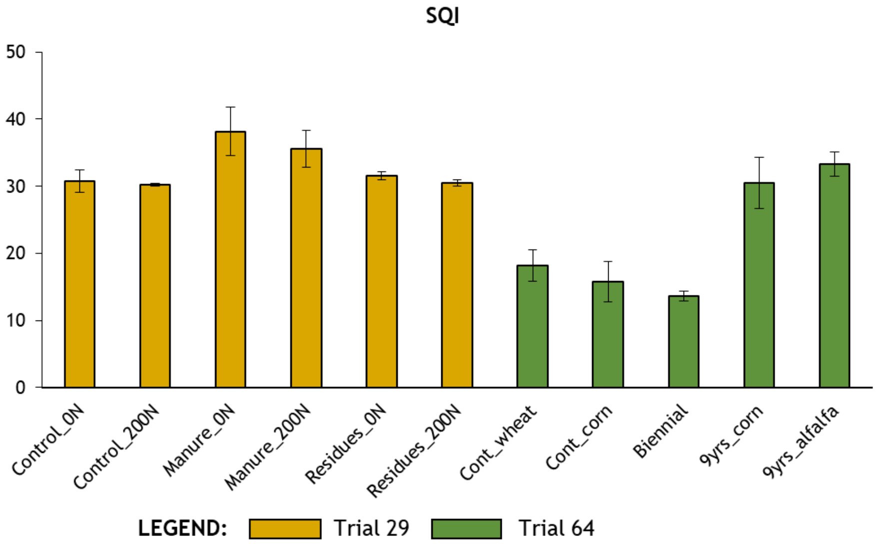


Figure 13. Soil Quality Index (SQI) for the sites investigated. Bars are standard deviation values.

The Soil Quality Index (SQI) was determined according to the procedure described in Figure 2 (section 2.2.3) and the minimum dataset identified by the statistical procedure consisted of MBN and TN content, CN ratio, and the specific NAG and β-cel activities. The SQI does not have standard values to refer to for discriminating between poor and good soil quality. In this case the SQI values have to be compared with a "control sample" as a reference. In any case, higher SQI values correspond to better soil quality.

In terms of the results obtained, the SQI showed a significant variation due to the CF techniques (Figure 13). Specifically, the highest SQI was measured in response to manure application in Trial 29 and the 9-year rotations in Trial 64.

In Box 3 a summary of how to proceed for carbon farming monitoring by SQI is reported.

Box 3 - HOW TO PROCEED FOR CF MONITORING BY SQI

When?

- The measurement of the enzymatic activities and the determination of the SQI should be carried out when investigation also regards the biochemical functionality of the soil.
- Soil sampling should be carried out in spring or in autumn, when soil conditions are optimal for the microbial biomass and its activity.

Where?

- One soil sample has to be collected and analysed for each experimental unit if field replicates are present.
- When field replications are not present in the experimentation the suggestion is to collect 2/3 soil samples for each experimental factor tested.

What?

- Work through the scheme (Figure 2) starting from a larger dataset (i.e., include all the chemical and biochemical parameters determined on soil samples).
- The selection of the minimum dataset, as well as, of the weight of each parameter are determined statistically by applying a PCA and a correlation in order to reduce redundance information/parameters.
- The complexity of the procedure is to assign, for biochemical parameters, the correct functions for the scoring (i.e., an increase of enzymatic activity could be either positive or negative depending on the dynamics that are occurring in that specific system; in this case the use of the specific enzymatic activities is suggested).
- For this SQI it does not exist reference standard values for results evaluation: it is suggested to always have a “no treated-control” sample as reference.

3.4 Advantages and disadvantages of soil indicators for CF monitoring supporting decision making

In Table 7 the linear correlation between the soil indicators for CF monitoring is reported, for both Trial 29 and Trial 64. As can be seen, in Trial 29 all indicators were correlated. In Trial 64, however, VSA and C stocks, both measured and predicted, were well positively correlated, while SQI was not significantly correlated.

Table 7. Correlation among soil indicators for CF monitoring (**: p<0.01; *: p<0.05; ns: not significant).

	VSA quality index	measured C stock	predicted C stock	SQI
TRIAL 29				
VSA quality index	1			
measured C stock	0.669**	1		
estimated C stock	0.741**	0.967**	1	
SQI	0.545*	0.951*	0.921**	1
TRIAL 64				
VSA quality index	1			
measured C stock	0.635*	1		
estimated C stock	0.643*	0.957**	1	
SQI	ns	ns	ns	1

Clearly, VSA reflects soil quality directly linked to the dynamics of soil organic matter, and in particular to the C cycle, regardless of the CF techniques applied (external organic fertilizer or crop rotation – Trial 29 and 64, respectively). On the contrary, as SQI is determined on parameters related to the C and nutrient cycles, such as N, it can provide complementary information on the cycle of elements other than C affected by CF techniques.

Finally, a roadmap explaining the advantages and disadvantages of each soil monitoring approach for CF is presented in Table 8.

Table 8. Roadmap for advantages and disadvantages of soil indicators for CF monitoring.

Monitoring stragegy	Final users	Information provided	Advantages	Disavantages	Links to other indicators/indexes
Visual Soil Assesement	Farmers and students	Soil quality based on crop performance and key soil condition related to SOC storage capacity.	User-friendly application, worldwide recognized method, existing reference value range. Independent of CF techniques applied, VSA provides information on soil C storage capacity. Both soil and plant parameters are considered.	Seasonal climatic conditions could change soil status (i.e., moisture, which in turn affects earthworm content and colour). No information is given on the quality of the C stored. Additional data on nutrient cycles are needed to assess soil health.	Root mass; C stock.
Soil C stocks	Farmers, agronomists, field technicians	Current soil storage C.	Based on a limited set of determinations (C and BD), it is easy to understand for a large audit.	BD is often missing in the dataset and the application of the PTF may require the intervention of a specialist. TOC is a soil parameter that can change very slowly in the soil. No information is given on the quality of the C stored. Additional data on nutrient cycles are needed to assess soil health.	C stock; Yield; Microbial biomass.
Soil enzyme activities and SQI	Independent audit, researchers	Soil functionality in relation to C and N cycles.	Provide very short-term (**monthly**) information being based on biochemical parameters that respond rapidly to changes in soil management and directly reflect the soil functionality, quality and health.	Determiantion of these parameters requires specialized laboratories, equipment and technicians. Interpretation of the results is not always easy and cannot be easily shared between different types of audit.	C stock (not generalizable to all the CF techniques); Microbial biomass content and activity.

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