

SPECIAL SECTION: AGROGEOPHYSICS: GEOPHYSICS TO INVESTIGATE SOIL-PLANT-ATMOSPHERE INTERACTIONS & SUPPORT AGRICULTURAL MANAGEMENT I

Soil apparent electrical conductivity-directed sampling design for advancing soil characterization in agricultural fields

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Abstract

The “4 per 1,000” initiative calls for land management practices that increase soil organic C (SOC). Despite an imperative for accurate SOC measurement, several methodological issues may complicate the verification of C sequestration. The aim of this work is to evaluate the potential advantages of using apparent electrical conductivity (EC_a)-directed sampling to deep (0–90 cm) SOC stock assessment. We compared simple random sampling (SRS) and stratified random sampling (StSRS), with either a fixed or optimized number of samples, in fields managed under conservation agriculture and conventional tillage. The stratification in StSRS was built from EC_a maps that showed two different soil conditions—the presence or absence (high-salinity conditions) of a strong correlation between EC_a and soil properties. Treatment and sampling design effects on SOC estimates were tested through a mixed-model approach. Sampling efficiency was calculated by classical and bootstrap methods. Results suggested that when EC_a has a strong relationship with soil properties, StSRS was more efficient than SRS, especially when using an optimal number of samples per stratum. Stratification was based on EC_a maps of the no-till site, which allowed a smaller minimum sample size. When stratification failed due to the effect of salinity on EC_a , StSRS efficiency was similar to SRS. These results suggest that EC_a -directed sampling, regardless of knowing the relationships between EC_a and soil properties, is a win-win solution to advance soil characterization and SOC stock estimation in agricultural fields of the low Venetian plain. However, further research should investigate EC_a -directed sampling where strong patterns not related to SOC could lead to inappropriate stratification or suboptimal sample allocation.

Abbreviations: BD, bulk density; CONS, conservation agriculture; CONV, conventional agriculture; EC_a , soil apparent electrical conductivity; F1, Farm 1; F2, Farm 2; SOC, soil organic carbon; SRS, simple random sampling; SRS_{fix} , simple random sampling using the same number of samples for each stratum; SRS_{opt} , simple random sampling using an optimal number of samples per stratum; StSRS, stratified simple random sampling; $StSRS_{fix}$, stratified simple random sampling using the same number of samples for each stratum; $StSRS_{opt}$, stratified simple random sampling using an optimal number of samples per stratum.

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1 | INTRODUCTION

Over recent years, accurate soil organic C (SOC) stock estimation in agricultural fields has become a key issue because of the potential impact of the C cycle on climate change. The “4 per 1,000” initiative (Minasny et al., 2017) calls for land management practices that raise SOC stocks at a rate of 4 per 1,000 per year (<https://www.4p1000.org/>). However, criticism has arisen as to the achievability of the initiative across soil types and its suitability in food-limited production systems (grasslands vs. croplands; Poulton, Johnston, Macdonald, White, & Powelson, 2018; Rumpel et al., 2020). Moreover, several methodological issues facing SOC inventory, such as soil profile depth, initial SOC content, and time after best management practice adoption, can complicate the aspiration.

Methods to quantify SOC in the soil profile depend on several factors, such as temporal and spatial resolution of the surveyed area, soil availability, land use, local or regional management data availability, the existence of harmonized monitoring networks, and others (Morari, Berti, Dal Ferro, & Piccoli, 2019). For this purpose, the Intergovernmental Panel on Climate Change (IPCC) has laid out a decision tree (Eggleston, Buenidia, Miwa, Ngara, & Tanabe, 2006) to identify the appropriate tiers to estimate soil C stock changes. To assess the nonlinear behavior of SOC in soils, the implementation of a measurement-based inventory or simulation model (e.g., CENTURY, Roth-C) has been suggested (Tier 3). Assessment using a measurement-based inventory approach requires the consideration of two methodological issues. One concerns SOC content measurement; the other relates to soil sampling optimization to maximize its sensitivity to field-level variation in soil C after changes in land use or management (Conant & Paustian, 2002; de Gruijter et al., 2016). Indeed, SOC spatial variability is often many times greater than its variability over time, which can introduce unavoidable uncertainties in the detection of changes in SOC stocks. In fact, the results of agronomic experiments are influenced by two types of factors: treatment and experimental error, including soil spatial variability and others. Although treatments reflect the objectives of the experiment, experiment error is not relevant to the objectives but does tend to mask treatment effects (Yang, 2010). Several efforts can be found in the literature to minimize and/or control experimental errors (Cochran & Cox, 1957; Petersen, 1994), but the mixed-models approach is the most recommended for use in agricultural experimentation (Yang, 2010). The classical approach regards the use of a soil property, such as granulometry fraction, N content, or moisture, as covariates to explain some of the variances. Unfortunately, soil properties may not always be known a

Core Ideas

- Stratification requires a correlation between covariates and soil properties.
- Stratified sampling based on EC_a is ideal in a no-tillage system.
- Stratified sampling allows a reduced sample size.
- Stratification is less effective in conventional tillage soil.

priori, and at times, their use is insufficient to describe the entire spatial variability.

With this context as background, it is best to rely on an optimized scheme at the sampling stage. Two main approaches can be used for this purpose, either a model-based approach or a design-based approach. In the former, the statistical inference is based on a model that describes the soil-forming process as a stochastic process, whereas the latter uses a predetermined random procedure to select sample locations and the statistical inference is based on the sampling design (Brus & De Gruijter, 1997). The approach choice is mainly dictated by study purposes. For the production of a SOC stock map, model-based sampling is preferable (England & Rossel, 2018; Priori et al., 2016). However, for accurate measures of SOC stock, design-based sampling may be most suitable, as the estimated quality does not depend on model assumptions (de Gruijter et al., 2016).

Simple random sampling (SRS) is one of the simplest sampling methods. A fixed number of samples are collected randomly and independently from within the study area. To reduce sampling variance, the stratified simple random sampling (StSRS) method is usually proposed. It divides the study area into subareas based on associated covariates. Although StSRS can improve design efficiency, two drawbacks can occur during the stratification step: (a) strata fail to partition variation of the target variable, and/or (b) samples are suboptimally allocated to strata (de Gruijter, Brus, Bierkens, & Knotters, 2006). There is also a risk that efficiency may be lost using StSRS, as compared with the simpler SRS. Further details about sampling designs for SOC estimation can be found in Allen, Pringle, Page, and Dalal (2010).

The first step in accurate StSRS is capturing soil spatial variability. An easy and quick method to do this is to measure soil apparent electrical conductivity (EC_a ; Martinez, Vanderlinden, Ordóñez, & Muriel, 2009) using the on-the-go electromagnetic induction techniques (Corwin & Lesch, 2003; Doolittle & Brevik, 2014). Early work by

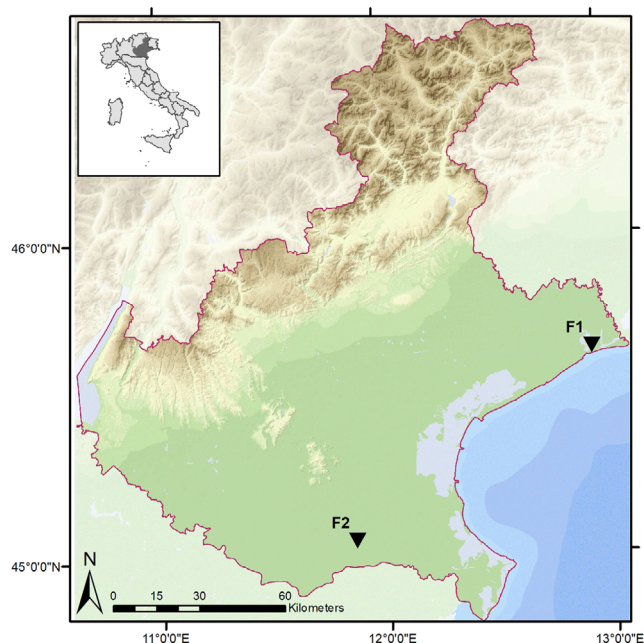


FIGURE 1 Experimental sites in the low plain of the Veneto Region in northeastern Italy. Farm positions are marked with triangles (F1 and F2)

Rhoades, Raats, and Prather (1976) focused mainly on the effect of soil salinity on EC_a . However, it is now understood that EC_a is influenced by other soil properties (e.g., SOC, texture, bulk density [BD], saturation percentage, water content, and cation exchange capacity; Corwin & Scudiero, 2016), which make EC_a use effective for soil–plant interaction studies (Cassiani et al., 2012). Despite its benefits, EC_a survey is recommended only when the EC_a /clay ratio < 5 (McBratney & Minasny, 2005) or $EC_a < 100 \text{ mS m}^{-1}$ (Corwin & Scudiero, 2016), except in salinity studies.

Within a general framework, to evaluate those agronomic practices best suited for the “4 per 1,000” initiative in northern Italy, we evaluated the potential advancements introduced by EC_a -directed sampling on deep SOC stock estimates under two different agronomic managements (conservation agriculture [CONS] and conventional tillage [CONV]). Two sampling strategies (SRS and StSRS) were compared in two types of soil conditions, the presence and the absence of a strong dependency of the covariate (i.e., EC_a) from the primary soil properties. Our initial hypothesis is that the stratified sampling design should be selected only when a strong relationship exists between EC_a and soil properties.

2 | MATERIALS AND METHODS

2.1 | Experimental sites

The experimental sites are located on two farms in Veneto Region (northeastern Italy, Figure 1, Table 1). Farm 1 (F1),

TABLE 1 Main soil physical and chemical characteristics (top 50 cm) at the experimental farms

Characteristic	Unit	Farm 1, Vallecchia	Farm 2, Sasse-Rami
Sand	$\text{g } 100 \text{ g}^{-1}$	34.2	18.4
Silt	$\text{g } 100 \text{ g}^{-1}$	42.6	57.8
Clay	$\text{g } 100 \text{ g}^{-1}$	23.2	23.8
pH		8.3	8.6
CO_3^{2-}	$\text{g } 100 \text{ g}^{-1}$	53.0	13.0
Active CO_3^{2-}	$\text{g } 100 \text{ g}^{-1}$	3.0	3.0
Organic C	$\text{g } 100 \text{ g}^{-1}$	1.0	0.8
Assimilable P	mg kg^{-1}	32.0	6.0
Exchangeable Ca	$\text{cmol}(+) \text{ kg}^{-1}$	24.7	15.5
Exchangeable Mg	$\text{cmol}(+) \text{ kg}^{-1}$	3.2	1.4
Exchangeable K	$\text{cmol}(+) \text{ kg}^{-1}$	0.5	0.2

“Vallecchia,” lies in a reclaimed area along the coastline of the Adriatic Sea ($45^\circ 38.350' \text{ N}$, $12^\circ 57.245' \text{ E}$, -2 m asl). The soil, characterized as either Calcari-Gleyc Fluvisols or Cambisols (WRB, 2006), originates from Tagliamento and Piave River sediment with textures ranging from silty-clay to sandy-loam. It is locally saline with a saturated paste extract soil electrical conductivity of $270 \mu\text{S cm}^{-1}$ and has a locally shallow groundwater electrical conductivity of $6,902 \mu\text{S cm}^{-1}$ (Agostini & Rosato, 1997), which is classified as “brine water” according to Rhoades, Kandiah, and Mashali (1992). Farm 2 (F2), “Sasse-Rami,” located in the southern low plain of the Po River ($45^\circ 2.908' \text{ N}$, $11^\circ 52.872' \text{ E}$, 2 m asl), is characterized by Hypocalcic Calcisols soil with a silty-clay loam or silt-loam texture.

The climate in the region (1981–2010) is subhumid and has a mean annual rainfall of 829 mm at F1 and 673 mm at F2. Average rainfall was highest in autumn (302 for F1, 187 mm for F2) and lowest in winter (190 for F1, 129 mm for F2), with average temperature rises from 3.5 (F1) and 3.1 °C (F2) in January to 23.3 (F1) and 23.6 °C (F2) in July. Reference evapotranspiration was 860 (F1) and 848 mm (F2), with the peak days of 4.9 (F1) and 4.8 mm d^{-1} (F2) occurring in July.

2.2 | The experiment

Experimental treatments were established at both farms in 2010 to compare the CONV and CONS management systems. The CONS protocol followed a set of practices outlined in Measure 214, Submeasure 1, “Eco-compatible management of agricultural lands” of the Rural Development Programme (RDP) supported by Veneto Region (Regione Veneto, 2013). It included such measures such as

no-tillage, soil surface retention of crop residues, and cover crop usage. The CONV management system used traditional tillage practices: moldboard plowing (35 cm), crop residue incorporation, and a second disk-harrow tillage to a depth of ~10 cm.

Each of the four rectangular experimental fields (two treatments \times two farms) covered a surface area averaging 1.2 ha (about 400 m long \times 30 m wide). Until 2014, the 4-yr crop rotation consisted of wheat (*Triticum aestivum* L.), oilseed rape (*Brassica napus* L.), maize (*Zea mays* L.), and soybean [*Glycine max* (L.) Merr.]. From 2014 and beyond, a simplified, 3-yr crop rotation (wheat–maize–soybean) was applied. In CONS, cover crops were grown between the main crops. Until 2014, sorghum (*Sorghum vulgare* Pers. var. *sudanense*) was grown during spring–summer, and a mix of vetch (*Vicia sativa* L.) and barley (*Hordeum vulgare* L.) was grown during autumn–winter. In the years after, only barley or winter wheat was grown in autumn–winter. In CONV, the soil remained bare between the main crops.

The base dressing fertilizer was applied 1–2 wk before sowing in CONV; subsurface band fertilization was applied at sowing in CONS. In both systems, mineral fertilization was integrated by side-dressing in maize (one treatment) and wheat (two treatments). Cover crops received no additional fertilization. Pesticide applications were applied based on need and crop for both treatments. The winter cover crop was suppressed with *N*-(phosphonomethyl) glycine; sorghum was suppressed by mechanical shredding. More details on the management systems are reported in Piccoli, Furlan, Lazzaro, and Morari (2019).

2.3 | Soil apparent electrical conductivity survey

The EC_a survey was carried out using a CMD-Mini Explorer conductivity meter (GF Instruments). The data were collected in December 2016 when the soil was bare in CONV and under a cover crop in CONS. Both soil EC_a (mS m^{-1}) measurements and the differential GPS (Trimble) positions were recorded at 4 km h^{-1} (1-s interval, 6-m transect distance) with both horizontal and vertical CMD configurations. The conductivity meter had three receiver coils positioned at 0.32, 0.71, and 1.18 m from the single transmitter coil. The effective depth ranges of the measurements were 0.5 (H_1), 1 (H_2), and 1.8 m (H_3) in the horizontal coplanar configuration, and 0.25 (V_1), 0.5 (V_2), and 0.9 m (V_3) in the vertical coplanar configuration (GF Instruments, 2011). Each of the EC_a depth range measurements was interpolated onto a 2-m regular grid using ordinary kriging with moving windows (de Gruijter et al., 2006).

2.4 | Soil sampling design

The SRS and StSRS sampling designs were used in this study. In SRS, sample positions were randomly placed inside the experimental field without any knowledge of the soil variability. In StSRS, EC_a was used as an ancillary variable for sample selection, which was based on six EC_a maps. We used the *k*-means clustering algorithm on the EC_a maps to identify field strata using R software (R Core Team). The *k*-means algorithm divides M points in N dimensions into K clusters to minimize the within-cluster sum of squares (Hartigan & Wong, 1979). The objective function was calculated using the formula by de Gruijter et al. (2016):

$$O_{KM} = \sum_{i=1}^N \sum_{h=1}^H d_{ih}^2 \quad (1)$$

where d is the component of a distance matrix, obtained by:

$$d_{ih}^2 = (x_i - c_h)' \mathbf{A} (x_i - c_h) \quad (2)$$

where c_h is the centroid of class h and \mathbf{A} is the distance norm matrix, which can be the inverse of variance-covariance matrix of \mathbf{X}' , called the Mahalanobis distance. The use of the Mahalanobis distance is because the six EC_a variables are correlated.

Two types of StSRS were performed; both used the same number of samples per field. In the first approach (StSRS_{fix}), an equal number of samples (six) were randomly placed inside each stratum for a total of 18 per field. In the second approach (StSRS_{opt}), an optimal number of samples were randomly placed inside each stratum. The sample number was calculated according to the following equation to consider the 0- to 90-cm instrumental configuration (i.e., V3):

$$n'_h = n \frac{N_h S_h}{\sum_{h=1}^H N_h S_h} \quad (3)$$

where n is the total sample size (18), H is the number of strata, N_h is the size of stratum h (i.e., stratum area), S_h is the standard deviation in stratum h , and n'_h is the sample size allocated to stratum h . Equation 3 allows more samples to be allocated to strata of larger area and higher variation. The StSRS_{fix} and StSRS_{opt} designs were used to produce SRS_{fix} and SRS_{opt}, respectively.

The procedure permitted the identification of 144 sampling points (i.e., 18 sampling points \times 2 treatments \times 2 farms \times 2 sampling designs). Considering the high number of needed soil samples, the time-consuming nature of

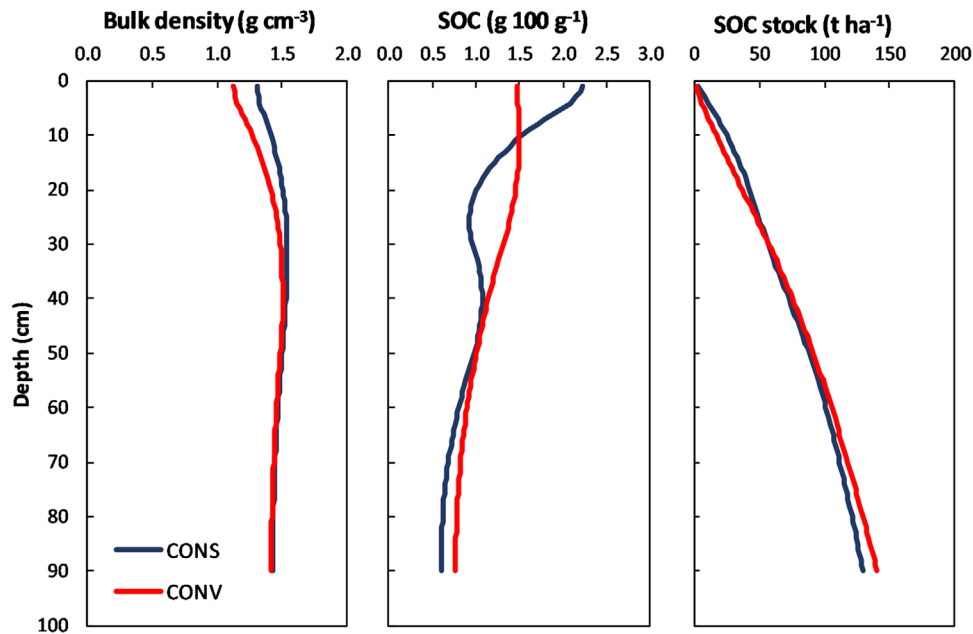


FIGURE 2 Example of bulk density and soil organic C (SOC) spline fitting procedures and the resulting cumulative C stock profiles in conservation agriculture (CONS) and conventional tillage (CONV)

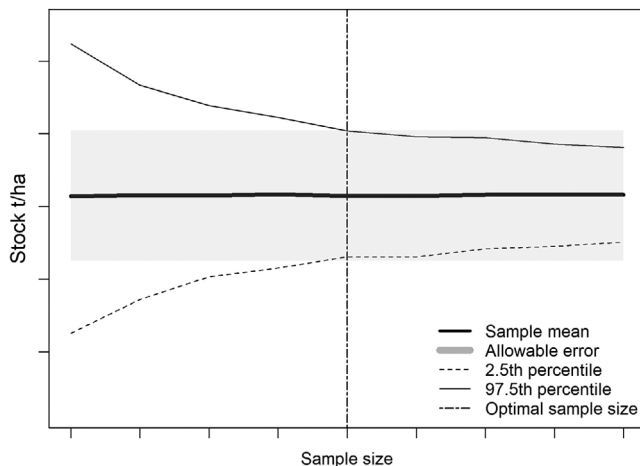


FIGURE 3 Description of optimal sample size estimation according to the bootstrap procedure. The points at which the 95% confidence lines just cross the allowable error range represent sample size (vertical dashed line)

core extraction, and the cost of analysis for the StSRS procedure, a modification was made to the process. Specifically, appropriate SRS points (i.e., inside strata) were reused as one of the possible infinite random configurations, while additional soil cores were collected to cover underrepresented strata. During winter 2017, sampling points were identified using differential GPS, and a total of 89 undisturbed soil cores (90-cm length, 7-cm diam.) were collected by a hydraulic sampler. The cores were then cut to expose four layers of differing depths: 0–5, 5–30, 30–50, and 50–90 cm.

2.5 | Soil physical and chemical analyses

A total of 356 samples were analyzed for BD, particle size distribution, and organic C content. After samples were weighed and a fraction (about half of the sample weight) of each sample was oven dried at 105 °C for 24 h, BD was determined by the core method (Grossman & Reinsch, 2002). The remainder of each sample fraction was air dried and sieved through a 0.5-mm mesh for the determination of C and N content via dry combustion method using a CNS elemental analyzer (Vario Max, Elementar Americas). To determine particle size distribution, samples were sieved through a 2-mm mesh, dispersed in 2% sodium hexametaphosphate solution as described in Gee and Or (2002), and analyzed using laser diffraction (Mastersizer 2000, Malvern Instruments). A dedicated algorithm was used to convert diffraction values into pipette values (Bittelli et al., 2019).

2.6 | Soil organic carbon stocks

The fixed minimum soil-mass approach of SOC stocks was calculated using a spline depth function as suggested by past studies (Gifford & Roderick, 2003; Orton, Pringle, Page, Dalal, & Bishop, 2014; Pringle et al., 2011). In brief, the method selects the reference mass based on the minimum soil mass approach in 0-a profiles ($m_{0-a \text{ ref}}$). Afterward, an equal-area spline was fitted to estimate the variation in BD along the soil profile (Bishop, McBratney, & Laslett, 1999; Malone, McBratney, Minasny, & Laslett,

TABLE 2 Statistical summary of apparent soil electrical conductivity at Farm 1 (F1) and Farm 2 (F2) for conservation (CONS) and conventional agriculture (CONV)

Treatment	Configuration ^a	F1				F2			
		Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
mS m ⁻¹									
CONS	V ₁	31.7	16.7	14.9	125.7	26.1	3.3	19.7	37.3
	V ₂	69.9	25	42.6	200.7	21.7	4.0	13.5	31.5
	V ₃	92.7	31.9	56.0	253.6	35.4	4.8	25.3	48.5
	H ₁	50.4	21.5	26.7	177.8	23.3	4.0	14.1	42.6
	H ₂	121.4	33.3	82	294.8	48.6	4.5	38.0	67.0
	H ₃	142.5	36.6	97.6	319.4	44.7	4.6	32.1	59.2
CONV	V ₁	29.4	9.5	9.0	92.5	22.1	3.2	12.2	33.7
	V ₂	60.1	14.5	28.5	130.1	18.6	5.0	4.3	32.8
	V ₃	82.0	19.4	38.5	167.2	34.1	6.3	16.1	48.7
	H ₁	41.8	11.5	14.5	100.8	25.1	3.6	13.1	37.5
	H ₂	107.7	19.2	61.0	219	48.7	5.6	30.1	63.7
	H ₃	125.7	23.0	71.9	242.7	45.7	6.3	24.7	60.3

^aV1, V2, and V3 represent the instrument vertical coplanar configuration with effective depth ranges of 0.25, 0.50, and 0.90 m, respectively. H1, H2, and H3 represent the instrument horizontal coplanar configuration with effective depth ranges of 0.50, 1.00, and 1.80 m, respectively.

2009). Given the BD (BD_i) profile, the spline was used to calculate cumulative soil mass (per unit of surface area) at incremental 1-cm depths to attain the depth $d_{0-a \text{ ref}}$ (BD_i , $m_{0-a \text{ ref}}$) of the reference mass ($m_{0-a \text{ ref}}$). Likewise, SOC concentration was used to determine SOC stocks at the 0- to 5-, 0- to 30-, 0- to 50-, and 0- to 90-cm soil profile layers. The splines were then fitted in R (R Core Team, 2017) using the 'mpspline' command of the GSIF package (Tomislav, Heuvelink, & Malone, 2019) (Figure 2).

2.7 | Statistical analysis

Data were analyzed for each farm using a linear mixed-effect model based on the REML (restricted maximum likelihood) estimation method. For BD and SOC concentration, both treatment and soil layer \times treatment interaction were considered as categorical variables, whereas clay content was considered continuous. For SOC stock, a mixed model that tested only the treatment effect was fitted at progressive soil profiles (0–5, 0–30, 0–50, and 0–90 cm). Mixed models were applied to all sampling designs (i.e., SRS_{fix} , SRS_{opt} , $StSRS_{\text{fix}}$, and $StSRS_{\text{opt}}$). In the SRS designs, same-field data were considered as subreplicates and treated as nested measures. In the StSRS designs, data from each treatment \times stratum combinations were nested, and the model was weighted by the strata surface in addition. Mixed model results were not corrected for spatial autocorrelation because both Moran's test I and semi-variogram analyses on the residuals confirmed the lack of spatial autocorrelation. Post-hoc pairwise comparison of

least-squares means (LSE) was performed using the Tukey method to adjust for multiple comparisons.

Pearson's correlation coefficients were calculated to estimate all possible linear relationships between EC_a , texture, SOC, and BD. The sampling efficiency in SOC stock estimation was evaluated by calculating the SRS equivalent sample size to obtain the same precision as in StSRS. At first, the StSRS sampling and spatial variances were determined according to Equations 7.15 and 7.16 in de Gruijter et al. (2006). The SRS sampling variance [$V(z_{si})$], StSRS sampling efficiency (V_r), and finally the equivalent sample size (N_{eq}) were calculated according to the following equations:

$$V(z_{si}) = \frac{\hat{s}^2(z)}{n} \quad (4)$$

$$V_r = \frac{V(z_{si})}{V(z_{st})} \quad (5)$$

$$N_{\text{eq}} = V_r n \quad (6)$$

where $\hat{s}^2(z)$ is the spatial variance, n is the actual sample size, $V(z_{si})$ is the SRS sampling variance, and $V(z_{st})$ is the StSRS sampling variance.

A $V_r > 1$ indicates that StSRS was more efficient than SRS, meaning that fewer soil samples were needed for StSRS to reach an accurate SOC stock estimate.

The optimal sample size within a 95% confidence level according to a fixed number of cores per stratum was also

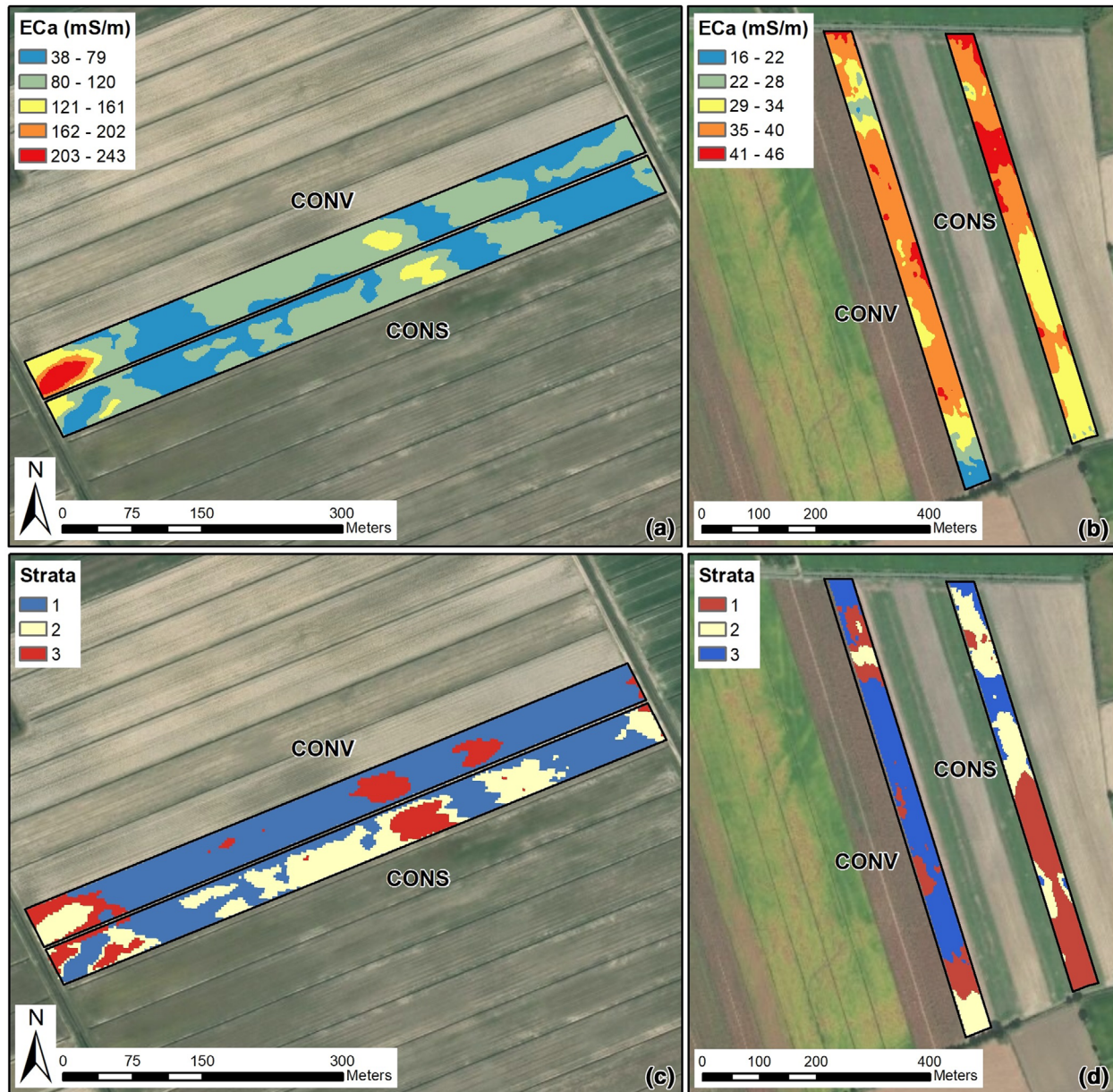


FIGURE 4 Ordinary kriging of soil apparent electrical conductivity (EC_a) at 0- to 90-cm depth through vertical coplanar configuration at (a) F1 and (b) F2. Panels c and d report EC_a -based stratification by k -means clustering. CONS, conservation agriculture; CONV, conventional tillage

TABLE 3 Percentage area covered by strata at Farm 1 (F1) and Farm 2 (F2) for conservation (CONS) and conventional agriculture (CONV)

Stratum	F1		F2	
	CONS	CONV	CONS	CONV
	%			
1	51	80	46	24
2	39	5	34	13
3	10	15	20	63

calculated, using both the bootstrap method (Dane, Reed, & Hopmans, 1986) and allowable error (E) criterion. The

latter is defined as

$$E = \frac{1.96\sigma}{\sqrt{c}} \quad (7)$$

where 1.96 corresponds to Z score at 95% confidence level, σ corresponds to the weighted standard deviation, and c corresponds to the number of observations. For each treatment \times farm, the resampling was applied 2,000 times to the original SOC stock dataset (all cores) at $d_{0-90 \text{ ref}}$ ($BD_{i, m0-90 \text{ ref}}$) for both SRS and StSRS designs. In SRS, resampling was applied from 1 to the maximum samples per field (18). In StSRS, resampling was applied, starting

TABLE 4 Sample size per stratum for stratified simple random sampling using the same number of samples for each stratum ($StSRS_{fix}$) and using an optimal number of samples per stratum ($StSRS_{opt}$) at Farm 1 (F1) and Farm 2 (F2) for conservation (CONS) and conventional agriculture (CONV)

Stratum	$StSRS_{fix}$				$StSRS_{opt}$			
	F1		F2		F1		F2	
	CONS	CONV	CONS	CONV	CONS	CONV	CONS	CONV
	no.							
1	6	6	6	6	9	12	9	5
2	6	6	6	6	6	2	5	3
3	6	6	6	6	3	4	4	10
Total	18	18	18	18	18	18	18	18

TABLE 5 Estimated means and statistical significance for bulk density (BD) and soil organic carbon (SOC) concentration. According to statistical model results, different letters indicate significant difference between estimated means according to Tukey test with $p < .05$

Property	Effect		SRS_{fix}		SRS_{opt}		$StSRS_{fix}$		$StSRS_{opt}$	
			F1	F2	F1	F2	F1	F2	F1	F2
BD	Treatment	<i>p</i> value	.3106	.0009	.3557	<.0001	.5239	.0929	.4021	.1727
		CONS	1.38	1.43a	1.39	1.45a	1.38	1.43	1.39	1.43
		CONV	1.36	1.35b	1.37	1.35b	1.37	1.36	1.37	1.37
	Treatment × layer ^a	<i>p</i> value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
		CONS L1	1.23c	1.27b	1.25d	1.31b	1.23c	1.27b	1.25d	1.30b
		CONV L1	1.33bc	1.02c	1.34bcd	1.12c	1.33bc	1.02c	1.34bcd	1.14c
		CONS L2	1.50a	1.50a	1.50a	1.48a	1.50a	1.50a	1.50a	1.47a
		CONV L2	1.43ab	1.40a	1.41abc	1.36b	1.44ab	1.40ab	1.41abc	1.38ab
		CONS L3	1.51a	1.52a	1.49a	1.53a	1.51a	1.52a	1.49a	1.52a
		CONV L3	1.46ab	1.49a	1.44ab	1.50a	1.46ab	1.49a	1.44ab	1.52a
CONS L4	1.29c	1.44a	1.32cd	1.46ab	1.29c	1.44a	1.32cd	1.45a		
CONV L4	1.23c	1.50a	1.30d	1.43ab	1.23c	1.50a	1.30d	1.45ab		
SOC	Treatment	<i>p</i> value	.0671	.0391	.0132	.5667	.1326	.3290	.1382	.4669
		CONS	1.62	1.49a	1.54a	1.23	1.62	1.50	1.55	1.37
		CONV	1.43	1.25b	1.30b	1.29	1.43	1.24	1.31	1.15
	Treatment × layer ^a	<i>p</i> value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
		CONS L1	2.65a	2.79a	2.53a	2.14a	2.65a	2.79a	2.54a	2.29a
		CONV L1	1.70b	1.74b	1.52b	1.55ab	1.70b	1.72b	1.52b	1.41ab
		CONS L2	1.33b	1.25bcd	1.36c	1.12bc	1.33b	1.26bcd	1.37b	1.27bc
		CONV L2	1.40b	1.45bc	1.38c	1.52b	1.40b	1.46bc	1.38b	1.38ab
		CONS L3	1.22b	1.09cd	1.08c	1.00bc	1.22b	1.10bcd	1.10b	1.14bc
		CONV L3	1.33b	1.02cd	1.20c	1.19bc	1.33b	1.02cd	1.20b	1.04bc
CONS L4	1.27b	0.83cd	1.19c	0.66c	1.27b	0.85cd	1.20b	0.80bc		
CONV L4	1.29b	0.78d	1.11c	0.90c	1.29b	0.76d	1.12b	0.75c		

^aL1, 0–5 cm; L2, 5–30 cm; L3, 30–50 cm; L4, 50–90 cm.

from 1 core to a maximum number of cores (6) per stratum at increments of 1. The optimal sample size was estimated when both confidence lines (i.e., 97.5th and 2.5th percentiles) crossed the allowable error range (Han, Zhang, Mattson, Zhang, & Weber, 2016), as reported in

Figure 3. The bootstrap test was not applied to $StSRS_{opt}$ because of the huge number of combinations in the resampling procedure. Mixed models were performed with SAS software version 5.1 (SAS Institute). Bootstrap tests used R software.

TABLE 6 Correlation matrix between soil properties in the study area for samples collected according to a fixed (*italics*) and an optimized number of samples (no italics). Underlined coefficients indicate significance for $p < .05$

Trait ^a	F1										F2									
	BD	SOC%	Sand	Clay	H1	H2	H3	V1	V2	V3	BD	SOC%	Sand	Clay	H1	H2	H3	V1	V2	V3
BD		-.37	.09	-.06	.24	.26	.26	.24	.25	.26		-.43	.15	-.17	-.21	-.15	-.14	-.01	-.08	-.11
SOC%	-.32		-.10	.11	-.24	-.26	-.28	-.24	-.26	-.26	-.44		-.26	.29	.34	.35	.31	.31	.35	.35
Sand	.07	-.03		-.96	-.04	-.04	-.04	-.05	-.05	-.05	.16	-.23		-.91	-.63	-.62	-.60	-.42	-.52	-.56
Clay	-.04	-.01	-.96		.08	.07	.07	.08	.08	.07	-.03	-.11	-.55		.66	.62	.60	.36	.48	.55
H1	.13	-.17	.03	.00		.99	.98	.99	.99	.99	-.25	.25	-.66	.36		.89	.86	.61	.76	.84
H2	.15	-.17	.04	-.01	.98		.99	.99	.99	.99	-.21	.28	-.66	.34	.89		.99	.82	.92	.97
H3	.14	-.18	.05	-.01	.95	.98		.97	.98	.99	-.20	.24	-.67	.39	.85	.99		.79	.89	.95
V1	.14	-.15	.02	.00	.99	.97	.93		.99	.99	-.05	.24	-.33	.05	.47	.73	.69		.95	.90
V2	.14	-.17	.02	.01	.99	.99	.97	.99		1.00	-.14	.28	-.49	.14	.71	.88	.84	.93		.98
V3	.15	-.17	.03	.00	.98	.99	.98	.97	.99		-.18	.29	-.58	.24	.84	.97	.95	.83	.96	

^aBD, bulk density; SOC%, percentage soil organic C. H1, H2, and H3 represent the instrument horizontal coplanar configuration with effective depth ranges of 0.50, 1.00, and 1.80 m, respectively. V1, V2, and V3 represent the instrument vertical coplanar configuration with effective depth ranges of 0.25, 0.50, and 0.90 m, respectively.

3 | RESULTS

3.1 | Soil apparent electrical conductivity and soil stratification

The overall average EC_a was higher at Farm 1 (F1) than at Farm 2 (F2) (79.6 vs. 32.8 mS m⁻¹), and maximum values were found in the H₃ configuration (242.7 mS m⁻¹ in CONS and 319.4 mS m⁻¹ in CONV) (Table 2, Figure 4). It is worth noting that at F1, EC_a peaked on the western side of the CONV field, despite the overall wider variability observed in the CONS field (Table 2). On the contrary, at F2, both EC_a variability and mean were similar between the two treatments (32.4 mS m⁻¹ in CONV and 33.3 mS m⁻¹ in CONS). Here, maximum values were recorded in the northern part of CONS field (67.0 mS m⁻¹ for H₂). For further details, see Supplemental Figures S1 and S2.

Stratification based on EC_a is shown in Figures 4c and 4d, and Table 3. At F1, Stratum 1 represented more than half of the field surface for both CONV (80%) and CONS (51%). The sample size range for StSRSopt was 3–9 in CONS and 2–12 in CONV (Table 4). Even in CONV at F2, a single stratum prevailed over the area (i.e., 63% in Stratum 3) to yield a sample size that ranged from 3 to 10, according to StSRSopt. Similarly, Stratum 1 in CONS accounted for 46% of the field and resulted in a sample size of nine (Tables 3–4).

3.2 | Bulk density and soil organic carbon concentration

Irrespective of sampling designs, BD results showed a similar effect from the treatment × layer interaction at both farms (Table 5). At F1, stratification was stronger in CONS, as evidenced by denser results in the 5- to 50-cm subsoil than in the 0- to 5-cm topsoil for fixed (1.50 vs. 1.23 g cm⁻³) and optimized (1.50 vs. 1.25 g cm⁻³) approaches, respectively (Table 5). On the contrary, in CONV, BD was more homogenous throughout the 0- to 50-cm profile (1.40–1.41 g cm⁻³, on average), although a progressing BD increase is detectable with depth. At F2, first-layer BD was dominant and denser in CONS than in CONV (Table 5), and its mean values varied with sampling design (1.27 vs. 1.02 g cm⁻³ in SRS_{fix} and StSRS_{fix}, and approximately 1.30 vs. 1.12 g cm⁻³ in SRS_{opt} and StSRS_{opt}). Clay content also affected BD, although the correlation was positive at F1 (0.003 slope, on average) and negative at F2 based on sampling design (+0.002 slope, on average, in SRS_{opt} and StSRS_{opt} and -0.003 slope, on average, in SRS_{fix} and StSRS_{fix}). For further details, see Supplemental Figures S3 and S4.

TABLE 7 Sampling variance [$V(z_{st})$], sampling efficiency (V_r), and simple random sampling (SRS) equivalent number (n_{eq} SRS) according to the stratified simple random sampling using the same number of samples for each stratum (StSRS_{fix}) and using an optimal number of samples per stratum (StSRS_{opt}) approaches

Variable	Sampling	Fixed approach				Optimal approach			
		F1		F2		F1		F2	
		CONS	CONV	CONS	CONV	CONS	CONV	CONS	CONV
$V(z_{st})$	SRS	141.51	70.97	88.22	82.57	115.50	61.27	86.05	87.51
	StSRS	176.48	152.51	37.43	67.90	93.56	60.53	50.38	96.43
V_r	–	0.80	0.47	2.36	1.22	1.23	1.01	1.71	0.91
n_{eq} SRS	–	14	8	42	22	22	18	31	16

Note. F1, Farm 1; F2, Farm 2; CONS, conservation agriculture; CONV, conventional agriculture.

The SOC concentration showed significant differences ($p < .01$, Table 5) depending on the treatment \times layer interaction only in the topsoil (0–5 cm) at both F1 (all sampling designs) and F2 (all but StSRS_{opt}). Indeed, CONS raised topsoil SOC concentration by about 61 (F1) and 56% (F2).

Regarding sampling approaches, the average SOC concentration was higher with SRS_{fix} and StSRS_{fix} than with SRS_{opt} and StSRS_{opt}, such that maximum values in the first layer of F2 were 2.79 and 1.74 g cm⁻³, respectively. At F2, clay content oppositely affected SOC according to sampling design, with a positive correlation with SRS_{fix} and StSRS_{fix} and negative correlation with SRS_{opt} and StSRS_{opt} (Table 5).

3.3 | Soil organic carbon stocks

The SOC stocks at 0–5 cm were generally greater in CONS than in CONV, regardless of farm and sampling design (Supplemental Figures S5 and S6). Notably, the relative difference between treatments ranged from 49 (SRS_{opt} at F2) to 74% (both SRS_{opt} and StSRS_{opt} at F1). Similarly, CONS 0- to 30-cm profile stocks were greater than CONV stocks, ranging from 50.56 t ha⁻¹ with SRS_{opt} at F2 to 62.42 t ha⁻¹ with StSRS_{opt} at F1. However, the observed variations and differences between the two treatments were significant ($p = .05$) only at F1 SRS_{opt}, with 62.00 (CONS) and 51.18 t ha⁻¹ (CONV). In the 0- to 50-cm and 0- to 90-cm soil profiles, no significant differences were observed between treatments, where average SOC stocks ranged between 140.47–152.85 t ha⁻¹ at F1 (SRS_{opt}) and 123.26–131.48 t ha⁻¹ at F2 (SRS_{fix}). For further details, see Supplemental Figures S2 and S3 and Supplemental Table S1.

3.4 | Relationship between soil apparent electrical conductivity and soil chemical–physical characteristics

In F1, EC_a did not show any dependency on the primary soil properties, with the exception of a very weak corre-

lation with both SOC and BD. Compared with F1, F2 had stronger correlations between EC_a and soil properties irrespective of sampling design. Significant positive relationships for EC_a–SOC and EC_a–clay, and significant negative relationships for EC_a–BD and EC_a–sand, were observed (Table 6).

3.5 | Sample size optimization

The sampling efficiency of SOC stock estimation in the 0- to 90-cm profile was evaluated initially by comparing the sampling variances of the four sampling schemes. Experimental site and sample allocation per stratum differences produced contrasting results. When considering variation related to sampling design (Table 7), the StSRS_{opt} method had the smallest variance (best result) at F1 in CONS (93.6 t C ha⁻¹) and CONV (60.5 t C ha⁻¹), whereas the StSRS_{fix} method had in the largest variance (worst result) in CONS (176.5 t C ha⁻¹) and CONV (152.5 t ha⁻¹). Conversely, F2 StSRS_{fix}, variances (37.4 and 67.9 t ha⁻¹ for CONS and CONV, respectively) were lower than other design averages (74.9 t ha⁻¹ in CONS and 88.8 t ha⁻¹ CONV). In general, a stratified design, both fixed and optimized, was more efficient in CONS than in CONV (Table 7). For example, at F2 with StSRS_{fix}, sampling efficiency (V_r) was 2.36 in CONS and 1.22 in CONV, and with StSRS_{opt}, sampling efficiency was 1.71 in CONS and 0.91 in CONV. Moreover, in CONS, StSRS_{opt} was more efficient (sampling efficiency >1) than SRS_{opt} at both farms (Table 7), given that the equivalent sample sizes with SRS_{opt} were larger by 4 (F1) and 13 (F2) than with StSRS_{opt}. On the contrary, in CONV, the efficiency of StSRS_{opt} was similar or slightly lower than that of SRS_{opt} (Table 7). Only when a fixed sample size per stratum was applied at F1 did sampling efficiency worsen with respect to SRS_{fix} in both treatments, particularly in CONV ($V_r = 0.47$). The contrary was observed at F2, where StSRS_{fix} yielded a SRS equivalent sample size equal to 42 (in CONS) and 22 (in CONV), +135.71% and +21.61% with respect to SRS_{fix}.

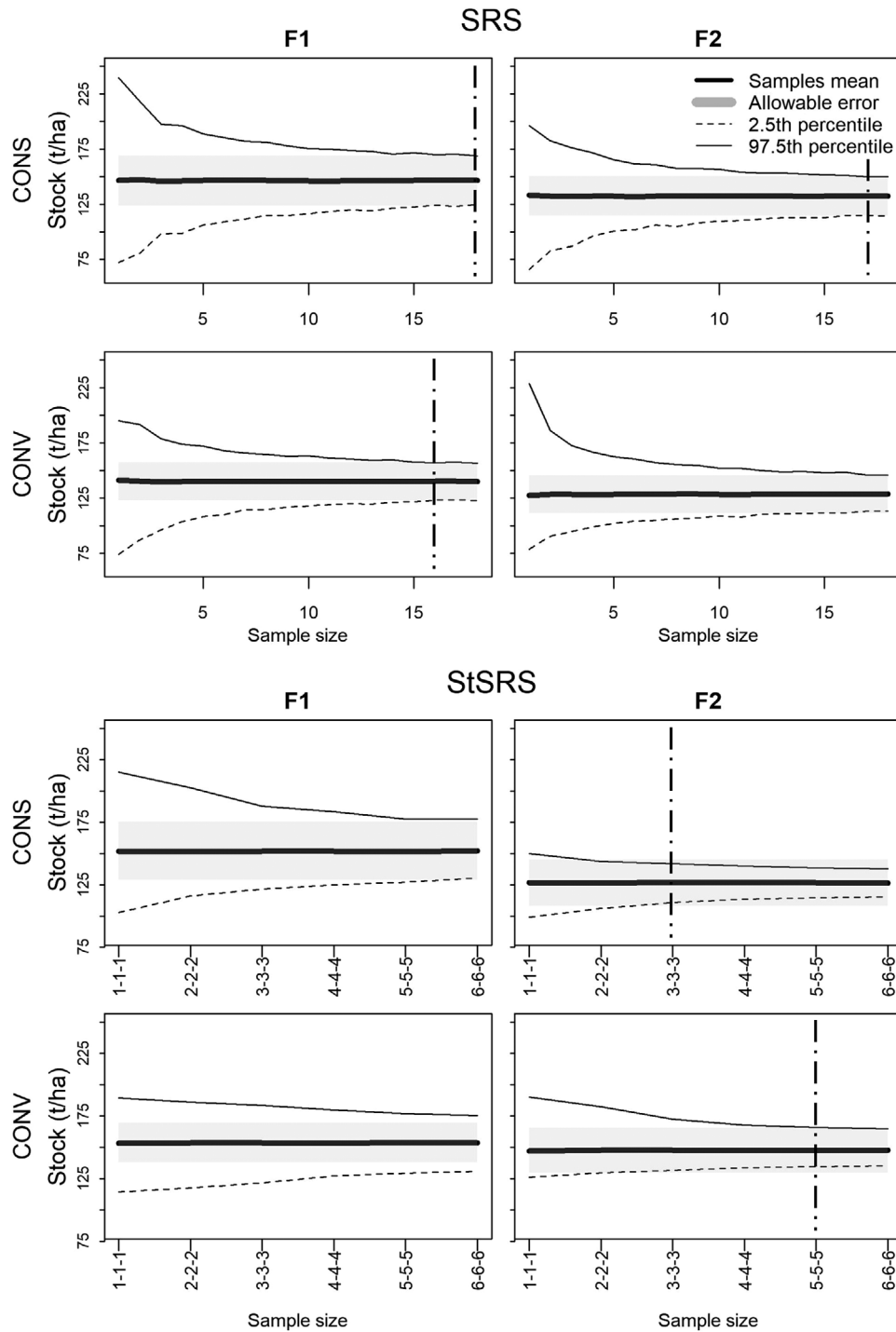


FIGURE 5 Decision criteria for sample size according to the bootstrap procedure in simple random sampling (SRS, italics) and stratified simple random sampling (StSRS, no italics) designs. The sample mean, allowable error, and 2.5th and 97.5th percentiles of soil organic C (SOC) stock estimates are reported for each sampling design \times farm \times treatment interaction. The points at which the 95% confidence lines just cross the allowable error range represent sample size (vertical dashed line). CONS, conservation agriculture; CONV, conventional tillage

Second, the StSRS_{fix} design was evaluated at a 95% confidence level according to the bootstrap method (Figure 5). In general, this method confirmed the equivalent sample size results, since SRS_{fix} required a more samples than StSRS_{fix} at F2, but not at F1. In the former, three sam-

ples per stratum (nine total) allowed means to be achieved at a 95% confidence level within the allowable *E* range in CONV; 17 were required with SRS. Similarly, CONV reached the optimal sample size with five samples per stratum, whereas even the maximum sample number (18) was

insufficient with SRS. At F1, the optimal sample size was equal to 18 (CONS) and 16 cores (CONV) with SRS, but the maximum core per stratum number (six) required means to be outside the allowable error range.

4 | DISCUSSION

The EC_a data from the two farms were grouped using the k -means clustering method. The goodness of the EC_a -directed stratification affected the sampling design performance. In the absence of a clear correlation between EC_a and soil properties, as in the case of F1, the stratification did not capture the spatial variability. As expected, the high-salinity conditions at F1 masked the influence of other soil properties on electromagnetic signal transmission, as shown by the weak correlations between EC_a and soil characteristics. The F1 results confirmed the recommendations made by McBratney and Minasny (2005) and Corwin and Scudiero (2016), as clay content averaged $26.4 \text{ g } 100 \text{ g}^{-1}$ and EC^a values were $>125 \text{ mS m}^{-1}$ for 65% of the total area. In general, stratified simple random sampling reduces uncertainty, improves the sampling efficiency, and reduces the sample size required for a given level of precision (de Gruijter et al., 2006). However, for an efficient StSRS design, the ancillary information must be spatially correlated with the variable to be sampled. In instances where this assumption was not met, the sampling efficiency is similar to a random sampling scheme. Indeed, the optimal F1 sampling scheme eliminated the effect of soil variability by strengthening the sampling points in heterogeneous areas. Similar results were described by Brus (1994) and Uribeetxebarria, Martínez-Casasnovas, Escolà, Rosell-Polo, and Arnó (2019), who attributed the failure of an StSRS scheme to using a fixed number of samples per stratum instead of an optimal number.

At F2, where a good EC_a -soil properties correlation was present, soil stratification captured the soil spatial variability. This may be because SOC and clay contents might have influenced soil bulk conductance and increased the number of exchangeable cations in the solid-liquid phase pathway (Corwin & Lesch, 2005; Sudduth et al., 2005). The significant correlation between EC_a and SOC appears to be more of an indirect consequence of the effect of texture on SOC than of a direct effect of SOC on electrical conductivity, considering its low content within the soil profile ($<15 \text{ g kg}^{-1}$). This is due to the “chemical stabilization” defined by Six, Conant, Paul, and Paustian (2002), in which SOC is protected by chemical or physicochemical binding with clay and silt particles. Indeed, a greater organic C input conversion efficiency and higher adsorption capacity are generally observed in clay soils, which act to stabilize the organic carbon limit and prevent mineralization

(Piccoli et al., 2016). In this way, EC_a can be used to assist the quantitative spatial characterization of SOC (Martinez et al., 2009). It is also possible that the opposite relationship (negative) between EC_a and sand might be explained by the lower water content usually recorded in the presence of high sand content. At F2, clustering allowed sampling variance to be reduced, even with a fixed sampling scheme.

Notably, irrespective of the goodness of the EC_a -directed stratification, the stratification procedure was particularly effective in CONS compared with CONV, as it always led to higher sampling efficiencies. The most likely explanation for this behavior is the absence of tillage operations, which preserved the original heterogeneity of the field and, in turn, increased the efficiency of the stratification. Alternately, CONV tillage mixed the soil layers and increased soil movement within the field, smoothing the soil spatial variability (Sibbesen, Skjøth, & Rubæk, 2000). The bootstrap method showed that StSRS_{fix} not only improved sampling efficiency by reducing the sampling variance with stratification, but it also failed when stratification was not correlated with the relevant soil properties.

5 | CONCLUSIONS

The SOC stock varied according to the sampling strategy and site-specific conditions. Results suggested that when the assumption of a strong EC_a -soil properties correlation was met, EC_a sensing was a valuable means by which to decrease the minimum sample size. However, when the stratification process failed because of the salinity effect on EC_a , StSRS efficiency was still comparable with that of SRS, especially when an optimal number of samples per stratum was used.

Most likely, this occurred because the salinity-driven EC_a did not show strong patterns unrelated to the SOC that resulted in a disproportional sample size with respect to the strata surface areas. From a practical point of view, we were able to reject our starting hypothesis in the specific conditions of the low venetian plain. Even without prior knowledge of the relationships between EC_a and soil properties, EC_a -directed sampling is a win-win solution to advance soil characterization and SOC stock estimation in agricultural fields. Of note, in different pedoclimatic conditions, StSRS could be less effective than SRS in optimizing sample size allocation when strong EC_a pattern would lead to inappropriate stratification or suboptimal samples allocation.

Further research should investigate the spatial relationship between EC_a and soil properties in different environments to improve the use of geophysical survey for directing the sample strategy.

CONFLICT OF INTEREST


The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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