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Carbon storage in agricultural topsoils and subsoils is promoted by including temporary grasslands into the crop rotation

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ABSTRACT

Atmospheric C sequestration in agricultural soils is viewed as one of the most promising negative emission technologies currently available. Nonetheless, it remains unclear how strongly soil organic carbon (SOC) stocks respond to agricultural practices, especially for subsoil. Here, we assess the SOC storage potential in croplands and how the presence of temporary grasslands (TG) in the crop rotation affects SOC stocks. We developed a new approach to correct for bias in bulk density (BD) induced by sampling conditions and land-use effects with a data-driven model to predict the BD of fine soil (<2 mm) for reference condition. Using 54 permanent grassland and cropland sites with various proportions of TG from a monitoring network in Switzerland, we showed that SOC stock differences down to 50-cm depth between cropland and permanent grasslands (maximum: 3.0 ± 0.8 kg C m^{-2}) depend on the TG proportion in the crop rotation, regardless of clay content and pH. An increase of the TG proportion by 10% would induce a SOC gain of 0.40 ± 0.13 kg C m^{-2}. The responses of topsoil (0–20 cm) and subsoil (20–50 cm) SOC stocks to TG proportion were linear and equivalent. The effect of TG on SOC storage would have been underestimated by 58% without accounting for subsoil stocks response and by 16% without BD corrections. The conversion of all croplands to permanent grasslands in the study region would potentially store a quantity of SOC equivalent to the anthropogenic greenhouse gas emissions generated by the same region during one year. Although the potential of agricultural soils as negative emission technology is relatively modest compared to former expectations, the findings demonstrate the potential to manage SOC and its associated ecosystem services at large scales and down to deep soil layers.

1. Introduction

To mitigate climate change and limit global warming to less than 2 °C, it is not only necessary to decrease greenhouse gas (GHG) emissions, but also to remove CO_2 from the atmosphere through negative emission technologies (NET) (Minx et al., 2018). Soil carbon sequestration in agroecosystems is a promising NET offering considerable mitigation potential (Bossio et al., 2020; Fuss et al., 2018) due to the important C-deficit of agricultural soils (Sanderman et al., 2017). Practices designed to increase soil organic carbon (SOC) are readily applicable at large scales by land managers because they do not require any new technological breakthrough (EASAC, 2018; Fuss et al., 2018; Minx et al., 2018).

Agricultural soils under croplands exhibit, in comparison to grasslands, a large deficit in SOC that has largely increased in the last 30 years (Guillaume et al., 2021, 2022; Launay et al., 2021), even though signs of recovery have been identified in areas where agri-environmental programs are in place (Dupla et al., 2021). Various agricultural practices in croplands, such as cover crops, optimum crop associations (e.g. legumes, perennial crop), soil C amendments (e.g. organic amendments, biochar), minimum soil tillage or improved crop rotations, can potentially improve SOC storage and result in net atmospheric GHG removal if the SOC accumulation surpass any potential GHG emissions induced by the practice (Bai et al., 2019; Chenu et al., 2019; De Stefano & Jacobson, 2018; Haddaway et al., 2017; Koishi et al., 2020; lard and Angers, 2014; Poeplau & Don, 2015). According to several modelling studies, the

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inclusion of temporary (ley) grasslands (TG) or the increase of their duration within the crop rotation shows the highest SOC accrual rate and GHG emission mitigation potential among those practices (Launay et al., 2021; Lugato et al., 2015).

The inclusion of TG within crop rotations has been a common practice in integrated crop-livestock farming systems, but it slowly disappeared in large areas of the world due to the specialization of agricultural systems (Franzluebbers et al., 2014; Lemaire et al., 2015). In the European Union, only 10% of the farms integrated crops and livestock in 2016, while two third were crop specialists (Schut et al., 2021). In parallel, temporary grasslands decreased the most since the 70′s in the regions that had already the lowest proportion (Schott et al., 2018). Temporary grasslands provide a large range of ecosystem services (e.g. soil conservation, nutrient provision, water regulation) whose benefits are also transferred directly to the subsequent crops of the rotation (Hoeffner et al., 2021; Martin et al., 2020; Panettieri et al., 2017). The positive effect of TG on cropland SOC stocks has been reported in long-term field experiments (Chan et al., 2011; Crème et al., 2018; Franzluebbers et al., 2014; Johnston et al., 2017; Martin et al., 2020; van Eekeren et al., 2008). However, agricultural management in these experiments and the resulting SOC dynamics do not always align with actual agricultural practice. More importantly, no relationship has been established yet between the proportion of temporary grasslands in the crop rotation and new equilibrium SOC stocks reached after TG inclusion. For example, it is unknown whether croplands with a TG proportion approaching 100% lead to SOC levels that are comparable with permanent grasslands (PG). Conflicting results indicate that SOC stocks could potentially be higher in croplands with high TG proportion compared to PG (Conant et al., 2017), or lower due to incomplete recovery of the agroecosystem after cropping perturbation (Mayer et al., 2019).

The potential of cropland subsoils to store additional C remains an open question as most studies focus on the top 20–30 cm-deep layer of the soil. The impact of agricultural practices on subsoil SOC stocks is so rarely documented. Subsoil SOC stocks are often absent in global soil C budgets (Crowther et al., 2019; van Gestel et al., 2018) despite the fact that they store a substantial proportion of the SOC (Balesdent et al., 2018; Poeplau et al., 2020). Studies have shown that the influence of land-use and management factors on SOC stocks decreases with soil depth while factors related to the nature of parent material and topography become more prominent (Funes et al., 2019; Mayer et al., 2019; Vos et al., 2019). Although SOC turnover is slower in subsoil than in topsoil, a substantial proportion of recently incorporated C in agricultural soil occurs below 20-cm depth, indicating that subsoil pools are not passive at decadal timescale (Balesdent et al., 2018; Quezada et al., 2019).

The impact of land-use type on subsoil SOC is not fully elucidated. A recent meta-analysis did not find significant effect of grassland conversion to cropland on subsoil SOC (Li et al., 2020) while some national scale monitoring surveys found differences in subsoil SOC stocks for these two land-use types (Gregory et al., 2014; Poeplau et al., 2020). The introduction of temporary grasslands in the crop rotation provides high belowground C inputs that favor SOC accrual (Panettieri et al., 2020; Rüegg et al., 2019). Also, the long grassland duration enables the rooting system to penetrate deeper into the soil, which may increase SOC storage in subsoil (Martin et al., 2020). However, this effect was not consistently observed in all studies (Börjesson et al., 2018; Jarvis et al., 2017; Johnston et al., 2017).

Identifying land-use type and management effects on SOC is further compounded by the small differences in SOC changes compared to the large and highly variable SOC stocks (Paustian et al., 2019) and by the methodological bias related to soil bulk density measurements. As SOC accrual tends to decrease bulk density, the effects of land-use type and management are systematically underestimated when SOC stocks are compared at fixed depths (Nye and Greenland, 1964). To overcome this limitation the comparison of stocks by equivalent soil mass is advocated (Ellert & Bettany, 1995; von Haden et al., 2020). This method reduces bias in experiments where land-use type and management effects for the same soil are compared. However, different soils under similar management do not have the same mass density due to texture or mineralogy differences (Martín et al., 2017). Hence, the equivalent soil mass approach may also introduce new bias especially when the effect of management is assessed across a soil monitoring network encompassing a variety of soils. A second, often overlooked bias, comes from sampling conditions under different soil moisture content due to soil swelling and shrinkage which, in turns, affect the soil bulk density (Fox, 1964). A third bias relates to the presence of coarse fragments (>2 mm). Accounting for differences in coarse fragments between soils is important for SOC stock calculation and comparison, since the SOC content is measured on fine soil < 2 mm (Poeplau et al., 2017).

The overall objective of this study was to determine the potential of croplands to store additional atmospheric C by increasing the proportion of TG in crop rotations and to assess whether subsoils represent a substantial contribution to the additional SOC storage under field conditions using a soil monitoring network in Switzerland. The conversion from croplands to grasslands and vice versa is a common practice in the study region where a diversity of crop rotations is characterized by a large variation of the proportion of TG in the crop rotation in terms of frequency and number of consecutive years (Guillaume et al., 2021). The agricultural system in the study region can be viewed as a cropland-grassland continuum where permanent grasslands represent the upper technical limit of SOC storage that can be achieved in croplands by increasing the proportion of TG in the crop rotation (Guillaume et al., 2022). Hence, the specific goals of this study were to: i) quantify SOC stocks in the topsoil (0–20 cm) and subsoil (20–50 cm) of croplands and grasslands; ii) establish the relationships between the proportion of TG within the crop rotation and the SOC stocks in the topsoil and subsoil; iii) compare the SOC storage potential in relation to the anthropic GHG emissions reported for the study region; and iv) determine the effect of bias of bulk density measurement on the estimation of SOC stocks.

2. Materials and methods

2.1. Study sites

The study was conducted in Western Switzerland on long-term soil monitoring sites from the network established in 1987 by the Agricultural Institute of the Fribourg Canton and called FRIBO (Guillaume et al., 2021; Levasseur et al., 2019). The Fribourg Canton (1670 km^2) is located north of the Alps (46° − 47°N, 7°E) and lies on the Swiss Midland between the Jura Mountains' piedmont (NW) and the Western Alps' foothills (SE). It presents a NW-SE gradient of soils, elevation and climate. The Midland elevation gradually increases from 429 m a.s.l. in the NW to 800–900 m a.s.l on the Alps' foothills. The geology is composed of Tertiary molasse (sandstone, marl) partly covered by moraine deposits. The lowest part of the NW area consists mostly of lake and alluvial sediments. This part is relatively flat, while the rest of the Midland presents a smooth, hilly topography. Soils are dominated by Cambisols, Gleysols and Fluvisols (Frau et al., 2020). The climate is temperate continental (Mean Annual Temperature (MAT): 8.9 °C; Mean Annual Precipitation (MAP): 1075 mm) with cold winters and mild summers. For every increase of 100 m in elevation, the MAP increases by about 80 mm and the MAT decreases by about 0.5 °C (Dumas, 2013; Sevruk, 1997). Agricultural land occupies 755 km^2. Of this, 67% is under permanent (PG) and temporary (TG) grasslands and 30 % is under annual crops, half of which are cereals (DIAF, 2019).

The FRIBO network is composed of 250 sites established on a 2 × 2 km grid (Julien and Morand, 1995). Out of the 184 well-drained cropland and grassland sites (Guillaume et al., 2021), 66 sites (Cambisols or Fluvisols) were purposely selected to represent the diversity of land-use and site conditions of well-drained soils. Since 1987, the beginning of the monitoring period, the main annual crops have been recorded for each site. Most croplands (CR) sites include temporary grasslands (TG), which are grasslands sown as part of the crop rotation for forage production and occasionally for grazing. Temporary grasslands last typically for 1 or 3 consecutive years, but this duration varies between farmers (Guillaume et al., 2021). The proportion of years with TG over the monitoring period (TG proportion) varies between CR sites (0–73%). The most intensive managed PG and TG are cut up to five times per year to produce forage and are fertilized with manure and slurry following the Swiss recommendations (Huguenin-Elie et al., 2017). Twenty-four sites under permanent grasslands (PG) and 30 sites under CR have remained under the same land-use type since the beginning of the monitoring in 1987. Only 12 sites have experienced one to several land-use changes (LUC) from CR to PG or vice versa. LUC sites were only included to develop bulk density models. According to Swiss legislation (OTerm 910.91), if TG at a site occurs for >6 consecutive years, the site is classified as PG. Thus, CR sites with TG > 6 consecutive years were considered to have experienced land-use change (LUC), even if they were subsequently converted back to croplands. Similarly, PG that were cultivated for at least 1 year were considered as sites that experienced LUC.

2.2. Soil sampling and analysis

Soil samples were collected in 2020. Permanent and temporary grasslands were sampled in May-June. Cereal (wheat, barley, and triticale) and rapeseed sites were sampled after harvest, i.e., in July-August, before any subsequent field operation (tillage, stubble ploughing, etc.) to minimize the effect of agricultural machinery on soil bulk density. Finally, sugar beet sites were sampled before harvest in October in between planting rows. Out of the 54 sampled sites, subsoil samples for 4 PG and 2 CR could not be collected because of the presence of bedrock or high stone content.

For each selected FRIBO site (10 × 10 m), topsoil (0–20 cm) and subsoil (20–50 cm) samples were collected separately at 4 sampling points that were 2 m apart from the center of the plot. The soil was sampled using a Humax drilling core sampler (5.3-cm diameter) with 25-cm plastic HumaxTube® (GreenGround AG, Burgdorf, Switzerland). This method enables the extraction of consecutive soil cores of known volume at fixed depth intervals from the same hole. A drilling tube is inserted down to the first desired depth. A 25-cm plastic tube within the drill metal sleeve retrieves the core from the drilling tube without removing the tube. To collect the subsequent core, the drilling tube is inserted down to the next desired depth with a new plastic tube. Topsoil samples were collected by inserting the drilling tube down to 20 cm. Subsequently, subsoil samples were collected using two consecutive cores of about 15 cm each to reach 50 cm depth and the two cores were mixed in a plastic bag. Soil samples were kept in closed plastic bags to retain soil water content at sampling time (SWC).

In the laboratory, samples were weighed before air-drying to determine SWC. Before sieving at 2 mm, samples were weighed again to determine total dry mass (Mt). Coarse fragments (>2 mm) were washed and weighted to determine their mass (Mc) and volume (Vc) assuming a density factor of 2.4 g cm^{-3} (Schwab & Gubler, 2016). Residual humidity (Rh) of fine soil (<2 mm) was determined based on drying a 10 g soil sample at 105 °C for 24 h. Dry, fine soil mass (Mf) was calculated as the difference between Mt and Mc corrected for Rh. The bulk density (g cm^{-3}) of fine soil (FD; excluding coarse fragment volume and mass) and the soil bulk density (BD, including coarse fragment volume, but not fragment mass) were calculated as follows:

$$FD = \frac{Mf}{Vt - (Mc^{*}2.4)} \tag{1}$$

$$BD = \frac{Mf}{Vt} = FD^{*}(1 - \frac{Vc}{Vt}) \tag{2}$$

where *Mf* is the dry fine soil mass (<2mm in g), *Vt* is the total volume of the sample (cm^3), *Mc* is the mass of the coarse fragments (g) and *Vc* is the volume of coarse fragments (cm^3).

After FD and BD determination, the four cores per site were combined to form one composite sample per site and per depth for SOC measurements. Soil organic C content (Corg) was determined by standard procedure using sulfochromic oxidation (NF ISO 14235).

2.3. Carbon stock corrections

Soil organic C stocks were calculated as follow:

$$Cs = FD^{*}(1 - fVc)^{*}Corg^{*}thickness \tag{3}$$

where *Cs* is the SOC stocks of a specific soil depth interval (kg C m^{-2}), *FD* is fine soil bulk density (g cm^{-3}), *fVc* is the fraction of the total volume occupied by coarse fragments (i.e., the stone content), *Corg* is the SOC content (mg C g^{-1} soil), and *thickness* is the soil thickness (m).

In order to compare the effect of land use on *Cs*, we developed an original method using corrections for three types of bias in bulk density measurement between sites, specifically bias due to: (i) different stone content (*fVc*); (ii) different soil water content (SWC) at sampling time and its influence on FD; and (iii) effect of land-use type on FD, especially due to changes in Corg.

For (i), Cs was adjusted by a constant value of coarse fragments volume (*fVc* value in eq. (3)) using the median value (0.03 for topsoil and 0.05 for subsoil) measured across all sites (*stone correction*). To correct for (ii) and (iii), a set of models were fitted and evaluated (see statistical analysis section) on all sampled sites (including LUC sites) to predict FD for each depth as a function of the main soil characteristics that affect soil density (i.e., SWC, Corg, clay content and land use at sampling time). Land use at sampling time was divided between CR (excluding TG) and grasslands (PG and TG) for topsoil models and between CR (including TG) and PG for subsoil models. To correct for the effect of SWC (ii), FD at each site was adjusted based on the median SWC values observed across all sampling sites (22% for topsoil and 17% for subsoil). The corrected FD were used in eq. (3) with fixed *fVc* to calculate SOC stocks corrected for coarse elements and sampling condition (*sampling correction*). Finally, to correct for land use (iii), and especially for Corg changes, PG sites were used as reference land-use type for bulk density. This approach is justified by the fact that, theoretically, PG has a maximum SOC storage, thus it can be used as reference for comparing SOC accrual and storage potential in CR and TG (Guillaume et al., 2021). Hence, SOC storage was compared on the basis of the FD that would have occurred after the conversion of CR sites and when they reached the new steady-state corresponding to PG conditions. For this purpose, a PG land-use type was considered for all sites. As maximum Corg in PG depends on soil texture (Guillaume et al., 2022), topsoil Corg was set to the level that would occur in PG depending on the clay content at each site. The Corg level was determined from the linear regression between Corg (%) and clay content (%) established from the data collected at PG sites: Corg = 1.90 × log(clay) − 2.44; $R^2_{adj} = 0.77$. As the regression was not significant for subsoil, the average Corg (1.2%) from all PG sites was used for subsoil. Finally, SOC stocks corrected for coarse elements, sampling condition and land-use type were computed with eq. (3) using FD values predicted with median SWC values, PG as land-use category, Corg estimated for all sites from the above regression, and median *fVc* value *(land-use correction)*.

2.4. Regional estimation

Land-use data for each category of agricultural land of the Canton of Fribourg in 2020 were obtained from the Swiss Federal Office of Agriculture. The potential surface area where temporary grassland could be implemented in the crop rotation was estimated from the surface area of TG plus the surface area of *open land* minus the surface area of crops typically not under rotation with TG (e.g., legumes, rice, berries, aromatic plants, etc.). The maximum additional SOC storage was estimated as the difference between the SOC stocks predicted at maximum theoretical TG proportion (i.e., 100%) and the SOC stocks predicted at the current regional proportion of CR under TG, multiplied by the potential surface area of CR that can include TG.

2.5. *Statistical analysis*

All statistical analyses were performed using R software 4.0.3 (R Core Team, 2020). Five models were fitted to predict FD as a function of SWC, Corg, clay and land-use type (grassland or cropland): i) multiple linear regression (function *lm*); ii) multiple linear regression with stepwise backward selection (function *stepAIC*); iii) least absolute shrinkage and selection operator (LASSO) penalized regression (function *glmnet*; alpha = 1); iv) partial least squares (PLS) regression (function *plsr*); and v) random forest (function *randomForest*). With stepwise and LASSO regressions, the most relevant explanatory variables are selected using the Akaike Information Criterion (with stepwise) or using a penalty parameter optimized by cross validation (with LASSO) (Tibshirani, 1996). PLS was used to deal with multicollinearity in the explanatory variables (Sharif et al., 2017). Finally, random forest models (implemented here with 500 trees) was used to handle possible nonlinear relationships between the predicted output and the inputs and complex interactions (Breiman, 2001). Accuracy (RMSE) and bias of each model were assessed by leave-one-out cross validation. The fitted models were then used to predict FD for each site with the R function *predict*, and the predicted values were used to calculate corrected SOC stocks (see subsection *Carbon stocks corrections*).

The effect of land-use type and the proportion of TG along with site characteristics (clay content, pH and elevation) on SOC stocks down to 50 cm depth in CR and PG (n = 48) were tested by linear multiple regression with stepwise backward selection (LRS) and the following R syntax: *SOC stock 0–50 cm* ∼ *land use* + *TG proportion*:*dummy* + *logClay20* + *logClay50* + *pH20* + *pH50* + *elevation* + *land use*:*logClay20* + *land use*:*pH20* + *land use*: *logClay50* + *land use*:*pH50.* As the relationship between clay and SOC was not linear (Guillaume et al., 2021), clay contents were $\log_{10}$-transformed for topsoils (*logClay20*) and subsoils (*logClay50*). The variable *dummy* is a numerical variable with 1 for CR and 0 for PG set to limit *TG proportion* effect to CR. The interaction between land-use type and soil parameters (clay content and pH) were also included in the model with the syntax *land use*:*soil parameter*. To determine the relative importance of *TG proportion* on SOC stocks in the topsoil and the subsoil of CR, one model was developed for each layer using only CR sites, excluding the variable *land use* and its interactions with soil parameters, as well as the clay content and pH of the layer that was not the one of interest. Deviation from normality of residuals was assessed by Shapiro Test. Semi-partial R^2 were determined by the R function *r2beta*. Data used in the partial regression plots were predicted using the coefficients of the respective LRS models and fixing the variables other than the one of interest to a constant reference value, i.e., the average found in all sites for *logClay50, logClay20*, *pH50 and pH20* and to the absence of TG in CR (*TG proportion* = 0 %). To determine the potential additional SOC storage due to TG in Fig. 2, the intercept of the model was subtracted from the SOC stocks. Data prediction (estimate and standard error) for specific values of model variables (e.g., for regional estimate) were done with the R function *predict*. If not specified, all discussed differences are significant at least at *p*-value < 0.05 and values are presented as mean ± standard error (SE).

3. Results

3.1. *Soil organic carbon stocks*

Soil organic C stock measured from surface to 50-cm depth without correction was higher in permanent grasslands (11.27 kg C m^{-2}; standard deviation (SD) = 1.71 kg C m^{-2}) than in croplands (7.84; SD = 1.54 kg C m^{-2}) (Table 1). The amounts of SOC stock in topsoil (0–20 cm) and subsoil (20–50 cm) were similar (49 ± 7%) in croplands (CR), but slightly less SOC was stored in permanent grasslands (PG subsoil (41 ± 5%) than in topsoil.

Besides SOC stocks, other soil characteristics affecting soil bulk density, namely fine soil density (FD), soil water content at sampling time (SWC), coarse fragments (>2mm), carbon content (Corg) and clay content, differed between CR and PG (Table 1). On average, CR topsoils and subsoils had higher FD but were also drier at the sampling time and exhibited lower clay and Corg. The five models built to correct for bias in FD due to sampling conditions affecting SWC and land-use effects had similar predicting performance for FD (RMSE < 0.117 g cm^{-3}) (Fig. S1, Table S1). Soil water content had no effect on FD for any soil layer according to the linear multiple regression models (LR) and the linear multiple regression with stepwise selection models (LRS) (Table S2). As the performances of all models were equivalent (Fig. 1), the LRS model was used to predict FD in the following calculations of corrected SOC stocks because of its parsimony and its good interpretability (Table S2).

While the model type had negligible effects on the calculation of SOC stocks, the correction type notably affected the effect size of land-use type on soil SOC stocks (Fig. 1, Table 2). The average SOC stocks down to 50 cm across all sites did not differ by >2% after corrections, but the difference in SOC stocks between PG and CR increased by 8% when the three corrections were applied (Table 2). Within CR sites, the slope between the proportion of temporary grasslands (TG) and the SOC stocks increased by 19% with the correction including land-use effects as compared to the absence of any correction (Table 2). From here on, only the SOC stocks corrected for all three biases on FD will be considered.

3.2. *Factors affecting corrected SOC stocks in agricultural soils*

The LRS model developed to explain the variation of SOC stock in the 0-to-50-cm soil layer of CR and PG (R^2_{adj} of 0.74) selected only four significant variables, namely *logClay50*, *land-use type*, *pH50* and *TG proportion*. The other variable, namely *elevation*, *logClay20*, *pH20* and the interactions between *land-use type* and soil properties were not selected in the model (Table 3). When in the model the pH and clay content values of the subsoil were replaced with the topsoil values, the R^2_{adj} (0.72) slightly decreased.

According to the model with the highest R^2_{adj} (0.74), SOC stock in the 0-to-50-cm layer of CR and PG was mostly explained by *logClay50* (semi-partial R^2: 0.41) followed by *land-use type* (semi-partial R^2: 0.26), *pH50* (semi-partial R^2: 0.16) and *TG proportion* in CR (semi-partial R^2: 0.14) (Table 3). SOC stocks increased with clay content but decreased with soil pH (Fig. 2). The SOC stocks down to 50-cm depth were lower by 2.96 ±

Table 1
Soil characteristics (mean ± SD) measured in topsoils and subsoils of croplands (CR) and permanent grasslands (PG).

	Topsoil (0–20 cm)		Subsoil (20–50 cm)	
	CR (n = 30)	PG (n = 24)	CR (n = 28)	PG (n = 20)
SOC stocks (kg C m^{-2})	4.0 ± 0.8	6.7 ± 0.9	3.8 ± 1.0	4.6 ± 1.1
Corrected SOC stocks [§] (kg C m^{-2})	3.9 ± 1.0	7.0 ± 1.1	3.6 ± 1.2	4.9 ± 1.4
Fine soil density (g cm^{-3})	1.25 ± 0.17	1.03 ± 0.15	1.52 ± 0.14	1.35 ± 0.14
Coarse fragments (%)	3.7 ± 2.5	3.6 ± 3.1	5.5 ± 3.9	6.0 ± 3.7
Soil water content (%)	18 ± 4	28 ± 3	15 ± 3	20 ± 3
Corg (mg C g^{-1})	17.1 ± 5.3	34.7 ± 9.0	9.1 ± 3.2	12.5 ± 3.4
Clay content (%)	15 ± 5	25 ± 13	15 ± 5	20 ± 6
pH H_2O	6.9 ± 0.6	6.1 ± 0.5	7.1 ± 0.7	6.1 ± 0.6
Elevation (m a.s.l.)	608 ± 106	802 ± 109	–	–

[§] SOC stocks corrected for the *stone*, *sampling condition* and *land-use* effects by linear multiple regression with stepwise selection model.

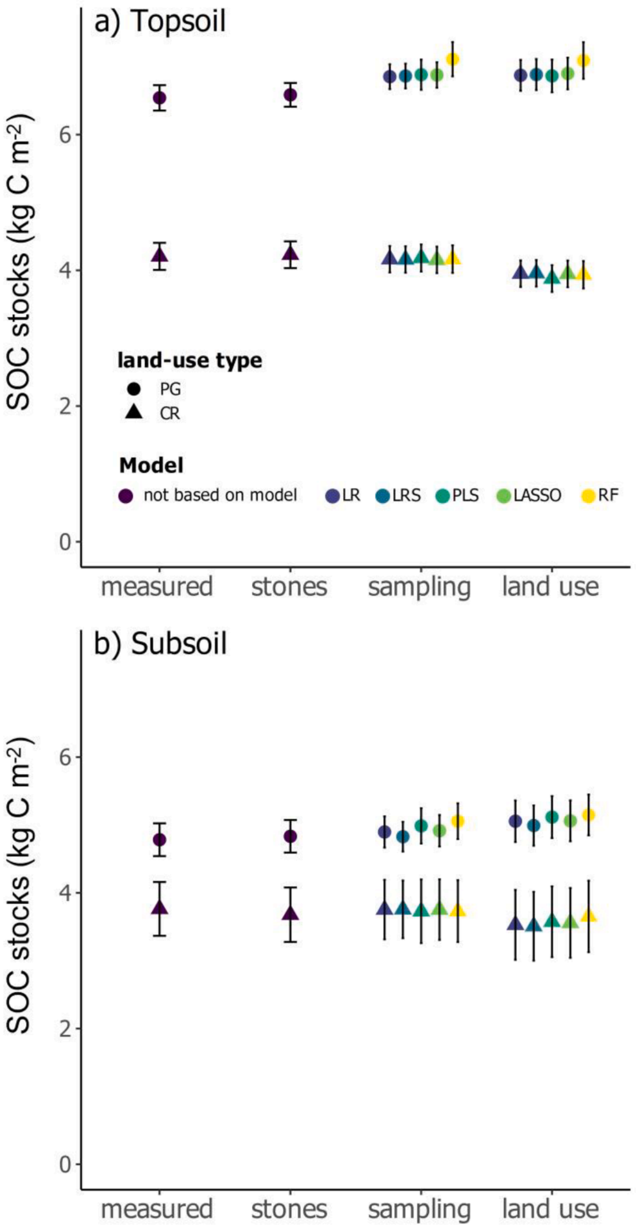

Fig. 1. Effect of soil bulk density corrections on SOC stocks for the topsoil and subsoil of permanent grasslands (PG) and croplands (CR). First, measured SOC stocks were corrected for differences in the volume of coarse fragments between sites (stones). Subsequently, additional corrections were made to account for differences in the bulk density of fine soil (FD) due to soil water content at sampling time (sampling) and land-use effects (land use) using five models to predict FD: linear multiple regression (LR), linear multiple regression with stepwise selection (LRS), partial least squares regression (PLS), least absolute shrinkage and selection operator regression (LASSO), and random forest (RF). Error bars represent the standard errors (topsoil PG: n = 24, CR: n = 30; subsoil PG: n = 20, CR: n = 28).

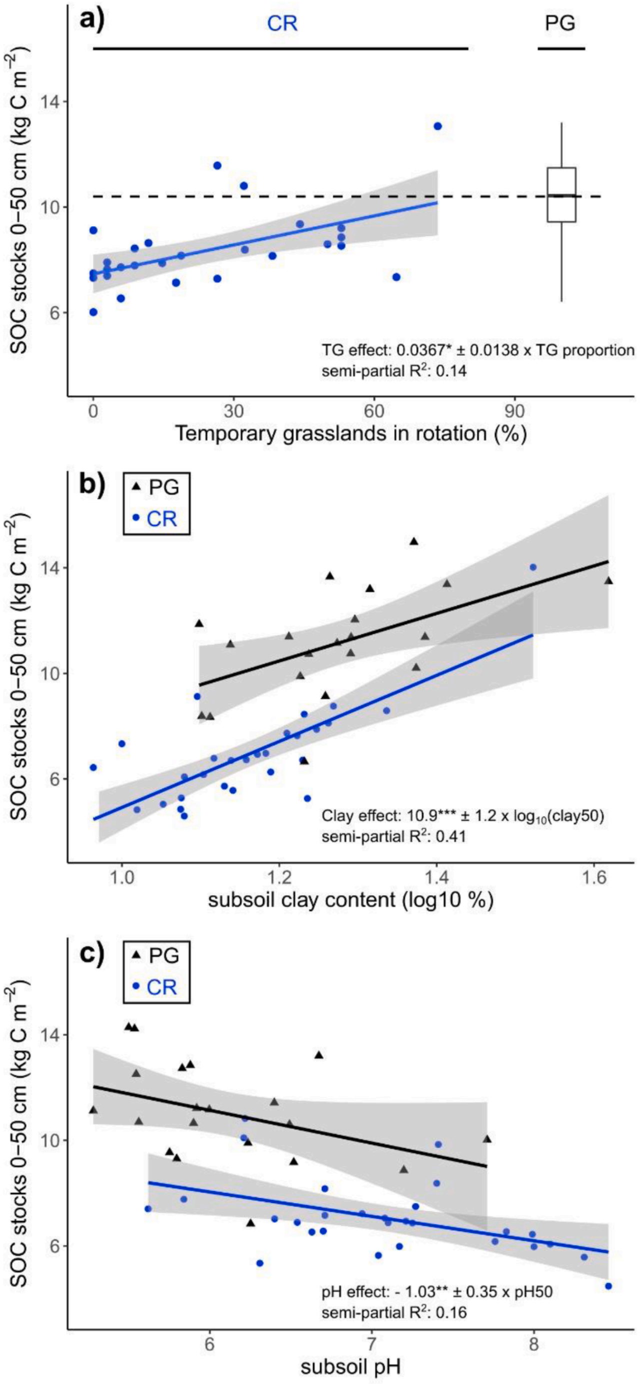

Fig. 2. Partial regression plots of SOC stocks (0–50 cm) in croplands (CR, dots) and permanent grasslands (PG, triangles) of the three numerical variables selected in the LRS model, i.e., (a) the proportion of temporary grasslands in the crop rotation (TG proportion), (b) the $\log_{10}$-transfromed clay content in the subsoil (logClay50), and (c) the pH H_2O in the subsoil (pH50). For each panel, the two variables other than the one of interest are fixed to a reference value, i.e., the average found in all sites for logClay50 (1.21) and pH50 (6.67), and to the absence of TG in CR (0%). The effects of land-use type (solid lines) and its 95% confidence interval (shaded area) as illustrated in the figure corresponds to the linear regression of SOC stocks with the variable of interest separately for CR and PG. In (a), PG (boxplot) were set at 100% for TG proportion for illustrative purpose. In each panel, the coefficient (± standard error) and the semi-partial R^2 from the LRS model for the variable of interest are included with stars indicating the level of significance.

0.77 kg C m^{-2} for CR without TG in the rotation compared to PG. This difference between land-use type was not affected by the clay content and the pH because of the absence of the interactions selected in the LRS model. Regardless of clay content and the pH, an increase by 10% of the TG proportion within the crop rotation would result in a SOC stock increase of 0.37 ± 0.14 kg C m^{-2} (Fig. 2).

Within the CR sites, the proportion of TG in the crop rotation for each site since the beginning of the monitoring was on average 24% (i.e., about one year out of four); with a minimum of 0% in four sites and a

Table 2
Effect of fine soil bulk density corrections on the average SOC stocks at all sites, on the difference between permanent grasslands and croplands, and on the effect of increasing the proportion of temporary grasslands in cropland rotation over the whole soil profile SOC stock (0–50 cm). Significance levels are represented by stars and were estimated by two-way ANOVA with the logarithm of clay content in the topsoil as co-variable for land-use and grassland effects.

Corrections	Mean C stock (kg C m^{-2})	Land-use effect (kg C m^{-2})	Grassland effect (kg C m^{-2} $\%^{-1}$ TG)
Measured	9.27 ± 0.30	−2.74 ± 0.65***	0.031 ± 0.012*
Stones	9.34 ± 0.30	−2.45 ± 0.63***	0.028 ± 0.011*
Sampling conditions	9.45 ± 0.33	−2.43 ± 0.63***	0.034 ± 0.011**
Land-use type	9.21 ± 0.39	−2.96 ± 0.77***	0.037 ± 0.014*

maximum of 73% (Fig. 2). The TG proportion within each CR site remained constant during the whole monitoring period with a difference of −2% on average between the first and second half of the monitoring period.

The TG proportion influenced SOC stocks for both topsoil and subsoil in CR (p-values < 0.05), along with the $\log_{10}$-transformed clay content (p-values < 0.001) and the pH (p-values < 0.05) in the respective layer while elevation was not selected (Table 3). The effect of TG proportion was 39% higher in the subsoil than in the topsoil (Fig. 3). Regardless of clay content or the pH, an increase by 100% of the TG proportion within the crop rotation would result in a SOC stock increase of 1.32 ± 0.62 kg C m^{-2} for the topsoil and 1.83 ± 0.81 kg C m^{-2} for the subsoil. The sum of the estimated SOC gain for topsoil and subsoil is 14 % lower than the estimation from the full soil profile (3.67 ± 1.38 kg C m^{-2}) but is 6 % higher than the SOC stock difference between CR and PG (2.96 ± 0.77 kg C m^{-2}).

3.3. *Regional estimates*

The potential surface area for the proportion of TG to be extended in 2020 in the Canton of Fribourg (35.3% of the area is already under TG) was 35,690 ha out of 75'657 ha. For each percent increase in the proportion of TG, an additional 0.013 ± 0.005 Mt of C would be stored in the top 50 cm of cropland soils in the Fribourg Canton. The maximum additional C storage in the study region, if all croplands were to be converted into grasslands, was calculated based on the difference between SOC stocks for CR with 35.3% of TG and the predicted theoretical maximum (i.e., 100%) of TG proportion. At the maximum SOC stocks in the system, the additional C storage in agricultural soil of the Fribourg Canton would reach 0.85 ± 0.38 Mt of C with a 95% confident interval (CI) between 0.11 and 1.59 Mt of C.

4. Discussion

4.1. *Impacts of temporary grasslands on SOC storage*

The current SOC stock difference between croplands and grasslands for the top 50 cm (i.e., 2.96 ± 0.77 kg C m^{-2}) represents the potential of croplands without TG (crop-only) to store additional SOC. This value is comparable to the 3.16 kg C m^{-2} difference between grasslands and croplands for the same soil depth across Germany (Poeplau et al., 2020). This similar difference between the two land use types over large regions like Germany or across a large range of site conditions like in our study is consistent with the negligible contribution of clay and pH variability to SOC stocks difference between land-use types (Fig. 2). The effects of clay and pH on SOC stocks has been already documented (Guillaume et al., 2021) and reflect the role of parent material on soil C-saturation (Six et al., 2002). This is supported by the fact that the condition of the subsoil, which is less prone to management effect and thus more representative of the site conditions (Vos et al., 2019), was selected in the LRS model instead of the topsoil pH and clay content. Nonetheless, a negative impact of pH on SOC stocks was unexpected because Corg has been shown to increase with soil pH favored by the stabilization mechanisms of SOC mediated by calcium (Rowley et al., 2018). It is possible that the impact of pH also reflects an effect of elevation, with cooler temperature affecting plant productivity, as both variables were correlated (r = 0.71).

The potential of subsoil to additional C was important (Fig. 1, Fig. 3). The increase of SOC stocks induced by TG tended to be even higher in the subsoil than in the topsoil even if the uncertainties are high (Fig. 3). Excluding the 20-to-50-cm layer from C accounting would have resulted in an underestimation by 58% of the potential of croplands to store additional SOC by introducing TG, an underestimation value that is higher than that reported by a Swedish study (27–39%) (Börjesson et al., 2018). The time necessary for achieving the new SOC equilibrium by

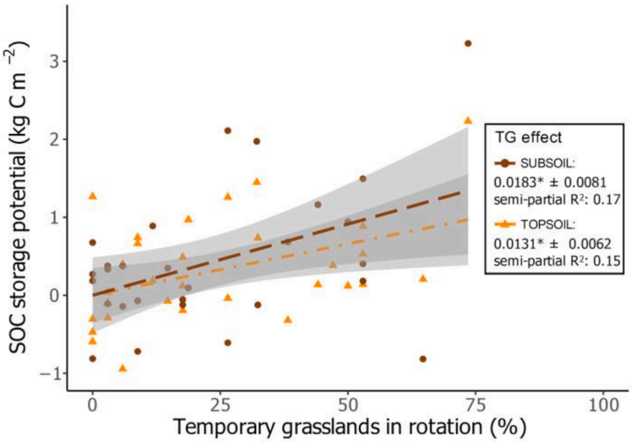

Fig. 3. Partial regression plot of the SOC storage potential induced by the introduction of temporary grasslands (TG) in the crop rotation in topsoil (0–20 cm, dots) and subsoil (20–50 cm, triangles) of croplands (CR) determined by LRS models. The coefficient (± standard error) and the semi-partial R^2 from the LRS models are included in the legend with stars indicating the level of significance. The effect of TG (solid lines) and its 95% confidence interval (shaded area) as illustrated in the figure corresponds to the linear regression of SOC stocks with TG proportion in topsoil (dot-dash) or subsoil (dashed). The level 0 kg C m^{-2} corresponds to the SOC stock for CR without TG having the average clay content and pH for the respective layer.

Table 3
Factors affecting SOC stocks (kg C m^{-2}) in all sites down to 50-cm depth, in cropland topsoil (0–20 cm) and subsoil (20–50 cm) separately as determined by linear multiple regression with stepwise selection (LRS).

	All sites 0–50 cm depth			Cropland topsoil			Cropland subsoil		
	Estimates	$R^{2\dagger}$	p-values	Estimate	R^2	p-values	Estimate	R^2	p-value
intercept$^{€}$	7.47 ± 0.51	0.74	< 0.001	4.19 ± 0.27	0.59	< 0.001	3.67 ± 0.32	0.50	< 0.001
land use	2.96 ± 0.77	0.26	< 0.001	NA	NA	NA	NA	NA	NA
TG proportion	0.037 ± 0.014	0.14	< 0.05	0.013 ± 0.006	0.15	< 0.05	0.018 ± 0.008	0.17	< 0.05
$\text{Log}_{10}\text{clay}^{¥}$	10.9 ± 1.99	0.41	< 0.001	5.41 ± 1.11	0.47	< 0.001	7.08 ± 1.56	0.46	< 0.001
$\text{pH}^{¥}$	−1.03 ± 0.35	0.16	< 0.01	−0.57 ± 0.22	0.20	< 0.05	−0.53 ± 0.26	0.15	< 0.05

increasing TG frequency remains an open question because our study compares stocks at equilibrium and not the rate of SOC accumulation. For croplands converted to grasslands it may take over a century to reach a new equilibrium, but the highest accumulation rates are expected to occur during the first decades (Poeplau et al., 2011; Smith, 2014). For example, by using the SOC accumulation rate determined by Launay et al. (2021) with a modelling approach (i.e., +0.0466 kg C m^{-2} on average over 30 years after the introduction of TG in the rotation), the additional SOC storage induced by a 50% increase of the proportion of TG in the rotation would be achieved in 39 years.

The hypothesis that permanent grasslands are the reference condition for maximal soil C storage in the current agricultural system is questioned by the fact that the expected SOC stocks under the 100% TG scenario appears to be higher than those currently present in permanent grasslands (Fig. 2a). The uncertainty of the model and the absence of sites with more than 73% of TG preclude the assessment of whether croplands can reach SOC stock levels similar to PG at TG proportion below 100% or that TG induces higher SOC stocks than permanent grasslands. Nonetheless, within the range of our study (0–73% TG), the relationships between SOC stocks and TG proportion in topsoil and subsoil appeared linear (Fig. 2a, Fig. 3). This is corroborated by an Australian experiment that found a doubling of SOC accrual rates with a doubling of TG proportion (Chan et al., 2011). Furthermore, the magnitude of the difference in SOC storage between crop-only sites (with no TG) and PG has increased in the last decades because about half of the permanent grasslands showed significant SOC gains in the last 30 years (Guillaume et al., 2021). Thus, the potential of croplands to store additional SOC may be slightly underestimated if the SOC dynamics observed in the last 3 decades will continue in the future.

In summary, despite the uncertainty in evaluating the maximal SOC storage for croplands with high proportion of TG, the study supports the concept that the agricultural system of the Fribourg Canton represents a cropland-grassland continuum, in which land-manager decisions to shift the land use in the direction of a crop-only or grass-only system by, respectively, reducing or extending TG will likely induce a proportional change of SOC storage.

4.2. Application of bulk density corrections

The effect of land use and TG on SOC stock changes were strong enough to be detected without applying corrections, even if the magnitude would have been underestimated by 7% and 16%, respectively (Table 2). The correction for stone content on SOC stocks within each land use was small (1%) and in line with another study that found little or no bias on SOC stock calculation with stone content below 5–10% (Poeplau et al., 2017). Nonetheless, when comparing PG and CR, the correction factor decreased the effects of land use and TG proportion on SOC stocks by 11% because the stone content was higher in PG than in CR (Table 1).

To avoid strong bias in cropland bulk density due to soil tillage, not all crop types can be sampled at the same period of the year, making almost impossible to avoid variations in sampling conditions, especially soil moisture content. Fortunately, soil moisture content had little predictive power for bulk density of fine soil when clay content and Corg were included in the model (Table S2). This supports the finding that while swelling and shrinkage may substantially affect bulk density for soils with high Corg or with a thick organic horizon like in forests, the soil bulk density of croplands and grasslands may not be affected substantially by variation in soil water content (Gubler et al., 2016). By contrast, the effect of land use, especially of Corg changes, on bulk density cannot be ignored, otherwise an important underestimation of the land-use effect on SOC will be obtained (von Haden et al., 2020; Wendt & Hauser, 2013).

Finally, it is important to highlight that the subsoil bulk densities were predicted with a mean average error of 5% using very few parameters, namely Corg and land use (Table S1). The application of the pedotransfer function may be an interesting alternative to estimate subsoil SOC stocks without investing substantial efforts into measuring fine soil density for the subsoil.

4.3. Relevance of SOC storage in climate mitigation

The technical C storage potential of agricultural soils in the cropland-grassland system of the study region (3.1 ± 1.4 Mt CO_2) corresponds roughly to one year of the total direct anthropogenic GHG emissions generated inside the region boundary and the indirect ones generated outside the region boundary (4.1 Mt CO_2-eq.), or to about 4 years of GHG emissions generated from the agricultural production inside the region (SEn, 2020). Such a comparison, however, remains uncertain given the dependency of the climatic effect of the different GHG's on the chosen time horizon (Lynch et al., 2021). Such technical potential corresponds to an upper boundary of C storage of agricultural soils in the region and thus provides an optimistic view of the potential for offsetting GHG emissions by soil C sequestration. However, all GHG emissions resulting from the efforts implemented to utilize the storage potential should also be accounted for in order to accurately determine the overall GHG balance. A study in France showed that area-related GHG emissions were actually reduced when temporary grasslands replaced croplands, mainly because of the decreased application of mineral fertilizers (Launay et al., 2021). On the other hand, the increase of cattle and the related GHG emissions that would be associated with a massive expansion of grasslands would likely offset a substantial part of the C sequestration (Hong et al., 2021). To avoid an increase of cattle, Launay et al. (2021) applied a much stricter scenario for France, in which TG only replaced maize for silage and, in sites already including TG in the rotation, the proportion was extended on average by +10%. As a result, the sequestration potential at national scale was lower for TG than for cover crops because of the smaller area that will be affected.

The linearity of the relationship of SOC stocks with TG proportion across the whole TG range means that soil C storage can be achieved anywhere with the same efficiency. Hence, the implementation of new TG or their spatial redistribution should be encouraged, especially where the level of organic matter is so low that soil functioning is impaired and soils are prone to degradation (Kuzyakov et al., 2020). Agricultural planning at landscape level could maximize the benefits of TG without affecting the overall agricultural production. Finally, because the proportion of temporary grasslands in the crop rotations is already high in the study region (one third on average), it can be foreseen that the potential of TG as negative C emission technology is much higher in areas where this practice is not yet as common (Schut et al., 2021).

5. Conclusions

We evaluated the potential for soil C storage in agricultural soils as a negative emission technology and found that soil C storage potential corresponds, roughly, to the total direct and indirect anthropogenic GHG emissions of the study region during one year only. The potential is far below the aspirational goal advocated by the "4 per mille" initiative. Nonetheless, at the field scale, SOC accrual can be much higher than 4 ‰ under some specific conditions, illustrating that agricultural systems still represent a potentially large C sink when agricultural practices are optimized. The estimation of the potential C sink by increasing the share of TG is affected by the soil bulk density. While bias resulting from different stone content and sampling conditions could be neglected, the correction of soil bulk density for land use, and especially for the increase of Corg, was necessary to avoid substantial underestimation of the C sink. Furthermore, the subsoils exhibit a substantial storage capacity that can be leveraged by managing the proportion of TG in the crop rotation. Reaching the upper limits of SOC storage implies a 100% shift from croplands to grasslands, which is neither realistic nor a desirable scenario. Hence, the increase of SOC storage would depend on the trade-offs between dietary needs and the environmental and economic constraints, most likely addressed at political level. Although the findings from this study seem to undermine some of the over-optimistic predictions about the role of agricultural soils to mitigate climate change, they highlight, at the same time, the importance of agricultural diversification to maintain or enhance the climate change adaptation capabilities and the ecosystem services of agroecosystems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2022.115937.

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