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Soil C dynamics after deforestation and subsequent conversion of arable cropland to grassland in humid temperate areas

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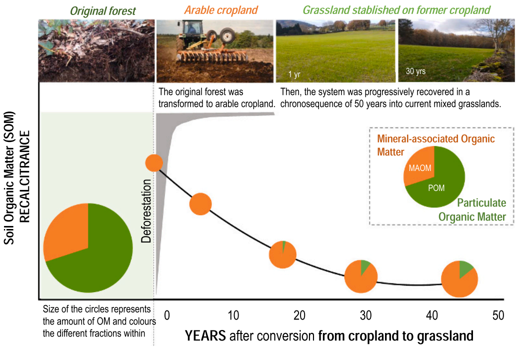
HIGHLIGHTS

- Low degree of physical protection explained large SOM losses after cultivation.
- Gradual gain in soil OM physical fractions during the restoration were monitored.
- Conversion of cropland to grassland led to replenishment of stabilized OM.
- Thermal analysis is useful complement to characterize the OM quality in different soil fractions.

GRAPHICAL ABSTRACT



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ABSTRACT

Land use and plant-soil management influence soil organic C stocks and soil properties. This study aimed to identify the main mechanisms by which these factors alter soil organic matter (SOM) dynamics and stocks. Changes in the organic C pools and biochemical quality in different OM compartments were assessed: a) after deforestation and intensive cultivation (SOM loss) and then, b) after the conversion of cropland to grassland (SOM replenishment) in a chronosequence of recovery (1–45 years). Topsoil samples were subjected to physical fractionation to assess the distribution of free particulate OM (POM) and mineral associated OM (MAOM). SOM quality was characterized by ^{13}C NMR spectroscopy, thermal analysis (DSC/TG), and microbial activity was monitored by isothermal microcalorimetry.

Deforestation and intensive cultivation led to the loss of 80 % of the C stored in the upper mineral soil (up to 30–35 cm). The POM was almost depleted, MAOM underwent significant losses (>40 %) and all OM compounds, including the aromatic C, were affected. The large and unexpected loss of MAOM can be attributed to the low specific surface soil area and also to the labile (biodegradable) nature of the OM in this fraction. After 45 years, conversion of cropland to grassland recovered 68 % of the C lost in the mineral soil (mainly as MAOM), at an annual rate of 1.25 Mg C ha^{-1}. The present findings showed that the persistence of long-term OM depends on

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how strongly organic compounds are adsorbed onto mineral surfaces (i.e., the specific surface area) and the biochemical nature of OM compounds. Adequate plant-soil management favoured the replenishment of the MAOM under these experimental conditions, and this fraction was an active pool in terms of C storage and biochemical quality. This study served to test current theories about changes in soil C fractions due to land use changes and soil-plant management.

1. Introduction

In most ecosystems, deforestation and intensive cultivation usually lead to important losses of soil organic carbon (SOC), degradation of soil and increased greenhouse gas emissions (Bennetzen et al., 2016; Gibbs and Salmon, 2015). This process can be reversed by the use of soil conservation techniques (Page et al., 2020) and by conversion of degraded land to forest and other, less intensive uses, such as grassland (Poeplau et al., 2011; Kurganova et al., 2019).

The impact of intensive cultivation on the amount and quality of the soil organic matter (SOM) and their replenishment by restoration techniques are governed by complex mechanisms of C sequestration and stabilization. Sequestration of SOC is determined by the composition of SOM and certain soil properties (texture, structure, pH, mineralogy, drainage), which regulate microbial activity and protect OM through organo-mineral interactions. Two main SOM functional fractions are usually distinguished: the particulate OM (POM) and the mineral associated OM (MAOM) (Schmidt et al., 2011; Cotrufo and Lavallee, 2022). The POM is made up of partly decomposed plant debris (similar to that in leaf litter), which is not adsorbed to soil minerals (Christensen, 2001). By contrast, the MAOM is thought to be made up of microbial by-products and even plant-derived compounds of low-molecular-weight adsorbed to mineral particles, mainly clay and silt (Zhang et al., 2022). Due to the physical protection, the MAOM is considered less bioavailable to decomposition by microorganisms, and it has a longer turnover time than the POM (residence time from decades to centuries; Six et al., 2002; Benbi et al., 2014). As the adsorption of OM to minerals is determined by the specific surface area of the soil, the pool of OM stored as MAOM is finite and is mainly determined by texture and mineralogy (Six et al., 2002; Lugato et al., 2021).

Knowledge of the OM distribution in POM and MAOM is important because it determines important factors such as the C sequestration capacity, the vulnerability of soil to OM loss in response to perturbations (deforestation, tillage, wildfire, drainage) and even soil conservation or plant nutrition (Lavallee et al., 2020). The potential distribution of SOM in POM and MAOM is primarily determined by the climate (which determines litter input and composition) and by pedogenic processes (which determine the mechanisms of OM protection) (Sokol et al., 2022). Land-soil management (tillage, artificial drainage, decreased plant biomass input and fertilization) usually has a negative influence on SOM content, but its effect is highly dependent on the distribution of SOM in the functional fractions.

Information about how SOC can be replenished in degraded soils by means of restoration strategies is even more scarce. Some strategies, including soil conservation practices (Hidalgo et al., 2019; Gao et al., 2019; Zhang et al., 2022), rewilding (Kurganova et al., 2019), conversion to grassland (Bai and Cotrufo, 2022, Baer et al., 2010; De et al., 2020) and reforestation (Poeplau et al., 2011; Pérez-Cruzado et al., 2014; England et al., 2016), have been shown to be effective for replenishing OM, but little is known regarding the distribution and quality of the sequestered OM. This information is important because the possible low rate of replacement of the MAOM may reduce the success of the restoration process (Tan et al., 2007; Ovsepyan et al., 2019).

Conversion of cropland to grassland (native or managed) and the inclusion of grassland areas in cropping systems is gaining attention as a strategy for restoring degraded cropland soils. Grasslands provide multiple ecosystem services, such as biodiversity, C sequestration, water regulation, water quality and soil conservation (Duchene et al., 2019; Zhao et al., 2020), and is considered a key strategy for mitigating global climate change via soil C sequestration (Paustian et al., 2016). As SOM plays a critical role in soil health and key environmental issues, different policies and strategies have been designed in the EU (Dupraz and Guyomard, 2019), USA (Morefield et al., 2016) and other regions (SANBI, 2020) to favour perennial grasslands and the inclusion of grasslands in cropping systems. In addition, about 220 million ha of land worldwide is classified as abandoned agricultural land, much of which evolves to grassland or scrubland (FAO, 2010).

The changes in OM that occur as a consequence of land use changes or plant-soil management are mainly determined by how strongly organic compounds are adsorbed onto mineral surfaces (i.e. specific surface area; Lavallee et al., 2020). However, current research shows that the biochemical qualities of both POM and MAOM also play a crucial role (Kleber et al., 2021; Giannetta et al., 2019). Although there remain many gaps in knowledge, it is thought that the organic composition of POM and MAOM varies widely in different type of soils as a result of the litter composition and different pedogenic processes, which affect microbial activity and therefore microbially synthesized metabolites (Kurganova et al., 2019). Some researchers have also reported changes in the composition of these fractions as a result of land use changes and plant-soil management (Boeni et al., 2014; Hidalgo et al., 2019; Kurganova et al., 2019; Zhang et al., 2022) and other major perturbations (Mastrolonardo et al., 2015; Merino et al., 2021).

Although the composition of the OM in the different functional fractions is known to play a role in C turnover and soil conservation, information on the topic remains limited, mainly due to technical limitations. This underlines the importance of identifying the most appropriate methods and strategies for the biochemical characterization of OM. Solid-state ^{13}C NMR and DRIFT spectroscopy have been used for this purpose (Boeni et al., 2014; Gao et al., 2019). However, when used individually, neither technique is able to characterize the SOM quality in terms of biological stabilization (Yeasmin et al., 2020). Sequential extraction procedures (Giannetta et al., 2019) and thermal analysis (Feng et al., 2014; Hidalgo et al., 2019; Giannetta et al., 2018; Merino et al., 2021) have been used to complement these instrumental techniques in order to overcome the above-mentioned limitations. Thermal analysis is based on the fact that the energy required for thermal oxidation is comparable to the energy required for SOM decomposition (Harvey et al., 2012; Siewert et al., 2012). It can thus be used to predict labile (biodegradable) and recalcitrant C pools (Kurganova et al., 2019) and microbial activity (Campo and Merino, 2016; Hidalgo et al., 2019; Kurganova et al., 2019).

In the present study, we use the term “biochemical quality” to indicate the combined information obtained from different techniques, including the chemical composition and biological stabilization and also involving the microbial decomposition of SOM. SOM stabilization must, by definition, be linked to biodegradability. Therefore, estimation of SOM chemical and thermal properties can be improved by using indices that parameterize the biodegradation rates. The most widely used method involves determining soil microbial respiration by measuring the rate of change in CO_2 concentrations. SOM biodegradability can also be determined by measuring the rate of CO_2 produced by soil microbial metabolism, by calorespirometry. This method is usually used for short term monitoring of soil respiration and metabolism, continuously and in real time (Barros et al., 2016; Chakrawal et al., 2020). It enables determination of the CO_2 from the heat dissipated by the reaction between the CO_2 and the NaOH inside the calorimeter. This has the advantage of measuring the heat and CO_2 produced by soil microbial metabolism, both which are involved in the bioenergetics of the soil system (Chakrawal et al., 2020).

The above information shows that although significant advances have been made in understanding the mechanisms of C sequestration and stabilization, there remain many uncertainties about how land use changes and plant-soil management affect the distribution and biochemical quality of SOM. Specifically, the changes in the mineral-associated OM after land perturbation or restoration are not well understood. The present study addresses specific questions regarding changes produced in the different fractions of the SOM by more (from forest to croplands) or less intensive changes (from cropland to grassland) in land use including a) how land use change affects the SOM replenishment rate and, b) what are the impacts on the quantitative and qualitatively composition of the different functional fractions of the SOM. Due to the slower C turnover of the MAOM, this fraction deserves special attention. Considering the limited information available on this topic, one of the aims of this study is to test the combination of different instrumental techniques that helps to overcome this constraint. The biochemical quality of the SOM was defined by the combined information obtained from ^{13}C NMR spectrometry and thermal analysis, which enabled characterization of the SOM in terms of both composition and biodegradability.

The study addresses specifically the replenishment of SOM as a consequence of the progressive conversion from cropland to grassland. To address this question, we took advantage of a uniform and extensive cropland which was gradually converted to grassland. In this land, a chronosequence (1–45 years) of 10 managed grassland plots established on former cropland was selected for study. Since this land is an example of a site where soil conservation measures were implemented by the national government in the 1980s to promote the recovery of degraded agricultural lands, it can serve as an example to assess the effect of these politics. The results (learnings) are thus applicable to other areas in which less intensive land uses are being implemented with the aim of restoring soil properties and increase C sequestration capacity.

2. Materials and methods

2.1. Study site

The study was carried out in the province of Lugo (NW Spain) (43° 01′49″N, −7° 59′09″W). The area is characterized by a highly fragmented mosaic territorial distribution, where peri-urban strips and scattered population centres are interspersed with small areas of land dedicated to livestock, forestry and small-scale rainfed agriculture, the economic pillars of the region. Despite the fragmented land use, the study area is homogeneous in terms of climate, parent material, soils and native vegetation (mesophytic deciduous forests dominated by *Quercus* spp.). The climate is subhumid Mediterranean, with a mean annual rainfall of 1300 mm, 60 % occurring between October and May. The mean annual temperature is 13 °C; the lowest mean temperature (5.8 °C) occurs in January, and the highest (18 °C) in August. The soil parent material is mica schist. Soils (Humic Cambisol; IUSS Working Group WRB, 2022) are of moderate depth (40–60 cm in depth) and of sandy loam to loamy sand texture and rapid-moderate hydraulic conductivity.

The A horizon has a low $pH_{(KCl)}$ (4.2), high organic matter (12 %) and intermediate C/N (15), effective cation exchange capacity (CEC) < 8 $cmol_{(+)}\ kg^{-1}$, Al saturation >80 % and low concentrations of available rock-derived nutrients (P, Ca, K and Mg). The soil moisture and temperature regimes can be described as Udic (mean period with partial drought, 2 months) and Mesic (mean frost-free period, 10 months) respectively.

2.2. Experimental design

The selected area (300 ha) is homogeneous in terms of topography, parent material, soil properties and microclimate. The experimental design included 4 fragments of semi-natural forest, 3 conventionally managed cropland plots and 13 mixed grassland plots. Cropland and grassland plots are part of a former 200 ha uniform cropland which was gradually converted to grassland. The four fragments of semi-natural forest (0.7–2 ha) were dominated by *Quercus robur* L. The cropland plots (*0.5–1 ha*) were previously cultivated with barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.) or corn (*Zea mays* L.), which have gradually been replaced with corn and occasionally turnip, *Brassica rapa* L. during the last three decades. The dominant method of post-harvest preparation of corn is silaging, which limits the input and supply of crop residues and thus of OM. The tillage depth of the cropland is 25–30 cm. Mineral fertilizers and lime are regularly applied. Liquid manure is usually applied once or twice a year. After harvesting (September), the soils usually lie fallow until the next spring (generally May), with no crop rotation or legume green manure. As a consequence, the soils suffer loss of large amounts of SOM as well as compaction and erosion.

The grassland plots (1–3 ha) were established on former cropland at different times, ranging from 6 to 50 years prior to the study. Grassland areas in the region are dominated by Yorkshire fog (*Holcus lanatus* L.), *Agrostis* sp., *Cynosurus cristatus* L. and *Plantago lanceolata* L., with a low abundance of legumes (*Trifolium* spp., *Lotus* spp., *Lupinum* spp.) and limited input of mineral fertilizers or liming. The main species used are Italian, hybrid and perennial ryegrasses (*Lolium perenne* L.) and white and red clover (*Trifolium repens* L. and *T. pratense* L., respectively). In these areas, root and shoot biomasses are 20–40 and 5–10 Mg ha^{-1}, respectively. The grassland plots are normally harvested to produce silage twice a year. Grass is not used for livestock grazing (or only occasionally). The grass is usually renewed every 8–10 years by rotovating the topsoil (0–20 cm). Liquid manure is applied regularly twice a year (10–20 $m^3\ ha^{-1}\ yr^{-1}$).

2.3. Soil sampling and analysis

Sample plots within the different three land use systems (i.e. semi-natural forest, cropland and grassland) were selected on the basis of availability, yielding a total of 4 semi-natural forest plots, 3 cropland plots and 13 grassland plots. In each plot, soil samples were collected within three 15 m × 20 m grids in April 2019. To ensure the pedological homogeneity between the sampling areas, these grids were selected after examining the soil with a hand auger. Topsoil (0–30 cm) was sampled for chemical and bulk density determinations at three different depths (0–5, 5–15 and 15–30 cm). For chemical analysis, 3 composite samples, each comprising three subsamples, were obtained for each soil depth per grid. Soil samples were mixed carefully, dried at room temperature and sieved (2 mm). Fine plant root and organic residues were removed by hand from the sieved soil samples.

2.3.1. Soil density fractionation

Soil samples were subjected to density fractionation (Golchin et al., 1994) to separate the free particulate OM (fPOM), occluded particulate OM (oPOM) and the mineral associated organic fraction (MAOM), as described by John et al. (2005). Briefly, 4 g of air-dried soil (<2 mm) was mixed in 20 mL of sodium polytungstate (SPT) solution (density $=$ 1.6 g cm^{-3}) and centrifuged at 5100 ×*g* (1 h). The pellet was then dispersed with 20 mL of the SPT by adding five glass beads of diameter of 3–5 mm to a tube containing the sample and shaking for 16 h (f $=$ 60 movements min^{-1}) to disperse the soil aggregates. The tube was again centrifuged at 5100 ×*g* (1 h). The supernatant with floating oPOM (density $<$ 1.6 g cm^{-3}) was vacuum filtered (0.45 μm) and washed. The pellet was then dispersed again by adding SPT solution of higher density (2 g cm^{-3}) and shaking for 10 min at 100 rpm and centrifuging at 4700 ×g (1 h). The supernatant with oPOM (density 1.6–2.0 g cm^{-3}) was vacuum filtered and washed.

2.3.2. Analysis of bulk soil samples and physical density fractions

Total C and N concentrations, pH, solid-state ^{13}C CP NMR spectroscopy, thermal analysis and microbial activity were analysed in the bulk soil samples. Total C and N concentrations and thermal analysis were also carried out in the three physical fractions.

The soil total C and N concentrations were determined in all soil depths using a LECO elemental analyzer (LECO Truspec Micro Elemental Analyzer CHNS, New York, NY, USA). Although SOC and total N pools were measured for the upper 30 cm in depth, the stocks referred to a specific depth are partially influenced by differences in bulk density of the soils under study. To prevent the interference of the bulk density on SOC and total N stocks, they were calculated on a fine mineral earth (FME) basis. We applied the "cumulative coordinates approach" proposed by Rovira et al. (2015). In this method, the amount of fine mineral earth was determined for each soil layer (0–5, 5–10, and 10–30 cm). In this study, we considered 300 kg m^{-2} of fine mineral earth for comparisons among the different sites (the average was 305 kg m^{-2} of FME down to 30 cm).

Thermal analysis (differential scanning calorimetry [DSC] and thermogravimetry [TG]) (DSC/TG) were used together with ^{13}C nuclear magnetic resonance (NMR) spectroscopy to assess OM biochemical quality in the uppermost soil layer (0–5 cm). In the present study, we use the term "biochemical quality" to indicate the combined information obtained from the different techniques, including the chemical composition and biological stabilization, and also the microbial decomposition of SOM. The DSC-TG analysis (Mettler Toledo Intl. Inc. TGA/DSC1, Zurich, Switzerland) was performed with 10 mg of soil (dry air at a flow rate of 2.1 kg cm^{-2} and a scanning rate of 10 °C min^{-1}). The temperature range was 50–600 °C. Two types of information can be obtained from the curves: the SOM content (from the area of the curves) and the SOM quality (from integration of different areas of the curves). Combustion heat (Q, in J per g) and SOM (SOM-LOI, in mg kg^{-1}) were measured by integration of the DSC and TG curves, respectively, over the exothermic region (150–600 °C). Weight losses and energy changes associated with moisture loss were excluded, as data recorded at <150 °C were not considered. The thermogravimetry curves were obtained by the first derivative of TG curves (DTG). Three groups representing different degrees of resistance to thermal oxidation were obtained from the DTG curves (Merino et al., 2014): (i) W_1 or labile OM, mainly carbohydrates and other aliphatic compounds (200–375 °C); (ii) W_2 or recalcitrant OM, i.e. lignin or other polyphenols (375–475 °C); and (iii) W_3 or highly recalcitrant OM, such as polycondensed aromatic forms (475–550 °C). The T_{50w}, i.e. the temperature at which 50 % mass loss of the SOM was released was also obtained.

To know the SOM composition, soil samples were analysed by solid-state ^{13}C CP NMR spectroscopy in one of the replicates of each selected land use type: one of the semi-natural forests, one of the arable lands, and 4 of the different grassland areas established at different times (6, 20, 30 and 50 years). The equipment (Agilent Varian VNMRS-500-WB spectrometer) was fitted with a zirconion rotor of volume 160 μL and operated at a proton resonance frequency of 500 MHz. Carbon chemical shifts were referenced to the C methylene signal of solid adamantane at 28.92 ppm. Cross-Polarization Magic Angle Spinning (1D CPMAS) analysis was carried out under the following conditions: contact time of 1 ms, inter-scan delay of 1 s (a proton T1 experiment was performed to check the suitability of the interval) and MAS rate of 12 kHz. The NMR spectra were processed using the MestReNova software 8.1.0 (Mestrelab Research Inc.) to quantify the area of the signals. For integration, each spectrum was divided into four regions representing different chemical environments of a ^{13}C nucleus: alkyl C (0–45 ppm), *O*-alkyl C (45–110 ppm), aromatic C (110–160 ppm) and carbonyl C (160–210 ppm). The contribution of the different C groups to total C was determined using MestReNova-7.0.3 (Mestrelab Research S.L., Santiago de Compostela, Spain). Aromaticity (aromatic C + alkyl C + *O*-alkyl C) and aliphaticity (alkyl C to *O*-alkyl C ratio) values were calculated as OM decomposition indices.

SOM decomposition was monitored using a TAM III calorespirometer (TA Instruments) with 4 mL stainless steel ampoules. Duplicates soil samples (1 g) at 60 % of their water holding capacity were placed in the ampoules with a vial containing 0.4 M NaOH for simultaneous measurement of the heat and the CO_2 rates associated with SOM microbial decomposition. The procedure is explained in detail elsewhere (Barros et al., 2016; Wadsö and Hansen, 2014). In brief, CO_2 rates are determined by the heat dissipated by the SOM microbial decomposition (μmol CO_2 g^{-1} h^{-1}) to facilitate comparisons between soil samples.

2.4. Statistical analysis

The arithmetic means and standard errors for different parameters were calculated. We examined the normality and heteroscedasticity of data. After that, the relationships between soil properties (total amount of OM, SOC, bulk density) were explored using the Pearson's correlation (F-test). Also, the relationship between the different parameters and the time elapsed during the chronosequence of ecosystem recovery (from degraded croplands to recovered grasslands) was studied through linear regression models that were applied to the dependent variables (SOC, bulk density, C:N, C_{org}, total N, T_{50w}, alkyl-C/O-alkyl ratio, aromaticity and RCO_2). The regression coefficients were estimated, the coefficient of determination (R_2) examined, and the significance of the model (r) represented. The significance of the regression coefficient ($p < 0.05$) was assessed analysis of variance (ANOVA). All statistical analyses were performed with STATISTICA 6 (StatSoft Inc., Tulsa, OK, USA) considering a significance level of $\alpha = 0.05$.

3. Results

3.1. Total SOC and N and soil C:N ratio (0–30 cm soil depth) in relation to land use change

The concentrations of SOC in the forest, cropland and grassland soils are shown in Fig. 1a. For grassland, the values were represented relative to the length of time since conversion of the cropland to grassland. The SOC and total N concentrations, and C/N ratios were higher in forest soils than in grassland and cropland soils (Fig. 1b). By contrast, the SOC, total N concentrations and C/N ratios were lowest in the intensively managed cropland soils. In the grassland chronosequence, SOC and N concentrations and C/N ratios increased with time. The rate of increase was highest in the uppermost soil layer (0–5 cm) and continued to increase after 45 years of grassland establishment from cropland. At greater depths, the rate slowed down 20 years after the change in land use.

In relation to the increases in SOC with the age of the grassland plots, soil bulk density decreased significantly, from 1.2 g cm^{-3} in the arable soils, to 0.90 g cm^{-3} in the longest-established grassland soils (Fig. 1c). Considering the whole set of samples, SOC concentration and bulk density were positively and significantly correlated ($r = 0.91$, $p < 0.05$).

As stated in the material and methods section, to prevent errors due to the differences in the bulk density, the SOC and total N stocks were not calculated for a fixed soil depth, and the values were expressed relative to 300 kg m^{-2} of fine mineral earth, for reliable comparison between the different sites (Fig. 2). Comparison of the SOC and total N pools in forest and cropland revealed that deforestation and cultivation led to loss of 9 kg C m^{-2} and 0.55 kg N m^{-2} (90 Mg C ha^{-1} and 5.5 Mg N ha^{-1}), relative to the semi-natural forests. When the forest floor was included in the calculation, an additional loss of 5 kg C m^{-2} (50 Mg C ha^{-1}) was observed. By contrast, the SOC and total N pools in the grassland increased with time after establishment. In accordance with these data, conversion of cropland to grassland implied replenishment of 7.5 kg C m^{-2} and 0.55 kg N m^{-2} (75 Mg C ha^{-1}, 5.5 Mg N ha^{-1}) in the mineral soil after a period of 50 years.

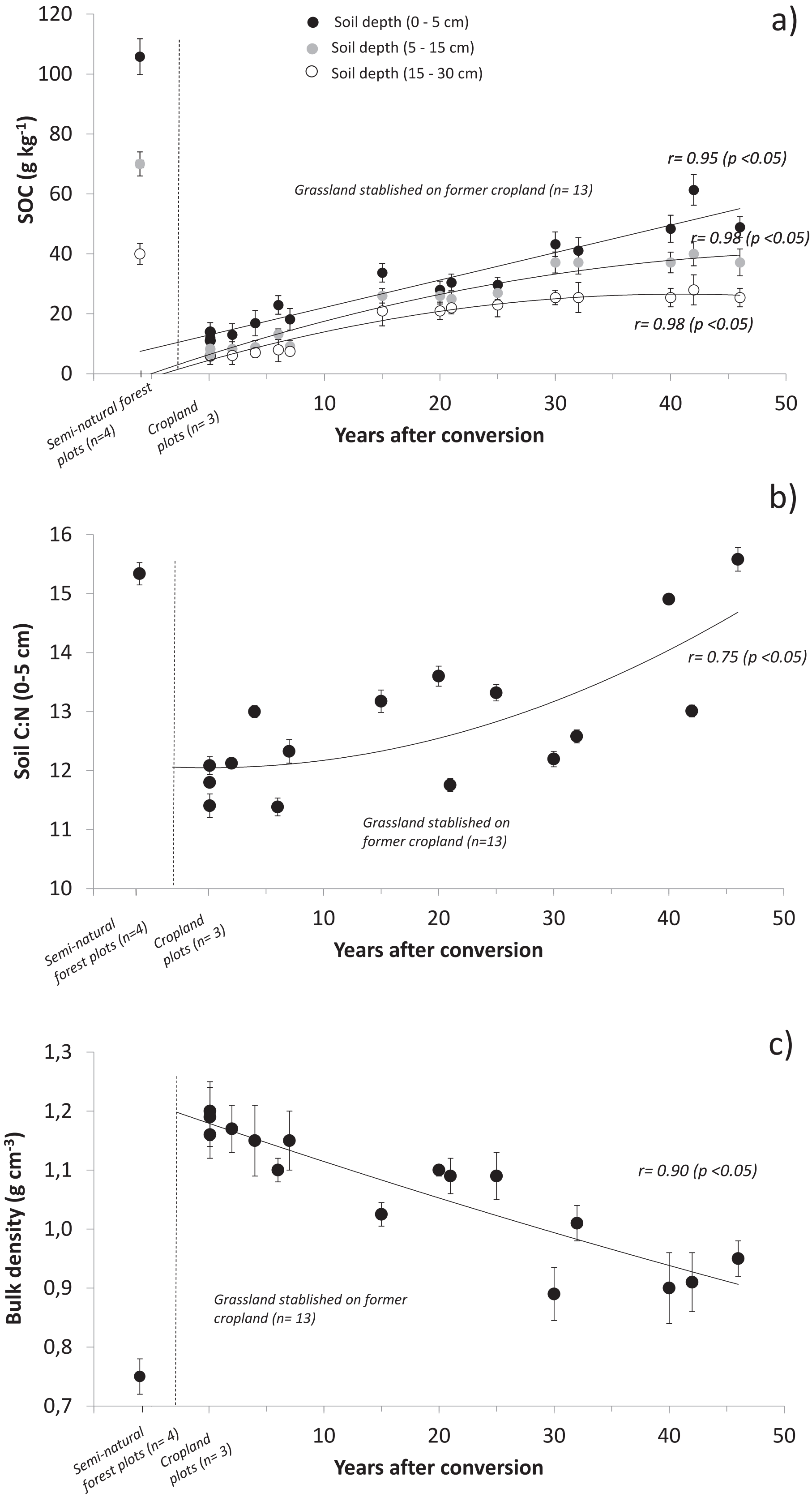

Fig. 1. Soil organic carbon (SOC), C/N ratios and bulk density in forest, croplands and grassland soils. For the grassland plots the values correspond to the length of time after conversion of cropland. SOC values are shown for the three depths (0–5, 5–15 and 15–30 cm), C/N ratios and bulk density values are shown for the uppermost mineral soil layer (0–5 cm) samples.

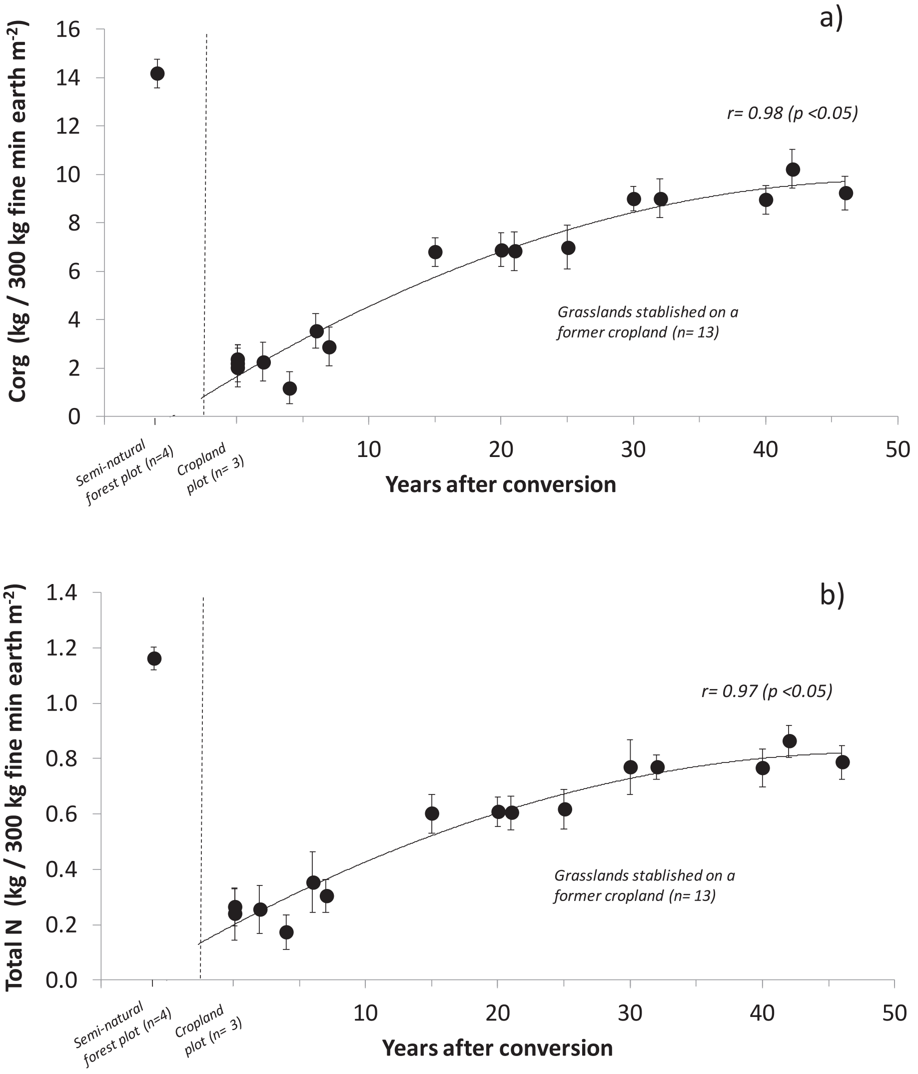

Fig. 2. Soil organic carbon (SOC) and total nitrogen (N) stocks in forests, croplands and grassland soils. For the grassland plots the values correspond to the length of time after conversion of cropland and are expressed relative to 300 kg m^{-2} of fine mineral earth (the average was 305 kg m^{-2} of FME to a depth of 30 cm).

3.2. *Effects of land use change on SOM biochemistry quality in the bulk soils*

The impact of land use changes on the SOM quality was investigated by thermal analysis (thermogravimetry) of the surface layer (0–5 cm soil depth), which was used in all studied plots. In addition, ^{13}C CP-MAS NMR spectroscopy was used to analyse the soils from the selected plots from different land uses (1 cropland, 1 grassland area established 6 years previously, 1 grassland established 30 years previously and 1 semi-natural forest, included for reference purposes). To complement this information, SOM decomposition was monitored by calorespirometry.

The derivative thermogravimetry (DTG) curves for representative samples are shown in Fig. 3a, and selected TG parameters are shown in Fig. 3b (SOC thermal fractions; W_1, W_2 and W_3) and 3c (T_{50w}; temperature at which 50 % of the total SOM is lost). The area under the curve corresponds to the total amount of OM (LOI), which was strongly correlated with the SOC content measured by a LECO autoanalyzer ($r = 0.99$, $p < 0.01$). The samples from the different types of land use (forest, cropland and grassland) showed notable differences in the shapes of the curves, suggesting significant changes in SOM quality. In the forest soils, the DTG curves shapes were characterized by a prominent peak at 300 °C and a secondary peak, a much less pronounced "shoulder" at 390 °C. In these soils, the most labile SOM fraction (W_1, weight lost at <375 °C, Fig. 3b) made up >60 % of the total SOM, whereas the recalcitrant fraction (W_2 and W3 weight lost at >375 °C, Fig. 3b) represented 25 %. The T_{50w} values in these soils were low, below 340 °C (Fig. 3c), reflecting a high proportion of SOM labile compounds.

The DTG curves corresponding to cropland and recently transformed grassland (<6 years) were smoother than those corresponding to the forest samples, with a very small peak of labile OM (<300 °C, Fig. 3a). In the cropland and recently transformed grassland soils, W_1 represented <50 % of the total SOM. The T_{50w} index values for croplands and young grasslands were significantly higher (370–390 °C) than for forestland, reflecting the lower proportion of labile OM (Fig. 3c). After conversion of cropland to grassland, the thermolabile fraction increased to approximately 60 % of the total SOM after 40 years. Most of the 80 % of the SOM gains in the newly established grasslands were due to thermolabile compounds (W_1), and the increase in recalcitrant OM (W2 and W3, Fig. 3b) was much lower. The T_{50w} increased from 350 to 360 °C in the recently established grasslands (similar to the current cropland areas) to 335–350 °C (similar to those in forest soils).

The ^{13}C CP-MAS NMR spectra of the mineral soil for the 5 selected representative study sites are shown in Fig. 4. The C distribution of various chemical-shift regions for the different plots studied are shown in Fig. 5a. *O*-alkyl C was the major functional group in the forest soils, representing 45 % of the total SOM. These samples were characterized

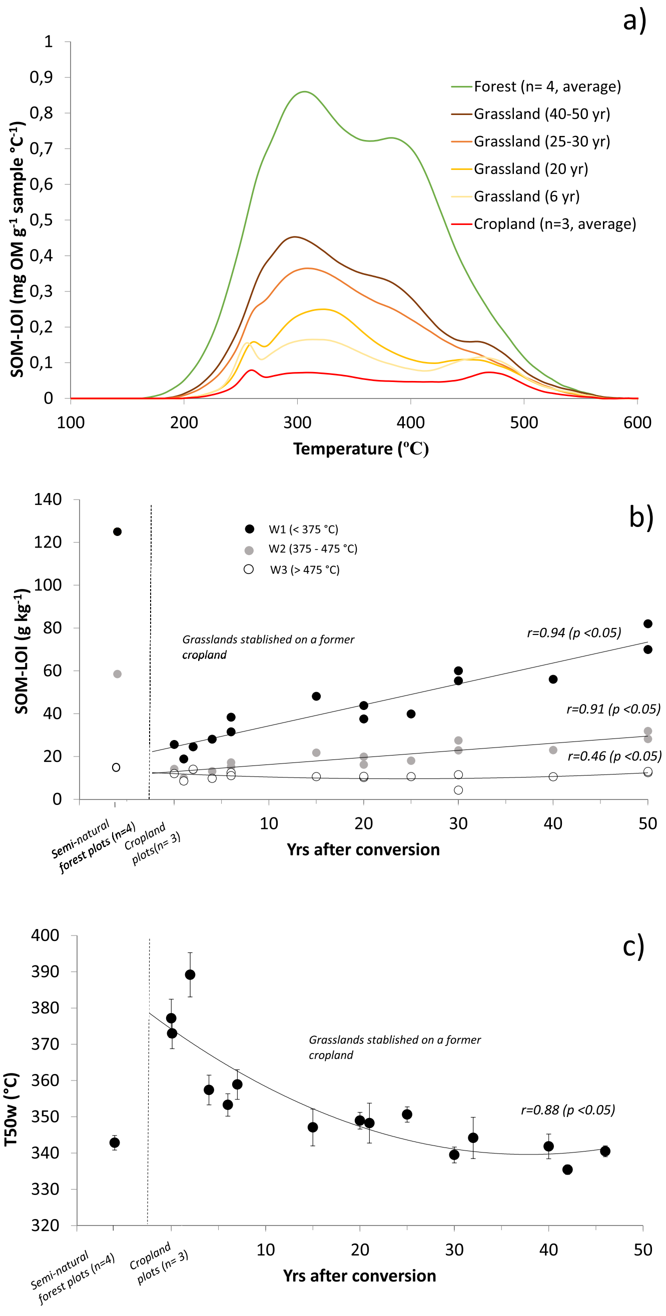

Fig. 3. Results of the thermogravimetry analysis of the uppermost mineral soil layer (0–5 cm) samples in forest, cropland and grassland soils. For the grassland plots, the values correspond to the length of time after conversion of the cropland. a) Derivative Thermogravimetry (DTG) curves (representative samples are shown as examples), b) distribution of the SOC thermal fractions; W_1, W_2, and W_3 are the SOC weight loss at <375 °C (labile), 375–475 °C (recalcitrant) and 475–600 °C (refractory C), respectively and c) changes in the T_{50w} (temperature at which 50 % of the weight is lost) for the different types of land use.

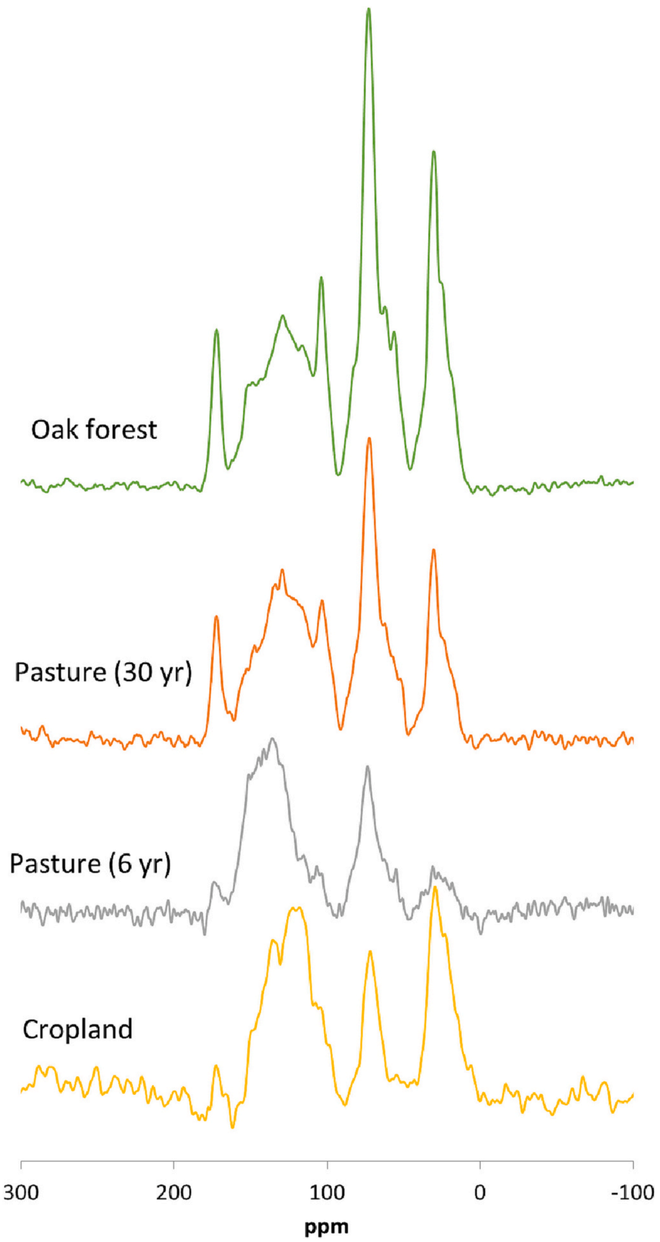

Fig. 4. Solid-state ^{13}C CP NMR spectroscopy spectra of the uppermost mineral soils (0–5 cm) samples from cropland, grassland established 6 years previously, grassland established 30 years previously and semi-natural forest (included for reference purposes).

by low aromaticity and high aliphaticity, in accordance with the higher proportion of labile OM detected in the thermal analysis (W_1 > 60 %; Fig. 3b). In this area, the signals at 73 and 105 ppm are attributed to the presence of celluloses and hemicelluloses respectively. Shoulders at 55 ppm, attributed to *O*-alkyl groups such as methoxyls in lignin-like structures, and at 63 ppm, due to carbon 6 in cellulose and amorphous cellulose, were also observed. The alkyl C region (30 ppm) was the second most abundant functional group (21 %). This peak was assigned to methylene C from lipids and aliphatic biopolymers, such as cutin and suberin. In the aromatic C region (27 % of the SOM), a prominent peak at 129 ppm, attributed to lignin, was also evident. In this area, the peaks at 131 and 155 ppm correspond to condensed tannins and total phenolics respectively.

Unlike the forest soil, the SOM in the croplands and in the novel grasslands was characterized by high aromatic C signals (>60 %) and very low amounts of *O*-alkyl C (<30 %). This was reflected by high thermolability (T_{50}w > 370 °C; Fig. 3c). Conversion of cropland to grassland led to a gradual increase in alkyl and *O*-alkyl signals (Fig. 5a), parallel with the decreased aromaticity. This coincided with increases in the thermolabile SOM, W_1 (r = 0.92, $P < 0.05$; Fig. 3b). The oldest grasslands showed high *O*-Alkyl signals and lower aromaticity, although the values were again different from those corresponding to the forests.

The CO_2 production rates associated with microbial decomposition

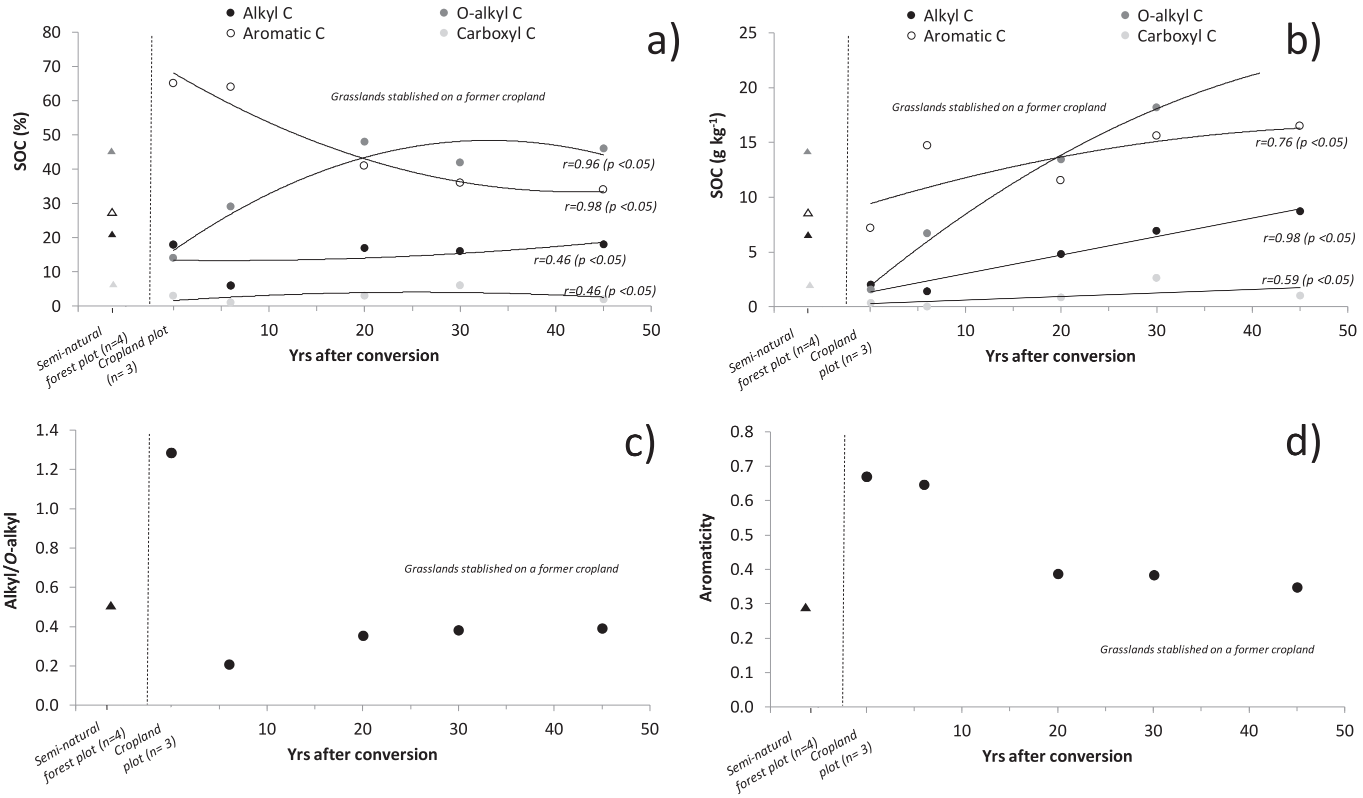

Fig. 5. Parameters obtained in the solid-state ^{13}C CPMAS spectroscopy analyses of the uppermost mineral soil layer (0–5 cm) samples from selected plots from in forests, croplands and grassland soils. For the grassland plots, the values correspond to the time after conversion of cropland. a) Relative intensities of different signals, b) absolute soil organic carbon concentrations relative to the soil organic carbon content and changes in c) aliphaticity (alkyl-C/*O*-alkyl), and d) aromaticity (aromatic C + alkyl C + O-alkyl C) for the different types of land use.

of the SOM are shown in Fig. 6. The rates (indicting soil respiration) were highest in the forest plots and lowest in the cropland plots. Thus, conversion to grasslands led to increases in the rate of SOM biodecomposition. In all grassland samples, the soil respiration values were lower than in the forest soil sample. Conversion to grassland initially increased respiration, up to 20 years, and thereafter remained stable or decreased between 25 and 45 years after the land use change.

3.3. *Distribution of SOM contents among density fractions*

SOM physical fractionation was carried out in the same samples (0–5 cm soil depth) that were analysed by ^{13}C NMR spectrometry (forest soil,

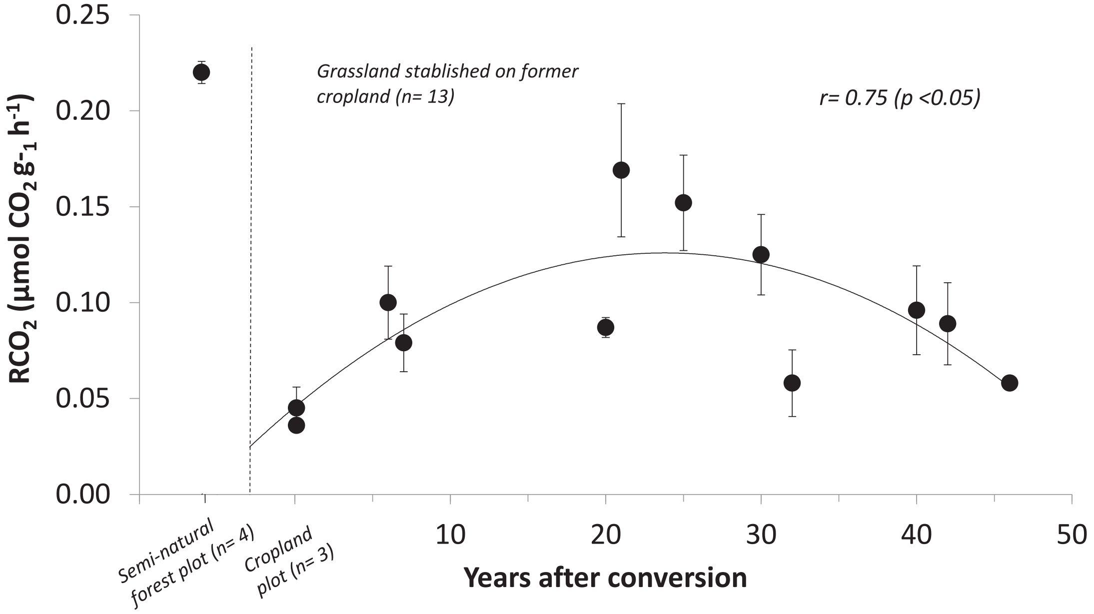

Fig. 6. Plot of the CO$_2$ rate (RCO$_2$) given in micro mol of CO$_2$ per gram of soil and hour (µmol CO$_2$ g^{-1} h^{-1}) from the soil samples, showing the evolution of the soil respiration in the grasslands after land use change, together with respiration rates in the forest plot and croplands.

cropland and grasslands established 6, 20, 30 and 50 years previously). The SOM contents in each fraction were estimated as the mass lost in the thermal analysis (<600 °C, LOI). The SOM distribution among physical fractions (fPOM, oPOM and MAOM) is shown in Fig. 7a. In the forest soil, the SOM fractions were distributed in the following order: unprotected free light fraction fPOM (50 % of the total SOM) > MAOM (40 %) > oPOM (10 %). Comparison of the forest and cropland soils showed that deforestation led to large losses in the three fractions: fPOM (95 %), oPOM (86 %) and MAOM (45 %). As a result, most of the SOM in the croplands was present as MAOM (>77 %).

Conversion of cropland to grassland led to gradual increases in the MAOM, which increased up to 30 years after conversion, reaching values of around 70 mg g^{-1}. Increases in fPOM and oPOM were only found after 30 years, and maximum values were much lower than the fPOM (Fig. 6a). After 45 years, 70 % of the OM was made up by MAOM.

3.4. Changes in SOM quality in the physical fractions induced by land use change

Regardless of the type of land use (forest, cropland or grassland), the abundance of the OM in the three density fractions (fPOM, oPOM and MAF) decreased in the following order: thermolabile fraction (W_1 < 375 °C) > slightly recalcitrant OM (W_2, 375–475 °C) > highly recalcitrant OM (W_3, 475–550 °C). The changes in T_{50w} (Fig. 7b) revealed that deforestation led to important increases in this value within the three physical fractions, mainly reflecting loss of W_1 and W_2. On the contrary, conversion of cropland to grassland implied large decreases in the T_{50w} in the three fractions. In the oldest grasslands (45 years), the T_{50w} values of the MAOM fraction were significantly lower with respect fPOM and oPOM.

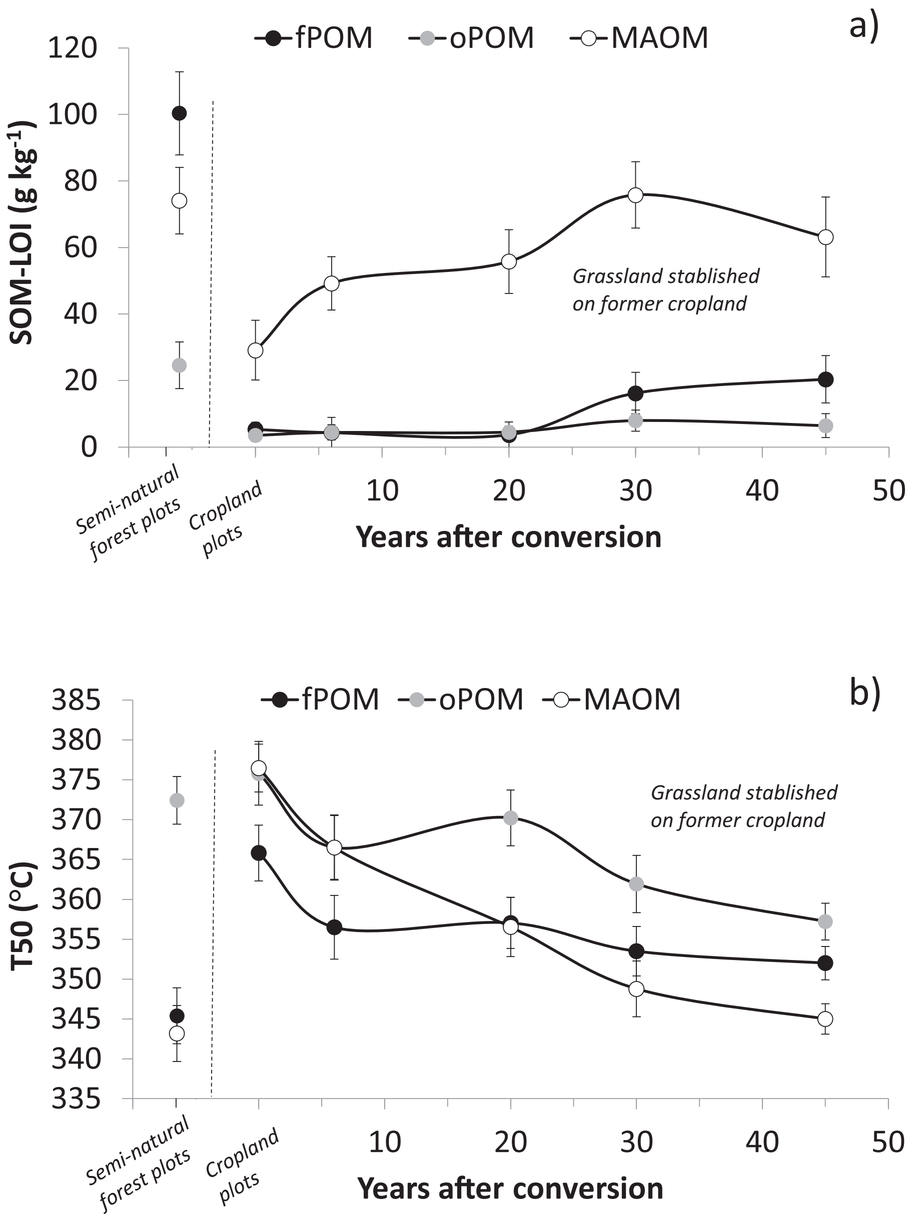

Fig. 7. a) Changes in the soil organic matter (calculated as weight loss in the TGA) pools and b) T_{50w} indexes in the different physical fractionations of the mineral topsoil (0–5 cm in depth) in forest, cropland, and grassland soils. fPOM: free particulate organic matter; oPOM: occluded particulate organic matter; MAOM: mineral associated organic matter fraction.

4. Discussion

This study assessed the mechanisms underlying the alterations in C dynamics after land use changes and plant-soil management. The experimental design, based on a uniform area subjected to historical changes in land use, together with the combination of advanced techniques and specific soil analysis, allowed us to evaluate the impact of deforestation and intensive cultivation, and, complementary, the effectiveness of restoring degraded cropland by conversion to grassland.

The amounts of C stored were calculated for soil plots showing a with a variable bulk density values (from 0.8 in forest soils to 1.3 g cm^{-1} in croplands). In order to calculate the changes in SOC, the SOC pools were therefore determined using an equivalent-mass approach, rather than being expressed for a fixed depth (Rovira et al., 2015, 2022).

4.1. Distribution of SOM physical fractions in the forest soils

In the forest soil, the high SOC contents in the upper 30–35 cm soil depth (expressed relative to 300 kg m^{-2} of fine mineral earth; 14 kg C m^{-2}) reflected the remarkable potential of these humid temperate soils to sequester C. A high proportion (70 %) of the SOM occurred as POM. The OM occluded in aggregates (oPOM, Fig. 6a) only made up 10 % of POM, possibly due to the low soil structural stability as a consequence of the sandy texture and low mineral charge. Only 30 % of the SOM occurred as mineral-associated OM (MAOM). Together these data show that most SOM in this soil is not protected by mineral adsorption and may therefore be vulnerable to decomposition (Lavallee et al., 2020).

Large pools of unprotected POM have been observed in other humid temperate forest soils (Sokol et al., 2022; Merino et al., 2021). The proportion of POM found in the present study is higher than usually reported in Mediterranean (Mastrolonardo et al., 2015) and tropical (Boeni et al., 2014) soils. However, it is lower than reported in colder areas (Mueller et al., 2015) and in areas that are frequently waterlogged (moors, heathlands), where POM accumulates due to strong microbial inhibition (Lugato et al., 2021).

The distribution of the OM in the physical fractions of the forest soil under study (high POM and low MAOM) may be related to the high inputs of plant OM due to the mild climatic conditions, along with the limited capacity of the mineral fraction to adsorb organic compounds. In these forests, the annual litterfall (6 and 11 Mg·ha^{-1}) and the forest floor (around 50 Mg C ha^{-1}) (Balboa-Murias et al., 2006) are much higher than in Mediterranean regions (Rapp et al., 1999). Regarding the capacity of the mineral fraction to adsorb organic compounds, in these soils, the clay content is <15 %, and the minerals are mainly 1:1 type and Al- and Fe oxides (Carballas et al., 2016). These data indicate a low specific surface area and CEC. According to Six et al. (2002), the protective capacity of the soils under study, based on the clay and soil contents, would range from 78 to 100 g silt + clay C kg^{-1} soil (Pérez-Cruzado et al., 2014). In accordance with these calculations, in this specific soil, SOC contents higher than 60 g C kg^{-1} would be close to or exceed the soil C saturation. These findings are consistent with those reported for temperate climate grasslands (Bai and Cotrufo, 2022). In addition to the large C inputs and low capacity of the mineral fraction to adsorb organic compounds, the strong acidity of the soil ($pH < 4.5$) could also favour a large POM pool. This parameter has a double effect through inhibition of microbial activity, thus preventing the decomposition of POM, and also by its effect on the reduction of the negative mineral charge and storage of MAOM (Yu et al., 2022).

4.2. SOM dynamics after deforestation and agricultural management

The SOC stocks in the intensively managed croplands amounted to around 2 kg C m^{-2}. The difference relative to the forest soil represents C losses of up to 80 % (11 kg C m^{-2}), indicating that these soils are highly susceptible to SOM loss after deforestation and cultivation. This process particularly affected the POM pool. Thus, the unprotected fPOM was almost depleted. Although the oPOM has a certain degree of protection, losses of this fraction were also considerable, possibly because the breakdown of aggregates during tillage could make available this occluded C available to microorganisms (Pikul et al., 2007; Jin et al., 2021). In addition to the lack of physical protection, most of the POM is made up by chemical compounds that are very susceptible to microbial activity (Von Lützow et al., 2006; Tan et al., 2007). The physical-chemical analysis (NMR spectroscopy analysis and the thermal analysis) revealed low alky/*O*-alkyl ratio values (around 2.0) and low T_{50w} values (around 340 °C) in these forest soils. Previous studies have shown that both of these indices indicate a high potential for microbial activity (Kurganova et al., 2019; Campo and Merino, 2016; Siewert et al., 2012; Stone and Plante, 2015).

Despite the assumed physic-chemical protection of SOM (Von Lützow et al., 2006), the present data also showed large losses of MAOM, e.g. of up to 40 % in the cropland soils relative to the original forest soils. Two factors working together may explain this important loss of mineral-protected OM. Firstly, mineral binding may be weak due to the sandy texture and the low charge of the clay minerals, as shown in other studies (Rasmussen et al., 2018; Giannetta et al., 2019). In addition, the thermal analysis revealed that the SOM in this fraction was mainly made up of thermolabile compounds (low T_{50w} values, around of 345 °C, Fig. 7d) and was therefore susceptible to decomposition. Other studies have also suggested that part of the MAOM is labile and subjected to relatively fast turnover (Giannetta et al., 2019; Yu et al., 2022).

In this regard, cultivation led to the depletion of *O*-alkyl compounds, which are considered highly available by microorganisms (Baldock and Skjemstad, 2000). These findings explain the low microbial activity in these soils indicated by microcalorimetry results (Fig. 6). Despite of the preferential loss of most labile OM compounds, the present study also revealed a significant loss of the aromatic C in this physical protected fraction. A previous study carried out in the same region (Merino et al., 2016) showed that even the recalcitrant pyrogenic aromatic C can be used by microorganisms, and the metabolism patterns revealed that the microorganisms adapted to use this aromatic-C as a C source.

4.3. C dynamics in the mixed grassland established on former cropland

4.3.1. Changes in the SOM pools in the functional fractions

Conversion of cropland to grassland led to the partial replenishment of SOM in terms of stocks and biogeochemical quality. After 45 years, the SOC sequestered amounted to around to 7.5 kg C m^{-2} for a depth of 30–35 cm (corresponding to 300 kg m^{-2} of fine mineral earth), which made up 68 % of the C lost in the mineral soil as a consequence of deforestation and cultivation. The study showed an average increase in SOC of 0.135 kg C m^{-2} yr^{-1} (1.35 Mg C ha^{-1} yr^{-1}), which is similar to that reported by Kauer et al. (2021; 1.26 Mg C ha^{-1} yr^{-1}) after the application of winter crops and composted manure to restore degraded cropland in Estonia. However, the rate is higher than reported in other areas, i.e. increases between 0.105 and 1 Mg C·ha^{-1}·yr^{-1} after conversion of cropland to grassland for a range of climates, managements and types of soils (Conant et al., 2017; Baer et al., 2010; Deng et al., 2014). The rate is also higher than observed in restored cropland after passive rewilding in continental areas of Russia (1 Mg C·ha^{-1}·yr^{-1}; Kurganova et al., 2014). Other studies, however, showed that after 40 years the conversion of cropland to grassland had little effect on C sequestration, possibly because the soils were already rich in SOM (De et al., 2020). The present study also showed faster increases in C at the early stages of cropland conversion, which is consistent with the trend observed by Hernández et al. (2013) in an area with a similar climate.

Most of the C sequestered in the newly established grassland was present as mineral-associated OM. Significant increases in POM were only recorded after 30 years, coinciding with the moment at which the MAOM stopped increasing. This pattern is consistent with the idea that once the OM reaches maximum levels in the soil, any new C input may be stored as POM (Six et al., 2002). The rapid replenishment of the

MAOM recorded in the present study could occur as the combined result of the following factors:

a) Increased production of easily decomposable litter. The mild climate along with the soil conservation practices (sowing legumes and application of slurry) probably determined the large inputs of C via decomposable herbaceous root litter (20–40 Mg ha^{-1}). Furthermore, the N inputs by slurry (1 Mg C ha^{-1} yr^{-1}) and N fixation by legumes together increase soil N inputs, stimulating the primary productivity of the grass and thus the production of root biomass and root exudates (Roa-Fuentes et al., 2015; Conant et al., 2017; Wu et al., 2017). Management of the grassland plots without livestock grazing (they are usually harvested for silage) is another factor leading to higher grass production. Some studies show that grazing generally reduces grass productivity (Byrnes et al., 2018), influencing the SOC stocks (Eze et al., 2018). Root litter inputs of up to around 0.95 Mg C ha^{-1} yr^{-1} have been reported for similar type of management (Guo and Gifford, 2002).
b) High microbial C efficiency. The findings showed increased microbial activity in grassland soils throughout the chronosequence, which could have promoted the rapid generation of microbial by-products able to be adsorbed to mineral particles (Cotrufo and Lavallee, 2022). In these mixed grasslands, the herbaceous root litter and the application of slurry implied a direct source of high-quality organic residues, very rich in highly available carbohydrates and N. Several studies have shown that these types of organic compounds support the microbial C efficiency and the build-up of mineral-associated OM, while also preventing OM accumulation in the POM (Córdova et al., 2018; Cotrufo and Lavallee, 2022; Wu et al., 2017).
c) Efficient protection exerted by the organo-mineral interaction and aggregation. The C sequestration potential in the soil is determined by the specific surface area and, in that sense, clay soils are able to restore greater amounts of SOM in a relatively short time (Baer et al., 2010). Despite the low specific surface area, the soils showed a high capacity for SOC replenishment. The rapid increase in MAOM, especially at the early stage of restoration, can be partly attributed to the initial low amount of OM in the cropland soils. Some studies have shown that soils far below their specific SOC storage capacity retain new C more efficiently, due to the larger number of reactive sites available for the OM sorption (Wiesmeier et al., 2014; Wu et al., 2017). In addition, in the grassland areas the lack of soil disturbance, the long root system and fibrous root architecture of the gramineous species favour soil aggregation and the formation of organo-mineral complexes (Hidalgo et al., 2019).

4.3.2. *Changes in the biochemical quality of the SOM*

Throughout the chronosequence, the most evident changes were the gradual increases in *O*-alkyl and alkyl C and to a lesser extent, aromatic C (Fig. 5). In the first 30 years after conversion, a gradual decrease in thermorecalcitrance was observed in the bulk samples and in the three physical OM fractions (fPOM, oPOM and MAOM). This effect would explain the increased soil respiration (Fig. 6). The lower respiration values found in the croplands could be due the lower POM and also the higher bulk density in the grassland, which surely prevented the microbial activity compared to the forest. Previous studies involving conversion of cropland to grassland and reforestation of cropland in the study region (Merino et al., 2004) reported increased microbial activity and also important changes in the patterns of GHG flux. The observed changes in this study (lower respiration and lower POM) indicate that soil microbial activity was partly restored, also suggesting a different pattern of microbial community development as a consequence of soil restoration.

In the MAOM, this trend can be attributed to metabolites of microbial origin (Baldock and Skjemstad, 2000) and also to plant-derived compounds of low molecular weight, which would be stabilized by adsorption on mineral surfaces (Yu et al., 2022; Wu et al., 2017). As POM quality is mainly determined by plant litter quality, the decreasing thermolability of this fraction would reflect the increased litter input by rhizodeposition and decomposition of roots (which are very rich in *O*-alkyl C) over time. In the longest-established grassland plots in the chronosequence, the T50w values decreased in the order oPOM > fPOM > MAOM, which coincides with the decreasing trend in plant-derived biomarkers (LFA, cutin, suberin and lignin) observed for these fractions by Zhang et al. (2022).

Although data obtained in the present study show a gain in labile OM compounds, different C dynamics may occur under other climatic conditions, soil types, land uses and soil management. In a study carried out in tropical Ferrasols, Boeni et al. (2014) observed higher amounts of *O*-Alkyl in the SOM of permanent pastures than in croplands. Kurganova et al. (2019) found decreased SOM recalcitrance after rewilding of cropland soils in Russia. However, in a study of Vertisols in a dry tropical region, Hidalgo et al. (2019) observed increased SOM recalcitrance after long-term application of soil conservation techniques; the increase was attributed to decreased microbial activity caused by lower aeration. Greater recalcitrance of SOM has also been observed in grassland areas reforested with pines and was attributed to the input of woody litter (Pérez-Cruzado et al., 2014). The different C dynamics observed across different environments and conditions show that SOC sequestration and biochemical quality are determined by multiple factors throughout the restoration process, including climate, biochemical composition of above−/belowground litter input, pedogenic processes, soil properties, initial low amount of SOM and plant-soil management. All of these factors exert a strong influence on microbial activity and microbial by-products, determining the rate of replenishment and the final quality of the SOM.

5. Conclusions

The experimental design of the present study allowed us, on the one hand, to determine how deforestation and intensive cultivation alter the different SOM compartments, in terms of OM pools and biochemical quality. On the other hand, the study assessed the efficiency of restoration of cropland soil by conversion to perennial managed grassland in relation to replenishment of SOM pools and quality.

The study revealed that the low degree of SOM physical protection (due to a low specific surface area) and the high SOM lability (biodegradability) in these soils explained the large SOM losses observed after deforestation and intensive cultivation. Both factors determined the depletion of the POM and substantial loss of the mineral-associated OM (MAOM). Large losses of aromatic C were also observed, due to the lack of physical protection.

Conversion of cropland to managed grassland, by incorporating soil conservation techniques, implied an annual rate of increase of 1.25 Mg C ha^{-1} yr^{-1} in the soil, most as MAOM. This high rate of replenishment was partly due to low initial SOM content. In addition, the plant-soil management and the mild climatic conditions increased decomposable root litter inputs rich in N, generating a large supply of microbial by-products able to be adsorbed by the minerals. Although most of the OM increases were due to increases in labile C, the protection due to mineral adsorption (most of the SOM compounds occurred as MAOM), reduced tillage and soil acidification could have prevented subsequent mineralization.

These findings highlight the benefits of establishing grasslands as a way of restoring degraded cropland through replenishment of OM and re-establishment of related properties Although this study focused on permanent grasslands, strategies such as establishing silvopastoral systems or including grassland areas in cropping systems may also be useful for recovering cropland soils degraded as a result of intensive management. These measures would contribute to recovering environmental benefits and therefore to contribute to designing a sustainable production model of food production.

The present study also showed that ^{13}C NMR spectroscopy and thermal analysis, applied in soil bulk soil samples and in physical fractions, can be used together to characterize the OM biochemical quality. This analytical approach provides valuable information about the mineral protection, chemical composition and biological stabilization of the SOM, which determine the C turnover in managed ecosystems.

To our knowledge, this is the first study to describe the gradual changes in soil functional OM fractions, considering pools and biochemical quality, in a degraded soil restored by a change in land use. This experimental design along with the analytical plan provided a unique opportunity to test and complement current theories in order to help understand the changes in OM that occur in soil as a consequence of land use changes and soil-plant management.

CRediT authorship contribution statement

AM obtained project funds, AM, BO, JC conceived the original idea of the paper and the experimental design. AM, BO, JC collected field samples. BO, VF, NB carried out the analytical analyses. AM, BO, PSA, NB critically reviewed the data and performed statistical analysis. AM, PSA discussed and developed manuscript structure and prepared a first version. All authors contributed to manuscript revision, read, and approved the submitted version.

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.

Data availability

Data will be made available on request.

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