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## Research Article

# Effect of Continuous Agriculture of Grassland Soils of the Argentine Rolling Pampa on Soil Organic Carbon and Nitrogen

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Long-term soil organic carbon (SOC) and soil organic nitrogen (SON) following cultivation of grassland soils (100/120-year tillage (T) + 20/30-year no tillage (NT)) of the Rolling Pampa were studied calibrating the simple AMG model coupled with the natural <sup>13</sup>C abundance measurements issued from long-term experiments and validating it on a data set obtained by a farmer survey and by long-term NT experiments. The multisite survey and NT trials permitted coverage of the history of the 140 years with agriculture. The decrease in SOC and SON storage that occurred during the first twenty years by a loss through biological activity was 27% for SOC and 32% for SON. The calibrated model described the SOC storage evolution very well and permitted an accurate simultaneous estimation of their three parameters. The validated model simulated well SOC and SON evolution. Overall, the results analyzed separately for the T and NT period indicated that the active pool has a rapid turnover (MRT ~9 and 13 years, resp.) which represents 50% of SOC in the native prairie soil and 20% of SOC at equilibrium after NT period. NT implementation on soils with the highest soil organic matter reserves will continue to decrease (17%) for three decades later under current annual addition.

## 1. Introduction

It is well established that grassland soils, particularly Molisols, originally rich in soil organic matter (SOM), rapidly lose important quantities of carbon (C) and nitrogen (N) after cultivation [1–10]. Long-term cultivation effects on soil organic carbon (SOC) and soil organic nitrogen (SON) provide necessary information to evaluate the sustainability of cropping systems and their effects on the environment. Assessment of SOM is a valuable step towards identifying the overall quality of a soil [11–13].

The agriculture of the Argentine Rolling Pampa consists of a sequence of arable crops for 100 to 120 years followed by two or three decades of cropping under no tillage (NT). Before the 1970s, maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and flax (*Linum usitatissimum* L.) were alternated with pastures for beef production. Since the 1970s, largely due to economic reasons, there has been an important increase in the area under arable crops, with the cropped area increasing relative to the pasture area at an annual rate

of 4% [14]. This resulted in an increase in tillage intensities. Furthermore, soybean was often double cropped with wheat (W/S) in the same year. Fertilizer use was relatively restricted until 1992 ( $<5 \text{ kg N ha}^{-1} \text{ año}^{-1}$ ) [7, 15–17], and liming is not practiced by farmers. Conservation tillage, based on chisel plow as primary tillage and no-tillage (NT) practices, was first introduced in the middle of the 1970s to provide several environmental benefits such as reduction of soil erosion, improvement of the soil structure and infiltration, and conservation of soil water. Until 1988, the agricultural area under NT was only 0.02% of the total national agricultural surface. After that NT has continued to develop and evolve, and the Rolling Pampa has become one of the world's fastest growing areas of NT adoption. Currently, the agriculture surface under NT represents 78.5% of the national agricultural area [18]. In the 1990s, the agricultural intensification advanced towards simplified production schemes under NT, with spring-summer species, especially soybean (70% of the agricultural surface) and, secondarily, maize (15% of the agricultural area) and wheat preceding soybean some years, or otherwise, the soil

remaining fallow between the two summer crops. About 80% of soybean, 61% of wheat and 72% of maize are cultivated under continuous NT [19]. This general adoption of NT occurred together with a high dependence on broad spectrum herbicides and increasing mineral nitrogen fertilization rates related to maize and wheat production [17]. These deep modifications of production systems seem to be the origin of the notable decrease in SOM in different zones of this region. In fact, Michelena et al. [20] documented reductions of 21, 56, and 10–84% in SOC, SON, and extractable phosphorus (P), respectively, of half unit in pH values, of 40–60% in stability structure, and of 54–73% in the infiltration rate, following cultivation. This evolution is generally considered to cause water erosion. On the other hand, the soils under NT with dominance of soybean also present a progressive decrease in their physical, chemical, and biological fertility [21]. The main causes of such decrease include the long periods of fall-winter fallow, the low annual C input to the soil, and the enhancement of the mineralization of SOM by products of the biological fixation of N. In addition, NT presents little soil coverage and low structure stability, tends to compaction, reduces the infiltration rate due to the presence of a laminar structure, and produces a significant contribution of N to the groundwater and surface waters [22–26].

SOM changes are likely to be slower in more temperate than in tropical climates [5, 27, 28]. Burke et al. [5] showed that SOC loss under cultivation increases with precipitation. The climate of the Rolling Pampa, with high annual rainfall and a soil temperature that infrequently reaches 0°C, favors a higher organic decomposition than North American or European climates. Among pedoclimatic characteristics, the amount and nature of clay and calcium carbonates participate in protecting SOM from decomposition by adsorption and aggregation, thus slowing turnover and effectively increasing SOM. Particularly, it was shown that montmorillonite presents a higher protecting role than kaolinite [29]. In the soils of the Rolling Pampa, the predominant mineral of the clay fraction is illite. Since calcium carbonates are removed from the overlying horizons, physical protection of the two latter components would be very low. The silt fraction (2–50  $\mu\text{m}$ ) of the Pampean soils has a high amount of phytolites (to 50% from 2–20  $\mu\text{m}$ ) [30]. This particularity leads to envisage an interaction between SOM and phytolites. Some authors proposed explaining SOM stability of grassland soils by a physical-chemical protection intervening preferentially adsorption mechanisms [31, 32].

During the first years of cultivation, decomposition of easily decomposable roots and crown tissue, soluble fractions, and prehumic substances, in which their production is much greater for native grasses than for cultivated crops, accounts for high initial SOM losses [27, 33–35]. SOM reduction upon cultivation is sensitive to organic management, and the difference relative to general trends depends on the farming practices, especially those involving crop residue utilization, animal manures, crop rotation, fertilizers, and tillage. Several mechanisms have been proposed for the organic matter losses following cultivation of prairie soils: mechanical disruption of previously unavailable organic matter available as substrates for microorganisms, a decrease in the amount and changes in

the type of residue applied [36], dilution with subsoil having less SOM [37], and soil erosion [3, 38].

To estimate and predict the evolution of soil fertility to cover the entire period of time since the beginning of agriculture in the Rolling Pampa, we need a model of long-term SOM evolution such as CENTURY, Roth-C, DAISY, or CN-SIM [39]. These models differ by the number of pools taken into account, the initialization and the size of such pools, and the number of parameters considered and their estimation. When we have minimal data input, it is necessary to abandon the explicit part of elementary processes and replace them by gross simulations indicating the average trends [40–42]. In a work on the Rolling Pampa, Andriulo et al. [43] modified the Hénin-Dupuis model [44] in combination with natural  $^{13}\text{C}$  abundance and applied it on a soil following cultivation and on a soil with a long cropping history both in medium-term studies (13 years). This model, which they named the AMG model, allowed them to obtain a very good prediction of the evolution of C from old and young SOC with an accurate simultaneous estimation of only three model parameters (humification coefficient,  $k_1$ , mineralization coefficient,  $k$ , and stable carbon,  $C_s$ ). AMG, a simple model designed to simulate SOC evolution, is embedded in the soil-plant simulation model STICS [45]. The model runs on an annual time step and assumes that fresh organic matter is either decomposed or humified in the soil after one-year decay. Three compartments are considered: crop residues and stable and active SOM [46].

In the Rolling Pampa, there are no long-term experiments with which we can systematically follow SOM stocks and residue inputs after cultivation and later NT of grassland soils. Since evolution is still relatively recent in Argentine and cultivation of natural grassland soils has developed progressively, there are fields with a different number of years under continuous agriculture since the beginning of the agriculture. Information regarding crop rotations, cultivation systems and yields, which can be obtained from each field by means of a survey, would compose a data set simulating the results of a long-term experiment. This approach has been used by Boiffin et al. [47].

Our objectives were (a) to calibrate the AMG model on the Pergamino soil series of the Rolling Pampa by applying the natural  $^{13}\text{C}$  abundance technique during two periods: a long crop cultivation (T) of native grassland and a more recent one under NT and (b) to validate it on other soils series of the same region by using the performing model version over the environmental functions proposed by Saffih-Hdadi and Mary [46], with some differences, in the same two mentioned periods where it was possible to reconstruct the agricultural history through survey and to obtain long-term information from NT experiments, respectively.

## 2. Materials and Methods

**2.1. Description of the Study Area.** This work was carried out in the Argentine Rolling Pampa (Figure 1). The soils are developed on deep loess sediment. The study area is located between 32° and 35°S and 58° and 63°W. Soils, formed



FIGURE 1: Argentine Rolling Pampa and study area.

from loess, are Typic, Vertic, and Aquic Argiudolls (US Soil Taxonomy) and are deep, relatively well drained, slightly acidic, originally well supplied with SOM, and very fertile. They usually have a silty-loam A horizon (19–26% clay, 55–74% silt, and 4–24% fine and very fine sand) followed by a silty-clay Bt horizon (30–55% in the Bt). Thickness of the Bt horizon is ~60 cm. The climate can be defined as temperate humid without a dry season and with a very hot summer [7]. Monthly mean temperatures range from 9°C in July to 24°C in February. Minimal soil temperature never reaches 0°C; therefore, soils do not freeze, and biological activity is never severely depressed. Rainfall varies from 900 to 1000 mm year<sup>-1</sup>, 70–75% of which occurs in spring and summer, when monthly rainfall erosivity is greatest. The relief is moderately undulating, with slopes of up to 3%. The combination between the degree of slopes and their length results in some water erosion susceptibility.

**2.2. Selection of Study Sites.** The data used to calibrate the model, applying the natural <sup>13</sup>C abundance technique, came from two sites (sites A and B). Site A consisted of a long-term soybean monoculture experiment, carried out at the Pergamino Experimental Station of the National Institute of Agricultural Technology (INTA) (33°01'S; 61°10'W), Buenos Aires province, Argentina, for 33 years, (1980–2012), where the soil is plowed with moldboard plow/double disk at a depth of 15 cm and disk and teeth harrowed at a depth of 10 cm. The experiment started from a known situation taken as a reference of the native prairie (adjacent to the former). Site B (33°58'S; 60°34'W), with 22 years under NT (16S + 2W/S + 2M), started after 80 years of cultivation (1991–2012). Both sites are developed on a fine, illitic, thermic Typic Argiudoll (US Soil Taxonomy), Luvis Phaeozem (WRB) of the Pergamino series without water erosion phases

(soil slope < 0.3%). The texture of the A horizon is silty loam with 25% and 64% clay and silt, respectively. The mean annual temperature is 16.7°C, and the mean annual rainfall for the 1910–2012 period was 965 mm (agroclimatological network database, INTA).

To validate the model during the T period, we used a survey, which consisted in selecting different sites in which time of continuous cultivation since natural grassland and crop successions were known. We opened profiles to start Bt2 horizon depth in the highest site of the landscape of each field. We considered mainly the pedological profiles of Argiudolls. Only two fields classified as Argialbolls were included because their A2 horizon was very thin. Hence, we excluded the surface horizons with a clay content lower than 19%, the clay soils truncated by erosion and without A horizon, and flooding soils. The soil series or soil phases as well as the soil slope of each site studied were identified by INTA soil survey maps [48–53]. Three sites (Pergamino, Correa, and Urquiza soil series) were selected to determine the time zero of the T period for SOC and SON (Table 1).

To validate the model during the NT period we used information issued during three long-term tillage systems experiments developed on the Pergamino series: a 34-year-old (1979–2012) doubled cropped wheat/soybean-maize (W/S-M) and a 25-year-old (1987–2012) soybean (SS) and maize monocultures (MM), both carried out at the Pergamino Experimental Station. We used only the NT systems from three long-term experiments. Each experiment presented a completely randomized block design. W/S-M started after a long alternating pasture/agriculture period, and the monocultures started 9 years after and were cultivated majorly with soybean conventionally tilled. The main plot was 45 m long by 14 m wide, and the tillage systems were randomized in the main plots. Weeds were chemically controlled, and no previous old plowed soil was recorded under the plow depth. Wheat and maize were fertilized with 90 and 100 kg N ha<sup>-1</sup>, respectively, as well as with 12 kg P ha<sup>-1</sup>.

**2.3. Sampling and Analysis.** For the calibration model, three composed soil samples were taken from three 2 m wide pits of sites A in 1990, 1993, 2003, 2007, 2010, and 2012 and of site B in 1990, 2000, 2010, and 2012. These soil samples were taken from at least two soil depths of the full A horizon and then C, N, and natural <sup>13</sup>C abundance were determined (except for <sup>13</sup>C in 2000). For the validation during the T period, soil samples were taken from a 2 m wide pit by site in 1990 for all sites (Table 1). Ten measurements of horizon thickness were obtained. Soils were collected in A11/Ap, A12, and A3/BA horizons. Ten 1 kg simple samples were sampled in each horizon to compose one soil sample. For the validation model during the NT period, in June, before carrying out the tillage previous to maize in W/S-M, SS, and MM (2004, 2008, and 2012), soil samples were taken at three depths: 0–5, 5–10, and 10–20 cm. Three sites were chosen at random for subsampling in each of the treatments, avoiding visible wheel tracks. In the selected sampling years, we covered the full A horizon thickness.



TABLE 1: Description of study sites used for the validation model during T and NT periods.

Soil series	Symbol	Soil type	Agriculture time years	Slope %	Sampling date year	Location	Fait
T period							
Pergamino	Pe	Typic Argiudoll	0	<1.0	1990	34°10'S 60°40'W	Calibration/validation
Correa	Cr	Typic Argiudoll	0	<1.0	1990	32°49'S 61°20'W	Validation
Urquiza	Ur	Typic Argiudoll	0	<1.0	1990	33°55'S 60°25'W	Validation
Rojas	Ro	Typic Argiudoll	2	<1.0	1990	33°56'S 60°55'W	Validation
Peyrano	Py	Vertic Argiudoll	5	1.5–3	1990	33°31'S 60°25'W	Validation
Rojas 5	Ro5	Typic Argiudoll	8	<1.0	1990	33°55'S 60°50'W	Validation
Pergamino	Pe	Typic Argiudoll	10	<1.0	1990	33°57'51.35"S 60°34'39.34"W	Calibration
Pergamino	Pe	Typic Argiudoll	13	<1.0	1993	33°57'51.35"S 60°34'39.34"W	Calibration
Pergamino	Pe	Typic Argiudoll	23	<1.0	2004	33°57'51.35"S 60°34'39.34"W	Calibration
Pergamino	Pe	Typic Argiudoll	27	<1.0	2008	33°57'51.35"S 60°34'39.34"W	Calibration
Pergamino	Pe	Typic Argiudoll	31	<1.0	2010	33°57'51.35"S 60°34'39.34"W	Calibration
Pergamino	Pe	Typic Argiudoll	33	<1.0	2012	33°57'51.35"S 60°34'39.34"W	Calibration
Las gamas	LG	Aeric Argialboll	34	<1.0	1990	33°44'S 60°48'W	Validation
Rojas	Ro	Typic Argiudoll	35	<1.0	1990	34°02'S 60°47'W	Validation
Pergamino 6 o Peyrano 2x	Pe6 o Py2x	Typic Argiudoll	40	1.5–3	1990	33°39'S 60°42'W	Validation
Villa Eloisa	Ve2	Typic Argiudoll	55	1.5–3	1990	33°00'S 61°10'W	Validation
Las gamas	LG	Aeric Argialboll	60	<1.0	1990	33°45'S 60°50'W	Validation
Arroyo dulce 2	AD2	Typic Argiudoll	64	<1.0	1990	34°08'S 60°22'W	Validation
Arroyo dulce 2	AD2	Typic Argiudoll	70	<1.0	1990	34°15'S 60°17'W	Validation
Urquiza	Ur	Typic Argiudoll	72	<1.0	1990	33°55'S 60°25'W	Validation
Arrecifes 2	Ar2	Typic Argiudoll	72	1–1.5	1990	33°52'S 60°17'W	Validation
Correa 1	Cr1	Typic Argiudoll	75	1–1.5	1990	32°49'S 61°20'W	Validation
Pergamino	Pe	Typic Argiudoll	80	<1.0	1990	33°46'S 60°38'W	Calibration/validation
Rojas 4	Ro4	Typic Argiudoll	80	1–1.5	1990	34°14'S 60°35'W	Validation
Arroyo dulce 2	AD2	Typic Argiudoll	83	<1.0	1990	34°10'S 60°30'W	Validation
Peyrano	Py	Vertic Argiudoll	84	<1.0	1990	33°22'S 60°47'W	Validation
Peyrano 1 o Peyrano 2x	Py1 ó Py2x	Vertic Argiudoll	87	1–1.5	1990	33°32'S 60°46'W	Validation
Pergamino	Pe	Typic Argiudoll	100	<1.0	2010	33°46'S 60°38'W	Calibration
Pergamino	Pe	Typic Argiudoll	102	<1.0	2012	33°46'S 60°38'W	Calibration
Peyrano 2x	Py2x	Vertic Argiudoll	112	1.5–3	1990	33°35'S 60°48'W	Validation
Casilda 1	Ca1	Vertic Argiudoll	113	1–1.5	1990	33°07'S 61°20'W	Validation
NT period							
Pergamino	Pe	Typic Argiudoll	0	<0.3	1979	33°57'36.26"S 60°33'50.25"W	Validation
Pergamino	Pe	Typic Argiudoll	0	<0.3	1987	33°57'32.06"S 60°33'41.58"W	Validation
Pergamino	Pe	Typic Argiudoll	0	<0.3	1987	33°57'31.54"S 60°33'44.85"W	Validation
Pergamino	Pe	Typic Argiudoll	17	<0.3	2004	33°57'32.06"S 60°33'41.58"W	Validation
Pergamino	Pe	Typic Argiudoll	17	<0.3	2004	33°57'31.54"S 60°33'44.85"W	Validation
Pergamino	Pe	Typic Argiudoll	21	<0.3	2008	33°57'32.06"S 60°33'41.58"W	Validation
Pergamino	Pe	Typic Argiudoll	21	<0.3	2008	33°57'31.54"S 60°33'44.85"W	Validation
Pergamino	Pe	Typic Argiudoll	25	<0.3	2004	33°57'36.26"S 60°33'50.25"W	Validation
Pergamino	Pe	Typic Argiudoll	25	<0.3	2012	33°57'32.06"S 60°33'41.58"W	Validation
Pergamino	Pe	Typic Argiudoll	25	<0.3	2012	33°57'31.54"S 60°33'44.85"W	Validation
Pergamino	Pe	Typic Argiudoll	29	<0.3	2008	33°57'36.26"S 60°33'50.25"W	Validation
Pergamino	Pe	Typic Argiudoll	33	<0.3	2012	33°57'36.26"S 60°33'50.25"W	Validation

T: tillage; NT: no tillage.

Samples were dried and sieved finer than 2 mm. Organic matter more coarse than 2 mm was taken into account in this study. Particle size was measured according to the pipette method [54]. C, N, and  $^{13}\text{C}$  contents were determined by dry combustion with a mass spectrometer (Fisons/Isochrom) coupled with a CN analyzer (Carlo Erba NA 1500). Soil bulk density (BD) measurements were used to transform mass-based measurements into volume-based ones. BD was determined by the cylinder method [55]. During the first period of validation, BD was measured with 50 or 200  $\text{cm}^{-3}$  cylinders, and four replications were sampled in each soil horizon. In the experiments, in a place adjacent to the disturbed sample, a small pit of 0–30 cm depth was opened, and a cylinder was extracted at each depth. The cylinder (58.9  $\text{cm}^3$ ) was placed vertically at 0–5 cm and horizontally at the other two soil depths. All the samples were dried in an oven at 105°C to constant weight. A mass of 2500  $\text{Mg ha}^{-1}$  was chosen to calculate SOC and SON stocks.

**2.4. Estimation of Organic Additions.** In order to estimate organic additions, culture succession, crop yield, residue removal by burning, and nitrogen fertilization were retained in each field. Before 1947, we used an average yield from typical maize-wheat or maize-linseed sequences in a proportion of 2 or 3 maize crops for each wheat or linseed, depending on the geographical zone and farmer declaration on the proportion. Values for the crop residue additions were based on measured straw [56] as well as on inputs from stem bases and roots calculated as follows: (1) straw harvest index (crop yield/total air dry matter) before 1947 were 0.20, 0.20, and 0.10 for maize, wheat, and linseed, respectively, between 1948 and 1990 were 0.40, 0.36, 0.23, 0.40, 0.36, 0.38, and 0.30 for maize, wheat, soybean, sunflower, sorghum, oat, pea, and lentil, respectively, and after 1990 were 0.50, 0.42, and 0.38 for maize, wheat, and soybean; (2) below-ground C biomass, including roots and rhizodeposits, was considered 0.30 of the total air dry matter for all crops [57]; (3) after harvest, aerial crop residue biomass was reduced to 15% of total aerial crop residue due to burning of maize, linseed, and wheat straws from start of cultivation to mechanical harvest and from W/S during the 1970s. This biomass was considered as black carbon; (4) the C/N ratios before 1990 were 87 for maize, 96 for wheat, 44 for soybean, 24 for sorghum, 12 for prairies, 55 for linseed, 74 for clover, 49 for sunflower, 7 for carrot, 17 for lentil, 29 for pea, 23 for vetch, and 67 for black carbon, whereas those after 1990 were 57 and 64 for maize and wheat, respectively, due to an increase in mineral nitrogen fertilization rate; (5) the C content of straw and below-ground biomass was assumed to be 40% of the total dry matter; and (6) the C biomass from weeds was assumed as 1  $\text{Mg ha}^{-1} \text{ year}^{-1}$  before mechanical weed control was replaced by chemical control (~1990).

In the experiments, annual yields and aerial biomass were used to estimate organic additions and the straw harvest index, below-ground C biomass, and C/N ratios found after 1990 were used. The C content was also 40% of the total dry matter, and no C biomass from weeds was considered.

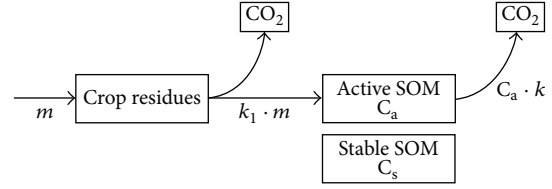


FIGURE 2: Diagram of AMG model.

**2.5. Three-Compartment Model.** The basic equations of AMG model (Figure 2) are the following [43]:

$$C = C_s + C_a,$$

$$\frac{dC_a}{dt} = m \cdot k_1 - k \cdot C_a, \quad (1)$$

where  $C$  is the SOC amount ( $\text{Mg ha}^{-1}$ ),  $C_s$  is the stable  $C$  amount ( $\text{Mg ha}^{-1}$ ),  $C_a$  is the active  $C$  amount ( $\text{Mg ha}^{-1}$ ),  $m$  is the mass of annual  $C$  input (represents the total mass of organic carbon returned to soil through all crop residues (straw, stubble, roots, and rhizodeposits, in  $\text{Mg ha}^{-1} \text{ year}^{-1}$ ),  $k_1$  is the humification coefficient (unitless), and  $k$  is the mineralization coefficient of the active pool ( $\text{yr}^{-1}$ ).

These equations can be integrated if  $m$  is considered constant every year. Then, the evolution of the carbon reserve may be described by the following equation:

$$C = C_s + C_{a_0} \cdot e^{-kt} + \frac{m \cdot k_1}{k} \cdot (1 - e^{-kt}), \quad (2)$$

$$C_{a_0} = C_0 - C_s, \quad (3)$$

where  $C_0$  is the initial SOC amount ( $\text{Mg ha}^{-1}$ ). In (2), the second term on the right side represents the decomposition of the “old carbon” (i.e., existing at time 0), while the third term represents the (net) newly humified carbon which reaches an asymptote:

$$C_{\max} = \frac{m \cdot k_1}{k}, \quad (4)$$

$$C_{\text{eq}} = C_{a_{\infty}} + C_s,$$

where  $C_{a_{\infty}}$  is the maximum quantity of soil  $C$  originated from crop sequences ( $\text{Mg ha}^{-1}$ ) and  $C_{\text{eq}}$  is the total quantity of soil  $C$  at equilibrium ( $\text{Mg ha}^{-1}$ ).

This model is traditionally used to describe the turnover of SOC. Here we also use it to simulate the turnover of soil total SON stock

$$N = N_s + NN_{a_0} \cdot e^{-kt} + \frac{m_N \cdot k_1}{k} \cdot (1 - e^{-kt}), \quad (5)$$

where  $N_s$  is the amount of stable  $N$  ( $\text{Mg ha}^{-1}$ ),  $N_{a_0}$  is the amount of initial active  $N$  ( $\text{Mg ha}^{-1}$ ),  $N_0$  is the amount of initial total  $N$  ( $\text{Mg ha}^{-1}$ ),  $m_N$  is the amount of  $N$  from crop residues annually added to soil ( $\text{Mg ha}^{-1} \text{ year}^{-1}$ ),  $k_1$  is the humification coefficient (unitless), and  $k$  is the mineralization coefficient of the active fraction ( $\text{year}^{-1}$ ).

**2.6. Calibration Procedure.** As stated previously, the data set obtained from two long-term experiments (sites A and B) was used to calibrate the AMG model. The second and third terms of (2) were determined separately by using the  $^{13}\text{C}$  natural abundance technique which allows obtaining a unique evaluation of the parameters  $k$ ,  $k_1$ , and  $C_s$  in long-term experiments [43, 58].

Since we needed measurements of natural  $\delta^{13}\text{C}$  abundance to establish the origin of the SOM in each site, we used the technique developed by Cerri et al. [59] and Balesdent et al. [60]. The proportion of soil C derived from C3 crops ( $\alpha$ ) was calculated as

$$\alpha (\%) = \frac{(\delta_m - \delta_1)}{(\delta_2 - \delta_1)} \cdot 100, \quad (6)$$

where  $\delta_m$  is the isotopic abundance of  $^{13}\text{C}$  in the soil at time  $t$  (‰),  $\delta_1$  is the isotopic abundance of  $^{13}\text{C}$  in the soil at time  $t = 0$  (‰) taken at the reference, and  $\delta_2$  is the isotopic abundance of  $^{13}\text{C}$  of the crop in the crop sequence (‰). These last values were  $-26.3$  and  $-24.98$ ‰ for A and B sites A and B, respectively [43].

When  $\alpha$  is known, the young and old carbon contents can be calculated. The C contents ( $\text{g kg}^{-1}$  soil) derived from crop sequence residues ( $C_{\text{DC}}$ ) and native prairie or after long-term cultivation period ( $C_{\text{DP}}$ ) in our case were calculated as

$$\begin{aligned} \text{young C : } C_{\text{DC}} &= \alpha \cdot C_m = \frac{m \cdot k_1}{k} \cdot (1 - e^{-kt}), \\ \text{old C : } C_{\text{DP}} &= (1 - \alpha) \cdot C_m = C_s + C_{a_0} \cdot e^{-kt}, \end{aligned} \quad (7)$$

where  $C_m$  represents the total C content of bulk soil ( $\text{g kg}^{-1}$  soil).

The SOC stocks (total, originated from crop sequences and native prairie, in  $\text{Mg ha}^{-1}$ ) were calculated using the following equation:

$$\text{SOC} = C_x \cdot d \cdot \text{BD} \cdot 10, \quad (8)$$

where  $C_x$  represents  $C_m$ ,  $C_{\text{DC}}$ , or  $C_{\text{DP}}$ ,  $d$  is the depth (m), and BD is the bulk density ( $\text{Mg m}^{-3}$ ) of the corresponding soil depth. SON stocks were also calculated at  $2500 \text{ Mg soil ha}^{-1}$ .

We tried to fit the two C models (young and old) at the same time, that is, by minimizing the sums of the square of deviations from the model for the variables  $C_{\text{DC}}$  and  $C_{\text{DP}}$ . While optimizing, we took account of the variance of the data by weighting the sums of squares of deviations. The following quantity was minimized as follows:

$$\text{SSQ} = \frac{\text{SSQ}_{\text{DC}}}{S_{\text{DC}}^2} + \frac{\text{SSQ}_{\text{DP}}}{S_{\text{DC}}^2}, \quad (9)$$

where  $\text{SSQ}_{\text{DC}}$  and  $\text{SSQ}_{\text{DP}}$  are the sums of squares of deviations (observed, simulated) of the variables  $C_{\text{DC}}$  and  $C_{\text{DP}}$ , respectively;  $S_{\text{DC}}^2$  and  $S_{\text{DP}}^2$  are the mean experimental variances obtained for the variables  $C_{\text{DC}}$  and  $C_{\text{DP}}$ . Fitting was performed by fixing  $C_0$  and  $m$  and optimizing three parameters:  $C_s$ ,  $k_1$ , and  $k$ .

In the case of  $N$ ,  $k$ ,  $k_1$ , and  $N_s$  were treated as follow.

(a)  $k$  values were optimized and fixed in T and NT period, respectively, and in this last period the same value estimated in SOC simulation was used; (b)  $k_1 = 1$  in the cultivation period and optimized in the NT period; (c)  $N_s$  values were optimized in both tillage periods.

The  $k_1$  values to simulate SON evolution are different from those used to simulate SOC evolution. The value  $k_1 = 1$  adopted for SON simulation for all residues during the cultivation period means that N harvest residue addition is completely humified. This hypothesis is supported by  $^{15}\text{N}$ -labeled harvest residue incubations mixed with the soil [61].

To judge the goodness of fit of the SOC and SON models, we used the absolute root mean square error (RMSE), in  $\text{Mg ha}^{-1}$ , defined as follows:

$$\text{RMSE}(j) = \sqrt{\frac{1}{n_j} \sum_{i=1}^n \Sigma (X_{ij} - \hat{X}_{ij})^2}, \quad (10)$$

where  $n_j$  is the number of observations of each data set  $j$ , and  $X_{ij}$  and  $\hat{X}_{ij}$  are the observed and simulated values of SOC (SON), respectively. The optimization was conducted using the Newton's method of Excel solver.

**2.7. Validation Procedure.** The proportions of stable pool to total SOC at the beginning of the simulations, estimated from the model calibration in the Pergamino soil series for sites A and B were applied in the soil series included in the T and NT validation periods. The proposed default value of the  $C_s/\text{SOC}_{\text{reference}}$  relationship (0.65) was not used because it can vary along the agriculture period and can be smaller in the native grassland [62]. The  $\text{SOC}_{\text{reference}}$  (native grassland) used for the sites included in the cultivation period were obtained from Michelena et al. [20]. The  $k_1$  values obtained by calibration were only applied for soybean culture. The  $k_1$  values used for the other crops were taken from international references, according to the evolution of different residue management practices (stubble burning, mineral N and P fertilization, tillage system, and weed control). As normally accepted, the value of  $k$  is highly dependent on the pedo-climatic conditions and tillage systems. Thus, we used the environmental functions proposed by Saffih-Hdadi and Mary [46] with the introduction of a third environmental factor (rainfall index) during the cultivation period. The  $k$  value obtained from NT calibration was used for the NT period.

The mineralization rate  $k$  depends particularly on soil temperature ( $T_e$ ), clay content ( $A$ ), and a simple rainfall index (RI):

$$k = k_0 f_1(T_e) f_2(A) f_3(\text{RI}), \quad (11)$$

where  $k_0$  is the potential mineralization rate ( $\text{year}^{-1}$ ) in reference conditions and  $f_1(T_e)$ ,  $f_2(C)$ , and  $f_3(\text{RI})$  are the temperature, clay, and rainfall functions, equal to 1 in reference conditions. The reference conditions are defined here as  $15^\circ\text{C}$  soil temperature, zero clay content, and  $900 \text{ mm}$

annual rain. The effect of clay content on mineralization of SOC is described by an exponential law:

$$f_2(A) = e^{-aA}, \quad (12)$$

where  $A$  is the clay content ( $\text{g g}^{-1}$  soil) and  $a$  is a constant ( $\text{g soil g}^{-1}$  clay) with  $a$  value of 2.72. The effect of temperature ( $T$ ,  $^{\circ}\text{C}$ ) on mineralization of humified organic matter is assumed to obey a logistic law:

$$f_1(T) = \begin{cases} \frac{c}{1 + (c-1)e^{-k(T-T_{\text{ref}})}} & \text{if } T \geq 0, \\ 0 & \text{if } T < 0. \end{cases} \quad (13)$$

The effect of water content on mineralization of SOM and residues was based on a very simple macroclimatic index since soil water data were not available. We assume the following relationship between mean annual precipitation ( $P$ ) and annual potential evapotranspiration calculated by Thornwhite method (PET), both expressed in mm, considering  $P$  and PET from the 1910–2012 period 965 mm and 870 mm, respectively (INTA, soil data network) (Table 2).

In the model, the humification coefficient  $k_1$  was only dependent on the quality of the residues. The  $k_1$  values of all crops included in crop sequences extracted from published values are shown in Table 3.

The values assumed for the  $C_{\text{stable}}/N_{\text{stable}}$  ratio were very close to those obtained during the model calibration. Besides,  $k_1$  values were fixed considering that all N additions are completely humified regardless of the type of tillage [61]. The initial  $\text{SON}_{\text{reference}}$  for T sites was estimated using a C/N ratio = 11 (we averaged SOC/NOC contents of the three sites without agriculture history of this paper (Table 1) and those reported by Michelen et al. [20]).

Finally, to obtain the goodness of fit of the AMG model simulated versus observed SOC and SON values were compared separately for the T and NT periods.

### 3. Results and Discussion

**3.1. Model Calibration.** Figure 3 shows the evolution of the average  $^{13}\text{C}$  values and their depth distribution for the two study sites. The effect of C incorporation issued from C3 species in the SOM was recorded by decreasing  $^{13}\text{C}$  values in time. These results allowed progress in monitoring the young and old SOC compartments. In site B, where maize crops occupied half of the cropping history, the previous agricultural period enriched the  $\delta^{13}\text{C}$  SOC ( $-18.3\text{‰}$  at 0–14 cm soil depth) which had started from mixed C3/C4 grasslands ( $-19.6\text{‰}$  at 0–14 cm soil depth).

The model fit to data using the  $C_s$ ,  $k_1$ , and  $k$  values issued from  $^{13}\text{C}$  measurements in sites A and B is presented in Figure 4. The parameter settings used worked very well, and the model described the general trends in the soil carbon data well (RMSE 1.13 and  $0.94 \text{ Mg SOC ha}^{-1}$  for sites A and B resp.) despite the small available number  $^{13}\text{C}$  measurements to obtain the parameter values during the NT period.

The  $C_s$  values found were very similar in both sites, both in a situation of recent agriculture and in one of old

TABLE 2

P/PET	RI
[0.5; 0.6)	0.5
[0.6; 0.7)	0.6
[0.7; 0.8)	0.7
[0.8; 0.9)	0.8
[0.9; 1.0)	0.9
$\geq 1$	1

agriculture (Table 4). Hence, the size of this pool can be considered as a valid indicator for the full period of the Pergamino soil series under agriculture. The SOC content of the A horizon was  $12.5\text{--}13.0 \text{ mg SOC g}^{-1}$ . A similar result had been previously obtained in soils of this region [43]. This value represents 47% at the beginning of agriculture and 67% at the beginning of NT, 80 years after cultivation. These values are in agreement with previous reports in different countries [46, 62, 63]. The rate constant of mineralization  $k$  was  $0.108$  and  $0.078 \text{ year}^{-1}$  and in agreement with the climate and soil conditions previously set, indicating a rapid transition to a new equilibrium under cultivation (mean residence time—MRT—of the active fraction  $\sim 9$  years) and a significant decrease under NT (MRT  $\sim 13$  years), respectively. In site A, the  $k_1$  value was the same as that been previously obtained [64] and higher than that obtained for cereal crop (0.21) combining the AMG model with the  $^{13}\text{C}$  technique [65]. Soybean residue decomposes relatively faster than cereal residues and stimulates the mineralization of SOM [66]. However, as it has higher lignin content, its soil stabilization is favored when it is incorporated into the soil [56]. The  $k_1$  value under NT was markedly smaller than under cultivation and slightly higher than that obtained by other authors [65, 66].

The estimated annual C addition in crop sequences of sites A and B, with a very high proportion of soybean, is characteristic in the Rolling Pampa [68]. In both sites, the technology was unable to maintain existing SOC stocks. Novelli et al. [69] found that the SOC storage was negatively associated with the soybean cropping frequency in the cropping sequences. After the introduction of agriculture in site A, there was a rapid loss of SOC (25%) during the first ten years, followed by a period with a lower loss (15%) in 23 years. In site B, NT implementation after a long history of continuous cultivation led to a loss of SOC of 15%. At the equilibrium, the active fraction would represent 23 and 19% in sites A and B, respectively.

The same general trends were observed for the soil nitrogen data which were well described by the model: RMSE  $0.04$  and  $0.05 \text{ Mg SON ha}^{-1}$  for sites A and B, respectively (Figure 5). The two crop sequences had similar N additions. However, the size of  $N_s$  was smaller in site B than in site A (Table 4). This difference can be explained by the previous agriculture history. In site B, the long cultivation period without N fertilization, where maize culture occupied half of the total crop sequence and the other crops such as wheat and linseed with high C/N ratios before the NT period, led to a poor N resistant pool ( $C_s/N_s = 12.7$ ). In contrast, soybean



TABLE 3: Crop  $k_1$  values used in the validation procedure as a function of the predominant soil residue management.

Culture	Default	Stubble burning from aerial root biomass	$k_1$ Mineral N/P fertilization	Mechanical weed control	NT
Maize	0.35 <sup>a</sup>	0.5 0.3 <sup>c</sup>	0.21 <sup>d</sup>		0.13 <sup>e</sup>
Leenseed		0.5 0.3			
Wheat	0.3 <sup>ab</sup>	0.5 0.3	0.21		0.13
Soybean	0.3				
Sunflower	0.3				
Sorghum	0.35 <sup>a</sup>		0.21		
Carrot	0.3				
Oat	0.3				
Pea	0.3				
Lentil	0.3				
Vetch	0.3				
Weed				0.21	

Taken from: <sup>a</sup>[43]; <sup>b</sup>[29, 47]; <sup>c</sup>[67]; <sup>d</sup>[46]; <sup>e</sup>[65].

NT: no tillage.

monoculture, implemented after a pristine prairie, showed a relatively high stable N pool ( $C_s/N_s = 10$ ). Besides, since under NT part of the crop residues are not in contact with the soil, the  $k_1$  value under NT was smaller than that in tilled soils. As a consequence, a similar  $m_N$  addition tended to higher  $N_{eq}$  in site A than in site B.

In site A,  $N_{a0}$  was  $3.2 \text{ Mg ha}^{-1}$ . Then, the nitrogen mineralized during the first period of cultivation was large enough for crop requirements and the nonused mineral nitrogen was likely leached out of the rooting zone and into subterranean water reserves. The optimized  $k$  values and the proportion of the stable fraction were close for SOC and SON. Similar results have been informed by Mary and Guérif [70] for the Rothamsted trials.

**3.2. Model Validation.** Mean SOC and SON contents and BD values of the sites surveyed and clay content at  $2500 \text{ Mg soil ha}^{-1}$  are shown in Table 5. Low variability of soil texture is observed among the study sites. Besides, some of the sites sampled during the T period did not meet the requirement of soil mass that is to have an A horizon with  $2500 \text{ Mg ha}^{-1}$ .

The AMG model was able to provide satisfactory simulation of the evolution of SOC and SON stocks in the sites that satisfied soil mass requirement in the cultivation period (Figure 6).

Figure 7 shows the evolution of SOC and SON stocks of all the sites surveyed during the T period. Some variability was observed in the data set partly due to the nature of this study (i.e., survey). A part of the mentioned variation can be attributed to the vertical and horizontal spatial variation of surface deposits. This was confirmed by considering the three virgin sites which showed values of 65, 68, and  $76 \text{ Mg SOC ha}^{-1}$ . These three values are in the content

range reported for the virgin soils of the study area [20, 71]. These reports showed SOC contents which translated in storage values ranging from  $60$  to  $80 \text{ Mg SOC ha}^{-1}$  for a soil mass of  $2500 \text{ Mg ha}^{-1}$ . So, we are confident that the current soil C storage of virgin soils corresponds to the C storage present in the last century. Also, certain cultivated sites with less than  $40 \text{ Mg SOC ha}^{-1}$  belong to soil series with high C contents in virgin state (Table 1 and Figure 7(a)). Therefore, variations in the observed SOC stocks cannot be completely explained by natural variability.

Water erosion can also explain part of the data variability, since it has been previously mentioned as a very important cause of losses in zones with high slopes [71]. Data showed that several sites older than 40 years are in slopes higher than 1% (Table 1). Six of these sites showed SOC stocks lower ( $\leq 40 \text{ Mg SOC ha}^{-1}$ ) than the average stock of all T sites (Figure 7(a)). On the other hand, one of the sites with five years of tillage and a slope higher than 1.5% showed a SOC stock lower than that of the other young sites (Table 1 and Figure 7(a)). Furthermore, a decrease in the A horizon mass was observed in the soils located in slopes higher than 1% (Table 1). On the other hand, soil survey maps classify the soils with slopes higher than 1% as eroded phases [48–53]. We are therefore confident that some of the losses for the sites with slopes higher than 1% are due to erosion. The sites with an A horizon soil mass smaller than  $2500 \text{ Mg ha}^{-1}$  were not taken into account in the validation (Table 5).

On average, during the first twenty years of cultivation, the SOC storage varied from  $70$  to  $51 \text{ Mg ha}^{-1}$  and SON storage varied from  $6.6$  to  $4.5 \text{ Mg ha}^{-1}$ ; this represents losses of 27 and 32% for SOC and SON, respectively (Figure 7). After the first twenty years, when water erosion was present ( $>1\%$  slope), SOC and SON loss increased with the years of tillage. For instance, a soil with a slope higher than 1% had about 47

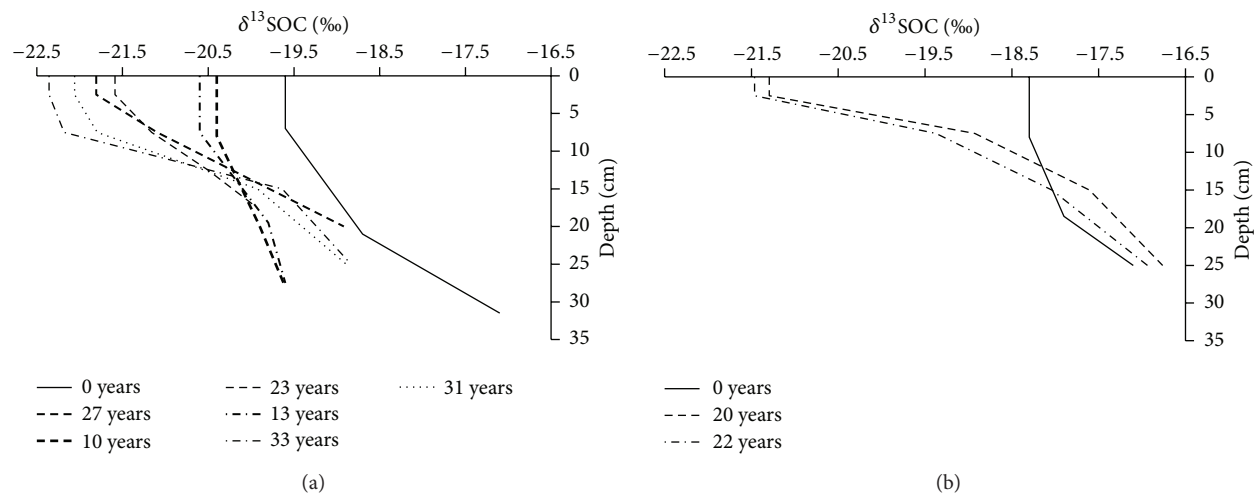


FIGURE 3: Distribution of mean 13COC values with depth and their evolution in sites A (a) and B (b).

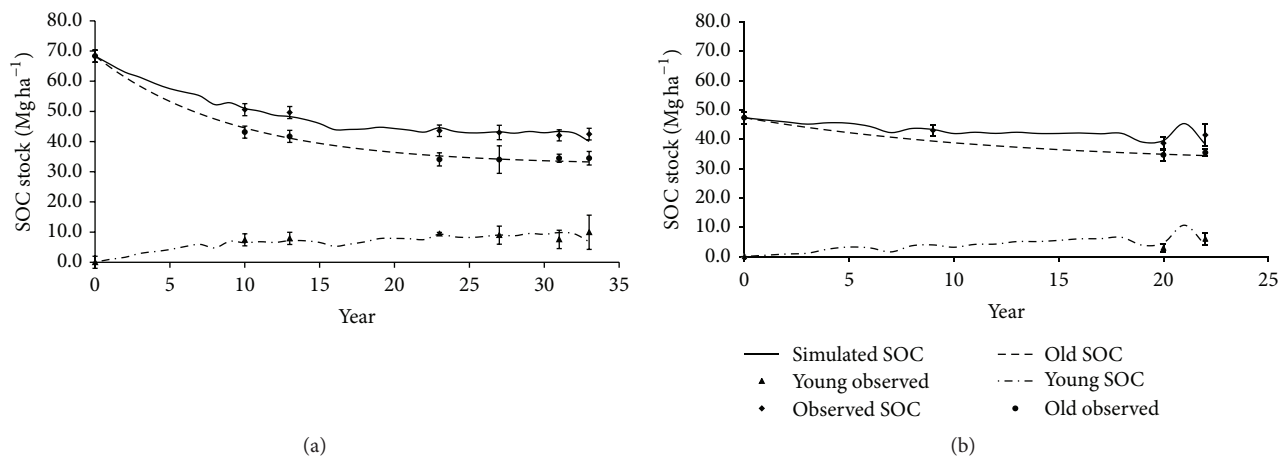


FIGURE 4: Observed and simulated total, young, and old carbon evolution in sites A (a) and B (b).

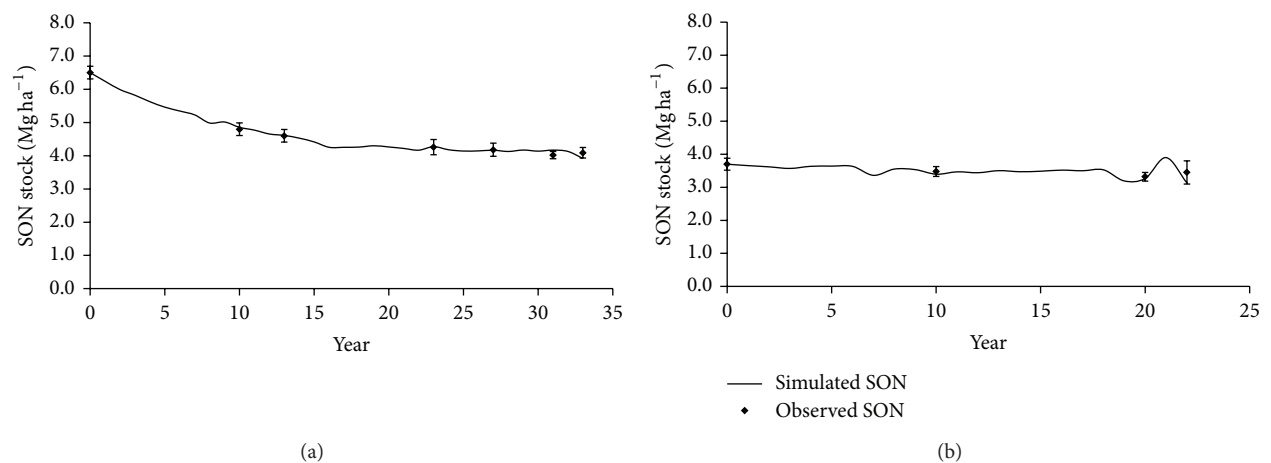


FIGURE 5: Observed and simulated SON evolution in sites A (a) and B (b).

TABLE 4: Values of model parameters ( $C_s$ ,  $k$ , and  $k_1$ ) obtained with the AMG model in sites A and B.

Site	Fixed parameters				Optimized parameters			Predicted values at equilibrium		
	$C_0$ Mg ha <sup>-1</sup>	$m$ Mg ha <sup>-1</sup> year <sup>-1</sup>	$k$ year <sup>-1</sup>	$k_1$	$C_s$ Mg ha <sup>-1</sup>	$k$ year <sup>-1</sup>	$k_1$	$C_{a \max}$ Mg ha <sup>-1</sup>	$C_{eq}$	
A	68.3	3.6	—	—	32.2	0.108	0.288	9.6	41.8	
B	47.3	3.5	—	—	31.7	0.079	0.167	7.3	40.4	
	$N_0$ Mg ha <sup>-1</sup>	$m_N$ Mg ha <sup>-1</sup> year <sup>-1</sup>	$k$ year <sup>-1</sup>	$k_1$	$N_s$ Mg ha <sup>-1</sup>	$k$ year <sup>-1</sup>	$k_1$	$N_{a \max}$ Mg ha <sup>-1</sup>	$N_{eq}$	
A	6.5	0.079	—	1	3.3	0.113	—	0.7	4	
B	3.7	0.082	0.079	—	2.5	—	0.827	1	3.4	

and 57% SOC and SON losses, respectively, after 40 years of tillage. A site located in the same soil series but after 112 years of tillage had 63 and 72% SOC and SON losses, respectively (dotted line). When erosion was not a factor, for instance the Pergamino soil series, SOC and SON losses were 30 and 43%, respectively. After 80 years of tillage, losses in the Pergamino soil series were in agreement with those in the other sites. This suggests that SOC and SON changes obtained by survey were the same as those obtained from long-term experiment evolution.

Average  $C_s$ ,  $k$ , and  $k_1$  validated values were  $32.9 \pm 2.6$  Mg ha<sup>-1</sup>,  $0.102 \pm 0.003$  year<sup>-1</sup>, and  $0.41 \pm 0.02$ , respectively, whereas average  $C_0$  and  $m$  values were  $70.0 \pm 5.6$  Mg ha<sup>-1</sup> and  $4.0 \pm 1.1$  Mg ha<sup>-1</sup> year<sup>-1</sup>, respectively (solid line of Figure 7(a)). The average crop residue C input was necessary to maintain the equilibrium C level in 48 Mg ha<sup>-1</sup> (19 mg C kg<sup>-1</sup> soil). The active fraction changed from 37 Mg ha<sup>-1</sup> at the beginning to 15 Mg C ha<sup>-1</sup> at equilibrium. Average  $N_s$  and  $k$  validated values were  $2.9 \pm 0.6$  Mg ha<sup>-1</sup> and  $0.102 \pm 0.003$  year<sup>-1</sup>, respectively, whereas average  $N_0$  and  $m_N$  values were  $6.6 \pm 0.6$  Mg ha<sup>-1</sup> and  $0.074 \pm 0.02$  Mg ha<sup>-1</sup> year<sup>-1</sup> (solid line of Figure 7(b)).

The  $k$  value obtained using the Saffih-Hdadi and Mary approximation was very close to the optimized value (0.106) and can be recommended to use under conventional tillage conditions. Overall, the MRT of the active compartment would be included in 9 years. This is twice the MRT of the North American prairie [72]. This model clearly shows that equilibrium is reached after the first 20 years following cultivation of grassland. The annual mineralization coefficient ( $k$ ) from the three-compartment model can be compared with the annual mineralization coefficient ( $k_2$ ) from the single-compartment model using the following relation:

$$k_2 = k \cdot \frac{C_a}{C_a + C_s} \quad (14)$$

$k_2$  values varied with tillage time, from 0.056 year<sup>-1</sup> at the beginning to 0.03 year<sup>-1</sup> at equilibrium. The  $k$  value from the active fraction was 2- to 3-fold higher than the  $k_2$  values. Our  $k_2$  values are intermediate between the Australian values (from 0.065 to 1.22 year<sup>-1</sup>) obtained by Dalal and Mayer [73] and the British value (0.008 year<sup>-1</sup>) obtained by Mary and Guérif [70] or French values (from 0.008 [74] to 0.03 year<sup>-1</sup>

[75]). Australian studies were carried out in a subtropical climate with average temperatures from 18.5 to 20.5°C, while British and French studies were carried out in a temperate climate with average temperatures from 9 to 13.5°C. In our study, the average temperature was 17°C.

The inclusion of the RI factor caused no changes in the estimated  $k$  value: the Saffih-Hdadi and Mary approximation without inclusion of the RI factor gave a mean value of  $0.106 \pm 0.002$ . As expected, the only model parameter that showed a difference with the value obtained for soybean in the calibrated model was  $k_1$  due to the inclusion of residue management recorded by the survey. The crop history of the cultivation period showed similar crop sequences in all the sites in the last twenty years (1970–1990s) (data not shown). In general, organic additions were greater than those in the preceding period (Figure 8).

Before the 1960s, typical crop rotations were maize wheat or maize linseed, sometimes sunflower, with a ratio 2-3 years of maize to 1 year of wheat or linseed or sunflower. Residue burning was a common practice until 1950 and the oldest farmers declared that crop yields were much lower before 1960. After 1960, there was important technical progress (fall tillage, improvement of the drilling technique, better selection of soils, advances in crop protection, better harvesting efficiency, and genetic progress), which resulted in significant yield improvements [7]. Hence, in the older sites, it is probable that crop additions and SOC contents progressively decreased with time (1960–1970s). At that moment, INTA reported that SOC contents under annual crops had decreased from 28.0 to 13.5 mg g<sup>-1</sup>, that is, about 30 Mg SOC ha<sup>-1</sup> after 70 years of crop cultivation [71]. Moreover, soybean introduction double cropped with wheat and the significant decrease of straw burning probably contributed to the increase in C additions into the soil. Thus, the decrease suffered by SOC and SON stocks observed in 1990 may have been higher before than after the 1960–1970s.

In general, the losses observed in our study are greater than those observed in other climatic regions with the same initial vegetation (grassland) [76]. Several reasons can explain these apparent faster losses in the soils of the Rolling Pampa.

One possible explanation is that the sites with less than twenty years of tillage (1970–1990s), as opposed to the oldest sites, have been, in general, cropped in soybean monoculture or soybean double cropped with wheat with many tillage

TABLE 5: Soil organic carbon and nitrogen contents ( $\text{mg g}^{-1}$ ) and bulk density ( $\text{Mg m}^{-3}$ ) of the different soil depths and A horizon clay content ( $\text{mg g}^{-1}$ ) at 2500 Mg soil  $\text{ha}^{-1}$  used in all the sites analyzed.

Symbol	Soil type	Agriculture time years	Depth cm			Bulk density Mg m <sup>-3</sup>			Carbon			Nitrogen g Kg <sup>-1</sup>			Clay	Requirement 2500 Mg ha <sup>-1</sup>
T period																
Pe	Typic Argiudoll	0	14	14	7	1.07	1.1	1.25	30.1	23.2	20.2	2.9	2.2	2.0	25.2	Pass
Cr	Typic Argiudoll	0	30		7	1.19		1.26	26.2		16.0	2.5		1.4	26.3	Pass
Ur	Typic Argiudoll	0	28		8	1.07		1.25	30.5		19.8	2.8		1.6	24.6	Pass
Ro	Typic Argiudoll	2	13	12	8	1.1	1.18	1.27	24.9	23.7	14.6	2.2	1.9	1.2	23.9	Pass
Py	Vertic Argiudoll	5	16	10	7	1.07	1.22	1.25	24.3	18.1	10.6	2.1	1.6	0.9	24.1	Pass
Ro5	Typic Argiudoll	8	22			1.17			23			1.9		0.0	23.9	Pass
LG	Aeric Argialboll	34	11	11	3	1.18	1.18	1.22	20	21.1	18.2	1.7	1.9	1.5	23.3	Pass
Ro	Typic Argiudoll	35	15	10	8	1.1	1.22	1.3	19.1	18.9	11.8	1.6	1.6	1.0	21.4	Pass
Pe6 o Py2x	Typic Argiudoll	40	18		4	1.19		1.31	15.2		12.9	1.2		1.0	19.0	Fail
Ve2	Typic Argiudoll	55	15		8	1.22		1.24	15.1		13.8	1.3		1.2	23.0	Fail
LG	Aeric Argialboll	60	16	5	8	1.23	1.28	1.3	19.1	19.4	11.2	1.4	1.8	0.8	24.7	Pass
AD2	Typic Argiudoll	64	20			1.25			18			1.4			22.1	Pass
AD2	Typic Argiudoll	70	16	5	7	1.23	1.41	1.35	16.1	15.9	14	1.3	1.1	1.1	21.2	Pass
Ur	Typic Argiudoll	72	12	6	5	1.15	1.36	1.38	18.7	18.5	16.7	1.4	1.5	1.2	20.8	Pass
Ar2	Typic Argiudoll	72	20			1.25			15.2			1.1			19.7	Fail
Cr1	Typic Argiudoll	75	16		8	1.19		1.3	19.4		17.3	1.5		1.4	24.1	Fail
Pe	Typic Argiudoll	80	16	5	7	1.25	1.32	1.28	19	18.4	18.5	1.5	1.4	1.5	21.5	Pass
Ro4	Typic Argiudoll	80	15			1.25			18.9			1.5			19.7	Fail
AD2	Typic Argiudoll	83	20		5	1.26		1.35	19.7		20.7	1.4		1.9	23.5	Pass
Py	Vertic Argiudoll	84	15	8	7	1.22	1.38	1.28	19.4	18.6	18.2	1.6	1.5	1.4	22.0	Pass
Py1 ó Py2x	Vertic Argiudoll	87	15		5	1.28		1.29	19.8		20.5	1.5		1.9	21.6	Fail
Py2x	Vertic Argiudoll	112	13			1.22			16.3			1.2			21.2	Fail
Cal	Vertic Argiudoll	113	20		6	1.24		1.3	12.7		12.2	1.0		0.9	23.2	Fail
NT period																
Pe	Typic Argiudoll	0	10	10		1.2	1.34		20	16.4		1.9	1.6			Pass
Pe	Typic Argiudoll	0	10	10		1.22	1.35		16.8	15.7		1.5	1.4			Pass
Pe	Typic Argiudoll	0	10	10		1.22	1.34		17.1	16		1.7	1.6			Pass
Pe	Typic Argiudoll	17	5	5	10	1.16	1.3	1.33	17.2	14.3	14.0	1.4	1.2	1.0	22.6	Pass
Pe	Typic Argiudoll	17	5	5	10	1.14	1.27	1.33	21.1	16.2	14.9	1.9	1.4	1.2	22.7	Pass
Pe	Typic Argiudoll	21	5	5	10	1.16	1.33	1.34	17.6	14.2	13.8	1.5	1.3	1.3		Pass
Pe	Typic Argiudoll	21	5	5	10	1.27	1.39	1.38	19.8	14.9	14.0	2.0	1.6	1.5		Pass
Pe	Typic Argiudoll	25	5	5	10	1.20	1.39	1.38	23.6	16.5	14.9	1.6	1.4	2.3	22.4	Pass
Pe	Typic Argiudoll	25	5	5	10	1.15	1.33	1.36	19.1	14.1	13.4					Pass
Pe	Typic Argiudoll	25	5	5	10	1.19	1.31	1.38	21.1	15.2	14.2					Pass
Pe	Typic Argiudoll	29	5	5	10	1.16	1.32	1.37	22.1	16.3	15.2	2.2	1.6	1.4		Pass
Pe	Typic Argiudoll	33	5	5	10	1.02	1.33	1.36	21.6	15.9	14.9					Pass

T: tillage; NT: no tillage.

operations per year, including mechanical weed control, and sometimes carried out under conditions, which favored mineralization. Besides, we have previously suggested that soybean accelerates SOM decomposition [77]. The comparison of the SOC stocks between soybean monoculture (site A) and crop rotations (T period) 33 years after the introduction of agriculture showed a smaller SOC stock with soybean monoculture than with crop rotation (Figure 7(a)).

Another factor which could explain the changes in SOC storage after grassland cultivation is the decrease in

the amounts of crop residue additions. Our calculations show that under native vegetation, organic additions are as important as under cultivation, that is, about 3–4  $\text{Mg C ha}^{-1} \text{ year}^{-1}$ . Aboveground production is presently substantially greater than the estimated production of native grassland. Hence, apparently, this factor cannot explain the differences observed.

SON losses were more important than SOC losses, except in the case of soybean monoculture of the site A (Figure 7(b)), where the C/N ratio increased with time of cultivation



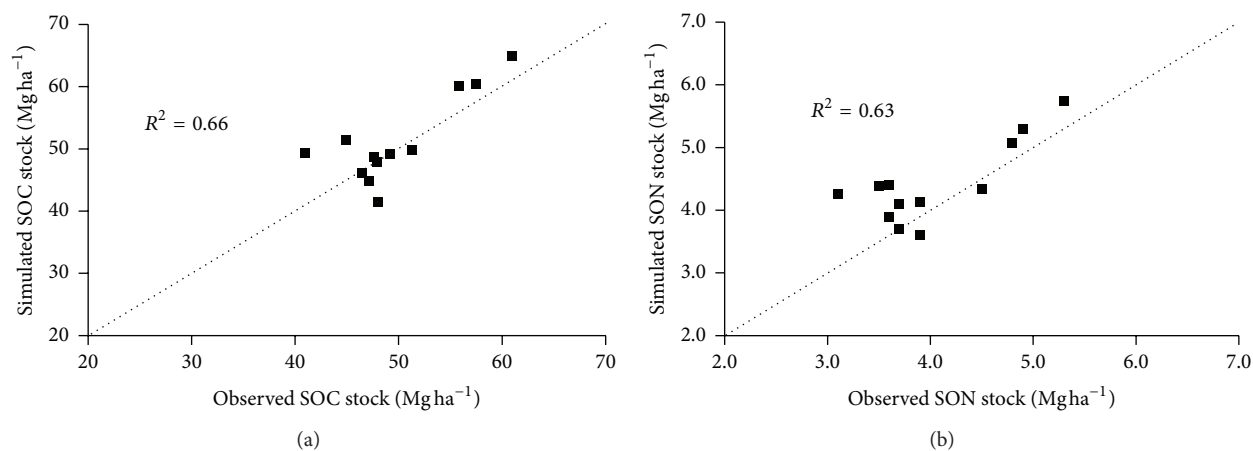


FIGURE 6: Comparison of observed and simulated SOC (a) and SON (b) in the T period.

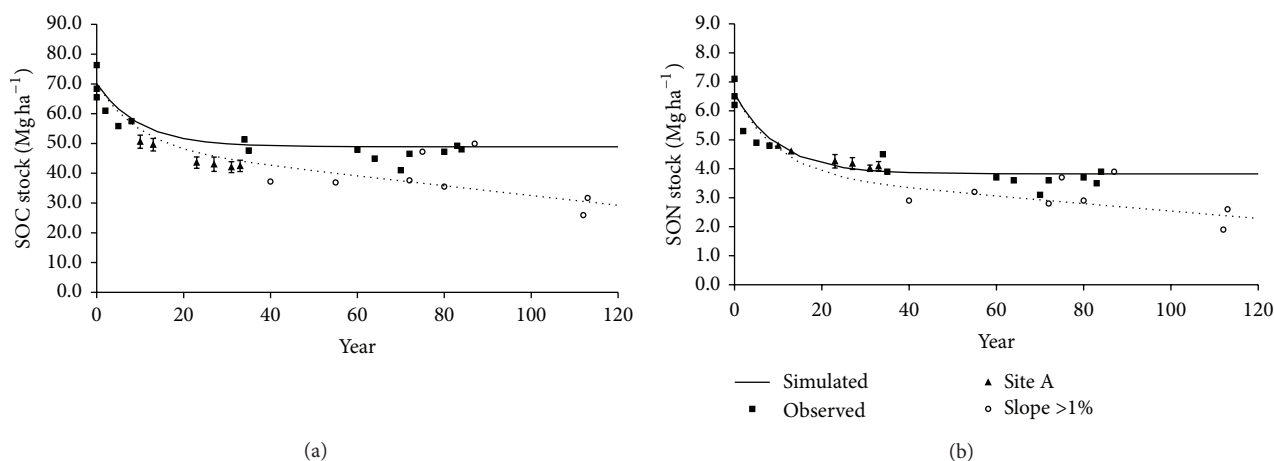


FIGURE 7: SOC (a) and SON (b) mean evolution during the T period of sites at 2500 Mg soil ha<sup>-1</sup> (solid line) and <2500 Mg soil ha<sup>-1</sup> (dotted line).

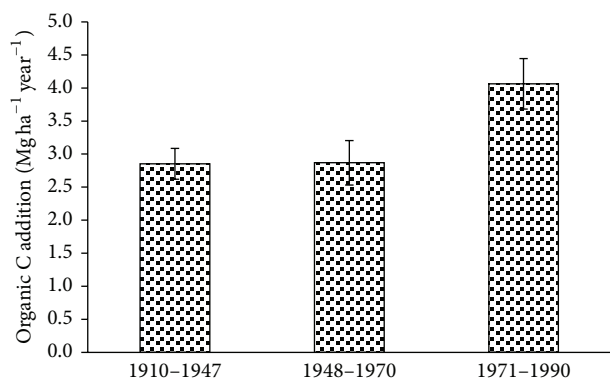


FIGURE 8: Organic C addition (Mg ha<sup>-1</sup> year<sup>-1</sup>) from three phases: manual harvest and stubble burning (1910–1947), mechanical weed control (1948–1970), and improved crop management (1971–1990).

(from 10–11 to 12–14), indicating the presence of physically protected SOM, with a low C/N ratio and a labile pool in the virgin soil with a faster mineralization rate than the rest

of SOM during cultivation. Apparently, in uncultivated soils, macroaggregation plays an important role in N protection [78–80]. Opposed trends in C/N have been reported in other studies where N fertilizers are regularly applied [2, 28]. We can assume that resistant organic fractions increased with time. Soils with a long period of cultivation would have a residual SOM that would mineralize less N. On the other hand, C/N ratios from sites with slopes >1% are not systematically greater than those from sites with slopes <1%. Therefore, we conclude that losses of N with the time under tillage were not due to erosion.

Farmer surveys not only have the advantage of their low cost but also provide the required information on equilibrium values of SOC and SON. The principal problem is that they mix sites with a different crop history. Despite the measure of the true Rolling Pampa crop history in the oldest sites, there is not the same history in the youngest sites.

Overall, the results in the Rolling Pampa after 120 years of tillage indicate that the SOM is composed of two pools: an active pool with a rapid turnover (MRT ~9 years) which represents 50% of SOC (SON) in the native prairie soil and

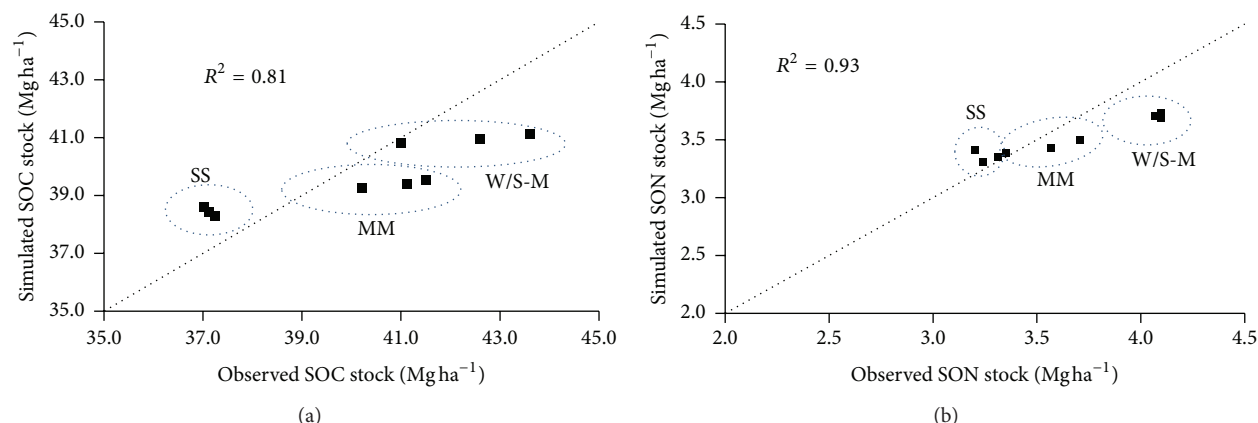


FIGURE 9: Comparison of observed and simulated SOC (a) and SON (b) in NT period. SS: soybean monoculture; MM: maize monoculture; W/S-M: doubled-cropped wheat/soybean maize.

a stable pool. SOM storage after 120 years of tillage is probably a result of an important decrease in the active fraction of a native soil, which would not be compensated by the inputs from the crops. At equilibrium, the active fraction would represent only 30% of the SOC and 20% of the SON.

In the NT period, the AMG model showed a better simulation of SOC and SON evolution than in the cultivation period (Figure 9). In this period, both the site used for calibration and that used for validation belonged to the same soil series and were implemented in similar times (1980–2012s). We could have done the calibration of this period using the long-term trials, even without sufficient information on <sup>13</sup>C measurements, but decided not to use them because there is no accurate information from the previous agricultural history cycles, corresponding to alternated agriculture and pasture cycles.

Despite the high  $R^2$  value obtained for SOC, the model tended to underestimate them in M-W/S and MM sequences and overestimate them in SS. This fate is correlated with the quantity of annual C addition: 2.9, 4.2, and 4.8 Mg ha<sup>-1</sup> year<sup>-1</sup> in SS, MM, and M-W/S sequences, respectively (Table 6). The crop sequences with more C addition tended to have higher observed SOC stocks, although observed SOC stocks in M-W/S and MM had a higher variation among years than simulated stocks. Another factor causing high variability was the size of the stable pool,  $C_s$ . The size of this pool was fixed for all the sequences evaluated (32 Mg SOC ha<sup>-1</sup>). However, it is likely that the size under NT tended to be a little higher in MM and M-W/S and relatively lower in SS. A  $C_s$  of 33.9, 32.6 and 30.4 Mg ha<sup>-1</sup> increases the  $R^2$  value to 0.92. In general, a similar behaviour was observed for SON simulations among the different crop sequences, although with better quality adjustment than with SOC. The  $k_1$  value was fixed at 0.83 for the three sequences. It is likely that in M-W/S the humification of N is higher than in SS and MM. Stemmer et al. [81] found that the mineralization of maize was delayed when straw was left on the surface and attributed it to the low contact of the residue with the soil. In MM, part of the maize stalks would be incorporated very slowly because they remain standing after the harvest and because

their nature and morphology offer a contact surface with the soil of about half of that of wheat stubble [82]. However, in M-W/S, during the W/S period, the fall of soybean leaves with annual frequency from the R6/R7 stage, which have very low C/N relationships, stimulates the breakdown of residues and becomes a nitrogen fertilization of low dose (around 25 kg N ha<sup>-1</sup>) [83] that leads to the formation of labile fractions very processed by fungal activity [84]. The  $k_1$  values of 1 and 0.63 for M-W/S and MM increased the  $R^2$  value to 0.95.

The SOC stocks at the beginning of the MM and SS (Table 6) were smaller than SOC stocks found at equilibrium of the T period (Figure 7(a)), whereas the SON stocks showed the opposite pattern (Table 6 and Figure 7(b)). Soybean conventionally tilled before to the start of monocultures probably decreased the SOC stocks but not the SON stocks as happened in the same Figure 7 at the site A.

All sequences tended to decrease the SOC and SON stocks by 5–9% and 25–27% (Table 6). The higher initial organic stocks increased further losses.

The  $C_a$  varied from 8.0, 9.0, and 13.3 Mg ha<sup>-1</sup> at the beginning to 7.2, 7.0, and 9.2 at equilibrium for SS, MM and M-W/S, respectively. When equilibrium is reached, this fraction would represent 18, 18, and 22% of SOC. It would be necessary to increase the annual C addition by 40–45% to maintain the  $C_0$  value in SS and M-W/S, respectively.

The active fraction  $N_a$  varied from 1.38, 1.43, and 1.50 Mg ha<sup>-1</sup> at the beginning to 0.63, 0.78, and 0.85 at equilibrium for SS, MM, and M-W/S, respectively. When equilibrium is reached, this fraction would represent 19, 22, and 23% of SON.

Overall, the results indicate that although the NT implementation in the rich SOM soils of the Rolling Pampa after a century of continuous agriculture contributed to a decrease in the SOM mineralization rate (overall MRT of active SOM compartment would be included in 13 years), the system also implies a low humification from harvest crop residues addition. The active fraction continued to decrease even in cases of high C and N input rates. At equilibrium, this fraction would represent between 20 and 25% of the overall SOM,

TABLE 6: Measured  $C_0$  ( $N_0$ ), annual C (N) additions,  $m$  ( $m_N$ ),  $C_{\max}$  ( $N_{\max}$ ), and  $C_{eq}$  ( $N_{eq}$ ) at equilibrium.

Crop sequence	$C_0$	$m$	$C_{\max}$	$C_{eq}$
	Mg ha <sup>-1</sup>	Mg ha <sup>-1</sup> year <sup>-1</sup>	Mg ha <sup>-1</sup>	
SS	40.5 (2)	2.8 (0.6)	0.47	37.9
MM	41.0 (2)	4.2 (1.8)	0.55	38.9
W/S-M	45.3 (0.8)	4.8 (0.8)	0.67	40.4
	$N_0$	$m_N$	$N_{\max}$	$N_{eq}$
	Mg ha <sup>-1</sup>	Mg ha <sup>-1</sup> year <sup>-1</sup>	Mg ha <sup>-1</sup>	
SS	4.05 (0.2)	0.068 (0.01)	0.057	3.3
MM	4.20 (0.2)	0.074 (0.03)	0.061	3.55
W/S-M	4.33 (0.1)	0.090 (0.01)	0.076	3.71

$C_0$  ( $N_0$ ): initial soil C (N) stock;  $m$  ( $m_N$ ): annual C (N) addition;  $C_{\max}$  ( $N_{\max}$ ): maximum humified C (N) stock;  $C_{eq}$  ( $N_{eq}$ ): soil C (N) stock at equilibrium. SS: soybean monoculture; MM: maize monoculture; W/S-M: doubled-cropped wheat/soybean maize.

corresponding to very high and low soybean frequency in crop sequences, respectively.

In 2010, we sampled fourteen new sites of Pergamino soils with a long time of continuous agriculture and at least five years under NT to obtain the SOC and NOC stocks at time zero of the future independent validation sites. The SOC and NOC values were  $42.8 \pm 3.3$  and  $3.6 \pm 0.2$  (data not shown). The AMG model previsions for the next 30 years are 15 and 20% in SOM loss in the sites where organic reserves are highest and annual C additions are 2.8 and 4.8 Mg ha<sup>-1</sup> year<sup>-1</sup>, respectively; meanwhile, SOM will remain steady and will tend to go up slightly for the same inputs in the sites where organic reserves are smallest. Using the Century model for the same region, Caride et al. [85] predicted a decrease of 15% in SOM for the next 60 years, in agreement with our results.

A strategy to balance the decreasing tendency in SOM reserves is to increase the residue addition to the soil by means of intensification of sequences, including the use of cover crops [86].

#### 4. Conclusions

The approach used to reconstitute the overall continuous agriculture history (T + NT periods) from grassland soils of the rolling Pampa was successful. During the cultivation period, the decrease in SOC and SON storage was due to two causes: a loss through biological activity and a loss through erosion. The samples were stratified (according to landscape position), allowing the selection of situations where biotransformation processes are dominant. The simple AMG model adequately simulated the evolution of SOC and SON stocks both during the T period and in the NT period when the erosion was not a major factor regarding N and C losses. The stable pool represented near 50% of total SOC and SON before cultivation and its proportion increased with the increase in agriculture time. The active fraction presented a rapid turnover during the T period (MRT = 9 years), which slowed down during the NT period

(MRT = 13 years). The environmental functions used over the active mineralization rate worked very well in tillage soils, and the values found were very close to those obtained with <sup>13</sup>C measurements. During the first twenty years, there was a loss of 27% for SOC and 32% for SON, and after that the equilibrium was apparently reached. The soils remained rich in SOM reserves. The NT applied in these conditions reduced not only the active mineralization rate but also the humification coefficient. In consequence, a slow new loss was produced: SOM storage after 140 years of continuous agriculture is probably the result of an important decrease in the active fraction, which would not be compensated by the inputs from the crops. A reduction of 20% or no reduction of the SOM reserves is expected in this region for the next 30 years with the average C (3.8 Mg C ha<sup>-1</sup> year<sup>-1</sup>) and N (0.08 Mg N ha<sup>-1</sup> year<sup>-1</sup>) annual addition, in the sites with the current highest (85.2 Mg SOM ha<sup>-1</sup> year<sup>-1</sup>) and the smallest (66.7 Mg SOM ha<sup>-1</sup> year<sup>-1</sup>) SOM, respectively.

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