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SOIL CARBON AND NITROGEN SATURATION IN CROP-PASTURE AGRICULTURAL SYSTEMS

A Dissertation in
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by
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ABSTRACT

Integrated crop-pasture systems can produce high grain yields without large inputs of external nitrogen (N). The extraction of N in grain and animal products, as well as gaseous, leaching and runoff losses should be offset by N fixation during the pasture phase of the integrated crop-pasture systems. The N would be timely delivered in moments of fast crop growth. The design of systems based on these intrinsic synergies offer a pathway for sustainable intensification of agriculture. In this dissertation, I propose that the carbon (C) and N cycling in crop-pasture rotations can be explained by soil C (C_s) saturation theory, and some degree of de-coupling of the C and N cycling. To this end, I developed hypotheses that integrate the concepts of C and N saturation. I tested these hypotheses by combining agroecosystem simulation models and soil incubations using stable isotopes tracing techniques. The systems modeled and the soils for incubations were obtained from long-term crop-pasture rotation experiments at the Instituto Nacional de Investigación Agropecuaria (INIA), Uruguay. Rotation systems that included perennial species showed greater N mineralization and immobilization when simulated with the Cycles agroecosystem model. Soil C stocks evolution and subsoil C_s distribution were more accurately simulated when the model included an algorithm for C_s saturation than when excluding it, indicating that this mechanism can explain C_s cycling. Soil sampling showed greater C_s saturation levels on the topsoil of systems including perennial species. The incubation of soils with residues enriched in ^{13}C and ^{15}N showed that C_s decomposition increased as C_s saturation increased, and C_s humification and N retention decreased. This accelerated turnover with increased C_s saturation underpins N supply in crop-pasture systems. Despite the link observed between C_s and N saturation mechanisms, some degree of decoupling was revealed by a decreasing C:N ratio of the retained or newly formed organic matter as C_s saturation increased. This lowering of the C:N ratio indicates higher N than C retention, buffering N losses in relatively C_s saturated soils. Overall, this dissertation supports soil C saturation theory and the implied reduction in the retention of C and acceleration of turnover as saturation increases, and suggests that C saturation is a fundamental pillar of the integrated crop-pasture systems function and sustainability.

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CHAPTER 1- INTRODUCTION

1.1. Crop-pasture systems and sustainable intensification

The agricultural footprint in the planet is as high as it has ever been in history, and is increasing by both the expansion of agriculture in the subtropics (e.g. Brazil, Paraguay, and Indonesia) and by the intensification of crop production in lands already under agricultural management. This two-front increase in agricultural production creates a challenge for the conservation of natural resources in the world as the demand for food, feed, fiber and bioenergy crops increases.

Sustainable intensification of agriculture requires balancing high productivity with soil, water, and biodiversity preservation (Matson et al. 1997; Cassman 1999).

One of the most successful cases of sustainable agricultural production might be integrated crop and livestock production systems that include a perennial grass-legume multi-year phase in the rotation. These systems increase landscape diversity while meeting or exceeding the performance of less diverse systems (Davis et al. 2012), and are still common in Australia (Hochman et al. 2013), and subtropical and temperate South-America (Carvalho et al. 2010, Moraes et al 2014, Franzluebbbers et al. 2014). In particular, long-term experiments established in Uruguay in 1963, 1993 and 1995 have shown that perennial pastures integrating legumes and grasses with grain cropping in a rotational cropping sequence improve physical and chemical soil properties, providing the basis for productive and diverse production systems (Díaz-Rosello, 1992a and b; Garcia-Préchac et al., 2004). Understanding the mechanisms by which integrated crop-pasture systems operate can provide pathways for the sustainable intensification of agriculture at the global scale.

Integrated crop-livestock systems diversify production and risk, reduce costs and stabilize farm economic returns (García-Préchac et al. 2004; Carvalho et al. 2010; Moraes et al. 2014). Rotation components are designed and managed to benefit from ecological synergies, where nitrogen (N) requirements of non-legume crops are partially fulfilled by biological N fixation (BNF) during the perennial pasture phase (Carvalho et al. 2010; Franzluebbbers et al. 2014; Soussana and Lemaire 2014).

In addition to economic benefits of beef or dairy production, livestock provide multiple environmental benefits through the inherent return of animal manure, enhancing soil tilth, fertility, and carbon (C) sequestration (Russelle et al. 2007). Due to the different phases of the

rotation in different fields, farms and watersheds are a mixture of annual and perennial crops that generate diverse mosaic landscapes. These landscapes contrast with the highly simplified and input-intensive agroecosystems that dominate areas like the United States Midwest with many undesired environmental outcomes, such as high vulnerability to pests and weeds, water pollution and greenhouse gas emission (Alexander et al, 2007; Matson et al. 1997; Robertson and Swinton 2005). However, in the last decades, there has been a sharp change in land use in several parts of South America. The high price for grain in combination with the technological progress of no-till agriculture enabled the establishment of continuous annual cropping under no-till, with a diminished role of the crop-pasture rotation. In this context, the local scientific community has expressed concerns about the potential damage to the soil in such pathway for agricultural intensification (Wingeyer et al 2015), and growers have perceived lower crop yields on lands that have shifted from crop-pasture rotations towards continuous cropping.

Based on growers' production records, Ernst et al (2016) has provided evidence of a reduced attainable wheat yield, i.e. that achievable with sound agronomic techniques, the longer the annual crop phase under no-till. This reduction can only be partially compensated for by additional N fertilizer which suggests that there is a structural loss in soil productivity. As reported for wheat (Ernst et al 2016), the longer the annual cropping phase the higher fertilizer N supply needed to achieve a decreasing yield. It seems that the benefits of integrated crop-pasture rotations are of major importance to sustain high yields, preserve soil productivity and reduce external inputs. No-till might be no substitute for the crop-pasture rotation.

Soil organic matter and the cycling of C, N and other nutrients underpin the sustainability of these integrated systems. Perennial pastures in the system shift C allocation from primarily ephemeral annual rooting systems with long periods without an active crop growing on the land and with most of the residues added in the top soil due to no-till, to a perennial and extensive rooting system of cool season grasses and legumes, and some warm season non-native invasive species such as Bermuda grass (*Cynodon dactylon* L.). The aim of this dissertation is to better understand the cycling of C and N in these systems.

1.2. Crop-pasture systems and internal nutrient cycling associated to soil C saturation

Integration of the crop and pasture systems allow high yielding and high N extracting grain crops without large quantities of external fertilizer N (Davis et al 2012). The reason is that biological N fixation during the pasture phase offsets N extraction with grain and animal products, as well as N

gaseous, leaching and runoff losses. Rotational systems including perennials minimize fallow periods, and provide a deep rooting system capable of reducing nitrate leaching by an order of magnitude (Randall et al., 1997). In addition, since N is delivered by microbial mineralization of residues and organic matter, it allows the system to deliver nutrients while the crops are growing rather than in one-time shot potentially minimizing the opportunity for N losses. The mechanism behind this timing or “kinetic hypothesis” has been traditionally understood as a built-up of a decomposable pool organic matter during the pasture phase that slowly decomposes during the crop phase, releasing nutrients (Díaz-Rossello, 1992a and b).

There is some logic to this concept, as the large amount of roots produced by perennial in comparison with annual species contribute three to seven times more C and N to the litter pool (Mapfumo et al. 2002, DuPont et al. 2014), building reservoirs in the soil that can be accessible to plant through microbial processes. These nutrient reservoirs are capable of conserving N in multiple pools and can contribute to an increase soil nutrient internal cycling as described by Drinkwater et al. (2008).

In the last decades, Hassink and Whitmore (1997) advanced the concept of soil C (C_s) saturation, which states that when C_s inputs are maximized, the stabilization of C from decomposing residues in organic matter is expected to approach a maximum limit or saturation content. This concept responds to the limited availability of mineral particles in the soil matrix with active surfaces for interaction where organic matter particles can be adsorbed (Baldock and Skjemstad 2000) and therefore protected from microbial enzyme access and consequent decomposition. As the mineral fraction adsorption sites are occupied by C_s inputs, it is expected that the accumulation of additional C in the mineral fraction would be reduced, with the consequent accumulation of C in labile fractions such as a particle organic matter, leading to an increase in overall decomposition rate. Consequences in C_s dynamics of approaching C_s saturation are an expected decrease in the stabilization of additional C inputs and an increase in net decomposition (Hassink et al. 1997).

Given the narrow range of organic matter C:N ratio, the concept of C saturation can be extended to N dynamics (Castellano et al., 2012). An increase in N availability in excess of N sinks in the soil system can lead to soil N saturation as described by Aber et al. (1989) for forest systems. The first stages of this process would favor plant productivity, providing enhanced N availability to satisfy plant demands, and may not necessarily cause a negative impact in ecosystem functions. A decline in the system occurs at advanced stages of N saturation, which may not be reached when N

availability increase is balanced by system harvest, or internal compensation by an adjustment in the C:N ratio of the soil reservoir.

The C and N saturation concept offers an alternative to the “kinetic hypothesis” or complimentary pathway to interpret C and N dynamics in crop-pasture rotations. It is plausible that soils in a crop pasture rotation are managed near saturation, at least in the top soil. As stated by Hassink et al (1997), as saturation increases, the turnover rate can increase (supplying more N) (Kemanian et al 2005), and the humification rate decrease reducing immobilization and increasing fresh residues N mineralization (White et al, 2014).

Applying the conceptual consequences of C_s saturation to crop-pasture systems in theory, one surmises that: (1) Soil profiles under perennial pasture may have a faster turnover of soil organic matter; (2) this dynamic may also express in deeper soil layers in systems with perennial plants compared with systems annual plant species; and (3) In addition, the fast turnover under saturated conditions may be attuned to increasing nutrient demand of growing crops.

A continuous annual cropping system would work exactly in the opposite direction, except perhaps for the topsoil. This process could lead to a slow reduction in C and N fluxes as the soil de-saturates. In fact, the concept of soil quality might be strictly related to the level of C saturation and its distribution with depth in the profile. Conceptually, C_s saturation might be one of the mechanisms behind C and N cycling in crop-pasture systems.

In this dissertation, I tested specific hypotheses derived from the concept of C_s and N saturation as applied to crop-pasture rotations using a combination of modeling and experiments that rely on dual labeling of soil organic matter with ^{13}C and ^{15}N isotopes. I propose that the C and N saturation level of a soil or a soil horizon is a critical property that has been overlooked and is important to develop sustainable agricultural systems.

1.3. Objectives

- 1) Test if the implementation of the C_s and N saturation concept can contribute to understanding variation in crop productivity and C_s accumulation in long-term experiments with contrasting frequencies of perennials as well as forage and grain crops for two decades.
- 2) Elucidate the relationship between C_s saturation and C decomposition rate for the bulk soil.

3) Elucidate the relationship between C_s saturation and the C retention or humification rate for the bulk soil.

4) Investigate the influence of C_s saturation on the decomposition and humification rate of C and N in the particulate and mineral associated organic matter fractions.

1.4. Approach

This dissertation has a modeling component and a soil incubation experiment component, both focusing on long-term experiments with contrasting crop-pasture systems in Uruguay at the Instituto Nacional de Investigación Agropecuaria (INIA-Uruguay).

In Chapter 2, I addressed the suitability of the model Cycles, which includes an algorithm to represent C_s and N saturation, for representing C_s and N dynamic in integrated crop pasture systems. Specifically, I evaluated if the consideration of C saturation allows simulating the observed dynamics of C and N as well as productivity in crop-pasture rotational systems. I use a 20-year old rotational system experiment located at the Experimental research Unit of “Palo a Pique” UEPP, Treinta y Tres, Uruguay.

The model Cycles was used for this purpose as it includes a saturation algorithm (Kemanian et al. 2005; Kemanian and Stöckle 2010). I tested if the inclusion or exclusion of the C_s saturation mechanism in the model alters the representation of both the C and N long-term evolution (20 years) and distribution with depth and crop productivity, comparing modeled and observed data for the UEPP long-term experiment. The hypotheses are that the inclusion of C saturation will enable the simulation of: (1) the long term evolution of C_s with better accuracy compared to a model that does not include C_s saturation, and (2) the distribution of C_s with depth.

In chapters 3, 4 and 5, using results from incubation experiments, I tested if soils with different saturation levels have C decomposition and retention rates consistent with hypotheses derived from C saturation theory. I did so by following the fate of C in soils incubated with variable levels of ^{13}C labeled C inputs (Balesdent et al, 1988; Personeni and Loiseau; 2004). I used two long-term experiments to cover a gradient in C_s conditions resulting from contrasting management practices in relation to crop-pasture systems. The soils were sampled from the same UEPP experiment addressed by modeling in Chapter 2 (20 years) and from a more than 50-years old experiment at INIA La Estanzuela (LE), the oldest long-term agroecosystems experiment in Latin America. The systems encompass from a 20-years old regenerated grassland at UEPP to a continuous cropping

system cultivated for 50 years without fertilizer or any amendment at LE. At both locations, A1, A2 and B horizons were sampled for incubation.

In Chapter 3, I addressed bulk C_s cycling, focusing on total soil decomposition as determined by the isotopic tracing of the old C remaining in the soil, as well as total new C incorporated in the soil from residue addition. In this chapter I tested the consequences of total C_s dynamics that can be derived from C_s saturation concept (Hassink and Whitmore 1997). I tested two hypotheses, that C_s decomposition rate should increase as the C_s saturation ratio increases, independently of the residue input rate, or in addition to any priming caused by residue inputs; and that the humification rate in the bulk soil should decrease as the saturation ratio increases, independent of the cropping history and the amount of C input.

In Chapter 4, I address the both decomposition and humification rates for particulate and mineral associated organic C. Since the C_s saturation concept specifically refers to soil organic matter (SOM) stabilization through adsorption to the mineral particles, the C retention in this fraction should decrease as saturation increases, and that of the particulate organic matter fraction should be independent of saturation.

In Chapter 5, I addressed soil N retention in the context of C_s saturation, linking the concepts of C_s and N saturation. If an N saturation mechanism is operating under C saturation, N retention should decrease as saturation increases. The stoichiometry of the C:N retained should show the level of coupling between C and N cycling.

A final chapter presents an interpretative overview of the results and the insights emerging from this dissertation in relation to both C_s and N saturation and their relation with the long-term sustainability and management of crop-pasture rotations.

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CHAPTER 2 - SOIL CARBON SATURATION, PRODUCTIVITY AND CARBON AND NITROGEN CYCLING IN CROP-PASTURE ROTATIONS

2.1. Abstract

Agricultural systems integrating perennial grass-legume pastures in rotation with grain crops sustain crop yields while preserving soil organic carbon (C_s) with low nitrogen (N) fertilizer inputs. I hypothesize that C_s saturation in the topsoil may explain the favorable C and N cycling in these systems. I simulated three contrasting crop and pasture rotational systems from a 20-year old no-till experiment in Treinta y Tres, Uruguay. The systems were: 1) Continuous annual cropping (CC); 2) crop-pasture rotation with two years of crops and four years of pastures (CP); and 3) perennial pasture (PP). I evaluated the inclusion or exclusion of a C_s saturation algorithm using the Cycles agroecosystem model. A new module was integrated in Cycles to simulate mixed grass-legume pastures and inter-seeding. Pasture forage, soybean, and sorghum grain yields were well-simulated with root mean square error (RMSE) of 1.5, 0.7 and 1.0 Mg ha⁻¹, respectively. Both field and simulation data showed a 10-year period of C_s accrual in the top 15-cm of soil for all systems (average 0.85 Mg ha⁻¹ y⁻¹), followed by a 10-year period of C losses for CC and CP (-0.64 Mg ha⁻¹ y⁻¹), and stable C_s in PP. Simulations with Cycles captured these management-driven C_s dynamics, although modeled rates of C_s change were less than those observed. The model performed better when using the C_s saturation algorithm than when excluding it (relative RMSE of 14% and 21%, respectively). When including the saturation algorithm, the model simulated well subsoil C_s distribution with depth, accrual of N during the legume-grass pasture phase along with faster N turnover and greater N availability in the subsequent grain crop phase. The results suggest that C_s saturation underpins the sustainability of crop-pasture rotations, and that modeling C_s saturation dynamics can be critical to reliably simulate complex crop-pasture rotational systems.

2.2. Introduction

Sustainable intensification of grain and livestock production requires balancing high productivity with soil, water, and biodiversity preservation (Matson et al. 1997; Cassman, 1999). Integrated crop and livestock production systems that include a perennial grass-legume multi-year phase in the rotation increase landscape diversity while meeting or exceeding the performance of less diverse systems (Davis et al. 2012). These systems are common in Australia (Hochman et al. 2013), and subtropical and temperate South-America (Carvalho et al. 2010, Moraes et al. 2014, Franzluebbers et al. 2014). Understanding the mechanisms by which integrated crop-pasture

systems operate can provide pathways for the sustainable intensification of agriculture in other regions.

Integrated crop-livestock systems diversify production and risk, reduce costs, and stabilize farm economic returns (García-Préchac et al. 2004; Carvalho et al. 2010; Moraes et al. 2014). Rotation components are designed and managed to express beneficial ecological synergies, where nitrogen (N) requirements of non-legume crops are partially fulfilled by biological N fixation (BNF) during the perennial pasture phase (Carvalho et al. 2010; Franzluebbers et al. 2014; Soussana and Lemaire, 2014). In addition to economic benefits of beef or dairy production, livestock provides environmental benefits through the inherent return of animal manure, enhancing soil tilth, fertility, and carbon (C) sequestration (Russelle et al. 2007). Due to the representation of different phases of the rotation in different fields, farms and watersheds are a mixture of annual and perennial crops that generate diverse mosaic landscapes. These landscapes contrast with the simplified and input-intensive agroecosystems that dominate areas like the Midwestern United States. Such simplified systems can produce many undesired outcomes, such as high vulnerability to pests and weeds invasion, water pollution, and greenhouse gas emission (Matson et al. 1997; Robertson and Swinton 2005; Alexander et al. 2007).

One of the keystones of crop-pasture systems is the relatively fast recovery of soil properties including both soil organic N (N_s) from BNF and soil organic C (C_s) during the perennial pasture phase (Díaz Rossello, 1992a and 1992b; Díaz-Zorita et al. 2002). This N gradually becomes available for subsequent annual crops, reducing fertilizer needs. This improved N supply / N uptake synchrony is believed to reduce nutrient losses and enable higher productivity. When crop-pasture systems in temperate South America are converted to continuous annual cropping systems, changes in N supply and other soil factors may result in a lower yield ceiling and a higher yield gap, as documented for wheat (Ernst et al. 2016).

Perennial pastures in the system can contribute to increased internal nutrient cycling capacity (Drinkwater et al. 2008) through maintenance of nutrient pools that can both conserve and provide these nutrients to plants through plant and microbe mediated processes. Perennial species can build these reservoirs because they contribute three to seven times more C and N to the litter pool than annual species through greater production of roots (Mapfumo et al. 2002, DuPont et al. 2014), and by reaching deeper soil layers (Monti and Zatta 2009).

A relatively large and continuous C and N input can maximize C_s storage given the limited capacity of C_s stabilization in silt and clay soil fractions (Hassink and Whitmore, 1997). By definition, the rate of C_s storage tends to zero when C_s approaches saturation. Under such conditions, new organic residue additions and relatively labile soil organic matter are exposed to microbial decomposition; the higher turnover rate also alters N mineralization dynamics (Castellano et al. 2012, White et al. 2014). A positive feedback between Ns supply and plant productivity on grassland soils (Baer and Blair, 2008) may accelerate progress towards C_s saturation, and the related N saturation (Aber et al. 1989, Lovett and Goodale 2011).

Alternatively, pasture C_s reservoirs can also act as N sinks, buffering against N losses even after years of N enrichment (Baer and Blair 2008). Large amounts of N can be immobilized quickly, retaining N in pools that are not readily accessible for microbial re-mineralization, leaching, and gaseous losses (Kaye et al. 2002). Soils in the pasture phase of the rotation have some of the attributes of grassland soils, and it is reasonable to assume that several desirable attributes of these systems derive from soil organic C and N cycling in a soil matrix with at least partial C saturation.

While traditionally the benefits of pastures in agricultural systems have been ascribed to diversification through livestock production, erosion control, and BNF, maintaining some soil layers near C_s saturation might be one of the critical services provided by perennials in the system. Despite its importance, modeling C and N saturation in systems that include pastures has not been previously attempted. Understanding the mechanisms by which C and N saturation operate and contribute to the long-term sustainability of crop-pasture rotations is fundamental to inform land management decisions towards sustainable intensification pathways.

The goal of this work is to test if the inclusion of C saturation in an agroecosystems simulation model allows more accurate modeling of the observed dynamics of C and N and plant productivity in crop-pasture rotational systems. I report C_s dynamics and crop and pasture performance from a 20-year experiment in Treinta y Tres, Uruguay with three rotational systems, and compare these results with those obtained with the simulation model Cycles. The cropping systems are an annual continuous system, a crop-pasture rotation, and a perennial pasture that have not been tilled since the beginning of the experiment. I tested if the inclusion or exclusion of a C_s saturation mechanism in the model Cycles altered the representation of both the C and N long-term evolution and crop productivity. Our hypotheses are that the inclusion of C saturation will enable

more accurate simulation of: 1) the long-term evolution of C_s ; and 2) the distribution of C_s with depth.

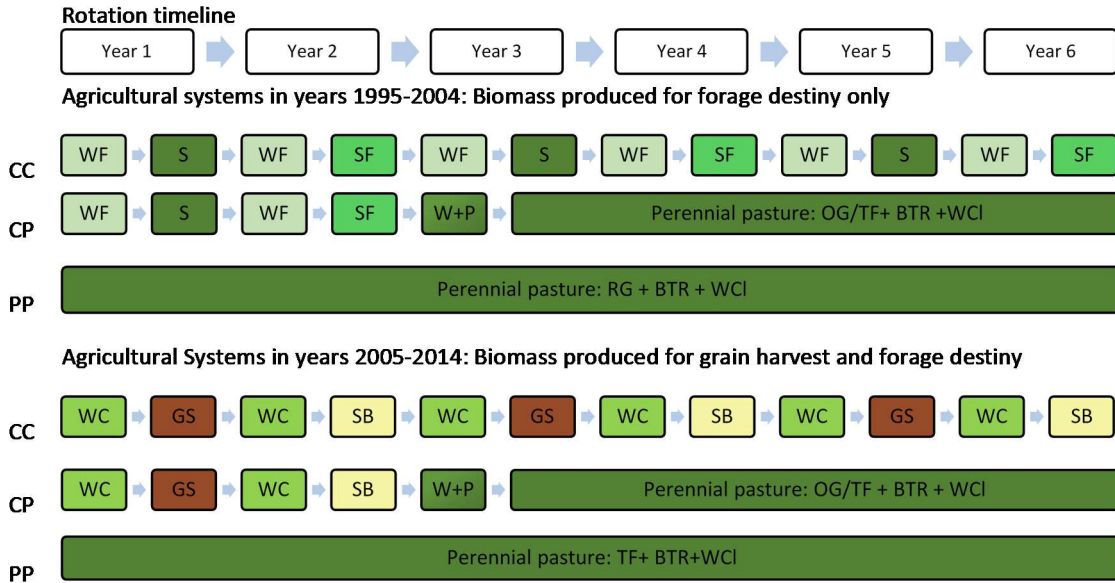
2.3. Methods

2.3.1. Long-term experiment

In 1995, the National Institute of Agricultural Research of Uruguay (INIA) established a long-term crop-pasture rotational experiment at the Palo a Pique Experimental Unit (UEPP; 33°15'44 S, 54°29' W). The UEPP is located in the South American subtropical humid region of the Pampas and Campos Biomas. The climate is mesothermic humid with a mean daily temperature of 23 and 11 °C in summer and winter, respectively, and a mean annual rainfall of 1300 mm distributed on average uniformly throughout the year, but with large inter-annual variations (Table 2-1). The research site has a 3% average slope. The loam soils are Oxyaquic Argiudolls according to USDA Soil Taxonomy, have moderate fertility with a well-developed Bt horizon. The soil has no rocks.

The site had a short history of soybean cropping with conventional tillage in the 1980s followed by a pasture of *Lolium multiflorum*, *Lotus corniculatus* and *Trifolium repens* invaded after few years by native and exotic species, mainly *Cynodon dactylon*. By the time the rotational experiment was started in 1995, the vegetation had transitioned back to the native grassland mixed C_3 and C_4 species composition. We used three of the four rotational systems compared under no-till: 1) CC: continuous cropping rotation of two years; 2) CP: six-year crop-pasture rotation that alternates four years of grass-legume pasture with two years of annual crops identical to CC; and 3) PP: perennial pasture of introduced perennial legumes and grasses that was renewed every six years (killed with glyphosate and no-till re-planted). All entry points of the crop-pasture system are present in the field every year (six fields) while there is only one field for the continuous cropping rotation, and one field for the perennial pasture, for a total of eight different fields. The average field size was originally 6 ha, allowing for cattle grazing within the fields.

The management of the rotational system varied over the years and a detailed record has been kept for each field. From 1995 to 2004, the systems were mostly cattle grazed and hayed. In 2005, fields were split in two and in one of the 3-ha halves annual grain crops substituted for annual forage crops. The data and simulations presented here focus on the fields that had grain production since 2005. The species planted in each system for each period are listed in Figure 2-1.



References: CC: continuous cropping, CP: crop-pasture, PP: permanent pasture, WF: winter forage crop for grazing: annual ryegrass (*Lolium multiflorum* Lam.), oat (*Avena sativa*) or wheat (*Triticum aestivum* L), S: sorghum (*Sorghum bicolor* L. Moench) for grazing, SF: summer forage crops for grazing or hay: sudangrass (*Sorghum x drummondii* (Steud.) Millsp. & Chase) or foxtail millet (*Setaria italica* (L.) P. Beauv.), WC: winter cover of ryegrass, avena or grain wheat, OG: orchardgrass (*Dactylis glomerata* L.), TF: tall fescue (*Lolium arundinaceum* (Schreb.) S.J. Darbyshire, or *Festuca arundinacea* Schreb.), BTR: birdsfoot trefoil (*Lotus corniculatus* L.), WCI: white clover (*Trifolium repens* L.), Rg: annual ryegrass, GS: Grain Sorghum, SB: Soybean (*Glycine max*). W+P: grain wheat interseeded with perennial pasture. Plus sign indicates interseeded species. Dash sign indicates alternative planting.

Figure 2-1. Cropping sequences at the rotation experiment. In the first decade (1995-2004), crops were produced for forage agriculture only, while the last decade (2005-2014) included both forage and grain cropping in CC and CP systems.

2.3.2. Weather database

Maximum and minimum daily temperature, dew point temperature, wind speed and downward solar radiation were taken from the closest weather station at Paso de la Laguna Experimental Unit, 30 km away from UEPP experimental site. Rainfall was recorded at the experimental site. Records from an automatic weather station located at UEPP during 2012-2014 indicated that the daily weather at these two locations was very similar. Solar radiation was available at Paso de la Laguna, although there were gaps in the data series. Existing records showed substantial agreement with those available at NASA-POWER; the latter database was used throughout the years for consistency.

Table 2-1. Weather monthly means for Palo a Pique experimental site between 1995 and 2014.

Month	Solar Radiation MJ m ⁻² d ⁻¹	Precipitation mm month ⁻¹	Penman-Monteith Reference ET mm month ⁻¹	Temperature			Wind speed m s ⁻¹
				Maximum	Minimum	Dew Point	
				----- °C -----			
January	24.8	91	182	30	17	14	2.5
February	20.7	131	137	28	17	14	2.3
March	17.7	104	124	27	15	13	2.1
April	13.3	128	85	23	12	9	1.9
May	9.9	133	60	20	8	5	1.7
June	8.2	116	50	17	6	3	1.9
July	9.0	96	58	16	6	3	2.2
August	11.7	103	75	18	7	4	2.3
September	15.6	105	96	19	8	5	2.7
October	19.3	100	128	23	11	8	2.8
November	23.7	86	157	26	13	10	2.7
December	25.3	106	180	28	15	12	2.6

2.3.4. Soil description

A complete description of the Oxyaquic Vertic Argiudolls at the UEPP site was available from soil survey records (Durán et al. 2006). This soil profile description named 'Uruguay Site 13' at the 1996 field survey was used to build the model soil input file (Table 2-2). Soil hydraulic properties were estimated within Cycles using pedotransfer functions (Saxton and Rawls 2006). Textural homogeneity in the top 18 cm reflects the history of tillage. Soil sampling of the top 15 cm of each field at the beginning of the long-term experiment in 1995 provided C_s information to initialize the eight simulated fields. Deeper soil layers were initialized with the same soil organic matter for all systems (Table 2-2). We also used the same bulk density for all treatments in the top 15 cm, despite differences in organic matter. That may overestimate (underestimate) the initial C_s mass in the top layer of the fields with the highest (lowest) C_s concentration. The saturation ratio calculation is described below.

Table 2-2. Soil properties at the beginning of the experiment.

System	All	All	All	CC	CP	PP	CC	CP	PP
Depth	Clay	Sand	Bulk density	Soil organic matter			Soil carbon saturation ratio		
cm	%	%	Mg m ⁻³	----- g kg ⁻¹ -----			----- kg kg ⁻¹ -----		
0 to 4	20.7	39.2	1.16	33.4	43.2	33.6	0.67	0.87	0.68
4 to 9	20.7	39.2	1.16	23.4	30.1	25.5	0.47	0.61	0.51
9 to 18	19.6	39	1.39	20.8	26.8	22.7	0.42	0.55	0.46
18 to 41	46.9	24.4	1.39	15.4	15.4	15.4	0.23	0.23	0.23
41 to 61	48.3	22.2	1.34	7.4	7.4	7.4	0.11	0.11	0.11
61 to 71	45.8	22.8	1.37	5.2	5.2	5.2	0.08	0.08	0.08
71 to 99	40.4	28.5	1.42	2.6	2.6	2.6	0.04	0.04	0.04
99 to 125	44.4	37.2	1.73	2.1	2.1	2.1	0.03	0.03	0.03
125 to 141	47.3	39.3	1.55	1.7	1.7	1.7	0.03	0.03	0.03
141 to 172	32.6	48.6	1.55	0.7	0.7	0.7	0.01	0.01	0.01
172 to 190	28.9	55.7	1.57	0.3	0.3	0.3	0.01	0.01	0.01

CC: continuous cropping rotation of two years; CP: six-year crop-pasture rotation that alternates four years of grass-legume pasture with two years of annual crops; PP: perennial pasture of introduced perennial legumes and grasses.

2.3.5. Agroecosystems model

All systems were simulated using the Cycles agroecosystems model. Cycles is a process-based, multi-year, multi-crop, and multi-soil layer simulation model that runs at a daily time step, with hydrology simulated with an adaptive sub-daily time step. Several modules of Cycles have been incorporated from CropSyst (Stöckle et al. 2003) and C-Farm (Kemanian and Stöckle 2010). The fundamental heat and water transport algorithms are adapted from Campbell (1985). Cycles has modules to represent plant growth based on radiation and transpiration use efficiency (Stöckle et al. 2008), coupled C_s and N_s cycling (Kemanian et al. 2005; Kemanian and Stöckle 2010; White et al. 2014), soil water infiltration and redistribution, and the effects of management practices on biogeochemical processes, although it does not specifically account for soil erosion losses. Cycles can simulate monoculture rotations, polycultures and relay crops. The inputs required to run Cycles are: 1) latitude, elevation, and daily weather data; 2) layer-by-layer initial soil profile properties (layer thickness, texture, bulk density, hydraulic properties, organic matter); 3) crop sequence; and 4) management operations (fertilization, irrigation, residue addition, tillage, harvest).

2.3.5.1. Soil organic carbon cycling simulation

The C_s dynamics module in Cycles considers the effect of C_s saturation capacity (Hassink and Whitmore 1997) in C stabilization. When microbes decay, a fraction of the microbial C loss is added to the C_s pool. This fraction is the humification coefficient in the Hénin and Dupuis (1945) representation of the C_s gain due to plant residue decomposition. The term humification acknowledges the C transfer from the residues pool to the soil organic matter pool (without any implications on its chemical composition as “humic” substances). In Cycles, this coefficient depends on the saturation ratio, i.e. the amount of organic C in the soil in relation to a putative C saturation capacity, or C_x (kg C kg⁻¹soil), sensu Hassink and Whitmore (1997):

$$C_x = 0.021 + 0.038 f_c \quad (1)$$

Where f_c is the fractional clay concentration of that layer.

When the soil is near saturation, the fractional stabilization of microbial detritus into C_s or humification rate approaches zero (Figure 2-2) using the following functional form:

$$h_c = h_x \left[1 - \left(\frac{C_s}{C_x} \right)^n \right] \quad (2)$$

where h_x is the maximum humification rate as a function of clay and h_c is the operative humification rate. Further discussion of the implications of this structure can be found in Kemanian et al (2011) and White et al (2014). Unique to Cycles, is the use of the saturation ratio to regulate the soil organic matter turnover rate, k_s (d⁻¹):

$$k_s = f_e f_t k_x f_s \quad (3)$$

$$f_s = 1 - 1 / \left[1 + \left(\frac{C_s / C_x}{0.22} \right)^3 \right] \quad (4)$$

where f_s is a factor that accelerates the turnover as the saturation ratio increases, f_e is the environmental factor (0-1), f_t is the tillage factor (>1), k_x is the maximum undisturbed C decomposition rate (0.00015 d⁻¹).

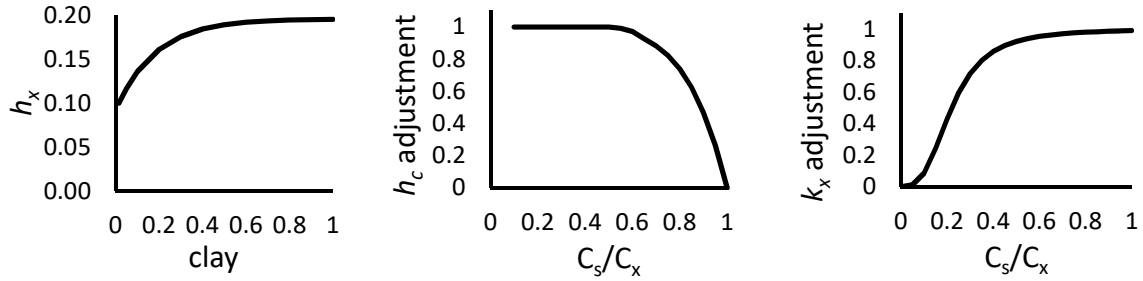


Figure 2-2. Humification rate (h_x) relationship to soil clay fraction and adjustment factors for h_c and decomposition rate (k_x) as a function of soil carbon saturation ratio (C_s/C_x).

2.3.5.2. Algorithm without carbon saturation

In order to test if saturation improves C_s dynamics simulation, I modified Cycles to represent the traditional non-saturating approach by setting $h_c = h_x$ and $f_s = 1$:

$$\frac{\partial C_s}{\partial t} = h_x C_i - f_e f_t k_x C_s \quad (5)$$

The cycling of N is coupled to that of C as presented by White et al. (2014), which includes explicitly the microbial pool.

2.4.3. Plant competition module

A new module was introduced in Cycles to simulate multi-species plant communities. This module enabled simulating grass-legume mixed pastures, and dual purpose and nursing crops. Wheat for instance is underseeded with perennial pasture species, can be grazed by beef cattle, and later harvested for grain while the perennial pasture remains. Each plant component has species-specific physiological parameters. The competition and acquisition of resource (i.e., water, nutrient, and solar radiation) among species as well as the community botanical composition are emergent properties of the interaction of species, environment and management. Algorithms for light interception in the competition module are presented in Appendix A.

2.3.5.3. Pastures and crop production calibration and evaluation

Parameters for forage and grain species were calibrated from information compiled from several sources (Table 2-3), and are provided for each of the 13 species in Appendix B.

Simulated forage yields were compared to field data from the experimental site reported by Terra et al. (2001), Rovira and Bermudez (2003), and measured by Pravia in the years 2008-2011

(unpublished). Pastures forage yield was summarized on an annual basis from comparison with the model, for a total of 28 field-year combinations through the 20 years of simulations. For grain crops, 12 soybean and 13 sorghum field-years were available for model evaluation.

Table 2-3. Sources of information for crop parameters of control.

Control Parameters	Crop species	Reference
Physiological relationships		
Radiation use efficiency	C3 and C4, perennial and annual	Stöckle and Kemanian, 2009.
	Perennial forage legumes	Riesinger et al. 2009; Torssell et al. 2007
Responses to temperature	Perennial forage legumes	Bowley et al 1984; Frame and Newbould 1986.
Stomatal conductance	C3 annual and perennial forage grasses	Holloway-Phillips and Brodribb 2011a and 2011b.
Nitrogen dilution curve	C3 and C4, perennial and annual	Lemaire and Gastal, 1997.
Local records		
Thermal time for phenology (Anthesis and maturity date when applicable), and forage and grain yield	Perennial forage legumes: white clover and birdsfoot trefoil	Díaz Lago et al 1996, Ayala et al 2010
	Perennial and annual C3 grass forages	García 2003, Ayala et al 2010, INIA-INASE National evaluation of cultivars
	Forage sorghum	National evaluation of cultivars (INASE-INIA)
	Sweet sorghum	Terra et al, 2007
	Foxtail millet	Terra et al, 2000
	Spring wheat and forage wheat	National evaluation of cultivars (INASE-INIA)
	Grain sorghum	Pravia et al 2008, National evaluation of cultivars (INASE-INIA)
	Soybean	National evaluation of cultivars (INASE-INIA)

2.3.6. Soil organic carbon model evaluation

2.3.6.1. Soil organic carbon evolution

Soil organic carbon model performance was evaluated using data from 15-cm deep and 2.5-cm diameter soil samples taken from each field in each year. Independent soil profile samples were taken in 2014 and 2015 using a soil core hydraulic cylinder probe of 4.7-cm diameter and 80-cm long. Forty soil profiles were sampled in 2014 on the middle slope topographic position of each

rotation, separating diagnostic soil horizons in the field (A1: 0-6 cm, A2: 6-18 cm, B: 18-40 cm). Samples were bulked by diagnostic horizon for laboratory analyses. In 2015, 60-cm deep soil samples were taken using the same probe. Samples were separated in the field at 15-cm depth increments at three topographic positions for each rotation. For calculation of C_s stocks, we used the average bulk density of these samples in the top 15 cm of soil for both observed and simulated data (1.33 Mg m⁻³). Below 15 cm we used the bulk density information reported in Table 2-2. Soil physical properties, particularly the hydrological properties, were kept constant during the simulations to isolate the putative effects of C and N cycling on crop performance.

2.3.6.2. Model statistical evaluation

Model performance was evaluated using the root mean square error (RMSE), relative root mean square error (rRMSE) and determination coefficient (r^2) between observed and simulated data. Rotational system and year effects on observed C_s were tested with an analysis of variance. All analyses used the R software (R Core Team, 2013).

2.4. Results

2.4.1. Forage and grain yield

There was good agreement between simulated and observed forage and grain yield. The model simulated forage yield fairly well, capturing both high and low yields, with a mild tendency to overestimate forage production in the 6 to 12 Mg ha⁻¹ y⁻¹ band (Figure 2-3). Soybean and sorghum grain yields were satisfactorily simulated, although three simulated soybean yields were much higher than those observed. These low observed yields on seasons of higher simulated yield potential correspond to the first two seasons of soybean in the long-term experiment, when insect damage reduced yield. It is noteworthy that each pairing of observed and simulated yield was obtained from the actual rotation sequence and reflects both the weather conditions during the corresponding growing season and the cumulative effects due to the corresponding crop rotation. (the model was not re-initialized every year). Considering the simulated forage and grain yield as proxies of total biomass production, the results indicate that the model represented well the C inputs to the soil.

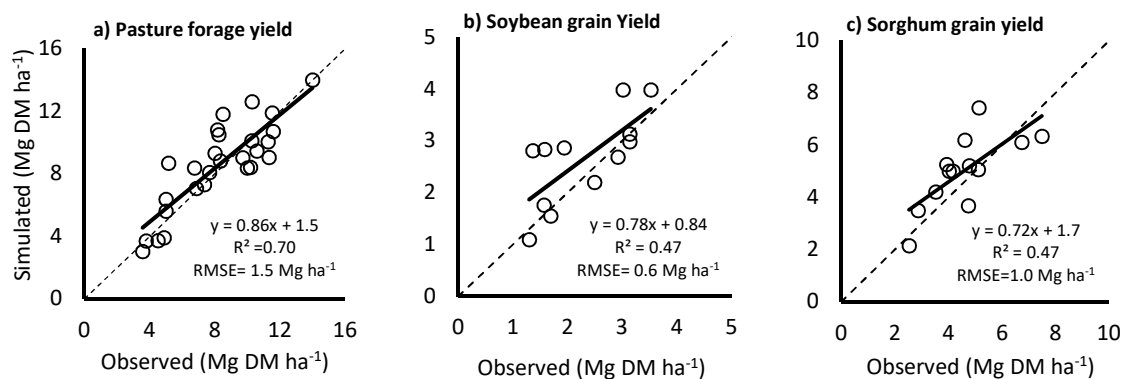


Figure 2-3. Comparison of simulated against observed pasture yield from long term experiments at Palo a Pique experimental unit (INIA Treinta y Tres, Uruguay), showing the 1:1 line and fitted regression.

In particular, the multispecies module allowed Cycles to simulate mixed pastures yield with $r^2 = 0.7$ and RMSE 1.5 Mg ha^{-1} , while O’Leary et al. (2016) reported an $r^2 = 0.48$ and RMSE 2.8 Mg ha^{-1} for pastures growth simulated over eight years using the APSIM-Agpasture module in Hamilton, Victoria (Australia).

2.4.2 Soil C stocks

The C_s evolution during the 1995 – 2014 period showed a time by system interaction (Table 2-4). There were two periods with distinctive time trends for C_s : increasing C_s in all systems from 1995 to 2004, ($P < 0.10$) and a subsequent decrease from 2004 to 2014 in the systems with annual crops in the rotation (Table 2-5). The C_s stock increases in the first period were 0.6 and 0.7 for CC and CP, and $1.3 \text{ Mg ha}^{-1} \text{ y}^{-1}$ for PP. In the second period, CC and CP lost C_s at a similar rate (respectively -0.7 and $-0.6 \text{ Mg ha}^{-1} \text{ y}^{-1}$), while C_s under PP remained stable. Over the 20-year period, PP was the only system that accrued C_s ($0.4 \text{ Mg ha}^{-1} \text{ y}^{-1}$); under CC and CP it decreased at an overall rate of -0.3 and $-0.2 \text{ Mg ha}^{-1} \text{ y}^{-1}$ (Table 2-5).

Table 2-4. Analyses of variance of the top 15 cm of soil carbon observed stocks for the period 1995-2014.

Effect	Degrees of freedom	F value	Pr(>F)
System	2	10.2	< 0.01
Year	1	5.4	< 0.03
Year quadratic term	1	29.9	< 0.01
System-year interaction	2	6.4	< 0.01
System-year interaction quadratic term	2	0.3	< 0.75
Error term ($17.5 \text{ Mg}^2 \text{ ha}^{-2}$)	120		

Table 2-5. Rate of change of soil carbon stocks in the top 15 cm of soil for the three long-term rotation systems between 1995 and 2014 ($\text{Mg ha}^{-1} \text{y}^{-1}$). Saturation and no saturation refers to the inclusion or not of the soil carbon saturation algorithm in Cycles model.

System	Observed C_s rates of change		Modeled C_s rates of change	
	----- $\text{Mg ha}^{-1} \text{y}^{-1}$ -----	p > F	Saturation ----- $\text{Mg ha}^{-1} \text{y}^{-1}$ -----	No saturation
	1995-2004			
Continuous Cropping	0.57	< 0.09	0.37	0.76
Crop Pasture	0.65	< 0.09	-0.03	0.42
Perennial Pasture	1.34	< 0.01	0.25	0.59
	2004-2014			
Continuous Cropping	-0.66	< 0.06	-0.17	0.01
Crop Pasture	-0.62	< 0.05	-0.15	0.30
Perennial Pasture	-0.37	< 0.50	-0.01	0.58

Although the measured data show high variability among samples, the simulations tracked reasonably well the general temporal trend of each rotational system (Figure 2-4). The CC lost C_s in the second half of the period considered, while PP remained almost stable. In these cases, the C_s from the last sampling (closed circle in Figure 2-4) agreed well with the simulation result. For the CP rotation, both Cycles modeled and measured data show a mild C_s decrease. Owing to a higher h , the no-saturation model accumulated C in the top 15 cm. When compared to measured data in the top 15 cm of soil, Cycles simulations with the saturation algorithm showed a better fit than the no-saturation algorithm. Overall the systems, the saturation and non-saturation algorithm had a respective RMSE of 4.7 and 6.8 Mg ha^{-1} , or rRMSE of 14% and 21% (Table 2-6). The RMSE obtained with the saturation algorithm in Cycles compares well with the RMSE of the statistical model ($17.5^{1/2} = 4.2 \text{ Mg ha}^{-1}$; Table 2-4).

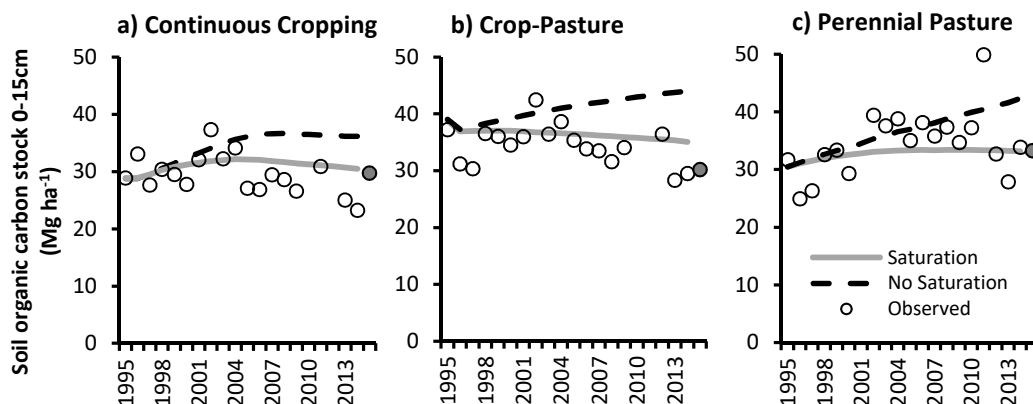


Figure 2-4. Observed and modeled soil organic carbon evolution in the top 15 cm of soil for three rotational systems at Treinta y Tres, Uruguay. (Saturation: simulated organic C stocks with Cycles model (includes C_s saturation algorithm); No Saturation: simulated stocks excluding C_s saturation algorithm. The closed circle in 2014 comes from an independent soil sample and averages 40 profiles).

Table 2-6. Comparison of observed and modeled soil organic carbon in the top 15 cm of soil for three rotational systems. Saturation and no-saturation refers to the inclusion or not of the saturation algorithm in the Cycles model.

System	RMSE		rRMSE	
	Model		Model	
	Saturation	No saturation	Saturation	No saturation
	----- Mg ha ⁻¹ -----		----- % -----	
Continuous Cropping	3.8	6.8	13	21
Crop Pasture Rotation	5.0	8.0	14	23
Permanent Pasture	5.3	5.5	15	19
Mean	4.7	6.8	14	21

When considering the entire soil profile, both the model with and without saturation predicted losses of C in the 20-year period, but the losses were much larger in the model without saturation (Table 2-7). In addition, the losses were distributed differently with depth. The model with the non-saturation algorithm predicted an increase in C_s in the top soil, but sharp losses in the subsoil. The model with the saturation algorithm preserved the subsoil C_s relatively stable (Figure 2-5).

Table 2-7. Profile soil organic carbon at the beginning and cumulative change in the stock at the end of the 20-year simulation for three rotational systems in Treinta y Tres, Uruguay. Saturation and no-saturation refers to the inclusion or not of the saturation algorithm in the Cycles model.

System	Initial stock	Twenty-years change in organic soil carbon	
		Model	
	---- Mg ha ⁻¹ ----	Saturation	No saturation
Continuous cropping	96	-3.7	-9.2
Crop pasture rotation	109	-6.5	-8.7
Permanent pasture	100	-0.7	-3.0

Comparing initial C_s contents to that in the 0-60 cm soil layer (2015 sampling, Macedo and Terra, unpublished), C_s distribution at 15 cm sampling interval shows increasing stratification only in PP, with greater topsoil C_s (19.6 g C soil kg⁻¹, P<0.10) compared to CP (14.9 g C soil kg⁻¹) and CC (12.1 g C soil kg⁻¹). Over all systems, final C_s distribution in the soil profile shows better agreement with the model with the saturation algorithm than with the non-saturation model, with respective RMSE of 0.15 vs 0.21 Mg ha⁻¹ of C_s.

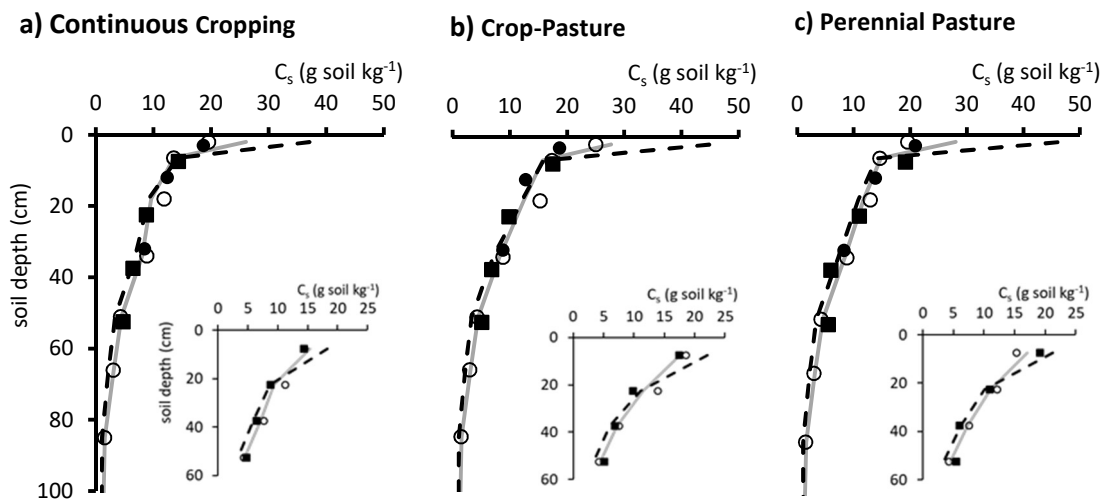


Figure 2-5. Soil organic carbon (C_s) distribution in the soil profile for the three rotation systems evaluated. Open circles represent C_s content in the soil profile survey in 1996. The closed circles represent C_s from samples taken on soil diagnostic horizons at the end of the simulation period, in 2014. Closed squares represent observed C_s on samples taken every 15 cm depth increments by Macedo and Terra in 2015 (unpublished). Grey solid lines represent C_s at the end of the 20 y simulation with Cycles model including saturation. Black broken lines represent C_s at the end of the 20 y simulation with no saturation algorithm. Inset figures show data at 15 cm depth increments up to 60 cm of soil depth.

2.4.3. Nitrogen balance

All systems lost N throughout the simulation period, in the order of 10 to 30 kg ha⁻¹ y⁻¹ of N for CC and CP and 3 kg ha⁻¹ y⁻¹ of N for PP (Table 2-8). Due to greater decomposition rate in deep soil layers, the losses of N in the simulation with the non-saturation algorithm were larger, in the order of 40 kg ha⁻¹ y⁻¹ of N for CC and CP and 10 kg ha⁻¹ y⁻¹ of N for PP. In CC, synthetic fertilizer was the main N input, while in the two systems with pastures it was BNF. The dominant losses were grain and forage harvest in CC, and N gaseous losses in the systems with pastures. Internal fluxes of N mineralization and immobilization were lower in CC, and increased with the pasture component in the rotation (Table 2-8).

Table 2-8. Simulated nitrogen balance. All components expressed in kg ha⁻¹ y⁻¹ of nitrogen. Saturation and no saturation refers to the inclusion or not of the soil carbon saturation algorithm in the Cycles model.

System	Saturation model			No Saturation model		
	CC	CP	PP	CC	CP	PP
N inputs						
Mean N fertilizer inputs	78	28	0	78	28	0
Biological Nitrogen Fixation (BNF)	34	56	72	31	52	64
Total inputs	112	85	72	109	81	64
N fluxes						
N mineralization	258	320	309	332	355	355
N immobilization	60	61	75	84	84	101
N outputs						
NO ₃ leaching	6	5	0.1	17	10	1
N gaseous losses (Includes N ₂ +N ₂ O+NH ₃)	41	59	75	47	57	72
N in harvest	77	50	0	82	50	0
Total outputs	124	114	75	147	117	73
Balance	-12	-29	-3	-38	-36	-9

CC: continuous cropping rotation of two years; CP: six-year crop-pasture rotation that alternates four years of grass-legume pasture with two years of annual crops; PP: perennial pasture of introduced legumes and grasses

2.5. Discussion

The control of both the humification rate and turnover rate through saturation in the model Cycles rendered a realistic representation of C_s gain and loss throughout the soil profile. When the model used the non-saturation algorithm, the C_s evolution with depth drifted away from that observed (Fig.5). There are two main controls in the saturation algorithm that explain this result. First, the reduction in *h* (the effective humification rate) in the topsoil due to higher C content (observed C_s/C_x range 0.67-0.87), prevented further accumulation of C_s. Second, the reduction in *k* (the

microbial decomposition rate) slowed down C_s decomposition in the subsoil ($C_s/C_x < 0.4$) and preserved the typically quasi-exponential decrease in C_s with depth (Fig. 2-5). Other models control this shape with forcing functions or by defining a vertical structure with a layer-specific k (Oleson et al. 2013). In Cycles, the shape of the C_s profile is an emergent property when including the saturation algorithm. One could argue that reducing h in the non-saturating model would prevent the simulated C_s accumulation in the topsoil. While this is true, this will cause an even more accelerated loss of C in the subsoil. Thus, it seems that the consideration of C saturation as a variable controlling not only h (Hassink and Whitmore 1997; Stewart et al. 2008) but also k is a necessary model property and a useful innovation in Cycles.

The observed C_s evolution in the top 15-cm of soil throughout the course of two decades of contrasting rotations showed that CC systems lost C_s at a rate of $-0.3 \text{ Mg ha}^{-1} \text{ y}^{-1}$ of C, while PP systems accumulated C_s at a rate of $0.4 \text{ Mg ha}^{-1} \text{ y}^{-1}$ (Figure 2-4). This long-term dynamic was adequately simulated with Cycles (RMSE = $3.8 \text{ Mg C ha y}^{-1}$, Table 2-6). Sharp year to year variations in measured C_s indicate that it can be difficult to track the short term variation in C_s , most likely due to year to year changes in sampling depth.

Cycles performance simulating C_s evolution compares well with that obtained with other agroecosystem models used in grassland or pasture soils. For example, simulating the C_s evolution in long term grazed pasture experiments in Hamilton, Australia, with APSIM-Agpasture resulted in a RMSE of $4.9 \text{ Mg ha yr}^{-1}$ (rRMSE 5%) (O'Leary et al. 2016). In simulations of grazed perennial pastures in Waga Waga, Australia C_s evolution estimated with RothC before and after inverse modeling calibration resulted in RMSEs of 3.2 (rRMSE 8%) and 2.1 (rRMSE 5%) Mg C ha^{-1} (Liu et al. 2011). In Brazil, simulations with the CENTURY model of top C_s in pastures that followed deforestation resulted in rRMSE of 16% (Cerri et al. 2004).

Potential losses of soil and C_s via erosion were not accounted for in the simulations. In the second half of the period considered, erosion could have been higher in CC than in other systems due to the higher frequency of soybean. Runoff plots installed by Terra and García-Préchac (2001) at the UEPP were used to calibrate USLE/RUSLE factors (Wischmeier and Smith, 1978; Renard et al., 1997) and integrated into the software EROSION 5.9 developed for Uruguay (García-Préchac et al. 2005). Calculations of erosion with this software in the soybean-cover crop sequence returns soil losses of $13 \text{ Mg ha}^{-1} \text{ y}^{-1}$. Multiplying this soil loss by the initial topsoil C_s concentration on CC returns C erosion losses of $0.43 \text{ Mg ha}^{-1} \text{ y}^{-1}$, matching observed C_s losses in CC. Although the RUSLE

simulation has a higher frequency of soybean than our simulations, erosion may explain why the simulated losses by Cycles seem to be lower than those observed (Figure 2-4). While the variability in the measured C_s exceeds the magnitude of the calculated erosion, it is clear that both erosion and C_s cycling need to be accounted for jointly to model C_s storage rates accurately.

Soil N inputs in the three systems studied ranged from a maximum modeled estimate of $112 \text{ kg ha}^{-1} \text{ y}^{-1}$ of N with 70% as synthetic fertilizer, to a minimum of $72 \text{ kg ha}^{-1} \text{ y}^{-1}$ on PP relying only on legumes BNF. The N recovery efficiency in harvest calculated considering all inputs was 0.7 and 0.53 for CC and CP. These efficiencies compare well with a mean of 0.35 (range 0.20-0.49) in the European Union (Godinot et al. 2016) and 0.5 in US (Conant et al. 2013), and with a mean of 0.37 for fertilizer N inputs in Midwest maize systems (Cassman et al. 2002). Considering only N fertilizer inputs, the ratio of N recovered in harvest to synthetic N fertilization was 0.99 and 1.72 for CC and CP, highlighting the critical importance of BNF on the N budget of these systems, and the low risk of groundwater and surface waters pollution due to the low fertilizer load in the crop-pasture rotation. While livestock systems can become a source of N pollution for groundwater (Sakadevan and Nguyen, 2017), the condition for nitrate leaching to occur requires grass-legume swards with high N fertilization ($>200\text{-}250 \text{ kg N ha}^{-1}$) in addition to N_2 fixation (Stout et al. 2000, Ledgard 2001). In contrast, average losses of N into the environment are generally low in grazed pastures relying on legume N_2 fixation only (Ledgard 2001). The three systems simulated in this study had very low N leaching losses.

The simulated gaseous losses of N as N_2O were low (range $1.4\text{-}3.2 \text{ kg ha}^{-1} \text{ y}^{-1}$), while the total gaseous losses of N were fairly large. Our simulations show relatively large volatilization losses of 35 and $74 \text{ kg ha}^{-1} \text{ y}^{-1}$ of N in CC and PP. These losses increased with the proportion of pastures in the rotation and derived from animal urine patches. These losses can be high on a patch basis (170 kg ha^{-1} of N, Ledgard 2001), but are restricted to the urine patch; averages over a paddock tend to be lower. For temperate South America, Piñeiro et al. (2010) reported ammonia losses of 17 to $25 \text{ kg ha}^{-1} \text{ y}^{-1}$ of N, somewhat higher than reports of $<17 \text{ kg ha}^{-1} \text{ y}^{-1}$ on grazed grass-legume swards by Ledgard (2001) and Saarijärvi et al. (2006). Mulvaney et al. (2008) estimated annual NH_3 volatilization of $20 \text{ kg ha}^{-1} \text{ y}^{-1}$ of N for dairy cows at a stocking density of 4 cattle ha^{-1} (range 5 to $48 \text{ kg ha}^{-1} \text{ y}^{-1}$ of N). In our simulations, higher volatilization losses were somewhat compensated for lower than expected denitrification losses (range $2\text{-}9 \text{ kg ha}^{-1} \text{ y}^{-1}$ of N). Nonetheless, the simulated N losses seem to be high, but do not limit the pasture productivity due to the presence of legumes

in the simulated pastures. For the same reason, these losses may indirectly cause higher than expected simulated BNF.

Both mineralization and immobilization fluxes increased along with an increase in the pasture proportion in the rotation (Table 2-8). The model including the saturation algorithm estimated 50 and 60 kg more of N mineralized in PP and CP than in CC, while the model excluding the saturation algorithm only showed a difference of 20 kg between these systems. This result is consistent with the hypothesis that as C_s stabilization capacity approaches saturation, N cycling turnover increases (Aber et al. 1989; Lovett and Goodale 2011). Aber et al. (1989) stated that as a soil approaches N saturation, an increase in N mineralization is observed before N losses increase. Internal N fluxes can be large, without N losses increasing proportionally.

A larger soil organic matter pool on crop-pasture systems can regulate N kinetics in two ways: through C accumulation in the system and through changes in the pool C:N ratio (Baer and Blair, 2008; Lovett and Goodale, 2011). As pool size and C:N ratio oscillate, it is possible to both buffer N_s losses and support large N mineralization from soil organic matter and residues. Nitrogen needs to be available in inorganic forms, but plants can also uptake simple organic N forms such as monomers (Schimel and Bennett 2004). The control of k by the saturation ratio as conceptualized in Cycles, implies that as the soil approaches the saturation condition, the ability of the physical matrix to protect organic substrates weakens. Since cropping systems integrating perennial pastures on grassland soils are close to saturation in the topsoil (C_s/C_x 0.67-0.87), it should enable faster C and N turnover that gradually supplies N from an organic pool, thus minimizing leaching losses. Consistently with this concept, the saturation model estimated lower NO_3^- leaching losses compared to the model excluding this algorithm (Table 2-8).

In all systems, the simulated mineralization was lower when including the saturation algorithm. Since texture controls of C_s saturation affect N dynamics (White et al. 2014), lower N mineralization rates reflect the C_s stability in deep soil layers at low saturation ($C_s/C_x < 0.4$). This emergent property of the model reproduces the typical distribution of C_s with depth.

The simulation results explain a declining N supply when converting crop-pasture systems to continuous cropping in no tillage systems of low inputs. This is consistent with a report by Ernst et al. (2016), who attributed part of the grain yield decline in wheat to a declining N supply as the annual cropping phase lengthens. In contrast, the C_s saturation in systems including perennial

grass-legume pastures results in an acceleration of the simulated N cycling dynamics in the topsoil and a greater availability of N for the crops in the sequence. The challenge for producers is to keep agricultural systems at a saturation level that secures this supply without triggering high N losses. It has been stated that integrated crop-livestock systems can recouple C and N cycling and play a critical role in sustainable intensification (Carvalho et al. 2010; Soussana and Lemaire, 2014); C_s and N saturation dynamics seem to underpin this concept.

The empirical relationships between saturation, C_s decomposition rate and fresh C inputs humification rate used in Cycles need to be thoroughly tested. The current model parameterization relies on interpretation of limited data (Hassink et al. 1997, Kemanian and Stöckle 2010; White et al. 2014), and therefore it is of critical importance to design and execute experiments that can help generalize and refine our understanding of these critical processes.

2.6. Conclusions

Incorporating C_s saturation into an agroecosystems model allowed simulating the long-term C_s evolution in crop-pasture rotational systems, reproducing with fidelity the C_s distribution with depth. In addition, it enabled a faster N turnover (mineralization-immobilization) when C_s saturation was higher, which occurred in systems with a higher proportion of perennial pastures in the rotation. A potential reason for the high productivity and low environmental footprint of crop-pasture rotations might be that they operate within the saturation band that provides sufficient nutrients in synchrony with annual crops demand, without facilitating N losses. A method to maintain a soil (or a soil layer) near the C_s saturation band is to integrate perennial pastures in the rotation. This integration might be the key for sustainable intensification in temperate and subtropical agricultural soils, even under no-till.

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CHAPTER 3 - SOIL CARBON SATURATION CONTROLS SOIL C DECOMPOSITION AND CARBON RETENTION OF DECOMPOSING RESIDUES

3.1. Abstract

The theory of soil organic carbon (C_s) saturation establishes that soils can store a limited amount of C_s . This theory has implications for the C turnover in soils that have not been thoroughly explored or addressed. Among them are an expected increase in the C_s decomposition rate (k , d^{-1}) and a reduction in the decomposing residue C retention or humification (h , $kg\ kg^{-1}$). In this research, soils with different saturation ratios derived from different sites (La Estanzuela = LE and Palo a Pique = UEPP, Uruguay), long-term management (from continuous annual cropping to regenerated pastures) and horizons (A1, A2, and B), were incubated with ^{13}C labelled fresh residues with a C:N ratio of 15. Residues were added at rates that vary from a typical input rate in field conditions (termed 1x) and at 3x, 9x and 27x reflecting larger inputs of fresh residues. The hypotheses to be tested were that k increases and h decreases as the saturation ratio increases. Results indicate that k increases linearly with the saturation ratio. In the range of saturation ratio (ca 0.2 to 0.8) in this experiment, k increased between 3- (LE) and 7-fold (UEPP). The humification rate depended on the saturation ratio as well, becoming larger the lower the saturation ratio. The effect was strong at UEPP, but only expressed at the C input rate of 1x at LE. The average h across sites was approximately $0.25\ kg\ kg^{-1}$. In conclusion, both models and empiric interpretation of C cycling in soil need to address the phenomena of carbon saturation, which strongly suggest a non-linear dependence of k and h on the existing level of C_s .

3.2. Introduction

Soil organic matter (SOM) influences the physical, chemical, and biological properties of soils and the biosphere. At a global scale, soil organic carbon (C_s) accounts for approximately 1,400 GT of carbon (C) globally, or about twice the amount of C in the atmosphere or in terrestrial vegetation (Schelsinger and Bernhardt, 2013), and can be an important sink or source C affecting the carbon dioxide (CO_2) concentration in the atmosphere and therefore climate (Lal, 2004). In a local scale, C_s is related to soil quality and soil productivity affecting both food production and the environment. Powered by C cycling, SOM retains nutrients in the soil, making C_s cycling the main driver of plant nutrient availability in an ecologically based management approach for sustainable agriculture (Drinkwater et al. 2008). The provision of nitrogen (N) to plants is intrinsically related to the C:N ratio of the decomposing substrates and the retention of C and N in SOM (Jenkinson et al 1990).

Our current framework for modeling the coupled C and N cycles reflects our understanding of biogenic processes of SOM formation, defined by Swift et al. (1979) as the “decomposition cascade”, where microbes oxidize organic inputs, release C as CO₂ and retain N with the remaining products gradually acquiring the properties of SOM. In the last two decades, it has become increasingly clear that SOM stabilization depends on the availability of mineral surfaces capable of adsorbing organic materials (Baldock and Skjemstad 2000). The association with soil mineral particles and further aggregation into micro-aggregates provide relative protection against biological decomposition or microbial attack as compared to similar unprotected organic materials (Hassink 1997).

Based on this conceptual framework, Hassink and Whitmore (1997) proposed that C_s stabilization capacity is limited by the soil matrix nature, and a “soil carbon saturation capacity (C_x, g C kg⁻¹ soil)” can be defined as a function of the properties of the mineral fraction representative of the soil specific surface, specifically the clay and silt particles content. Recent reviews by Dungait et al. (2012) and Lehmann and Kleber (2015) summarize the cumulative evidence built in the last decades.

Within this paradigm, humification (h , kg kg⁻¹) can be strictly defined as the fraction of the decomposing residues C (C_r) that is retained as C_s, i.e. it is protected from further biological oxidation by their chemical and physical interaction with the soil mineral matrix. The protected SOM may have different degrees of chemical resemblance to the original organic material according to the decomposition process suffered.

Based on empirical research, Hassink (1997) proposed that C_x is linearly related to the soil clay concentration. Although the protective capacity of the soil is affected by clay specific surface area and flocculation by oxy-hydroxides of Al and Fe (Baldock and Skjemstad 2000), clay mineralogy (1:1 versus 2:1) and variation within textural classes (Six et al. 2002), Hassink (1997) established that C_x increases by 0.37 g C kg⁻¹ soil per % increase in the soil particles < 20 μm for temperate and tropical soils worldwide (including soil of European grasslands, North American prairies, African savannas and forests, Central and South American forests). This relationship has been introduced in C_s cycling models C-Farm, Cycles and CropSyst (Kemanian et al. 2005; Kemanian and Stöckle 2010), adopting the equation proposed by Hassink and Whitmore (1997) ($C_x = 21.1 + 0.037 \times f_c$, where f_c is clay content in g kg⁻¹).

The consideration of soil C_s saturation affects substantially not only our understanding but the dynamic simulation of C and N cycling in soil (White et al 2014). Soil C_s saturation would affect both humification and decomposition rates for: (1) as C_s increases and approaches saturation the humification rate will tend to zero because there is no more storage capacity in the soil (Hassink and Whitmore, 1997; Kemanian et al 2005), and (2) the stored C_s is increasingly unprotected, theoretically increasing the C_s decomposition rate (k, T^{-1}). While further experimental evidence supports C saturation theory and particularly the inability of soils to store C_s in mineral associated form (Stewart et al. 2008), there is no experimental evidence supporting an increase in the turnover rate. Nonetheless, these concepts have been introduced in the models C-Farm and Cycles (Kemanian and Stöckle, 2010), and seem to provide a relatively simple but robust framework to simulate both the C_s balance and the distribution of C_s with depth as discussed in Chapter 2 of this dissertation.

The concept of C_s saturation and its influence on N cycling has direct implications for soil and cropping systems management. Given a residue input to the soil, the threshold at which the decomposition of this residue elicits N mineralization or immobilization is strictly dependent on the saturation ratio (C_s/C_x): the closer to 1 (more saturated) the higher the C:N ratio of the residue that causes N net mineralization. This N net mineralization can be seen as N becoming available for crops thus reducing fertilizer needs, or N available for losses, thus facilitating N leaching or gaseous losses. In this formulation, a soil with higher C_s is more fertile not only because it has more SOM (or C_s) but because it is closer to saturation. If, in addition to the saturation effect, the soil decomposition rate is faster as the saturation ratio increases as proposed in the C-Farm and Cycles model, then the saturation effect is even more pronounced. As explained in Chapter 2, rotational systems that include a perennials that allocated large amount C to the roots (Monti and Zatta 2009) generate soils with higher saturation ratio, and with higher C_s in all particle size fractions in the soil profile, including mineral-associated (<53) and soil profile deep layers (Beniston et al., 2014).

Given the importance of the concept of C_s saturation to understand C and N cycling in soils, I elaborated the following hypotheses: 1) The C_s decomposition rate should increase as the saturation ratio increases; 2) this increase should be independent of the residue input rate, or additional to any priming caused residue inputs; and 3) humification rate should decrease as the saturation ratio increases, independent of the cropping history of a soil and the amount of C input.

I tested these hypotheses following the fate of C in soils incubated with variable levels of ^{13}C labeled C inputs (Balesdent and Mariotti 1987, Personeni and Loiseau 2004). The soils were taken from two long-term experiments (>50 and >20 years each) that have had contrasting crop-pasture rotation systems that generated a gradient of C_s in the same soil matrix. This dissertation chapter addresses the bulk C_s cycling, without discriminating the behavior of particulate organic carbon (C_p) or mineral associated organic carbon (C_m), which are addressed in the following chapter. For reference, a list of abbreviations used in this chapter is provided in Table 3-1.

Table 3-1. List of abbreviations used in Chapter 3

Abbreviation	Description
A1	Topsoil 6 cm with most roots and a darker color
A2	Topsoil A diagnostic soil horizon remaining after removing A1.
B	Subsoil B horizons packed separated from transitional horizons
C	Carbon
C_m	Mineral associated organic carbon passing a 53 μm mesh after soil dispersion on 0.5 % (m/v) sodium hexametaphosphate solution
C_p	Particulate organic carbon retained in a 53 μm mesh after soil dispersion on 0.5 % (m/v) sodium hexametaphosphate solution
C_r	Carbon added as sorghum residue
C_s	Soil organic carbon
C_s^i	Initial C at time zero of incubation
C_s^f	Final C at the end of incubation
C_s/C_x	Soil carbon saturation ratio. Ratio between actual soil carbon and saturation contents
C_x	Soil carbon saturation content (kg C kg^{-1} soil)
CC	Continuous annual cropping rotational system
CC no F	Continuous cropping no fertilizer
CP	Crop-pasture rotation
^{13}F	Isotope Molar fraction
F_t	^{13}F of C the soil + residue sample
F_r	^{13}F of C the added residue
f_c	Fractional clay concentration in the soil layer
h	Soil C retention rate or "humification" rate after accounting for simultaneous decomposition
h_a	Apparent retention of added C for the corresponding C_s fraction
INIA	National Institute for Agricultural Research in Uruguay (Instituto Nacional de Investigación Agropecuaria)
k	Soil carbon decomposition rate
LE	"La Estanzuela" experimental site
N	Nitrogen
N_s	Soil nitrogen
New C_s	new Carbon coming from residues
Old C_s	carbon present in the soil before the addition of residues
^{13}R	Isotope Molar ratio
RG	Regenerated pasture
SOM	Soil organic matter
UEPP	"Palo a Pique" experimental unit.
x_r	The proportion of residue-derived C stabilized in the soil calculated as the molar fraction using the mass balance equation
$\delta^{13}\text{C}$	Isotopic composition of C in ‰ units, as calculated in equation (3)

3.3 Methods

3.3.1 Soil saturation gradient

The soils selected for the incubations control for variables that can otherwise produce results difficult to interpret or generalize in the evaluation of saturating or non-saturating responses. There are three sources of variation to generate a saturation gradient: two locations, three contrasting cropping systems, and three soil horizons. At each location, the soils are uniform and share the same climate and therefore, excluding erosion and tillage mixing, differences in C_s are related to the total C inputs and their distribution with depth. Two long-term crop-pasture rotational experiments at the National Institute for Agricultural Research in Uruguay (INIA) serve this purpose well, presenting different SOM content in the same soil as a consequence of different rotations and managements. The experiments are located at “La Estanzuela” (LE) and “Palo a Pique” (UEPP). The LE experiment started in 1962 and is the oldest long-term rotational experiment in South America. The UEPP experiment started in 1995. At each location, three contrasting crop rotations were selected (there are more at LE) whose characteristics are summarized in Table 3-2.

Table 3-2. Rotational systems, location, and soil classification at each experimental site

Site name and year established	La Estanzuela (1963)	Palo a Pique (1995)
Location coordinates (lat, long)	34°20'34 S, 57°43'25 W	33°15'50 S, 54°29'30 W
Local classification system and group unit from (DSF, 1979)	Vertisoles, brunosoles, planosoles halomórficos Unidad Ecilda Paullier-Las Brujas	Brunosles y Planosoles Unidad Alférez
Corresponding USDA Soil Taxonomy (Duran et al. 2005)	Hapluderts, Argiudolls, Argiabolls and Natraquolls	Oxyaquic Argiudolls and Argiaquolls
Annual system sequence without fertilizer (CC no F)	Oats-Sorghum, Wheat-sunflower, Fallow-Corn	N/A
Annual system sequence (CC)	Oats-Sorghum, Wheat-sunflower, Fallow-Corn	Ryegrass or wheat - Sorghum Ryegrass or wheat - Soybean
Crop-pasture sequence (CP)	Oats-Sorghum Wheat-sunflower Fallow-Corn, three-year mixed pasture (Tall fescue, white clover, birdsfoot trefoil)	Ryegrass-Sorghum Ryegrass-Soybean, four-year mixed pasture (Tall fescue or orchardgrass, white clover, birdsfoot trefoil)
Regenerated Grassland (RG)	N/A	Natural grassland C_3 and C_4 species (soil not cultivated for 20 years)

There was no a priori consideration regarding the locations except that soils are on a qualitative basis more fertile at LE (the temperate South American Pampas). Regarding cropping systems, soils with a larger proportion of perennial crops are expected to have a higher saturation ratio, and a cropping system at LE receiving no fertilizer and maintained in annual cropping should have a low saturation throughout. Finally, saturation ratio was expected to decrease with depth for two reasons: lesser C inputs and higher clay with depth in the Argiudolls (argillic B horizon should have more less C_s than the topsoil of a mollic epipedon). Therefore, soils should be near saturation in the soils surface and well below saturation in the B horizon.

3.3.2 Soil sampling

The A and B genetic soil horizons were sampled in spring 2014 using a hydraulic probe cylinder of 4.2 cm diameter that rendered 0.8 m deep soil cores. For each system, 40 cores were taken and gently packed in individual sample plastic bags, separating each soil horizon in the field (Figure 3-1). The topsoil was divided in A1 and A2 soil horizon. The top 6 cm with most roots and a darker color was identified as A1, letting A2 as the remaining A diagnostic soil horizon. Subsoil B horizons were packed separated from transitional horizons retaining only the B horizon. Samples were stored in a 4°C refrigerated room until processing. Once in the lab, samples were manually and gently broken and air dried to pass a 2 mm sieve.



Figure 3-1. Soil profile sampling at LE and UEPP

3.3.3 Soil C saturation estimation

Soil C saturation ratio C_s/C_x was estimated for each soil rotation and horizon by applying the equation developed by Hassink and Whitmore (1997) according to soil particle size analyses. The soil C saturation ratio was estimated using the C_s obtained from the soils samples at the beginning of the incubation.

$$C_x \text{ (g C soil kg}^{-1}\text{)} = 21.1 + 0.037 \times \text{clay (g clay soil kg}^{-1}\text{)} \quad (1)$$

$$\text{Soil saturation ratio (kg kg}^{-1}\text{)} = C_s/C_x \quad (2)$$

3.3.4 Labeled plant residues

Plant residues highly enriched in ^{13}C were prepared in greenhouse pots by leaf feeding sorghum (*Sorghum bicolor* Moench L.) plants with a ^{13}C urea solution (Schmidt and Scrimgeour 2001). Beginning at three leaves stage, plant leaves were sprayed every other day until plant harvesting at anthesis using a solution of 97% atom ^{13}C urea (2 g/L). Plants were kept in a sealed plastic chamber for two hours to prevent CO_2 losses after spraying. The advantages of the leaf feeding method for ^{13}C labeling over the traditional chamber method are the relatively low cost and minor infrastructure requirements (Figure 3-2). Labeled residues C:N was 12.9 with an isotopic composition of $\delta^{13}\text{C} = 199$, contrasting with the soil range of $\delta^{13}\text{C} -18$ to -22 .



Figure 3-2. Greenhouse set up for sorghum plant labeling with urea ^{13}C solution

3.3.5 Incubations

Each incubation treatment consisted of three replicates of 100 g soil samples of A1, A2 and B soil horizons mixed with ^{13}C and ^{15}N sorghum litter for 120 days at the following ratios: 0, 1, 3, 9, and 27 g C soil kg^{-1} , and with a C:N of 12.9. Soil and residues were carefully mixed in glass jars and

slowly wet to soil field capacity. Incubation jars were maintained at 23 °C during the 120-day incubation period. Moisture was replenished twice a week by adding distilled water to maintain soils slightly below field capacity.

3.3.6 Sample physical fractionation and isotopic composition determination

Before and after the incubation, the soil was physically separated in two fractions (soil retained or passing a 53 µm sieve) and the C_s determined for each one of them separately: the particulate organic matter (C_p , > 53 µm), and the mineral associated organic matter (C_m , < 53 µm). Soil fractions were obtained by sieving 10 g of soil through a 53 µm mesh after soil dispersion on 0.5 % (m/v) sodium hexametaphosphate solution, following the procedure described by Marriott and Wander (2006). Both C_p and C_m were added to obtain C_s for each sample.

After physical fractionation C isotopic composition were tested on a continuous flow IRMS-EA at Penn State's Geochemistry and Mineral Sciences isotope lab. The C concentration of each sample was determined at Penn State's Soil Research Cluster Laboratory.

3.3.7 Calculation of h and k

Soil and residues molar fractions (^{13}F) were calculated using the $\delta^{13}C$ and C concentration of soil samples before and after soil incubation. The fraction ^{13}F in each sample was calculated from $\delta^{13}C$ as delivered by the analyzer, according to the following equations:

$$\delta^{13}C_{(‰)} = \left[\frac{^{13}R_{\text{sample}}}{^{13}R_{\text{standard}}} - 1 \right] 1000 \quad (3)$$

$$^{13}R = \frac{^{13}C}{^{12}C} \quad (4)$$

$$^{13}F = \frac{^{13}R}{(1 + ^{13}R)} \quad (5)$$

where $^{13}R_{\text{standard}}$ is the international Pee Dee Belemnite standard.

The proportion of residue-derived C stabilized in the soil (x_r) was calculated as the molar fraction using the mass balance equation:

$$x_r = (F_t - F_s) / (F_r - F_s), \quad (6)$$

where $F_t = ^{13}F$ of C the soil + residue sample at time t , $F_r = ^{13}F$ of C the added residue, which was calculated based on the IRMS – EA $\delta^{13}C$ as described above.

The quantity of the residue derived C_r in the soil (as C_s) at the end of the incubation period was calculated as:

$$\text{New } C_s = C_s \times r \quad (7)$$

$$\text{Old } C_s = C_s (1 - r) \quad (8)$$

where C_s is the total soil C of the respective C_s pool at the end of the incubation, New C_s is the new carbon coming from residues and Old C_s is the carbon present in the soil before the addition of residues.

An underlying assumption in these calculations is that isotopic fractionation in microbiological mediated process can be ignored when working with such highly labeled residue material.

Soil C decomposition is assumed to follow first order decay, with the decomposition rate k calculated using the initial and final C_s as indicated by the superscript i and f, respectively, in Eq. 9:

$$k = -\ln(C_s^f/C_s^i)/120 \quad (9)$$

Data processing was done with k on daily basis, but results are annualized if numerically convenient. Apparent humification of added C (h_a) for each soil fraction and its corrected to account for decomposition during the incubation period was calculated relative to C inputs (C_r):

$$h_a = \text{New C} / C_r \quad (10)$$

$$h = (\text{New C} + k \cdot 0.5 \cdot \text{New C}) / C_r \quad (11)$$

3.3.8. Statistical analysis

The effect of system, horizon, saturation ratio (C_s/C_x) and C inputs on C_s , k and h were tested by analysis of variance within each site using the SAS statistical software package. The C_s/C_x term was included as a covariable.

3.4. Results

3.4.1 Soil carbon balance

Soils initial C_s content exhibited a wide range of saturation ratios (range 0.22 to 0.78) across sites, systems and horizons (Table 3-3 and 3-4). At LE both system and horizon had similar share of the variance, while at UEPP horizon was the dominant cause of variance with B horizons consistently showing low saturation ratio regardless of the cropping system. Topsoils in systems with perennial pastures had the highest saturation ratio (LE crop-pasture rotation and UEPP regenerated

pasture). Remarkably, the B horizon in crop-pasture rotation had a saturation ratio similar to that of the A1 horizon in continuous cropping but not fertilized system. No condition showed full saturation.

Table 3-3. Analysis of variance of soil carbon saturation (C_s/C_x) before incubations

Site Source	----- LE -----		----- UEPP -----	
	DF	F	DF	F
System	2	18.6 ***	2	3.5 *
Horizon	2	22.3 ***	2	1139.7 ***
System*Horizon	4	0.3	4	3.6 *
Error (MSE)	18		18	
Mean saturation (C_s/C_x)	0.51		0.54	
RMSE	0.09		0.02	

P>F values is indicated as p<0.1· , p<0.05 * , p<0.01 ** , p<0.001***
 LE = La Estanzuela experimental site; UEPP = Palo a Pique experimental site.
 C_s/C_x Soil carbon saturation ratio. Ratio between actual soil carbon and saturation contents

Table 3-4. Mean soil carbon saturation (C_s/C_x) for rotation system before incubation

Site	----- LE -----			----- UEPP -----		
	System	horizon	C_s/C_x	System	horizon	C_s/C_x
CC no F	A1	0.47	CC	A1	0.72	
CC no F	A2	0.47	CC	A2	0.59	
CC no F	B	0.22	CC	B	0.27	
CC	A1	0.61	CP	A1	0.76	
CC	A2	0.51	CP	A2	0.61	
CC	B	0.32	CP	B	0.26	
CP	A1	0.77	RG	A1	0.78	
CP	A2	0.70	RG	A2	0.63	
CP	B	0.48	RG	B	0.24	

P>F values is indicated as p<0.1· , p<0.05 * , p<0.01 ** , p<0.001***. LE = La Estanzuela experimental site; UEPP = Palo a Pique experimental site; System: CC no F = continuous cropping with no fertilizer; CC =continuous cropping with fertilizer; CP = crop-pasture rotation; RG = regenerated grassland.

Soil horizon and system were the main effect determining C_s initial stocks at both LE and UEPP sites (Table 3-5). As expected, greatest soil C_s contents were found in top soil A1 layer and systems including perennials, while deeper soil layers and continuous cropping systems showed lower soil C contents. Final C contents were affected by these same variables, and also a significant effect of residues treatments reflected the addition of C inputs as plant residues increasing average total C_s .

at the end of incubation by slightly over 10% on average for all treatment (1.6 g C kg⁻¹ soil). This effect of residues added interacted with system in UEPP, where the mean C_s in the topsoil of regenerated pasture system was the same before and after residue addition.

Table 3-5. Analysis of variance and least square means for soil carbon content before (C_sⁱ) and after incubation (C_s^f).

Site	LE				UEPP			
	DF		F Values		DF		F Values	
Source	C _s ⁱ	C _s ^f	C _s ⁱ	C _s ^f	C _s ⁱ	C _s ^f	C _s ⁱ	C _s ^f
C _r	NA	4	NA	46.4 ***	NA	4	NA	137.3 ***
System	2	2	120.0 ***	70.8 ***	2	2	9.1 **	26.6 ***
horiz (System)	6	6	86.9 ***	56.6 ***	6	6	86.7 ***	193.9 ***
C _r *System	NA	8	NA	0.6	NA	8	NA	2.4 *
C _r *horiz (System)	NA	24	NA	0.7	NA	24	NA	1.5
Error (MSE)	18	90	1.1	7.1	18	90/70	1.2	1.5
Mean, g C soil kg ⁻¹			15.2	16.9			13.5	15.0
RMSE, g C soil kg ⁻¹			1.1	2.7			1.1	1.2

Soil carbon least squares means by rotation system and soil horizon

System	Horiz	C _s ⁱ	C _s ^f	System	Horiz	C _s ⁱ	C _s ^f
		--- g C soil kg ⁻¹ ---				--- g C soil kg ⁻¹ ---	
CC no F	A1	13.6	15.7	CC	A1	17.1	18.9
CC no F	A2	13.6	15.2	CC	A2	12.4	14.1
CC no F	B	7.7	9.8	CC	B	8.1	10.4
CC	A1	18.7	20.6	CP	A1	19.3	19.4
CC	A2	15.6	18.2	CP	A2	12.9	15.0
CC	B	9.5	11.5	CP	B	7.8	10.1
CP	A1	26.9	27.3	RG	A1	22.4	22.4
CP	A2	20.1	12.0	RG	A2	13.6	16.1
CP	B	11.0	13.5	RG	B	8.2	10.8

P>F values is indicated as p<0.05 *, p<0.01 **, p<0.001***

C_r=Carbon added as sorghum residues, C_sⁱ=initial soil C at time zero of incubation, C_s^f=final soil C at the end of incubation; Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland; Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B; NA = not applicable.

3.4.2 Soil decomposition rate

Soil C decomposition (*k*) for each treatment was calculated based on the loss of “old” C_s. The mean *k* for LE and UEPP were 0.11 and 0.05 y⁻¹, respectively (Table 3-6). The per year time unit makes number more readable but may not reflect realistic average rates, for such loss cannot be sustained for a long period of time (hence the short term incubation). The variability in the estimation of *k* made detecting statistically significant variations due to treatments challenging. However, soil horizon, system and amount of residues added effects were detected at UEPP. Soil

horizon was the main effect, reflecting the higher k in topsoil layers (Table 3-6). In addition, A1 horizons from perennial systems (RG and CP) showed the largest k .

Table 3-6. Analysis of variance and least square means for C_s decomposition exponential decay rate (k).

Site	LE			UEPP				
Source	DF	F		DF	F			
C_r	4	1.25		4	3.12	*		
System	2	1.30		2	3.62	*		
hor(System)	6	1.84		6	5.43	***		
C_r *System	8	0.43		8	0.81			
C_r *hor(System)	24	0.89		24	1.17			
Error (MSE)	90	0.00000152		69	0.00000029			
		k (d ⁻¹)	k (y ⁻¹)		k (d ⁻¹)	k (y ⁻¹)		
Mean k		0.000287	0.11		0.000133	0.05		
RMSE		0.001233			0.000540			
Decomposition least squares means by rotation system and soil horizon								
	System	Horizon	k (d ⁻¹)	k (y ⁻¹)	System	Horizon	k (d ⁻¹)	k (y ⁻¹)
	CC no F	A1	-0.000075	-0.03	CC	A1	-0.0001531	-0.05
	CC no F	A2	0.000394	0.15	CC	A2	0.0000369	0.01
	CC no F	B	0.000233	0.09	CC	B	-0.0001043	-0.04
	CC	A1	0.000444	0.18	CP	A1	0.0008532	0.37
	CC	A2	-0.000064	-0.02	CP	A2	0.0001812	0.07
	CC	B	0.000065	0.02	CP	B	-0.0002662	-0.09
	CP	A1	0.000680	0.28	RG	A1	0.0005408	0.22
	CP	A2	0.001095	0.49	RG	A2	-0.0000114	0.00
	CP	B	-0.000193	-0.07	RG	B	0.0000215	0.01

P>F values is indicated as p<0.05 *, p<0.01 **, p<0.001***; C_r =Carbon added as sorghum residues, C_s^i =initial soil C at time zero of incubation, C_s^f =final soil C at the end of incubation; Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland; Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B; NA = not applicable

The horizon fixed effect as class variable hides the within horizon variation in saturation ratio. Substituting C_s/C_x as a covariance term for soil horizon as a fixed effect showed that increasing C_s/C_x resulted in an increase in k at both experimental sites (Figure 3-3, Table 3-7, $p > F = 0.1$ at LE and $p > F = 0.001$ at UEPP).

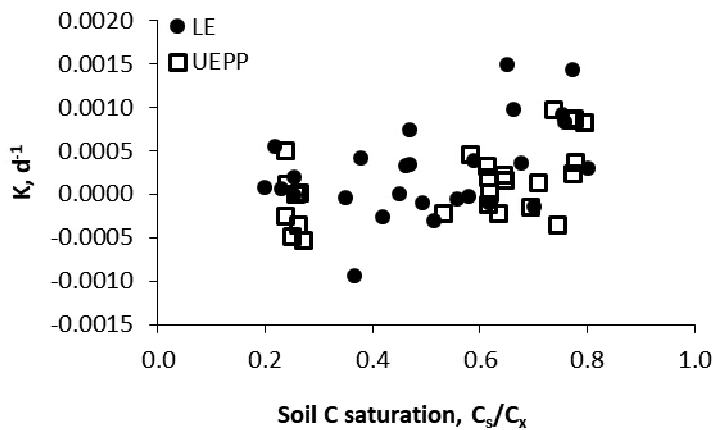


Figure 3-3. Soil carbon decomposition (K, d^{-1}) after 120 days of incubation averaged for all C_r levels.

Table 3-7. Analysis of variance for C decomposition (k) during soil C incubation with soil saturation as a covariate.

Site	----- LE -----		----- UEPP -----	
	DF	F	DF	F
C_r	4	0.3	4	3.1 *
System	2	0.2	2	3.1 ·
C_s/C_x	1	3.0 ·	1	12.9 ***
C_r *System	8	0.5	8	0.6
C_s/C_x * C_r	4	0.4	4	2.0
Error (MSE)	115	0.00000156	94	0.00000034
	$k (d^{-1})$	$k (y^{-1})$	$k (d^{-1})$	$k (y^{-1})$
Mean	0.000287	0.11	0.000133	0.05
RMSE	0.001248		0.000580	
Slope of k vs C_s/C_x	0.00099		0.00175	

P>F values is indicated as $p < 0.1$ ·, $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***; C_r =Carbon added as sorghum residues; C_s/C_x = Soil carbon saturation ratio calculated as the ratio between actual soil carbon and saturation contents.

The amount of C inputs as residues and the system also affected C_s decomposition at UEPP, although the effect was of lower magnitude. Increasing level of residues added resulted in lower k values (negative priming). Continuous annual cropping systems showed virtually no decomposition while perennial systems decomposed C_s in the period at a mean annual k rate of 0.10 and 0.06 (y^{-1}) for CP and RG respectively (Table 3-8).

Table 3-8. Mean daily and respective annual decomposition rates for different levels of C inputs added as residues and systems in UEPP.

Variable	$k, (d^{-1})$
C_r	
0	0.000367
1	0.000134
3	0.000181
9	0.000071
27	-0.000188
System	
CC	-0.000073
CP	0.000260
RG	0.000152

C_r=Carbon added as sorghum residues; UEPP = Palo a Pique experimental site; Rotational Systems: CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland.

3.4.3 Residues retention rate

Mean retention rate of the residues C input was 0.27 and 0.24 kg kg⁻¹ at LE and UEPP, respectively (Table 3-9). Correcting estimated humification rates to account for simultaneous decomposition of the humified C returned “corrected” h figures that were similar to the apparent h . Soil horizon, system and the amount of C inputs affected total C humification only at UEPP, where B horizon seemed to have a higher h , but the effect of system was less clear.

Substituting the saturation ratio C_s/C_x for soil horizon rendered a similar residual error at UEPP, indicating that saturation may explain differences in total soil C humification observed between soil horizons (Table 3-10). At LE, this analysis revealed that total soil C humification was also affected by the system, and also that soil C saturation interacts with the amount of soil C inputs. This interaction refers to the fact that the saturation effect was only statistically significant at low C inputs (1 g C soil kg⁻¹ added as residues), with a gradual decrease in saturation effect for larger amounts of C added. In other words, the addition of larger amounts of residues removed the saturation effect.

Assuming that the C_r used to estimate h were already in stable form (it can be in a transient pool like microbial biomass or particulate organic matter that will undergo further microbial cycles), the

slope of the saturation effect is relatively modest. For the corrected h , its variation in the full range of saturation will be only 0.03 units, for an average h of near 0.24 kg kg⁻¹.

Table 3-9. Analysis of variance and least square means of soil C apparent (h_a) and corrected (h) humification rate.

Site	LE			UEPP		
		h_a	h		h_a	h
Source	DF	F	F	DF	F	F
C_r	3	1.0	1.2	3	3.0 *	2.7
System	2	0.6	1.8	2	4.5 *	5.5 **
hor(System)	6	0.5	0.6	6	4.8 ***	4.6 ***
C_r *System	6	0.9	0.8	6	0.5	0.4
C_r *hor(System)	18	1.1	1.4	18	1.5	1.5
Error (MSE)	72	0.0054	0.0048	55	0.0043	0.0040
Mean h		0.273	0.275		0.239	0.239
RMSE		0.07	0.07		0.07	0.06

Soil carbon retention rates least square means by rotation system and soil horizon

System	Horz	h_a	h	System	Horz	h_a	h
CC no F	A1	0.26	0.26	CC	A1	0.16	0.16
CC no F	A2	0.25	0.25	CC	A2	0.20	0.20
CC no F	B	0.29	0.29	CC	B	0.27	0.27
CC	A1	0.26	0.26	CP	A1	0.26	0.25
CC	A2	0.28	0.27	CP	A2	0.25	0.25
CC	B	0.27	0.27	CP	B	0.25	0.25
CP	A1	0.27	0.28	RG	A1	0.20	0.20
CP	A2	0.29	0.31	RG	A2	0.29	0.29
CP	B	0.29	0.29	RG	B	0.29	0.29

P>F values is indicated as For p<0.1 · , p<0.05 * , p<0.01 ** , p<0.001***

h_a =apparent retention of added C for the corresponding soil C fraction, h = Soil C retention rate or "humification" rate after accounting for simultaneous decomposition; C_r =Carbon added as sorghum residues; Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland; hor(System)=soil horizon effect within system; Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B.

Table 3-10. Analysis of variance and least square means of soil C apparent (h_a) and corrected (h) humification rate with the saturation ratio C_s/C_x as a covariate.

Site	LE			UEPP		
		h_a	h		h_a	h
Source	DF	F	F	DF	F	F
C_r	3	1.7	2.2	3	0.2	0.2
System	2	1.5	2.38	2	4.2 *	4.94 **
C_s/C_x	1	1.7	1.32	1	12.4 ***	10.22 ***
C_r *System	6	1.7	1.61	6	0.4	0.31
C_s/C_x * C_r	3	2.4	2.89 *	3	0.3	0.52
Error (MSE)	92	0.0051	0.0047	75	0.0052	0.0048
Mean h		0.273	0.275		0.239	0.239
RMSE		0.071	0.069		0.072	0.070
C_s/C_x		ns	ns		-0.0646	-0.0362

Soil carbon retention rates least square means by rotation system and residue input

	System	h_a	h	System	h_a	h
	CC no F	0.26	0.26	CC	0.21	0.21
	CC	0.27	0.27	CP	0.25	0.25
	CP	0.29	0.30	RG	0.26	0.26
C_r		h_a	h	C_r	h_a	h
	1	0.25	0.26	1	0.21	0.21
	3	0.28	0.29	3	0.23	0.23
	9	0.28	0.28	9	0.25	0.25
	27	0.28	0.27	27	0.27	0.26

P>F values is indicated as $p<0.1$ · , $p<0.05$ * , $p<0.01$ ** , $p<0.001$ ***; ns: not significant

h_a =apparent retention of added C for the corresponding soil C fraction, h = Soil C retention rate or “humification” rate after accounting for simultaneous decomposition; C_r =Carbon added as sorghum residues; C_s/C_x = Soil carbon saturation ratio calculated as the ratio between actual soil carbon and saturation contents; Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland

3.5. Discussion

A wide range of C_s saturation ratios were obtained via the choice of sites, cropping systems and soil horizons. The A1 horizons are near saturation at almost 0.8 saturation ratio (Table 3-4) and with $C_s \approx 28$ g C kg⁻¹ soil. I would expect that if the overall proposition about soil C saturation is correct, soils that are near saturation should have a lower change in C_s storage during incubation. Indeed, and on average, adding fresh residue C inputs increased C_s but this increase was subdued or not evident at all in topsoils (Table 3-5 and Figure 3-4.), a first indication that the saturation

ratio may affect the ability to retain more organic C. In fact, there seems to be a sharp decrease in C retention after the saturation ratio is over 0.75.

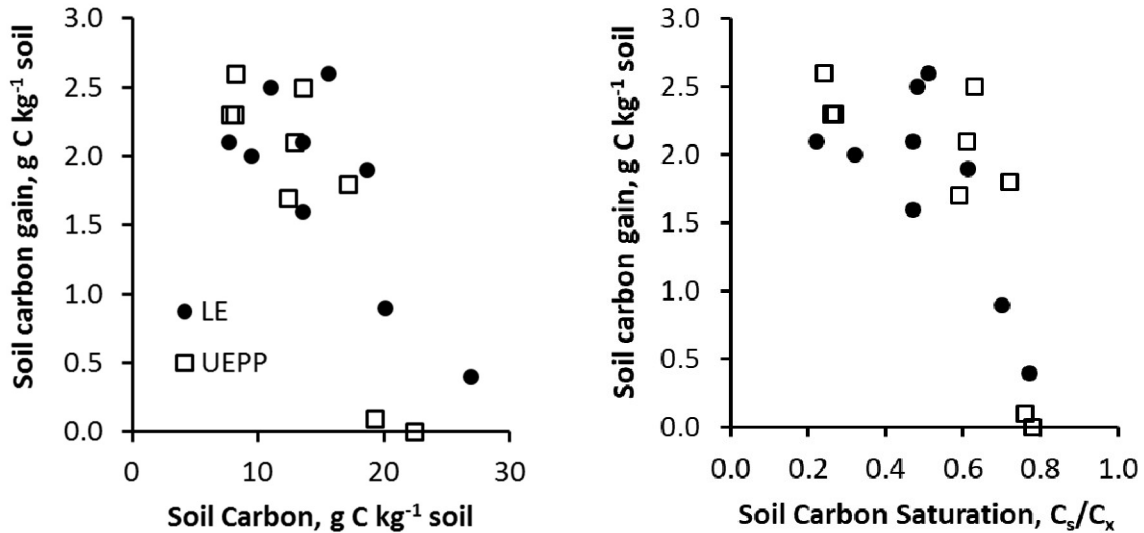


Figure 3-4. Soil carbon gain after 120 days of incubation averaged for all C_r levels. Left and right panels use the actual initial soil carbon or the saturation ratio, respectively, in the abscissa axis.

In the introduction I formulated three hypotheses: that soils near saturation should have higher k (Hypothesis 1), that this increase should be present regardless of priming due to the addition of fresh residues (Hypothesis 2), and that h should decrease the higher the saturation ratio (Hypothesis 3). The results strongly suggest that Hypotheses 1 (Table 3-7) and 3 (Table 3-10) are generally correct, that hypothesis 2 requires further interpretation, and that all hypotheses require considerations that pertain to other controls of the C turnover.

The C_s turnover, quantified via k (the turnover or non-residue-labeled C_s), was greater in topsoils of perennial systems closer to saturation at UEPP (Table 3-6). Furthermore, an acceleration in C_s decomposition due to soil C saturation was evidenced at both sites (Table 3-7), firmly supporting the first hypothesis. Quantitatively, if I applied the estimated slopes of the response of k to saturation to the overall means on Table 3-6, k at LE could vary between 0.11 y^{-1} for an unsaturated soil ($C_s/C_x = 0$, virtually no C_s), to 0.60 y^{-1} in a saturated soil ($C_s/C_x = 1$) (a five-fold increase). Similar calculations for UEPP render 0.05 y^{-1} for $C_s/C_x = 0$ and 0.98 y^{-1} for $C_s/C_x = 1$, a 20-fold increase. The actual magnitude of the annualized values should be carefully considered, for the incubations were made on soil manually de-aggregated (tilled in a sense) and in relatively

optimal moisture, temperature, and aeration conditions. Clearly, a soil turning over at rates of 0.6 y^{-1} would quickly become desaturated. But that is exactly the conceptual basis of the hypothesis: that as the soil desaturates in C, the C turnover (or k) decreases sharply. This agrees with the formulation of the model C-Farm (Kemanian et al. 2005; Kemanian and Stöckle 2010) which to our knowledge is the only model including such control on the soil C turnover rate.

The complementary hypothesis (our hypothesis 3) already proposed by Hassink and Whitmore (1997) is that when soils are C saturated, the inability to store new C will leave decomposing residues more exposed to further microbial cycling. This would reflect experimentally in a lower h which I clearly detected considering both an apparent h and a corrected h that considers the losses of newly formed C during the incubation period (Table 3-10). This means that the decrease in C humification observed here and previously reported by other researchers (Stewart et al. 2008) is not an apparent effect derived by an increase in decomposition but a true reduction in the retention of decomposing C. The mechanisms need to be conceived at the molecular and micro-aggregate scales, when short chains of peptides and other microbial debris interact with the mineral matrix, particularly the charged clay faces forming organo-mineral associations that further aggregate into micro-aggregates (Chenu and Plante, 2006). The experimental data reported here provide evidence that this effect occurring at the microscale level can be measured in a lumped form without addressing aggregation scales. Since the average h was near 0.25, assuming a maximum microbial efficiency of around 0.6 for residues of low C:N ratio (e.g. Figure 4c in Manzoni et al 2012) would require about 2.7 microbial cycles to reach an average h of 0.25.

One caveat of this interpretation is that the saturation effect on h was strong at UEPP, but difficult to detect at LE where it was statistically significant only when adding a low amount of inputs (1x, Table 3-10). A possible reason may be that when considering the bulk soil and bulk C_s , there is no discrimination between stabilized C in the mineral fraction, and new C kept in unprotected fractions like particulate organic matter or microbial biomass. Thus, large pools still undergoing microbial turnover may hide the retention signal. As suggested by the increase in k , new C_s may be accumulated in unprotected pools. The distribution of new C among mineral associated or particular pools may be critical to further understand soil C humification as a response to saturation and is addressed in the following chapter.

Interestingly, the system effect on soil C humification persisted in UEPP after including saturation as a covariate, indicating that there is a system effect rather independent from soil C saturation.

Soil C retention in the bulk soil was greater in systems including perennial pastures. A possible mechanism behind this result could be related to soil re-aggregation through the incubation period, where new C_s could have been physically protected from further decomposition more effectively in systems that had already a better aggregation status. However, this is speculative and not supported by the results in LE. The takeaway from such result is that there remains a portion of the variation in turnover that is explained either by interactions or factors other than C_s saturation that were not considered in this research but that can be locally relevant.

The interaction of k and priming can be more difficult to disentangle. By definition, priming states that k is a function of the amount and composition of the residues being decomposed (Fontaine et al., 2003; Blagodatskaya and Kuzyakov, 2008). The operational mechanism proposed is that C-rich substrates promote the growth of a microbial biomass that “mines” the N-rich mineral associated organic matter with exo-enzymes that de-polymerize N-rich compounds associated with C in the soil mineral fraction (Talbot et al., 2008; Guenet et al., 2012). By extension of this concept, adding increasing amounts of N-rich substrates (ie. $C/N < 25$ for example), like the ones used in this experiment, can cause “negative priming” (Kuzyakov et al. 2000). This should reflect in reduced k in treatments with large amounts of residue added. This was exactly the results obtained at UEPP (Table 3-8). The magnitude of the k suppression for the treatment 27x is fairly large, for the calculated decomposition rate is negative which obviously impossible. It suggest a rather stable C_s . The only plausible interpretation is that the microorganism had a strong preference for the fresh residues, and that possibly, the decomposition of fresh residues also enabled a greater aggregation of existing soil further suppressing decomposition.

These results are consequential for the interpretation and management of agroecosystems. Systems with low saturation levels have in theory room to store more C (and N) if the C supply via residues or perhaps manure additions can be increased. The storage rate can be high because the retention efficiency is high and the decomposition rate is low. The drawback is that low saturation may reflect degradation and therefore a lower intrinsic productivity, and perhaps lower responsiveness to nutrient and other inputs (for example due to lower water storage). This is likely the case in the continuous cropping and not fertilized system in LE, but also in the fertilized system. On the contrary, systems near saturation have higher resilience because they can provide a steady supply of nutrients, provided of course that they are replenished through higher productivity. Pertaining C, it can be a virtuous cycle in which higher C_s saturation causes high C

inputs. The drawback is the lesser ability to store C, except to transfer of C to subsoil layers. Clearly, the crop-pasture rotation in LE has a higher C_s saturation through the soil profile, highlighting the importance of the subsoil as compartment for C storage.

3.6. Conclusion

Increasing soil saturation increased the C_s decomposition rate and reduced residue C stabilization into C_s . This provides strong evidence supporting the conceptual model of C_s turnover dependence on C_s saturation. To our knowledge this is the first time that the decomposition rate is associated to the saturation ratio. The implications for agroecosystem management are that soils or soil horizon near saturation are able to provide a steady supply of nutrients to growing plants and micro-organisms for the retention of C (and N) is reduced due to the lower humification rate, and due to the higher mineralization of existing SOM due the higher k. Further insights into these processes require understanding the apportioning of decomposing C into pools that are or are not physically protected in the soil.

3.7. References

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CHAPTER 4 – THE RELATIONSHIP BETWEEN SOIL CARBON SATURATION AND THE RETENTION OF CARBON FROM DECOMPOSING RESIDUES IN SOIL FRACTIONS

4.1. Abstract

The theory of soil organic carbon (C_s) saturation establishes that soils can store a limited amount of C_s in the mineral fraction. In this research, soils with different saturation ratios derived from different sites (La Estanzuela = LE and Palo a Pique = UEPP, Uruguay), long-term management (continuous annual cropping to regenerated pastures) and horizons (A1, A2, and B), were incubated with ^{13}C labelled fresh residues with a C:N ratio of 13. Residues were added at rates that vary from a typical input rate in field conditions of 1 g C kg⁻¹ soil, and at 3, 9 and 27 g C kg⁻¹ soil reflecting larger inputs of fresh residues. The remaining C_s from the beginning of the incubation, and the C from residues (C_r) retained in C_s after 120 days of incubation were determined for the following soil fractions. Three soil fractions were considered: particulate organic C (C_p) and mineral-associated organic C (C_m), with each fraction associated to soil particles larger (C_p) or smaller (C_m) than 53 μ m, and a fraction nested within C_m obtained after removing fine soluble compounds by leaching with KCl and centrifuging (C_{mL}). The hypotheses were that: 1) as the saturation ratio increases the fractional C_r retention (h) as C_m or C_{mL} (h_m or h_{mL} , kg kg⁻¹) decreases, 2) C_r retention in C_p (h_p) is not affected by the saturation ratio; 3) the addition of C residues may affect the turnover rate of C_p (k_p), but 4) not that of C_{mL} (k_m). The h in different fractions across C_r inputs levels and saturation ratios after incubation was similar between sites (e.g. h_{mL} of 0.10 and 0.11 kg kg⁻¹, h_p of 0.15 and 0.13 kg kg⁻¹ for LE and UEPP, respectively). The h_{mL} decreased linearly with the saturation ratio at a rate of -0.07 and -0.05 (kg kg⁻¹) for LE and UEPP, but did not affect h_p . The larger the C_r amount the lower k_p , with k_m remaining constant. In conclusion, as the saturation ratio increased, the stabilization of decomposed C in the soil mineral matrix decreased providing evidence that C-saturated soils have lesser capacity to store C. While C not stabilized as C_m may favor an increase in C_p , the turnover rates of this pool is much faster providing little buffering capacity for C retention, but perhaps playing a pivotal role in providing a temporary of storage of nitrogen and other elements in an easily decomposable fraction.

4.2. Introduction

Soil C (C_s) cycling is a fundamental process at global and local scales, influencing the physical, chemical and biological properties of soils and the biosphere. In Chapter 3, I analyzed C cycling in soils with different levels of C_s after decades of contrasting crop-pasture systems implemented in

long-term experiments. The soils used for that study represent a gradient of C_s saturation ratio, i.e. the C content with respect to a theoretical maximum or saturation content as defined by Hassink and Whitmore (1997). The fate of ^{13}C added in fresh residues (C_r) enriched in ^{13}C was traced after a period of 120 days of incubation, determining both the amount of C_r incorporated in the bulk soil as well as the amount of C_s lost in this period. Results indicated that increasing C_s saturation caused a faster decomposition of C_s in the bulk soil, and simultaneously reduced the stabilization of C from decomposing residues (the fractional C retention of decomposing residues as C_s is also called humification throughout the text). Results obtained in Chapter 3 indicate that the generic C_s equation (1) to estimate the rate of change of C_s (Hénin and Dupuis, 1945):

$$\frac{\partial C_s}{\partial t} = h_c C_i - k_s C_s \quad (1)$$

where C_s is soil organic C (kg ha^{-1}), t is time (d^{-1}), h_c is the humification rate (kg kg^{-1}), C_i is the C_r input rate ($\text{kg C ha}^{-1} \text{d}^{-1}$), and k is the soil decomposition rate (d^{-1}), should include C_s saturation controls on C mineralization and humification rates, rather than rates that are only modulated by temperature, moisture and other abiotic factors.

That the rates h and k depend on the C_s saturation ratio fit within predictions based on C saturation theory, support the current mathematical structure of the models C-Farm and Cycles (Kemanian et al. 2005; Kemanian and Stöckle 2010), and have clear implications for N cycling and availability for crops (White et al. 2014). As explored in Chapter 3, these features contribute to explain soil N cycling in crop-pasture systems with low external inputs and topsoil near C saturation, and the synchrony between high crop N demands with soil nutrient supply.

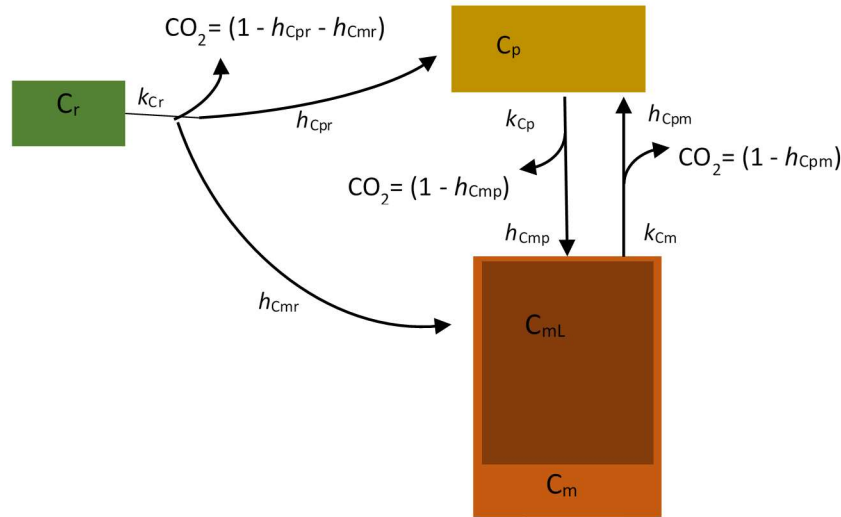
Nonetheless, the saturation effect on C_s retention was strong at UEPP, but it was difficult to detect at the LE experiment, which suggests that the definition of saturation needs to be refined, or that factors not accounted for in this analysis may explain site to site variations.

As explained in Chapter 2, rotational systems that include perennial species that allocate large amount C to the roots (Monti and Zatta 2009) generate soils with higher saturation ratio, and with higher C_s in all particle size fractions in the soil profile (Beniston et al., 2014). The increase in C_s decomposition (k) observed in Chapter 3 when assessing the bulk C_s , may suggest that new C_s is accumulated in unprotected pools of high turnover rate such as particulate organic matter fractions or microbial biomass, compensating for a decrease in new C stabilization in the mineral fraction.

A process that may interact with C saturation is priming. It is defined as an stimulation of microbial activity and therefore C_s turnover rate when adding decomposable residues that are rich in C (relatively high C:N ratio) (Fontaine et al. 2003). It has been proposed that priming may stimulate decomposition of old C_s in deep soil layers, or that in other words, the typical low supply of fresh C may explain the stability of C_s accumulated in deep soil layers (Fontaine et al. 2007). That priming may trigger decomposition of C_s considered stabilized in unsaturated soil layers is somewhat in opposition to the major mechanism of C_s stabilization. The adsorption of organic particles to soil mineral surfaces is the major mechanism of C_s stabilization (Baldock and Skjemstad 2000), which supports the C_s saturation theory (Hassink and Whitmore 1997).

The C_s saturation (Hassink and Whitmore 1997) specifically refers to a maximum capacity of C_s stabilization due to the formation of organo-mineral aggregates (Oades, 1988), while allowing for an unprotected pool due to desorption as well as microbial turnover. Considering the microscale at which the stabilization and decomposition processes occur, one could argue that priming may actually act only on this unprotected pool, which should gradually increase as the C_s stabilization capacity saturates.

Both principles could then be reconciled if a differential response in C_s dynamics on physically protected and unprotected C_s pools were observed after the addition of fresh C residues. First, these observations would support the increase in k observed in the bulk C_s of relatively saturated soils due to an increase in the size of the unprotected pool available for immediate microbial attack. Second, these observations will also support the saturation theory, if the decomposition of the mineral associate organic C remains unaffected by the addition of fresh residues, and the stabilization of new C decrease with the availability of adsorption sites in the C_s mineral fraction. Therefore, the distribution of new C among physically protected and unprotected soil pools may be critical to further understand C_s retention as a response to saturation. The particle size and density of soil fractions can be used as criteria to approximate these soil functional soil pools (Christensen 1987; Cambardella and Elliot 1993; Six et al. 2002; Stewart et al. 2008). Particles of greater size ($> 53 \mu\text{m}$) and low density (typically $< 1.6\text{-}1.8 \text{ kg kg}^{-1}$) constitute the particulate organic matter (C_p) and highly decomposable light fractions respectively, representing fresh organic material with a low degree of decomposition, and the small (C_m) and dense (C_{ml}) particles are considered associated to the mineral fractions in a stabilized form (Figure 4-1).



References: C_r = C in residues, C_p =C in soil fraction > 53 μ m, C_m =C in soil fraction < 53 μ m, C_{mL} = C in C_m after leaching with KCl and centrifuging, k_{Cr} =C decomposed from residues, h_{Cpr} =humification in C_p from decomposing residues, h_{Cmr} =humification in C_m from decomposing residues, k_{Cp} =C decomposed from C_p , h_{Cmp} = humification in C_m from C_p , k_{Cm} =C decomposed from C_m , h_{Cpm} = humification of C in C_p from C_m decomposition.

Figure 4-1. Diagram of soil C pools and fluxes representing soil organic matter.

The goals of this chapter are to provide further proof of the C_s saturation framework by exploring the C retention and turnover in the protected and unprotected C_s fractions using the gradient generated by soils under crop-pasture systems, and explore the interaction of the saturation and priming concepts. Specifically, I tested the following hypotheses: 1) the retention or humification of fresh C residues in the soil mineral fraction is affected by the degree of C_s saturation; 2) the incorporation of fresh C residues in the soil unprotected pool is not affected by C_s saturation; 3) the turnover of C in the unprotected C_s pools will be accelerated by the addition of fresh residues; and 4) the turnover of the mineral associated C is not affected by the addition of fresh C residues.

I tested these hypotheses following the fate of ^{13}C in soils incubated with variable levels of ^{13}C labeled C inputs (Balesdent and Mariotti 1987, Personeni and Loiseau 2004), in the same soils and incubation treatments as described in Chapter 3. The soils were taken from two long-term experiments (>50 and >20 years each) that have had contrasting crop-pasture rotation systems that generated a gradient of C_s in the same soil matrix. This dissertation chapter addresses the soil organic matter in two soil particle size fractions: particulate organic carbon (C_p) representing the C_s unprotected pool, and the mineral associated organic carbon (C_m) representing the C_s pool protected by adsorption to mineral surfaces (Figure 4-1). For reference, a list of abbreviations used in this chapter is provided in Table 4-1.

Table 4-1. List of abbreviations used in Chapter 4.

Abbreviation	Description
A1	Topsoil 6 cm with most roots and a darker color
A2	Topsoil A diagnostic soil horizon remaining after removing A1.
B	Subsoil B horizons packed separated from transitional horizons
C	Carbon
C_m	Mineral associated organic carbon passing a 53 μm mesh after soil dispersion on 0.5 % (m/v) sodium hexametaphosphatesolution
C_{mL}	Mineral associated organic carbon passing a 53 μm mesh after soil dispersion on 0.5 % (m/v) sodium hexametaphosphate solution; retained after leaching KCl soluble compounds and centrifuging.
C_p	Particulate organic carbon retained in a 53 μm mesh after soil dispersion on 0.5 % (m/v) sodium hexametaphosphate solution
C_r	Carbon in residues
C_s	Soil organic carbon
C_s/C_x	Soil carbon saturation ratio. Ratio between actual soil carbon and saturation contents
C_x	Soil carbon saturation content (kg C kg^{-1} soil)
CC	Continuous annual cropping rotational system
CC no F	Continuous cropping with no fertilization
CP	Crop-pasture rotation
^{13}F	Isotope Molar fraction
F_t	^{13}F of C the soil + residue sample
F_r	^{13}F of C the added residue
f_c	Fractional clay concentration in the soil layer
h (kg kg^{-1})	Soil C retention rate or "humification" rate after accounting for simultaneous decomposition
h_a (kg kg^{-1})	Apparent retention of added C for the corresponding C_s fraction
h_m (kg kg^{-1})	the fractional C_r retention as C_m
h_{ma} (kg kg^{-1})	the apparent fractional C_r retention as C_m
h_{mL} (kg kg^{-1})	the fractional C_r retention as C_{mL}
h_{mLa} (kg kg^{-1})	the apparent fractional C_r retention as C_{mL}
h_p (kg kg^{-1})	the fractional C_r retention in C_p
h_{pa} (kg kg^{-1})	the apparent fractional C_r retention in C_p
INIA	National Institute for Agricultural Research in Uruguay (Instituto Nacional de Investigación Agropecuaria)
k	Soil carbon decomposition rate
k_p	Decomposition rate in the particulate organic fraction
k_m	Decomposition rate in the mineral fraction
LE	"La Estanzuela" experimental site
N	Nitrogen
N_s	Soil nitrogen
New C_m	At the end of the incubation, C in soil fraction < 53 μm from residues
New C_{mL}	At the end of the incubation, C in soil fraction < 53 μm from residues after KCl leaching and centrifuged
New C_p	At the end of the incubation, C in soil fraction > 53 μm from residues
New C_s	new Carbon coming from residues
Old C_m	At the end of the incubation, C in soil fraction < 53 μm from soil
Old C_{mL}	At the end of the incubation, C in soil fraction < 53 μm from soil after KCl leaching and centrifuged
Old C_s	carbon present in the soil before the addition of residues
RG	Regenerated grassland
SOM	Soil organic matter
superscript i	initial C at time zero
superscript f	final C at the end of incubation
UEPP	"Palo a Pique" experimental unit.
x_r	The proportion of residue-derived C stabilized in the soil calculated as the molar fraction using the mass balance equation

4.3. Methods

4.3.1. Saturation gradient and integrated crop-pasture systems

The soils selected for the incubations are described in Chapter 3. There are three sources of variation to generate a saturation gradient: two locations, three contrasting cropping systems, and three soil horizons. The two locations are “La Estanzuela” (LE) and “Palo a Pique” (UEPP). At each location, the soils are uniform and share the same climate and therefore, excluding erosion and tillage mixing, differences in C_s are related to the total C inputs and their distribution with depth. The LE experiment started in 1962 and is the oldest long-term rotational experiment in South America. The UEPP experiment started in 1995. At each location, three contrasting crop rotations were selected (there are more rotational treatments at each location) whose characteristics are summarized in Chapter 3, Table 3-2. In the text I will refer to these cropping rotations or agricultural production systems as “systems”. At each location, the plots have different SOM content in the same soil as a consequence of different systems and managements.

There was no a priori consideration regarding the selection of these locations except that soils are on a qualitative basis more fertile at LE (the temperate South American Pampas). Regarding cropping systems, soils with a larger proportion of perennial crops are expected to have a higher saturation ratio, and a cropping system at LE receiving no fertilizer and maintained in annual cropping should have a low saturation throughout. Finally, saturation ratio was expected to decrease with depth for two reasons: lesser C inputs and higher clay with depth in the Argiudolls (argillic B horizon should have less C_s than the topsoil of a mollic epipedon). Therefore, soils should be near saturation in the top of the A horizons and well below saturation in the B horizons, with a gradation within horizon given by the proportion of perennials and other factors in the rotation.

4.3.2. Soil sampling

The A and B genetic soil horizons were sampled in spring 2014 using a hydraulic probe cylinder of 4.2 cm diameter that rendered 0.8 m deep soil cores. For each system, 40 cores were taken and gently packed in individual sample plastic bags, separating each soil horizon in the field (Figure 3-1). The topsoil was divided in A1 and A2 soil horizon. The top 6 cm with most roots and a darker color was identified as A1, letting A2 as the remaining A diagnostic soil horizon. Subsoil B horizons were separated from the transitional horizons retaining only the B horizon. Samples were stored in a 4°C refrigerated room until processing. Once in the lab, samples were manually and gently broken and air dried to pass a 2 mm sieve.

4.3.3. Soil C saturation estimation

Soil C saturation C_x was estimated for each soil rotation and horizon by applying the equation developed by Hassink and Whitmore (1997) according to soil particle size analyses. The C_s saturation ratio was estimated by dividing the actual C_s concentration at sampling timing by the C_x value (ratio = C_s/C_x), where C_x was estimated from [Eq. 2] with C_x in (g C soil kg^{-1}) and clay in g clay kg^{-1} soil. The C_s saturation ratio before incubation ranged between 0.2 and 0.8; specific soil horizons and systems are described in Chapter 3 (Table 3-4).

$$C_x = 21.1 + 0.037 \times \text{clay} \quad (2)$$

4.3.4. Labeled Plant Residues

Plant residues enriched in ^{13}C were prepared in greenhouse pots by leaf-feeding sorghum (*Sorghum bicolor* Moench L.) plants with a ^{13}C urea solution (Schmidt and Scrimgeour 2001). Beginning at three leaves stage, the leaves were sprayed every other day until plant harvesting at anthesis using a solution of 97% atom ^{13}C urea (2 g L^{-1}). After spraying the plants were kept in a sealed plastic chamber for two hours to prevent CO_2 losses and facilitate the ^{13}C uptake. The advantages of the leaf feeding method for ^{13}C labeling over the traditional chamber method are the relatively low cost and minor infrastructure requirements (Figure 3-2). Labeled residues C:N was 12.9 with an isotopic composition of $\delta^{13}\text{C} = 199$, contrasting with the soil range of $\delta^{13}\text{C} -18$ to -22 .

4.3.5. Incubations

Each incubation treatment consisted of three replicates of 100 g soil samples of A1, A2 and B soil horizons mixed with ^{13}C and ^{15}N sorghum litter for 120 days at the following ratios: 0, 1, 3, 9, and 27 g C soil kg^{-1} , and with a C:N of 12.9. Soil and residues were carefully mixed in glass jars and slowly moistened to soil field capacity. Incubation jars were maintained at 23 °C during the 120-day incubation period. Moisture was replenished twice a week by adding distilled water to maintain soils slightly below field capacity.

4.3.6. Sample physical fractionation

Before and after the incubation, the soil was physically separated in two fractions and the C_s determined for each one of them separately. The fractions are the particulate organic matter ($C_p > 53 \mu\text{m}$), and the mineral associated organic matter ($C_m < 53 \mu\text{m}$). Soil fractions were obtained by sieving 10 g of soil through a 53 μm mesh after soil dispersion on 0.5 % (m/v) sodium

hexametaphosphate solution, following the procedure described by Marriott and Wander (2006). Both C_p and C_m were added to obtain C_s for each sample. Soluble compounds were removed from the mineral fraction using a KCl solution and centrifuging (Kaye et al., 2003). Briefly, 25 ml of KCl 2M solution were added to 2 g of C_m fraction in a 50 ml falcon centrifuge tube, and placed on a shaker for 30 min. The tubes were centrifuged for 10 minutes at 4000 RPM after adding with 15 drops of $CaCl_2$ 0.25 M for clay flocculation. This procedure was performed twice using the KCl solution, and a third time with distilled water. Leachate was removed keeping the solid phase for oven-drying at 60 °C for 48 hours.

After physical fractionation C isotopic composition were tested on a continuous flow IRMS-EA at Penn State's Geochemistry and Mineral Sciences isotope lab. The C concentration of each sample was determined at Penn State's Soil Research Cluster Laboratory.

4.3.7. Soil ^{13}C retention calculations

Soil and residues molar fractions (^{13}F) were calculated using the $\delta^{13}C$ and C concentration of soil samples before and after soil incubation. The fraction ^{13}F in each sample was calculated from $\delta^{13}C$ as delivered by the analyzer, according to the following equations:

$$\delta^{13}C_{(‰)} = \left[\frac{^{13}R_{\text{sample}}}{^{13}R_{\text{standard}}} - 1 \right] 1000 \quad (3)$$

$$^{13}R = \frac{^{13}C}{^{12}C} \quad (4)$$

$$^{13}F = \frac{^{13}R}{(1 + ^{13}R)} \quad (5)$$

where $^{13}R_{\text{standard}}$ is the international Pee Dee Belemnite standard.

The proportion of residue-derived C stabilized in the soil (x_r) was calculated as the molar fraction using the mass balance equation:

$$x_r = (F_t - F_s) / (F_r - F_s), \quad (6)$$

where $F_t = ^{13}F$ of C the soil+residue sample at time t, $F_r = ^{13}F$ of C the added residue, which was calculated based on the IRMS – EA $\delta^{13}C$ as described above.

The quantity of the residue derived C_r in the soil (as C_s) at the end of the incubation period was calculated as:

$$\text{New } C_s = C_s x_r \quad (7)$$

$$\text{Old } C_s = C_s (1-x_r) \quad (8)$$

where C_s is the total C_s of the respective C_s pool at the end of the incubation, New C_s is the new Carbon coming from residues and Old C_s is the carbon present in the soil before residues addition.

An underlying assumption in these calculations is that isotopic fractionation in microbiological mediated process can be ignored when working with such highly labeled residue material.

Since humification and decomposition are simultaneous process, the ratio of the C from residues in organic matter to the added C in residues is just an apparent humification (h_a) because it accounted for the decomposed C during the period of interest. The h_a was calculated as

$$h_a = \text{New } C_s / C_r \quad (9)$$

In attempting to obtain a corrected h , it was discovered that there were non-biological transfers of material from Old C_p to Old C_m , likely through re-aggregation of the soil. This impeded calculating a corrected h . Therefore, only h_a is investigated in this chapter for each C fraction. It must be understood that h_a underestimates the instantaneous humification due to the inability to account for simultaneous decomposition.

4.3.8. Statistical analysis

The effect of system, horizon, saturation ratio (C_s/C_x) and C inputs on C_s , k and h were tested by analysis of variance within each site using the SAS statistical software package. The C_s/C_x term was included as a covariable.

4.4. Results

4.4.1. Soil C pools

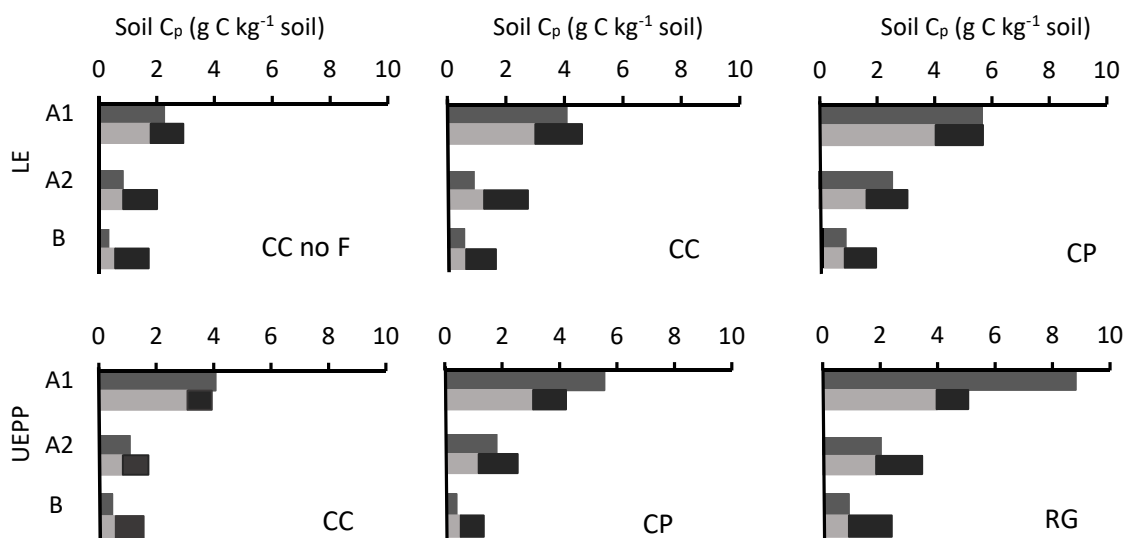
Initial C_p varied with system and horizon in the same pattern as C_s as described on Chapter 3. Before the incubation C_s in the C_p fraction decreased with soil depth, and among cropping systems, those including perennial species showed higher C_p at both experimental sites (CP and RG) when compared to CC (Table 4-2, Figure 4-2). After the incubation, total C_p still showed these system and soil horizon prints, but the main variable explaining the observed variation in final C_p was the amount of C input added as residues.

Table 4-2. Analysis of variance of soil initial and final C content for particular organic matter size fraction (> 53 μm).

Site		LE							
Source	Df ⁱ	Df ^f	C _p ⁱ	C _p ^f	New C _p ^f	Old C _p ^f	F values		
							C _r		4
System	2	2	13.55 ***	5.57 **	0.66	15.39 ***			
Horizon (System)	6	6	18.41 ***	9.24 ***	0.7	27.48 ***			
C _r *System		8		0.97	0.92	0.85			
C _r *horizon(System)		24		0.59	0.6	0.64			
Error (MSE)	18	90	0.58	3.50	1.19	0.87			
Mean (g C kg ⁻¹ soil)			2.0	2.9	1.3	1.6			
RMSE			0.8	1.9	1.1	0.9			

Site		UEPP							
Source	Df ⁱ	Df ^f	C _p ⁱ	C _p ^f	New C _p ^f	Old C _p ^f	F values		
							C _r		4
System	2	2	8.4 **	14.9 ***	7.2 ***	17.5 ***			
Horizon(System)	6	6	25.5 ***	22.9 ***	1.7 ***	74.3 ***			
C _r *System		8		3.4 **	4.7 ***	1.35			
C _r *horizon(System)		24		1.03	1.48	0.72			
Error (MSE)	18	90	1.15	1.00	0.32	0.33			
Mean (g C kg ⁻¹ soil)			2.8	2.7	1.0	1.7			
RMSE			1.1	1.0	0.6	0.6			

Superscripts "i" and "f" indicate initial (before incubation) and final (after incubation) respectively. Df= degrees of freedom, C_r= C added in residues; C_p= total C in soil fraction > 53 μm , New C_p=C in soil fraction > 53 μm from residues, Old C_p=C in soil fraction > 53 μm from soil, P>F values is indicated as p<0.1 , p<0.05 * , p<0.01 ** , p<0.001***



References: Dark Grey: C_p initial, Light Grey: Old C_p final, Black: New C_p final. Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland; Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B.

Figure 4-2. Soil C content in the particulate organic fraction (C_p > 53 μm)

New C in the C_p fraction was mainly determined by the amount of C inputs added as residues, resulting on a mean accumulation of 1.3 and 1.0 g C soil kg⁻¹ at LE and UEPP respectively. Old C_p remaining at the end of the incubation was similar among sites (1.6 and 1.7 g C soil kg⁻¹ for LE and UEPP respectively), showing the same soil horizon and systems patterns as the initial values. Total soil C_p at the end of the incubation was greater on topsoil horizons and systems with perennials. The addition of C inputs affected both New C_p and Old C_p. An internal C_s flux between C_p and C_m soil fractions was revealed in A2 and B horizons by similar final and initial C_p contents, and even a slight increase in C_p in the A2 horizon of LE CC (Figure 4-2).

Initial C_s in the mineral fraction at LE showed the same pattern as C_p, with greater initial C on CP systems, and decreasing C_s content with soil depth (Table 4-3, Figure 4-3). In contrast, the initial C_m content at UEPP depended only on soil horizon without a clear cropping system effect (Table 4-3). Two decades of contrasting cropping systems affected C_p but not C_m levels at UEPP. Final C_m content varied with the amount of C inputs, although most of the variation was still explained by the differences between systems and soil horizons. New C stabilization in the C_m fraction was mainly determined by the amount of residues C inputs at both sites, but also affected by the soil

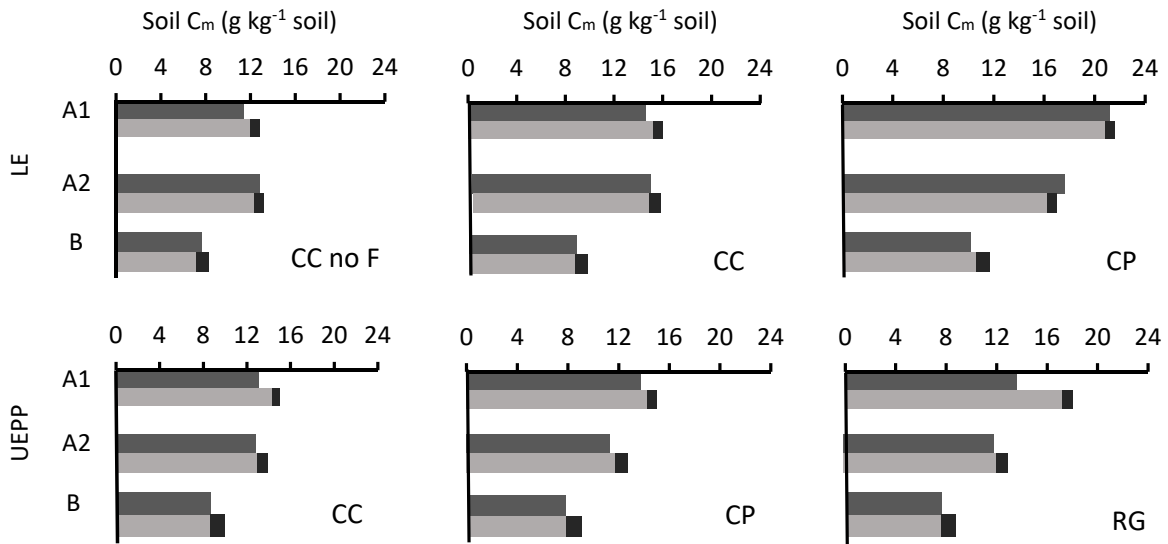
horizon in each system, as B horizons seemed to incorporate more C. This effect also depended on the amount of C inputs, although this interaction was minor compared to the effects of C inputs. Observed increases in the Old C_m fraction after the incubation can be explained by the decrease in Old C_p , showing internal C_s flux between C_s pools likely indicating re-aggregation of C_p into C_m , or decomposition of C_p and stabilization in C_m (Figures 4-2 and 4-3).

Table 4-3. Analysis of variance of soil initial and final C_s content for the mineral-associated organic matter size fraction ($< 53 \mu\text{m}$) at LE site and UEPP.

Site		LE									
Source	Df ⁱ	Df ^f	C_m^i		C_m^f		New C_m^f		Old C_m^f		
C_r		4			7.7	***	1519.4	***	1.2		
System	2	2	244.6	***	106.9	***	2.9	.	109.3	***	
hor(System)	6	6	160.7	***	72.6	***	9.7	***	77.9	***	
C_r *System		8			0.5		1.2		0.5		
C_r *hor(System)		24			1.3		4.6	***	1.3		
Error (MSE)	18	90	0.31		3.1		0.03		3.07		
Mean			13.2		13.9		0.9		13.0		
RMSE			0.56		1.75		0.2		1.8		

Site		UEPP									
Source	Df ⁱ	Df ^f	C_m^i		C_m^f		New C_m^f		Old C_m^f		
C_r		4			20	***	2615.8	***	3.0	*	
System	2	2	0.3		11.2	***	5.7	**	12.4	***	
hor(System)	6	6	145.5	***	180.6	***	46.5	***	205.8	***	
C_r *System		8			0.3		1.6		0.4		
C_r *hor(System)		24			1.7	*	9.6	***	1.3		
Error term (MSE)	18	70	0.19		1.00		0.01		0.97		
Mean			10.8		12.3		0.8		11.5		
RMSE			0.4		1.0		0.1		1.0		

Superscripts "i" and "f" indicate initial (before incubation) and final (after incubation) respectively. Df= degrees of freedom, C_m = total C in soil fraction $< 53 \mu\text{m}$, New C_m =C in soil fraction $< 53 \mu\text{m}$ from residues, Old C_m =C in soil fraction $< 53 \mu\text{m}$ from soil, P>F values is indicated as $p<0.1$., $p<0.05$ *, $p<0.01$ **, $p<0.001$ ***



References: Dark Grey: C_m initial, Light Grey: Old C_m final, Black: New C_m final. Rotational Systems: CC no F= continuous cropping with no fertilization, CC= continuous annual cropping rotational system, CP= crop-pasture rotation, RG= regenerated grassland; Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B.

Figure 4-3. Soil C content in the mineral fraction (C_m < 53 μm).

Removing soluble compounds from C_m by KCl leaching did not affect soil horizon and system effects on stabilized C. Variation in C_{mL} contents before incubation was mainly explained by soil horizon at both sites, while at LE a system effect was observed (Table 4-4). Soil initial C in C_{mL} was greater on systems including fertilization and pastures at LE, while all systems showed similar C_{mL} at UEPP (Figure 4-4). A system effect on New C incorporated in this fraction (C_{mL}) was detected at LE (Table 4-4), where CP systems showed less New C stabilized in the mineral fraction, particularly at low levels of inputs as shown by the interaction term.

Table 4-4. Analysis of variance of soil initial and final C_s content for C_{mL} at LE and UEPP site.

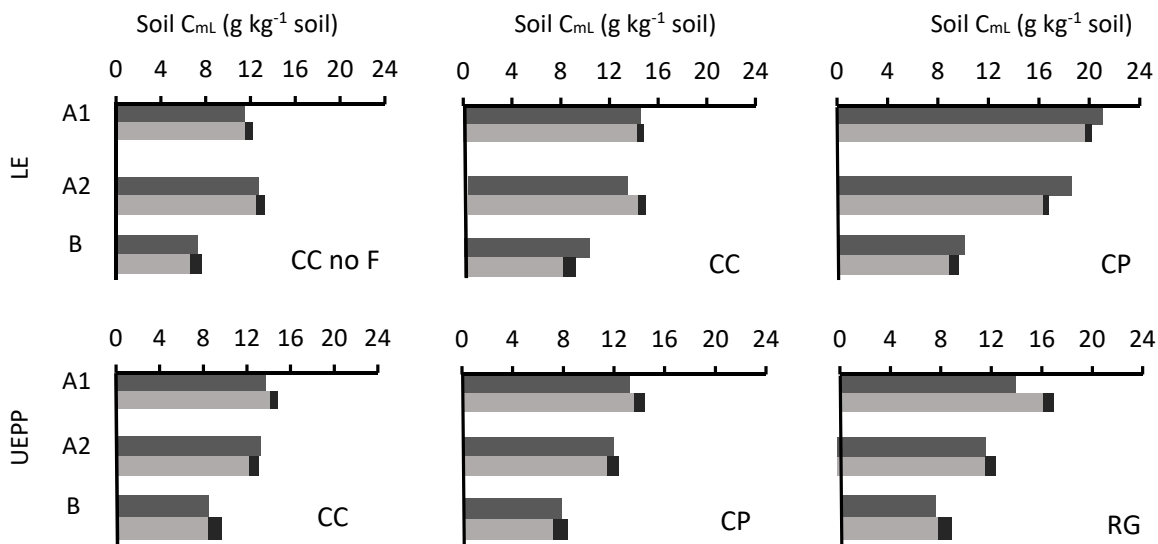
Site	LE										
	Source	Df		C _{mL} ⁱ	C _{mL} ^f		NewC _{mL} ^f	OldC _{mL} ^f			
				F values							
	C _r		4		10.2	***	869.1	***	2.4		
	System	2	2	27.2	***	115.0	***	14.3	***	124.0	***
	hor(System)	6	6	13.7	***	110.8	***	13.6	***	120.3	***
	C _r x System		8			0.9		4.0	***	0.8	
	C _r x hor(System)		24			1.0		3.0	***	0.9	
	Error (MSE)	18	90	3.28		2.13		0.04		2.16	
	Mean			13.2		13.1		0.71		12.4	
	RMSE			1.81		1.46		0.21		1.47	
Site	UEPP										
	Source	Df		C _{mL} ⁱ	C _{mL} ^f		NewC _{mL} ^f	OldC _{mL} ^f			
				F values							
	C _r		4		22.9	***	2243.3	***	0.9		
	System	2	2	0.1		9.6	***	5.9	**	10.4	***
	hor(System)	6	6	38.5	***	123.3	***	28.0	***	143.6	***
	C _r *System		8			0.9		3.7	***	0.9	
	C _r *hor(System)		24			1.2		6.3	***	1.0	
	Error (MSE)	18	90	0.76		1.5		0.02		1.40	
	Mean			10.9				0.9		11.0	
	RMSE			0.87				0.14		1.20	

Superscripts "i" and "f" indicate initial (before incubation) and final (after incubation) respectively. Df= degrees of freedom, C_{mL}= total C in soil fraction < 53 μm after KCl leaching, New C_{mL}=C in soil fraction < 53 μm from residues after KCl leaching, Old C_{mL}=C in soil fraction < 53 μm from soil after KCl leaching, P>F values is indicated as for p<0.1 , p<0.05 * , p<0.01 ** , p<0.001***

Variation in old C_{mL} at the end of the incubation was mainly explained by the soil horizon and system effects, following the patterns for the initial C contents (Table 4-4). Soil C in C_{mL} was greater on topsoil horizons and at LE systems including fertilization and pastures, while at UEPP all systems showed similar C_{mL} (Figure 4-4). Interestingly, C_{mL} in RG and CP showed more old C_s at the end of the incubation than at the beginning. This is another indication of internal C_s fluxes between soil fractions, particularly transfers from C_p to C_m, consistent with the observation of decreasing C_p through the incubation. Part of the C_p lost during the incubation would seem to be retained in the mineral fraction.

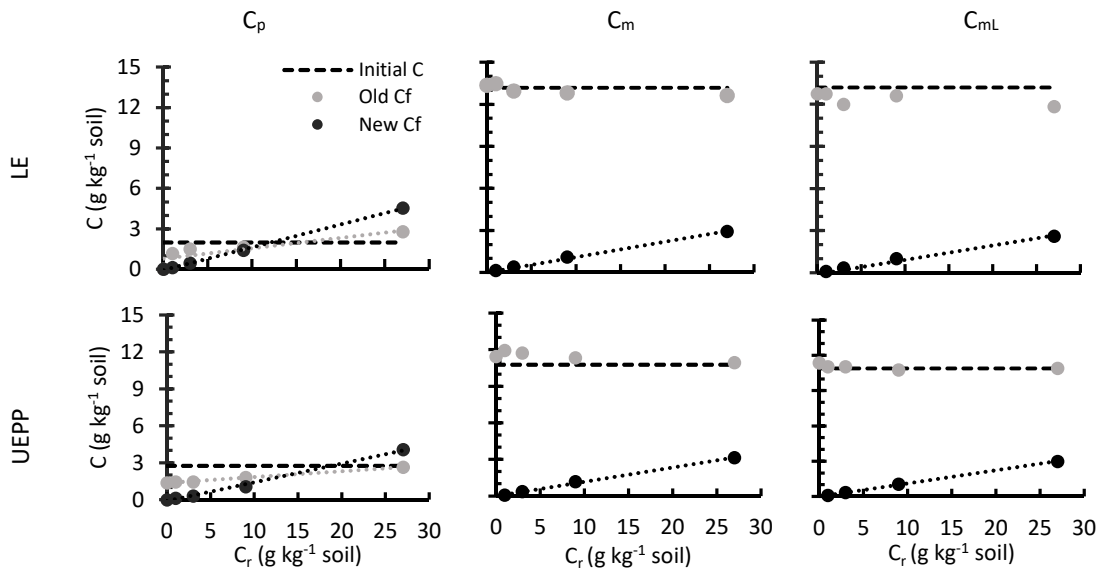
The amount of C inputs only affected the amount of New C stabilized in the C_{mL}, but did not affect the C_{mL} content of Old C at both sites (Table 4-4). On average, an increase in C_r increased New C in

all soil fractions, while Old C was only affected by C_r in the C_p fraction, associated to a decrease in Old C_p losses (Figure 4-5).



References: Dark Grey: C_{mL} initial, Light Grey: Old C_{mL} final, Black: New C_{mL} final. Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland; Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B.

Figure 4-4. Soil C in the mineral fraction after leaching with KCl solution ($C_{mL} < 53 \mu m$).



References: C_p = C in soil fraction $> 53 \mu m$, C_m = C in soil fraction $< 53 \mu m$, C_{mL} = C in soil fraction $< 53 \mu m$ after leaching with KCl solution, dashed lines represent initial C content before incubation, black dots represent final C content coming from the added residues (New C_f), and grey dots represent final C contents from original soil C (Old C_f).

Figure 4-5. Mean soil C content for different soil physical fractions according to C_r inputs added as residues at LE and UEPP sites. Plotted values show mean C contents by fraction, site and C input level averaging A1, A2 and B horizons, rotation systems and replications for each experimental site.

4.4.2. Soil C humification

Soil C humification in C_p (h_{pa}) was similar between experimental sites, with a mean of 0.148 kg kg⁻¹ in LE and 0.125 kg kg⁻¹ in UEPP (Table 4-5). The system only affected the C retention in New C at UEPP, where CC system incorporated less C. The h_{pa} was independent of C_r and the soil horizon.

In line with these results, C_s saturation did not affect h_{pa} (Table 4-6), consistent with the idea that C in this fraction has little or no interaction with the mineral particles. An interaction between saturation and the amount of C in LE shows that for low inputs, saturation may decrease C retention in C_p .

Table 4-5. Soil C humification in particulate organic matter and mineral-associated organic matter size fractions h_{pa} , h_{ma} and h_{mLa} , considering soil horizons in LE and UEPP.

Site	LE			UEPP				
	h_{pa}	h_{ma}	h_{mLa}	h_{pa}	h_{ma}	h_{mLa}		
Source	DF	F values			DF	F values		
C_r	3	2.37	9.43 ***	12.3 ***	3	1.65	37.0 ***	5.0 **
System	2	0.73	0.42	29.7 ***	2	3.26 *	5.6 ***	1.6
hor(System)	6	0.32	5.93 ***	38.0 ***	6	2.78 *	54.6 ***	36.3 ***
C_r x System	6	0.83	0.5	8.4 ***	6	0.55	0.4	0.6
C_r x hor(System)	18	1.04	0.72	4.4 ***	18	1.83 *	4.6 ***	4.0 ***
Error (MSE)	72	0.006	<0.001	<0.001	55	0.004	<0.001	<0.001
r^2		0.32	0.53	0.86		0.51	0.91	0.81
RMSE		0.074	0.021	0.015		0.062	0.017	0.019
Mean (kg kg ⁻¹)		0.148	0.125	0.102		0.125	0.113	0.112

Least square means

System	hor	h_{pa}	h_{ma}	h_{mLa}	System	hor	h_{pa}	h_{ma}	h_{mLa}
CC no F	A1	0.146	0.115	0.098	CC	A1	0.100	0.063	0.074
CC no F	A2	0.121	0.126	0.111	CC	A2	0.099	0.098	0.102
CC no F	B	0.147	0.141	0.135	CC	B	0.124	0.149	0.147
CC	A1	0.153	0.108	0.078	CP	A1	0.153	0.080	0.088
CC	A2	0.152	0.123	0.102	CP	A2	0.141	0.108	0.113
CC	B	0.133	0.137	0.134	CP	B	0.085	0.164	0.147
CP	A1	0.158	0.112	0.059	RG	A1	0.108	0.088	0.084
CP	A2	0.168	0.120	0.087	RG	A2	0.193	0.101	0.100
CP	B	0.150	0.143	0.119	RG	B	0.146	0.148	0.151

P>F values is indicated as For $p<0.1$, $p<0.05$ * , $p<0.01$ ** , $p<0.001$ ***. h_a =apparent retention of added C for the corresponding soil C fraction; C_r =Carbon added as sorghum residues; Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland; hor(System)=soil horizon effect within system; Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B.

Mean C_s apparent humification in the mineral fraction was similar between LE and UEPP sites, with respective values of 0.125 and 0.113 kg kg^{-1} for C_m and 0.102 and 0.112 kg kg^{-1} for C_{mL} (Table 4-5). Soil C humification in the mineral fraction was mainly determined by the soil horizon, with greater humification in B horizons. At LE, the CP system had lower humification than CC on A1 horizons, while CP showed similar humification than CC on B horizon. A system effect was not observed at UEPP, which seems reasonable considering that the initial C_m and C_{mL} were similar among systems at this site.

There was also an effect of C inputs which interacted with horizon at both sites and also system at LE (Table 4-5). The interaction is explained by lower h_{mLa} humification at the lowest C_r (1 g C kg^{-1} soil), particularly at A1 horizons, and also in CP system in LE. This effect of C_r inputs was not observed on A2 and B horizons, which showed a similar mean humification for different amounts of C_r inputs. Apparent C_{mL} humification on A1 for the lowest input treatment was 0.05 compared to a mean 0.10-0.12 for the rest of the soil horizon and input combinations ($p < 0.001$).

The effects of system and horizon on h_{ma} and h_{mLa} are consistent with the gradient in C_s saturation determined by these sources of variation. At both sites, h_{mLa} decreased as saturation increased (Figure 4-6).

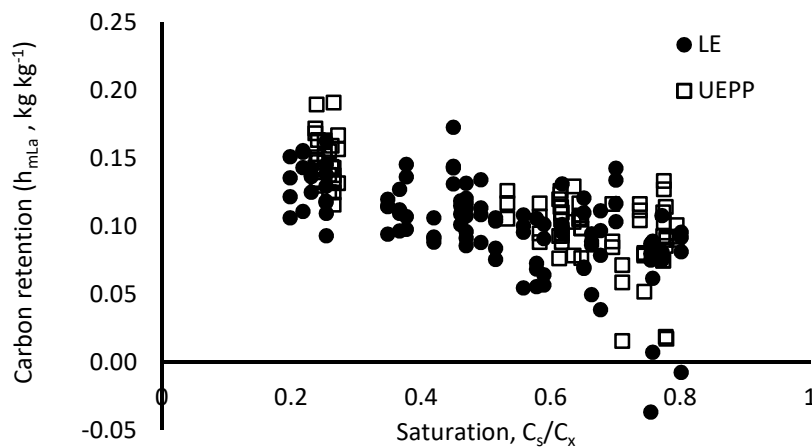


Figure 4-6. Apparent humification in C_{mL} at LE and UEPP sites as a function of the C_s saturation ratio.

Including C_s saturation as a covariate and substitute for soil horizon in the analysis of variance of humification returned statistical models with similar r^2 and RMSE than those including soil horizon (Table 4-6). In addition, a system effect could not be detected on h_{ma} , and the interaction with C_r

were only minor effects when compared to saturation, as most of the variance was explained by the saturation regression term. This indicates that saturation may explain much of the soil horizon and system effects. The saturation effect interacted with the amount of residues, because at the lowest C_r the saturation effect in decreasing C stabilization was even stronger with a negative slope of greater magnitude than for the rest of the treatments.

Table 4-6. Soil C humification in C_p , C_m and C_{mL} soil fractions (h_{pa} and h_{ma} and h_{mLa}) considering soil saturation in LE and UEPP.

Site Source	LE				UEPP			
		h_{pa}	h_{ma}	h_{mLa}		h_{pa}	h_{ma}	h_{mLa}
	DF		F		DF		F	
C_r	3	1.83	1.1	10.4 ***	3	0.67	6.7 ***	6.0 ***
System	2	0.28	1.5	5.8 **	2	2.9	4.9 *	0.5
Sat	1	0.12	34.4 ***	167.7 ***	1	0.27	333.7 ***	179.2 ***
C_r *System	6	0.5	0.5	2.7 *	6	1.15	1.8	0.9
sat* C_r	3	2.81 *	1.7	10.5 ***	3	0.82	20.7 ***	9.4 ***
sat* C_r *System	8	0.37	0.4	2.8 **	8	1.53	3.3 **	1.3
Error (MSE)	84	0.005	0.034	<0.001	67	0.005	<0.001	<0.001
r^2		0.23	0.47	0.80		0.28	0.89	0.74
RMSE		0.070	0.020	0.016		0.070	0.017	0.021
Mean (kg kg ⁻¹)		0.15	0.125	0.102		0.13	0.113	0.112
Sat estimate			-0.115	-0.077			-0.055	-0.053
Least square means (kg kg⁻¹)								
		h_{pa}	h_{ma}	h_{mLa}		h_{pa}	h_{ma}	h_{mLa}
System					System			
	CC no F	0.131	0.118	0.100	CC	0.107	0.100	0.105
	CC	0.148	0.120	0.100	CP	0.126	0.118	0.116
	CP	0.152	0.140	0.116	RG	0.148	0.113	0.113
C_r					C_r			
	1	0.120	0.140	0.104	1	0.125	0.079	0.100
	3	0.151	0.132	0.112	3	0.110	0.118	0.115
	9	0.149	0.124	0.111	9	0.122	0.129	0.119
	27	0.155	0.109	0.095	27	0.151	0.116	0.111
sat*C_r					sat*C_r			
	1	-0.28 *	-0.13	-0.40 ***	1	0.05	-0.23 ***	-0.24 ***
	3	0.00	-0.04	-0.17	3	-0.03	-0.09	-0.12
	9	0.14	-0.15	-0.13	9	-0.04	-0.07	-0.08
	27	0.31	-0.12	-0.08	27	-0.08	-0.06	-0.05

P>F values is indicated as p<0.1. , p<0.05 * , p<0.01 ** , p<0.001***. h_a =apparent retention of added C for the corresponding soil C fraction; C_r =Carbon added as sorghum residues; Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland, sat=saturation ratio (C_s/C_x)

4.5. Discussion

The first hypothesis of this chapter was to test if the humification rate decreased as saturation increases, following the C_s saturation concept (Hassink 1997). This hypothesis is accepted. The concept of C_s saturation relates the C_s stabilization capacity to the amount of C present in the soil mineral fraction, represented here as C_m and C_{mL} . The amount of new C stabilized in the mineral fraction increased with amount of C inputs (Figure 4-5 and Table 4-4). However, this effect at LE interacted with soil horizon and system, associated to C_s saturation in topsoil horizons, particularly at CP systems. When analyzing h_{mL} , data showed that C_s humification in the C_m fraction significantly decreased with C_s saturation (Figure 4-6), providing further evidence of the C_s saturation control on humification.

The C_s saturation seems to be general, as it underpins the soil horizon and system effects, as determined by the model comparison on tables 4-5 and 4-6. At LE, the lower humification in CP compared to CC on A1 horizons could be driven by the fact that A1 horizon on CP is closer to saturation than A1 horizon of CC. Greater humification in B horizons with no differences between systems could be driven by low saturation. An important part of the horizon effect may be due to the higher C accumulation associated to a higher C inputs in the topsoil layers. Similarly, the system effect in C_s humification may be given by the extent to which the system is able to support C_s saturation of the mineral fraction. Tall fescue, one of the species planted in LE CP systems evaluated here, has been reported to contribute as much as three times C_s accrual down the soil profile in comparison with annual systems (Carter and Gregorich 2010). Under field conditions at La Estanzuela experimental location root characterization of perennial species planted in typical forage systems of Uruguay report rooting depth of more than 1 m (Gentile et al 2003). Perennial species in the systems tested may be supporting C_s saturation through greater root mass production than annual cropping systems, and then this saturation in turn explain a decrease in C_s humification in systems and horizons with greater C_s .

The h_{pa} showed no relationship to saturation (Table 4-6), supporting the second hypotheses which stated that C_r retention in C_p is not affected by the C_s saturation ratio. This is reasonable, considering that the C_p fraction represents the unprotected soil organic matter pool in theory with little or no interaction with mineral surfaces, and C stabilization in the context of C saturation theory is based on the interaction of organic compounds with mineral surface active sites (Baldock and Skjemstad 2000). The organic C increase in this unprotected fraction depended on the

addition of C inputs at both sites with relatively minor effect of system interacting with the amount of residues at UEPP. This interaction in C_p accumulation in UEPP is given by a larger C_p accumulation on RG respect to CP and CC. A possible mechanism to explain this effect could be soil aggregation. The accumulation of organic compounds during soil incubation such as polysaccharides resulting from microbial activity and fungal hyphae can act as binding agents for soil aggregation, providing transient protection from further decomposition (Tisdall and Oades, 1982). The greater initial C_p in RG than CP and CC (Table 4-2) may have provided more substrate for this process, resulting in higher accumulation of added C inputs as new C_p in RG system.

Apparent decomposition of C_p , observed as the difference between final old C_p and C_p initial content decreased for increasing amounts of C_r (Figure 4-5). This may be an indication that the increase in C inputs added as residues results in an increase in soil aggregation (Tisdall and Oades, 1982). Another explanation to explore would be that decomposing C_p competes for limited available adsorption sites for C stabilization in the mineral fraction. The larger the C inputs, the larger the pool competing to enter the same limited stabilization locations in the mineral fraction, and the lower the chances for decomposing C_p to find a place for stabilization. This effect of the increasing additions of fresh residues resulting in progressively lower Old C_p losses could be interpreted as a negative priming effect as described by Kuzyakov et al. (2000), supporting the third hypothesis, which stated that C_p turnover is affected by the amount of C in residues. A reduction in Old C_p to C_m transfer due to limited adsorption sites should in theory increase C_p decomposition. However if C_m adsorption sites availability is limited, the only way for C_p to be lost would be full mineralization to CO_2 . However, with the increase in C_r , the provision of all nutrient for microbial processes may not be balanced for microbial growth and a limiting factor other than C and N may be limiting decomposition. This would explain the effect of C_r in reducing C_p losses (Figure 4-5) and that at LE saturation effect in decreasing h_{pa} is significant at low C_r ($C_r = 1 \text{ g C soil kg}^{-1}$), but not when increasing C_r inputs (Table 4-6). Although this interaction between C_r and saturation was not evident in UEPP and therefore h_{pa} making this point less clear.

The amount of C inputs added as residues did not affect the amount of C that was stabilized in the mineral fraction, supporting the last hypothesis. Even if the amount of old C that was retained in C_m and C_{mL} slightly varied respect to initial amounts showing fluxes between C_s pools, this variation did not respond to the amount of C inputs (Tables 4-4 and 4-5). This is an indication that although the addition of fresh residues may affect C dynamics of the non-stabilized C pool, as the negative

priming effect observed on Old C_p , the pool of C that is actually stabilized by adsorption to the soil mineral fraction surface active sites may still remain unaffected because the physical protection would make C_m relatively independent of priming. However, this contrast with the priming effect reported by Mazzilli et al (2014), and may suggest that positive priming was not a factor because of the low C:N ratio of the residue added (C:N=13).

Mean C humification rates in C_{mL} (0.10 kg kg⁻¹ for LE and 0.11 kg kg⁻¹ for UEPP), and the response to soil saturation (-0.08 for LE and -0.05 for UEPP) were similar among sites, resulting in similar humification models. This is an indication that not only the concept, but also the approximation equation developed by Hassink and Whitmore (1997) to estimate maximum C stabilization capacity as a function of the soil content of fine particles seems to provide a workable foundation to approximate C_x in a variety of soils. Despite some exceptions have been reported (Hassink 1997; Feng et al. 2014), and more complex models have been suggested (Feng et al. 2013; Beare et al. 2014), soils investigated here from the Pampas region of South America under crop-pasture systems support C_x estimates by this simple function. The concept of C saturation provides a strong basis to modeling C cycling, as those proposed by Hassink and Whitmore (1997), and other mathematical approaches along this line, such as the C-Farm and Cycles models (Kemanian et al. 2005). This modeling approach of including a saturation mechanism of the soil mineral fraction clearly contrasts with that of models with multiple pools such as Century (Parton et al., 1987) or Roth-C (Jenkinson et al. 1990). As suggested by White et al (2014), these multiple pool models may have indirectly accounted for C_s saturation controls through differential microbial efficiency as a function of clay.

The explicit inclusion of a saturation mechanism has direct implications on N cycling, explored by a modeling comparison approach by White et al (2014) and discussed in Chapter 2 in the context of crop-pasture systems. Cumulative evidence in the last decades supports C saturation as a fundamental mechanism in C_s cycling. Experimental evidence to further develop the suggested implications of the C_s saturation concept on N cycling in the soil would consolidate the C_s saturation theory as a fundamental mechanism explaining soil organic matter cycling in the soil, perhaps providing a unifying theory to benchmark soil productivity.

4.6. Conclusions

Soil C saturation across sites and systems was associated to a decrease in C_s humification in the soil mineral fraction, without any association on C accrual on the unprotected C_s pool in the C_p fraction. Soil C humification explained by C_s saturation absorbed most of the system and soil horizon effects, supporting saturation theory as a major mechanism behind these observed effects. The addition of C inputs as fresh residues affected C_s accumulated in the unprotected fraction, without disturbing C_s stabilized in the soil mineral fraction. This research outcomes support C_s saturation concept based on the principle of mineral matrix physical protection to C_s decomposition as a major mechanism explaining C_s sequestration and cycling operating in agricultural soils under crop pasture systems. One of the implications at the global scale is the limited capacity of soils to sequester C in a stabilized form, and a second important aspect is the fact that the addition of fresh residues does not seem to alter C sequestered through this physical stabilization mechanism. At the local scale, the third relevant aspect of C_s saturation theory as a fundamental mechanism in soil organic matter cycling is the direct implications on N cycling, which is a fundamental aspect to consider for agricultural systems management.

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CHAPTER 5 –SOIL CARBON SATURATION AND THE CONTROLS OF NITROGEN RETENTION FROM DECOMPOSING RESIDUES

5.1. Abstract

According to soil C (C_s) saturation theory, as the maximum C_s storage capacity of the soil mineral matrix is reached, the retention of new C is gradually reduced, and C_s decomposition increases with the accumulation of unprotected soil organic matter. Soil organic matter cycling establishes a clear connection between C_s and nitrogen (N) pools, and therefore an increase in C decomposition can be translated to an increase in soil N availability. An increase in N inputs exceeding N sinks can lead to soil N saturation. The first stages of this process would favor plant productivity, due to enhanced N available to satisfy plant demands. Nevertheless, reaching advanced stages of N saturation would lead to increased environmental losses and a system decline. In this chapter, I explore the connection between C_s and N saturation concepts by following the fate of N added as residues enriched in ^{13}C and ^{15}N in soils under a gradient of C_s saturation ratios. The hypotheses to be tested were that: 1) if C saturation is linked to N saturation, the retention of N in the soils should decrease as the C_s saturation ratio increases; 2) The C:N ratio of the new organic material retained in the soil should decrease with saturation, showing some degree of decoupling between C and N cycling to support buffering capacity of C saturation on N losses. Soils with different C_s saturation ratios were obtained from different long-term management (continuous annual cropping to regenerated grassland) and horizons (A1, A2, and B) at two sites (La Estanzuela = LE and Palo a Pique = UEPP, Uruguay). Increasing levels of dual labeled residues with a C:N ratio of 12.9 determined increasing N inputs ($N_r=0.078, 0.234, 0.701, 2.103 \text{ kg N soil kg}^{-1}$). The retention of N was determined in the particulate ($N_p > 53 \mu\text{m}$) and mineral soil fractions after leaching inorganic compounds ($N_{mL} < 53 \mu\text{m}$). After 120 days of incubation, mean N retention and its distribution in soil physical fractions was similar among sites, with a mean of 0.09 kg kg^{-1} retained in N_p and 0.21 kg kg^{-1} in N_{mL} . Soil C saturation reduced N retention in the N_{mL} fraction (mean rates - 0.13 and -0.10 kg kg^{-1} at LE and UEPP), supporting the conceptual model of N cycling dependence on C_s saturation. The C:N ratio of the organic material stabilized in the soil was reduced with saturation ratio, ranging between 8.5 and 0. This indicates that C and N cycling are not fully coupled and although both C and N stabilization rates are reduced with saturation, there is a buffering capacity for N losses in C saturated soils. The inclusion of perennial pastures in crop rotations may play a key role in sustainable nutrient management providing an opportunity for managing C_s and N saturation.

5.2. Introduction

The main mechanism for organic C stabilization in the soil is the adsorption of organic particles to mineral surfaces and their aggregation (Baldock and Skjemstad 2000). Mineral surfaces available for adsorption are limited in a given soil according to the soil clay and silt content, and therefore a limited capacity of the soils to stabilize soil organic matter ultimately depends on soil texture, following the concept of C_s saturation as proposed by Hassink and Whitmore (1997). Given the narrow range of organic matter C:N ratio, if a maximum capacity for C_s stabilization can be reached, it is reasonable to expect that the same principle would stand for the retention of N. Following this concept, a maximum N stabilization capacity model would follow the C model divided by the soil C:N ratio. It is not surprising then, that the ratio that can be obtained by dividing the models developed by Hassink (1997) for C and N maximum stabilization in $< 20 \mu\text{m}$ particles is close to 10, matching the typical soil C:N ratio.

Soil organic matter (SOM) cycling establishes a clear connection between C and N dynamics, determining that C_s saturation has consequences on N cycling and N estimated availability for biological processes in the soil, as observed when implementing this mechanisms in simulation models (White et al. 2014) and discussed in Chapter 2. Extending the concept of C_s saturation to N dynamics (Castellano et al. 2012) implies that soils that show little or no capacity for C stabilization in the silt + clay soil fraction would be expected to exhibit N saturation symptoms, particularly for mineral-associated organic matter.

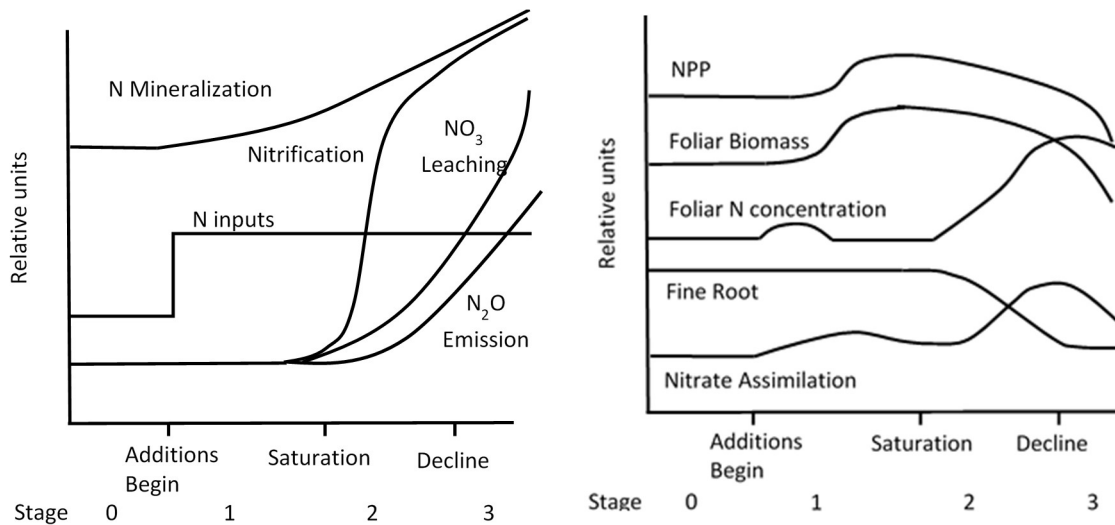


Figure 5-1. N saturation stages. Adapted from Aber et al (1989)

When the soil capacity to retain N is close to saturation, a higher concentration of ammonium and nitrate is available in the soil solution. When availability of these mineral forms is in excess of combined plant and microbial nutritional demand, the ecosystem is considered to be saturated in N (Aber et al., 1989). Initial saturation symptoms include reduced N translocation from senescing foliage, leading to higher N content in litter and faster decay rates. Then, ammonium levels increase, increase nitrate leaching and N₂O emissions, as well as reducing fine roots formation (Figure 5-1). The first stages of N saturation would favor plant productivity through enhanced nutrient cycling and N availability, and the last stages of saturation would lead to N losses to the environment.

The concept of N saturation has been mostly explored in forest soils where most of N inputs come from deposition (Aber et al 1989; Lovett and Goodale, 2011), however, there is evidence that supports its application to grassland systems and other N inputs. After eight years of annual N enrichment, Baer and Blair (2008) observed that the fertilized grassland soil contained a similar microbial biomass C to the ambient soil, but with higher N content and lower C:N ratio. The higher biomass N content and net nitrification rates in the fertilized soil compared to the ambient soil in their experiment were consistent with patterns of N saturation described by Aber et al (1989).

Two components have been recognized in N saturation dynamics: *capacity* N saturation, in which the sinks in the vegetation and soil are zero or negative, and *kinetic* N saturation, in which the sinks are positive but lower than the N input rate (Lovett and Goodale, 2011). Sink strengths governing *kinetic* N saturation in vegetation and soil N dynamics have also two components, one due to C accumulation in the system and the other due to change in the stoichiometry (C:N ratio) of the pool (Kaye et al. 2003, Baer and Blair, 2008; Lovett and Goodale, 2011).

According to these concepts, degraded soils not only need more N inputs because of the low capacity to provide it, but also to fulfill the soil stabilization capacity. When systems are far from saturation low N availability could be expected as a consequence. Plants and microorganisms demand may not be fulfilled, while the soil matrix could stabilize part of N inputs. Plants would have to compete with these other N sinks in a system that is far from saturation capacity, since added N can flow simultaneously to all sinks and losses in the ecosystem and the fate of the added N as well as its temporal flow patterns would depend on the strength of the sinks (Lovett and

Goodale, 2011). This implies that external N applications would be less efficient in soils in which the N content is far from their saturation level, as the soil stabilization capacity would compete as a sink with the plants for N absorption.

Integrated crop-pasture systems could provide conditions for N saturation of the soil *capacity* of stabilization. A positive feedback between soil N supply and plant productivity may promote enhanced long term N availability as SOM accumulates in plant and soil pools of grassland soils showing N saturation dynamics (Baer and Blair, 2008). Therefore, as long as not applied in excess of plant demand, N fertilization is likely to be more efficient when applied in systems close to their saturation level, as the soil would provide less competition to plant uptake of the ready available N.

When legume species are included in pasture systems, biological dinitrogen fixation contributes to build N reservoirs in the soils in addition to C_s as observed in chapter 3 and 4 for soils of Uruguay. Legume species used in mixed pastures of Uruguay fix dinitrogen with an efficiency ranging between 29 and 40 kg N/Mg DM of above ground biomass (Díaz Rosello 1992; Garcia et al. 1994). The second year of a perennial pasture is usually the most productive, where 180 to 390 kg N/ha can be fixed according to this range of efficiency and the biomass produced for different legume-gasses mixtures (Labandera et al., 1988; Mallarino et al., 1990a). About 35kgN/ha/yr can be transferred to the grass component in mixed pastures (Mallarino et al., 1990b), increasing the retention of the incorporated N compared with pure legume pastures. However, during the lifetime of a pasture the proportion of legumes in mixed pastures slowly decays, and the incorporation of N is then reduced every year. The total N incorporated in the soil during 4 years of mixed pastures in Uruguay has been estimated in 500kgN/ha (Díaz Rosello, 1992).

Conditions for C_s and N saturation seem to provide several advantages to integrated crop-pasture systems in terms of ecosystem services related to C and N cycling. Production systems close to or at their soil N saturation level can provide a faster and greater N supply than soils far from the saturation levels, as well as a more efficient use of fertilizer. In addition, depending on the relationship between C_s and N saturation mechanisms, provision of N may not necessarily be accompanied by C losses to the environment, allowing both N supply and C sequestration at the same time instead of showing decay in C_s . In contrast, annual cropping systems far from saturation levels, not only would provide less N, but also show a less efficient use of fertilizers, providing a greater soil sink for stabilization of external N supply, by adjusting the soil C:N ratio. Grassland

systems can stabilize large amounts of N incorporated into SOM pools, providing a pathway for rapid soil N immobilization (Kaye et al., 2002). This accumulation of SOM may provide a mechanism to avoid large seasonal N losses in grassland systems when inorganic N fertilizer is applied. In these systems, a larger amount of external instant N supply may be required for a kinetic saturation and short term losses.

A variable stoichiometry between C and N content in the soil could determine that a certain soil supply of N may not necessarily mean losses of C in a 10:1 C:N ratio. Moreover, considering that plants not only absorb inorganic N but are also able to absorb different N containing monomers resulting from microbial depolymerization (Schimel and Bennett, 2004), calculating corresponding C_s losses with a corresponding C:N fixed ratio does not seem to be reliable. Rather than calculating a fixed proportion of C released to the environment, a change in C:N ratio should be considered. The possibility of a faster N cycling compared to the C cycling may be considered in this context. This implies that soils that are close to N saturation should be able to supply more N, but may not lose a proportional amount of C as a consequence of N supply.

Within this conceptual framework, implications in N and C economy of the design of integrated crop-pasture or pure grain systems have an impact on farming profitability and the environment. The use of external sources of N could be reduced if C and N contents in the soil are maintained close to saturation levels, maximizing the advantages of natural feedback processes in the soil, showing a pathway for sustainable intensification. As exposed in Chapter 3, the higher the level of organic matter retained in the soil, the higher its turnover would be, allowing a timely delivery of N as observed in Chapter 2. It seems that the N saturation mechanism may operate in relation to C saturation in these systems, possibly resulting in accelerated dynamics but also buffered N losses at the same time. If N saturation were proven to work simultaneously with C saturation but with a dynamic stoichiometry, integrated crop-pasture systems would deserve attention not only because large amounts of N are added through N_2 fixation, but also may have a key role for sustainable nutrient management through the dynamics of N timely delivery and buffering of the N losses.

I elaborated the following hypotheses that link the concepts of C_s saturation and N saturation to better understand C and N cycling in soils under crop-pasture system management. 1) If C saturation is linked to N saturation, the retention of N in the soils should decrease as the saturation ratio increases. 2) The C:N ratio of the new organic material retained in the soil should

decrease as C_s saturation increases, showing some degree of decoupling between C and N cycling that results in buffering N losses as suggested in Chapter 2. I tested these hypotheses following the fate of C and N in soils incubated with variable levels dual labeled inputs, enriched in both ^{13}C and ^{15}N (Balesdent et al, 1987; Personeni and Loiseau; 2004). The soils were taken from two long-term experiments (>50 and >20 years each) that have had contrasting crop-pasture rotation systems that generated a gradient of C_s in the same soil matrix. For reference, a list of abbreviations used in this chapter is provided in Table 5-1.

Table 5-1. List of abbreviations used in Chapter 5.

Abbreviation	Description
A1	Topsoil 6 cm with most roots and a darker color
A2	Topsoil A diagnostic soil horizon remaining after removing A1.
B	Subsoil B horizons packed separated from transitional horizons
C	Carbon
C_s	Soil organic carbon
C_s/C_x	Soil carbon saturation ratio. Ratio between actual soil carbon and saturation contents
C_x	Soil carbon saturation content (kg C kg^{-1} soil)
CC	Continuous annual cropping rotational system
CC no F	Continuous cropping with no fertilization
CP	Crop-pasture rotation
^{15}F	Isotope Molar fraction
h	Soil N retention rate after accounting for simultaneous decomposition
h_{mLa} (kg kg^{-1})	Apparent fractional N_r retention as N_{mL}
h_{pa} (kg kg^{-1})	Apparent fractional N_r retention in N_p
h_a	Apparent retention of added N for the corresponding N_s fraction
INIA	National Institute for Agricultural Research in Uruguay (Instituto Nacional de Investigación Agropecuaria)
K	Soil Nitrogen decomposition rate
LE	“La Estanzuela” experimental site
N	Nitrogen
N_r	Nitrogen in residues
N_p	N in the particulate soil fraction retained in a 53 μm mesh after soil dispersion on 0.5 % (m/v) sodium hexametaphosphatesolution
N_m	N in the mineral soil fractions < 53 μm
N_{mL}	Mineral associated organic carbon passing a 53 μm mesh after soil dispersion on 0.5 % (m/v) sodium hexametaphosphatesolution; retained after leaching KCl soluble compounds and centrifuging
N_s	Soil nitrogen
New N_{mL}	At the end of the incubation, N in soil fraction < 53 μm from residues after KCl leaching and centrifuged
New N_p	At the end of the incubation, N in soil fraction > 53 μm from residues
New N_s	New Nitrogen coming from residues
Old N_{mL}	At the end of the incubation, N in soil fraction < 53 μm from soil after KCl leaching and centrifuged
Old N_p	At the end of the incubation, N in soil fraction > 53 μm from soil
Old N_s	Nitrogen present in the soil before the addition of residues
RG	Regenerated grassland
SOM	Soil organic matter
superscript i	Initial N at time zero
superscript f	Final N at the end of incubation
UEPP	“Palo a Pique” experimental unit.
x_r	The proportion of residue-derived N stabilized in the soil calculated as the molar fraction using the mass balance equation

5.3 Methods

5.3.1 Soil saturation gradient

The soils selected for the incubations are described in Chapter 3. There are three sources of variation to generate a saturation gradient: two locations, three contrasting cropping systems, and three soil horizons. At each location, the soils are uniform and share the same climate and therefore, excluding erosion and tillage mixing, differences in C_s are related to the total C inputs and their distribution with depth. Two long-term crop-pasture rotational experiments at the National Institute for Agricultural Research in Uruguay (INIA) serve this purpose well, presenting different SOM content in the same soil as a consequence of different rotations and managements. The experiments are located at “La Estanzuela” (LE) and “Palo a Pique” (UEPP). The LE experiment started in 1962 and is the oldest long-term rotational experiment in South America. The UEPP experiment started in 1995. At each location, three contrasting crop rotations were selected (there are more at LE) whose characteristics are summarized in Chapter 3, Table 3-2.

There was no a priori consideration regarding the locations except that soils are on a qualitative basis more fertile at LE (the temperate South American Pampas). Regarding cropping systems, soils with a larger proportion of perennial crops are expected to have a higher saturation ratio, and a cropping system at LE receiving no fertilizer and maintained in annual cropping should have a low saturation throughout. Finally, saturation ratio was expected to decrease with depth for two reasons: lesser C inputs and higher clay with depth in the Argiudolls (argillic B horizon should have more less C_s than the topsoil of a mollic epipedon). Therefore, soils should be near saturation in the soils surface and well below saturation in the B horizon.

5.3.2 Soil sampling

The A and B genetic soil horizons were sampled in spring 2014 using a hydraulic probe cylinder of 4.2 cm diameter that rendered 0.8 m deep soil cores. For each system, 40 cores were taken and gently packed in individual sample plastic bags, separating each soil horizon in the field (Figure 3-1). The topsoil was divided in A1 and A2 soil horizon. The top 6 cm with most roots and a darker color was identified as A1, letting A2 as the remaining A diagnostic soil horizon. Subsoil B horizons were packed separated from transitional horizons retaining only the B horizon. Samples were stored in a 4°C refrigerated room until processing. Once in the lab, samples were manually and gently broken and air dried to pass a 2mm sieve.

5.3.3 Soil C saturation estimation

Soil C saturation ratio C_s/C_x was estimated for each soil rotation and horizon by applying the equation developed by Hassink and Whitmore (1997) according to soil particle size analyses. The C_s saturation ratio was estimated using the C_s obtained from the soils samples at the beginning of the incubation.

$$C_x \text{ (g C soil kg}^{-1}\text{)} = 21.1 + 0.037 \times \text{clay (g clay soil kg}^{-1}\text{)} \quad (1)$$

$$\text{Soil saturation ratio (kg kg}^{-1}\text{)} = C_s/C_x \quad (2)$$

5.3.4 Labeled plant residues

Plant residues highly enriched in ^{13}C were prepared in greenhouse pots by leaf feeding sorghum (*Sorghum bicolor* Moench L.) plants with a ^{13}C urea solution (Schmidt and Scrimgeour 2001). Beginning at three leaves stage, plant leaves were sprayed every other day until plant harvesting at anthesis using a solution of 97% atom ^{13}C urea (2 g/L). Plants were kept in a sealed plastic chamber for two hours to prevent CO_2 losses after spaying. The advantages of the leaf feeding method for ^{13}C labeling over the traditional chamber method are the relatively low cost and minor infrastructure requirements (Figure 3-2). Dual isotope labeling of plant tissue was obtained by fertilizing ^{13}C labeled sorghum pots with 10% atom ^{15}N NH_4NO_3 for ^{15}N enrichment. Labeled residues C:N was 12.9 with an isotopic composition of $\delta^{13}\text{C} = 199$ and $\delta^{15}\text{N} = 3520$, contrasting with the soil range of $\delta^{13}\text{C} = -18$ to -22 , and $\delta^{15}\text{N} = -4$ to 8 .

5.3.4 Incubations

Each incubation treatment consisted of three replicates of 100 g soil samples of A1, A2 and B soil horizons mixed with ^{13}C and ^{15}N sorghum litter for 120 days at the following ratios: 0, 1, 3, 9, and 27 g C soil kg^{-1} , and with a C:N of 12.9. Respectively for each treatment, N added in residues were 0.078, 0.234, 0.701, 2.103 g N soil kg^{-1} . Soil and residues were carefully mixed in glass jars and slowly wet to soil field capacity. Incubation jars were maintained at 23 °C during the 120-day incubation period. Moisture was replenished twice a week by adding distilled water to maintain soils slightly below field capacity.

5.3.5 Sample physical fractionation and isotopic composition determination

Before and after the incubation, the soil was physically separated in two fractions (soil retained or passing a 53 μm sieve) and the Ns determined for each one of them separately: the particulate

organic matter (N_p , $> 53 \mu\text{m}$), and the mineral associated organic matter (N_m , $< 53 \mu\text{m}$). Soil fractions were obtained by sieving 10 g of soil through a $53 \mu\text{m}$ mesh after soil dispersion on 0.5 % (m/v) sodium hexametaphosphate solution, following the procedure described by Marriott and Wander (2006). Soluble compounds were removed from the mineral fraction using a KCl solution and centrifuging (Kaye et al., 2003). Briefly, 25 ml of KCl 2 M solution were added to 2 g of N_m fraction in a 50 ml falcon centrifuge tube, and placed on a shaker for 30 min. The tubes were centrifuged for 10 minutes at 4000 RPM after adding with 15 drops of CaCl_2 0.25 M for clay flocculation. This procedure was performed twice using the KCl solution, and a third time with distilled water. Leachate was removed keeping the solid phase for oven-drying at 60°C for 48 hours. The N in the sample obtained after removing KCl soluble compounds is referred to as N_{mL} . Both N_p and N_{mL} were added to obtain N_s for each sample.

After physical fractionation N isotopic composition were tested on a continuous flow IRMS-EA at Penn State's Geochemistry and Mineral Sciences isotope lab. The N concentration of each sample was determined at Penn State's Soil Research Cluster Laboratory.

5.3.6 Calculation of h and k

Soil and residues molar fractions (^{15}F) were calculated using the $\delta^{15}\text{N}$ and N concentration of soil samples before and after soil incubation. The fraction ^{15}F in each sample was calculated from $\delta^{15}\text{N}$ as delivered by the analyzer, according to the following equations:

$$\delta^{15}\text{N}_{(\text{‰})} = \left[\frac{^{15}\text{R}_{\text{sample}}}{^{15}\text{R}_{\text{standard}}} - 1 \right] 1000 \quad (3)$$

$$^{15}\text{R} = \frac{^{15}\text{N}}{^{14}\text{N}} \quad (4)$$

$$^{15}\text{F} = \frac{^{15}\text{R}}{(1 + ^{15}\text{R})} \quad (5)$$

where $^{15}\text{R}_{\text{standard}}$ is the air standard.

The proportion of residue-derived N stabilized in the soil (x_r) was calculated as the molar fraction using the mass balance equation:

$$x_r = (F_t - F_s) / (F_r - F_s), \quad (6)$$

where $F_t = ^{15}\text{F}$ of C the soil + residue sample, $F_r = ^{15}\text{F}$ of N in the added residue, which was calculated based on the IRMS – EA $\delta^{15}\text{N}$ as described above.

The quantity of the residue derived N_r in the soil (as N_s) at the end of the incubation period was calculated as:

$$\text{New } N_s = N_s x_r \quad (7)$$

$$\text{Old } N_s = N_s(1-x_r) \quad (8)$$

where N_s is the total soil N of the respective N_s pool at the end of the incubation, x_r is the proportion of residue-derived N stabilized in the soil, New N_s is the new nitrogen coming from residues and Old N_s is the old nitrogen.

An underlying assumption in these calculations is that isotopic fractionation in microbiological mediated process can be ignored when working with such highly labeled residue material.

Soil N decomposition is assumed to follow first order decay, with the decomposition rate k calculated using the initial and final N_s as indicated by the superscript i and f, respectively, in Eq. 9:

$$k = -\ln(N_s^f/N_s^i)/120 \quad (9)$$

Data processing was done with k on daily basis, but results are annualized if numerically convenient. Apparent humification of added N (h_a) for each soil fraction and its corrected form to account for decomposition during the incubation period was calculated relative to N inputs (C_r):

$$h_a = \text{New } N_s / N_r \quad (10)$$

$$h = (\text{New } N_s^f + k \cdot 0.5 \text{ New } N_s^i) / N_r \quad (11)$$

5.3.7. Statistical analysis

The effect of system, horizon, saturation ratio (C_s/C_x) and N inputs on N_s , k and h were tested by analysis of variance within each site using the SAS statistical software package. The C_s/C_x term was included as a covariable.

5.4. Results

5.4.1 Soil nitrogen contents

Mean N_s content after leaching of inorganic compounds was similar among sites, with an average of 1.4 g N soil kg^{-1} (range 2.4 to 0.9 g N soil kg^{-1}) considering the sum of N_p and N_{ml} (Table 5-2). The main factor explaining the variation in observed N initial contents was explained by system at LE, where systems including perennial pastures and fertilization showed greater N_s contents, while no system effect was observed at UEPP. Soil horizon was the second variable affecting N contents at LE, and the main effect at UEPP, where A1 horizons showed the greatest N_s contents and B

horizons showed lower amounts of N. These data are consistent with observations on total C contents in Chapter 3. Final N contents were affected by the same variables, in addition to a significant effect of residues treatments reflecting the addition of N_r according to different levels of inputs.

Table 5-2. Analysis of variance for soil nitrogen total content ($N_s=N_p+N_{mL}$) before (N_s^i) and after (N_s^f) incubation (NA = not applicable; LS Means = least squares means).

Site	LE				UEPP			
	N_s^i	N_s^f	N_s^i	N_s^f	N_s^i	N_s^f	N_s^i	N_s^f
Source	DF	F Values	DF	F Values	DF	F Values	DF	F Values
N_r	NA	4	NA	79.2 ***	NA	4	NA	93.0 ***
System	2	2	73.0 ***	102.6 ***	2	2	2.5	14.1 ***
horizon(System)	6	6	35.3 ***	81.3 ***	6	6	51.8 ***	130.4 ***
N_r *System	NA	8	NA	0.57	NA	8	NA	0.6
N_r *hor(System)	NA	24	NA	0.74	NA	24	NA	1.3
Error (MSE)	18	90	0.016	0.023	18	71	0.014	0.013
Mean, g N soil kg^{-1}			1.464	1.405			1.405	1.354
RMSE, g N soil kg^{-1}			0.128	0.152			0.119	0.115
Least squares means by treatment			N_s^i	N_s^f	N_s^i	N_s^f		
System	Horiz	g N soil kg^{-1}		System	Horiz	g N soil kg^{-1}		
CC no F	A1	1.249	1.292	CC	A1	1.784	1.648	
CC no F	A2	1.182	1.281	CC	A2	1.340	1.276	
CC no F	B	0.922	1.032	CC	B	0.906	1.022	
CC	A1	1.720	1.618	CP	A1	1.859	1.747	
CC	A2	1.447	1.384	CP	A2	1.412	1.360	
CC	B	1.124	1.078	CP	B	0.939	0.993	
CP	A1	2.428	2.178	RG	A1	2.086	1.910	
CP	A2	2.000	1.662	RG	A2	1.364	1.358	
CP	B	1.103	1.120	RG	B	0.956	1.103	

P>F values is indicated as p<0.1· , p<0.05 * , p<0.01 ** , p<0.001***. N_r =Nitrogen added as sorghum residues; N_s =Nitrogen in the soil; Superscript i= initial content, superscript f= final content after incubation, Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland; horizon(System)=soil horizon effect within system; Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B.

The amount of N_s in the particle size fractions followed variation patterns consistent with those observed for C in Chapter 4. Initial N_s contents in the N_p fraction varied with soil horizon and system at both sites, where systems including perennial pastures and topsoil horizons showed greater amounts of N (Table 5-3, Figure 5-2). The greatest N_p was observed on A1 horizon of the RG, with 0.7 g N soil kg^{-1} , followed by CP A1 horizons at both sites with 0.45 g N soil kg^{-1} . It is remarkable that A1 horizon of the CC no F system showed lower N_p than A2 at LE CP, with N contents close to those observed on B horizons.

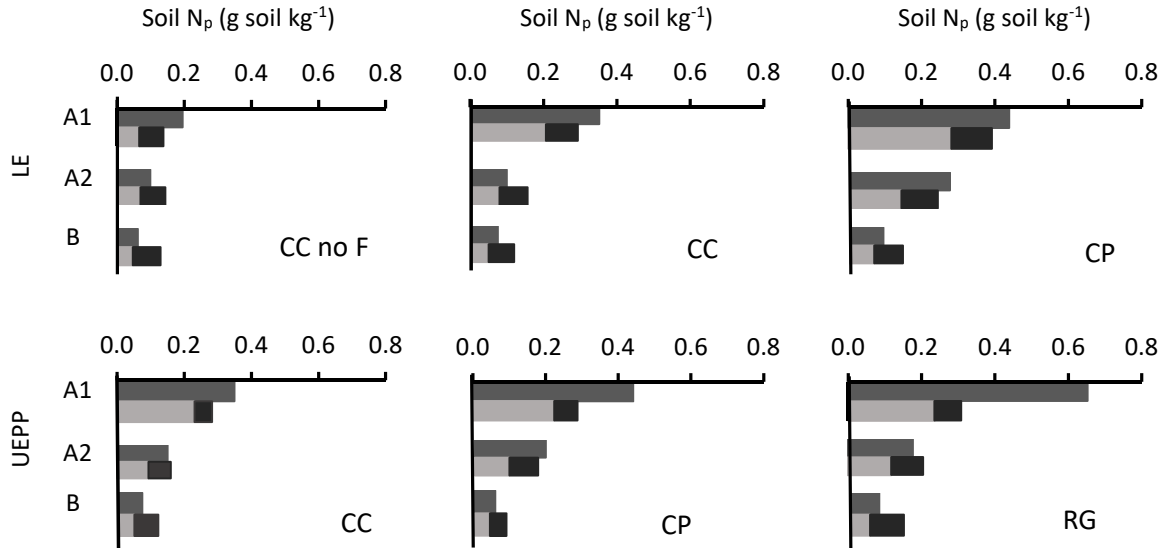
Table 5-3. Analysis of variance for soil Nitrogen content in the particulate organic matter fraction ($N_p > 53 \mu\text{m}$) before and after incubation (NA = not applicable; LS Means = least squares means).

Site	LE					
Source	DF initial	DF final	N_p^i	N_p^f	New N_p^f	Old N_p^f
N_r		3		52.0 ***	114.7 ***	7.6 ***
System	2	2	14.4 ***	9.0 ***	1.0	12.0 ***
hor(System)	6	6	19.7 ***	15.1 ***	0.8	22.3 ***
N_r *System		6		1.1	0.6	1.5
N_r *hor(System)		18		0.8	0.6	0.9
Error (MSE)		72	0.003	0.009	0.002	0.004
Mean N (g soil kg^{-1})			0.182	0.174	0.081	0.116
RMSE			0.056	0.093	0.049	0.064

Site	UEPP					
Source	DF initial	DF final	N_p^i	N_p^f	New N_p^f	Old N_p^f
N_r		3		96.1 ***	195.6 ***	9.9 ***
System	2	2	6.1 **	6.7 **	7.3 **	2.9 .
hor(System)	6	6	28.3 ***	51.1 ***	1.9 .	95.5 ***
N_r *System		6		3.9 ***	5.0 ***	1.8
N_r *hor(System)		18		0.9	1.3	0.9
Error (MSE)		55	0.005	0.002	0.001	0.001
Mean N (g soil kg^{-1})			0.074	0.171	0.061	0.123
RMSE			0.240	0.046	0.028	0.030

P>F values is indicated as $p < 0.1$. , $p < 0.05$ * , $p < 0.01$ ** , $p < 0.001$ ***.

N_r =Nitrogen added as sorghum residues; N_p =Nitrogen in the soil fraction $> 53 \mu\text{m}$; Superscript i= initial content, superscript f= final content after incubation, New N_p = N in soil fraction $> 53 \mu\text{m}$ retained from residues, Old N_p = N in soil fraction $> 53 \mu\text{m}$ from soil, hor(System)=soil horizon effect within system.



References: References: Dark Grey: N_p initial, Light Grey: Old N_p final, Black: New N_p final LE: La Estanzuela Experimental Site, UEPP: Palo a Pique experimental site, Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland; Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B.; Soil N_p : Nitrogen in the soil fraction $> 53 \mu m$.

Figure 5-2. Nitrogen content in the soil particulate organic matter fraction ($N_p > 53 \mu m$).

The amount of N in the N_{mL} before the addition of residues also varied with system and horizon following the same system and horizon patterns at LE, but only with soil horizon at UEPP (Table 5.3, Figure 5-3). Observed N contents in t N_{mL} fraction varied between 2.0 and 0.9 g N soil kg^{-1} , where greatest values were observed in A1 and A2 horizons of CP systems at LE, and the lowest values on B horizons. Five decades of contrasting management at LE affected N contents in N_{mL} fraction in the same way as N_p . Interestingly, the A2 horizon at CP in LE showed similar N_{mL} content as A1. On the other hand, A1 horizon of CC no F showed similar N contents as low as the B horizons. At UEPP however, two decades of different system management were not reflected in N contents in the N_{mL} fraction, where the variation in N_{mL} was only explained by the soil horizon.

After the incubation of soils with fresh residues, variation in N_s contents was explained by the amount of residues added, as well as maintaining the initial system and horizon effects. At the end of the incubation, system effects on N contents were detected at both sites, where systems including perennial pastures showed greater N contents at the end of the incubation. Variation due to N in residues affected final N_s contents in both N_p and N_{mL} , mainly by the effect of an

increase in new N incorporated in the soil. Increasing N_r inputs resulted greater mean N retention (Tables 5-3 and 5-4, figure 5-4).

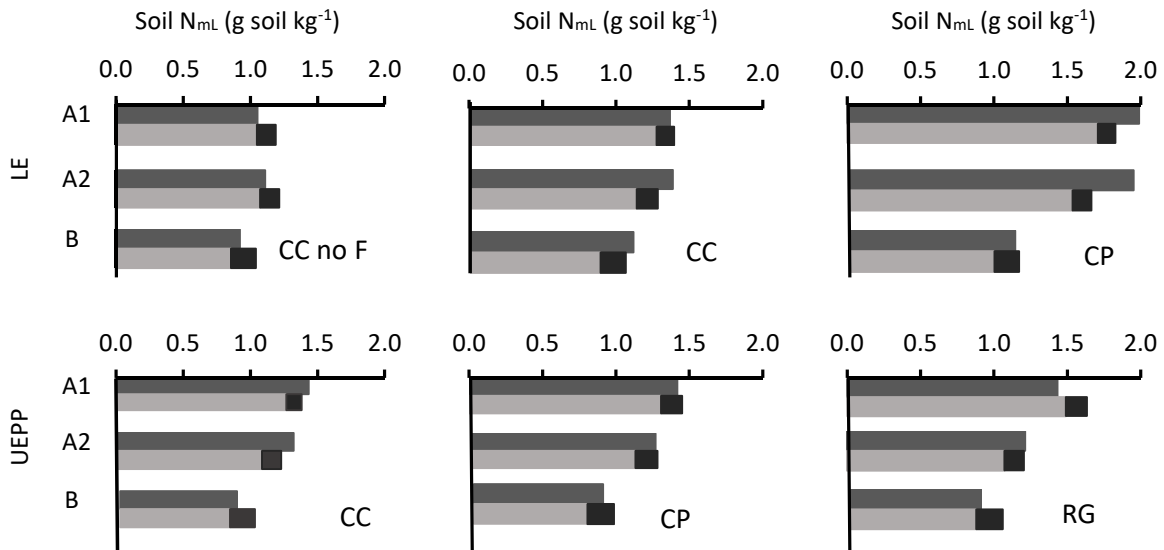
Table 5-4. Analysis of variance for soil Nitrogen content in the N_{mL} fraction (> 53 μm) before and after incubation (NA = not applicable; LS Means = least squares means).

Site		LE					
Source	DF		N _{mL} ⁱ	N _{mL} ^f	New N _{mL} ^f		Old N _{mL} ^f
	initial	final			F values		
N _r		3		35.2 ***	1863.8 ***	2.9 *	
System	2	2	62.9 ***	115.3 ***	11.8 ***	139.4 ***	
hor(System)	6	6	24.5 ***	78.9 ***	15.5 ***	101.4 ***	
N _r *System		6		1.4	7.3 ***	1.0	
N _r *hor(System)		18		1.0	5.7 ***	0.9	
Error (MSE)		72	0.012	0.014	0.0003	0.009	
Mean N			1.282	1.231	0.141	1.117	
RMSE			0.111	0.117	0.018	0.096	

Site		UEPP					
Source	DF		N _{mL} ⁱ	N _{mL} ^f	New N _{mL} ^f		Old N _{mL} ^f
	initial	final			F values		
N _r		3		48.7 ***	2267.3 ***	0.8	
System	2	2	0.1	11.7 ***	6.7 **	8.8 ***	
hor(System)	6	6	31.7 ***	76.7 ***	27.2 ***	73.5 ***	
N _r *System		6		1.0	3.1 **	1.4	
N _r *hor(System)		18		1.4	6.3 ***	1.2	
Error (MSE)		72	0.008	0.015	0.0003	0.013	
Mean N			0.087	1.192	0.150	1.065	
RMSE			1.165	0.121	0.018	0.114	

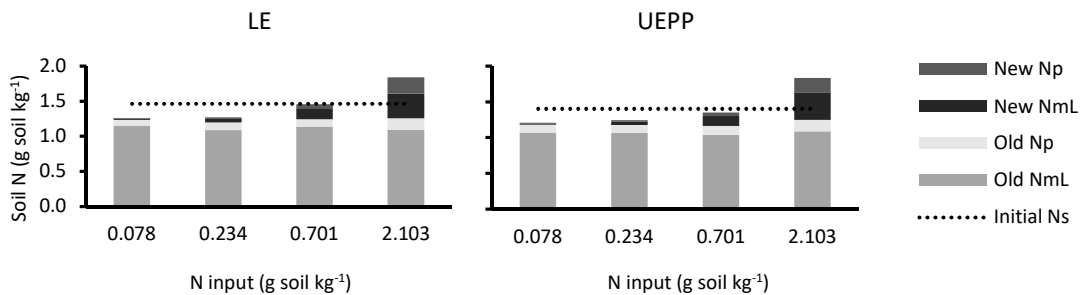
P>F values is indicated as p<0.1 · , p<0.05 * , p<0.01 ** , p<0.001***

LE= La Estanzuela Experimental site, UEPP= Palo a Pique experimental site; N_r=Nitrogen added as sorghum residues; N_{mL}=Nitrogen in the soil fraction <53 μm after KCl leaching and centrifuging; Superscript i= initial content, superscript f= final content after incubation, New N_{mL}= N in soil fraction < 53 μm retained from residues after KCl leaching and centrifuging, Old N_{mL}= N in soil fraction < 53 μm from soil after KCl leaching and centrifuging; hor(System)=soil horizon effect within system.



References: Dark Grey: N_{ML} initial, Light Grey: Old N_{ML} final, Black: New N_{ML} final; LE: La Estanzuela Experimental Site, UEPP: Palo a Pique experimental site, Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland; Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B.; Soil N_{ML}: Nitrogen in the soil fraction < 53 μm retained after KCl leaching and centrifuging.

Figure 5-3. Nitrogen content in the soil mineral fraction after leaching inorganic compounds (N_{ML}< 53 μm).



References: LE: La Estanzuela Experimental Site, UEPP: Palo a Pique experimental site, N_s= Nitrogen in the soil (N_p+N_{ML}); New N_p= N in soil fraction > 53 μm retained from residues; New N_{ML}= N in soil fraction < 53 μm retained from residues after KCl leaching and centrifuging; Old N_p= N in soil fraction > 53 μm from soil; Old N_{ML}= N in soil fraction < 53 μm from soil after KCl leaching and centrifuging.

Figure 5-4. Mean N contents after soil incubation of LE and UEPP experimental sites soils for each residues treatment, discriminated by soil mineral fractions and by origin according to isotopic composition.

5.4.2 Residues N retention rate

Apparent total nitrogen retention (h_a) of N added as fresh residues was similar among sites, with a mean of 0.300 kg kg⁻¹ expressed in terms of retained N amounts in relation to N inputs (Table 5-5 and 5-6). Correcting estimated rates to account for simultaneous mineralization (k) returned “corrected” h of 0.322 kg kg⁻¹ at LE and 0.352 kg kg⁻¹ at UEPP. At both experimental sites, the variation observed in total N retained was explained by the amount N added in residues and soil

horizon. Low N additions showed greater N retention rates than those observed at high inputs, and the retention of N inputs in B horizons was 15% and 25% greater than N retained in A horizons, respectively for LE and UEPP. These effects were explained by the N retained in N_{mL} fraction at both sites, which accumulated 70% of the total N retained from N added as residues (mean 0.210 kg kg⁻¹). The variation of N retained in N_p fraction showed no significant effects of N_r inputs or horizons at LE and no clear patterns at UEPP. The continuous cropping system with no fertilization inputs at LE showed an apparent N retention rate in N_{mL} which on average for all horizons was 10% higher than CC and CP (Table 5-5). In such unsaturated systems as CC with no fertilizer inputs, the soil mineral fraction seems to act as a stronger sink for N inputs than in fertilized CC and CP systems.

Table 5-5. Analysis of variance and least square means of apparent N retention (h) in N_p , N_{mL} , total N_s , and corrected value for k , at LE

Source	DF	h_{pa}	h_{mLa}	F values		h_a	h	
N_r	3	1.46	39.0 ***	2.8 *	4.8 **			
System	2	1.54	10.4 ***	2.6 .	1.9			
hor(System)	6	0.8	21.9 ***	2.8 *	4.3 ***			
N_r *System	6	1.09	1.1	1.0	1.0			
N_r *hor(System)	18	0.72	1.0	0.9	1.0			
Error (MSE)	72	0.002	0.0005	0.003	0.002			
Mean (kg kg ⁻¹)		0.096	0.204	0.300	0.322			
RMSE		0.049	0.022	0.051	0.048			
Least Square Means		h_{pa}	h_{mLa}	h_a	h			
	Horz	kg kg ⁻¹						
	A1	0.108	0.178	0.286	0.305			
	A2	0.087	0.199	0.286	0.308			
	B	0.093	0.235	0.327	0.354			
	N_r	h_{pa}	h_{mLa}	h_a	h			
	-- g N soil kg ⁻¹ --	kg kg ⁻¹						
	0.078	0.081	0.228	0.365	0.333			
	0.234	0.099	0.215	0.335	0.340			
	0.701	0.096	0.206	0.341	0.323			
	2.103	0.109	0.167	0.261	0.294			

P>F values is indicated as p<0.1 . , p<0.05 * , p<0.01 ** , p<0.001***

LE= La Estanzuela Experimental site, N_r =Nitrogen added as sorghum residues; h_{pa} = apparent fractional N_r retention in soil fraction > 53 μ m; h_{mLa} = apparent fractional N_r retention in the soil fraction passing a 53 μ m mesh after leaching KCl soluble compounds and centrifuging, h_a = Apparent retention of added N in the soil, h = Soil N retention rate after accounting for simultaneous decomposition; hor(System)=soil horizon effect within system. Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B.

Table 5-6. Analysis of variance and least square means of apparent N retention (h) in N_p , N_{mL} , total N_s , and corrected value for k , at UEPP

Source	DF	F value			
		h_{pa}	h_{mLa}	h_a	h
N_r	3	5.09 **	11.5 ***	10.2 ***	10.4 ***
System	2	2.53 .	1.3	2.6 .	2.6 .
hor(System)	6	2.94 *	8.1 ***	6.2 ***	4.7 ***
N_r *System	6	1.81	0.2	0.5	0.6
N_r *hor(System)	18	1.48	0.8	0.9	1.1
Error (MSE)	55	0.001	0.003	0.005	0.006
Mean (kg kg ⁻¹)		0.081	0.217	0.300	0.358
RMSE		0.027	0.056	0.068	0.078
Least Square Means		h_{pa}	h_{mLa}	h_a	h
	Horz	----- kg kg ⁻¹ -----			
	A1	0.075	0.191	0.271	0.325
	A2	0.083	0.192	0.276	0.343
	B	0.091	0.267	0.364	0.419
	N_r	h_{pa}	h_{mLa}	h_a	h
	-- g N soil kg ⁻¹ -	----- kg kg ⁻¹ -----			
	0.078	0.092	0.268	0.376	0.447
	0.234	0.073	0.215	0.287	0.346
	0.701	0.071	0.201	0.272	0.328
	2.103	0.097	0.184	0.279	0.328

P>F values is indicated as p<0.1 . , p<0.05 * , p<0.01 ** , p<0.001***. UEPP= Palo a Pique Experimental site, N_r =Nitrogen added as sorghum residues; h_{pa} = apparent fractional N_r retention in soil fraction > 53 μ m; h_{mLa} = apparent fractional N_r retention in the soil fraction passing a 53 μ m mesh after leaching KCl soluble compounds and centrifuging, h_a = Apparent retention of added N in the soil, h = Soil N retention rate after accounting for simultaneous decomposition; hor(System)=soil horizon effect within system. Soil Horizons: A1= Topsoil 6 cm with most roots and a darker color, A2= Topsoil A diagnostic soil horizon remaining after removing A1, B= Subsoil B.

Substituting the saturation ratio C_s/C_x for soil horizon rendered a similar residual error, indicating that C_s saturation may explain differences in total soil N stabilization observed between soil horizons (Tables 5-7 and 5-8). The variation in total N retention due to C_s/C_x mirrored the variation in N retention observed in the mineral fraction, in which N retention rates significantly decreased as saturation increased (Figure 5-5). The second variable related to N retention variation was the amount of N added, where higher N inputs showed lower N retention rates.

Table 5-7. Apparent N retention in N_p , N_{mL} , $N_{p mL}$, and corrected value for k , LE

Source	DF	h_{pa}	h_{mLa}	h_a	h
		----- F values -----			
N_r	3	0.7	4.5 **	2.8 *	4.8 **
System	2	1.3	2.0	2.9	6.7 **
Sat	1	0.4	95.4 ***	16.5 ***	24.1 ***
N_r *System	6	1.2	1.1	1.2	1.5
Sat* N_r	3	1.4	0.4	1.7	2.9 *
Error (MSE)	92	0.0022	0.0006	0.0024	0.0022
Mean (kg kg ⁻¹)		0.096	0.204	0.300	0.322
RMSE		0.047	0.024	0.049	0.047
Saturation estimate			-0.129	-0.042	-0.041
Least Square Means		h_{pa}	h_{mLa}	h_a	h
System		----- kg kg ⁻¹ -----			
CC no F		0.099	0.201	0.300	0.310
CC		0.086	0.199	0.285	0.308
CP		0.104	0.211	0.314	0.348
N_r		h_{pa}	h_{mLa}	h_a	h
-- g N soil kg ⁻¹ -		----- kg kg ⁻¹ -----			
0.078		0.081	0.228	0.309	0.333
0.234		0.099	0.215	0.314	0.340
0.701		0.096	0.206	0.301	0.323
2.103		0.109	0.167	0.276	0.294

P>F values is indicated as p<0.1 . , p<0.05 * , p<0.01 ** , p<0.001***; N_r =Nitrogen added as sorghum residues; h_{pa} = apparent fractional N_r retention in soil fraction > 53 μ m; h_{mLa} = apparent fractional N_r retention in the soil fraction < 53 μ m after KCl leaching and centrifuging, h_a = Apparent retention of added N in the soil, h = Soil N retention rate after accounting for simultaneous decomposition; sat=saturation ratio (C_s/C_x), Rotational Systems: CC no F= Continuous cropping with no fertilization, CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation.

Table 5-8: Apparent N retention in N_p , N_{mL} , total N_s , and corrected value for k at UEPP

Source	DF	h_{pa}	h_{mLa}	F values		h
Nr	3	1.8	4.2 **	3.1 *		3.9 *
System	2	2.2	1.9	2.9		2.8
Sat	1	3.2	40.4 ***	28.5 ***		19.7 ***
Nr *System	6	1.5	0.2	0.5		0.6
Sat* Nr	3	1.2	0.9	0.7		1.0
Error (MSE)	75/92	0.0009	0.0031	0.0048		0.0064
Mean (kg kg ⁻¹)		0.081	0.217	0.300		0.358
RMSE		0.030	0.056	0.069		0.080
Saturation estimate		0.002	-0.097	-0.109		-0.074
Least Square Means		h_{pa}	h_{mLa}	h_a		h
	System	----- kg kg ⁻¹ -----				
	CC	0.077	0.202	0.278		0.333
	CP	0.080	0.223	0.312		0.379
	RG	0.092	0.225	0.319		0.373
	Nr	h_{pa}	h_{mLa}	h_a		h
	-- g N soil kg ⁻¹ -	----- kg kg ⁻¹ -----				
	0.078	0.092	0.268	0.372		0.443
	0.234	0.073	0.215	0.288		0.347
	0.701	0.071	0.201	0.272		0.329
	2.103	0.097	0.184	0.279		0.328

P>F values is indicated as p<0.1 . , p<0.05 * , p<0.01 ** , p<0.001***; Nr=Nitrogen added as sorghum residues; h_{pa} = apparent fractional N_r retention in soil fraction > 53 μ m; h_{mLa} = apparent fractional N_r retention in the soil fraction < 53 μ m after KCl leaching and centrifuging, h_a = Apparent retention of added N in the soil, h = Soil N retention rate after accounting for simultaneous decomposition; sat=saturation ratio (C_s/C_x); Rotational Systems: CC= Continuous annual cropping rotational system, CP= Crop-pasture rotation, RG= Regenerated grassland.

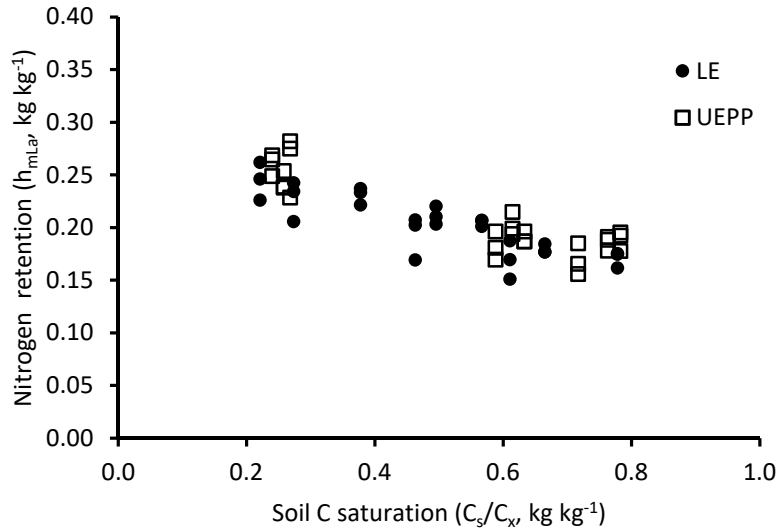
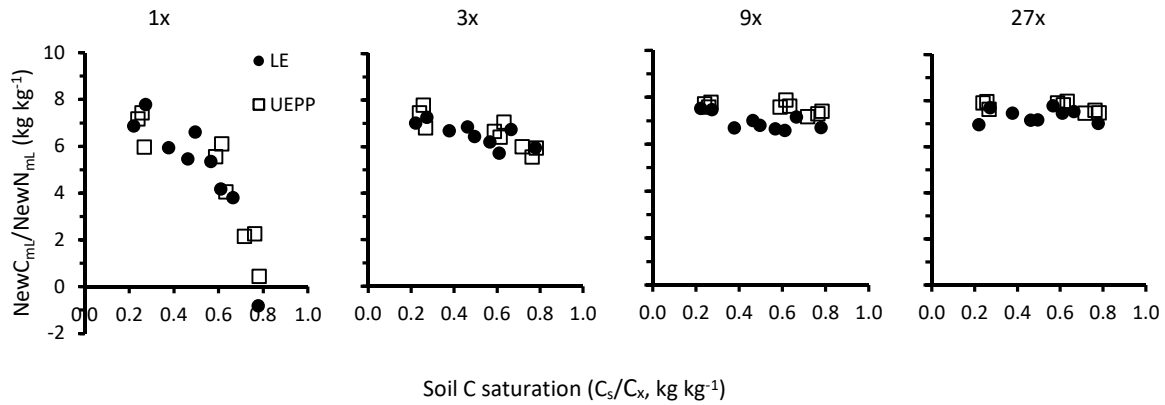


Figure 5-5. Nitrogen retention from residues addition in the soil mineral fraction (< 53 μm) after leaching with KCl. The figure includes individual data for each replication, averaging observed values for all systems and residues treatments.

5.4.3. Relationship between C and N retained from fresh residues

The ratio between new C and new N stabilized in the soil mineral fraction decreased with C saturation ratio. This means that even though the amount of N stabilized in the mineral fraction decreased with saturation, C and N cycling are not fully coupled.

There was an interaction between the N_r added and C_s saturation, where low inputs showed a steeper decrease in C:N ratio of stabilized compounds with C_s saturation increase (figure 5-6). At low residues inputs (1x), C and N cycles were most uncoupled, where soil at 0.8 saturation ratio was capable of retaining N without stabilizing new C. The slope of this relationship decreased with increasing N_r inputs, until showing very little change in C:N ratio due to saturation for large inputs. This indicates that for large inputs systems stabilization the reduction observed in N is shows a similar shape as that observed for C.



Source	DF	Residue inputs			
		1x	3x	9x	27x
		----- F values -----			
Site	1	0.7	0.6	0.6	5.8 *
System(site)	4	1.1	0.8	0.1	6.5 ***
Sat	1	47.1 ***	25.7 ***	6.8 *	4.4 *
Sat x Site	1	0.5	0.2	0.9	0.2
R-Square		0.61	0.42	0.40	0.58
Root MSE		1.74	0.65	0.47	0.24
Mean (C:N)		4.70	6.58	7.26	7.55
Intercept		8.51	8.38	7.93	8.06
Sat Slope		-8.51	-2.84	-0.62	-0.50

References: From left to right, C and N inputs: 1x=1g C soil kg⁻¹ and 0.078 g N soil kg⁻¹, 3x=3g C soil kg⁻¹ and 0.234 g N soil kg⁻¹, 9x=9g C soil kg⁻¹ and 0.701 g N soil kg⁻¹, 27x=27g C soil kg⁻¹ and 2.103 g N soil kg⁻¹. New C_{mL}= carbon in soil fraction < 53 μm retained from residues after KCl leaching and centrifuging; New N_{mL}= N in soil fraction < 53 μm retained from residues after KCl leaching and centrifuging; Sat= C_s saturation (C_s/C_x). Data points shown are averaged among systems and replications

Figure 5-6. Relationship between New C and New N retained in the soil mineral fraction of LE and UEPP experimental sites and C_s saturation for different amounts of fresh residues inputs (1x, 3x, 9x, and 27x), and their respective analysis of variance.

5.5. Discussion

The first hypothesis to test was that C_s saturation concept as proposed by Hassink and Whitmore (1997) and observed in the soil of crop-pasture systems was linked to N saturation (Aber et al. 1989). Data showed that soil N incorporated in the soil followed similar patterns as observed in C_s in a previous chapter, supporting this concept. The new N retained in the N_p fraction, mostly depended on the N_r inputs, while N retained in the mineral fraction depended also on the horizon. Topsoil horizons were a weaker sink for new N, while B horizons retained more of the N added. These results agree with the concept of a limited capacity of N retention determined by the soil mineral fraction as proposed by Hassink (1997), and with the concept of N saturation as described by Aber et al (1989), where N saturation is described in stages where increasing degrees of

saturation reduce N retention. In addition, the fact that the retention of soil N decreased as soil saturation increased (figure 5.5), shows similar dynamics for N retention as observed by Stewart et al (2008) on C_s , and provides further quantitative evidence supporting that N retention in the soil shares C_s saturation controls on soil mineral particles as proposed by Hassink (1997). These results are in line with those presented by Castellano et al (2012) on forest soils, where the retention of inorganic N additions was related to C_s saturation when tested in a gradient of C_s saturation.

The fraction of N retained through the saturation gradient studied followed a similar gradual pattern as that observed for C_s retention (Chapters 3 and 4). The quantitative relationship observed in figure 5-5 shows the potential impact that may be observed in moving from a soil near C saturation to a low saturation, in terms of N retention. A change in land use where system transit from a RG or CP to a system like CC no F could imply that the N sink would increase from 0.15 to 0.25 kg kg⁻¹ of the N added. Following this pathway a soil that gradually desaturates (looses organic C and N) may be trapped in a megative cycle of poor N availability for the plants, with the consequent poor productivity that leads to low residues returning to the soil that keep the system in a low saturation condition. If plant N requirements were to be fulfilled in this context, N applied should not only take into account plant requirements, but increase according to the increase in the soil N sink, with the consequent risk of environmental losses and increased farming cost. This pathway could be one of the mechanisms behind the decrease observed in wheat grain yields as farms move away from crop-pasture rotations and accumulate years in continuous cropping (Ernst et al. 2016).

The second hypothesis was that C to N ratio of the new organic material retained in the soil should vary with saturation, showing some degree of decoupling between C and N cycling and buffering N losses as suggested in Chapter 2. The variation observed in the C:N ratio of the material retained in the mineral fraction (Figure 5-5) suggests that even if C_s saturation can be related to both a decrease in C and N retention, these relationships work within a range that allows some buffering capacity to N losses on soils of high C saturation ratio. In other words, the slopes of the C and N retention as C_s saturation increases are different, with that for C being steeper than that for N.

This observed C:N stoichiometry adjustment seems to allow systems relatively saturated in C to retain some additional N. However, this N buffering capacity is clearly limited and eventually saturated, since increasing inputs show a gradual increase in the C:N ratio of the incorporated material together with a decrease in the N relative retention. This principle may explain the N

dynamics in crop-pasture systems as observed in Chapter 2, where greater N mineralization observed in crop-pasture systems were buffered by an increased in N immobilization. The increase in turnover observed in saturated soils allows a timely N delivery, while the increase in N immobilization prevents N losses to the environment. This behavior can be explained by the shift in C:N observed here. The combination of C_s and N saturation in crop-pasture systems may be one of the key principles underpinning nutrient cycling. The C:N adjustment seems to operate mostly at low albeit realistic residues input rates. One of the differences between CC and CP systems is exactly related to this aspect. Annual cropping provides high amounts of residues after every harvest, while perennial species grown in crop-pasture systems provide a constant input through the growth and death of perennial rooting system.

5.6. Conclusion

This chapter provides evidence that N retention is reduced with C_s saturation, supporting the conceptual model of N cycling dependence on C_s saturation, and N saturation as an operating process in soils under crop-pasture systems management. The implications for agroecosystem management are relevant in terms of nutrient use efficiency of added N and the possibility of buffering N losses by maintaining soils or soil horizons near saturation. Soil organic matter in this context acts not only as a reservoir of N eventually available to the plants through microbial decomposition, but also may define if soil organic matter pool acts as a sink or source of N. The inclusion of perennial pastures in crop rotations may play a key role in sustainable nutrient management through the opportunity of managing C_s and N saturation to benefit production systems. One of the challenges is keeping the systems at early stages of N saturation (near 80% of saturation) to benefit from enhanced nutrient supply but avoiding nutrient losses to the environment.

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CHAPTER 6 – CONCLUSIONS

Integrated crop-pasture systems in which perennial species rotate with crops provide several advantages related to soil C (C_s) and N cycling exposed in this dissertation and summarized below, which fit within the theoretical framework of soil C and N saturation (Aber et al. 1989; Hassink and Whitmore 1997). These advantages in C and N cycling support high crop yields in the long term as such measured during two decades of crop-pasture rotations in long-term experiments at INIA-Uruguay (Chapter 2).

The traditional rationality behind integrated crop-pasture systems is that crops should benefit from system design synergies to sustain high crop yields with relatively low external inputs as fertilizer (Russelle et al. 2007; Carvalho et al. 2010). A key issue for the supply of sufficient nutrients to high demanding crops in the system is the N fixed by legumes during the pasture phase and nutrient recycling in manure returned to the soil and its later availability for the next crop through soil organic matter cycling. In addition to N supply from biological dinitrogen fixation, systems including perennial pastures should exhibit larger mineralization and immobilization fluxes than continuous cropping systems, as revealed by the application of agroecosystems models in Chapter 2.

An increase in N mineralization rate results from the expected increase in soil organic matter decomposition in soils that have accumulated large quantities of C_s (Hassink 1997). In the long term, soils under agricultural systems including perennial species contain more C_s than those managed under continuous annual cropping. Since the soil capacity to retain C in a stable form is limited to the soil matrix adsorption sites (Hassink and Whitmore 1997; Baldock and Skjemstad 2000), differences in C_s content due to rotational systems determine differences in soil C saturation of this theoretical maximum storage capacity (C_x). This concept of crop pasture systems rendering more saturated soils is supported by the range in C_s saturation obtained in Chapter 3 for long-term rotational systems experiments at INIA- Uruguay. A gradient in soil C saturation measured as the C_s/C_x ratio was greater with the participation of perennial pastures in the systems at La Estanzuela (LE) and Palo a Pique (UEPP) sites, and also at the top soil horizons, as expected due to differences in soil texture and soil C inputs allocation. Overall, moving from a continuous cropping system to crop-pasture systems with perennials determined increasing C_s saturation, particularly at the topsoil.

The quantitative reproduction of two decades of crop pasture and continuous cropping system management in Cycles model in Chapter 2 also showed that the increase in N availability through an increase in N mineralization in crop-pasture systems did not determine a proportional increase in N losses to the environment. An increase in N availability without a proportional increase in N losses is what Aber et al (1989) describe in forest soils as the first stages of the process of N saturation. This behavior showed a buffering capacity of the systems including perennial pastures evidenced by the simultaneous increase in N immobilization; given by the large capacity of grassland soils demonstrated to retain large quantities of N not readily available for decomposition (Kaye et al. 2002). These results support the idea that N saturation is a mechanism operating in crop-pasture systems.

Addressing C stocks evolution for two decades of different systems operating in the field in the long term (Chapter 2) also determined that the implementation of C_s saturation in the model Cycles resulted in lower error when estimating C_s stocks at the topsoil, and reproduced the C_s natural distribution with depth as an emerging property. This is another indication that the mathematical representation of the mechanism of C_s saturation unique to Cycles and C-Farm (Kemanian et al. 2005; Kemanian and Stöckle 2010) contributes to explain soil C dynamics in the soil.

After tracing soil ^{13}C in soil incubations, evidence presented in Chapter 3 shows that increasing C_s saturation determines an increase in C_s turnover, measured as the positive relationship between C_s/C_x and the C_s decomposition rate k . The increase in C_s also determined a decrease in the retention of C relative to the residues inputs, which was more evident at UEPP site. This relationship supports the mathematical approach (Kemanian et al 2005) build in Cycles and C-Farm models, which provided mathematical shape to the expected effect of C_s saturation in C_s decomposition envisioned by Hassink (1997). It is clear from this dissertation results that simulation models should include the C_s saturation mechanism in order to estimate soil C stocks and fluxes more accurately, as a conclusion from combining the theoretical approach summarized in simulation models with experimental evidence.

The negative relationship between soil C saturation and total soil C retention observed in bulk soil was then confirmed from experimental results to rise from the limitation of C stabilization in the soil mineral fraction (Chapter 4), add on to evidence provided earlier by Stewart et al. (2008). This

relationship was also observed for N retention, linking soil N and C saturation concepts in crop-pasture systems, building up on evidence presented by Castellano et al. (2012) on forest soils.

Another conclusion from Chapter 4 and 5 is that the soil mineral fraction represented by particles < 53 μm and the particulate organic fraction represented by >53 μm are clearly different soil functional pools. The organic fraction dynamics clearly are independent of the soil mineral matrix, evidenced by the lack of response to C_s saturation in C and N retention.

Overall, this dissertation provides additional evidence supporting soil C saturation theory (Hassink and Whitmore 1997), and suggest this as a major mechanism underpinning observed soil C and N dynamics in crop-pasture systems. Soil C saturation dynamics on crop-pastures systems seem to accelerate C dynamics, and in turn, support high N mineralization as observed in crop-pasture system. At the other end, intensification towards continuous cropping following soil C de-saturation may shrink in C and N cycling fluxes, with the consequence loss of soil fertility and buffering capacity. Systems pushed to this end would increase their dependence on external inputs supply, as reported for wheat (Ernst et al 2016), enhancing chances of low efficiency use of fertilizers with consequent unintended environmental and economic consequences.

The integration of crop and livestock systems can benefit from enhancing nutrient cycling as a result of synergies between the components of the system (Carvalho et al. 2010; Franzluebbers et al. 2014; Soussana and Lemaire 2014). However, this integration can occur at different scales and dimensions with different outcomes (Russelle et al, 2007). Managing the systems to increase nutrient cycling by promoting soil saturation has the risk of triggering environmental losses when soil nutrient supply may exceed the system sinks. Even if the last stages of saturation process as described by Aber et al (1989) may not be reached, undesired outcomes and inefficiencies may occur due to kinetic saturation (Lovett and Goodale, 2011) when the rate nutrient supply exceeds that of retention in of the system sinks. Crop and livestock production intensification without this consideration has led to environmental pollution with surpluses of on-farm nitrogen and phosphorus inputs, affecting water resources in different parts of the world (Alexander et al., 2007, Sakadevan and Nguyen, 2017). Therefore, in the context of the concepts of soil C and N saturation theory, when crop and livestock production is intensified a challenge for sustainable intensification is to manage the soil within the first stages of this process.

In this dissertation I provide evidence that relate the role of perennials in integrated crop-pasture systems to C and N dynamics through soil C saturation of the soil profile, which add to the multiple traditional roles of perennials in crop-pasture systems. An acceleration of C and N cycling through soil C saturation seems to be a keystone of these systems success to balance productive and environmental tradeoffs. When internal nutrient cycling substitutes external inputs plant nutrient supply by managing soil C saturation, it appears as an interesting strategy to pursue sustainable intensification of agricultural systems. The design of systems for sustainable intensification need to balance high productivity with soil, water and biodiversity preservation (Matson et al. 1997; Cassman 1999), where soil C saturation should be considered a fundamental mechanism to manage C and N cycling.

6.1. References

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APPENDIX A. ALGORITHMS FOR LIGHT INTERCEPTION IN THE COMPETITION MODULE OF CYCLES

For a given species growing alone, the fractional transmitted light by the canopy of species i growing alone is

$$\tau_i = 1 - \phi_{0i}, \quad (\text{A.1.1})$$

where ϕ_{0i} is the fractional intercepted light by the canopy of species i growing alone. As

$$\tau_i = \exp(-kL_i), \quad (\text{A.1.2})$$

where k is the light extinction coefficient ($= 0.5$), and L_i is the leaf area index of species i .

Therefore

$$L_i = \frac{-\log \tau_i}{k}. \quad (\text{A.1.3})$$

Ignoring the differences in height, the total transmitted light is

$$\tau_T = \exp\left(-\sum_i d_i k L_i\right) \quad (\text{A.1.4})$$

where d_i is the planting density (0–1), and L_i is calculated using Equation (3). Then

$$\phi_T = 1 - \tau_T \quad (\text{A.1.5})$$

The fractional intercepted light by the canopy of species i is

$$\phi_i = \frac{d_i k L_i}{\sum_i d_i k L_i} \phi_T \quad (\text{A.1.6})$$

APPENDIX B. PARAMETERS CALIBRATED FOR FORAGE AND GRAIN SPECIES

NAME	Foxtail Millet	Forage Sorghum	Forage wheat	Lotus	Oats	Orchardgrass	Annual Ryegrass	Sorghum	Soybean	Spring wheat	Sweet Sorghum	Tall Fescue	White Clover
FLOWERING_TT	738.25	1115.5	1506.26	1857.08	2589.48	1937.9	1331.78	1115.49	1174.86	900	1550	1408.36	916.81
MATURITY_TT	1522.3	1755.92	1844.16	2316.99	3470.95	2825.47	2213.25	1755.92	2250.5	1250	1900	2206.5	2199.21
MAXIMUM_SOIL_COVERAGE	100	100	100	100	100	100	100	100	100	100	100	100	100
MAXIMUM_ROOTING_DEPTH	0.6	1	0.7	0.5	0.7	1	1	1	0.75	0.7	1	1	0.3
AVERAGE_EXPECTED_YIELD	8000	5500	4000	8000	6600	7300	7300	5500	2500	4000	16000	7400	6700
MAXIMUM_EXPECTED_YIELD	14000	10000	6000	12000	5000	9900	9000	10000	4500	6000	20000	11000	8000
MINIMUM_EXPECTED_YIELD	6600	4000	1500	4000	8600	2700	5000	4000	800	1500	6000	3000	2000
COMMERCIAL_YIELD_MOISTURE	0	14	0	0	0	0	0	14	13	0	14	0	0
STANDING_RESIDUE_AT_HARVEST	30	60	30	50	30	30	30	60	30	30	60	30	50
RESIDUE_REMOVED	70	40	70	70	70	70	70	40	70	70	75	70	70
CLIPPING_BIOMASS_THRESHOLD_UPPER	5	5	5	15	5	5	5	25	20	5	6	15	15
CLIPPING_BIOMASS_THRESHOLD_LOWER	0.5	0.5	0.5	0.5	0.5	0.5	0.5	20	15	0.5	0.5	0.5	0.5
HARVEST_TIMING	-999	60	50	60	60	60	-999	-999	-999	-999	80	60	60
CLIPPING_BIOMASS_DESTINY	REMOVE	GRAZING	GRAZING	GRAZING	GRAZING	GRAZING	GRAZING	RETURN	RETURN	REMOVE	REMOVE	GRAZING	GRAZING
MIN_TEMPERATURE_FOR_TRANSPIRATION	-1	3	-1	8	-1	0	-1	3	3	-1	3	0	2
THRESHOLD_TEMPERATURE_FOR_TRANSPIRATION	8	15	8	10	8	9	8	15	15	8	15	9	7
MIN_TEMPERATURE_FOR_COLD_DAMAGE	-10	-5	-10	-7	-10	-10	-10	-5	-5	-10	-5	-10	-10
THRESHOLD_TEMPERATURE_FOR_COLD_DAMAGE	0	5	-1	0	0	-2	-1	5	3	-1	5	-2	0
BASE_TEMPERATURE_FOR_DEVELOPMENT	6	5	3	5	2	2	2	5	5	3	5	2	4
OPTIMUM_TEMPERATURE_FOR_DEVELOPEMENT	26	28	20	25	26	24	26	28	28	20	28	24	20
MAX_TEMPERATURE_FOR_DEVELOPMENT	40	46	25	35	40	38	40	46	43	25	46	38	35
INITIAL_PARTITIONING_TO_SHOOT	0.45	0.45	0.45	0.35	0.45	0.35	0.45	0.45	0.45	0.45	0.45	0.35	0.35
FINAL_PARTITIONING_TO_SHOOT	0.95	0.95	0.95	0.75	0.95	0.75	0.95	0.95	0.95	0.95	0.95	0.75	0.75
RADIATION_USE_EFFICIENCY	1.9	2	1.7	1.43	1.65	1.43	1.65	2	1.32	1.7	2	1.43	1.43
TRANSPIRATION_USE_EFFICIENCY	7.8	7.9	6	4.62	6	5.5	6	7.9	4.5	6	7.9	5.6	4.62
MAXIMUM_HARVEST_INDEX	0.52	0.8	0.52	0.04	0.52	0.08	0.52	0.8	0.4	0.52	0.8	0.08	0.04
MINIMUM_HARVEST_INDEX	0.31	0.2	0.31	0.01	0.31	0.01	0.31	0.2	0.15	0.31	0.2	0.01	0.01
HARVEST_INDEX	2	1.13	2	1	2	1.5	2	1.13	2	2	1.13	1.5	1
THERMAL_TIME_TO_EMERGENCE	100	100	100	90	100	100	100	100	70	100	100	100	90
N_MAX_CONCENTRATION	0.055	0.055	0.07	0.07	0.07	0.07	0.07	0.055	0.07	0.07	0.055	0.07	0.07
N_DILUTION_SLOPE	0.39	0.4	0.47	0.35	0.47	0.4	0.47	0.4	0.4	0.47	0.4	0.4	0.35
KC	1.1	1.2	1	1.05	1.1	1.1	1.1	1.2	1.05	1	1.2	1.1	1.05
ANNUAL	1	1	1	0	1	0	1	1	1	1	1	0	0
LEGUME	0	0	0	1	0	0	0	0	1	0	0	0	1
LWP_STRESS_ONSET	-1200	-1200	-1200	-1100	-1200	-1200	-1200	-1200	-1000	-1200	-1200	-1200	-900
LWP_WILTING_POINT	-2200	-2200	-1800	-2200	-2000	-2300	-1900	-2200	-1800	-1800	-2200	-2300	-1700
TRANSPIRATION_MAX	8	8	6	6	6	6	6	8	8	6	8	6	6

VITA

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Virginia Pravia was born in Montevideo, Uruguay. She graduated from High school at the Colegio del Sagrado Corazón de Jesús (ex-Seminario) in 1998, and earned a Bacalaureate degree in Agronomy at the Universidad de la República (UdelaR, Uruguay) in 2004. Her interest in agronomy developed from enjoying farming at her family farm to finding interest in biological principles of agricultural sciences, eventually evolving as a research vocation. After working as a consultant agronomist for a couple of years, she applied for an internship at Pioneer Hi-Breed international, working as a research intern in Ontario, Canada. She returned to her home country under a fellowship award from the Instituto Nacional de Investigación Agropecuaria (INIA-Uruguay) as the First Student in her Agronomy Class of 2004 at UdelaR, continuing her studies at the UdelaR to pursue a Master in Agricultural Sciences degree that she earned in 2009. Her Master's thesis at INIA Treinta y Tres Research Station focused on incorporating precision agriculture tools and simulation models into agricultural research at the field scale. Virginia integrated INIA research team as an Assistant Researcher at the Pasture and Forages Program in 2007. She started her PhD in Agronomy at The Pennsylvania State University College of Agricultural Sciences in 2012 in a joint program with her position at INIA-Uruguay.