PRIMING EFFECTS ON SOIL ORGANIC CARBON DECOMPOSITION INDUCED BY HIGH C:N CROP INPUTS

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1- Abstract.

Carbon budgets in soils can be computed by a simple equation in which inputs are accounted by the humification of residues added to the soil, and outputs are estimated by the respiration of soil organic carbon (C_S). It is generally accepted that physical protection in soil aggregates and chemical recalcitrance limit the rate of C_S decomposition. We propose that soybean-based crop sequences will have lower steady state C_S stocks than those based on corn, because of lower C inputs and enhanced soil respiration of C_S due the so-called priming effect. To address this hypothesis, we studied C_S turnover from $\delta^{13}C$ natural abundance changes in no-till corn and soybean crops. Soybean and corn crops were sown with a complete randomized design with three replicates on an old pasture with an intermediate soil $\delta^{13}C$ signal of -16.34. Soils were sampled at the beginning and after two years of continuous no till cropping. Aboveground and belowground C inputs were measured each year for corn and soybean crops. Soil and plant samples were analyzed for C, nitrogen and $^{13}C/^{12}C$ ratio. Corn C inputs where significantly higher than soybean (10.7 and 7.0 Mg ha^{-1}), but no significant changes in C_S where observed among treatments (68 Mg ha^{-1} in the first 0.3 m). However, the Cs turnover and humification rates under corn (0.015 and 0.18 y^{-1}) were higher than under soybean (0.006 and 0.11 y^{-1}). Contrary to our predictions high C:N corn residues enhanced C_S decomposition, but also the stabilization of fresh residues into C_S, suggesting that soil microbes mine the soil organic matter while feeding on high C:N crop residues. Possible explanations for such result are discussed.

2- Introduction.

Soil organic carbon (C_S) is a major determinant of agricultural soils productivity. The storage of C_S has been proposed as part of a strategy to reduce the accumulation of carbon (C) dioxide in the atmosphere (Lal, 2004; Bernoux et al., 2006). The annual organic C balance of a soil layer can be accounted for by a simple equation in which inputs are represented by the amount of residues and roots added to the soil and a retention or humification coefficient, and outputs are represented by the respiratory losses from C_S using first order kinetics. This model was formalized by Hénin and Dupuis (1945) who established that for a given time step a fraction of the decomposed residues becomes C_S with a humification coefficient h (T^{-1}) and a fraction k (T^{-1}) of C_S is lost through microbial respiration.

This equation and the coefficients therein allow for a straightforward characterization of the factors affecting the C_S balance. Estimates of the coefficient h reported by Henin and Dupuis (1945) were approximately 0.3, 0.08, 0.1 and 0.15 for farmyard manure, corn, and small-grain cereal straw, and roots of corn and small-grain cereals, respectively. Other estimates are within the range originally provided by these authors. In a 12-yr study, Barber (1979) estimated an h ranging from 0.08 to 0.11 for aboveground corn in Indiana, USA, while Plénet et al (1993) reported h between 0.055 and 0.065 for aboveground biomass and 0.16 and 0.30 for belowground biomass of corn in the southwest of France. Recent estimates from a 50-yr study in Sweden-Upsala, Kätterer et al. (2011) report h of 0.17 for aboveground biomass and 0.39 for belowground inputs. All of these estimates were made under the assumption that C inputs from rhizodeposition are similar to those of the root biomass.

The magnitude of k depends on temperature, humidity, and soil factors such as soil aggregation controlled by texture and tillage. Soils that are cool, dry or too wet have slower decomposition rates than soils that have conditions more favorable for microbial growth. These conditions are affected by the crop sequence and tillage system (Wildung et al., 1975; Bunnell et al., 1977; Kowalenko et al., 1978; Buyanovsky and Wagner, 1986; Power et al., 1986; Hendrix et al., 1988; Gregorich et al., 1998).

In South America and in particular in the temperate region that includes Uruguay and Argentina, two trends converged in the last decade: a transition to no-till cropping and the expansion of corn and soybean as summer crops. In Uruguay, which has approximately one million hectares of rainfed summer annual crops, about 10 and 85 % of the area is dedicated to no-till production of corn and soybean, respectively (DIEA 2011).

In no-till systems aboveground residues remain in the soil surface. Soil mechanical disturbance is minimal and restricted to the top 5 to 8 cm of the soil profile, which reduces soil erosion and C_S turnover rate compared to conventionally tilled systems. It has been reported and customarily assumed that the balance of these effects causes C_S to increase in the top layer (West and Post, 2002); however, a net gain of C_S is not necessarily detectable when the entire soil profile is accounted for (Baker et al., 2007; Blanco-Canqui and Lal, 2008). Residue type may also play a role at determining the C_S balance since residues from corn and soybean have different properties. For a given soil and climate, the corn residue mass is ≈1.5 larger than that returned by soybean (Buyanovsky and Wagner, 1986; Paustian et al., 1997; Andriulo et al., 1999; Allmaras et al., 2000; Studdert and Echeverría, 2000; Al-Kaisi et al., 2005; Huggins et al., 2007) with a much higher C:N ratio, ranging from 60 to 105 for corn and 25 to 50 for soybean (Broder and Wagner, 1988; Ernst et al., 2002; Al-Kaisi et al., 2005; Oelbermann and Echarte, 2011). The lower inputs of C through soybean residues have raised concerns on the ability of soybean-based system to sustain existing C_S levels (Buyanovsky and Wagner, 1986; Andriulo et al., 1999; Al-Kaisi et al., 2005; Huggins et al., 2007).

Isotopic tracing of natural ^{13}C is a suitable method for identifying the source of C in C_S and its turnover rate. The method is particularly useful when a system originally managed with C3 plants ($\delta^{13}C$ = -26 ‰) is substituted by C4 plant ($\delta^{13}C$ = -12 ‰) (Balesdent et al., 1988), or in systems where the soil exhibits an intermediate isotopic composition ($\delta^{13}C$ = -18 to -21 ‰) allowing to follow both the decline of $\delta^{13}C$ C in soil following the introduction of C3 plants and its enrichment after the introduction of C4 plants (Balesdent et al., 1988; Desjardins et al., 1994; Andriulo et al., 1999; Bayala et al., 2006; Desjardins et al., 2006).

In the agricultural soils of Uruguay C_S levels are relatively high, commonly in the order of 50 Mg C ha^{-1} in the top 0.2 m of the soil profile (Garcia-Prechac et al., 2004; Ernst et al., 2009; Salvo et al., 2010). We hypothesize that under no-till, corn and soybean will affect C_S dynamics differently. Specifically, we propose that soybean-dominated systems will have lower steady state C_S for two reasons: first, lower C inputs through residues and roots, and second due to the so-called priming effect, i.e. accelerated decomposition of the existing C_S due to the lower C:N ratio of the residue inputs (Hendrix et al., 1988; Andriulo et al., 1999). To address this hypothesis, we studied the turnover of C_S utilizing $\delta^{13}C$ natural abundance isotopic techniques in no-till corn and soybean systems in which aboveground and belowground C inputs were manipulated. Our specific objective is to estimate the C_S apparent turnover and residue humification in response to the amount and quality of residues in the first years of an old pasture conversion to no-till agriculture.

3- Materials and methods.

3.1- Plot Establishment and Field Measurements.

The experiment was located 10 km south of Paysandú (31° 21' S and 58° 02'W; 61 m elevation) in the northwest of Uruguay, which corresponds broadly with the eastern edge of the South American Pampas. The climate is meso-thermal sub-humid climate with a mean daily temperature of 25 and 13 °C for summer and winter, respectively, and annual precipitations of 1200 mm distributed on average uniformly within the year, but with a large intra- and inter-annual variation. The soil at the site is a fertile Typic Argiudol with a slope of about 1.0%. Soil particle-size distribution in the first 0.3 m is 280, 440 and 280 g kg^{-1} of sand, silt, and clay, respectively.

Between 1940 and 1970 the study site was under continuous annual cropping of wheat (one crop per year) under conventional tillage (inversion tillage plus several secondary operations). From 1970 to 1993 annual crops were rotated with pastures in a six-year rotation consisting of three years of white clover (*Trifolium repens L.*), birdsfoot trefoil (*Lotus corniculatus L.*), and tall fescue (*Festuca arundinacea* L.) and three years of crops at 1.7 crops per year (Ernst et al., 2009). From 1993 until de beginning of the experiment in 2007 the site was not cropped and was gradually colonized by bermudagrass (*Cynodon dactylon* L. – C4 perennial), maintaining a variable abundance of annual ryegrass (*Lolium multiflorum* L.) and white clover.

The experiment was established in April of 2007. The experimental area was treated with glyphosate at a rate of 3.0 kg a.i. ha^{-1} and subsequently at a rate of 1.5-2.0 kg a.i. ha^{-1} depending on weed infestation and weather conditions. Crops were sown on December 6 in 2007 and November 28 in 2008. Pre- and post-emergent herbicides were applied in all treatments to control weeds as needed, and insects and diseases controlled chemically based on regular insect population monitoring. Prior to sowing, all plots were fertilized with 150 kg ha^{-1} of ammonium phosphate (27 kg N ha^{-1} and 30 kg P ha^{-1}). At V6 corn was side dressed with 69 kg N ha^{-1} as urea. All crops are no-till planted in 30 x 5.2 m plots with 0.52-m row spacing.

The experiment has four treatments arranged in randomized blocks with three replications. The treatments are: continuous corn (CC), continuous soybean (SS), and two exchange stubble treatments: continuous corn with soybean stubble (SC), and continuous soybean with corn stubble (CS). For the exchange plots, the aboveground residue from the corn and soybean plots are removed and exchanged within each replicate.

Soils were sampled before sowing in November 2007 and November 2009. Samples were taken at seven depths (0-5, 5-10, 10-20, 20-30. 30-50, 50-70 and 70-100 cm). In 2007, a sample consisted of a single 7-cm diameter core per plot. In 2009, each sample was a composite of four 2-cm diameter cores per plot. Samples were weighed and approximately a third of the sample was dried to 105 °C to estimate soil moisture. The soil moisture was used to estimate the soil mass and bulk density (BD). The reminder of the samples were lightly crushed and sieved through a 2 mm mesh and dried to 60 °C. In this report we only show data from the CC and SS treatments in the first 0.3 m depths.

3.2- Carbon and δ ^{13}Carbon Natural Abundance Analyses

Inputs of C from aboveground and belowground biomass were measured each year. Grain yield and total aboveground biomass of each plot was determined after physiological maturity by harvesting 2 m of two adjacent center rows (2.08 m^2). Roots were measured at flowering using the soil core method. In each plot, two soil cores (diameter = 5 cm; depth = 100 cm) were taken on the row and in the inter-row space at the same depth intervals used for the soil samples. The intact soil cores were frozen immediately after sampling and kept at -20 °C. To recover the roots, the samples were thawed, and roots were separated from the soil using water and three sieves of 2, 0.5, and 0.05 cm. The roots were collected individually with tweezers. The plot root mass was calculated by averaging the row and inter-row samples.

Sub-samples of corn and soybean aboveground and root mass, and samples of soil from each sampling time were ball-milled and analyzed for C, N and $^{13}C/^{12}C$ ratio with an elemental C-N analyzer interfaced with an isotope ratio mass spectrometer (Carlo Erba, model NA 1500and Fisons, Optima model; Fisons, Middlewich-Cheshire, UK) at Duke University, USA. The C isotope ratios were expressed as $\delta^{13}C$:

$$\delta^{13C} \text{‰=RsamRstd-1x 103}$$

where R_{sam} = $^{13}C/^{12}C$ ratio for the sample, and R_{std} = $^{13}C/^{12}C$ ratio of the Pee Dee Belemnite standard.

C_S was partitioned into original or native C (i.e. present at the beginning of the experiment) and new C using a mixing equation:

$$\text{CSfnew=}\delta\text{-}\delta\text{0(}\delta\text{v-}\delta\text{0)}$$

$$\text{CSnew= CSfnew x Ci}$$

$$\text{Cor=(1-CSfnew) x Cf}$$

where C_{Sfnew} is the fraction of total C derived from the new vegetation; C_{Snew} (Mg ha^{-1}) is the C derived from the new vegetation (soybean or corn), δ is the δ ^{13}C of soil at sampling time, δ_0 is the $\delta^{13}C$ of soil at the beginning of the experiment,; δ_v is the $\delta^{13}C$ of the residue input from new vegetation, C_f is the amount of C at the end of the time interval considered, and C_{or} is the amount of original C at the end of the time interval considered. The C stock of each plot was calculated on a constant soil mass basis. C mineralization was calculated assuming an exponential decrease of the original amount as follows:

$$Cort= Co \times exp-kt.$$

This is the integral of Henin and Dupuis (1945) differential equation when C_i = 0. The constant k can be readily solved. The humification coefficient (h) for each crop was first computed by bulking aboveground and belowground inputs and ignoring inputs from rhizodeposition. However inputs by rhizodeposition will change the measured $\delta^{13}C$ signature of C_S and is therefore included in our estimates of the fraction of total C derived from the new vegetation. Thus, our estimates of humification represent an upper boundary and are estimated as: h= Cf-Ci+k x CS /Si

3.3- Statistical analysis

Variables were analyzed using a split plot design with treatments (CC and SS) as main plot and time of sampling as subplot. We focused our analysis in the effect of time of sampling, and the interactions between time and treatments. The software used was Infostat 2011/p.

4- Results.

4.1. Residue dry matter production from crops and its $\delta^{13}C$ signal.

Crop biomass production in both years was high in relation to that expected for rainfed crops in this region. Carbon inputs per year from corn were on average 4.4 and 0.9 Mg ha^{-1} for aboveground and belowground, respectively. These inputs were approximately 1.2 and 0.5 $Mg.ha^{-1}$ higher than those for soybean for aboveground and belowground, respectively.

Table 1: Carbon production, carbon and nitrogen concentration and $\delta^{13}C$ mean and standard error in above and belowground biomass.

	Crop	Aboveground†	Belowground‡
Crop Production (Mg of C ha^{-1})	Soybean	6.5 ± 0.4	0.9 ± 0.1
	Corn	8.8 ± 0.1	1.9 ± 0.1
Crop Biomass Carbon Concentration (g g^{-1})	Soybean	0.50 ± 0.1	0.39 ± 0.04
	Corn	0.44 ± 0.1	0.37 ± 0.04
Crop Biomass Nitrogen Concentration (g g^{-1})	Soybean	2.87 ± 0.03	1.15 ± 0.05
	Corn	0.57 ± 0.07	0.62 ± 0.08
Crop Biomass C:N ratio	Soybean	17.4 ± 0.3	33.3 ± 1.5
	Corn	77.5 ± 5.0	57.3 ± 1.5
Crop Biomass $\delta^{13}C$ (‰)	Soybean	-27.10 ± 0.18	-25.07 ± 0.25
	Corn	-13.00 ± 0.18	-13.63 ± 0.25

† Grain is not included; ‡ In the first 30 cm of soil.

There were differences in the C concentration between crops and between above and belowground biomass. Carbon concentrations in both crops were quite different than some figures used in the literature (Andriulo et al., 1999; Clapp et al., 2000; Studdert and Echeverría, 2000) but are in line with reports by Buyanovsky and Wagner (1986) and Bono et al. (2008) particularly for roots. The average concentration of C of roots is around 0.38 g g^{-1}, while that of the aboveground residues averaged 0.50 and 0.44 g g^{-1} for soybean and corn. Thus, in terms of C inputs, 1 Mg of soybean aboveground biomass is equivalent to 1.14 Mg of corn biomass. The C isotopic composition of above and belowground biomass was similar within crops and diverged between crops as expected from C4 and C3 plants. In terms of C:N ratio as was expect there were important differences between crops and aboveground and belowground inputs, particularly for soybean.

4.2. Soil organic carbon dynamic and soil δ^{13}C.

The initial amount of C was high and in agreement to that reported by Salvo et al. (2010) in a neighboring site. Total initial C_S δ^{13}C exhibits an intermediate isotopic composition between C3 and C4 signatures in all the profiles analyzed.

Table 2. Average C_S stock and initial δ^{13}C signal per depth and in CC and SS treatments in T_2.

Depth	C_S Stock	δ^{13}C - T_0	δ^{13}C - T_2 CC	δ^{13}C - T_2 SS
cm	Mg ha^{-1}		‰	
0 – 5	13.21	-18.08	-17.80	-18.45
5 – 10	12.62	-16.87	-16.90	-16.96
10 – 20	23.24	-16.36	-16.21	-16.72
20 – 30	18.84	-15.08	-15.09	-14.76
0 – 30	67.88	-16.34	-16.25	-16.46

After two growing seasons, there were no significant differences in bulk density (BD) ($p<0.6453$), C_S stock ($p<0.8491$) and δ^{13}C of C_S ($p<0.5947$), but as expected δ^{13}C signal of soil with soybean became more negative and corn soil became more positive. Changes in soil δ^{13}C allow calculating the native or original C humification and apparent turnover rate (Table 3).

Table 3. Decomposition rates (k), residue humification (h) and percentage of new carbon (C_{Sfnew}) for CC and SS treatments.

Variable	Units	CC	SS
Decomposition rate (k)	yr^{-1}	0.015	0.006
Humification (h)	%	18.X	11.X
C_{Sfnew}	%	2.9X	1.1X

There were differences between crops in the apparent turnover rate (*k*). Soils with corn (CC) had a two and a half-fold greater *k* than the soil with soybeans (SS). Since total C_S stock remained stable in the two-year period for both CC and SS, the CC treatment had a higher C inputs and humification rates that compensated the C_S losses by decomposition (Table 3).

5- Discussion.

The temperature and hydrologic regime of the region favors a rapid turnover of soil organic matter. In these conditions, the turnover of soil organic matter should be approximately one and a half times faster than cooler and wetter regions like such as the U.S. Midwest (e.g. Huggins et al., 1998) and five times faster than cooler and drier regions like eastern Oregon (e.g. Rasmussen and Smiley, 1997), based on the limitation to microbial activity by temperature and soil water potential used in the model C-Farm (Kemanian and Stöckle, 2010). Therefore and for comparison, two years in this region corresponds theoretically to approximately three to eight years of an experiment in cooler regions.

In the first two years of this experiment we did not detect a significant change in the size of C_S in any treatment. This implies that the C_S respired was balanced by inputs from humified aboveground and belowground inputs. We did, however, detect a change in the δ^{13}C signature of soils under different treatments. Soil under CC acquired a more C4 signal and soil under SS acquired a more C3 signal. However, soil under CC had a higher turnover rate than under SS (k of 0.015 and 0.006 y^{r-1}, respectively). Inputs of C from CC were 40% higher than from SS and yet, the humification coefficient for corn inputs was also higher than that of soybean (h of 0.18 and 0.11 yr^{-1} for CC and SS, respectively). Therefore, our hypothesis that soybean inputs could have a priming effect that would accelerate C_S mineralization should be rejected. Furthermore, our results suggest that the opposite happened, and that corn inputs had a priming effect on C_S turnover.

While unexpected, there are several possible explanations for such result. First, soil under corn could have been moister than under soybean. The corn and soybean stubble in this experiment was cut at the ground level and laid flat while in farm conditions a large fraction of the corn stubble remains upright. Due to the large inputs of corn stubble, the residues fully cover the soil surface well into the following spring, which prevents soil drying by evaporation thus maintaining the soil at a high and stable water potential. In soybean ground, the stubble is barely visible four months after harvest (Ernst et al., 2002) and the soil surface undergoes more pronounced drying and wetting cycles. Second, rhizodeposition can enhance soil organic matter turnover (Cheng and Coleman, 1990; Sanchez et al., 2002) and corn C flux through rhizodeposition seems to be significant (Amos and Walters, 2006); however we did not find much information for soybean and this point remains moot. Third, the mineral N fertilizer applied in corn (70 kg N ha^{-1}) could have enhance native soil organic matter mineralization (Khan et al., 2007); yet information in this regard is not conclusive and the amount of N applied is small in relation to the size of organic N pool or that removed in the grain. In addition, it can be argued that the lower C:N ratio of soybean could compensate for such effect.

A fourth possible explanation is based on the ideas proposed by Fontaine et al. (2003). Based on the C:N ratio of the corn residue, the microbial decomposition of the corn residues requires a significant input of N. The addition of low C:N soybean residues could promote the growth of R-strategist microorganisms (Fontaine et al., 2003) that grow quickly and rely primarily on the soybean input biomass per se. In contrast, the addition of large amounts of corn biomass with high C:N ratio would promote the turnover of C_S by other specialized microorganisms called K-strategist by Fontaine et al. (2003) that may benefit from polymerized substrates which have a long residence time in soil. In these conditions, K-strategists populations could increase the amount of C_S decomposing enzymes released in the soil, thus accelerating the decomposition of the native C_S. While the low turnover rate under soybean seems to fit this hypothesis, the data collected is clearly inconclusive. We lack however the evidence to accept this hypothesis with certainty. Yet, future analysis of this experiment should shine some light on these intriguing results.

7- Conclusions.

After two years of high input of corn and soybean established under no-till we found no changes in C_S. This balance was achieved through higher turnover rate and humification in corn, and through lower turnover rate and humification in soybean. Contrary to our predictions, high C:N corn residues accelerated the C_S turnover, yet corn residues also augmented residue humification. These results warrant further research and the continuation of this experiment.

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9- Keywords.

Soil carbon, soybean, corn, priming effect, 13C abundance.

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