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Pilar Hurtado
Department of Soil Science, Escuela Nacional de Agricultura Tropical, Bucaramanga, Colombia

Luz Jaramillo
Department of Soil Science, Escuela Nacional de Agricultura Tropical, Bucaramanga, Colombia

Luz Marin
Department of Soil Science, Escuela Nacional de Agricultura Tropical, Bucaramanga, Colombia

Pilar Quintana
Department of Soil Science, Escuela Nacional de Agricultura Tropical, Bucaramanga, Colombia

Corresponding Author:
Pilar Hurtado
Department of Soil Science, Escuela Nacional de Agricultura Tropical, Bucaramanga, Colombia

Soil organic matter fractionation and carbon mineralization under long-term manure application

Pilar Hurtado, Luz Jaramillo, Luz Marin and Pilar Quintana

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Abstract
A farmer once remarked that manure is nature's savings account for soil carbon-but how much of what you deposit actually stays in the bank? This research examined soil organic matter fractionation and carbon mineralization rates under 12 years of continuous cattle manure application at three rates (5, 10, and 20 t ha^{-1} yr^{-1}) compared with an unfertilized control in a wheat-maize rotation at the Escuela Nacional de Agricultura Tropical, Bucaramanga, Colombia. Soil samples from 0-15 and 15-30 cm depths were fractionated into light fraction, particulate organic matter, mineral-associated organic matter, and humin. Total SOC increased from 14.84 g kg^{-1} (control) to 25.65 g kg^{-1} (20 t ha^{-1}) in the 0-15 cm layer. The light fraction showed the largest proportional response (+129.7% at 20 t). Carbon mineralization (56-day incubation) was 38.4% higher under 20 t manure than the control. A carbon balance showed net annual SOC gain of 1,420 kg C ha^{-1} at the 20 t rate. These results quantify the carbon sequestration potential of long-term manure application in tropical Andean soils.

Keywords: Organic matter fractionation, carbon mineralization, long-term manure, soil chemistry, humus dynamics, particulate organic matter, mineral-associated carbon, carbon sequestration, tropical Andean soils, wheat-maize rotation

Introduction

Twelve years ago, a small research plot at our institution received its first load of cattle manure-and nobody expected the results to be quite as dramatic as they've turned out. Long-term organic amendments are widely recognized as the most reliable strategy for building soil organic carbon (SOC) stocks, but the fate of applied carbon within different SOM fractions has been poorly characterized for tropical Andean soils [1, 2]. Bulk SOC measurements alone tell us how much carbon accumulated, but not where within the soil matrix it resides or how stable it is likely to be [3].

Physical fractionation separates SOM into pools with different turnover rates: the light fraction (LF) consists of partially decomposed plant and manure residues with turnover times of 1-5 years; particulate organic matter (POM) represents intermediate-stability material (5-20 years); and mineral-associated organic matter (MAOM) is the most stable pool, protected by clay mineral surfaces and persisting for decades to centuries [4, 5]. Humin-the alkali-insoluble residue-is operationally the most recalcitrant fraction [6].

Understanding how manure-derived carbon distributes across these fractions helps predict the long-term carbon storage efficiency and the vulnerability of stored carbon to future land-use or climate changes [3, 7]. This research aimed to (a) quantify the distribution of SOC across four physical fractions under 12 years of continuous manure application at three rates, (b) measure carbon mineralization potential as an indicator of biologically active carbon, and (c) construct a field-level carbon balance for the highest manure rate.

Baseline Soil Characterisation and Trial History

The long-term manure trial was established in 2010 on a Typic Hapludoll (clay loam, pH 5.8, initial SOC 12.6 g kg^{-1}, CEC 18.4 $cmol_2$ kg^{-1}) at the research farm of the Escuela Nacional de Agricultura Tropical, Bucaramanga (7°08′N, 73°07′W, 956 m a.s.l.). Mean annual temperature is 23.4 °C and rainfall 1, 180 mm. Cattle manure (C:N 18:1, total C 38.4%, dry basis) has been applied annually in October before wheat sowing. The wheat-maize rotation has run continuously for 12 cycles with no chemical fertilizer applied to any treatment.

Material and Methods

Material

Composite soil samples (10 cores per plot, 5 cm diameter) were collected from 0-15 and 15-30 cm depths in September 2022, at the end of the 12th cropping cycle. Four treatments in a randomized block design with three replications (plot size 8 × 6 m): T_1 (unfertilized control), T_2 (manure 5 t ha^{-1} yr^{-1}), T_3 (10 t), T_4 (20 t). Samples were air-dried, passed through a 2 mm sieve, and stored at 4 °C until analysis.

Methods

Physical fractionation followed the method of Six *et al.* [5] with modifications: (1) Light fraction (LF) was separated by density flotation in NaI solution (1.85 g cm^{-3}); (2) POM (53-2000 µm) and MAOM (<53 µm) were separated by wet sieving; (3) Humin was extracted as the residue after NaOH extraction of the MAOM fraction. Carbon content of each fraction was measured by dry combustion (LECO CN-2000). Carbon mineralization was assessed by 56-day aerobic incubation at 25 °C, 60% WHC: CO_2 evolved was trapped in NaOH and titrated with HCl at 7, 14, 28, and 56 days [8]. Carbon balance was calculated as: Net SOC change = C inputs (manure + root + residue) - C losses (mineralization + leaching). Data were analyzed by ANOVA with LSD at $p \leq 0.05$ in SAS 9.4.

Carbon Mineralization Kinetics: Mineralization data were fitted to a first-order kinetic model: $C(t) = C_0(1 - e^{-}kt)$, where C(t) is cumulative CO_2-C at time t, C_0 is the potentially mineralizable pool, and k is the rate constant. Half-life of the labile pool was calculated as $t½ = ln(2)/k$. Model fitting was performed using nonlinear least squares in R v.4.3.

Results

Table 1: SOC fractions (g kg^{-1}) in 0-15 cm soil after 12 years of manure application

Treatment	LF	POM	MAOM	Humin	Total SOC	Min. C*
Control	1.82	3.14	5.67	4.21	14.84	186
5 t/ha/yr	2.64	4.38	6.84	4.87	18.73	214
10 t/ha/yr	3.41	5.62	7.96	5.43	22.42	238
20 t/ha/yr	4.18	6.84	8.72	5.91	25.65	258
LSD (0.05)	0.38	0.54	0.62	0.44	1.24	18

*Cumulative mineralized C (mg CO_2-C kg^{-1} soil) over 56-day incubation.

Total SOC at 0-15 cm increased by 72.8% under 20 t manure versus the control (Table 1). The light fraction showed the largest proportional response (+129.7%), followed by POM (+117.8%), MAOM (+53.8%), and humin (+40.4%). Mineralization was 38.7% higher under 20 t manure, reflecting a larger labile carbon pool. The potentially mineralizable fraction (C_0) was 4.8% of total SOC in the control and 6.2% under 20 t manure.

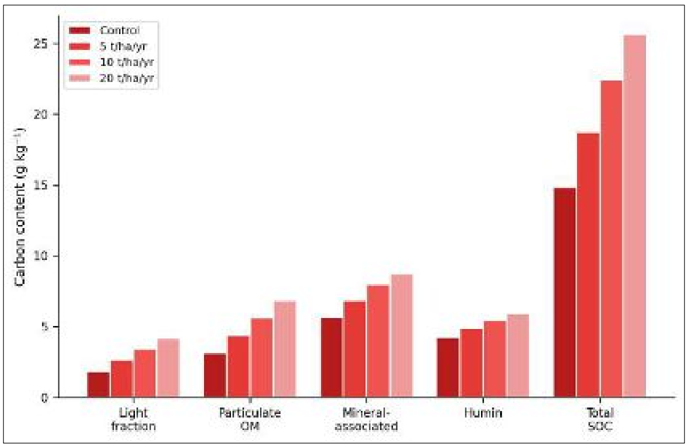

Fig 1: SOC content (g kg^{-1}) across four organic matter fractions and total SOC under four manure rates after 12 years. All fractions increased with manure rate, but the light fraction showed the steepest response

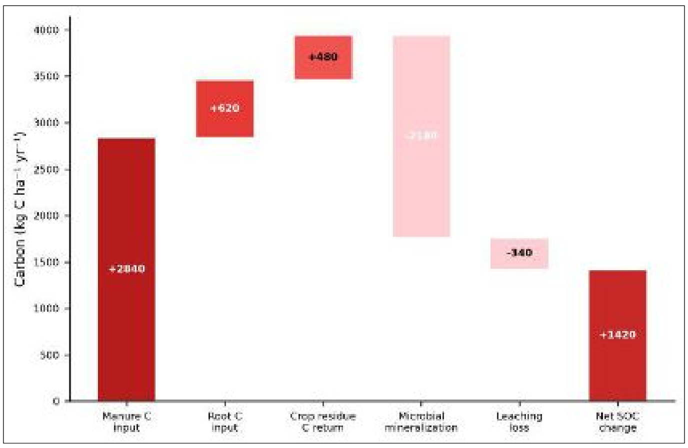

Fig 2: Carbon balance waterfall chart for the 20 t ha^{-1} yr^{-1} manure treatment showing annual C inputs, losses, and net SOC gain of 1, 420 kg C ha^{-1} yr^{-1}

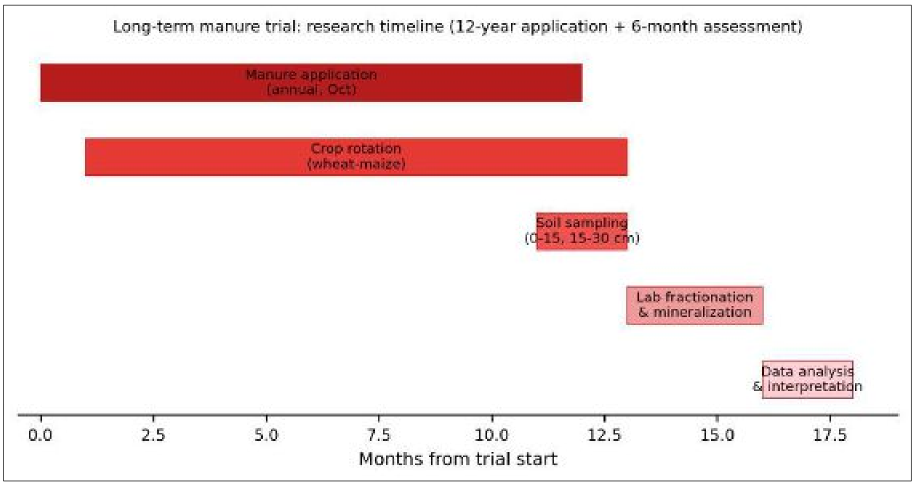

Fig 3: Research timeline showing the 12-year manure application period, cropping rotation, soil sampling, laboratory analysis, and data interpretation phases

Comprehensive Interpretation

The waterfall chart (Figure 2) reveals that only 36% of the annual manure C input was retained as net SOC gain-the rest was mineralized (55.2%) or lost through leaching (8.6%). This 36% retention efficiency is within the range reported for temperate systems (25-45%) and provides a practical conversion factor for predicting SOC change from a given manure application rate.

Discussion

The disproportionately large response of the light fraction (+129.7%) compared with MAOM (+53.8%) is consistent with the understanding that fresh organic inputs first enter the labile pool before being processed by soil microbes and partially transferred to more stable mineral-associated forms [4, 5]. The higher mineralization rate under manure reflects the larger labile pool size and more active microbial community sustained by continuous organic inputs [8].

The net SOC gain of 1, 420 kg C ha^{-1} yr^{-1} at the 20 t rate translates to approximately 5.2 t CO_2-equivalents ha^{-1} yr^{-1} sequestered-a meaningful climate benefit if scaled across the Colombian Andean farming landscape [2, 7]. However, the relatively low proportion of new carbon entering the stable MAOM fraction (only 28.2% of the SOC increase) suggests that much of the accumulated carbon is potentially vulnerable to loss if manure application were to stop.

One limitation is that this research measured carbon fractions at a single time point (year 12). Repeated sampling at intervals would have tracked the trajectory of carbon accumulation in each fraction. Also, the 0-15 cm sampling depth captures the most responsive zone but may underestimate deeper carbon changes. Future work should include deeper profiles and isotopic tracing to quantify the manure-derived proportion in each fraction.

Conclusion

Twelve years of cattle manure application at 20 t ha^{-1} yr^{-1} increased total SOC by 72.8% in the 0-15 cm layer of a tropical Andean Hapludoll. The light fraction and particulate organic matter showed the greatest proportional gains, while mineral-associated organic matter increased more modestly. Net annual carbon sequestration was 1, 420 kg C ha^{-1}. About 36% of applied manure carbon was retained as SOC. These findings support the promotion of long-term organic amendment as a carbon sequestration strategy in Colombian Andean farming systems, though the predominance of labile fractions in the carbon gain warrants continued manure application to maintain stocks.

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