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Sequestration of native soil organic carbon and residue carbon in complex agroecosystems

Amanda Bichel, Maren Oelbermann, Paul Voroney* and Laura Echarte†

School of Environment, Resources and Sustainability, University of Waterloo, Waterloo, Ontario, Canada

ABSTRACT

Knowing short-term gains and losses of soil organic carbon (SOC) is crucial for understanding the role of different land management practices in climate change mitigation. This study evaluated the flow of carbon (C) in soil from two differently configured intercrops [1:2 (one row of maize and two rows soybean); 2:3 (two rows of maize and three rows of soybean)] compared to a maize and soybean sole crop as a result of residue addition. Addition of soybean or maize residues significantly increased ($p < 0.05$) SOC, light fraction (LF-C), and soil microbial biomass (SMB). Soil organic C from native sources was significantly greater ($p < 0.05$) than C from new (residue) sources. The LF had a significantly greater ($p < 0.05$) C content from new sources. Treatments amended with soybean residue had a significantly greater ($p < 0.05$) contribution from new C sources for SOC and LF than treatments amended with maize residue. The SMB-C was significantly greater ($p < 0.05$) in the 2:3 intercrop. Cumulative soil CO_2 emission was significantly lower ($p < 0.05$) in intercrops than in sole crops. CO_2 emissions derived from new C sources was significantly greater ($p < 0.05$) than that derived from native sources in maize amended treatments; and not significantly different ($p < 0.05$) for treatments amended with soybean residues.

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CO_2 emissions; crop residues; intercrops; soil microbial dynamics; soil organic carbon

Introduction

Soil organic matter (SOM) is the largest terrestrial carbon (C) reservoir that actively interacts with the atmosphere [1]. In this dynamic soil–atmosphere interaction, C is continuously added via photosynthesis and lost by respiration [2]. These opposing processes respond differently among ecosystem types and are strongly influenced by management practices in agricultural landscapes, resulting in C accumulation or CO_2 emission.

In intensively managed agricultural soils, crop residues are the major sources of C input. Agricultural practices supporting the return of plant residues to soil are recommended by the Intergovernmental Panel for Climate Change (IPCC) as a means to mitigate the accumulation of atmospheric CO_2 [3]. Additionally, the input of fresh organic matter increases SOC stocks and enhances the soil's nutrient status [4]. However, the quantity and quality of residue, and therefore C, added to the soil depends on the type of crop produced and the management system implemented [5]. Since current interest in enhancing SOC stocks continues as a climate change abatement option, it is crucial to understand the dynamics between new C, derived from added residues, and that of native C housed in SOM.

Conservation agriculture and/or the diversification of agroecosystems through increasing the quantity of crop species and mixing low- and high-quality residues, by integrating legumes, can address future challenges of agroecosystem productivity and enhance SOC stocks [6–8]. Higher crop diversity and input of crop residues from mixed sources can be achieved through the establishment of complex agroecosystems, including agroforestry [4] and cereal–legume intercrops [6]. Intercropping, where crop intensification occurs in both time and space, is defined as the simultaneous growth of more than one species in the same field [9]. In temperate regions, where crops are commonly produced in single stands (sole crops), intercropping is of particular interest because of complementary resource use and a greater potential to sequester C [8,10].

Interaction among chemical, physical and biological processes that regulate the long-term accumulation of C in soil remains poorly understood [1]; and knowledge on the underlying influences of mixed residues on C dynamics in cereal–legume intercrops remains scarce. In sole crops, recently added C (e.g. crop residue) follows a different pathway of transformation compared to native SOC [11]. However, the synchronous input of residues from cereal and legume plants in intercrops

CONTACT Maren Oelbermann moelbermann@uwaterloo.ca
*Present address: School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada; †CONICET, EEA INTA Balcarce, Ruta 226 km 73.5, C.C. 276 (7620) Balcarce, Argentina.

causes interspecific interactions in the soil–plant system that stimulate a more active microbial community and affect soil C transformations differently compared to sole crops [12]. These interspecific interactions are governed by temporal and spatial complementarity through the acquisition of nitrogen (N) by the cereal crop and atmospheric N_2-fixation by the legume [6]. Additionally, N derived from the soil and crop residues from previous cropping seasons (legacy N) also influences C dynamics differently in intercrops than in sole crops [13].

To date, research in temperate intercrop systems has focused on crop metrics [14], fertilizer requirement, erosion control and nutrient leaching [15, 16]. Oelbermann and Echarte [8] noted that temperate cereal–legume intercrops remain an under-recognized option in their potential to sequester C; and knowledge on C transformation and partitioning in these land management systems remains incomplete. The objective of this study was to evaluate the flow of C from added soybean or maize residues in soils from two differently configured cereal–legume intercrops and in a cereal and a legume sole crop. This was achieved through a 140-day incubation study using $\delta^{13}C$ natural abundance to quantify changes in SOC and its associated fractions (light fraction, microbial biomass), CO_2 emission rates, and the quantity of C derived from new and native sources.

Materials and methods

Field site and sample collection

The research site was located in the southern Argentine Pampa, outside the city of Balcarce (37°45′S, 58°18′W). The climate in this area was classified as mesothermal subhumid–humid (Thornthwaite classification) or as temperate humid without dry season (Köpen classification). The mean annual rainfall, potential evapotranspiration, and annual mean air temperature (1980–2012) were 860 mm yr^{-1}, 856 mm yr^{-1} and 14.3 °C (maximum 24.2 °C and minimum 7.6 °C), respectively (Unidad Integrada Balcarce Weather Station, 37°45′S, 58°18′W, 130 m above sea level). The soil was classified as a Typic Agriudoll (US Soil Taxonomy) or Luvic Phaeozem (FAO Soil Classification) and was part of the *Mar del Plata* series, with a soil texture of 41.1% sand, 35.8% silt and 23.1% clay [17]. The soil (0–20 cm) was moderately acidic with a pH of 5.77, with a low available phosphorus (P) of 7.83 mg kg^{-1} (Bray-extractable P), and a high soil organic C (SOC) content of 30.6 g kg^{-1}. The slope of the site was $< 2\%$, suggesting little to no potential for water erosion.

Experimental intercrop and sole crop plots were established in 2007, and soil used in the present study was collected in May 2011. The experimental plots were established on land previously under alternating sunflower (*Helianthus annuus* L.) and pasture. The previous crop was 2 years of sunflower and the soil was prepared using a disk harrow followed by a spike harrow. The intercrop study was a randomized complete block design (RCBD) with four treatments: maize sole crop, soybean sole crop, 1:2 intercrop (one row of maize and two rows of soybeans) and 2:3 intercrop (two rows of maize and three rows of soybeans). Each treatment was replicated 3 times, and each treatment plot was 8.8 m $\times$ 12 m. The maize and soybean sole crops were rotated annually, but the intercrops were not. Plant density (plants m^{-2}) was 4.3 (1:2 intercrop), 5.3 (2:3 intercrop), 8.0 (maize sole crop) and 29 (soybean sole crop), with a 0.52-m distance between crop rows in all treatments. The soil was disk harrowed 3 times and spike harrowed once before each crop seeding. Weeds were controlled with N-phosphonomethyl glycine (Glyphosate). All crops received P fertilizer (35 kg P ha^{-1}). Maize in the sole crop and in the intercrops received N fertilizer (150 kg N ha^{-1}) in the form of urea. Fertilizer was applied by hand at the bottom of the maize stems at the 6th leaf stage in the intercrops. Soybeans were inoculated with *Bradyrhizobium japonicum*. Maize was seeded in late October to early November and harvested in April; soybeans were seeded in November and harvested in May. Crop residues were returned to all treatments after each harvest.

Five soil samples (0–20 cm) were extracted from the center of each treatment replicate to avoid edge effects, using a soil corer with a 5-cm inner diameter. In the intercropped treatments, soil was extracted between all possible combinations of rows, including between two maize rows, between two soybean rows and between maize and soybean rows. Soil from each treatment replicate was combined, air dried and sieved to < 2 mm. In 2011, after crop harvest, soybean and maize stems and leaves were randomly collected from each treatment replicate, representative of crop residues retained on the field after harvest. Approximately 100 g of soybean and maize residue from each treatment replicate was combined, dried at 65 °C for 48 h, and a subsample was ground to a fine power using a ball mill (Retsch® ZM1, Haan, Germany) and analyzed for C and $\delta^{13}C$. Mean values, in all treatments, of C and N concentrations of crop residue were 422 g kg^{-1} (C) and 6.6 g kg^{-1} (N) for maize, and 448 g kg^{-1} (C) and 14 g kg^{-1} (N) for soybeans. The $\delta^{13}C$ of soybean residue was $-$ 28.62 ‰ and $-$ 11.89 ‰ for maize residue.

Experimental design

Prior to soil incubation, air-dried soil was pre-conditioned for 7 days at 21 °C by adding deionized water to reach a water holding capacity of 60% (wt/wt) of field capacity. After pre-conditioning, 60 g of soil from the soybean sole crop, 1:2 and 2:3 intercrops were placed into 1000-mL glass jars and mixed thoroughly, using a glass rod, with 1.5 g ground (2-mm) soybean

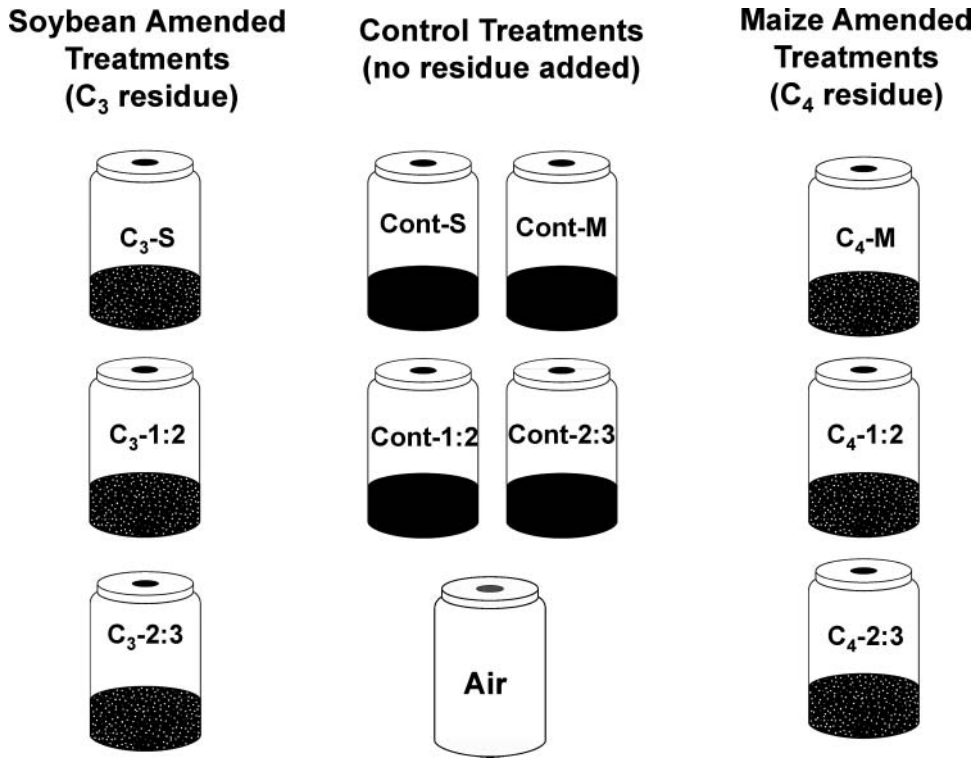

Figure 1. Schematic diagram of soil from sole crop and intercrop treatments amended with soybean (S) or maize (M) residue, and non-residue amended control (Cont) treatments.

residue. An additional set of jars containing 60 g pre-conditioned soil from the maize sole crop, 1:2 and 2:3 intercrops was mixed thoroughly, using a glass rod, with 1.5 g ground (2-mm) maize residue. A set of jars with no added residue for the intercrops and sole crops was used as a control (Cont). The Cont soil was also mixed thoroughly to generate similar conditions to soils that received residue. Blank jars, containing no soil or residue, were also included (Figure 1). Each residue-amended treatment, the Cont and the blank jars were replicated 3 times. All jars were sealed with lids containing septa for gas sampling, and kept in the dark at 21 °C for 140 days. Throughout the incubation, soil moisture was maintained at 60% (wt/wt) of field capacity by adding deionized water. The quantity of residue application for all treatments was based on above-ground residue input for the 2010/2011 crop season for the intercrops.

Soil carbon characteristics

Soil carbonates were removed by adding 150 mL 0.5 M HCl to 2 g of soil [8]. The mixture was stirred 3 times over a 24-h period, and subsequently washed by pipetting the HCl from the settled soil and adding ultrapure water to the soil. This washing procedure was repeated daily for 4 d after which the soil was dried in an oven at 40 °C for 2 d [18]. The acid-treated soil was ground in a ball mill (Retsch® ZM1, Haan, Germany) and analyzed for SOC on a Costech 4010 elemental analyzer (Cernusco, Italy).

Soil free light fraction C (LF-C) was quantified by shaking 10 g of air-dried soil with 35 mL of NaI (1.7 g cm^{-3}) at 400 rpm and left for 48 h to separate from mineral particles. After 48 h, the floating LF was removed from the surface of the NaI and rinsed with 0.01 M $CaCl_2$ and 75 mL of distilled water to remove the NaI. The recovered LF was dried at 60 °C for 48 h, ground in a ball mill and analyzed for its C concentration [19].

Soil microbial biomass C was evaluated using chloroform fumigation extraction (CFE) [20]. Organic C was extracted from the fumigated and non-fumigated soils by adding 35 mL of 0.05 M K_2SO_4 to each sample, shaking the mixture for 1 hr at 400 rpm and filtering the extract using Whatman GF934-AH filter paper. The extracted samples were freeze dried and analyzed for organic C, and soil microbial biomass (SMB) was quantified as the difference between fumigated and non-fumigated samples using a conversion factor of 0.35 [20].

Soil CO_2 emission rates

The concentration of CO_2 in the headspace of the jar was measured every 4 days during the first 2 weeks and weekly thereafter using an Agilent HP6890N gas chromatograph (Santa Clara, CA, USA). The daily CO_2 emission rate was determined according to Hogg et al. [21]:

$$R = (C_S - C_A) \times VD/(M/t) \quad (1)$$

where R (μg CO_2 g^{-1} d^{-1}) is the amount of CO_2 (μg) evolved per gram of (dry) soil per day; C_S is the

concentration of the CO_2 evolved from the soil ($\mu L\ L^{-1}$); C_A is the concentration of CO_2 ($\mu L\ L^{-1}$) from the control (blank jar); V is the volume of the effective headspace (0.962 L); D is the density of CO_2 adjusted for temperature, pressure and humidity ($g\ L^{-1}$); M is the dry mass of the soil sample (g); and t is the sampling time interval in days. After each gas sampling, the jars were flushed with ambient air for 20 min and then resealed.

Stable carbon isotope ($\delta^{13}C$) analysis

Plant and soil samples were analyzed for $\delta^{13}C$ (Tracermass Isotope Mass Spectrometer, Europa Scientific, Crewe, UK), and C derived from soybean or maize residue, and from the soil (SOC, LF-C) was quantified using a two-end-member mixing model [22]:

$$\text{Applied C} = (\delta^{13}\text{C}_{\text{SOC}} - \delta^{13}\text{C}_{\text{cont}})/(\delta^{13}\text{C}_{\text{RESIDUE}} - \delta^{13}\text{C}_{\text{cont}}) \quad (2)$$

and

$$\text{Native Soil C} = 1 - \text{New C} \quad (3)$$

where $\delta^{13}C_{SOC}$ is the SOC from soils with added residue; $\delta^{13}C_{cont}$ is the SOC from the corresponding control treatment with no residue added; and $\delta^{13}C_{RESIDUE}$ is the $\delta^{13}C$ value of the added soybean or maize residue. The contributions from applied residue C and soil C sources to SOC concentrations were quantified by multiplying applied and soil C fractions by the SOC concentration.

The SMB was extracted as described above, and prior to freeze drying and $\delta^{13}C$ analysis, samples were treated with 1 M HCl until a pH of 6 was reached to remove the carbonates. The $\delta^{13}C$-SMB was estimated as the difference in $\delta^{13}C$ of the C extracted from the fumigated and unfumigated samples [23]:

$$\delta^{13}\text{C}_{\text{SMB}} = [(\delta^{13}\text{Cf} \times \text{Cf}) - (\delta^{13}\text{Cnf} \times \text{Cnf})]/(\text{Ct} - \text{Cnf}) \quad (4)$$

where Cf is the amount of C extracted from the fumigated and unfumigated (Cnf) samples, and δ^{13}Cf and δ^{13}Cnf are the ^{13}C-natural abundances of the fumigated and non-fumigated extracts. From the values of δ^{13}C-SMB-C, the fraction of SMB-C derived from new and native C sources when soybean or maize residues were added, was quantified using Equations 2 and 3.

The fraction of CO_2 emission rates when soybean residue (fCO_2-C_3) was added to the soil was quantified using a two-end-member mixing model [22]:

$$f\text{CO}_2 - \text{C}_3 = (\delta^{13}\text{C} - \text{CO}_{2\text{Soy}} - \delta^{13}\text{C}_{\text{Cont}})/(\delta^{13}\text{C}_{\text{C3Soy}} - \delta^{13}\text{C}_{\text{Cont}}) \quad (5)$$

where δ^{13}C-CO_{2Soy} was the δ^{13}C of the respired CO_2 from soils with added soybean residue; $\delta^{13}C_{Cont}$ was the δ^{13}C of the respired CO_2 from control treatments with no added residue; and $\delta^{13}C_{C3Soy}$ was the mean δ^{13}C value of soybean residue. The fraction of CO_2 emission rates derived from maize (fCO_2-C_4), when soybean residue was added, was quantified using the following equation [22]:

$$f\text{CO}_2 - \text{C}_4 = 1 - f\text{CO}_2 - C_{3\text{Soy}} \quad (6)$$

To determine the fraction of CO_2 emission rates when maize residue (fCO_2-C_4) was added, Equation 2 substituted fCO_2-C_3 with fCO_2-C_4; δ^{13}C-CO_{2Soy} was substituted with δ^{13}C-CO_{2Maize} which was the δ^{13}C of CO_2 emission rate from soils with added maize residue; and $\delta^{13}C_{C3Soy}$ was substituted with $\delta^{13}C_{C4Maize}$. SOC, LF-C, SMB-C and their respective stable isotopes were analyzed in samples taken after t = 1, 35, 70, 105 and 140 days of the incubation. Results reported in Tables 1–3 represent the mean value over 140 d of incubation for each of the evaluated soil characteristics, since most values were not significantly different with time.

Statistical analysis

Prior to any statistical analyses, all data were examined for normality (Shapiro–Wilk test of normality) and homogeneity of variance (Levene test of equality of variances). For SOC, LF-C, SMB-C, CO_2 emissions rates and stable isotope analyses, a two-way repeated-

Table 1. Mean values, over 140 days of incubation, of soil organic carbon (SOC), soil light fraction carbon (LF-C), soil microbial biomass carbon (SMB-C) and soil microbial biomass carbon as a percentage of soil organic carbon (SMB-C/SOC) in sole crop and intercrop treatments amended with soybean residue (C_3-S, C_3-1:2, C_3-2:3) or maize residue (C_4-M, C_4-1:2, C_3-2:3), and non-residue amended control treatments (Cont-S, Cont-M, Cont-1:2, Cont-2:3). Standard errors are given in parentheses.

	SOC (g C kg^{-1})	LF-C (g C kg^{-1})	SMB-C (mg C kg^{-1})	SMB-C/SOC (%)
C_3-S	31.55 (2.47)^{a*}	8.51 (0.15)^{a*}	376.29 (16.18)^{a*}	1.29 (0.18)^{ab}
C_3-1:2	31.59 (2.01)^{a*}	8.92 (1.08)^{a*}	416.56 (15.61)^{a*}	1.39 (0.09)^{a*}
C_3-2:3	32.06 (0.90)^{a*}	9.19 (0.36)^{a*}	396.70 (16.76)^{a*}	1.45 (0.15)^{a*}
C_4-M	30.08 (1.03)^{a*}	6.99 (0.15)^{a*}	370.15 (8.67)^{a*}	1.38 (0.09)^{b}
C_4-1:2	31.25 (1.32)^{a*}	7.44 (0.13)^{a*}	377.90 (10.40)^{a*}	1.20 (0.38)^{ab}
C_4-2:3	31.34 (1.41)^{a*}	7.66 (0.58)^{a*}	333.25 (8.01)^{a}	1.12 (0.12)^{ab}
Cont-S	22.44 (1.39)^{y}	1.13 (0.19)^{y}	284.81 (17.20)^{y}	1.32 (0.09)^{y}
Cont-M	21.83 (1.63)^{y}	1.07 (0.03)^{y}	274.72 (23.05)^{y}	1.30 (0.05)^{y}
Cont-1:2	22.93 (1.75)^{y}	0.96 (0.13)^{y}	290.23 (14.45)^{y}	1.26 (0.08)^{y}
Cont-2:3	24.43 (1.44)^{y}	1.01 (0.17)^{y}	302.13 (21.57)^{y}	1.19 (0.02)^{y}

Values followed by the same lower case letters, comparing among treatments (a–c) and controls (y), are not significantly different at $P < 0.05$.
Values followed by an asterisk (*) are significantly different treatments from their corresponding control (Cont).

Table 2. Mean values, over 140 days of incubation, of soil organic carbon (SOC), soil light fraction carbon (LF-C), soil microbial biomass carbon (SMB-C) derived from new and old carbon sources in sole crop and intercrop treatments amended with soybean residue (C_3-S, C_3-1:2, C_3-2:3) or maize residue (C_4-M, C_4-1:2, C_3-2:3). Standard errors are given in parentheses.

	SOC (g C kg^{-1})		LF-C (g C kg^{-1})		SMB-C (mg C kg^{-1})	
	New C	Old C	New C	Old C	New C	Old C
C_3-S	9.59 (1.06)^{B,a}	22.33 (1.51)^{A,a}	6.73 (0.41)^{A,a}	1.71 (0.17)^{B,d}	124.63 (24.72)^{A,b}	96.81 (5.71)^{B,a}
C_3-1:2	8.26 (0.46)^{B,a}	25.38 (1.92)^{A,a}	7.41 (0.64)^{A,b}	2.03 (0.07)^{B,c}	146.95 (10.32)^{A,b}	111.34 (16.70)^{B,a}
C_3-2:3	8.59 (0.53)^{B,a}	25.98 (1.09)^{A,a}	7.29 (0.41)^{A,a}	1.74 (0.24)^{B,d}	129.07 (20.37)^{B,b}	145.30 (17.33)^{A,a}
C_4-M	7.47 (0.82)^{B,ab}	24.10 (1.17)^{A,a}	5.13 (0.43)^{A,c}	2.05 (0.07)^{B,c}	168.00 (16.61)^{A,b}	131.23 (24.51)^{B,a}
C_4-1:2	7.02 (1.13)^{B,ab}	24.19 (2.05)^{A,a}	5.23 (0.24)^{A,c}	2.21 (0.13)^{B,b}	207.87 (15.77)^{A,a}	112.32 (18.38)^{B,a}
C_4-2:3	7.02 (0.28)^{B,c}	27.26 (1.70)^{A,a}	5.86 (0.24)^{A,d}	2.52 (0.09)^{B,a}	128.32 (11.14)^{B,b}	137.72 (16.72)^{A,a}

Values followed by the same upper case letters, comparing between new and old C sources, are not significantly different at $P < 0.05$.
Values followed by the same lower case letters, comparing among treatments, are not significantly different at $P < 0.05$.

Table 3. Mean values, over 140 days of incubation, of respired carbon dioxide (CO_2-C) from new and old carbon sources in sole crop and intercrop treatments amended with soybean residue (C_3-S, C_3-1:2, C_3-2:3) or maize residue (C_4-M, C_4-1:2, C_3-2:3). Standard errors are given in parentheses.

	Respired CO_2-C (μg CO_2-C g^{-1} d^{-1})	
	New C	Old C
C_3-S	104.60 (20.62)^{A,b}	82.86 (11.66)^{A,a}
C_3-1:2	89.31 (11.31)^{A,b}	84.38 (13.26)^{A,a}
C_3-2:3	83.48 (13.04)^{A,b}	102.56 (7.60)^{A,a}
C_4-M	145.21 (6.24)^{A,a}	14.77 (0.82)^{B,c}
C_4-1:2	133.42 (2.62)^{A,ab}	24.70 (0.53)^{B,b}
C_4-2:3	115.70 (5.60)^{A,ab}	12.45 (3.97)^{B,c}

Values followed by the same upper case letters, comparing between new and old C sources, are not significantly different at $P < 0.05$.
Values followed by the same lower case letters, comparing among treatments are not significantly different at $P < 0.05$.

measures analysis of variance was used to determine differences among treatments on each day, among sampling days for each treatment and for overall means. Sampling day was used as the within-subject repeated measures, and treatment type was used as the between-subjects main factor [24]. Significantly different main effects were tested using the Tukey's multiple comparison test [24]. Significant simple effects were tested with the estimated marginal means function using the least significant difference (LSD) test [24]. The threshold probability level for determining significant differences for all statistical analyses was set at $P < 0.05$. All data analyses were carried out in IBM SPSS Statistics (version 21, 2012).

Results

Soil carbon characteristics

The interaction effect of treatment-by-day was not significant for SOC [F(4,36) = 77, p = 0.81], and SOC content was not significantly different among residue-amended treatments or among Cont treatments (Table 1). However, SOC was significantly greater in each residue-amended treatment compared to its respective Cont treatment. The interaction effect of treatment-by-day was not significant for SOC derived from new or native C sources [F(10,22) = 0.87, p = 0.57]. Treatments amended with soybean residues had a significantly greater SOC content from new sources compared to treatments amended with maize residues (Table 2). However, C content from native C sources was not significantly different among all treatments for SOC.

The interaction effect of treatment-by-day (g C kg^{-1}) was not significant for LF-C [F(36,60) = 1.29, p = 0.19], and LF-C was not significantly different among residue-amended treatments or among Cont treatments (Table 1). LF-C was significantly greater in each residue-amended treatment compared to its respective Cont treatment (Table 1). The interaction effect of treatment-by-day was not significant for LF-C (g C kg^{-1}) from new and native C sources [F(10,18) = 1.67, p = 0.20]. The content of LF-C derived from new sources was significantly greater compared to that from native sources (Table 2). New C sources in soybean-amended treatments had a significantly greater LF-C content compared to treatments with maize-amended residues. However, this trend was reversed for native C sources.

The interaction effect of treatment-by-day was significant for SMB-C [F(27,42) = 3.57, $p < 0.0001$)]. SMB-C decreased significantly with time only in treatments amended with maize residues. Over 140 days of incubation, SMB-C was not significantly different among residue-amended treatments or among Cont treatments (Table 1). However, SMB-C was significantly greater in each residue-amended treatment compared to its respective Cont treatment, except for C_4-2:3, where no such difference was observed (Table 1). The interaction effect of treatment-by-day was not significant for SMB-C (g C kg^{-1}) from new and native C sources [F(10,10) = 3.60, p = 0.08]. Soil microbial biomass C derived from new C sources was significantly greater compared to native C sources, except for the 2:3 intercrop in both maize and soybean residue-amended treatments (Table 2). Only the 1:2 intercrop treatment amended with maize residue had a significantly greater C content from new sources, but there was no significant difference among treatments from native C sources.

The interaction effect of treatment-by-day was significant [F(27,39) = 1.24, p = 0.001] for SMB-C as a percentage of SOC (SMB-C/SOC). Intercrop treatments amended with maize residue had a significant

decrease in SMB-C/SOC with time. The SMB-C/SOC in treatments amended with soybean residue was significantly greater compared to treatments amended with maize residue (Table 1). Only the intercrop treatments amended with soybean residue had a significantly greater SMB-C/SOC compared to its respective Cont treatments.

Soil CO_2 emission rates

The interaction effect of treatment-by-day for CO_2 emission rate ($\mu g\ CO_2\text{-}C\ g^{-1}\ d^{-1}$) with time was significant [$F(176,308) = 3.72$, $p < 0.0001$], where CO_2 emission rates decreased with time. Over the 140 days of incubation, treatments amended with soybean or maize residues had a significantly greater rate of CO_2 emissions than their respective Cont treatments (Figure 2a and b). Cumulative CO_2 emission was significantly greater in sole crop treatments amended with soybean or maize residue compared to intercrop and Cont treatments (Figure 3a and b).

The interaction effect of treatment-by-day was not significant for respired CO_2 ($\mu g\ CO_2\text{-}C\ g^{-1}\ d^{-1}$) from new and native C sources [$F(10,24) = 5.38$, $p = 0.12$]. The rate of C respired from new sources was significantly greater in treatments amended with maize residues. However, the rate of C respired from native sources was significantly greater in treatments amended with soybean residues (Table 3). There was no significant difference between new and native C sources in treatments amended with soybean residues; but for new C sources, treatments amended with maize residue had a greater rate of C respired from new C sources.

Discussion

Although addition of soybean or maize residues in sole crops and intercrops caused a greater incorporation of C into the LF, compared to Cont treatments, enhanced microbial activity led to the rapid mineralization of the added residue and minimized decomposition of native C sources [25]. In contrast, a lack of residue input in Cont treatments caused decomposition of native SOC and LF-C that remained in the soil from previous crop seasons [26,27]. Given the low C/N ratio (32) of soybean residue, we expected a significantly lower quantity of new C sources in treatments amended with soybean residues. Instead we found a significantly lower SOC and LF-C content from new C sources in treatments amended with maize residue (C/N ratio of 64). This was due to an N legacy effect from the previous crop seasons [28]. At this study site, maize and soybean sole crops were rotated annually. As such, treatments referred to as maize sole crop were under maize in 2009 and 2011, and under soybean sole crop in 2008 and 2010; and treatments referred to as soybean sole crop were under soybean in 2009 and 2011, and under maize in 2008 and 2010 [13]. As such, legacy N from the previous season's (2010) soybean sole crop, and N from soybeans in the intercrops, caused a greater rate of decomposition of new C sources in maize amended treatments. This was also observed by Nguyen et al. [29], who found that legacy residue with a low C/N ratio stimulated microbial growth and decomposition after the addition of residue with a high C/N ratio.

Residue addition, and the availability of new C sources, enhanced microbial community activity, leading to a significantly greater accumulation of SMB-C at the beginning of the incubation [30,31]. During the early phase of the incubation, the SMB preferentially incorporated labile C sources derived from the added residue over native and more recalcitrant material [32]. However, a lack of residue addition in Cont treatments decreased the SMB significantly compared to residue-amended treatments. This is because recalcitrant C sources in the Cont treatments were not able to supply the same quantity of energy to the microbial community [33]. It was expected that residue type and/or management practices would strongly influence SMB-C because microbial biomass is a sensitive short-term indicator that can detect changes in land-use and/or management practices [34]. For example, microbial activity is stimulated and decomposition occurs more readily when residues with a lower lignin content and a low C/N ratio, such as soybeans, are added to the soil [28]. However, when agroecosystems receive input from mixed residue sources, such as that in cereal–legume intercrops, the SMB responds differently than in sole-crop agroecosystems [35]. Although no significant impact on the SMB in our study was observed with respect to residue type or management practice, intercrops (C_3-2:3 and C_4-2:3) showed a trend of a greater concentration of SMB-C from native C sources. This is because microbes from intercrop-derived soil evolved in a system with a more species-rich residue input causing an increase in microbial activity compared to sole crops [36]. Additionally, Regehr et al. [12] found that 2:3 intercrops had a significantly lower N limitation compared to sole crops. Therefore, microbes in the 2:3 intercrops utilized new C sources more efficiently, minimizing the decomposition of native C and thereby increasing SOC stocks. The SMB-C/SOC ratio is a useful measure that quantifies the contribution of microbial biomass to SOC. Therefore, it is a more sensitive indicator to changes in land management than SOC [37]. Intercrop treatments amended with soybean residue had a significantly greater SMB-C/SOC ratio compared to their respective Cont treatments. Combining a high-quality residue, such as soybean, with soil derived from cereal–legume intercrops enhanced microbial activity, causing a larger proportion of the incoming C to be incorporated into microbial C [38,39].

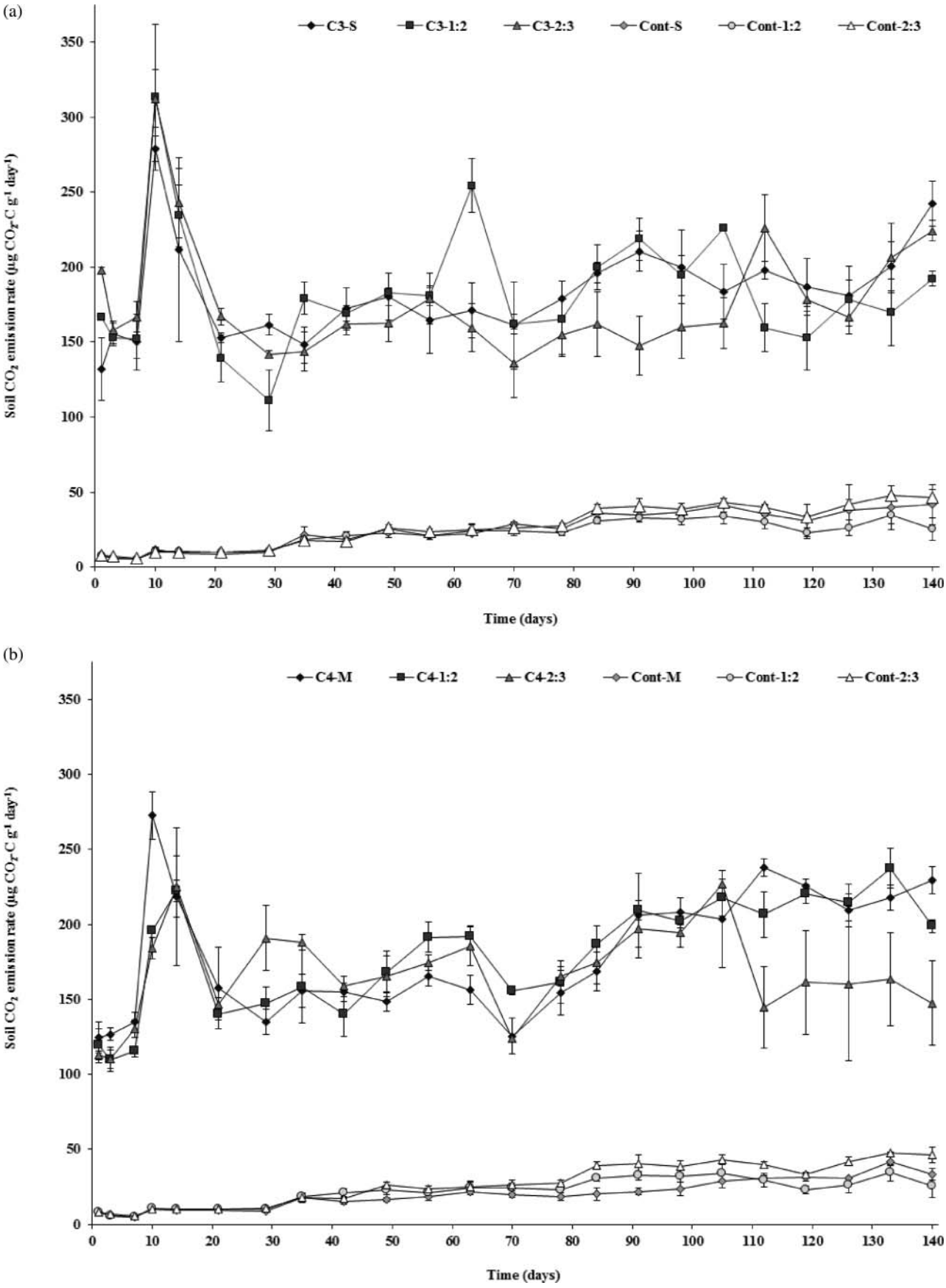

Figure 2. (a) CO_2 emission rates (μg CO_2-C g^{-1} day^{-1}) of soil derived from soybean and maize sole crops and 1:2 and 2:3 intercrops over a 140-day incubation with added soybean residue (C_3-S, C_3-1:2, C_3-2:3), and in control (Cont-S, Cont-1:2, Cont-2:3) treatments. (b) CO_2 emission rates (μg CO_2-C g^{-1} day^{-1}) of soil derived from soybean and maize sole crops and 1:2 and 2:3 intercrops over a 140-day incubation with added maize residue (C_4-M, C_4-1:2, C_3-2:3), and in control (Cont-M, Cont-1:2, Cont-2:3) treatments.

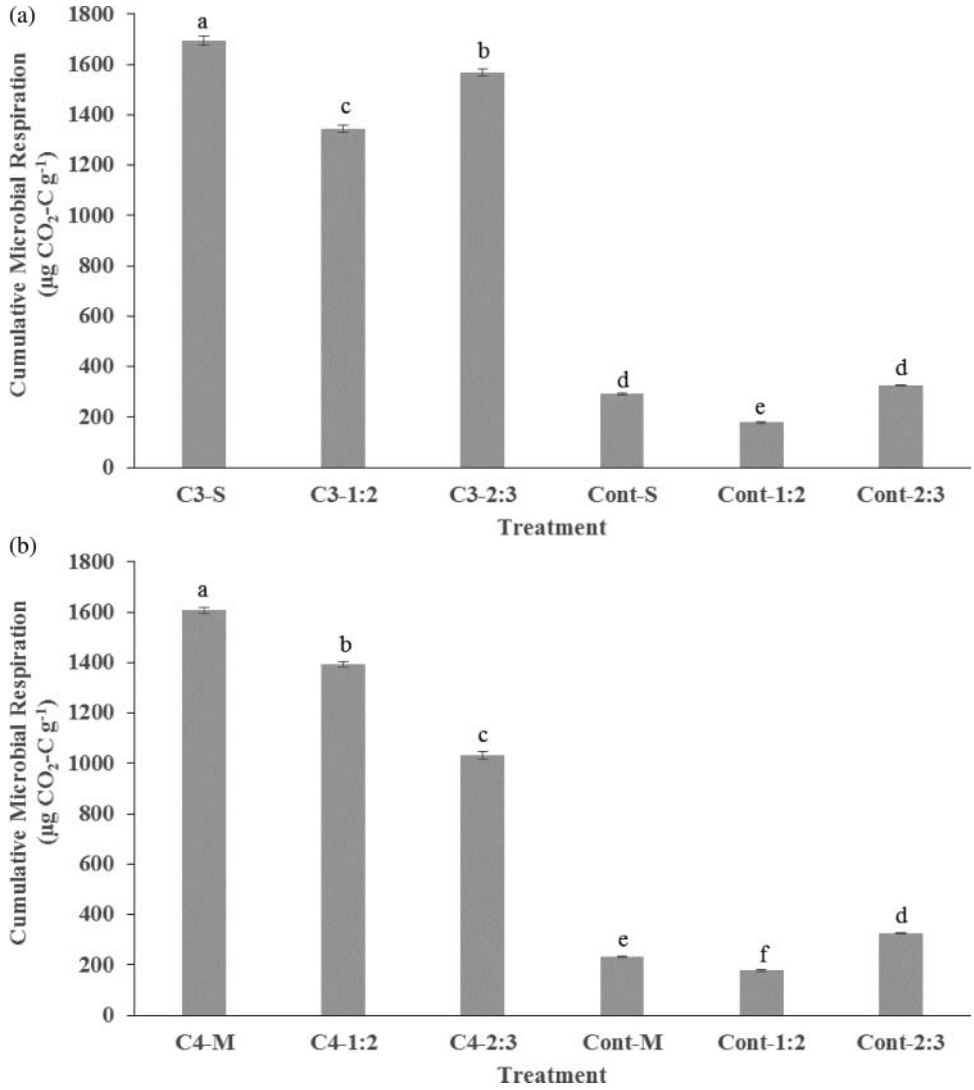

Figure 3. (a) Cumulative respiration (μg CO_2-C g^{-1}) from soil derived from soybean and maize sole crops and 1:2 and 2:3 intercrops over a 140-day incubation with added soybean residue (C_3-S, C_3-1:2, C_3-2:3), and in control (Cont-S, Cont-1:2, Cont-2:3) treatments. (b) Cumulative respiration (μg CO_2-C g^{-1}) from soil derived from soybean and maize sole crops and 1:2 and 2:3 intercrops over a 140-day incubation with added maize residue (C_4-M, C_4-1:2, C_3-2:3), and in control (Cont-M, Cont-1:2, Cont-2:3) treatments.

Recently added residue or newly formed soil organic matter (SOM) follows a different pathway of mineralization and stabilization than C from native sources [11], because the succession of microbial communities is different between labile and recalcitrant sources [3]. Residue decomposition is dependent not only on substrate composition but also on the ability of the existing microbial community to decompose the available substrates [40]. Carbon sources that were recently added (e.g. fresh residue input) contain structural components that are retained within SOC, and therefore take longer to decompose [41]. For example, Bichel [42], using the same experimental treatments as our study, found a distinct difference in C source metabolism among all treatments (residue-amended and Cont) after 1 day of incubation. After 140 days of incubation, Bichel [42] found a shift in the microbial community, and the C sources metabolized in the intercrops (e.g. C_3-1:2 and C_4-2:3 treatments) were distinctly different than the remaining treatments. These shifts were caused by changes in the process of residue decomposition and microbial diversity [43], where microbial richness (R) and diversity (Shannon–Weaver) were greater in C_3-1:2 and C_4-2:3 treatments [40]. This suggests that intercrop treatments have a greater potential to increase SOC stocks, given that their cumulative CO_2 emission was also significantly lower than that in the sole crops.

CO_2 emission rates in residue-amended treatments showed that ample substrate was available to support decomposition, given the relatively constant respiration rates over the 140 days. Carbon from legumes was slightly more prone to microbial degradation and synthesis (Figure 3a), and therefore more accessible to the microbial community than that from maize residues [1]. However, a small and slightly labile soil C pool previously protected from microbial decomposition became available in the Cont treatments. Previous

studies found that SOC content in Luvic Phaeozens from this region of Argentina were affected by disturbance such as contrasting tillage systems [44]. Such disturbance influenced the distribution of the labile fraction and their exposure to mineralization among various aggregate size categories [44]. Therefore, physical disruption during soil processing in this study (e.g. sieving) metabolized fragments of the LF from the native C pool. This stimulated microbial activity, and caused a minimal increase (17 to 38 μg CO_2-C g^{-1}) in respiration over the 140 days in the Cont treatments.

In conclusion, our results suggest that adding different residue types affected short-term SOC dynamics and this influence differed between sole crops and intercrops. The addition of residue C to soil derived from intercrops caused complex interactions at the soil–residue scale. These interactions stimulated a more active microbial community and altered soil C transformations differently compared to the sole crops. Both intercrop configurations were demonstrated to be more sustainable land management options compared to sole cropping, but the greatest potential for enhancing SOC stocks occurred in the 2:3 intercrop configuration. Therefore, our data contributed new knowledge on C dynamics in intercrops and how these can be influenced by the design of the intercrop. This study highlighted that over the long term, cereal–legume intercrops will play an important role in the process of enhancing SOC stocks and in the mitigation of greenhouse gases.

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Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Maren Oelbermann http://orcid.org/0000-0003-0996-4140

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