Increasing soil organic matter and short-term nitrogen availability by combining ramial chipped wood with a crop rotation starting with sweet potato

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Abstract

Increasing soil organic matter is essential for enhancing agricultural soil quality and ecosystem services, including crop yields. Ramial chipped wood (RCW), a pruning by-product, has great potential in this regard, yet its short-term effects on soil organic carbon (C) retention, nitrogen (N) availability, and crop yields remain unclear. This study aimed to rapidly increase soil organic matter and nutrient stocks by applying RCW combined with sweet potato, a starter crop suitable for low-nutrient soils. We monitored soil organic C and N stocks, crop yields, and N use in soils recently enriched with high or low doses of RCW, comparing them to organically managed soils that were regularly tilled and fertilized with either organic granulate or plant residue compost. For the first time, we show that RCW application rapidly increased N stocks in the fine earth fraction, particularly at the high dose. At two months in high-dose plots, 61% of the remaining C was retained in the organic debris fraction, while 73% of N was incorporated into the fine earth. After one year, 22% of the added C was retained in soil with the high RCW dose, whereas neither the low dose nor compost application led to significant C increases. In contrast, N retention was nearly 100% for both RCW doses and compost. Agronomic production and crop performance were maintained or slightly improved with the high RCW dose, suggesting that the increased N stocks supported crop nutrition. Additionally, RCW enhanced biological N fixation in sweet potato. These results indicate that high-dose RCW incorporation into soils with sweet potato cultivation is a promising catalytic strategy to boost soil organic matter and N reservoirs while achieving good crop yields.

This practice also promotes a circular economy by repurposing a locally available C-rich resource and aligns with sustainable agriculture principles.

Keywords: Organic agriculture, wood by-products, carbon and nitrogen cycling, local resources

1. INTRODUCTION

Agricultural intensification is depleting soil organic matter (SOM) (Kopittke et al., 2019), a key driver of soil degradation (Gregory et al., 2015). Even with mineral fertilization, low SOM levels can limit crop yields (Loveland & Webb, 2003). In the face of ongoing climate change, most agricultural soils are of insufficient quality to meet the rising global food demand projected for the coming decades (FAO, 2015). Moreover, there is a pressing need to modify agricultural practices to achieve the United Nations Sustainable Development Goals for 2030 (Eyhorn et al. 2019). In this context, it has been proposed that the ecosystem services associated with increased organic matter in agricultural soils may help address the challenges of climate change and feeding a growing population (Sandhu et al., 2008).

Zero-tillage and the application of organic amendments are among the most widely adopted practices for increasing SOM. In addition to mitigating climate change, enhancing SOM in agricultural soils can promote crop resilience to drought by improving water retention (Kallenbach et al., 2019; Tisdall & Oades, 1982) and nutrient availability (Chen et al., 2014; Wild et al., 2019). However, for SOM to improve soil function, it must be readily decomposed by microbial activity (Janzen, 2006). In agroecosystems, microbial activity can benefit C sequestration, nutrient cycling, and overall soil health (Banerjee & van der Heijden, 2022; Larkin, 2015). Plant residues provide energy for soil microorganisms, stimulating carbon (C) and nutrient cycling through a priming effect (Gunina & Kuzyakov, 2022). Although farming practices using organic materials as energy sources for microorganisms may temporarily reduce nitrogen (N) availability for plants, they ultimately increase soil organic N stocks (Coonan et al., 2020).

In agricultural systems, organic N in soil is not considered an important nutrient source for crops. This aligns with the widely held view that plants primarily rely on inorganic N and compete poorly with soil microbiota for N (Schimel & Bennet, 2004). However, in conditions of low or moderate nutrient availability, soil microbiota can mobilize retained nutrients in SOM (Clarholm et al., 2015; Daly et al., 2021), especially in N-poor environments (Bonner et al., 2021). On the other hand, low N availability can enhance biological N fixation in legume nodules (Romanyà & Casals, 2019). Plant-associated nitrogen fixation can also occur in the rhizosphere and/or endophytically in non-legume crops (Kennedy et al., 2004; Yoneyama et al., 2017; Yoneyama & Suzuki, 2019); both processes involve a large number of bacteria, but little is known about how they are affected by N availability. A well-known non-legume N-fixing crop is sweet potato (*Ipomoea batatas* LAM.) (Terakado-Tonooka et al., 2008, 2013), which is suitable for horticultural rotations. Due to its high vigor, sweet potato can mobilize large amounts of nutrients from the soil and significantly increase N input through biological nitrogen fixation. Indeed, sweet potato is considered a low nutrient-demanding crop in the Mediterranean region and is notably resilient to drought (Agili et al., 2012; Sapakhova et al., 2023). The use of N-fixing crops after the application of high-energy sources such as woody residues could be a strategy to counteract N starvation caused by increased microbial growth and activity.

Ramial chipped wood (RCW), a pruning by-product, is reported to be suitable for soil conservation under field conditions (Gholami et al., 2016), including in horticultural practices that benefit soil aggregation (Tahboub et al., 2008). Its application also contributes to the circular economy by repurposing woody waste (Hague et al., 2023; Lemieux, 1992). Nonetheless, increased microbial activity after the addition of these residues may transiently reduce nutrient availability for plants (Barthès et al., 2010). Even though the agricultural application of RCW was developed several decades ago to recycle woody residues, little research has been done on its effect on soil organic C dynamics (Lemieux, 1992; Lemieux G & Germain D, 2001). Most scientifically validated studies in this field have been performed in Canada, focusing on the burial of RCW in coarse-textured soils, and some have been carried out in tropical areas (Barthès et al., 2010). While typical application rates of 1.2 and 15 kg/m^2 improve soil quality, higher doses pose logistical challenges, including availability and the costs of chipping and transportation (Barthès et al., 2015). In temperate climate zones, such as the Mediterranean region, there is still a knowledge gap regarding the effects of RCW on agricultural systems. Although plant residues with a high C:N ratio are expected to decompose slowly (Berg, 2000, 2012), the incorporation of RCW can increase soil respiration due to favorable environmental conditions for microbial activity due to high carbohydrate contents. At the same time, this increased microbial activity promotes the retention of both organic and mineral N (Becker & Ladha, 1997; Homyak et al., 2008). This newly retained N may subsequently be remobilized by priming processes triggered by RCW-derived carbohydrates, potentially contributing to crop nutrition.

We hypothesized that adding large amounts of RCW would rapidly increase soil stocks of organic C and N. By enhancing microbial activity and microbial demand for N, this practice would increase organic N mobilization and plant-associated biological N fixation. These processes could benefit the yield of N-fixing and non-fixing crops and potentially stabilize a significant proportion of SOM. The aim of this study was to monitor soil stocks of organic C and N, crop yields, and crop N use in soils recently enriched with RCW, comparing their agronomic performance with organically managed regularly tilled soils fertilized with either organic granulate or plant residue compost.

2. MATERIAL AND METHODS

2.1. Experimental design

The study was carried out at Masia Cal Notari, an organic horticultural farm in the peri-urban area of Barcelona (Sant Boi de Llobregat, Barcelona, Spain, 41°19’4.8’’ N, 2°3’3.6’’ E, 2 m.a.s.l.). The soil is a silty clay loam (Sand:Clay (5.5:28.5)) with 36.17% of calcium carbonate and a pH of 8.6. The location has a typically Mediterranean climate. Average temperatures during the study were as follows: 17.0 ± 0.4ºC in May 2021, when the experiment began, 24.0 ± 2.5ºC in summer, 17.2 ± 1.8ºC in autumn, 9.6 ± 1.0ºC in the winter months, 15.2 ± 1.6ºC in spring 2022, and 25.7 ± 2.7ºC in summer. Accumulated precipitation for these periods was 32, 23, 179, 21, 138 and 92 mm, respectively. The meteorological data were obtained from the Servei Meteorològic de Catalunya (https://www.meteo.cat/).

In May 2021, four organic management systems were established. The first system was an unamended control (Control), whereas the second system (Compost) involved the application of 1.28 kg/m^2 of compost derived from woody plant residues with 2.2% N and a C:N ratio of 13. Both systems involved soil tillage. Subsequently, two other organic management systems were established using RCW with a C:N ratio of 40.5, applied in two doses: a low dose of 7.5 kg/m^2 (RCW-LD) and a high dose of 15 kg/m^2 (RCW-HD), following the recommendations of (Lemieux and Germain 2001). The RCW-LD was selected given the potential N shortages after the massive application of RCW (Barthès et al., 2010). In these two organic management systems, tillage was discontinued after the incorporation of RCW. The RCW material consisted of C-rich peri-urban pruning residues from the municipality of Sant Boi de Llobregat (Fig. S1). The experimental layout comprised 16 plots (1.5 × 7.5 m each), spaced 1 m apart, and located along two 2 m-wide pathways. Management systems were interspersed so that in each pathway there were two replicates of each system. Thus, the plots were divided into four blocks, each with one replicate of the four management systems. At the start of the experiment, all plots were tilled twice with a rotary tiller to incorporate the organic amendments into the top 20 cm of soil. The soil surface was then covered with a plastic geotextile, and a drip irrigation system was installed.

The cultivated crops were sweet potato (*Ipomoea batatas* cv Beauregard) as the starter crop for soil regeneration, followed by a combination of spinach (*Spinacia oleracea* L.) and faba bean (*Vicia faba* L.), and lastly tomato (*Solanum lycopersicum* L.). Sweet potatoes were grown from May to October 2021; four sweet potatoes were planted per square meter, which resulted in 45 sweet potato plants per plot. Two rows of spinach and one of faba bean were cultivated from October 2021 to April 2022 and then tomatoes from May 2022 to August 2022 (Fig. S1). In May 2022, prior to planting the tomatoes, 210 kg of an N-rich commercial organic fertilizer (C:N of 4) was applied to the Control plots and 120 kg of N-rich fertilizer was added in the RCW management systems to ensure nutrient availability for tomato production. In the Compost system, an additional dose of compost was incorporated at this stage. At the same time, the Control and Compost plots were tilled once with the rotary cultivator according to local organic farming practices. Tomatoes were harvested from July to August 2022. See Table 1 for the fertilization schedule and the C and N inputs associated with each amendment. The RCW used in the experiment had a density of 181.8 kg / m^3. The amount of air-dried RCW incorporated into the soils was measured by volume. From this, and the C and N content in air-dried RCW, the C and N inputs were calculated.

Table 1. Fertilization and crop rotation calendar. The amount of amendment used for each organic management system along with the corresponding C and N inputs are shown.

Date	Crop	Intervention	Amount of amendment	C and N inputs	Details
May 2021 (0 months)	--	Application and incorporation of compost and C-rich residues	Compost: 1.28 kg/m^2 (C/N 13); RCW-LD (C/N 40.5): 7.5 kg/m^2; RCW-HD (C/N 40.5): 15 kg/m^2	Compost: 0.39 kg/m^2 of C and 0.03 kg/m^2 of N; RCW-LD: 3.6 kg/m^2 of C and 0.09 kg/m^2 of N; RCW-HD: 7.1 kg/m^2 of C and 0.18 kg/m^2 of N	Incorporation with rotary tilling at a depth of 20 cm.
May 2021	Sweet potato	Planting	--	--	Nodal explants of cv Beauregard.
October 2021	Sweet potato	Final harvest	--	--	--
October 2021	Spinach and faba bean	Planting	--	--	--
April 2022	Spinach and faba bean	Harvest	--	--	--
May 2022	--	Application of compost, N-rich fertilizer, and C-rich residues	N-rich fertilizer (C/N 4): 0.021 kg/m2 in Control; Compost: 1.28 kg/m^2 (C/N 11); 0.012 kg/m^2 in RCW-LD and RCW-HD	Control: 0.084 kg/m^2 of C and 0.021 kg/m^2; Compost: 0.33 kg/m^2 of C and 0.03 kg/m^2 of N; RCW-LD: 0.048 kg/m^2 of C and 0.012 kg/m^2 of N; RCW-HD: 0.048 kg/m^2 of C and 0.012 kg/m^2 of N	In the RCW management systems, the material was applied as a mulch, whereas the N-rich fertilizer and compost were incorporated with rotary tilling.
May 2022	Tomato	Planting	--	--	--
August 2022	Tomato	Final harvest	--	--	--

2.2. Soil sampling and analysis

2.2.1. Carbon and nitrogen balances

In May 2021 (i.e., 0 months), before applying the management systems, two soil samples were collected from each plot using a volumetric soil sampling probe (5 × 5 cm section). Samples were taken at depths of 0 to 20 and 20 to 25 cm and bulked to produce one sample per plot per depth. Subsequent samples were taken at the end of July 2021 (i.e., at 2 months, when sweet potatoes were flowering) and in July 2022 (i.e., at 14 months, before the tomato harvest).

The bulk density of the soil was calculated by dividing sample weight by probe volume. The bulk density of fine earth (< 2 mm) was determined by separating it from larger elements, including organic debris, gravel, and stones. To evaluate the C balance, organic debris (> 2 mm) was included – this fraction contained the remaining woody plant residues, hereafter referred to as organic debris. The weight of organic debris per volume of soil was also calculated at this stage. Fine earth and organic debris were included in all soil analyses to assess the incorporation of the amendments into the soil.

The bulk density of soil was calculated by dividing the weight of fine earth (g) by its volume (cm^3). Fine earth was obtained by subtracting the volume of gravel and stones (assumed density of 2.65 g/cm^3) from the total volume and the volume of the fine woody residues used (assumed density of 0.66 g/cm^3).

2.2.2. Equivalent-mass criterion

Due to soil decompaction in the RCW management systems, the amount of fine earth sampled in the second sampling was significantly lower. To determine the C and N stocks of each plot, this difference was corrected using the equivalent-mass criterion proposed by Gifford and Roderick (2003) and Rovira et al (2015). Results were normalized by the amount of mineral matter (MM) in the initial fine earth and adding the amount of differential MM in the final samples. To calculate the MM of each sample, we subtracted the weight of organic matter from the fine-grained soil (weight of fine-grained soil – (1.724*%C of fine-grained soil). Additional MM was assigned the C, N, and woody plant residue content >2 mm of the 20-25 cm samples.

The following equations were used to calculate C and N stocks in fine earth and organic debris contained in the equivalent mass of soil. This allowed us to quantify the changes in C and N stocks over time.

(1)

$$\text{C and N stocks (kg/ha)} = \left(\left(\frac{\text{g C or N}}{\text{100 g dry soil}}\right) \times \text{BDfe} \times \text{(1-vGi)} \times \text{20 cm}\right) + \left(\Delta\text{MM} \times \frac{\%C \text{ or } \%\text{N}_{\text{fine earth 20-25 cm}}}{\text{100-(Cx1.724)}_{20-25}}\right) \times 10000$$

where:

BD_{fe} is the bulk density of fine earth (g/cm^3)

vGi is the fraction of the volumetric content of coarse fragments (mineral+organic) in the 0-20 cm layer.

$MM_{0\text{-}20}$ mineral matter at 0-20 cm depth (gMM/cm^2 = g $soil/cm^2$ – g organic C/cm^2 × 1.724)

$\Delta MM_{0\text{-}20\ cm}$ is differential mineral matter between the initial soil (base line) and sample.

(2)

$$\text{C and N stocks } (\text{kg}/ha) = \left(\left(\frac{\text{g C or N}}{\text{g organic debris}} \times \frac{\text{g of organic debris}}{\text{cm}^3 \text{ of soil}}\right) + \left(\Delta\text{MM} \times \frac{\text{g C or N in organic debris}}{\text{g MM}_{20-25}}\right)\right) \times 10000$$

where:

ΔMM is differential mineral matter between the base line and sample.

To calculate C and N balances, the initial soils were used as a baseline. For the initial soils differential MM was zero.

2.2.3 Soil organic carbon and total nitrogen analyses

Organic C in the fine earth fraction was determined colorimetrically using the dichromate oxidation method (Walkley & Black, 1934) with some modifications (Heanes, 1984). Briefly, 50–80 mg of air-dried soil was mixed with 2 mL of 1/6 M potassium dichromate and 3 mL of concentrated sulfuric acid, followed by digestion at 155°C for 30 minutes. After digestion, 5 mL of distilled water was added, and the samples were allowed to cool before centrifugation. The supernatants were transferred to clean tubes, vortexed, and the absorbance was measured at 600 nm. The concentration of organic C was quantified by the spectrophotometric procedure using a glucose standard curve.

Total N and C concentrations in the organic debris fraction and organic amendments, as well as leaf N content, were determined using an elemental analyzer coupled to an isotope ratio mass spectrometer (CF IRMS, Flash 2000 HT, Thermo Fisher Scientific Bremen, Germany).

2.3. Crop sampling, agronomic production, leaf N content, and biological N fixation

Sweet potatoes were harvested twice, at the end of September 2021 and in early October 2021. In each harvest, a 2 × 2 m area was sampled, discarding shoot biomass, and harvesting tuberous roots with a pitchfork. Fresh and dry weights of shoot biomass were determined. Spinach and faba bean were harvested in March 2022 and April 2022, respectively. For both crops, two 2 × 0.5 m areas were sampled in each plot. Sample weight was recorded, and all remaining biomass was returned to the plots except for the faba bean biomass, which was commercialized. Tomatoes were harvested from July 2022 to the end of August 2022. Tomatoes were sampled twice per week, and their weights were recorded. Tomatoes were commercialized and the shoot biomass was returned to the plots. In each harvest, the crops were classified as commercial or non-commercial.

Biological N fixation (BNF) from atmospheric N_2 (% $N_{\text{derived from the atmosphere}}$) was determined in sweet potato and faba bean based on the $\delta^{15}N$ natural abundance method (Boddey et al., 2001; Unkovich et al., 2008).

$$\%N_{dfa} = 100\ (\delta 15Nref - \delta 15Nfixing\ plant)/(\delta 15Nref - B)$$

A mixture of weeds (*Cyperus rotundus* L., *Amaranthus retroflexus* L., *Beta vulgaris* L., *Sonchus tenerrimus* L., *Sonchus oleraceus* L., *Portulaca oleracea* L., *Abutilion theophrasti* MEDIK.) was used as a reference for sweet potato (collected in the same plots) using 0 as the b value as suggested by Boddey et al. (2001). Spinach was used as a reference for faba bean, for which a b value of -0.36 ‰ was used.

2.4. RGB vegetation indices

RGB (red, green, and blue) vegetation indices were calculated for each plot from conventional digital images using free-access BreedPix software (Casadesús et al. 2007) based on the methodology of Vergara-Díaz et al. (2016). The green area (GA), green green area (GGA), and crop senescence index (CSI) were assessed. Four pictures per plot were taken using a Canon EOS M5 camera (55 mm lens, 18 mm 1:3 5-6.3 IS STM) at *ca*. 50 cm above the plant canopy.

2.5. Statistical analyses

Statistical analyses and graphs were produced using R (version 4.3.0) and R Studio (version 2023.6.0.421). Linear mixed models were used to assess organic management system effects on the response variables after 2 months and 14 months for the soil analyses. The *lme* function from the *nlme* package was used (Pinheiro & Bates, 2023), with 'Organic management system' fitted as a fixed factor and 'Block' as a random factor to account for any spatial variability. Maximum likelihood was used to fit the models during model selection and the model with the lowest Akaike information criterion was selected. Restricted maximum likelihood was used to fit the selected models. Effects of the organic management systems on crop production, biological N fixation parameters, and vegetation indices were analyzed by linear mixed models as described above.

For all models, homoscedasticity and normality of residuals were checked visually (Zuur et al., 2010) and if necessary, the response variables were transformed to meet these criteria. The *varIdent* variance structure (*nlme* package) was used to account for variances in 'Organic management system' and was fitted in the model with the argument 'weights' if residual homoscedasticity could not be met by data transformations. The *p*-values for the effect of the response variables were obtained using the *anova* function. Significant effects were further analyzed by conducting post-hoc tests using estimated marginal means with the function *emmeans* using the Tukey adjustment (Lenth, 2023). All *p*-values were considered statistically significant at $p \leq 0.05$. Differences between times within the organic management system were tested using a Welch Two Sample t-test with the function *t.test*.

To further analyze the data of fine earth and organic debris, a factor analysis of mixed data was performed using the packages *FactoMineR* (Lê et al., 2008) and *factoextra* (Kassambara and Mundt 2020). Graphs with error bars were generated using *ggplot2* and *Rmisc* (Hope, 2022). Correlation matrices were produced using the custom function *corstars* (Vallet, 2015). Spearman's correlations were performed with scaled data.

3. RESULTS

3.1. Bulk density

Two months after the addition of RCW, soil bulk density was lower compared to the Compost and Control organic management systems (Table 2), particularly in RCW-HD plots. A year later, however (July 2022), the bulk density in the RCW-HD system had slightly increased, although no significant differences among management systems or between sampling times were found (linear mixed effects model, Time: $F_{df=15}= 2.67$, $p >$ 0.05).

Table 2. Soil bulk density after 2 and 14 months in the different organic management systems. Different letters indicate statistically significant differences (if present) between groups at $p \leq 0.05$. HD, high dose; LD, low dose; RCW, ramial chipped wood.

Sampling	**Organic management system**	Bulk density (g/cm^3)
2 months	Control	1.25 ± 0.06 a
	Compost	1.28 ± 0.02 a
	RCW-LD	1.14 ± 0.04 ab
	RCW-HD	1.07 ± 0.06 b
14 months	Control	1.28 ± 0.03
	Compost	1.25 ± 0.02
	RCW-LD	1.23 ± 0.03
	RCW-HD	1.17 ± 0.02

3.2. Soil C and N content and stocks

At two months, soil organic carbon (SOC) content in the fine earth fraction had increased only for the high dose of RCW, while the Compost system showed the lowest levels (Fig. 1, % C). One year later, RCW-HD plots continued to maintain higher SOC contents than Compost and RCW-LD. In the organic debris fraction, both RCW systems showed higher SOC levels than Compost after 2 months (Fig. 1), with RCW-HD differing significantly from the Control. However, after one year, only RCW-HD maintained higher SOC levels in organic debris compared to the Control (Fig. 1).

Regarding N content in the fine earth fraction, two months after amendment application, RCW-HD showed higher values than Compost (Fig. 1). After one year, N levels had increased in both Compost and RCW-HD, being significantly higher in Compost compared to the Control and RCW-LD. In the organic debris fraction, no differences in N content were observed among management systems at any time point. After two months, the Compost and Control management systems showed higher variability in N content compared to the RCW systems, which had lower N levels than the original fresh RCW material.

The application of RCW increased the fine earth C:N ratio in the short term, but this effect was no longer evident after 14 months (Fig. 1). Compared to the organic debris from previous crops, the C:N ratio decreased in all management systems, with Compost showing the most pronounced reduction. Notably, in RCW systems, the C:N ratio of the organic debris fraction showed a transient increase during the first two months, followed by a return to near-baseline values (C:N of 40.5) the following year.

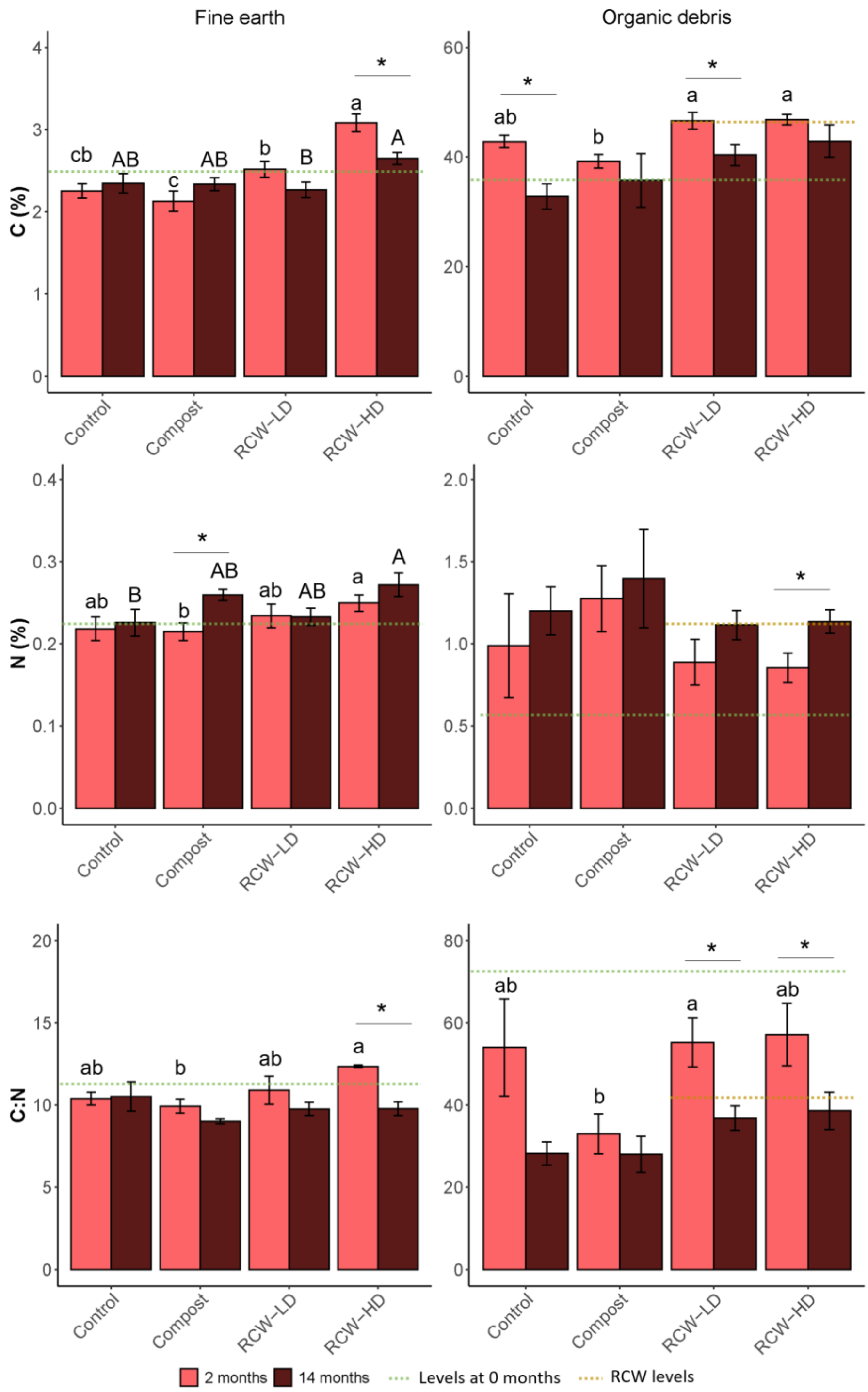

Figure 1. Concentrations of C (%) and N (%), and the C:N ratio in fine earth and organic debris fractions in the different organic management systems at 2 and 14 months. Bars correspond to the mean ± SE of four replicates of soil samples. The green dotted line represents baseline levels (0 months), while the yellow dotted line indicates levels in RCW systems. Lower case letters (a, b, c) denote any significant differences between management systems at 2 months; upper case letters (A, B, C) indicate any significant differences at 14 months. Asterisks indicate differences between sampling times within an organic management system. All p values were considered significant at $p \leq 0.05$). HD, high dose; LD, low dose; RCW, ramial chipped wood.

The addition of RCW increased SOC stocks (Fig. 3), with the greatest increase observed in the RCW-HD system, as expected. It is noteworthy that at two months, most of the added C in the RCW systems was retained in the organic debris fraction, while most of the N had already been incorporated into the fine earth. Over the first year, substantial C losses were observed in both RCW systems due to decomposition, with only RCW-HD showing a net increase in C stocks. In contrast, N stocks remained stable across all systems. After one year, N increased significantly in both RCW and Compost systems, the latter having received two amendments by this time. In the RCW systems, N in the organic debris decreased significantly during the first year: by 460.7 kg N/ha in RCW-HD and 247.7 kg N/ha in RCW-LD. In RCW-HD, this reduction closely matched the concurrent N increase in the fine earth fraction. After one year, the N content in organic debris in the RCW-HD system (145.7 kg N/ha) was still significantly higher than at baseline.

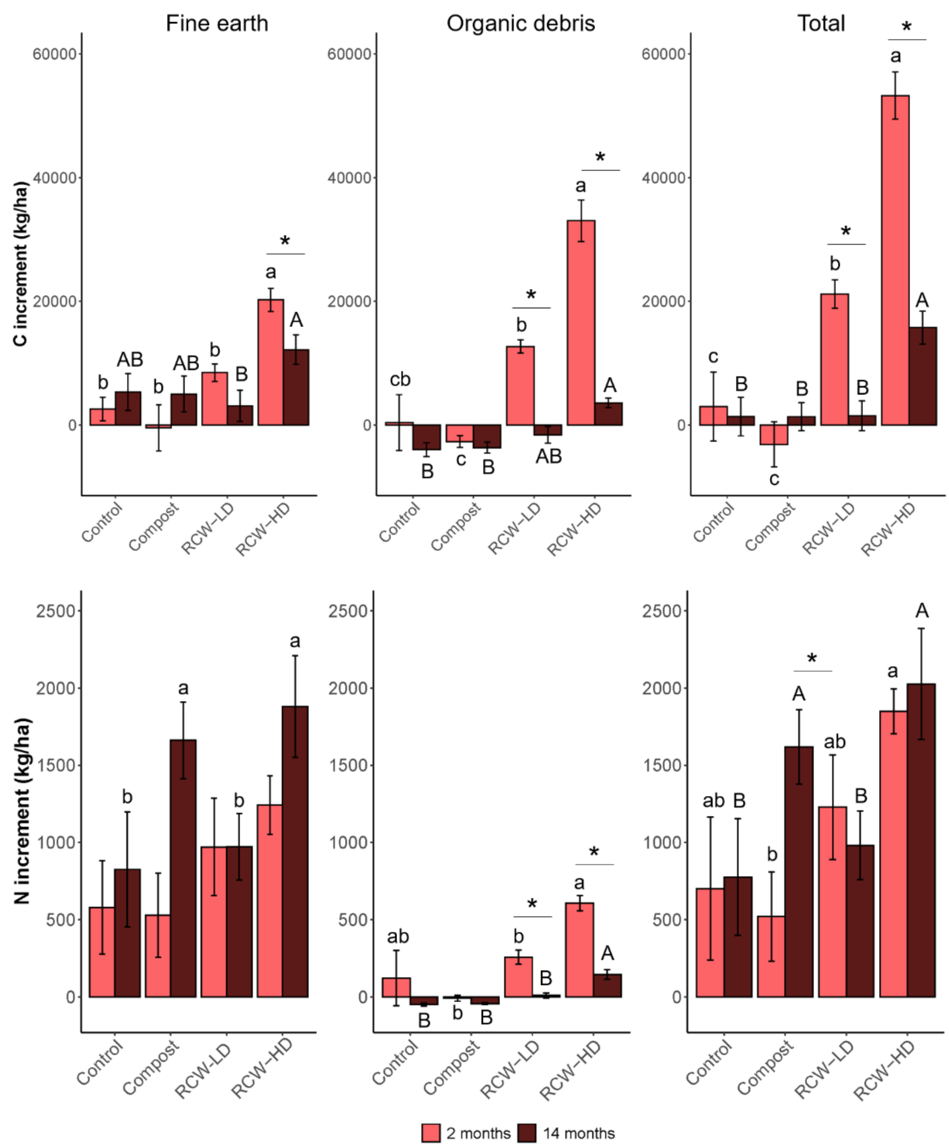

Figure 2. Changes in C and N in fine earth and organic debris fractions in the different organic management systems at 2 and 14 months. Increments (kg/ha) at 2 months are the difference between values from July

2021 and baseline values from May 2021, while increments at 14 months are the difference between values from July 2022 and May 2021. Bars correspond to the mean ± SE of four replicates of soil samples. Lower case letters (a, b, c) denote any significant differences between management systems at 2 months; upper case letters (A, B, C) indicate any significant differences at 14 months. Asterisks indicate differences between sampling times within an organic management system. All *p* values were considered significant at $p \leq 0.05$. HD, high dose; LD, low dose; RCW, ramial chipped wood.

3.4. Overall soil changes

Two-factor analyses of mixed data were computed with the soil data of 2021 and 2022 for fine earth and organic debris to further explore relationships and underlying patterns in the dataset, incorporating both categorical and continuous variables (Fig. 4A). In fine earth, dimension 1 accounted for 49.3% of the variance, while dimension 2 explained 26%. Using the *fviz_contrib* function, we observed that C-related variables (*e.g.*, increment of C mass per area, %C, and kg of C per area) contributed most to dimension 1. In contrast, the C:N ratio and increment of N mass per area were the primary contributors to dimension 2 (Fig. 4B).

The ellipses represent the estimated confidence interval with a probability of 95% for the dispersal of observations around group centroids. As shown by the separation along dimension 1, the management system with the higher dose of RCW stood out from the other groups, being positioned on the positive side of dimension 1, which indicates higher C-related variable values (Fig. 4B). Compost was on the negative side of dimension 2, indicating reduced C:N values and an increased kg N/ha.

In the organic debris, dimension 1 accounted for 50.7% of the variance, whereas dimension 2 explained 18% (Fig. 4A). The primary contributors to dimension 1 were kg of C and N per area and the increments of C and N per area, whereas N concentration and the C:N ratio contributed most to dimension 2 (Fig. 4B). RCW-HD was distinctly separated from the other organic management systems, located on the positive side of both dimensions, indicating higher C and N contents. Compost was positioned on the positive side of dimension 2 and the negative side of dimension 1, reflecting positive values for the N concentration and C:N ratio but negative values for the kg of C and N per area and C and N increments per area. RCW-LD fell on the negative side of dimension 2 and around 0 of dimension 1, indicating negative values for the N concentration and C:N ratio and either positive or negative values for the kg of C and N per area and C and N increments per area (Fig. 4B).

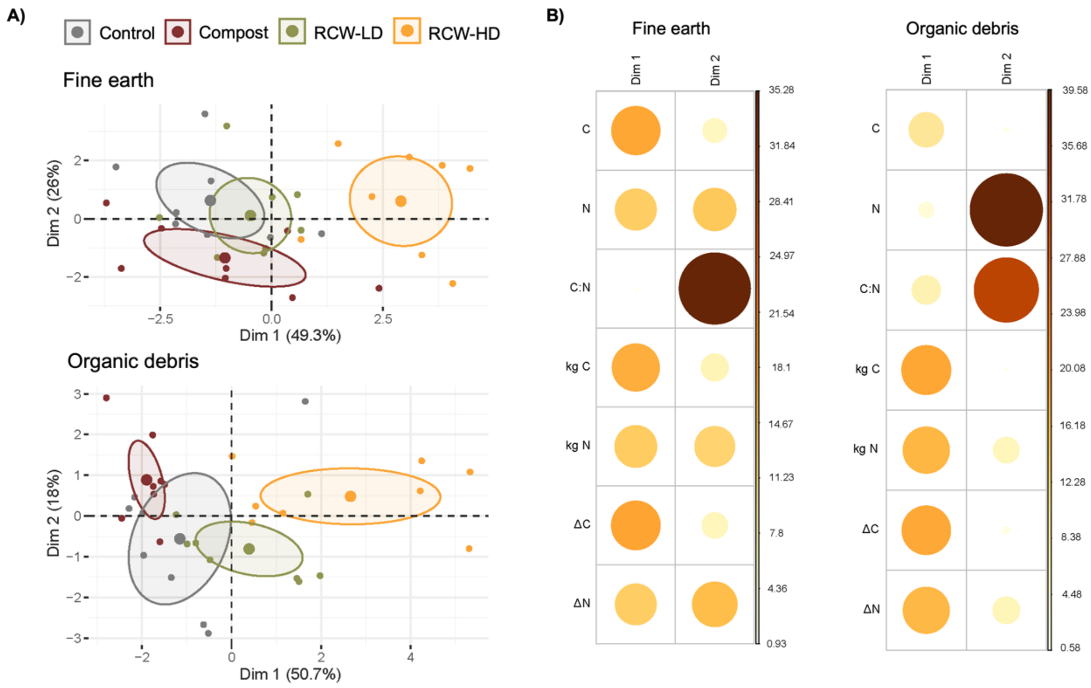

Figure 4. Results of Factor Analysis of Mixed Data (FAMD) for fine earth and organic debris fractions of soil and variable contributions to the first two dimensions. A) Scatter plot showing the distribution of observations based on Dimensions 1 and 2. Each point represents an observation, with colors indicating the corresponding organic management system. Ellipses represent 95% confidence intervals, illustrating the dispersion within each group. B) Correlation plots showing variable contributions to Dimensions 1 and 2 in the FAMD of fine earth and organic debris. Percentage contributions are displayed within the cells, with darker colors and larger circles indicating higher variable contributions to each dimension. Δ, increment; Dim 1, dimension 1; Dim 2, dimension 2; HD, high dose; LD, low dose; RCW, ramial chipped wood.

3.5. Crop production, N requirements, and RGB indices

Agronomic production varied significantly among organic management systems for spinach, but not for tomato (Table 3). Spinach yield averaged 3.9 t/ha and was highest in RCW-HD, where it reached a notable 5.1 t/ha. The mean production of tomato was 16.4 t/ha, with no significant differences among systems. The mean yield of sweet potato over two harvests was 52 t/ha, being lowest in the Control (42 t/ha), although the difference was not statistically significant. The average plant biomass of faba bean was 14.7 t/ha, with 8.0 t/ha of harvested beans.

Table 3. Agronomic production of the crops cultivated during the experiment in the different organic management systems. Values correspond to the mean ± SE of four replicates. Different letters indicate any statistically significant differences between groups at $p \leq 0.05$. HD, high dose; LD, low dose; RCW, ramial chipped wood.

Organic management system	**Production (t/ha)** Sweet potato	Spinach	Faba bean	Tomato
Control	42.75 ± 2.07	2.88 ± 0.60 b	6.57 ± 1.10	79.47 ± 12.24
Compost	46.08 ± 1.30	3.44 ± 0.85 ab	7.18 ± 2.37	60.33 ± 5.20
RCW-LD	41.70 ± 3.47	3.71 ± 0.41 b	7.60 ± 2.19	53.70 ± 2.50
RCW-HD	51.66 ± 3.86	5.09 ± 0.73 a	10.45 ± 2.26	69.40 ± 5.23

Leaf N content ranged from 2 to 6% (Table S2) with no statistically significant differences among management systems in any of the crop species. On average, sweet potato had the lowest leaf N concentration at 2.38%, whereas faba bean had the highest at 5.21%, possibly due to its ability to fix N from the atmosphere (Klippenstein et al., 2022). Indeed, we found a correlation between leaf N and the kg of N derived from the atmosphere in faba bean (Table S5).

Canopy RGB indices (GA and GGA) at the end of the harvest season varied among management systems for each crop (Table 4). In sweet potato, GA and GGA ranged from 0.70 to 0.98, with RCW-HD showing the highest values: its GA was higher compared to Compost and GGA higher compared to the Control and Compost, indicating that RCW-HD induced a bigger and greener canopy. In spinach, GA and GGA were higher in Compost compared to RCW-LD, which had a smaller canopy and more yellow leaves. For faba bean, GA and GGA ranged from 0.79 to 0.97, both indices being higher in RCW-HD and RCW-LD versus the Control, with statistically significant differences. In tomato, the lowest GA and GGA values were observed in RCW-LD, whereas Compost showed the highest GA, with statistically significant differences from RCW-LD, and RCW-HD had the highest GGA.

CSI, an indicator of senescence, varied among management systems in each crop except for faba bean (Table 4). CSI values ranged from 13 to 25 in sweet potato, 36 to 50 in spinach, and 30 to 35 in tomato. The high dose of RCW reduced CSI compared to the other management systems, although statistically significant differences were not observed between RCW-HD and RCW-LD in sweet potato or between RCW-HD and the Control in spinach (Table 4).

Table 4. RGB-image-based vegetation indexes for the crops cultivated during the experiment in the different organic management systems. Values correspond to the mean ± SE of four replicates. Different letters indicate any statistically significant differences between groups at $p \leq 0.05$. CSI, crop senescence index; HD, high dose; GA, green area; GGA, green green area; LD, low dose; RCW, ramial chipped wood.

Organic management system	Sweet potato			Spinach		
	GA	GGA	CSI	GA	GGA	CSI
Control	0.95 ± 0.02 ab	0.75 ± 0.10 b	21.91 ± 8.46 a	0.72 ± 0.17 ab	0.45 ± 0.23 ab	39.82 ± 15.99 ab
Compost	0.94 ± 0.47 b	0.70 ± 0.12 b	25.44 ± 9.22 a	0.80 ± 0.10 a	0.51 ± 0.14 a	36.84 ± 10.81 b
RCW-LD	0.97 ± 0.02 ab	0.79 ± 0.05 ab	18.39 ± 4.10 ab	0.63 ± 0.08 b	0.32 ± 0.07 b	50.03 ± 5.82 a
RCW-HD	0.98 ± 0.01 a	0.85 ± 0.04 a	13.28 ± 3.88 b	0.66 ± 0.05 b	0.39 ± 0.06 ab	41.08 ± 6.77 b

Organic management system	Faba bean			Tomato		
	GA	GGA	CSI	GA	GGA	CSI
Control	0.92 ± 0.05 b	0.79 ± 0.12 b	13.94 ± 8.15	0.92 ± 0.05 ab	0.61 ± 0.07 ab	33.29 ± 5.40 a
Compost	0.94 ± 0.06 ab	0.84 ± 0.12 ab	10.82 ± 8.24	0.90 ± 0.08 a	0.59 ± 0.75 ab	34.67 ± 6.35 a
RCW-LD	0.97 ± 0.02 a	0.91 ± 0.03 a	6.42 ± 1.40	0.88 ± 0.06 b	0.58 ± 0.05 b	34.08 ± 5.15 a
RCW-HD	0.97 ± 0.02 a	0.92 ± 0.05 a	5.99 ± 2.98	0.90 ± 0.06 ab	0.63 ± 0.05 a	30.02 ± 6.48 b

3.6. Biological N fixation

Both sweet potato and faba bean fixed atmospheric N in their aerial tissues (Table 5). Sweet potato averaged 12.5% of N derived from the atmosphere, fixing 10.5 kg of N per hectare, while faba bean had 97% of N_{dfa}, fixing an average of 157 kg of N per hectare (considering both plant biomass and beans). The faba bean Control resulted in higher N_{dfa} compared to RCW-amended soils (regardless of dose), whereas N_{dfa} in sweet potatoes grown with a high RCW dose was higher compared to the Control (Table 5).

Table 5. Biological N fixation in sweet potato and faba bean expressed as the concentration of N derived from the atmosphere (%N_{dfa}) and kg of N_{dfa} per hectare. Values correspond to the mean ± SE of four replicates. Different letters indicate any statistically significant differences between groups at $p \leq 0.05$. dfa, derived from the atmosphere; HD, high dose; LD, low dose; RCW, ramial chipped wood.

Organic management system	Sweet potato		Faba bean		
	%N_{dfa}	kg N_{dfa}/ha	%N_{dfa}	kg N_{dfa}/ha plant biomass	kg N_{dfa}/ha beans
Control	8.92 ± 1.66 b	7.47 ± 1.86	98.22 ± 0.34 a	169.09 ± 30.54	91.12 ± 16.27
Compost	12.51 ± 0.91 ab	11.88 ± 2.23	97.26 ± 0.46 ab	189.17 ± 63.13	103.69 ± 34.93
RCW-LD	13.47 ± 3.07 ab	9.49 ± 2.27	96.00 ± 0.71 b	194.21 ± 55.52	102.96 ± 29.01
RCW-HD	14.91 ± 1.21 a	12.97 ± 1.75	96.38 ± 0.46 b	261.22 ± 60.52	143.45 ± 30.85

4. DISCUSSION

The high-dose application of RCW was the only organic management system that increased both soil C and N pools in both fine earth and organic debris fractions. In contrast, compost amendments had a positive effect only on N pools. At 14 months, C recovery in the RCW-HD system was much lower than N recovery, indicating increased respiration during this period and N enrichment in SOM. Two months after RCW application, most of the added N was recovered in the fine earth fraction, whereas most of the added C remained in the organic debris. Shortly after the incorporation of RCW, we also observed improved crop growth, enhanced plant-associated N fixation, and no signs of N limitation in spinach, a high N-demanding crop. After one year, most of the N recovered in the organic debris had transferred to the fine earth fraction, with no apparent N losses during this period. This suggests rapid N mobilization from RCW, contrasting with the conventional view that N retained in SOM contributes minimally to crop nutrition (Schimel & Bennett, 2004).

4.1. Effects of RCW on soil characteristics

RCW incorporation influenced soil physical properties by reducing bulk density, as reported in other studies (Barthès et al., 2010; Fontana et al., 2023). Given that bulk density is an indicator of soil compaction (Blake & Hartge, 1986), its reduction implies improved soil aeration, which can enhance root development and water infiltration (Dexter, 1988; Logsdon & Karlen, 2004). Compared to the initial values (see Table S1 and Fig. 1), RCW-HD reduced soil compaction by 17%, suggesting that RCW is an effective organic amendment for improving soil physical properties.

4.2. High-dose RCW application increases soil organic C and N reservoirs

Although most of the C added through RCW was respired, RCW application still led to an increase in SOC, an indicator of soil quality (Reeves 1997). While SOC had increased after two months with both RCW doses, only the high-dose RCW system maintained elevated SOC levels after one year (2022). A rapid increase in SOC following plant litter application is commonly observed in low-C soils (Han et al., 2016; Larney & Angers, 2012), particularly when incorporated at high doses (Arona & Samba, 2001). For example, a recent study on a Swiss cereal cropping system in Switzerland using lower doses of woodchips than in our experiment found a 10% SOC increase five years after a single application, while the control showed no change (Fontana et al., 2023). In our experiment, the high-dose RCW application increased SOC by 7.8% after one year, corresponding to a 22% retention of the added C, 17% of which was retained in the fine earth fraction. Fontana et al. (2023) reported slightly higher retention in fine earth after five years. Our lower retention may reflect more rapid mineralization due to warmer climatic conditions and intensive summer horticultural crop production. Our C retention rates are much higher than the general litter-derived C sequestration index reported in another study, which ranged from 0.4 to 5% per year (Gunina & Kuzyakov, 2022), likely due to the high lignin content in RCW.

The 0.74 kg C/m^2 supplied with compost did not lead to a measurable increase in SOC stocks. This aligns with findings in other studies, which report short-term increases in SOC only when compost is applied at significantly higher doses (Tambone et al., 2007). Additionally, tillage in the Compost system failed to enhance SOC, consistent with observations by Haddaway et al. (2017).

In the RCW management systems, we observed highly dynamic N cycling between organic debris and fine earth fractions. Notably, N was incorporated into the fine earth more rapidly than C. For both sampling periods, the low RCW dose resulted in the highest proportion of the added N being incorporated into the fine earth, suggesting that lower application rates accelerate N transfer to this soil fraction. Such rapid incorporation of N from high C/N ratio materials contrasts with previous findings in studies where surface-applied litter retained N in the decomposing material for at least two years (Montané et al., 2010). However, our results are consistent with other research using buried leaf litter or roots, which similarly found rapid N migration from litter bags into fine earth (Garcia-Pausas et al., 2012; Rovira & Ramón Vallejo, 2002). These studies also noted that N incorporation was faster in the subsoil, likely due to differing environmental conditions. In our experiment, the low-dose RCW application may have improved direct contact between organic residues and fine earth, thereby accelerating N incorporation into this soil fraction.

These outcomes indicate that RCW incorporated into the soil may accelerate N mobilization significantly more than mulching materials incubated aboveground. A meta-analysis found that the use of RCW increases cereal yields, suggesting that high inputs of organic C stimulate soil microbiota and enhance soil fertility (Félix et al. 2018). Nevertheless, a transient reduction in N availability may occur during the initial months after RCW incorporation (Barthès et al., 2010). During this period, complex organic molecules in the wood by-products are broken down into simpler forms by microbes such as saprotrophic fungi (Clocchiatti et al., 2020). These smaller C compounds are then transformed into more stable molecules (humic substances) that will eventually enrich the soil, improving its structure, water retention, and nutrient availability (Adams, 1973; Murphy, 2015; Saxton & Rawls, 2006).

4.3. The N reservoir increases in the short-term after RCW application and then stabilizes

Two months after RCW application, a substantial increase in N was observed, most of which was recovered in the fine earth fraction (Fig. 2). In contrast, the application of compost did not increase soil N at this stage. One year later, after the second compost application, N levels in both compost- and RCW-amended soils were higher compared to baseline values (Fig. 3). Although N exports in subsequent harvests were 270 kg N/ha, the increase in soil N was comparable to the amount of N added through RCW or compost, primarily retained in fine earth (see Fig. 2 and Table 1). This indicates a rapid incorporation of N into fine earth and a high N retention capacity of the studied soils, which is in line with our hypothesis. Organic N from materials with a high lignin:N ratio, such as RCW, is expected to be largely retained in the soil (Becker & Ladha, 1997). Our data show that N applied through compost with a much lower C:N ratio was also retained in the soil.

Changes in the N cycle following the addition of organic amendments are associated with shifts in the microbial community (González-Coria et al., unpublished). These shifts are driven by alterations in soil physicochemical conditions, particularly SOM stocks and composition (Siedt et al., 2021). In our study, high doses of RCW resulted in high C stocks that were sustained until the end of the experiment (14 months post-application). Although the C:N ratio at that point was similar to the Control, the presence of a significant amount of cellulose-rich organic debris may have promoted saprophytic fungal activity, thereby boosting long-term soil fertility (De Vries et al., 2011; Ning et al., 2021).

4.4. Agronomic performance is maintained with RCW

Overall, agronomic production was maintained following the addition of RCW, which is consistent with the results of Fontana et al. (2023). In our RCW management systems, no additional N was applied for the first two crops (sweet potato and faba bean/spinach), while a 57% dose of N relative to the Control was used for the third crop (tomato). Fontana et al. (2023) also incorporated supplementary fertilizer after a wood chip application. For spinach, the high RCW dose resulted in higher yields compared to RCW-LD and the Control. Spinach production correlated positively with soil C and N stocks (Table S4), demonstrating the beneficial effects of RCW on crop yield. While various C-rich amendments have been shown to promote spinach growth (Liu et al., 2022), we found that RCW application negatively affected spinach crop cover, greenness, and senescence. This was reflected in lower GA and GGA values, and a higher CSI compared to the Compost system (Table 4). Specifically, the lower RCW dose resulted in the highest senescence index, although this did not correlate with either spinach production or leaf N content (Table S4). As a leafy green, spinach has high N requirements, and potential N immobilization due to woody organic amendments could affect its growth (Bourdon et al., 2024). However, in our study, higher N immobilization due to RCW can be ruled out, as leaf N content was consistent across all organic management systems (Table S2) and the high RCW dose resulted in the highest yield.

Although not statistically significant, higher yields were also observed for sweet potato and faba bean with the RCW-HD amendment. The production of sweet potato has been shown to increase when the soil is covered with a woody mulch compared to bare soil (Richardson et al., 2023), whereas faba bean yields were unaffected by woodchip mulching (Xu et al., 2018). However, other studies have reported improved legume production when using wood-derived mulches compared to bare soil (e.g. (Gruda, 2008). Wood-chip mulching has also been found to positively affect tomato yield (Horimoto et al., 2022), although Soumare et al. (Soumare et al., 2002) observed reduced tomato growth in the first cropping season. In our study, the highest tomato yield was obtained with the application of an N-rich fertilizer, consistent with the high N requirements of this crop (Zotarelli et al., 2009). However, neither tomato production nor leaf N content in the Control system were statistically different from those of RCW-HD, suggesting that sufficient N was available with the high RCW dose. This is further supported by consistent crop cover and GGA values (Table 4 and Table S2). Tomatoes grown with RCW-HD showed a lower CSI, and we notably found a negative correlation between tomato CSI and kg of C in the soil (Table S6). Moreover, there was a positive correlation between soil C stocks and GGA (Table S6), suggesting that RCW can improve tomato plant growth conditions.

Altogether, these results alleviate concerns that growing crops such as sweet potato, spinach, faba bean, and tomato with high doses of RCW will lead to lower yields compared to soils amended with N-rich granulate fertilizer or compost. However, our findings suggest that low RCW doses may potentially limit the growth and/or yield of the spinach and tomato cultivars used in this study. Further research is needed to determine the optimal RCW dosage, nutrient richness, and application methods to maximize the benefits of RCW while minimizing any negative effects on crop productivity.

4.5. Addition of RCW increases N fixation in sweet potato and reduces it in faba bean

Sweet potato can fix N_2 with the help of diazotrophic endophytes (Terakado-Tonooka et al., 2013; Ueda & Yano, 2023), which allows it to thrive in low-nutrient soils. Our results showed that higher atmospheric N fixation was correlated with increased sweet potato production (Table S3). We also observed positive correlations between GA and GAA values and soil C stocks, and a negative correlation between soil C stocks and the CSI (Table S3). Additionally, a positive relationship was found between N_{dfa} and soil N stocks. Although the proportion of N derived from N_2 was highest in soils amended with RCW, the values were rather low (less than 20% N_{dfa}), suggesting that sweet potatoes mainly used soil-derived N. Unlike legumes, biological N fixation (BNF) of pot-grown sweet potato has been shown to increase with the addition of readily available N and other nutrients (Ueda & Yano, 2023). Thus, it is likely that the application of large amounts of

RCW contributed to soil nutrient mobilization. In fact, increases in leaf P content have been observed in sweet potato fertilized with RCW (Jaime-Rodríguez et al., 2025), in agreement with Fontana et al. (2023), who reported the remobilization of P, N, and Mg. The rapid incorporation of N into the fine earth fraction within two months of RCW application may have enhanced nutrition availability for sweet potato and increased its BNF capacity. These results indicate that high doses of RCW helped meet the N requirements of sweet potato, allowing for optimal overall crop performance in the short term.

Unlike sweet potato, legumes primarily rely on BNF to obtain N (Kebede, 2021; Vasconcelos et al., 2020). The amount of N fixed per hectare varies depending on the legume species, climatic conditions, soil characteristics, and soil management. Among legume crops, faba bean stands out for its high BNF capacity. Vasconcelos et al. (2020) reported BNF rates ranging from 118 to 300 kg of N/ha, which is consistent with the 203 kg of N/ha found in our study for aboveground faba bean biomass. Generally, the lower the N input in a cropping system, the higher the BNF in legumes (Preissel et al. 2015). However, in our experiment, RCW application reduced BNF in faba bean, leading to an increased use of soil-derived N. This could be an indicator of enhanced N availability in the RCW management systems, consistent with the higher amount of applied N (Table 1). Interestingly, the lower BNF in RCW systems did not negatively impact crop production or leaf N content (Table 3 and Table S2). In fact, RCW-amended plots showed improved crop cover and greenness compared to the Control (Table 4), suggesting a better crop performance after the addition of RCW.

In this work, we addressed a complex agronomic challenge through a focused case study involving a single soil type, a defined RCW source, a specific crop rotation, and consistent climatic conditions, including the season of RCW application. We assessed the combined effects of RCW application and no-till management within a single treatment over one year. While this approach captures short-term responses to RCW, it limits our ability to isolate RCW-specific effects. Nevertheless, despite these limitations, the study provides valuable insights. To better understand the synergies and trade-offs among these interacting factors, future research should investigate different RCW by-products, application timings, and crop combinations over an extended experimental timeframe.

5. CONCLUSIONS

The incorporation of high doses of RCW into a crop rotation starting with sweet potato, without subsequent tillage, enhanced soil organic matter. For the first time, we show that

RCW application rapidly increased N stocks in the fine earth fraction. N accumulation continued over the following year, especially at the high RCW dose, due to the N contribution from the added organic debris. Crop performance indicates that these N stocks improved crop nutrition, resulting in good yields. At the end of the experiment, only high RCW doses significantly increased SOC (by 22%), with 5% recovered as > 2 mm RCW (i.e., organic debris) and a C:N ratio very close to that of the original material. Conversely, low RCW doses or compost amendments resulted in negligible C retention.

We propose that combining high RCW doses with sweet potato as a starter crop is an effective strategy for rapid soil regeneration due to their synergistic effects. Immediately after RCW incorporation, sweet potato showed adequate leaf N levels and enhanced BNF. All rotation crops performed well, although spinach showed reduced canopy cover, especially at low RCW doses. The implementation of RCW amendments aligns with sustainable agricultural practices and supports the circular economy by using a locally available carbon- and nutrient-rich resource. Further research should elucidate the specific mechanisms underlying these effects and evaluate broader ecosystem and economic impacts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary data

The online version contains supplementary material available at xxx.

Data availability

Code and datasets are available from the corresponding author upon reasonable request.

Acknowledgements

We would like to thank the Scientific and Technological services of the University of Barcelona (CCiT-UB) for the CF IRMS analyses. We would also like to thank Masia Cal Notari for providing the experimental plot and labor, and ADV Horta del Baix Llobregat for their technical support throughout the experiment.

CRediT authorship contribution statement

Anna Vallverdú-Queralt: Validation, Funding acquisition. **Carolina Jaime-Rodríguez**: Investigation, Methodology, Data curation, Writing – review and editing. **Joan Romanyà**: Conceptualization, Investigation, Methodology, Writing – original draft, Supervision, Validation, Funding acquisition. **Johana González-Coria**: Investigation, Methodology, Data curation. **Maria Pérez**: Writing – review and editing, Validation, Funding acquisition. **Marina Pérez-Llorca**: Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Olivier Chantry**: Conceptualization, Investigation, Methodology. **Rocío Hernández**: Conceptualization, Investigation, Methodology. **Rosa Maria Lamuela-Raventós**: Writing – review and editing, Validation, Funding acquisition.

Funding sources

This research was funded by the Ministerio de Ciencia, Innovación y Universidades (AEI/FEDER, UE) with the projects (TED2021-130783B-C21 and PID2023-146650OB-I00); by the Instituto de Salud Carlos III, with the project CIBEROBN (CB12/03/30020); and, by the Generalitat de Catalunya + European Funds of Rural Development (56 30126 2021 P4). INSA-UB is María de Maeztu Unit of Excellence which is funded by MICIN/AEI/FEDER, UE (CEX2021-001234-M). R.L-R wants to thank ICREA Academia. C.J.-R thanks MinCiencias-Ministry of Science, Technology and Innovation of Colombia, call for PhD applications 885-2020. J.G-C. thanks the National Scholarship Program of Paraguay "Carlos Antonio López" (BECAL 183/2021).

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