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ORIGINAL PAPER

Enhancing Soil Carbon Sequestration with C-Rich Carrier Materials from Spent Mushroom Substrate and Composted Wheat Straw: Implications for Smart Fertilizer Design

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Abstract

This study aimed to evaluate the potential of two carbon (C)-rich carrier materials derived from agricultural residues, spent mushroom substrate (SMS) and composted wheat straw (CWS) for enhancing soil carbon stabilization in an Andisol, with a view towards their future application in smart fertilizer design. We investigated how their contrasting nitrogen contents and application rates affect soil organic carbon dynamics and contribute to sustainable soil management and climate change mitigation. Soil incubations were conducted over 365 days, during which C mineralization, enzymatic activities, and C and N sequestration were assessed. SMS addition at both low and high rates (0.5% and 1% soil C increase, respectively) enhanced soil organic carbon (SOC) stabilization, increasing mean residence times (40.6 and 48.8 years) and half-life times (28.1 and 33.8 years) of the stable C pool compared to unamended soil (35.9 and 24.9 years). High-rate CWS application (1% soil C increase) promoted native SOC decomposition, increasing C losses (5.8%) and reducing C sequestration potential (96%). However, low-rate CWS application (0.5% soil C increase) showed promise, increasing mean residence time (46.8 years) and half-life time (32.4 years) of the stable C pool. Spearman correlations revealed positive associations between electrical conductivity, total N, humification indices, and C stabilization parameters, highlighting the importance of nutrient availability and humification potential for C stabilization. Incorporating C-rich carrier materials with balanced nutrient content, such as SMS, can enhance soil C stabilization and support climate-smart agriculture goals. Low-rate CWS application also shows potential as an alternative C-rich carrier material. However, careful consideration of application rates and material properties is crucial to avoid adverse effects on native SOC mineralization.

Keywords Spent mushroom substrate · Composted wheat straw · Soil enzyme activity · Soil nitrogen sequestration · Food security · Soil organic matter

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1 Introduction

Human activities have perturbed the planetary boundaries established as a guideline for preserving the Earth's natural systems (Rockström et al. 2009; Steffen et al. 2015; Richardson et al. 2023). As a result, six of the nine boundaries are transgressed, suggesting that Earth is outside of the safe operating space for humanity (Richardson et al. 2023). In order to provide food security, agriculture practices have been one of the primary causes of these transgressions (Campbell et al. 2017; Richardson et al. 2023). Indeed, agriculture is currently facing severe challenges due to growing population pressure, with increased competition for land, water, energy, and other inputs for production (Campbell et al 2014; Frelat et al. 2016). Hence, it is imperative to achieve sustainable intensification of food production on existing agricultural land by the implementation of innovative approaches and the fostering of collaboration across various sectors (Chabbi et al. 2017). Climate-smart agriculture (CSA) is one of these approaches that provides guide actions to transform agricultural systems towards sustainable and climate resilient practices (FAO, 2013). One of the major objectives of CSA is to reduce greenhouse gas (GHG) emissions and enhance soil C sequestration and soil health (Campbell et al. 2014; Lipper et al. 2014). Soil health includes the role of soil in water quality, climate change and human health (Lehmann et al. 2020). Agricultural soils therefore are of special attention as being a major source of GHG emissions, mainly as N_2O and CH_4 and additionally they are relatively C-depleted. Nevertheless, enhanced soil management (e.g., as outlined by the Intergovernmental Panel on Climate Change (IPCC); Arneth et al. 2019) can be implemented to substantially reduce GHG emissions and sequester C (Paustian et al. 2016; Bai et al. 2019; Chowdhury et al. 2020). Organic carbon storage in agricultural soils depends on a range of biophysical processes including incorporation/decomposition of organic residues, fertilizer application and environmental management (Muñoz et al. 2010; Doetterl et al. 2015).

Post-harvest residues (e.g., straw, stubble and root) of agricultural crops are a major C source for arable lands (Ma et al. 2021). In this context, agricultural waste attracts increasing global attention to reduce GHG emissions for C neutrality (Yrjälä et al. 2022). Crop residues returned to the soil offer multiple crucial functions on soil quality. This includes promotion of biodiversity by providing food and habitat for soil organisms, enhanced soil structure, increased concentration of soil organic carbon (SOC), improved water holding capacity, strengthening of soil nutrient recycling and enhanced soil fertility (Ma et al. 2021; Smerald et al. 2023; Sukhoveeva 2022). However, and in some circumstances, return of crop residues may also decrease SOC (Fontaine et al. 2004; Kirkby et al. 2014), because priming effects and loss of existing soil organic matter (SOM) can exceed freshly generated SOC. Studies have shown that fresh organic matter inputs accelerate SOM mineralization (Lenka et al. 2019) with consequence for increased CO_2 emissions. Various studies over long periods have shown though that increased residue inputs in soil can result in an increase in SOC (Soon 1998; Rumpel 2008). Kirkby et al. (2013) showed that whilst crop residues incorporated into soil were mainly emitted as CO_2 in a short period, a small but variable proportion was retained as stable SOM. Crop residues differ in quality, defined by chemical composition (e.g., lignin content) and stoichiometry (C/N ratio), which plays an important role in SOC mineralization through the priming effect (Ma et al. 2021). As a result, variation in the mineralization induced by residues with different qualities has been reported, as evidenced from short-term incubations, where rate of residue application, N availability and soil characteristic may explain these variations (Lim et al. 2012; Ma et al. 2021). Longer-term incubations are needed however to better understand the dynamics of C and C mineralization derived from crop residues, considering that residue quality changes during decomposition and recalcitrance may increase over time (Fang et al. 2019). In recent decades, several studies have focused on an integrated management approach for crop residue incorporation that consider exogenous nutrient supply (particularly N) as a key factor to stimulate microbial activity, which can both enhance the decomposition of residue and propitiate the longer-term stabilization of soil C (Kirkby et al. 2013; Kirkby et al., 2016; Fang et al. 2019; Grzyb et al. 2020).

A recent meta-analysis by Duque-Acevedo et al. (2020) examining approaches for use and transformation of agricultural wastes, concluded that there is need to enhance and broaden the techniques for recovering agricultural waste in order to ensure resource efficiency, sustainable production, and the reduction of emissions and negative environmental impact. In this context, it is evident that lignocellulosic wastes exhibit varying characteristics (e.g., ion exchange, surface adsorption, complexation-chelation, nutrient composition) as carrier materials (Bilal et al. 2020). Furthermore, there is need for information regarding the significance of lignocellulosic wastes as effective carriers for enzyme immobilization and their considerable potential as more environmentally-friendly biocatalytic systems (Calabi-Floody et al. 2018). New fertilization strategies and smart fertilizer design based on organic materials and biotechnological approaches have potential to limit and/or reduce the adverse effects on the environment as result of the extensive utilization of chemical fertilizers (Calabi-Floody et al. 2018). The development of 'smart fertilizers' in order to increase soil C sequestration and to improve soil

quality and nutrient use efficiency is in line with CSA goals. Such approaches may not only help to reduce waste accumulation and to mitigate climate change but also add to increasing food security by improving soil fertility, which is a win–win scenario (Paustian et al. 2016).

In this paper we hypothesize that the implementation of lignocellulosic C-rich carrier materials for smart fertilizer development depends on their N content in order to promote soil C stabilization instead of CO_2 emission. Enhancing the understanding of how C-rich carrier materials influences soil C dynamics is essential to create efficient crop residue management techniques to avoid negative effects on SOC mineralization. The aim of this study therefore was to evaluate the potential of two C-rich agricultural residue-carrier materials, derived from either spent mushroom substrate (SMS) or composted wheat straw (CWS) with contrasting N contents and application rates, on the short-term dynamics of the added C and the longer-term stabilization of soil C in an Andisol.

2 Methodology

2.1 Source of C-Rich Waste Material

Wheat straw was collected during the post-harvest stage at the end of season 2020/2021 from farms located in the La Araucanía region, specifically in Cunco (38°55057, 90'-071°59021,10') and Curacautín (38°24025,80′ 071°55022,20'). Spent mushroom substrate was provided by the company *Milagros del Sur Ltd.* and collected after 3 harvested periods. For this study, an air-dried composite sample of the 3 collections was used.

To obtain CWS, approximately 45 kg of the wheat crop residue was composted in 3 batches according to Calabi-Floody et al. (2019). Briefly, the wheat straw was ground to obtain a particle size < 1 cm and divided into equal parts that were then inoculated with 28 mycelia discs (10 mm diameter) of whole growth *Trichoderma harzianum* per kg of straw and 0.85 g kg^{-1} of ammonium nitrate (NH_4NO_3). The moisture was adjusted to ~ 60% with distilled water and the compost was aerated by manual turning at a regular interval of two weeks. The SMS was processed through physical modification, where the raw SMS material was ground in a BIOBASE Disintegrator MPD-102 at mesh 30 and 1,400 rpm.

2.2 Characterization of C-Rich Carrier Materials

2.2.1 Physicochemical Determinations

Total C and N contents were determined by dry combustion using an EuroEA3000 series instrument. Total and Olsen phosphorus (P) were determined according to Sadzawka et al., (2007). A glass electrode was used to measure pH and electrical conductivity (EC) in water extracts (1:10 ratio; dry weight basis) of the carrier materials. (Sadzawka et al. 2007). Water holding capacity (WHC) was determined by gravimetric difference (Yu et al. 2013; Springer and Heldt 2016).

The potential humification degree of the carrier materials was determined by E_4:E_6 ratio as outlined by Filcheva et al., (2018). This description is based on spectrometric analysis (Zbytniewski and Buszewski 2005; Gaind and Nain 2010; Filcheva et al. 2018; Medina et al. 2020b), whereby 1 g of oven dried material was extracted with 50 ml of 0.5 M NaOH, shaken overnight at 20 °C, and centrifuged at 4500 × g for 25 min. Absorbances of the supernatant were then determined at 280 (E_2), 472 (E_4) and 664 (E_6) nm. According to reported literature, high E_4:E_6 values (> 5) are characteristic of fulvic acids and indicate low humification, and values < 5 indicate higher humification and presence of humic acids (Pansu and Gautheyrou 2006). On the other hand, the E_2:E_4 ratio reflects the proportion between lignins and other materials at the beginning of humification, and the content of materials at the beginning of transformation. The E_2:E_6 ratio denotes the relation between non-humified and strongly humified material.

2.2.2 Fourier-Transform Infrared (FTIR) Spectroscopy

The CSW and SMS carrier material samples were freeze-dried before analysis. The Fourier Transform Infrared (FTIR) adsorption spectra of the materials were recorded across a wave number range of 4000–500 cm^{-1}. Samples were analysed at the Universidad de La Frontera using a Bruker Tensor 27 spectrometer (Bruker Corporation, Germany). For analysis, 500 mg of oven-dried samples (at 105 °C) were mixed with approximately 50 g of spectroscopic-grade KBr at ambient temperature. The resulting pellets were then subjected to Infrared spectroscopy, performed at a resolution of 5 cm^{-1} and cumulating 32 scans.

2.2.3 Phytotoxicity Determination

Phytotoxicity of extracts from composted materials was determined by percentage of germination index (GI) as outlined by Zucconi et al. (1981). The extraction solutions were obtained using a ratio of 1:10 (w/v) carrier:water by shaking 5 g samples with 50 ml deionized water for 1 h using a horizontal shaker. The extracts were then centrifuged at 3000 rpm for 20 min and filtered. Two concentrations (100% and 50% diluted) were used for subsequent seedling assays (Diacono et al. 2012). Fifty surface sterilized seeds of *Lolium perenne* were placed on sterile filter paper soaked with 20 ml of each extract and incubated in

dark at 25 ºC. The number of seeds germinated and root length were recorded after 5 days. Seeds germinated in distilled deionized water was used as a control. The GI was measured according to the Eq. (1)

$$GI = \frac{Number\ of\ seed\ germinated\ in\ treatment}{Number\ of\ seed\ germinated\ in\ control} \times \frac{Mean\ root\ length\ in\ treatment}{Mean\ root\ length\ in\ control} \times 100 \quad (1)$$

2.3 Soil Incubation Procedure and Analysis of C Mineralization

Five kilograms of soil was collected to a depth of 30 cm from a Chilean Andisol under agricultural management. The Andisol was a series Freire (Soil Survey Laboratory Staff, 1996) located in Southern Chile (39°05′ S and 072°31′ W). Fresh soil was air dried, sieved (< 2 mm) and basic properties were determined in triplicate (Table 1).

The potential of the carrier materials to increase soil C sequestration was determined by incubation assays that were conducted in mason jars over a one-year period (Rahman 2013; Li et al. 2019). Destructive samples for analysis of soil C and N were taken at 0, 45, 90, 135, 180, and 365 days. Incubations were set up using 100 g of air-dried soil in 1000-ml glass flasks with a 30% of water content, for pre-incubation at 25 ± 1 °C for 7 days to activate microbial activity (Li et al. 2019). Thereafter, carrier materials were added at either of two rates (Teutscherova et al. 2017; Li et al. 2019). The Low dose rate corresponded to an addition of 0.5 g C per 100 g of SOC (18.7 g SMS and 12.2 g CWS per kg soil) and the High dose corresponded to an addition of 1 g C per 100 g of SOC (37.5 g SMS and 24.3 g CWS per kg soil). The soil water content was then adjusted to 60% of water-holding capacity and maintained by regular addition of distilled water through the incubation period. Soil respiration was monitored thereafter using 1 M NaOH trap solutions (20 ml in 50 ml Falcon tubes) placed inside the incubation jars which were air-tight sealed and incubated in dark conditions at room temperature (~ 22 °C). Soil respiration was monitored every 3 to 4 days by decrease in electrical conductivity of NaOH solutions (Hanna instrument HI5522) as induced by absorption of CO_2 (Nordgren 1988). After each measurement period the NaOH solutions were replaced and empty mason jars were included as controls.

Table 1 Physical–chemical properties of soil used for the incubation experiment

Parameter	Value[a]
Organic matter (%)	19.5 ± 0.7
pH (in water)	5.4 ± 0.11
Total C (g kg^{-1})	111.3 ± 1.2
Total N (g kg^{-1})	8.4 ± 0.1
C:N ratio	13.2 ± 0.2
Extractable P (mg kg^{-1})	4.5 ± 0.7
Extractable N (mg kg^{-1})	35.5 ± 0.7
Extractable K (mg kg^{-1})	103.8 ± 8.1
Al saturation (%)	2.6 ± 0.1
CEC ($cmol^{+}$ kg^{-1})	3.4 ± 0.1
∑Bases ($cmol^{+}$ kg^{-1})	3.4 ± 0.1

[a]Values of mean ($n = 3$) ± standard deviation

CEC = Cation Exchange Capacity; ∑ Bases = sum of bases.

Analytical techniques according to the CNA standards of the Chilean Soil Science Society, CEC as exchangeable Ca + Mg + K + Na + Al determined following extraction with 1 mol L^{-1} KCl; Al saturation (as Al exchangeable × 100)/CEC; and ∑ Bases determined following extraction with 1 mol L^{-1} CH_3COONH_4 at pH 7.0

According to Knicker et al. (2013), the cumulative C loss due to respiration was calculated by normalizing the CO_2 production to the C content of the sample and using a calibration constant. This constant converts the decrease of the conductance to accumulated CO_2 under consideration of the temperature. The obtained data were fitted to a double exponential decay model (pools with fast and slow turnover) using Sigmaplot version 14.0 (Systat software, Inc.) according to Eq. (2).

$$A(t) = A_1 \times e^{-k1t} + A_2 \times e^{-k2t} \quad (2)$$

where, A(t) = remaining C (as the % of total soil organic C (C_{org})); A_1 = the amount of C which is relatively labile against mineralization (% of total C_{org}); A_2 = the amount of C which is more stable against degradation (% of total C_{org}); t = incubation time; k_1 and k_2 = apparent first order mineralization rate constants for the labile and refractory pool (y^{-1}). Accordingly, the mean residence times (MRT_1 and MRT_2) of the first-order reactions are equal to $1/k_1$ and $1/k_2$, whereas the half-life time of A_2 was determined by $t_{1/2long} = 0.693/k_2$.

2.4 Determination of Soil C Sequestration

The C sequestration was determined according to the procedure of Mahmoodabadi and Heydarpour, (2014), where the native SOC was measured initially and the different amounts of organic C were added to each treatment according to type and addition rate. Therefore, the SOC content in each soil treatment was calculated prior to the incubation (OC_i) and as the final SOC (OC_f) at the end of the experiment. C

sequestration percentage (CSP) was thus calculated for each treatment as:

$$CSP = (OCf \div OCi) \times 100$$

The same calculation procedure was used to determine the possible N sequestration percentage (NSP).

2.5 Fluorescein Diacetate (FDA) Hydrolysis and Urease Activity

Microbial activity was evaluated at the end of soil incubation period, using the FDA hydrolysis assay as outlined by Prosser et al. (2011) which is a wide-ranging assessment of soil hydrolase activity. Briefly, 1 g fresh soil samples were shaken with 10 ml of 0.06 M potassium phosphate buffer (at pH 7.6) and 200 µL FDA stock solution (2 mg ml^{-1}) for 1 h at 25 °C. Reactions were then terminated by addition of 10 ml of acetone and the FDA activity was measured colorimetrically at 490 nm wavelength using a microplate spectrophotometer (EPOCH2) and expressed as µg fluorescein g^{-1} of soil per hour (Sánchez-Monedero et al. 2008; Khadem et al. 2021).

The enzymatic activity of urease was determined using the methodology described by Kandeler and Geber (1988), where release of ammonium (NH_4) was measured after soils were incubated with urea as substrate. Briefly, soil was incubated with a KCl:HCl (1 M) extractant solution with urea (79.7 mM) added as substrate for 3 h at 37ºC. The NH_4 released was measured in an aliquot that was treated with sodium nitroprusside and salicylate, as well as with dichloroisocyanuric acid (Kandeler and Geber, 1988). The colorimetric assessment was performed at 690 nm absorbance and results were expressed as µg NH_4 g^{-1} dry soil per hour.

2.6 Statistical Analysis

All analyses were performed in R statistic version 4.1.1 (R Core Development Team, 2021, Vienna, WU, Austria). ANOVA and Tukey HSD tests were performed with the package "agricolae" after verifying the normality and homoscedasticity assumptions through the Shapiro-Wilks test and Levene test, respectively. Spearman correlations were carried out to evaluate the relationship between the physicochemical and biological properties. Additionally, a principal component analysis (PCA) was generated using the "FactoMineR" and "factoextra". PERMANOVA based on Euclidean distance that was used to evaluate multivariate differences between treatments with the package "vegan".

3 Results and Discussion

3.1 Characterization of C-Rich Carrier Materials for Soil Application

The two carrier materials (CWS and SMS) used in this study differed in various properties that included, nutrient content (N, P), solution pH, electrical conductivity (EC) and water holding capacity (WHC) (Table 2). SMS had higher total N and P contents (21.2 ± 0.7 and 3.3 ± 0.1 g kg^{-1}, respectively) and thus an associated lower C:N (and C:P) ratio

Table 2 Physical–chemical properties of C-rich carrier materials derived from agricultural wastes spent mushroom substrates (SMS) and composted wheat straw (CWS)

	SMS			CWS		
pH	6.1	±	0.0	8.1	±	0.1
EC (mS cm^{-1})	7.5	±	0.4	3.9	±	0.1
WHC (%)	71.8	±	1.0	87.7	±	0.4
Total C_1 (g kg^{-1})	316.9	±	5.3	417.2	±	2
Total N (g kg^{-1})	21.2	±	0.7	9.7	±	0.1
Extractable P (mg kg^{-1})	871.1	±	31.7	99.3	±	7.5
Total P (g kg^{-1})	3.3	±	0.1	0.5	±	0.1
C:N ratio	14.7	±	0.4	42.9	±	0.6
Humification index						
E_4:E_6	6.3	±	0.1	5.7	±	0.1
E_2:E_6	192.9	±	10.8	198.9	±	17.5
E_2:E_4	16.4	±	0.7	18.6	±	1.4
Germination index						
GI_{100} (%)	85.0	±	10.6	89.6	±	16.4
GI_{50} (%)	111.6	±	12.1	90.3	±	10.7

Values are expressed as mean ± standard deviation ($n = 4$). *EC*: Electrical conductivity; *WHC*: water holding capacity; *GI*: Germination index; Humification index is presented as absorbance ratios in 0.5 M NaOH solution: E_2 :E_4 (280:472 nm), E_2 :E_6 (280:664 nm), E_4 :E_6 (472:664 nm)

which suggests greater potential as a biofertilizer product. By contrast higher WHC and a more moderate basic pH of CWS supports its potential utilization as a soil conditioner or organic amendment.

Both carrier materials showed high GI values that were equivalent to 100% (ie., 85% to 111%) at either dilution indicating absence of phytotoxicity. According to Bernal et al. (1998) and Pera et al. (1991) GI values > 60% are indicative of suitability for being used in soil–plant systems and thus be appropriate for the development of biofertilizers.

According to spectroscopic analysis of the extractable organic C, both composts similarly showed relatively low E_2:E_4 and E_4:E_6 ratios which is indicative of a high degree of organic matter humification (i.e., based on aromatic condensation). Although being > 5, also suggests that humification is not complete (Zbytniewski et al. 2002). However, this operational measure can be limited as E_4:E_6 ratios are only a proxy for an operational measurement of humification in these kinds of substrates (Medina et al. 2020a).

The FTIR spectra of the C-rich carriers CWS and SMS displayed similar signals indicative of their lignocellulosic origin and composition. Prominent absorbances observed at 3400–3500 cm^{-1} were attributed to H-bonded OH and NH groups likely originating from the carbohydrates present in both carriers. Additionally, peaks with lower intensities at 2920–2925 cm^{-1} (aliphatic CH_3 stretch), a weak peak at 2849 cm^{-1} (CH_2 aliphatic C) and a smaller less pronounced peak at 2300 cm^{-1} were also observed (Medina et al. 2020a).

The adsorption bands observed between 2840 and 2900 cm^{-1} were associated with carboxylic OH and have been previously interpreted as being derived from lipids, particularly in composts derived from lignocellulosic and manure mixtures. The shoulders observed at 1700–1720 cm^{-1} (C–O stretch of C = O and COOH of uronic acids), pronounced peaks in the 1630–1640 cm^{-1} region (C–O stretch of COO^{-1}), and less pronounced signals in the 1515–1580 cm^{-1} region correspond to conjugated C = O bonds in aromatic rings being indicative of lignin and lignin byproducts of decomposition (Medina et al. 2020a, b). Small peaks observed at 1450–1465 cm^{-1} (-CH_3, –CH_2, -asymmetric bending), 1420–1425 cm^{-1} (C–OH deformation of COOH and COO- symmetric stretch), 1375–1385 cm^{-1} (C–CH3, C–CH2 deformations), and at 1235 cm^{-1} (C–O stretch or OH-deformations of COOH) were also identified (Fig. 1).

Additionally, a pronounced peak observed in both materials, with a signal between 1000 and 1100 cm^{-1}, can be attributed to C–O stretching in cellulose. The signal at 1030 cm^{-1} has also previously been assigned to silica (Si–O stretching), which is associated with the high silica content in wheat straw and other lignocellulosic materials (Medina et al. 2020a, b). Furthermore, the signal recorded in the 880–910 cm^{-1} region for both materials can be linked to cellulose, with a higher intensity observed in the SMS substrate (Fig. 1).

3.2 Decomposition of C-Rich Carriers in Soil Incubations

The cumulative CO_2 emission and C-loss from soil and C-rich carriers-amended soil over time exhibited a polyphasic response as shown in Fig. 2. Notably, the SMS-H and CWS-H treatments showed high initial rates of CO_2 evolution and loss of soil C, with the CWS treatment persisting at higher levels across the entire incubation. The SMS-L and CWS-L treatments showed comparable rates of CO_2 evolution and C-loss to each other, that in both cases were different to that of the unamended soil control (Fig. 2). Similar trends in C loss were reported by Lim et al. (2012) who evaluated C mineralization on a compost-amended Andisol with different levels of compost stabilization.

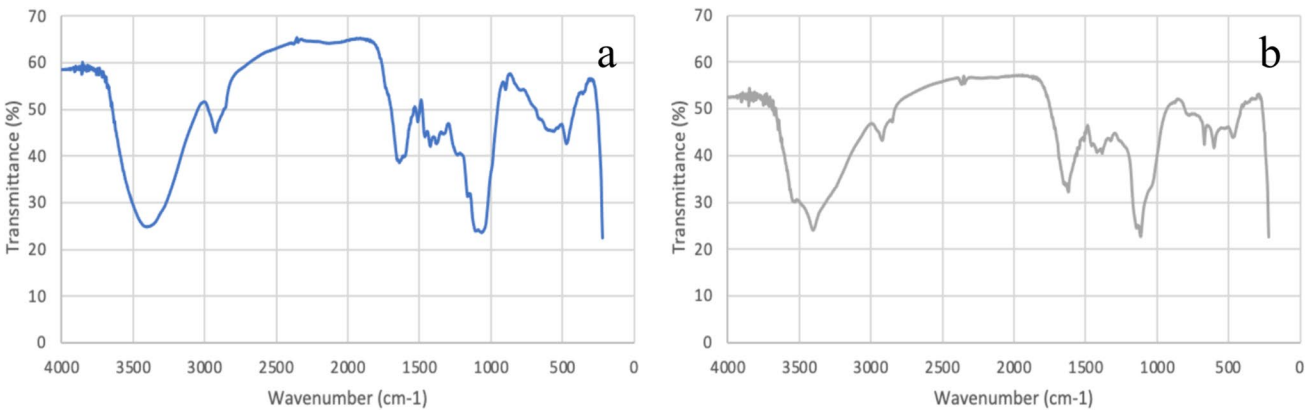

Fig. 1 Fourier-transform infrared spectroscopy (FT-IR) spectra of carrier materials (**a**) composted wheat straw (CWS) and (**b**) spent mushroom substrate (SMS)

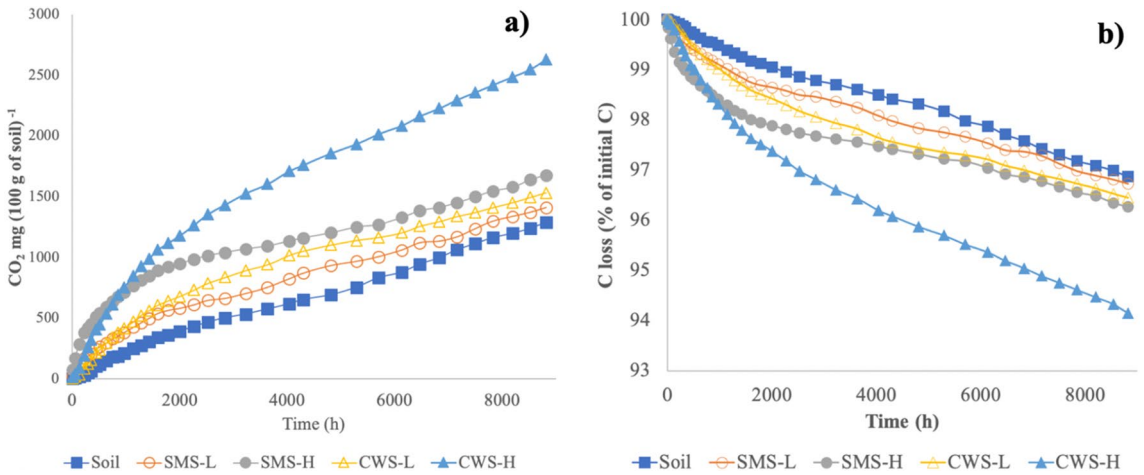

Fig. 2 Cumulative CO_2 emission (**a**) and C loss as a % of initial C content (**b**) determined from soil-C-rich carrier material incubations as a function of time (365 days). Treatments correspond to Soil; as unamended control; SMS-L and SMS-H; as spent mushroom substrate incorporation at low and high rate, respectively and CWS-L and CWS-H; composted wheat straw incorporation at low rate and high rate, respectively

Table 3 Total carbon content and cumulative amount of CO_2-C emission after the addition of low and high doses of C-rich carrier materials derived from agricultural wastes

	Carrier application	C_{input}	$C_{initial}$	C_{final}	CO_2-C cumulative
	g kg^{-1} soil	g C kg^{-1} soil			
Soil	0	0	116.1 ± 1.0^{c}	123.9 ± 0.5^{ab}	3.5 ± 0.17^{d}
SMS-L	18.7	5	119.5 ± 1.3^{bc}	125.0 ± 0.8^{ab}	3.8 ± 0.20^{cd}
SMS-H	37.5	10	122.0 ± 1.6^{b}	125.6 ± 1.0^{ab}	4.6 ± 0.21^{b}
CWS-L	12.2	5	118.7 ± 1.4^{bc}	121.7 ± 1.0^{b}	4.2 ± 0.16^{bc}
CWS-H	24.3	10	128.5 ± 1.5^{a}	126.9 ± 1.5^{a}	7.2 ± 0.19^{a}

Values are expressed as mean ± standard deviation ($n=4$). Soil = unamended soil as control; SMS-L = low dose of spent mushroom substrates; SMS-H = high dose of spent mushroom substrates; CWS-L = low dose of composted wheat straw; CWS-H = high dose of composted wheat straw. C_{input} = theoretical C content supplied at the experiment establishment; C_{inital} = C content at day one of incubation; C_{final} = C content at day 365 of incubation. Significant differences ($p \leq 0.05$) between treatments are indicated by different superscript letters within each column.

The amount of cumulative CO_2-C emission from soil and treatments across the incubation period, ranged from 3.5 to 7.2 g C kg^{-1} (Table 3) over 365 days incubation. These results are consistent with the C loss during the respiration experiment, which ranged from 3.1 to 5.8% (Table 4) of the initial C content per treatments (Table 3. Mineralization of C-rich carrier materials observed in the present study were in the range of values (i.e., from 1.6 to 6.6%) reported by Lim et al. (2012) for composts derived from swine and cattle manures combined with sawdust and/or rice hull when incubated in an Andisol over 100 days. The proportion of the fast SOM pool (A_1) ranged from 0.3 to 2.2% (Table 4) of the total initial C suggesting that all treatments affected the existing SOM component compared to the unamended soil, with the CWS-H treatment being the most significant.

In the CWS-H treatment, the high prevalence of aromatic structures, resulting from partial decomposition of lignin and polysaccharides during the wheat straw composting process (Calabi-Floody et al. 2019). Combined with the material's low N content, likely restricted its decomposition at the high incorporation rate (Li et al. 2011). This would lead to N deficiency and a stimulation of microbial-mediated decomposition of native SOM (Chen et al. 2014; Grzyb et al. 2020), and thus an associated increase in net C mineralization. Across all soil treatments the mineralization rate of the fast SOM pool (k_1) ranged from 7.0 to 18.4 years^{-1}, with no significant differences being observed between unamended soil and the SMS treatment at both incorporation rates, suggesting a similar degradation rate of fast C pool over the initial ~ 25 days. By comparison the CWS at both rates of incorporation showed a slower rate of C degradation from the fast pool (7.0 to 8.3 years^{-1}), reinforcing the effect observed for native SOM decomposition due to less N availability. In this sense, the mean residence time of the fast SOM pool (MRT_1) observed for unamended soil and the SMS treatment at both rates of incorporation ranged from 0.05–0.07 years (Table 4)

Table 4 Organic matter decomposition of amended and unamended soils with two levels of C-rich carrier materials (SMS and CWS) with low and high (L and H, respectively) incorporation rates. Mean values of the proportion of the fast (A_1) and slow (A_2) soil organic matter (SOM) pools, degradation rate constants (k_1, k_2), mean residence times (MRT_1 and MRT_2) and the half-life time of the slow pool ($t_{1/2long}$) are shown as determined from respiration experiments

	Total C loss (% of C_{org})			A_1 (% of C_{org})			k_1 ($year^{-1}$)			MRT_1 (year)			A_2 (% of C_{org})			k_2 ($year^{-1}$)			MRT_2 (year)			$t_{1/2long}$ (year)		
Soil	3.1	±	0.2^{c}	0.3	±	0.05^{d}	14.9	±	4.9^{ab}	0.07	±	0.03^{bc}	99.7	±	0.05^{a}	0.028	±	0.002^{b}	35.9	±	2.8^{cd}	24.9	±	1.9^{c}
SMS-L	3.2	±	0.2^{bc}	0.7	±	0.10^{c}	16.6	±	5.9^{a}	0.07	±	0.03^{c}	99.2	±	0.10^{b}	0.025	±	0.002^{bc}	40.6	±	3.0^{bc}	28.1	±	2.1^{bc}
SMS-H	3.5	±	0.2^{bc}	1.5	±	0.02^{b}	18.4	±	1.2^{a}	0.05	±	0.00^{c}	98.4	±	0.03^{d}	0.021	±	0.002^{c}	48.8	±	5.2^{a}	33.8	±	3.6^{a}
CWS-L	3.6	±	0.2^{b}	1.5	±	0.03^{b}	7.0	±	0.0^{c}	0.14	±	0.00^{a}	98.6	±	0.02^{c}	0.021	±	0.002^{c}	46.8	±	3.6^{ab}	32.4	±	2.5^{ab}
CWS-H	5.8	±	0.1^{a}	2.2	±	0.21^{a}	8.3	±	0.5^{bc}	0.12	±	0.01^{ab}	97.9	±	0.21^{e}	0.038	±	0.004^{a}	26.6	±	2.5^{d}	18.5	±	1.7^{d}

Values are expressed as mean ± standard deviation ($n = 4$). Treatments correspond to Soil: unamended soil as control; SMS-L: spent mushroom substrate incorporated at low rate; SMS-H: spent mushrooms substrate incorporated at high rate; CWS-L: composted wheat straw incorporated at low rate and CWS-H: composted wheat straw incorporated at high rate. Within each column different superscript letters show significant differences ($p < 0.05$) according to an ANOVA and Tukey HSD or Kruskal–Wallis in cases when statistical assumptions were not met

with no significant difference. These MRT_1 values are comparable to those reported by Leal et al. (2019) (i.e., in the order of ~ 0.05 year), where the effect of C stable materials (charcoal fine residues) on SOM incorporated into a Cambisol soil were evaluated. Furthermore, our findings align with those of Lim et al. (2012), who evaluated 3 soil types (Andisol, Inceptisol, and Ultisol) under different compost applications with varying stabilization degrees. They found that the fast mineralizable C pool was determined primarily by soil characteristics that mediated SOM stabilization, rather than by compost quality. Similarly, our results suggest a protective effect of the Andisol through the formation of SOM-mineral complexes that limit microbial decomposition. This protective effect is attributed to the soil's mineral composition (clays and nano-clay fractions with high reactive and amorphous fractions) and the formation of Al and Fe-organic matter complexes (Matus et al. 2008; Calabi-Floody et al. 2011; Hernández and Almendros 2012).

The slow SOM pool of untreated (control) soil revealed a k_2 degradation rate constant (0.028 $year^{-1}$) in the Andisol soil that was between 2 and fourfold lower than that obtained by others using an agricultural soil (Leal et al. 2019) and fortes soil (Knicker et al. 2013). Moreover, no differences were observed between the control soil and the SMS-L treatment k_2 values (Table 4) such that the two soils did not differ in the mean residence time (MRT_2) of the slow SOM pool (i.e., 35.9 ± 2.8 and 40.6 ± 3.0 years respectively, and a half-life time ($t_{1/2long}$) of 24.9 ± 1.9 and 28.1 ± 2.1 years for both treatments). This indicates a similar decomposition of SOM when total soil C was increased by 0.5% with addition of the SMS carrier material. On the other hand, at the higher rate of SMS incorporation (1%; SMS-H) and for the lower rate of incorporation for CWS (0.5% increase in total C content; CWS-L) lesser values of k_2 were observed compared to the unamended soil (control). Thus, indicating a slower degradation rate of the SOM pool, where the MRT_2 and $t_{1/2long}$ were increased to by 11 and 7.5 years with respect to the unamended soil, respectively (Table 4). Several studies have reported that the addition or availability of nutrients such as N, P, and K can effectively promote the stabilization of exogenous C in soil from organic materials and contribute to C sequestration. In such cases soil microorganisms are able to adequately acquire nutrients directly from soil (i.e., soil solution) instead of expending energy to produce enzymes capable of mineralizing pre-existing stable SOM (Kirkby et al. 2014; Ma et al., 2021; Jesmin et al., 2021). In this sense, incorporation of SMS at both rates into the soil and containing higher amount of N and P (Table 2; as well as K and Fe (data not shown)), showed a lower decomposition rate that was associated with increased C sequestration. However, in the case of CWS with lower nutrient availability (Table 2) and the presence of more highly stabilized C (Calabi-Floody et al. 2019), there was greater potential to conserve the

natural dynamics of SOM (Mulumba and Lal 2008; Wei et al. 2015), especially at the low rate of application. For such substrates, consideration of the rate of application is crucial because high levels can promote the decomposition of native SOM with an associated release of CO_2 instead of C sequestration; for example, as was observed for the CWS-H treatment (Fig. 2, Table 3). Fontaine et al. (2004), showed that excessive incorporation of non-stabilized residues into soils favours a priming effect from existing OM that leads to a net loss of soil C. Consistent with this, the k_2 value for CWS-H was 1.4-fold higher than that for the control soil and the mean residence time (MRT_2) declined from 35.9 to 26.6 years (albeit without significant difference), which suggests that the slow SOM pool was degraded faster than in the unamended soil. Furthermore, the $t_{1/2long}$ slow SOM pool in the CWS-H treatment showed a significant reduction of over 6 years compared to the unamended soil, suggesting a change in the SOM dynamic that led to the observed increase in CO_2 release (Fig. 2).

3.3 Effect of Carrier Materials on Soil C and N Sequestration

The addition of the CWS-L and SMS-H as C-rich carrier materials to the soil as amendments to increase total soil C by either 0.5% or 1% C increased the conservation of C over the 365-day incubation experiment. Despite this modest C addition, the results, with exception of the CWS-H treatment, showed an increase across the treatments from 4.6 to 2.5% soil C (Table 3) despite the observed CO_2 emissions (Fig. 2). For the CWS-H treatment, a final C loss around 1.2% instead of sequestration was observed, which was significantly different to the other treatments including the unamended soil control (Table 3). A final increase in C content of 6.6% (with respect to the initial C, Table 3) in the control soil, whilst surprising, may be attributed to the fact that soil management prior to incubation (dried and sieved < 2 mm process) induced an initial C destabilization without loss. Therefore, after long-term soil incubation under optimal conditions for microbial activity (including moisture and temperature), the resultant microbial biomass and subsequent detritus could contribute substantially to the total SOM (Coonan et al. 2020). Several mechanisms have been reported for the C sequestration capacity in Andisols and could be related to the observed increase in final soil C, which includes; formation of organo-mineral coatings providing protection against microbial degradation (Hernández and Almendros 2012; Matus et al. 2014), intense sorption processes at the soil matrix level on specific surfaces of short-range minerals protecting SOC fractions (Dahlgren et al. 2004; Hernández et al. 2012), and the role of the fractal structure of allophanic nanoclays in the physical and chemical protection of SOC (Woignier et al. 2008; Chevallier et al. 2010; Calabi-Floody et al. 2011).

The C sequestration percentage (CSP) expressed relative to the initial soil C content after 365-days incubation ranged from 108 to 96% across the different soil treatments (Fig. 3). Sequestration was highest for the unamended soil and SMS-L treatments, which were not significantly different as was observed for the dynamics and mineralization rates of soil C in these two treatments (Table 3 and 4). Mahmoodabadi and Heydarpour, (2014), similar reported highest levels of CSP in control soil that was attributed to the stability of native SOC. Notably the CSP in the SMS-H and CWS-L treatments was significantly lower than unamended soil. However, the SOC stabilisation provided by these treatments increased the MRT_2 and $t_{1/2long}$ as compared to the unamended soil (Table 4). On the other hand, lower CSP was evident in the CWS-H treatment with a net C loss of ~ 5%. These

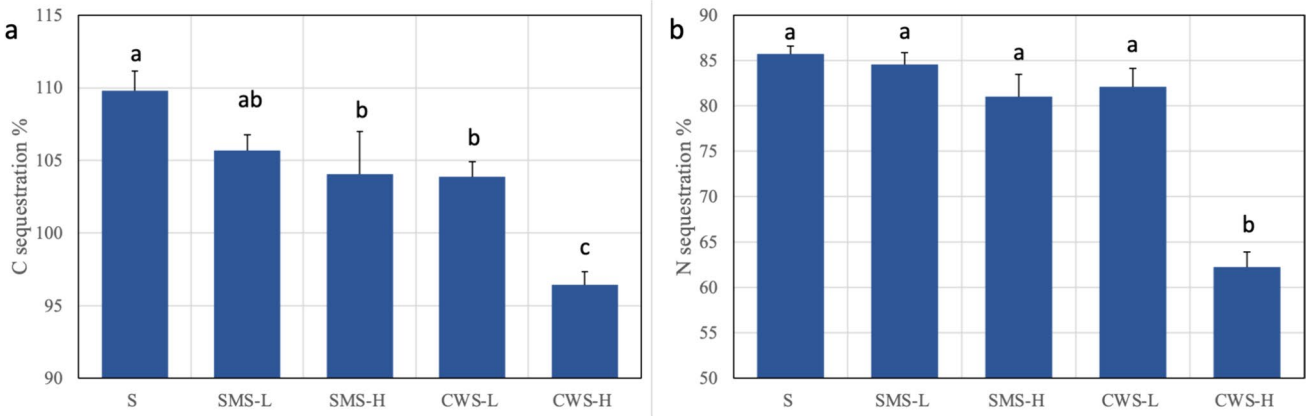

Fig. 3 Sequestration of C and N after 365 days of soil-C-rich carrier material incubation where (**a**) soil C is expressed as a percentage of the initial C content for each treatment and (**b**) soil N is expressed as a percentage of the initial content in the different treatments. The bars show means ($n = 3, \pm$ standard error) with significant differences ($p \leq 0.05$) between treatments indicated by different letters

results are consistent with those obtained from the analysis of the degradation rate and turnover of C pools and carrier materials as presented in Table 4 and Fig. 2, where the CWS-H treatment showed the highest degradable C pools.

In difference to that for soil C, soil N sequestration expressed as a percentage (NSP) of the initial soil N contents decreased to between 86 to 62% (Fig. 3b). Thus, showing that a net loss of N occurred regardless of treatment, that could also be associated with the C mineralization (Teutscherova et al. 2017; Grzyb et al. 2020). Teutscherova et al. (2017) showed similar N losses from control soil and compost amended soil of between 6 and 19%, with immature compost showing the highest loss. Significantly, the highest loss of N in our study was in the CWS-H incorporation treatment with the widest C:N ratio. Lower N availability for microbial residue degradation in this treatment (Table 2), would be expected to increase the mineralization (i.e., mining) of N from native organic materials and SOM to meet N requirement, thus increasing CO_2 emission and net loss of soil N (Teutscherova et al. 2017; Xie et al. 2022).

3.4 Microbial Activity and Soil Quality

In our study after 365-days of soil incubation the FDA values ranged from 193 to 232 µg fluorescein g soil^{-1} h^{-1} and were significantly higher in response to the addition of CWS at both rates of application, as compared to the unamended control soil (Fig. 4a). No significant differences ($p > 0.05$) however were observed between the unamended soil and SMS treatments (Fig. 4a). This indicates an increase in soil microbial activities when soil was amended with CWS carrier materials, and is consistent with the observed inadequate supply of N to cover the requirements of microorganisms and therefore a stimulation of N mineralization from the soil (Ma et al. 2021; Thangarajan et al. 2013). In comparison, no significant differences were observed between unamended soil and the SMS treatments at both rates of application that could be attributed to higher N availability through external N supply (Table 2). This would be expected to reduce the metabolic need for enzyme production and thus decrease the N mining from SOM and associated reduced priming effect (Ma et al. 2021). As a result, improved stabilization of SOM could be achieved (Table 3) without marked alteration of microbial activity and C dynamic leading to a potential increase in soil C sequestration (Fig. 3a and Table 4).

A similar trend was observed for the urease activity that across the soil treatments ranged from 6 to 13 µg N-NH_4 g soil^{-1} h^{-1} (Fig. 4b). The treatments with both levels of CSW addition were significantly affected, increasing the activity by ~ 80% in comparison to the SMS and control soil treatments. These results support higher N mineralization from native SOM in order to decompose residue in the CSW treatments. Similarly, higher urease activity in soils has been observed where fresh organic matter is added with lower N content (Torres et al. 2015; Teutscherova et al. 2017; Joniec et al. 2022). According to Roy et al. (2006) the dynamics of N in soils are complex, where soil mineral N (NO_3^- and NH_4^+) is primarily derived from the SOM mainly in upper

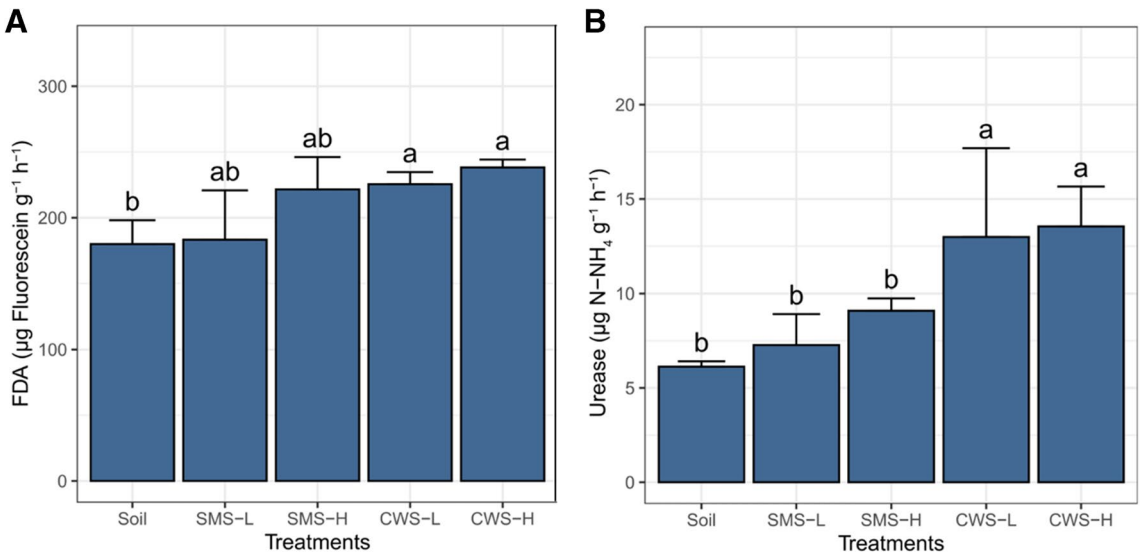

Fig. 4 Enzyme activities after 365 days C-rich carrier material soil incubations. The activities correspond to (**a**) Fluorescein diacetate hydrolysis (FDA) and (**b**) Urease activities for unamended control soil (soil) and soils amended with SMS and CWS carrier materials at two rates of application. Bars shown mean ($n = 8 \pm$ standard deviation with significant differences ($p \leq 0.05$) between treatments indicated by different letters in each panel

soil horizons. Nitrogen added in amide forms (NH_2) from organic residues and as in urea is first hydrolysed to NH_4^+ through the action of urease enzyme. It can then be absorbed by roots, converted to nitrate or in some cases lost through ammonia volatilization particularly in alkaline environments (Roy et al. 2006). The high urease activity in CWS treatments is consistent with the N losses that we observed especially at higher rate of application due to the possible increase of the alkaline environment (pH 8.1, Table 2). Similar adverse phenomenon of soil N loss from manure-amended soil and release of gaseous N catalysed reactions by urease has been observed by Joniec et al. (2022). The low urease activity found in SMS treatments did not show significant differences with respect to the control that could be attributed to the high N content of the carrier (i.e., 21.2 g N kg^{-1}) which was over twofold than that for CWS (9.7 g N kg^{-1}). The availability of inorganic forms of N would suppress the synthesis of the enzyme with lesser dependence for mineralization (Marcote et al. 2001). In this sense, several studies reported a decrease in urease activity as a consequence of N supply in soil under different organic residues or biochar amendment (Marcote et al. 2001; Rozas et al. 2023; Joniec et al. 2022). These changes would necessarily affect the dynamics of soil and residue N through mineralization and immobilization (Jesmin et al., 2021).

3.5 Relationships Between Soil Properties and Measures of Soil C Dynamics

In order to explore the effect and the relation between the analyzed parameters, a linear correlation analysis was conducted (Fig. 5). Significant positive correlations of soil treatments were generally observed between EC, TN, humification indexes, and positive correlations were observed with C stabilization parameters (CSP%, MRT_2 and $t_{1/2long}$). This suggests that higher nutrient availability, especially N, and a greater degree of organic matter humification favoured C stabilization in the soil (Lützow et al., 2006; Dungait et al. 2012; Kallenbach et al. 2016).

Percentage total C loss showed a negative correlation with NSP (r = -0.78), CSP (r = -0.74) and negative correlation with C stabilization parameters (MRT_2 and $t_{1/2long}$). This

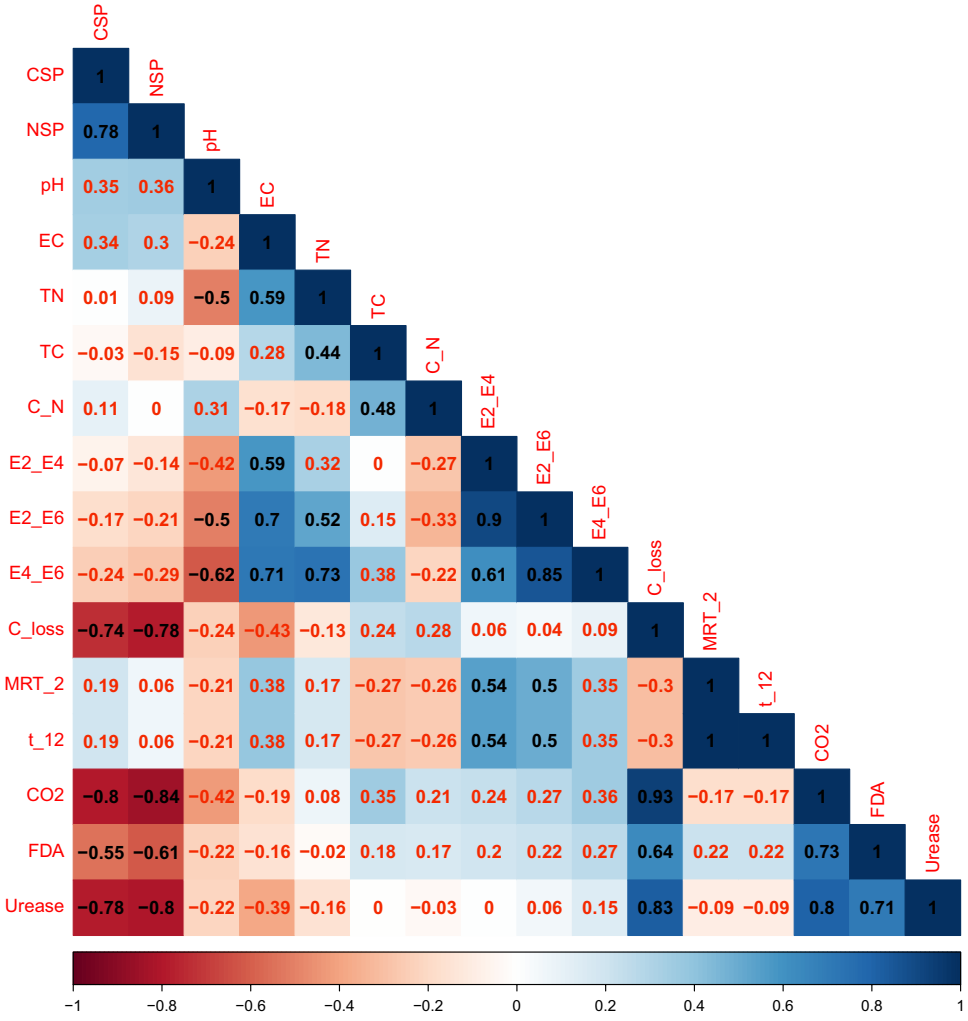

Fig. 5 Spearman correlation coefficient matrix of soil and C-rich carrier material properties across soil treatments after 365 days incubation. The showed soil properties correspond to; pH; EC; total nitrogen (TN); total carbon (TC); carbon:nitrogen ratio (C:N); fluorescein diacetate hydrolysis (FDA); urease activity, and humification index properties of carrier materials as; absorbance ratios of E_2:E_4 (280:472 nm), E_2:E_6 (280:664 nm), E_4:E_6 (472:664 nm), soil C and N stabilization mean residence time of stable C pools (MRT2); half-life time of the stable C pool($t_{1/2long}$); N sequestration percentage (NSP); C sequestration percentage (CSP); cumulative C emitted as CO_2 (C-CO_2_Cum); and percentage of total C losses from total SOC (C loss%). Red boxes indicate negative correlations, while blue boxes indicate positive correlations. Correlations with $p < 0.05$ are considered significant and are shown in boxes with black numbers, while non-significant correlations appear as red numbers

indicates potential trade-offs between C and N sequestration processes, where greater N availability could increase C sequestration (Kirkby et al. 2014; Ma et al., 2021; Jesmin et al., 2021). Meanwhile, percentage total C loss presented a significant positive correlation with microbial activity represented by enzymatic activities (urease ($r = 0.83$), FDA ($r = 0.64$)) and CO_2 cumulative emission ($r = 0.93$). Likewise, CO_2 cumulative emission has high significant correlations ($r > 0.73$) with microbial activity expressed as urease and FDA. This confirms that higher microbial activity could be associated with lower C stabilization in the soil (Fontaine et al. 2003; Luo et al. 2017). CSP and NSP showed a significant positive correlation with each other, as well as a negative correlation with the parameters associated with the destabilization of C and N (microbial activity, C losses and emission).

The PCA shows the clustering of treatments and analyzed variables into two principal components that explained 64% of the total variance (Fig. 6). These treatments, corresponding to the incorporation of SMS at low and high rates, notably clustered in the positive quadrant of PC1. This indicates a strong association with humification and spectroscopy indices, total N, and C stabilization parameters (MRT_2, $t_{1/2long}$). The higher nutrient content, especially N, in the SMS treatments would be expected to favour the humification of the added OM and its subsequent stabilization in the soil (Chen et al. 2014; Lützow et al., 2006). Additionally, the high N content in the SMS could suppress microbial enzymatic activity involved in the decomposition of native SOM, and thus avoiding the "priming effect" to promote C stabilization (Fontaine et al. 2003; Averill and Waring 2018).

The CWS-L treatment showed an intermediary position in the PCA plot without a clear association with PC1 or PC2. This position of CWS-L treatment suggests that at low application rate C stabilization was favoured (i.e., higher MRT_2 and $t_{1/2long}$) compared to the high rate of addition. Several studies have demonstrated that the humification of OM added to soil is closely related to its stabilization and subsequent sequestration (Lützow et al., 2006; Kallenbach et al. 2016). Furthermore, the N content in the OM applied to the soil can influence microbial activity and, therefore, the rate of decomposition and C stabilization (Chen et al. 2014; Averill and Waring 2018). In the case of the CWS-L treatment, the lower N content compared to SMS treatments (Table 2) may have partially limited the humification and stabilization of the added C, despite the lower application rate. Furthermore, the physicochemical properties and composition of the carrier materials can influence their degree of humification and stabilization in the soil (Medina et al. 2020a; Woolf et al. 2021). It is possible that the more lignified and recalcitrant nature of the CWS compared to the SMS affected its rate of humification and stabilization, even at low application rates. The CWS-H treatment was located in the positive quadrant of PC2, and was associated with total C losses, CO_2 emissions, and microbial enzymatic activity. This can be attributed to the nutrient imbalance and low N availability in the CWS carrier material, which would be

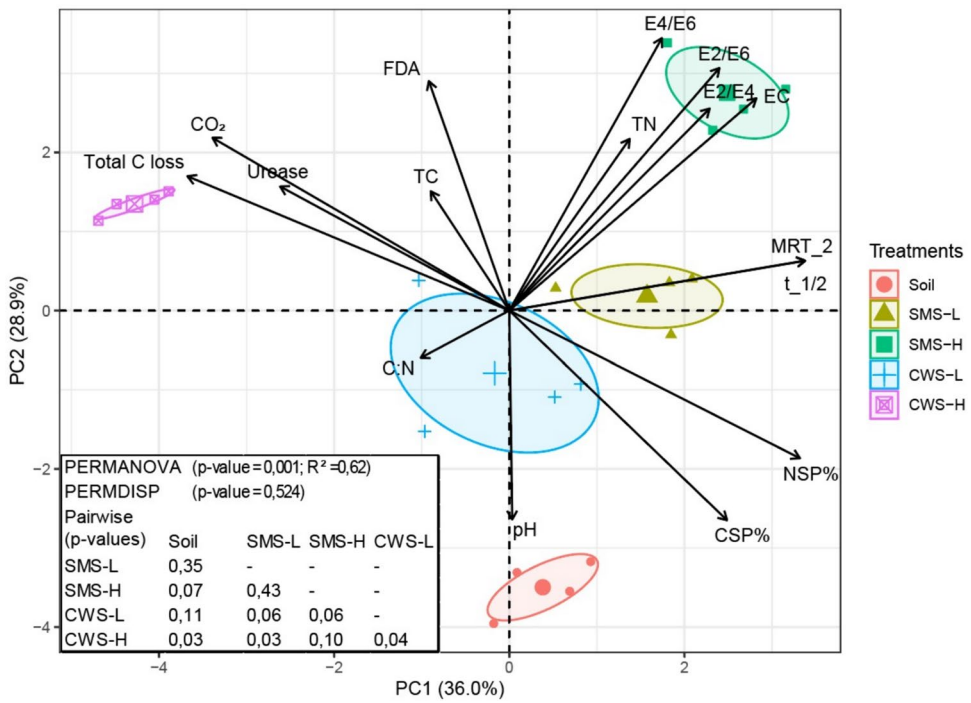

Fig. 6 Principal components analysis (PCA) biplot for the soil properties concerning C-rich carrier materials across soil treatments at the end of the 365-day incubation period. Incubation includes unamended soil as control (Soil), spent mushrooms substrate incorporation at low (SMS-L) and high rate (SMS-H), and composted wheat straw incorporation at low rate (CWS-L) and high rate (CWS-H). Permanova analysis is indicated as insert box and vectors show strength and direction of significant correlations

expected to stimulate the mineralization of native SOC by microorganisms in search of N (Grzyb et al. 2020; Kirkby et al. 2013). The high C:N ratio of this material (Table 2) could have promoted a "priming effect," and thus accelerated the decomposition of stabilized OM in the soil (Fontaine et al. 2004). This is consistent with the results of Grzyb et al. (2020) and Kirkby et al. (2013) who found that the incorporation of residues with high C:N ratios (such as wheat straw) can promote the mineralization of native SOC. Finally, unamended soil (control) showed a separation to the carrier treatments. The absence of exogenous organic material inputs could explain the lack of significant influence on the variables represented by PC1 (humification, total N, and C stabilization) and PC2 (C losses, CO_2 emissions, and enzymatic activities) where the treatments were mainly grouped. However, it may also be related to the CSP and NSP, attributable to the stabilization of native OM through physico-chemical protection mechanisms that are unique to volcanic Andisol soils (Dahlgren et al. 2004; Hernández and Almendros 2012).

4 Conclusions

Our study demonstrates that incorporating C-rich carrier materials derived from agricultural residues can significantly enhance soil C stabilization, with effectiveness dependent on nutrient balance and application rates. Spent mushroom substrate, with its higher N content, promoted SOC stabilization at both application rates, while composted wheat straw showed promise only at lower rates. These findings highlight the critical importance of optimizing application strategies to maximize C sequestration potential.

We observed complex interactions between soil properties, enzymatic activities, and C stabilization, revealing both potential synergies and trade-offs. This underscores the need for a nuanced approach to soil C-rich carrier applications. The incorporation of these materials, particularly spent mushroom substrate, offers a promising strategy for enhancing soil C sequestration while contributing to climate-smart agriculture goals.

Future research should focus on long-term effects under field conditions and explore other agricultural residues as potential carriers for smart fertilizer design. This study provides valuable insights for developing effective climate change mitigation strategies and promoting sustainable agriculture through enhanced soil C stabilization.

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