

RESEARCH ARTICLE

Manure induced transformations in soil nutrient stocks, microbial activity and multifunctional diversity: A six year long study in Chernic Phaeozem

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Abstract

Manure amendment is a viable strategy to counteract declining soil fertility and degradation. However, existing studies often fail to comprehensively evaluate how different manure types and application methods affect soil health and crop yields. To address this gap, a six-year-long field experiment was conducted on Chernic Phaeozem soil, examining the effects of manure amendments in four experimental variants: a control field with only mineral fertilizers (NPK) and three fields treated with poultry, cattle and swine manure in combinations with NPK. Soil biochemical properties such as carbon (C) and nitrogen (N) contents, dehydrogenase activity (DHA), basal respiration (BS) and substrate-induced respirations (SIR) were assessed at two depth intervals (0–10 cm and 10–20 cm). All manure treatments significantly increased C and N contents in the 0–10 cm as compared to the control. Both poultry and swine manures increased C, N contents in 0–10 cm and 10–20 cm by approximately +24%, +22% and 13%, 10%, respectively, while the effect of cattle manure was rather intermediate (+10%, 11% and +1%, 4%, respectively). In the 0–10 cm, DHA was higher in plots treated with the poultry (+80%) and cattle (+125%) manures, as compared to the swine manure treatment. In the 10–20 cm, poultry manure resulted in higher C (+6%) and N (+12%) contents, compared to swine manure, while the contents in cattle

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manure-treated plots were similar to the control. Swine manure showed the highest increase in manure-related C and N stocks across both depth intervals (+18% and 69%, respectively), despite no significant effects on soil biological activity (DHA, BR and SIR). Over the entire experiment period, all manure treatments significantly increased crop yields by 0.5 t cereal units ha⁻¹ year⁻¹. The poultry and swine manure amendments of soil biochemical properties and crop yields emphasize their value for sustainable soil management. We emphasize the need for site-specific strategies to maximize the benefits of manure amendments for sustainable agriculture.

KEYWORDS

manure amendment, microbial activity, soil degradation, soil depth, soil health, sustainable agriculture

1 | INTRODUCTION

Contemporary agriculture is grappling with significant challenges to maintain sustainability in meeting the World's food and other essential needs of mankind. Among the most pressing issues are decreased soil fertility and the degradation of croplands (Lal, 2015), which threaten global food security. Intensive farming practices, the overuse of synthetic fertilizers and unsustainable land management have resulted in the depletion of essential nutrients and organic matter, impairing the capacity of soils to support high-yielding crops (Feller et al., 2012; Obalum et al., 2017). As the demand for food increases, the search for sustainable solutions that restore soil health and fertility has gained urgency (Zavattaro et al., 2017). One promising approach is the addition of manure to improve soil chemical, physical and biological properties, which has shown to benefit nutrient cycling in the plant–soil system and thus enable higher crop yields (Hammerschmidt et al., 2021; Mustafa et al., 2020; Mustafa et al., 2021), despite even a suboptimal nitrogen to phosphorus ratio (Szogi et al., 2020).

The quality and composition of various manure types depend on the source of livestock production (Kafle & Chen, 2016) or animal category (cattle, horse, swine, poultry, etc.), production technology, type of bedding (Lendelová et al., 2016), the used litter and animal feeding management practices (Dahlin et al., 2021). As reported by Lejon et al. (2007), different types of manure, and organic amendments in general, affect the quality of soil organic matter (SOM) through changes in soil organic carbon and nitrogen content. These effects are further linked to variations in the quantity and composition of soil microbial communities, highlighting the influence of manure type on microbial processes (Lejon et al., 2007). Similarly, Dinesh et al. (1998) observed that various organic manure

types increased C and N availability and turnover to different extents, accompanied by different microbial and enzymatic activity.

The detrimental effects of increasing greenhouse gases produced by livestock production on global climate change (Grossi et al., 2018) have prompted growing calls to down-regulate animal farming practices and shift toward more sustainable land use strategies (Cassandro, 2020). Such shifts are associated with several environmental benefits (Eisen & Brown, 2022). Among the livestock sector, cattle have experienced the most markable decline in both animal numbers and meat production (MacLeod et al., 2019), compared to chicken and swine farming (OECD/FAO, 2021), which are associated with lower emissions (Shapiro et al., 2017).

This anticipated shift in the composition of livestock production, with increasing emphasis on poultry and pork farming, underscores the growing importance and potential for utilizing the specific waste products generated, namely poultry and pork manures. Therefore, it is rational to evaluate the effects of these manures on soil quality.

Apart from the differences resulting from the distinct origin of the waste organic matter, varied approaches to the bedding litter processing in the stables (e.g. the length of keeping the material without removal) and later during maturation (on the manure heap (Gómez-Brandón et al., 2008)), determine the properties (Tiquia, 2003) of final products and the stability of manures in soil (de Melo et al., 2019). Conclusively, different stable management (i.e. varied handling of the litter in stables (Pepper & Dunlop, 2021)) practices during poultry, swine and cattle breeding are the secondary reasons why the respective manures differ in their parameters.

While previous research has highlighted the potential of manure to enhance soil fertility, many studies focus on a narrow range of factors, such as specific animal waste

types or single application methods (Liu et al., 2022; Sadet-Bourgeteau et al., 2022; Shu et al., 2022; Zhang, Gao, et al., 2022). The complexity of manure composition, including differences in nutrient content and organic matter derived from various animal production systems, along with bedding materials and processing techniques, can have variable effects on soil properties. Moreover, application practices, particularly the depth at which manure is incorporated into the soil, may influence nutrient cycling, microbial dynamics and root access to nutrients (Anandakumar et al., 2022; Epelde et al., 2018; Gryta et al., 2020; Li et al., 2018; Zhang et al., 2018), but the number of reports about the dependence of soil microbial degradation activity and potential on both manure type and soil depth factors is still limited (Mkhonza et al., 2022; Thakur et al., 2022; Zhran et al., 2020). These intricacies underscore the need for more detailed studies that account for the interaction between manure types, application depth, and their compositional differences on soil health and crop performance. However, there is still a gap in this research field to answer the questions how the stability of organic carbon in manure, determined by the origin of livestock waste and processing during composting (stable management, maturation conditions), affects the permeation of nutrients into deeper layers and its effects on their availability to soil microbes.

Given the diverse factors influencing the success of manure amendments, ranging from the source of livestock production to the handling of manure in stables and on heaps, the objectives of this experiment were (i) to evaluate the impact of different manure types on microbial functional diversity and enzyme activity, with a focus on depth-dependent responses, and (ii) to relate variations in microbial activity to differences in soil carbon and nitrogen content, as well as the stability of these nutrients, with the aim of elucidating the relationships between organic matter decomposition indicators, such as soil respiration and dehydrogenase activity.

It was hypothesized that: (i) different manure sources and their handling practices would lead to variable soil microbial activity, which correlates with C and N contents at both depths, and (ii) microbial functional diversity, reflected in dehydrogenase activity and substrate-induced

respiration, would be enhanced by varying levels of total carbon and nitrogen inputs.

2 | MATERIALS AND METHODS

2.1 | Experimental site and treatments description

This field experiment was carried out near the town Sloveč (Czech Republic; (50°14.256' N, 15°20.705' E)) between 2014 and 2020. The experimental field is located in the beet production area, characterized as clayic (according to USDA Textural Triangle) highly fertile chernic Phaeozem according to World Reference Base for Soil Resources (WRB of IUSS Working Group, version from 2015). The initial basic soil physico-chemical properties were as displayed in Table 1.

The altitude averages 212 m above sea level, with a mean annual temperature of 9.4°C and annual rainfall ranging between 550 and 600 mm. In autumn 2014, four experimental variants were established, each on three plots (replications) 0.25 ha in size (i.e. 0.75 ha per a variant), in sum 12 plots for purposes of soil and crop sampling. These included a control variant, fertilized solely with mineral NPK fertilizer throughout the experiment to meet the standard crop nutrition requirements (Section 2.2) for intermediate yield levels. Additionally, three variants amended with different types of manure (poultry, cattle, swine.). Throughout the experiment, these manure-treated experimental plots received the total nutrient elements specified in (Table 2) corresponding to each manure type. In addition, all manure-amended variants were supplied with mineral NPK to achieve the same total (manure- and NPK-derived) nitrogen dose as applied to the control variant adjusted according to the crop-specific requirements (Figure 1).

All manure types originated from organic wastes of the respective livestock breeding (poultry, cattle, swine), housed on deep litter bedding systems. The higher amount of straw added to the bedding likely contributed to a higher final C:N ratio in the manures. Cattle dung was removed from stables every 2 months, swine dung remained in stables longer and removed once a year, while poultry dung was removed

TABLE 1 Physico-chemical properties of soil from experimental site Sloveč.

Property	Value/unit	Property	Value/unit
Clay (%)	48%	pH/KCl	7.18
Soil organic matter	4.76%	Humic/fulvic acid ratio	1.02
Total carbon	1.81%	C/N (organic C:total N)	5.65
Total nitrogen	0.32%	Cation exchange capacity	278 mmol·kg ⁻¹
Available K	456 mg·kg ⁻¹	Available Mg	244 mg·kg ⁻¹
Available Ca	6114 mg·kg ⁻¹	Available P	73 mg·kg ⁻¹

TABLE 2 Doses of manure and the contained macroelements (carbon^a, nitrogen, phosphorus) added to soil during the whole experiment at each treatment.

Treatment	Manure [t·ha ⁻¹]	C _{input}	N _{input}	P _{input}	C:N input ratio
Poultry manure (PM)	40	18.64 ± 0.07	1.19 ± 0.12	0.32 ± 0.10	16:1
Cattle manure (CM)	160	69.77 ± 2.01	1.04 ± 0.02	0.27 ± 0.00	67:1
Swine manure (SM)	120	48.92 ± 1.37	0.83 ± 0.04	0.31 ± 0.01	59:1

^aThe manure organic carbon (C) content was calculated from the organic matter (OM) content (measured as loss on ignition LOI) using the coefficient 1.917, which determines the ratio between OM/C according to Larney et al. (2005). The nitrogen content (N) was determined according to ISO 11261:1995, phosphorus content (P) was determined after extraction with Mehlich III reagent (Mehlich, 1984), using an atomic absorption spectrometer (AAS), specifically Agilent 55B AA (Agilent Technologies, Santa Clara, CA, USA).

SM1 = silage maize var. Subito FAO 260, 90000 seeds·ha⁻¹

SM2 = silage maize var. SY Kardona FAO 250, 90000 seeds·ha⁻¹

SB = spring barley var. Laudis 550, 170 kg·ha⁻¹

WW2 = winter wheat var. Dagmar, 190 kg·ha⁻¹

WW1 = winter wheat var. Secese, 190 kg·ha⁻¹

WW3 = winter wheat var. Frisky, 140 kg·ha⁻¹

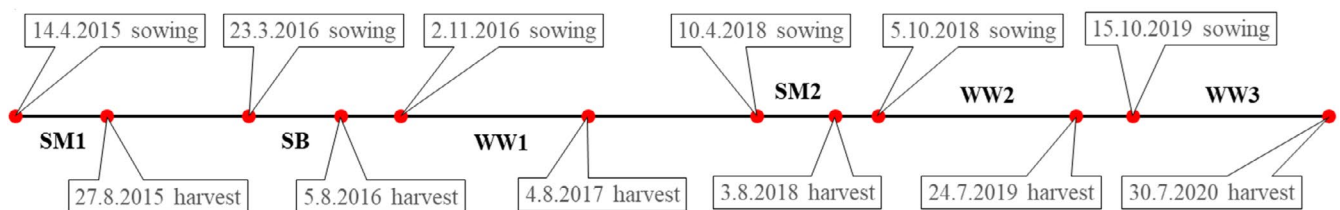


FIGURE 1 Schematic diagram of the crop growing timeline of the experiment.

monthly. All types of manure were applied to the soil in the fall using a manure spreader and KÖckerling Vario 480 tine cultivator, which operated at depths of up to 10 cm.

2.2 | Crop establishment, fertilizer application, harvest and sampling

During the experiment conducted from 2014 to 2020, the following crops were grown, as shown in Figure 2 and described below:

- Silage maize (*Zea mays* L., variety Subito FAO 260, sowing density 90,000 seeds·ha⁻¹, sowing/harvest: 14 April–27 August 2015).
- Spring barley (*Hordeum vulgare* L., variety Laudis 550, sowing density 170 kg·ha⁻¹, sowing/harvest: 23 March–5 August 2016).
- Winter wheat (*Triticum aestivum* L., variety Secese, sowing density 190 kg·ha⁻¹, sowing/harvest: 2 November 2016–4 August 2017).
- Silage maize (variety SY Kardona FAO 250, sowing density 90,000 seeds·ha⁻¹, sowing/harvest: 10 April–3 August 2018).

- Winter wheat (variety Dagmar, sowing density 190 kg·ha⁻¹, sowing/harvest: 5 October 2018–24 July 2019).
- Winter wheat (variety Frisky, sowing density 140 kg·ha⁻¹, sowing/harvest: 15 October 2019–30 July 2020).

Standard pre-sowing soil preparation was performed before planting each crop. Fertilizer application was applied to meet nutrient requirements based on crop-specific norms (in kg·ha⁻¹):

- Silage maize: 185 N, 30 P, 190 K.
- Spring barley: 140 N, 30 P, 80 K.
- Winter wheat: 180 N, 35 P, 95 K.

Crop sampling was conducted annually during harvest using a standard self-propelled combine harvester. Each of the three subplots per variant was harvested entirely, and the produce was collected in a tractor trailer. The trailer was weighed using a mobile axle scale with a capacity of 0–15 t and an accuracy of ±1 kg.

The yield (in tonnes per hectare) for each variant was recalculated into cereal units (CU), which are based on the animal feeding value and standardized to a grain moisture content of 14%. Grain moisture was determined

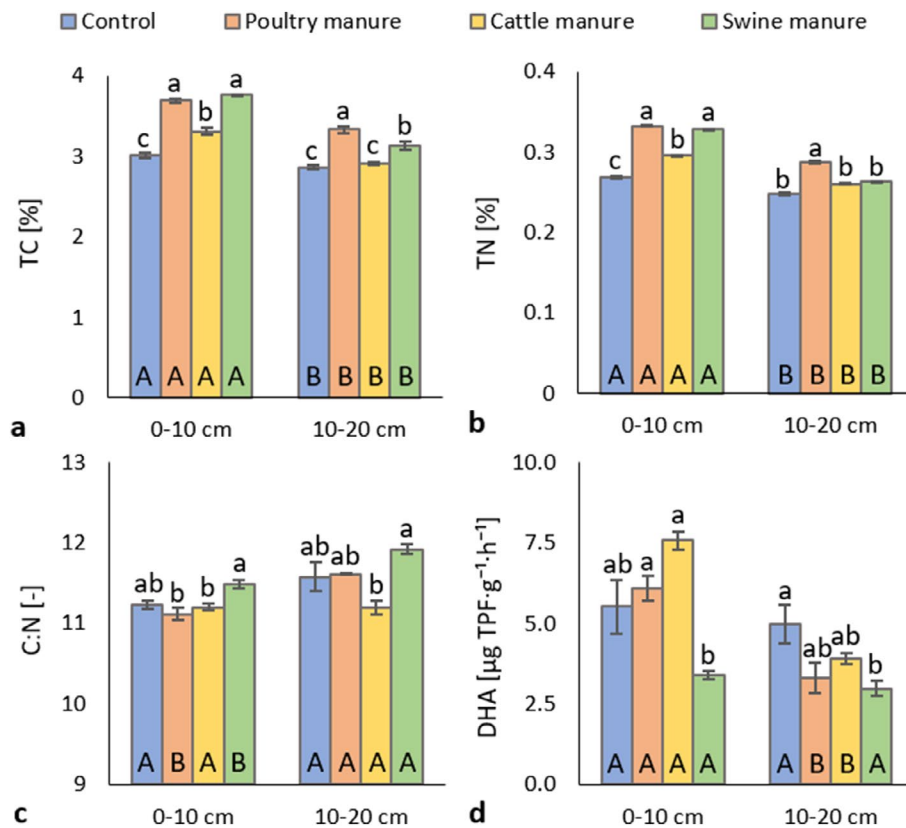


FIGURE 2 Soil nutrient content and dehydrogenase activity in L_{0–10} soil (0–10 cm) and L_{10–20} soil (10–20 cm) of experimental manure-amended variants. Mean values \pm SE of soil total carbon (a) total nitrogen (b), the total carbon to total nitrogen ratio (c), and dehydrogenase activity (d) at different manure treatments and sampled depths ($n=3$). Small letters indicate differences among variants at one depth at a statistical level of significance $p \leq .05$ (Tukey's HSD test); capital letters indicate differences between sampled depths (one-way ANOVA).

gravimetrically by weighing the harvested grains after drying at 60°C to a constant weight.

CU enables comparison of different agricultural products and was calculated by multiplying yield values (in tonnes per hectare) by coefficients provided by Mönking et al. (2010) such as silage maize: 0.3; winter wheat: 1.04; and spring barley: 1.0.

2.3 | Soil sampling and laboratory analyses

After the harvest of winter wheat in 2020, a composite soil sample was taken from every experimental subplot with an auger by merging 8 individual soil cores (per a replication subplot) ranging (25 mm) diameter and height (200 mm) from the depth 0–10 cm (abbreviation L_{0–10}) and 10–20 cm (L_{10–20}), the marking of soil samples according to variant and depth is displayed in Table 3.

Bulk density (BD) was determined by using a soil sample ring kit (Eijkelkamp, Giesbeek Netherlands). The volume of each ring was 100 cm³, and sampling was

TABLE 3 Experimental and sampling variants.

Amendment	Abbreviation	0–10 cm	10–20 cm
Control	CK	CK_0–10	CK_10–20
Poultry manure	PM	PM_0–10	PM_10–20
Cow manure	CM	CM_0–10	CM_10–20
Swine manure	SM	SM_0–10	SM_10–20

performed in three repetitions per variant, according to the national standard (ISO_17892-2:2014). Soil samples were transported immediately to the laboratory, homogenized, sieved to fine earth (≤ 2 mm fraction, through Retsch Test Sieves) and subsampled for further analyses. Air-dried fine samples were analysed for total carbon (TC) and total nitrogen (TN) using the Vario Macro Cube (Elementar Analysensysteme GmbH, Langensfeld, Germany). Fresh fine samples were stored at 4°C and analysed for dehydrogenase activity (DHA) using 2,3,5-triphenyltetrazolium chloride (TTC) method (Doi & Ranamukhaarachchi, 2009), basal respiration (BR) and respiration induced by D-glucose (Glc-IR), D-mannose (Man-IR), protocatechuic acid (Pro-IR), D-trehalose

(Tre-IR), N-acetyl- β -D-glucosamine (NAD-IR), L-alanine (Ala-IR) and L-arginine-IR (Arg-IR) using MicroResp[®] device (The James Hutton Institute, Scotland) according to (Campbell et al., 2003). Each (of three) composite soil sample which corresponded to each of 3 subplots (per each one of 4 experimental plots, see details above) was measured repeatedly (3 analytical replicates for TC, TN, DHA; 4 replicates for BR and IRs) to reduce measurement uncertainty (mainly due to the high internal variability of the soil biological properties of the samples).

The values of induced respiration were used to calculate the microbial functional diversity (MFD) expressed as a Shannon's index (H'), defined by Staddon et al. (1997) and by Iovieno et al. (2021). Shannon's index was in the respective study used as a parameter to describe richness and evenness in community level physiological profiling (CLPP) using Biolog sole C source utilization tests. In this study, the respective approach designed for Biolog sole C source utilization tests was adopted and modified to MicroResp[®] and MFD was calculated according to Equation 1.

$$\text{MFD} = -\sum p_i \ln p_i \quad (1)$$

where p_i is the ratio of the activity on a particular substrate with respect to the sum of activities on all substrates, and \ln is the natural logarithm.

The soil bulk density values [$\text{g}\cdot\text{cm}^{-3}$] and the TC and TN contents [%] were multiplied with the sampling depth [cm] to calculate the soil TC and TN stocks [$\text{t}\cdot\text{ha}^{-1}$] of a particular soil layer at each experimental plot, using Equations 2 and 3:

$$\text{TC}_{0-10} \text{ (or } \text{TC}_{10-20}) [\text{t}\cdot\text{ha}^{-1}] = 10.000 \text{ m}^2 \times 0.1 \text{ m} \times \text{BD} [\text{g}\cdot\text{cm}^{-3}] \times \text{TC}^* [\%]/100 \quad (2)$$

$$\text{TN}_{0-10} \text{ (or } \text{TN}_{10-20}) [\text{t}\cdot\text{ha}^{-1}] = 10.000 \text{ m}^2 \times 0.1 \text{ m} \times \text{BD} [\text{g}\cdot\text{cm}^{-3}] \times \text{TN}^* [\%]/100 \quad (3)$$

*values determined in depth 0–10 cm or 10–20 cm.

2.4 | Data treatment and statistical analysis

One-way ANOVA was used to test the treatment effects on selected soil properties as well as the differences between the two sampling depths. Tukey's HSD (honestly significant difference) test was used to test the pairwise differences among treatments in each depth and among the total crop yields of each variant within 6 years of the experiment. Principal component analysis (PCA) was

used for the characterization of relationships among selected soil properties (R Core Team, 2020). The results were graphically presented with a Rohlf biplot for standardized PCA to display significant synergy or antagonism among soil properties. All statistical analyses were performed in R (R Core Team, 2020).

3 | RESULTS

3.1 | Soil physico-chemical properties and dehydrogenase degradation activity

Bulk density (BD) considerably differed among the variants, as shown in Table 4. The organic unamended (NPK fertilized) variant showed the highest BD at both soil depths, manure treatments decreased the bulk density to 5% (PM₀₋₁₀, PM₁₀₋₂₀, CM₀₋₁₀, CM₁₀₋₂₀) and 13% (SM₀₋₁₀, SM₁₀₋₂₀) lower values.

TC and TN of all tested variants were significantly ($p \leq .05$) higher (10%–43% and 8%–27%, respectively) in L₀₋₁₀ compared to L₁₀₋₂₀. All manure-treated variants had significantly ($p \leq .05$) increased TC and TN in L₀₋₁₀ (10%–25% and 11%–22%, respectively) in comparison to CK₀₋₁₀ (Figure 2a,b), PM-, SM-treated soil had significantly ($p \leq .05$) higher TC and TN compared to CM-treated variant. L₁₀₋₂₀ TC values of PM and SM variants were significantly ($p \leq .05$) higher than CK and CM variants (9%–16% and 8–15%), while TN was only significantly higher in the PM (12%–16%) variant. However, the SM-treated soil had a significantly higher C:N ratio (2%–3%) compared to the other manure-treated variants in L₀₋₁₀ and compared to the CM variant in the L₁₀₋₂₀ soil, indicating a potentially lower bioavailability of N following the application of SM. Moreover, the SM-treated soil had significantly ($p \leq .05$) lower DHA (80%–125%) compared to the other manure-treated variants in L₀₋₁₀ and compared to CK₁₀₋₂₀ (40% less) in L₁₀₋₂₀ soil, indicating the reduced degradation activity in soils treated with SM. DHA was negatively related to the C:N ratio in L₀₋₁₀ soil (Figure S1a).

3.2 | Nutrient stock in soil and the total crop yield

SM-treated variant had the highest total manure-derived nutrient stocks during the whole experiment in L₀₋₁₀ for both carbon and nitrogen (10%–16% and 8%–14% more; Table 4). PM-amended soil 10–20 cm showed as the only variant positive nitrogen stock and the second highest C stock (2% and 1% more than CK; Table 4),

TABLE 4 The bulk density, total carbon (TC) and total nitrogen (TN) stocks and the manure-derived soil TC and TN stocks in the experimental manure-amended variants.

0–10 cm				10–20 cm				
Variant	BD [g·cm ⁻³]			Variant	BD [g·cm ⁻³]			
CK_0–10	1.327			CK_10–20	1.353			
PM_0–10	1.158			PM_10–20	1.287			
CM_0–10	1.262			CM_10–20	1.284			
SM_0–10	1.259			SM_10–20	1.181			
Variant	TC _{0–10}	TC _{10–20}	TN _{0–10}	TN _{10–20}	TC st _{0–10}	TC st _{10–20}	TN st _{0–10}	TN st _{10–20}
	[t·ha ⁻¹]							
CK	39.93	38.08	3.58	3.32	–	–	–	–
PM	42.74	38.69	3.82	3.36	2.81	0.61	0.24	0.04
CM	41.78	36.73	3.79	3.28	1.84	–	0.20	–
SM	47.47	39.54	4.16	3.27	7.54	1.46	0.57	–

Note: TC_{0–10} (TC_{10–20}), TN_{0–10} (TN_{10–20}) = total stock of carbon (nitrogen) in upper (deeper) soil layer on area 1 ha; TCst_{0–10} (TCst_{10–20}), TNst_{0–10} (TNst_{10–20}) = increment in carbon (nitrogen) stock in manure-treated variants in comparison to control variant in upper (deeper) soil layer; TC and TN element stocks were calculated using average bulk density values for each treatment variant. Manure-related stock (TCst, TNst) was calculated as difference between soil TC (TN, respectively) stocks of each manure-amended variant and of NPK-fertilized control.

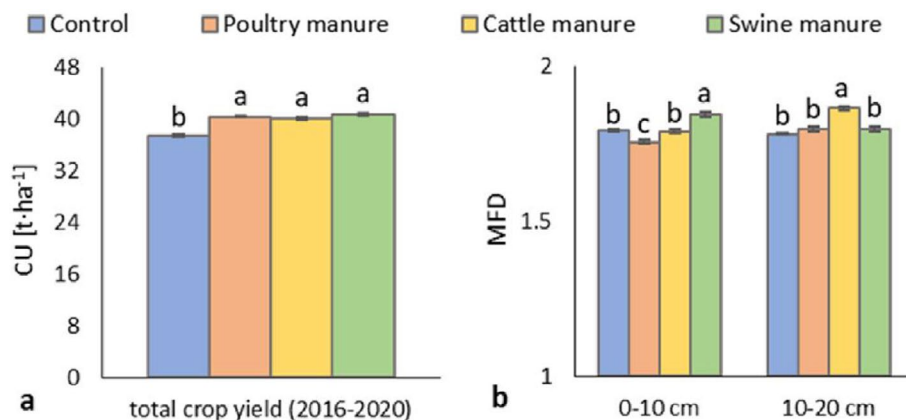


FIGURE 3 Annual and total crop yield per hectare and microbial functional diversity (MFD) in the experimental control and manure-amended soil variants. Displayed are average values \pm standard error of mean (SEM) of (a) total crop yield (2016–2020), calculated as cereal units (CU), and (b) MFD values, calculated from the values of SIRs; small letters indicate differences among variants at a statistical level of significance $p \leq .05$ (Tukey's HSD test).

albeit it acquired the smallest C input via applied manure (Table 1). The lowest N input via swine manure (compared to other manures, Table 1) mediated the highest nitrogen stock (65% more than CM) in L_{0–10} of the respective soil variant.

Each manure-treated variant showed significantly ($p \leq .05$) higher crop yield (7%–9%), calculated for the whole experimental period, compared to the NPK-fertilized control, but statistically comparable between PM, CM and SM (Figure 3a). However, it showed a trend positively related to the results of nutrient stock (Table 4), the highest value was found in the swine manure-amended variant.

3.3 | Respiration indicators of soil functional diversity

DHA showed markable negative relation with Arg-IR in L_{0–10} (PCA biplot, Figure S1a). In L_{10–20}, DHA showed antagonism with three substrate-induced respirations (Glc-, Pro-, Man-IR; PCA biplot, Figure S1b). Basal respiration of PM was significantly ($p \leq .05$) increased in both depths (0–10, 10–20 cm) in comparison to CK (42% and 119% more) and unaffected by depth. On the contrary, CM was comparable to CK in L_{0–10} and increased in L_{10–20} (6% and 105% higher BR), while the SM variant

was increased (52% of CK value) in L₀-10 while decreased in 10-20 cm (comparable to CK; Figure 4a). Glc-IR (employing versatility of D-glucose) and other saccharide-induced IR types (Tre-, NAG-, Man-IR) were significantly increased compared to CK all in L₀-10 (54%–245% more than control), but not each of them (the highest values 123% for Tre-IR while no decrease for Glc-IR in CM₁₀-20) at L₁₀-20 (Figure 4b,d-f). Significantly lowered Glc-, Tre-, NAG-, Man-IR values of all variants in 10-20 cm in comparison to 0-10 cm indicated a negative effect of depth on induced soil respiration (Figure 4). Pro-IR was significantly ($p \leq .05$) increased only by PM treatment (80% and 135% compared to CK) in both depths, whereas SM decreased its value in 0-10 cm (35%) and was comparable in

10-20 cm (but 66% higher in average; Figure 4c). Pro-IR of other variants (CK, PM, CM) decreased with depth (47%–110%).

Amino acid-induced IR types—Ala-IR (Figure 4g) and Arg-IR (Figure 4h)—in L₀-10 were significantly ($p \leq .05$) increased (71% and 250% compared to CK) only in the SM variant. In L₁₀-20, Ala-IR was higher (59% than CK) in CM and Arg-IR higher (133%–300%) in all manure-amended variants (Figure 4g,h). Whereas all variants showed decreased Ala-IR with depth (39%–89%), the PM value of Arg-IR was unaffected by depth and the CM value was increased (38%). TC and TN were expectedly synergic with basal respiration and almost all IR types (except of Pro-, Man-, Arg-IR) in the

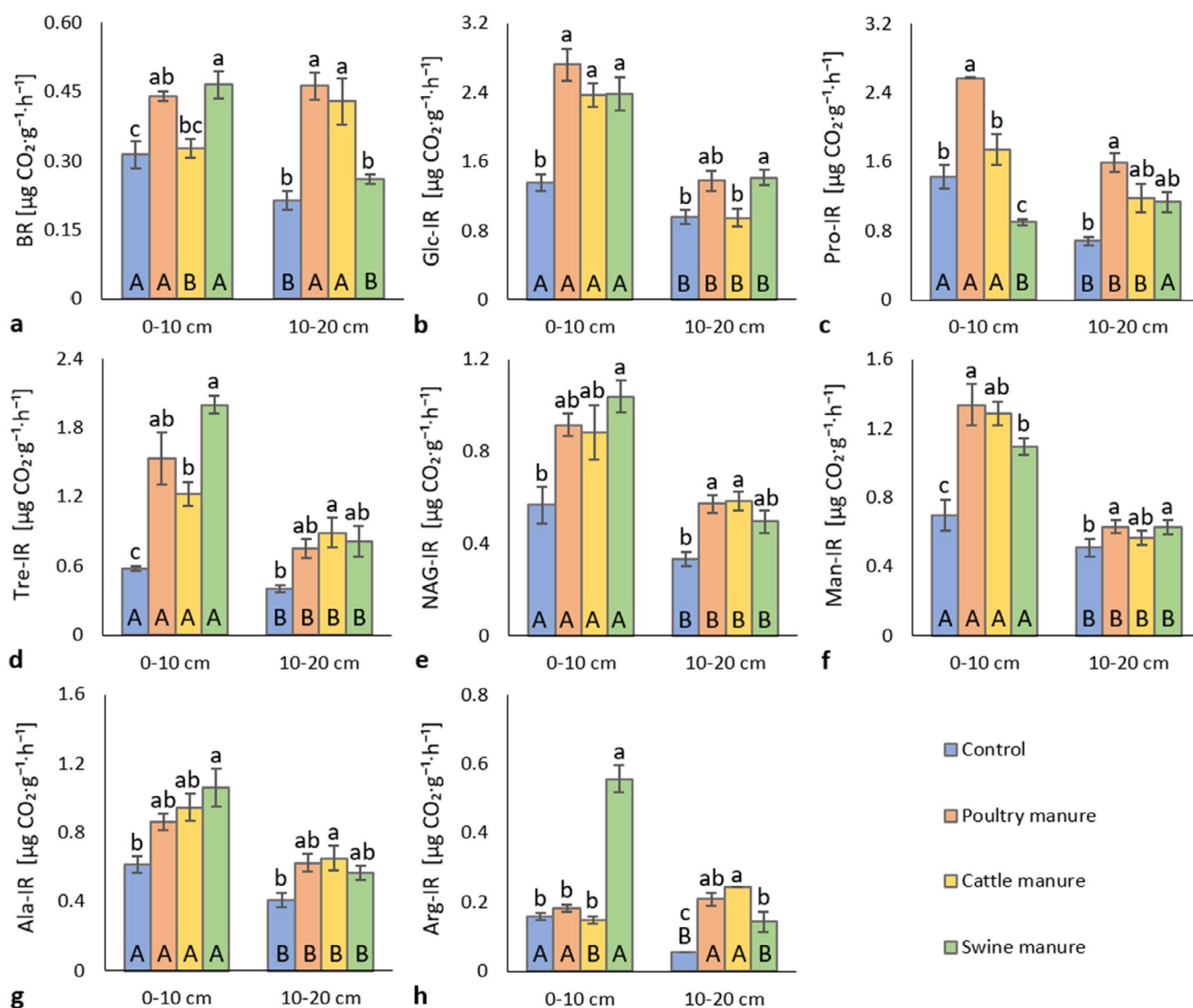


FIGURE 4 Soil respiration types in L₀-10 soil (0-10 cm) and L₁₀-20 soil (10-20 cm) of experimental manure-amended variants. Displayed are average values ($n = 3$) of basal respiration (a), Glc-IR (b), Pro-IR (c), Tre-IR (d), NAG-IR (e), Man-IR (f), Ala-IR (g) and Arg-IR (h); error bars represent standard error (SE). Small letters indicate differences among variants at one depth at a statistical level of significance $p \leq .05$, and capital letters indicate differences between two depths in one variant at a statistical level of significance $p \leq .05$.

L₀–10 (Figure S1a), while in L₁₀–20 the synergy of TC and TN was found only with Glc-, Pro- and Man-IR (Figure S1b). BR and IRs were significantly impacted by both manure types and soil depth. PCA biplots showed the strongest synergy for BR, Tre-, NAG- and Ala-IR in L₀–10, while in L₁₀–20 for BR, Tre-, NAG-, Ala- and also Arg-IR (Figure S1a,b).

Values of MFD (microbial functional diversity (Figure 3b)) showed that the application of swine manure had a positive effect on the functional diversity of the L₀–10 soil microbiome (3% more than CK₀–10), while in the 10–20 cm layer, only the amendment of cattle manure led to markedly (5%) increased microbial functional diversity (Figure 3b).

4 | DISCUSSION

4.1 | Soil total carbon, nitrogen, degradation activity and nutrient stock

In this experiment, the amendment of varying amounts of manure with diverse properties was expected to alter soil nutrient content, directly depending on the total input of carbon and nitrogen (Table 1). Each manure type increased TC content due to the significant addition of external organic matter (EOM) to the soil, consistent with the findings of Yost et al. (2022). Despite the highest C input from cattle manure (CM), the CM treatment resulted in lower TC content (compared to PM and SM) in both L₀–10 and L₁₀–20 (Figure 2a). However, it exhibited the highest DHA activity in the L₀–10, indicating higher C losses through SOM mineralization. This high DHA activity suggests that CM contributed the largest input of less recalcitrant organic matter compared to PM and SM.

The high C:N ratio in CM (and SM) (Table 1) may indicate an incomplete composting process of cattle manure, as previously noted by (Atallah et al., 1995). Nitrogen unavailability and immobilization due to the high C:N ratio could have contributed to this issue (Azim et al., 2014; Zhan et al., 2021). In the line with Atallah et al. (1995), incompletely composted cattle manure likely retained a significant proportion of labile, easily mineralizable carbon sources, which, under NPK fertilizer application, led to enhanced organic matter degradation and DHA activity. As a result, cattle manure contributed to the lowest manure-derived carbon stocks among all manure treatments, both in the upper (1.84 t·ha⁻¹) and lower soil layers (not positive balance of stocks) (Table 4).

Manure properties, as highlighted by Wan et al. (2021), are influenced by the source livestock species (poultry,

cattle or swine) and breeding management practices such as bedding type retention time in stables. These differences likely contributed to the significant variations in carbon stocks between cattle and swine manure, despite comparable total carbon inputs. Frequent removal of bedding in cattle stables contrasts with the deep litter housing of swine, which allows for longer and more complete composting (Didier, 1999). Subsequently, SM with more stabilized EOM likely had a lower biodegradation index in soil compared to CM, as much of the labile carbon in swine manure have been consumed during composting in the stables Miller et al. (2014).

Differences in carbon mineralization during manure maturation were likely mediated by microbial consortia from stable management practices, influencing the composting process (Aguilar-Paredes et al., 2023; Khatibi & Hassani, 2021). These differences, along with variations in compost composition, contributed to distinct long-term carbon and nitrogen stocks in soil treated with three types of manure, as previously observed by Griffin and Hutchinson (2007).

These findings support hypothesis (i), although the relationship between microbial functional diversity (as indicated by SIR) and C and N content was demonstrated only in the L₀–10 via PCA Rohlf's biplot (Figure S1a). Moreover, C and N contents were positively related to NAG_SIR, Tre_SIR and Ala_SIR, which are indicators of soil fungal biomass turnover, suggesting that fungal communities responded most closely to nutrient levels in the soil.

Soil depth further influenced carbon distribution, with limited TC accumulation in L₁₀–20. However, the PM variant exhibited the highest TC in this layer (Figure 2a). It is possible that complex carbon compounds from poultry manure were gradually incorporated into SOM or decomposed into dissolved organic carbon (DOC). These processes may have contributed to carbon leaching and deposition in the 10–20 cm soil, similarly as referred by (Andersson et al., 2000).

Soil total nitrogen (TN) content also appeared related to manure-derived nitrogen inputs, which decreased from poultry manure to cattle manure and swine manure (Table 1). Despite differences in applied N doses, the final TN pattern in L₀–10 mirrored TC trends: poultry and swine manure enriched soil nitrogen significantly more than cattle manure, while all manure treatments increased TN compared to the control (Figure 2b).

The largest differences in N stocks were observed between CM and SM, despite higher inputs of C and N in CM. This discrepancy is likely due to incomplete composting of CM, which can lead to rapid mineralization and high losses of mineral N through volatilization or

leaching, as suggested by Cambardella et al. (2003). These findings support the hypothesis (i).

On the contrary, swine manure, with a higher C:N ratio and more stabilized compost (Cotrufo et al., 2019), likely promoted gradual N mineralization, improving nitrogen use efficiency (NUE) and reducing losses. These results align with findings by Qian and Schoenau (2002) who found that manures with a higher C:N ratio (over 15) decreased N availability due to retarded N mineralization in the short term. This gradual and delayed N mineralization likely prevented excessive losses of mineral N and improved nitrogen use efficiency (NUE) in swine manure-treated soil compared to cattle manure-treated soil, as similarly reported by (Nishida, 2011; Stumborg et al., 2007). However, the moderate and gradual N mineralization in the SM treatment likely did not produce sufficient leachable nitrogen forms in the surface soil to enable significant transport into deeper soil layers. Consequently, in the L_{10–20}, only the PM treatment significantly increased TN compared to CM. The enhanced ability of poultry manure to deliver N to deeper soil layers, as shown in Figure 2b, has also been reported previously (Hoover et al., 2019; Mohamed Am et al., 2010).

4.2 | Total crop yield

The highest nutrient stock (TCst and TNst) in the swine manure-treated variant (Table 4) was in line with the calculated highest average total crop yield (in tonnes per hectare and cereal units, Figure 3a). A possible explanation for this most beneficial effect of swine manure could be the differences in nutrient mineralization rates and associated nutrient losses (particularly nitrogen) through leaching or volatilization.

As discussed in the previous (Section 4.1), the higher recalcitrance of swine manure organic matter, compared to other manure types, as evidenced by its lower DHA activity (a key SOM-degrading enzyme), is likely a contributing factor. This effect is generally observed in soils with higher inputs of stabilized C and reduced availability of dissolved organic carbon (DOC) or labile C forms (Zheng et al., 2016).

The finding that swine (and other livestock-derived) manure application supplemented with auxiliary NPK amendments can achieve comparable or higher crop yields than long-term application of NPK alone aligns with reported advantages of swine manure (Mustafa et al., 2021). Manure, particularly swine manure, releases its nutrients gradually, providing a steady supply over time, unlike synthetic fertilizers, which are prone to rapid leaching from the soil (Adekiya et al., 2019). This positive effect of swine

manure treatment may even be more beneficial due to the long-term simultaneous increase in soil nutrient storage, which is advantageous both economically and environmentally for sustainable crop production joint with gradual soil quality improvement and carbon enrichment as highlighted by Jiang et al. (2018), who recommended regular use of manure with mineral fertilizers as essential for the long-term dual functions of soil for food production and soil carbon sequestration. Regular amendment of swine manure may also serve to rational agro-waste disposal (Moraes Tavares et al., 2019). Moreover, the observed higher yields under manure application could be attributed to manure's ability to increase the soil's water-holding capacity, reducing water stress during dry periods and ensuring that plants have sufficient moisture for growth (Hasnain et al., 2020).

4.3 | Degradation, respiration indicators, soil functional diversity

DHA activity, an indicator of SOM degradation, showed no significant relationship with TC and TN in the L_{0–10} and exhibited an antagonistic relationship in the L_{10–20} (Figure S1a,b). Furthermore, DHA responded differently to manure amendments compared to most respiration indicators. Although no manure amendment significantly increased DHA compared to the unamended control in the L_{0–10}, poultry and cattle manure differed in their effects on DHA activity compared to swine manure (Figure 2d), supporting hypothesis (i).

In the L_{10–20}, the DHA value for the SM variant was significantly lower than that of the control, suggesting that swine manure suppressed DHA activity in the amended soil. This suppression could be attributed to the higher proportion of stabilized organic carbon in the EOM of SM. Additionally, the lower BR value and higher C:N ratio observed in the SM variant in the L_{10–20} layer indicated a tendency toward carbon stock accumulation. These findings suggest an altered soil microbiome with distinct diversity and specificity, aligning with hypothesis (ii).

In contrast, the SM-amended soil exhibited the highest rate of aerobic carbon mineralization (indicated by BR) in the upper layer, coupled with the highest microbial functional diversity (MFD) among all experimental variants (Figure 3b). This finding pre-determined the most efficient nutrient acquisition from manure-derived EOM. Domeignoz-Horta et al. (2020) similarly reported that microbial diversity drives carbon use efficiency.

Soil basal respiration (BR) and substrate-induced respirations (SIRs) serve as indicators of carbon mineralization and the soil microbiome's ability to utilize variable carbon

sources. These parameters are strongly influenced by soil community diversity. The highest TC and TN in PM_{0–10} and SM_{0–10} indicate that poultry and swine manure amendments enhanced basal respiration. Significantly higher BR values in PM and CM, not only compared to control, but also to SM in the L_{0–10}, further support this observation.

These findings indicate that DOC and other labile, easily oxidizable carbon fractions from the EOM in PM and CM were distributed into deeper layers, where they increased TC content and enhanced respiration activity. This provides verification of hypothesis (i). Additionally, the greater involvement of aerobic microbes in SOM degradation activities in the L_{10–20} of variants PM and CM suggests altered microbial community diversity and specificity. This was supported by the highest MFD value observed in the CM_{10–20} variant (Figure 3b), corroborating hypothesis (i).

Demir and Gülser (2008) reported a similar trend of increased soil organic carbon (SOC) content at deeper soil depths and decreased SOC in the surface layer after the application of organic waste-based amendments (Demir & Gülser, 2008). In addition, Jones et al. (2018) observed rapid microbial activity in the L_{10–20} following the addition of DOC and DON substrates, which aligns with the enhanced microbial activity in deeper layers of the PM and CM variants.

However, higher respiration potential to mineralize easily degradable carbon sources (such as saccharides) was still detectable in the L_{0–10} soil than in the L_{10–20} soil which finding was expectable with respect to the soil aeration, carbon content composition and conditions which determine the thriving of aerobic microbes in deeper soil layers (Fang et al., 2015; Fang & Moncrieff, 2005). The largest proportional decrease in Glc-IR with soil depth was found for cattle manure-treated soil (−59.9%), compared to PM-treated (−49.3%) and SM-treated soil (−40.8%). Probably, aerobic microbes in 10–20 cm were more active (see results of BR), but less abundant and thus weakly inducible. Trends in Glc-IR and Man-IR values were similar, as both of them indicate aerobic microbial potential to degrade soil plant waste matter components, cellulose and hemicelluloses. PM variant showed concurrently the highest average Glc-, Man-, as well as Pro-IR in the L_{0–10} soil (Figure 4b,c,f), which finding assumed that poultry manure derived more abundant degraders of straw in the soil microbial community and corroborated the hypothesis (ii). Higher content of straw in bedding used for production of poultry manure could be ascribed from these results, as Hassan (2013) reported higher cellulose and hemicellulose content in straw-derived organic matter amended to soil (Hassan, 2013). Zhang (2022), (2022) also

referred to in lignin distribution, structure and morphology in wheat straw (Zhang, Larsson, et al., 2022), which could promote enhanced lignin degradation (to protocatechuic acid) provided by soil fungi, as they represent leading and the most efficient lignin degraders in nature (Silva et al., 2021). Higher content of D-trehalose, an excretion product of fungi (Thevelein, 1984), and N-acetyl- β -D-glucosamine as a degradation product of chitin could both indicate increased fungal biomass in soil. Both compounds were found most efficient in respiration induction of SM L_{0–10} soil, which finding could indicate the previously assumed higher content of more stable organic carbon in EOM, for example in the form of highly recalcitrant lignin residues, which only could be degraded under the effect of lignocellulolytic fungi (Sánchez, 2009).

Also, results of Ala- and Arg-IR determination indicated that the functional composition of the soil microbial community in the SM variant markedly differed from the other two variants, PM and CM (Figure 4g,h). Whereas all 10–20 cm manure-amended variants exerted comparable Ala-IR values (significantly higher than control), the value of L_{0–10} soil SM variant was again the significantly highest. This was in line with the findings of Lima et al. (2009), who reported about the contribution of amended farmyard manure to both lignin and lignin-like as well as protein and protein-like content in SOM (Lima et al., 2009). Presumably, the increased content of more recalcitrant lignin and soil protein in swine manure-amended L_{0–10} was ascribed and evidenced by the highest values of Ala-IR, and by the markedly highest respiration induced by the nitrogen-rich substrate, L-arginine (Figure 4h). L-arginine utilization is an indicator of microbial activity in soil (Giacometti et al., 2013), reported to be positively related to increased microbial biomass and N mineralization due to farmyard manure application (Kandeler et al., 1999). As L-arginine transformation (determined mostly as arginine deaminase) is an important indicator of nitrogen (N) mineralization, the positive impact of swine manure addition on long-term, gradual soil nitrification could be ascribed from these observations. This ascription corroborates hypothesis (i) and if increased abundance of soil nitrifiers was presumed, it verified also hypothesis (ii). The still high nitrogen mineralization rate at the end of the experiment (compared to e.g. CM variant) could indicate slower progress in swine m. EOM-derived nitrogen utilization during the whole period (2016–2020), which might have mitigated presumable losses of nitrogen from the amended soil, for example, via volatilization (in the ammonia form) or leaching. Thus, the SM variant sequestered (in contrast to the variants PM and SM) the highest amount of soil TN despite the lowest manure-derived input of nitrogen during the

whole experiment (Table 1). Moreover, the functional microbial diversity in the SM variant was the highest, in contrast to the narrowed functional diversity in the PM and CM variants. This reduction in MDF for PM and CM could be ascribed to a presumed shift toward copiotrophic taxa, as described for soils with increased access to labile carbon sources (DOC) (Ma et al., 2021; Yang et al., 2022). Similarly, Lin et al. (2019) reported strong effects of swine manure on SOM enrichment and microbiome diversity, with a shift toward taxa associated with recalcitrant SOM, particularly in microaggregates.

5 | CONCLUSIONS

This study confirmed that manure types (swine, cattle, poultry) influence soil microbial activity through differences in soil total carbon, nitrogen content and their degradability. Higher carbon and nitrogen inputs boosted decomposition activity due to the diversity of microbial species. Poultry manure significantly increased nutrient content and basal respiration, whereas swine manure had the highest carbon and nitrogen stock, upper soil microbial diversity and total crop yield (2016–2020), most likely due to its higher recalcitrant organic matter concentration. Cattle dung suffered rapid nutritional losses due to varied bedding litter processing. The third hypothesis, predicting more decomposition activity in the upper soil layer (0–10 cm) than the deeper layer (10–20 cm), was partly validated, as total carbon and nitrogen levels, dehydrogenase activity, basal respiration and most substrate-induced respirations declined with depth. It was concluded that, nevertheless, soil depth influenced manure treatment effectiveness, manure characteristics had a greater impact on soil quality. Different manure types had varying effects on nitrogen stock and crop yields, indicating that other manure forms other than swine manure could improve soil fertility.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Adekiya, A. O., Agbede, T. M., Aboyeji, C. M., Dunsin, O., & Simeon, V. T. (2019). Effects of biochar and poultry manure on soil characteristics and the yield of radish. *Scientia Horticulturae*, 243, 457–463. <https://doi.org/10.1016/j.scienta.2018.08.048>
- Aguilar-Paredes, A., Valdés, G., Araneda, N., Valdebenito, E., Hansen, F., & Nuti, M. (2023). Microbial community in the composting process and its positive impact on the soil biota in sustainable agriculture. *Agronomy*, 13, 542. <https://doi.org/10.3390/agronomy13020542>
- Anandakumar, S., Bakhoun, N., Chinnadurai, C., Malarkodi, M., Arulmozhiselvan, K., Karthikeyan, S., & Balachandar, D. (2022). Impact of long-term nutrient management on sequestration and dynamics of soil organic carbon in a semi-arid tropical Alfisol of India. *Applied Soil Ecology*, 177, 104549. <https://doi.org/10.1016/j.apsoil.2022.104549>
- Andersson, S., Nilsson, S. I., & Saetre, P. (2000). Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. *Soil Biology and Biochemistry*, 32, 1–10. [https://doi.org/10.1016/S0038-0717\(99\)00103-0](https://doi.org/10.1016/S0038-0717(99)00103-0)
- Atallah, T., Andreux, F., Chone, T., & Gras, F. (1995). Effect of storage and composting on the properties and degradability of cattle manure. *Agriculture Ecosystems & Environment*, 54(3), 203–213. [https://doi.org/10.1016/0167-8809\(95\)00595-j](https://doi.org/10.1016/0167-8809(95)00595-j)

- Azim, K., Ouyahia, K., Amellouk, A., Perissol, C., Thami Alami, I., & Soudi, B. (2014). Dynamic composting optimization through CN ratio variation as a startup parameter. In G. Rahmann, & U. Aksoy (Eds.) *4th ISOFAR Scientific Conference 'Building Organic Bridges'*. Organic World Congress.
- Cambardella, C. A., Richard, T. L., & Russell, A. (2003). Compost mineralization in soil as a function of composting process conditions. *European Journal of Soil Biology*, 39(3), 117–127. [https://doi.org/10.1016/s1164-5563\(03\)00027-x](https://doi.org/10.1016/s1164-5563(03)00027-x)
- Campbell, C. D., Chapman, S. J., Cameron, C. M., Davidson, M. S., & Potts, J. M. (2003). A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Applied and Environmental Microbiology*, 69, 3593–3599. <https://doi.org/10.1128/AEM.69.6.3593-3599.2003>
- Cassandro, M. (2020). Animal breeding and climate change, mitigation and adaptation. *Journal of Animal Breeding and Genetics*, 137, 121–122. <https://doi.org/10.1111/jbg.12469>
- Cotrufo, M. F., Ranalli, M. G., Haddix, M. L., Six, J., & Lugato, E. (2019). Soil carbon storage informed by particulate and mineral-associated organic matter. *Nature Geoscience*, 12, 989–994. <https://doi.org/10.1038/s41561-019-0484-6>
- Dahlin, A. S., Mukangango, M., Naramabuye, F. X., Nduwamungu, J., & Nyberg, G. (2021). Effect of grass-diet and grass-legume-diet manure applied to planting holes on smallholder maize production in Rwanda. *Field Crops Research*, 263, 108057. <https://doi.org/10.1016/j.fcr.2021.108057>
- de Melo, T. R., Figueiredo, A., Machado, W., & Tavares Filho, J. (2019). Changes on soil structural stability after in natura and composted chicken manure application. *International Journal of Recycling of Organic Waste in Agriculture*, 8, 333–338. <https://doi.org/10.1007/s40093-019-0250-1>
- Demir, Z., & Gülser, C. (2008). Changes in organic carbon, NO₃-N, electrical conductivity values and soil respiration along a soil depth due to surface application of organic wastes. *Asian Journal of Chemistry*, 20, 2011–2021.
- Didier, M. (1999). Composting of pigs waste in deep litter systems. *Revue De Medecine Veterinaire*, 150(6), 499–510.
- Dinesh, R., Dubey, R. P., & Prasad, G. S. (1998). Soil microbial biomass and enzyme activities as influenced by organic manure incorporation into soils of a rice-rice system. *Journal of Agronomy and Crop Science*, 181, 173–178. <https://doi.org/10.1111/j.1439-037X.1998.tb00414.x>
- Doi, R., & Ranamukhaarachchi, S. L. (2009). Soil dehydrogenase in a land degradation-rehabilitation gradient: observations from a savanna site with a wet/dry seasonal cycle. *Revista de Biología Tropical*, 57, 223–234. <https://doi.org/10.15517/rbt.v57i1-2.11317>
- Domeignoz-Horta, L. A., Pold, G., Liu, X. A., Frey, S. D., Melillo, J. M., & DeAngelis, K. M. (2020). Microbial diversity drives carbon use efficiency in a model soil. *Nature Communications*, 11(1), 3684. <https://doi.org/10.1038/s41467-020-17502-z>
- Eisen, M. B., & Brown, P. O. (2022). Rapid global phaseout of animal agriculture has the potential to stabilize greenhouse gas levels for 30 years and offset 68 percent of CO₂ emissions this century. *PLOS Climate*, 1, e0000010. <https://doi.org/10.1371/journal.pclm.0000010>
- Epelde, L., Jauregi, L., Urrea, J., Ibarretxe, L., Romo, J., Goikoetxea, I., & Garbisu, C. (2018). Characterization of Composted Organic Amendments for Agricultural Use. *Frontiers in Sustainable Food Systems*, 2, 44. <https://doi.org/10.3389/fsufs.2018.00044>
- Fang, C., & Moncrieff, J. B. (2005). The variation of soil microbial respiration with depth in relation to soil carbon composition. *Plant and Soil*, 268, 243–253. <https://doi.org/10.1007/s11104-004-0278-4>
- Fang, X.-M., Chen, F.-S., Wan, S.-Z., Yang, Q.-P., & Shi, J.-M. (2015). Topsoil and Deep Soil Organic Carbon Concentration and Stability Vary with Aggregate Size and Vegetation Type in Subtropical China. *PLoS One*, 10, e0139380. <https://doi.org/10.1371/journal.pone.0139380>
- Feller, C., Blanchart, E., Bernoux, M., Lal, R., & Manlay, R. (2012). Soil fertility concepts over the past two centuries: the importance attributed to soil organic matter in developed and developing countries. *Archives of Agronomy and Soil Science*, 58, S3–S21. <https://doi.org/10.1080/03650340.2012.693598>
- Giacometti, C., Demyan, M. S., Cavani, L., Marzadori, C., Ciavatta, C., & Kandeler, E. (2013). Chemical and microbiological soil quality indicators and their potential to differentiate fertilization regimes in temperate agroecosystems. *Applied Soil Ecology*, 64, 32–48. <https://doi.org/10.1016/j.apsoil.2012.10.002>
- Gómez-Brandón, M., Lazcano, C., & Domínguez, J. (2008). The evaluation of stability and maturity during the composting of cattle manure. *Chemosphere*, 70, 436–444. <https://doi.org/10.1016/j.chemosphere.2007.06.065>
- Griffin, T. S., & Hutchinson, M. (2007). Compost Maturity Effects on Nitrogen and Carbon Mineralization and Plant Growth. *Compost Science & Amp Utilization*, 15, 228–236. <https://doi.org/10.1080/1065657x.2007.10702338>
- Grossi, G., Goglio, P., Vitali, A., & Williams, A. G. (2018). Livestock and climate change: impact of livestock on climate and mitigation strategies. *Animal frontiers: the review magazine of animal agriculture*, 9, 69–76. <https://doi.org/10.1093/af/vfy034>
- Gryta, A., Frąc, M., & Oszust, K. (2020). Genetic and Metabolic Diversity of Soil Microbiome in Response to Exogenous Organic Matter Amendments. *Agronomy*, 10, 546. <https://doi.org/10.3390/agronomy10040546>
- Hammerschmiedt, T., Holatko, J., Pecina, V., Huska, D., Latal, O., Kintl, A., Radziemska, M., Muhammad, S., Gusiatin, Z. M., Kolackova, M., Nasir, M., Baltazar, T., Ahmed, N., & Brtnicky, M. (2021). Assessing the potential of biochar aged by humic substances to enhance plant growth and soil biological activity. *Chemical and Biological Technologies in Agriculture*, 8, 1–2. <https://doi.org/10.1186/s40538-021-00242-7>
- Hasnain, M., Chen, J., Ahmed, N., Memon, S., Wang, L., Wang, Y., & Wang, P. (2020). The Effects of Fertilizer Type and Application Time on Soil Properties, Plant Traits. *Yield and Quality of Tomato. Sustainability*, 12, 9065. <https://doi.org/10.3390/su12219065>
- Hassan, W. (2013). C and N mineralization and dissolved organic matter potentials of two contrasting plant residues: effects of residue type, moisture, and temperature. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science*, 63, 642–652. <https://doi.org/10.1080/09064710.2013.846398>
- Hoover, N. L., Law, J. Y., Long, L. A. M., Kanwar, R. S., & Soupir, M. L. (2019). Long-term impact of poultry manure on crop yield, soil and water quality, and crop revenue. *Journal of Environmental*

- Management*, 252, 109582. <https://doi.org/10.1016/j.jenvman.2019.109582>
- Iovieno, P., Scotti, R., & Zaccardelli, M. (2021). Functional Diversity of Soil Microbial Community after Conversion of a Chestnut Forest to an Agricultural System. *Agriculture*, 11, 43. <https://doi.org/10.3390/agriculture11010043>
- ISO 11261:1995. *Soil quality-Determination of total nitrogen-Modified Kjeldahl method*. International Organization for Standardization.
- ISO 17892-2:2014. *Geotechnical investigation and testing-Laboratory testing of soil-Part 2: Determination of bulk density*. International Organization for Standardization.
- Jiang, G., Zhang, W., Xu, M., Kuzyakov, Y., Zhang, X., Wang, J., Di, J., & Murphy, D. V. (2018). Manure and mineral fertilizer effects on crop yield and soil carbon sequestration: a meta-analysis and modelling across China. *Global Biogeochemical Cycles*, 32, 1659–1672. <https://doi.org/10.1029/2018gb005960>
- Jones, D. L., Maghlab, E. A., Gleeson, D. B., Hill, P. W., Sánchez-Rodríguez, A. R., Roberts, P., Ge, T., & Murphy, D. V. (2018). Microbial competition for nitrogen and carbon is as intense in the subsoil as in the topsoil. *Soil Biology and Biochemistry*, 117, 72–82. <https://doi.org/10.1016/j.soilbio.2017.10.024>
- Kafle, G. K., & Chen, L. (2016). Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. *Waste Management*, 48, 492–502. <https://doi.org/10.1016/j.wasman.2015.10.021>
- Kandeler, E., Stemmer, M., & Klimanek, E.-M. (1999). Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management. *Soil Biology and Biochemistry*, 31, 261–273. [https://doi.org/10.1016/s0038-0717\(98\)00115-1](https://doi.org/10.1016/s0038-0717(98)00115-1)
- Khatibi, H., & Hassani, A. (2021). Effective management and composting of organic wastes using new developed consortia. *Environment, Development and Sustainability*, 23, 16891–16910. <https://doi.org/10.1007/s10668-021-01383-3>
- Lal, R. (2015). Restoring Soil Quality to Mitigate Soil Degradation. *Sustainability*, 7, 5875–5895. <https://doi.org/10.3390/su7055875>
- Larney, F. J., Ellert, B. H., & Olson, A. F. (2005). Carbon, ash and organic matter relationships for feedlot manures and composts. *Canadian Journal of Soil Science*, 85, 261–264. <https://doi.org/10.4141/s04-060>
- Lejon, D. P., Sebastia, J., Lamy, I., Chaussod, R., & Ranjard, L. (2007). Relationships between soil organic status and microbial community density and genetic structure in two agricultural soils submitted to various types of organic management. *Microbial Ecology*, 53(4), 650–663. <https://doi.org/10.1007/s00248-006-9145-6>
- Lendelová, J., Žitňák, M., Bošanský, M., Šimko, M., & Piterka, P. (2016). Testing of property changes in recycled bedding for dairy cows. *Research in Agricultural Engineering*, 62, S44–S52. <https://doi.org/10.17221/45/2016-rae>
- Li, L., Xu, M., Eyakub Ali, M., Zhang, W., Duan, Y., & Li, D. (2018). Factors affecting soil microbial biomass and functional diversity with the application of organic amendments in three contrasting cropland soils during a field experiment. *PLoS One*, 13, e0203812. <https://doi.org/10.1371/journal.pone.0203812>
- Lima, D. L. D., Santos, S. M., Scherer, H. W., Schneider, R. J., Duarte, A. C., Santos, E. B. H., & Esteves, V. I. (2009). Effects of organic and inorganic amendments on soil organic matter properties. *Geoderma*, 150, 38–45. <https://doi.org/10.1016/j.geoderma.2009.01.009>
- Lin, Y., Ye, G., Kuzyakov, Y., Liu, D., Fan, J., & Ding, W. (2019). Long-term manure application increases soil organic matter and aggregation, and alters microbial community structure and key-stone taxa. *Soil Biology and Biochemistry*, 134, 187–196. <https://doi.org/10.1016/j.soilbio.2019.03.030>
- Liu, H., Du, X., Li, Y., Han, X., Li, B., Zhang, X., Li, Q., & Liang, W. (2022). Organic substitutions improve soil quality and maize yield through increasing soil microbial diversity. *Journal of Cleaner Production*, 347, 131323. <https://doi.org/10.1016/j.jclepro.2022.131323>
- Ma, X.-L., Liu, J., Chen, X.-F., Li, W.-T., Jiang, C.-Y., Wu, M., Liu, M., & Li, Z.-P. (2021). Bacterial diversity and community composition changes in paddy soils that have different parent materials and fertility levels. *Journal of Integrative Agriculture*, 20, 2797–2806. [https://doi.org/10.1016/s2095-3119\(20\)63364-0](https://doi.org/10.1016/s2095-3119(20)63364-0)
- MacLeod, M., Leinonen, I., Wall, E., Houdijk, V. E., Burns, J., Vosough Ahmadi, B., & Gómez-Barbero, M. (2019). *Impact of animal breeding on GHG emissions and farm economics*. European Commission JRC. <https://doi.org/10.2760/731326>
- Mehlich, A. (1984). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Communications in Soil Science and Plant Analysis*, 15, 1409–1416. <https://doi.org/10.1080/00103628409367568>
- Miller, J. J., Beasley, B. W., Drury, C. F., Hao, X., & Larney, F. J. (2014). Soil properties following long-term application of stockpiled feedlot manure containing straw or wood-chip bedding under barley silage production. *Canadian Journal of Soil Science*, 94, 389–402. <https://doi.org/10.4141/cjss2013-087>
- Mkhonza, N. P., Muchaonyerwa, P., & Buthelezi-Dube, N. N. (2022). Carbon dioxide emission, nitrogen mineralisation and spinach dry matter yield in a loamy humic soil amended with lime and poultry manure. *Environmental Monitoring and Assessment*, 194, 110. <https://doi.org/10.1007/s10661-021-09730-7>
- Mohamed Am, M., Sekar, S., & Muthukrish, P. (2010). Prospects and Potential of Poultry Manure. *Asian Journal of Plant Sciences*, 9, 172–182. <https://doi.org/10.3923/ajps.2010.172.182>
- Mönking, S. S., Klapp, C., Abel, H., & Theuvsen, L. (2010). Überarbeitung des Getreide- und Vieheinheitenschlüssels—Endbericht zum BMELV-Forschungsprojekt 06HS030 [Revision of cereal unit and livestock unit—Final report on research project 06HS030 BMELV] (06HS030). Retrieved from Göttingen, 312. <http://download.ble.de/06HS030.pdf>
- Moraes Tavares, R. L., Assis, R. L., Ferreira, R. V., Menezes, J. F. S., Simon, G. A., Boldrin, P. F., & Cantão, V. C. G. (2019). Long term application of pig manure on the chemical and physical properties of Brazilian Cerrado soil. *Carbon Management*, 10, 541–549. <https://doi.org/10.1080/17583004.2019.1676095>
- Mustafa, A., Hu, X., Abrar, M. M., Shah, S. A. A., Nan, S., Saeed, Q., Kamran, M., Naveed, M., Conde-Cid, M., Hongjun, G., Ping, Z., & Minggang, X. (2021). Long-term fertilization enhanced carbon mineralization and maize biomass through physical protection of organic carbon in fractions under continuous maize cropping. *Applied Soil Ecology*, 165, 103971. <https://doi.org/10.1016/j.apsoil.2021.103971>
- Mustafa, A., Minggang, X., Ali Shah, S. A., Abrar, M. M., Nan, S., Baoren, W., Zejiang, C., Saeed, Q., Naveed, M., Mehmood, K., & Núñez-Delgado, A. (2020). Soil aggregation and soil

- aggregate stability regulate organic carbon and nitrogen storage in a red soil of southern China. *Journal of Environmental Management*, 270, 110894. <https://doi.org/10.1016/j.jenvman.2020.110894>
- Nishida, M. (2011). Nitrogen dynamics of organic materials applied to paddy fields: Direct evaluation using organic materials labelled with nitrogen-15. *Japan Agricultural Research Quarterly: JARQ*, 45, 31–38. <https://doi.org/10.6090/jarq.45.31>
- Obalum, S. E., Chibuike, G. U., Peth, S., & Ouyang, Y. (2017). Soil organic matter as sole indicator of soil degradation. *Environmental Monitoring and Assessment*, 189, 1–9. <https://doi.org/10.1007/s10661-017-5881-y>
- OECD/FAO. (2021). *OECD-FAO agricultural outlook 2021–2030*. FAO, Rome/OECD Publishing. <https://www.fao.org/3/cb5332en/Meat.pdf> Accessed 22 Apr 2023.
- Pepper, C.-M., & Dunlop, M. W. (2021). Review of litter turning during a grow-out as a litter management practice to achieve dry and friable litter in poultry production. *Poultry Science*, 100, 101071. <https://doi.org/10.1016/j.psj.2021.101071>
- Qian, P., & Schoenau, J. J. (2002). Availability of nitrogen in solid manure amendments with different C:N ratios. *Canadian Journal of Soil Science*, 82(2), 219–225. <https://doi.org/10.4141/s01-018>
- R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.
- Sadet-Bourgeteau, S., Djemiel, C., Chemidlin Prévost-Bouré, N., & Feder, F. (2022). Dynamic of bacterial and archaeal diversity in a tropical soil over 6 years of repeated organic and inorganic fertilization. *Frontiers in Microbiology*, 13, 943314. <https://doi.org/10.3389/fmicb.2022.943314>
- Sánchez, C. (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnology Advances*, 27, 185–194. <https://doi.org/10.1016/j.biotechadv.2008.11.001>
- Shapiro, B. I., Gebbru, G., Desta, S., Negassa, A., & Nigussie, K. (2017). Rwanda Livestock Master Plan. In *International Livestock Research Institute* (pp. 1–124). Ministry of Agriculture. <https://cgspace.cgiar.org/handle/10568/104049> Accessed 22 Apr 2023.
- Shu, X., He, J., Zhou, Z., Xia, L., Hu, Y., Zhang, Y., Zhang, Y., Luo, Y., Chu, H., Liu, W., Yuan, S., Gao, X., & Wang, C. (2022). Organic amendments enhance soil microbial diversity, microbial functionality and crop yields: A meta-analysis. *Science of the Total Environment*, 829, 154627. <https://doi.org/10.1016/j.scitotenv.2022.154627>
- Silva, J. P., Ticona, A. R. P., Hamann, P. R. V., Quirino, B. F., & Noronha, E. F. (2021). Deconstruction of Lignin: From Enzymes to Microorganisms. *Molecules (Basel, Switzerland)*, 26, 2299. <https://doi.org/10.3390/molecules26082299>
- Staddon, W. J., Duchesne, L. C., & Trevors, J. T. (1997). Microbial diversity and community structure of postdisturbance forest soils as determined by sole-carbon-source utilization patterns. *Microbial Ecology*, 34, 125–130. <https://doi.org/10.1007/s002489900042>
- Stumborg, C., Schoenau, J. J., & Malhi, S. S. (2007). Nitrogen balance and accumulation pattern in three contrasting prairie soils receiving repeated applications of liquid swine and solid cattle manure. *Nutrient Cycling in Agroecosystems*, 78, 15–25. <https://doi.org/10.1007/s10705-006-9071-5>
- Szogi, A. A., Takata, V. H., & Shumaker, P. D. (2020). Chemical Extraction of Phosphorus from Dairy Manure and Utilization of Recovered Manure Solids. *Agronomy*, 10, 1725. <https://doi.org/10.3390/agronomy10111725>
- Thakur, A., Sharma, R. P., Sankhyani, N. K., & Sepehya, S. (2022). Effect of 46 years' application of fertilizers, FYM and lime on physical, chemical and biological properties of soil under maize-wheat system in an acid Alfisol of northwest Himalayas. *Soil Use and Management*, 39, 357–367. <https://doi.org/10.1111/sum.12821>
- Thevelein, J. M. (1984). Regulation of trehalose mobilization in fungi. *Microbiological Reviews*, 48, 42–59. <https://doi.org/10.1128/mr.48.1.42-59.1984>
- Tiquia, S. M. (2003). Evaluation of organic matter and nutrient composition of partially decomposed and composted spent pig litter. *Environmental Technology*, 24, 97–107. <https://doi.org/10.1080/09593330309385540>
- Wan, J., Wang, X., Yang, T., Wei, Z., Banerjee, S., Friman, V.-P., Mei, X., Xu, Y., & Shen, Q. (2021). livestock manure type affects microbial community composition and assembly during composting. *Frontiers in Microbiology*, 12, 621126. <https://doi.org/10.3389/fmicb.2021.621126>
- Yang, Y., Sun, K., Liu, J., Chen, Y., & Han, L. (2022). Changes in soil properties and CO₂ emissions after biochar addition: Role of pyrolysis temperature and aging. *Science of the Total Environment*, 839, 156333. <https://doi.org/10.1016/j.scitotenv.2022.156333>
- Yost, J. L., Schmidt, A. M., Koelsch, R., & Schott, L. R. (2022). Effect of swine manure on soil health properties: A systematic review. *Soil Science Society of America Journal*, 86, 450–486. <https://doi.org/10.1002/saj2.20359>
- Zavattaro, L., Bechini, L., Grignani, C., van Evert, F. K., Mallast, J., Spiegel, H., Sandén, T., Pecio, A., Giráldez Cervera, J. V., Guzmán, G., Vanderlinden, K., D'Hose, T., Ruyschaert, G., & ten Berge, H. F. M. (2017). Agronomic effects of bovine manure: A review of long-term European field experiments. *European Journal of Agronomy*, 90, 127–138. <https://doi.org/10.1016/j.eja.2017.07.010>
- Zhan, Y., Wei, Y., Zhang, Z., Zhang, A. K., Li, Y., & Li, J. (2021). Effects of different C/N ratios on the maturity and microbial quantity of composting with sesame meal and rice straw biochar. *Biochar*, 3(4), 557–564. <https://doi.org/10.1007/s42773-021-00110-5>
- Zhang, J., Li, M., Li, P., Kang, W., Shi, J., Yang, L., & Wang, S. (2018). Effect of pig manure amendments on microbial biomass and functional diversity in three agricultural soils. *IOP Conference Series: Earth and Environmental Science*, 186, 12061. <https://doi.org/10.1088/1755-1315/186/3/012061>
- Zhang, L., Larsson, A., Moldin, A., & Edlund, U. (2022). Comparison of lignin distribution, structure, and morphology in wheat straw and wood. *Industrial Crops and Products*, 187, 115432. <https://doi.org/10.1016/j.indcrop.2022.115432>
- Zhang, Y.-J., Gao, W., Luan, H.-A., Tang, J.-W., Li, R.-N., Li, M.-Y., Zhang, H.-Z., & Huang, S.-W. (2022). Effects of a decade of organic fertilizer substitution on vegetable yield and soil phosphorus pools, phosphatase activities, and the microbial community in a greenhouse vegetable production system. *Journal of Integrative Agriculture*, 21, 2119–2133. [https://doi.org/10.1016/s2095-3119\(21\)63715-2](https://doi.org/10.1016/s2095-3119(21)63715-2)
- Zheng, J., Chen, J., Pan, G., Liu, X., Zhang, X., Li, L., Bian, R., Cheng, K., & Jinwei, Z. (2016). Biochar decreased microbial metabolic quotient and shifted community composition four years after a single incorporation in a slightly acid rice paddy from southwest China. *Science of the Total Environment*, 571, 206–217. <https://doi.org/10.1016/j.scitotenv.2016.07.135>

Zhran, M., Ge, T., Tong, Y., Deng, Y., Wei, X., Lynn, T. M., Zhu, Z., Wu, J., & Gunina, A. (2020). Assessment of depth-dependent microbial carbon-use efficiency in long-term fertilized paddy soil using an $18\text{O}\text{-H}_2\text{O}$ approach. *Land Degradation & Amp Development*, 32, 199–207. <https://doi.org/10.1002/ldr.3708>

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