Soil Use and Management

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Influence of Organic and Inorganic Amendments on Aggregate Development in a Sodic Dispersive Soil

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ABSTRACT

Improvement of soil structure is critical for efficient nutrient and water transport to plants, particularly in sodic, dispersive soils. In addition to inorganic amendments such as gypsum, organic amendments (OAs) can enhance soil aggregation and stability through biochemical processes, but it is unclear how OAs composition [carbon (C) content, C: nitrogen (N) ratio and C functional groups] influences aggregation in sodic subsoils. To address this, we conducted a 17-month soil incubation study in which 19 organic and inorganic amendments were vertically banded in an alkaline sodic subsoil (collected from 20 to 40 cm depth). The study examined how OA properties and their decomposition affected aggregate formation and stability (indicated by wet mean weight diameter; WMWD), via changes in soil physiochemical and biological properties at 3 and 17 months. At 3 months, aggregate stability correlated positively with total C content and C quality of the OAs (i.e., aliphatic C, C/N ratio), and soil microbial biomass C. The highest increase in aggregate stability (2–2.5-fold) was observed in soils amended with plant residues, including reed (*Phragmites australis*), faba bean (*Vicia faba* L.) and wheat (*Triticum aestivum* L.). By 17 months, these beneficial effects on aggregation largely diminished. Chicken manure improved soil aggregation in the short term (3 months) and maintained higher N availability in the long term (17 months), indicating its potential use to address multiple subsoil constraints. In contrast, neither gypsum nor polyacrylamide improved soil aggregation. Our findings support the use of vertically banded plant and animal manure-based OAs to improve sodic subsoil structure and biological functions of sodic subsoils, with implications for soil function and agricultural productivity.

1 | Introduction

Soil structure has a major effect on the flux and efficiency of nutrient and water transport to plants (Oades 1984; Neal et al. 2020). Poor structure of agricultural soils is one of the major threats to soil health and function (Pagliai et al. 2004; Rabot et al. 2018; Hartmann and Six 2023). Alkaline sodic dispersive soils, defined by exchangeable sodium percentage (ESP) > 6.0

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and pH > 8.5, have poor structure and soil-water-air relations due to slaking, swelling and clay dispersion, which limit root proliferation and crop yield (Rengasamy 2002). Early estimates suggested that about 436 million ha of the world's land area have sodicity issues, with 60% (249 million ha) located in Asia, the Pacific and Australia regions (Rengasamy 2002; Leogrande and Vitti 2019). More recent global assessments indicate that 1.4 billion ha (11% of the world's land area) are affected by salinity and sodicity (FAO 2024).

Sodic subsoils, especially those deeper than 20 cm, pose significant challenges for management in arid and semi-arid regions, as there is insufficient rainfall to leach excess sodium (Na^+) from these zones (Rengasamy and Olsson 1991). To improve soil fertility in such soils, agricultural practices such as the use of organic amendments (OAs) and/or gypsum have been widely adopted (Liang et al. 2025). Gypsum ($CaSO_4 \cdot 2H_2O$) has been shown to reduce clay dispersion and increase flocculation by replacing Na^+ with calcium (Ca^{2+}) on soil clay surfaces (Rengasamy et al. 2016). However, most studies have focused on improving the surface layers (i.e., < 10 cm) (Lebron et al. 2002; Leogrande and Vitti 2019; Green et al. 2023). Deep incorporation of amendments into subsoils has gained attention, as it can directly alter subsoil biological, physical and chemical properties (Clark et al. 2007; Sale et al. 2019; Wang et al. 2020). However, little is known about the effects of deep placement of OAs and/or gypsum on aggregate formation and stability in alkaline sodic dispersive subsoils, despite reports of yield improvements (Tavakkoli et al. 2019).

Soil aggregation is a key component of soil structure, constantly changing and restructuring due to biological, chemical and physical processes (Abiven et al. 2009). Soil aggregation plays a crucial role in soil carbon (C) persistence and storage (Verchot et al. 2011; Lehmann et al. 2020), and is influenced by the organic matter decomposition processes (Fang et al. 2021), as well as soil texture, clay mineralogy, cation content and climate (Abiven et al. 2009; Bai et al. 2020). Two main conceptual models describe organic matter (OM)–aggregate linkages: a bottom-up model, which proposes that microbially-derived OM acts as a binding agent of clay particles and silt-clay fractions, leading to the formation of micro-aggregates and eventually macro-aggregates (Vidal et al. 2018; Rabbi et al. 2024). On the other hand, the top-down model proposes that plant-derived OM, such as fresh or partly decomposed plant residues, nucleates macro-aggregates that subsequently break down into micro-aggregates and the silt-clay fraction during decomposition (Golchin et al. 1997). These competing theories continue to be explored by researchers seeking to understand the complex interactions between soil organic matter and soil structure.

The role of microbial processing in aggregate dynamics has been well established (Gupta and Germida 2015; Rabbi et al. 2020). Increased microbial biomass and the associated microbial polysaccharides (the main component of fungal cell walls) can enhance the formation of stable soil aggregates (Chenu et al. 2002; Cania et al. 2020). The OAs, however, have varied qualities (e.g., total and labile C contents, C functional groups, nitrogen (N) content) which can directly and indirectly affect soil aggregation (Baldock et al. 1997; Fang et al. 2021). For example, the labile C content (e.g., carbohydrate) in OAs has been shown to be positively correlated with soil aggregation (Sarker et al. 2022). Since most labile C in OAs decomposes in < 3–6 months and is facilitated by nutrient inputs (Fernandez et al. 2016; Fang et al. 2018, 2020), the benefits of OAs to soil aggregation may diminish over time. However, it lacks quantitative information on how OA quality controls aggregation during microbial decomposition over longer time frames (i.e., > 1 year).

This research aimed to evaluate the application strategy (such as vertically banded placement) and mechanisms of OAs that improve soil structure of sodic subsoils, where dispersive clay particles can lead to a quick breakdown of aggregates. Specifically, in a controlled incubation experiment, we examined (i) the effects of a range of OAs with varying C quality (including crop residues, green manure and animal manures), humate, inorganic amendment (gypsum) and polyacrylamide on aggregate formation and stability; and (ii) the role of microbial processing in aggregate formation and turnover after deep placement of amendments in a sodic subsoil. We hypothesized that:

i. Application of high-C and/or low-quality-C OAs (such as crop residues) would rapidly increase water-stable aggregates in soil adjacent to the amendment band, via enhanced microbial growth and associated bottom-up aggregation.
ii. OAs would increase soil aggregation in the short term (e.g., a few months) but have limited effects over longer periods (e.g., > 1 year) due to microbial death and turnover.
iii. Gypsum would have long-term positive effects on aggregate stability, primarily by reducing clay dispersion.

2 | Materials and Methods

2.1 | Soil and Amendments

An alkaline sodic dispersive subsoil with ESP of 12.9 and pH_{H2O} of 8.9 was collected at 20–40 cm depth from a semi-arid region near Grogan, New South Wales, Australia (34.3524°S, 147.7126°E). The site has a mean annual rainfall of 560 mm and mean minimum and maximum temperatures of 8.7°C and 23.6°C, respectively (https://www.bom.gov.au/climate/data). The site had a crop rotation history of wheat, canola and legumes with summer fallow. The soil is classified as a Solonetz (IUSS Working Group 2006), equivalent to a Sodosol in the Australian Soil Classification (Isbell 2002). The dominant clay mineral in the soil is kaolinite (Fang et al. 2020). The soil was air-dried, gently crushed and sieved to < 4 mm, with the stones and recognizable plant debris (> 2 mm) manually removed prior to the experiment.

Soil organic carbon (SOC) was measured after removing carbonate C by acid-washing (Midwood and Boutton 1998). Soil inorganic carbon (SIC) was calculated based on the loss of C after acid-washing. The soil was then dried at 60°C, ground to < 0.05 mm and analysed for C and N concentration using a LECO TruMac Carbon and Nitrogen Elemental Analyser (CA, USA). Total phosphorus (P) was determined by the molybdenum-blue method after Kjeldahl digestion (Kjeldahl 1883), and four cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+) were extracted from the soil using 0.1 M $BaCl_2$ and 0.1 M NH_4Cl solution (Gillman and Sumpter 1986) and then quantified using an Agilent 280FS Atomic Absorption Spectrophotometer (CA, USA). The basic soil chemical properties are summarised in Table 1.

Total C and N contents of OAs were analysed using the LECO Analyser (CA, USA) after drying and grinding at 60°C to < 0.1 mm. Inorganic C in the OAs was determined by a titration method using 1 M HCl and was calculated based on the weight loss due to acidification/CO_2 emission. The water extractable C (dissolved organic C, DOC) and dissolved N (DN) in the OAs were extracted using Milli-Q water (1:5 w/v) and filtered through a 0.45 μm Millex-HV Syringe filter (Durapore PVDF membrane), and then analysed using a Total Organic Carbon Analyser (TOC-V CSH/CPN; Shimadzu, Japan). The chemical composition of both the soil and OAs were determined by solid-state ^{13}C cross-polarisation magic angle spinning (CP/MAS) nuclear magnetic resonance (NMR) spectroscopy, as detailed in Baldock et al. (2021). The spectra were divided into eight chemical shift regions, representing alkyl C (0–45 ppm), methoxyl (45–60 ppm), O-alkyl C (60–90 ppm), di-O-alkyl C (90–110 ppm), aryl C (110–145 ppm), O-aryl C (145–160 ppm), carboxylic/amide/ester C (160–180 ppm) and ketone/aldehyde C (180–210 ppm) (Chen et al. 2004). The aliphatic C to aromatic C (aliphatic C (0–110 ppm)/aromatic C (110–160 ppm)) ratio in the OAs was also calculated.

TABLE 1 | The basic chemical properties of the soil (20–40 cm soil depth). Data are mean ± standard error ($n = 4$).

Properties	Solonetz
pH (1:5 H_2O)	8.92 ± 0.01
EC (μS cm^{-1})	426 ± 49
ESP	12.9 ± 0.60
Sand (%)	40.3 ± 0.5
Silt (%)	8.5 ± 0.8
Clay (%)	51.3 ± 0.9
SOC (g kg^{-1})	5.77 ± 0.18
SIC (g kg^{-1})	0.85 ± 0.02
Dominant clay minerals	Kaolinite

Note: ESP was calculated as exchangeable Na^+ divided by the sum of exchangeable Ca^{2+}, Mg^{2+}, K^+ and Na^+.
Abbreviations: EC, electrical conductivity; ESP, exchangeable sodium percentage; SIC, soil inorganic C; SOC, soil organic C.

2.2 | Experimental Design

Soils were incubated in PVC containers (15 cm diameter × 20 cm height) in the dark at 20°C ± 0.5°C for over 17 months, with or without organic and inorganic amendments (Figure 1). A total of 19 treatments and one control (nil amendments) were included, each with 4 replicates (Table 2). The study included 12 different OAs, encompassing plant residues, animal manures, compost, compost-biochar/zeolite mixtures, biosolids and humate (Sigma-Aldrich, USA), covering a range of C:N (7 ~ 109) and C:P (phosphorus) (7 ~ 1088) ratios (Table 3). The amendments (< 2 mm, apart from humate) were applied at 15 t dry weight ha^{-1}, equivalent to 12 g kg^{-1} of dry soil. Gypsum was used at 5 and 15 t dry weight ha^{-1}, and polyacrylamide (PAM; Sigma-Aldrich, USA) at 5 kg ha^{-1}, applied either alone or in combination with gypsum at 5 t ha^{-1}. A control soil without any amendments was also included. Two nutrient rates

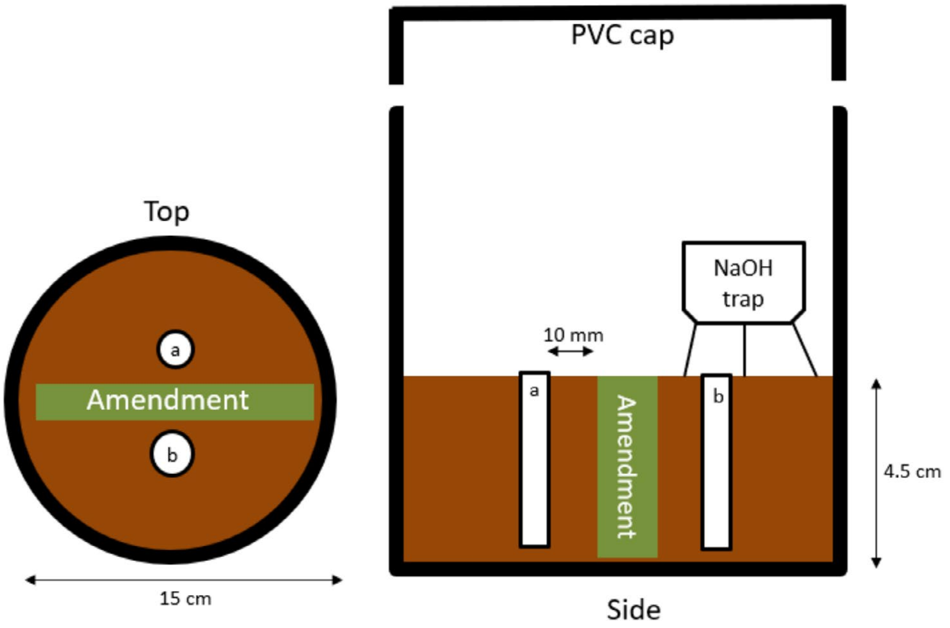


FIGURE 1 | Diagram of the soil incubation setup, CO_2 sampling approach (NaOH trapping) and soil sampling positions. Amendments were vertically banded along the central axis of each PVC column. PVC caps were closed only during CO_2 sampling periods and remained open at all other time. Sampling positions a and b indicate the locations where soil was destructively collected using a stainless-steel micro-corer (2.5 cm diameter × 5 cm height), approximately 1 cm from the amendment band (i.e., 1–3.5 cm lateral distance from the band) over the 0–4 cm depth.

TABLE 2 | Organic and inorganic amendments and nutrient additions in the treatments.

No	Amendments	Application rate ($t\,ha^{-1}$)	Application rate ($g\,kg^{-1}$ dry soil)	Notes
1	Bean residues	15	12	*Vicia faba* L.
2	Common reed residues	15	12	*Phragmites australis*
3	Wheat residues	15	12	*Triticum aestivum* L.
4	Wheat residues + Nut1	15	12	*Triticum aestivum* L.
5	Wheat residues + Nut2	15	12	*Triticum aestivum* L.
6	Green waste + Pig manure	15	12	Immature pig bedding material compost
7	Chicken manure composted	15	12	Mature poultry litter compost
8	Chicken manure uncomposted	15	12	Uncomposted poultry litter; Charcoal was presented at sampling site
9	Green organics compost	15	12	Mature municipal green waste compost
10	Pig manure	15	12	Uncomposted
11	Cow manure	15	12	Uncomposted
12	Compost + Biochar	15	12	49:1 w/w mature green waste compost: green waste biochar ratio; blended post-composting
13	Compost + Zeolite	15	12	9:1 w/w mature green waste compost: zeolite ratio; blended post-composting
14	Biosolids	15	12	Taken from a small suburban wastewater treatment plant receiving domestic commercial and industrial inflows.
15	Humate	15	12	
16	Gypsum_low	5	4	
17	Gypsum_high	15	12	
18	Polyacrylamide (PAM)	$5\,kg\,ha^{-1}$	$4\,mg\,kg^{-1}$	
19	PAM + Gypsum	$5\,kg\,ha^{-1}$ PAM $+5\,t\,ha^{-1}$ gypsum	$4\,mg\,kg^{-1}$ PAM + $4\,g\,kg^{-1}$ gypsum	
20	Control	Nil	Nil	

Note: Two nutrient rates were added to wheat residues in the amendment band: (1) 'wheat residues + nut1', receiving 65 mg $(NH_4)2SO_4/NH_4NO_3$–N kg^{-1} soil, 15 mg KH_2PO_4–P kg^{-1} soil and 11 mg $(NH_4)2SO_4$–S kg^{-1} soil and (2) 'wheat residues + nut2', receiving 130 mg N kg^{-1} soil, 30 mg P kg^{-1} soil and 22 mg S kg^{-1} soil.

were added to wheat residues in the amendment band for facilitating decomposition and transformation of wheat residues into stabilized SOC: (1) 'wheat residues + nut1', receiving 65 mg $(NH_4)_2SO_4/NH_4NO_3$–N kg^{-1} soil, 15 mg KH_2PO_4–P kg^{-1} soil and 11 mg $(NH_4)_2SO_4$–S kg^{-1} soil and (2) 'wheat residues + nut2', receiving 130 mg N kg^{-1} soil, 30 mg P kg^{-1} soil and 22 mg S kg^{-1} soil (Table 3).

To apply the amendments, a vertical band was created in the middle of the subsoil and buried to a depth of about 4.5 cm within PVC containers. Each container was filled with 300 g of subsoil mixed with 12 g of OAs (15 $t\,ha^{-1}$) or 4 or 12 g of inorganic amendments (5 or 15 $t\,ha^{-1}$), and this mixture was packed to achieve a soil bulk density of 1.3 $g\,cm^{-3}$. The remaining 700 g subsoil was then carefully added around the amendment band. Soil moisture was adjusted to 70% water holding capacity and maintained by weekly re-watering with reverse osmosis water to store their initial weight over the 17-month incubation. To evaluate the effects of the OAs bands on soil physicochemical and biological properties while minimising disturbance, we sampled soils destructively using a stainless-steel micro-corer (2.5 cm diameter × 5 cm height) about 1 cm from the amendment band, that is, at 1–3.5 cm lateral distance to the amendment bands and over the 0–4 cm depth (Figure 1).

TABLE 3 | Characteristics of the basic properties of soil and organic amendments. The soil was HF-treated before the NMR analysis.

	TC (%)	TN (%)	TP (%)	C/N ratio	C/P ratio	CO_3^{2-} (%)	DOC (%)	DN (%)	Alkyl	N-alkyl	O-alkyl	di-o-alkyl	Aryl	O-aryl	Carboxyl	Ketone	Aliphatic/Aromatic
Sodosol	0.7	0.05	0.010	13	70	0.1	0.02	0.002	20.0	9.8	19.5	9.1	21.3	5.6	8.9	5.9	2.2
Bean (*Vicia faba* L.) residues	42.2	2.5	0.043	16.9	981	DL	5.37	0.22	6.4	5.0	56.7	12.8	8.8	3.9	5.4	1.0	6.4
Reed residues	43.5	0.5	0.040	87.0	1088	DL	1.25	0.09	4.9	5.3	55.6	13.1	10.8	4.6	4.7	1.0	5.1
Wheat (*Triticum aestivum* L.) residues	43.5	0.4	0.112	108.8	388	DL	4.23	0.13	5.8	5.6	57.2	13.5	9.0	3.6	4.8	0.4	6.5
Green waste + Pig manure	31.4	1.7	0.654	18.5	48	8.0	2.97	0.31	8.7	6.1	49.9	11.7	11.6	5.2	5.9	1.0	4.6
Chicken manure composted	31.0	4.2	1.712	7.4	18	2.0	7.58	1.68	13.1	6.2	41.7	9.1	13.1	6.4	9.3	1.1	3.6
Chicken manure uncomposted	37.3	3.2	1.186	11.7	31	1.2	0.32	0.06	15.5	3.0	5.0	3.7	52.6	12.5	5.1	2.6	0.4
Green organics compost	20.1	1.6	0.399	12.6	50	7.0	1.27	0.16	14.8	7.2	37.2	8.8	15.3	6.1	9.1	1.6	3.2
Pig manure	38.6	2.4	0.999	16.1	39	1.6	3.36	0.56	9.9	7.6	44.7	10.9	12.4	6.2	7.3	1.0	3.9
Cow manure	18.0	2.1	0.480	8.6	38	0.9	0.33	0.23	21.6	9.1	21.4	5.6	18.5	8.4	13.2	2.3	2.1
Compost + biochar	21.9	1.3	0.237	16.8	92	1.6	0.47	0.14	15.0	7.0	20.8	5.6	31.8	9.9	7.8	2.1	1.2
Compost + zeolite	14.4	1.3	0.267	11.1	54	1.1	0.43	0.11	18.7	8.4	26.6	6.5	19.1	8.3	10.3	2.0	6.4
Biosolids	23.1	3.2	3.224	7.2	7.2	DL	0.42	0.18	23.5	9.4	20.1	4.4	17.1	6.6	16.7	2.1	2.4
Humate	47.5	0.9	0.004	52.8	11,875	5.2	40.5	0.71	20.3	3.5	4.5	3.7	42.7	11.1	11.2	2.9	0.6

Note: 'DL' means it was below the detection limit.
Abbreviations: DN, dissolved N; DOC, dissolved organic C; TC, total C; TN, total N; TP, total P.

2.3 | Aggregate Wet Mean Weight Diameter and Analyses of Dispersion

Soils sampled adjacent to the amendment bands (at 3 and 17 months) were air-dried, gently crushed along natural planes of weakness, and sieved to <6.5 mm before wet sieving. The soil fractions were separated into macro-aggregates (>0.25 mm), micro-aggregates (0.05–0.25 mm) and silt-clay fraction (<0.05 mm) following the method described by Chan et al. (2002). About 20 g of air-dried soil was placed in a wet-sieving apparatus (McAuliffe Engineering, Wagga Wagga, Australia), submerged in reverse osmosis water in a 2 L cylindrical container (17 cm diameter × 13.5 cm height), and pre-soaked for 5 min before wet sieving. A stroke length of 38 mm at a frequency of 30 strokes min^{-1} was used. The fractions were washed carefully, dried at 60°C and weighed. The 2–6.5 mm size fraction was combined with the 0.25–2 mm size fraction as the macro-aggregates (>0.25 mm) due to the high dispersion of the clay particles (Sarker, Singh, et al. 2018). After wet sieving, the <0.05 mm fraction in suspension was determined using the pipette sampling technique (Yoder 1936; Chan et al. 2002), and the micro-aggregate (0.05–0.25 mm) fraction was calculated by the difference in bulk soil weight and the combined fractions. The soil mass proportion distribution in aggregate-size classes is provided in Table S1. Wet mean weight diameter (WMWD) of soil aggregates, an index of aggregate stability, was calculated by multiplying the mass proportion of soil in each aggregate-size class by the mid-point of the size class and then summing those values (Six et al. 1998).

The amount of dispersible clay (g clay kg^{-1} soil) was determined using a Hach Turbidimeter 2100 N (HACH, Colorado, USA). A calibration curve, established using $<2\,\mu$m clay fractions obtained by standard dispersion–sedimentation, was used to convert turbidity readings to clay content. Mechanical clay dispersion of soils was measured following the method described by Zhu et al. (2016): 1.0 g of the air-dried soil (<2 mm) was placed in a turbidimeter cell with deionized water at a 1:5 soil:water ratio, the cell was gently inverted 20 times and turbidity was measured after 4 h of sedimentation.

2.4 | Total CO_2 Emissions From the Amended and Unamended Soils

The CO_2 emission fluxes from the amended and control soils were measured using the NaOH trapping method on 2, 10, 13, 15, 18, 24, 52, 90, 102, 131, 164, 193, 234, 270 and 500 days. In brief, a jar to trap CO_2 (30 mL of 2 M NaOH) was placed on a stainless-steel tripod stand in each soil container with a cap to seal for 24 h at each measuring time. To account for headspace CO_2, 3 blank PVC containers (without soil but with a closed cylinder equivalent to the soil volume in the treatments) were established in the same manner as the soil containers. The total CO_2-C trapped in the NaOH solution was determined using phenolphthalein indicator and acid–base titration (Fang et al. 2018). A linear interpolation of CO_2 emissions between the sampling points (Singh et al. 2015). Cumulative CO_2 emissions were calculated by summing the linearly interpolated emissions at different time points over the 17-month incubation.

2.5 | Soil Microbial Biomass C

Soil microbial biomass C (MBC) was measured using the chloroform fumigation-extraction method (Vance et al. 1987) at 90 and 500 days' incubation. A 10 g subsample of fresh soil was mixed with 40 mL of 0.5 M K_2SO_4 solution and shaken for 1 h. A paired 10 g fresh soil subsample was fumigated with ethanol-free chloroform in a desiccator for 24 h in the dark at 22°C, then extracted with K_2SO_4 using the same procedure. The resulting extracts were filtered through glass-fibre filter paper (Whatman GF/C), and dissolved organic C (DOC) was measured using a TOC Analyser. Microbial biomass C was determined as the difference between the DOC values of the fumigated and non-fumigated soils, divided by a conversion factor (kec) of 0.45 (Vance et al. 1987).

2.6 | Soil Enzyme Activity

Activities of four soil enzymes: β-glucosidase (BG), β-1,4-N-acetylglucosaminidase (NAG), L-leucine aminopeptidase (LAP) and phosphatase (PHOS) were measured to represent C, N and P acquisition. Air-dried soil (2.5 g) was weighed into 50 mL polypropylene centrifuge tubes and adjusted to 70% water holding capacity. Samples were incubated at 25°C for 5 days to allow the microbial community to re-equilibrate. Enzymatic activity was then determined following Deng et al. (2011). Approximately 0.5 g of soil was mixed with a modified buffer solution (pH 9) at a ratio of 1:100 (w/v). An aliquot of the slurry (100 μL) was pipetted into black microwell plates together with Methylumbelliferyl (100 μL) and the appropriate enzyme substrate (50 μL). Three blanks containing all reagents, but no soil, were included to correct for substrate autohydrolysis. The soil-substrate mixtures were incubated in the dark at 37°C for 2–4 h (depending on enzyme activity), and fluorescence was measured using a FLUOstar Omega (BMG labtech) plate reader (Bell et al. 2013).

To quantify the enzyme stoichiometry of C-to-N acquisition versus C-to-P acquisition, we followed the approach of Fang et al. (2022). Vector length was calculated as the square root of the squared sum of the values of x and y, where x represents the C-to-P acquiring enzyme activities, and y represents the C-to-N acquiring enzyme activities (Fanin et al. 2016; Moorhead et al. 2016). Vector angle was calculated as the arctangent of the point (x, y). A larger vector length indicates greater C acquisition compared to nutrient (N and P) acquisition, whereas a larger angle indicates greater P limitation relative to N limitation (Fanin et al. 2016; Moorhead et al. 2016).

2.7 | Statistical Analysis

A linear mixed model was used to analyse all responses using the ASReml-R package (Butler 2021) within R (R Core Team 2024). The model included fixed effects of amendments, time and their interaction, and random effects of replicate and replicate by time for WMWD and MBC; WMWD and MBC were inverse and square-root transformed, respectively, to stabilize variance. To account for within-unit correlation in the repeated measures, we specified an unstructured residual variance–covariance matrix. For cumulative CO_2-C mineralised, enzyme vector length and angle and physicochemical properties (mineral N, EC, pH, CEC, ESP and dispersible clay), the model comprised fixed amendment effects and random replicate effects. When the Wald F-statistic for amendments (or amendment by times) was significant, treatment means were compared with the control at the 5% significance level, and LSD values were reported for other pairwise comparisons.

Relationships among WMWD, MBC, cumulative C mineralised (Cmin), OA C functional groups and OA C, N and C/N ratio at 3 and 17 months we evaluated using partial least squares path modelling (PLS-PM) using the 'plspm' package (Sanchez et al. 2013). PLS-PM, a regression-based method suitable for small samples and non-normal, was used to estimate path coefficients and coefficients of determination (R^2), which quantify the strength and direction of direct linear effects between variables (Hair Jr et al. 2014). Model performance and alternative path structures were compared using the Goodness-of-Fit (GoF) statistic, which summarises overall predictive ability.

3 | Results

3.1 | Chemical Properties of the Organic Amendments

The dominant C functional groups varied among the different OAs, with alkyl, O-alkyl and aryl being the most prevalent, accounting for 4.9%–23.5%, 4.5%–57.2% and 8.8%–52.6% of the total C functional group signal area in the solid-state ^{13}C NMR spectra, respectively (Table 3). Notably, the plant residues of common reed (*Phragmites australis*), faba bean (*Vicia faba* L.) and wheat (*Triticum aestivum* L.) exhibited lower proportions of alkyl (4.9%–6.4% vs. 13.1%–23.5% in the other OAs) but higher O-alkyl (55.6%–57.2% vs. 4.5%–44.7%) (Table 3). In terms of aliphatic-aromatic ratios, the plant residues (reed, bean and wheat) showed higher ratios (5.1–6.5) compared to the other OAs (0.42–4.6) (Table 3).

Total C contents of plant residues, including reed, bean and wheat residues, ranged from 42.2% to 43.5%, which were higher than pig manure, chicken manure (un)composted and green waste + pig manure amendments (31%–38.6%) (Table 3). The other OAs, including green organics compost, cow manure, compost + biochar, compost + zeolite and biosolids, had lower C contents, ranging from 14.4% to 23.1%. The C content in humate was 47.5%. The CO_3^{2-} contents in green waste + pig manure and green organics compost were the highest among all amendments, with contents of 8% and 7%, respectively. The DOC contents in the OAs varied from 0.42% (Biosolids) to 7.58% (chicken manure composted). The N contents in the OAs ranged from 0.4% (wheat residues) to 3.2% (chicken manure). The C/N ratios of the OAs varied between 7.2 (biosolids) and 109 (wheat residues), while the C/P ratios ranged between 7.2 (biosolids) and 1088 (reed residues).

3.2 | Wet Mean Weight Diameter

The effects of amendments, time and their interaction on WMWD were significant ($p < 0.001$), and WMWD decreased significantly over time (Table 4). At 3 months, the application of plant residues (reed residues, bean residues and wheat residues with or without nutrients) increased WMWD ($p < 0.05$) by 2–2.5 times (i.e., 0.48–0.60 mm), compared to the control (0.24 mm) (Table 4). Green waste + pig manure, Chicken manures (uncomposted and composted) and green organics compost also had a significant positive effect on WMWD at 3 months (i.e., 0.39–0.50 mm), compared to the control. However, the beneficial effects of amendments on WMWD diminished over time. At 17 months, WMWD ranged between 0.22 and 0.31 mm across treatments and control (Table 4).

3.3 | Cumulative CO_2 Emissions

The cumulative C mineralised over a 17-month period ranged from 139 to 385 g CO_2-C m^{-2} across the control and treatments, as shown in Table 4 and Figure S1. Amendments significantly affected cumulative C mineralisation ($p < 0.001$), with values 50%–180% higher than the control, in soils amended with wheat residues with or without nutrients, reed residues, bean residues, chicken manure composted, biosolids (214–385 g CO_2-C m^{-2}). However, no significant difference was detected in the cumulative C mineralisation for the soils treated with gypsum (low and high rates), green waste + pig manure, green organics compost, cow manure, humate, PAM + gypsum, compost + zeolite and PAM alone (142–194 g CO_2-C m^{-2}), compared to the control (139 g CO_2-C m^{-2}) (Table 4 and Figure S1).

3.4 | Soil Biological and Physicochemical Properties

Amendments significantly affected ($p < 0.001$) soil MBC over time, as evidenced by the main effects of amendments, time and their interaction (Table 4). While the MBC decreased over time, the rate of decrease varied depending on the type of amendment used. At 3 months, soils treated with wheat residues (with or without nutrient additions), chicken manure (uncomposted and composted) and bean residues had significantly higher MBC ($p < 0.05$) compared to other treatments and the control soil.

At 17 months, amendments significantly affected enzymatic stoichiometry (C:N vs. C:P), altering the vector angle ($p < 0.001$), which ranged between 5.3° and 16.8°. Treatments with bean residues, manures (compost + zeolite, compost + biochar, chicken manure composted, green organics compost, pig manure, green waste + pig manure and cow manure), biosolids, gypsum (gypsum_low, PAM + gypsum) and PAM had significantly lower vector angles ($p < 0.05$) compared to the control soil (16.8°). The vector length (C vs. nutrient acquisition) was not significantly affected by amendments and ranged from 0.19 to 0.35 (Figure 2a–c).

After 17 months, several amendments significantly reduced dispersive clay ($p < 0.05$) to 7.9–9.6 g kg^{-1} soil, including gypsum (low and high rates), PAM + gypsum, green waste + pig manure, biosolids, chicken manure composted, reed residues, cow manure, bean residues and wheat residue + nut2, as compared to the control soil (10.1 g kg^{-1} soil). On the other hand, the input of humate resulted in an increase in dispersive clay

TABLE 4 | Wet mean weight diameter (WMWD), microbial biomass C (MBC) and cumulative C mineralised (C_{min}) in the amendment treated soils (1–3.5 cm distance from the amendment bands) and the control soil on 3 months and 17 months.

	WMWD		MBC		C_{min}	
	3 months	**17 months**	**3 months**	**17 months**	**3 months**	**17 months**
Bean residues	0.56 (1.77)*	0.27 (3.71)	814.5 (28.5)*	90.5 (9.5)	91.3*	233.8*
Reed residues	0.55 (1.83)*	0.29 (3.41)	181.6 (13.5)	79.6 (8.9)*	54.5	259.2*
Wheat residues	0.44 (2.29)*	0.31 (3.28)	575.5 (24)*	90.5 (9.5)	96.9*	385*
Wheat residues + Nut1	0.5 (1.99)*	0.27 (3.71)	1139.4 (33.8)*	77.4 (8.8)*	147.1*	352.8*
Wheat residues + Nut2	0.5 (1.98)*	0.25 (3.98)	1135.8 (33.7)*	85.6 (9.3)	144.8*	368*
Green waste + Pig manure	0.47 (2.13)*	0.27 (3.7)	135.8 (11.7)	74.6 (8.6)*	49.1	182.8
Chicken manure composted	0.38 (2.66)*	0.26 (3.91)	644.2 (25.4)*	90.8 (9.5)	78.9*	227.7*
Chicken manure uncomposted	0.38 (2.6)*	0.27 (3.65)	834.4 (28.9)*	76.5 (8.7)*	78.5*	194.5
Green organics compost	0.38 (2.66)*	0.27 (3.72)	157.8 (12.6)	81.1 (9)	38.3	153.5
Pig manure	0.28 (3.62)	0.25 (4.04)	144.8 (12)	85.6 (9.3)	60.6	197.2
Cow manure	0.29 (3.44)*	0.27 (3.65)	99.3 (10)	80.1 (8.9)	35.7	169.9
Compost + biochar	0.27 (3.67)	0.26 (3.88)	132.6 (11.5)	77.8 (8.8)*	47.8	195.2
Compost + zeolite	0.27 (3.75)	0.25 (4.05)	144.1 (12)	68.3 (8.3)*	43.7	181.6
Biosolids	0.28 (3.61)	0.27 (3.68)	162.6 (12.8)	72.5 (8.5)*	48.6	214*
Humate	0.17 (5.76)*	0.22 (4.56)*	174.2 (13.2)	75.3 (8.7)*	24.6	157.8
Gypsum_low	0.3 (3.38)*	0.26 (3.85)	191.2 (13.8)	87.3 (9.3)	44.4	187.8
Gypsum_high	0.32 (3.12)*	0.26 (3.78)	189.8 (13.8)	78 (8.8)*	35.3	142.4
PAM	0.22 (4.5)	0.25 (3.94)	162.1 (12.7)	77.3 (8.8)*	38.6	184.1
PAM + gypsum	0.22 (4.5)	0.26 (3.87)	191.8 (13.8)	80.6 (9)	40.2	171.9
Control	0.24 (4.17)	0.27 (3.64)	129.7 (11.4)	86.6 (9.3)	36.6	139
$LSD_{0.05}$	(0.63)	(0.31)	(2.8)	(0.3)	21.7	56

Note: The cumulative C mineralisation over time is presented in Figure S1. The $LSD_{0.05}$ indicates the least significant differences at the 5% level. WMWD and MBC were inverse and square root transformed prior to analysis, respectively. The values in the brackets are the values after transformation. The $LSD_{0.05}$ indicates the least significant differences at the 5% level. The asterisk indicates the significant difference between treatment and control.

content to 11.0 g kg^{-1} soil, as well as ESP, relative to the control (Table 5). Furthermore, soil mineral N was significantly higher in the manures and composts treated soils. In treatments with chicken manure (composted and uncomposted), biosolids, pig manure, compost + biochar and cow manure, mineral N was about 1–3 times greater than in the control (29 mg kg^{-1} soil) (Table 5). Applications of gypsum (low and high rates) led to significant reduction in soil pH and ESP ($p < 0.05$), relative to the control (Table 5). Electrical conductivity across the control and amended soils ranged from 322 to 697 μS cm^{-1} after 17 months (Table 5). Cation exchange capacity across the control and amended soils ranged between 28.5 and 31.2 cmol (+) kg^{-1} soil (Table 5).

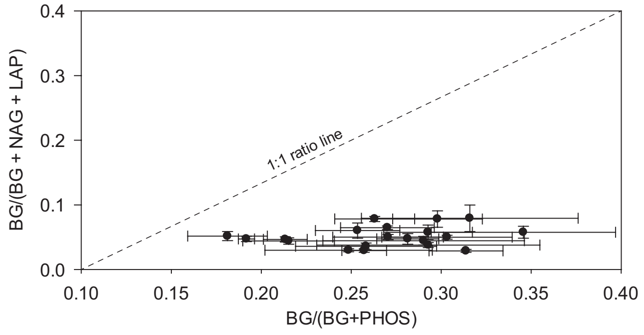
3.5 | The Effect of Biochemical Function and Properties of OAs on Soil Aggregation

The path models showed that the aggregate stability, measured as WMWD, was significantly associated with MBC, both directly and indirectly via total C content and C functional groups in OAs, particularly at 3 months after application (Figure 3). Specifically, at 3 months, WMWD was positively ($p < 0.05$) correlated with the aliphatic C content in OAs and soil MBC (with path coefficients of 0.54 and 0.77, respectively). At 3 months, soil MBC was positively ($p < 0.05$) correlated with C content in the OAs (path coefficient = 0.43), but negatively ($p < 0.05$) correlated with carboxylic C content in the OAs (path coefficient = −0.60). At 17 months, WMWD was positively ($p < 0.05$) influenced by cumulative C mineralised (0.51), which was enhanced with increasing C/N ratio (0.51) in the OAs, as well as total C (0.34). However, at 17 months, MBC did not significantly affect WMWD (Figure 3b).

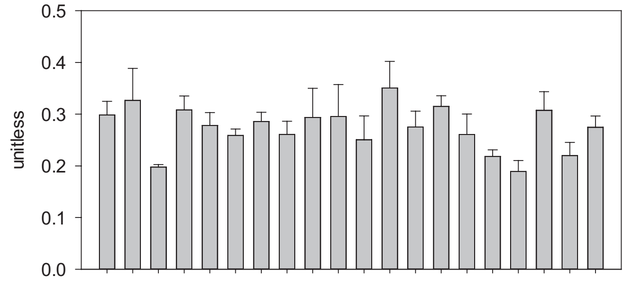
4 | Discussion

To our best knowledge, this study is one of few to simulate common management practices, such as vertically banded amendments in subsoils (Jin et al. 2025; Uddin et al. 2025), and elucidating their effects on soil structure and functions.

(a) Enzyme stoichiometry



(b) Length



(c) Angle

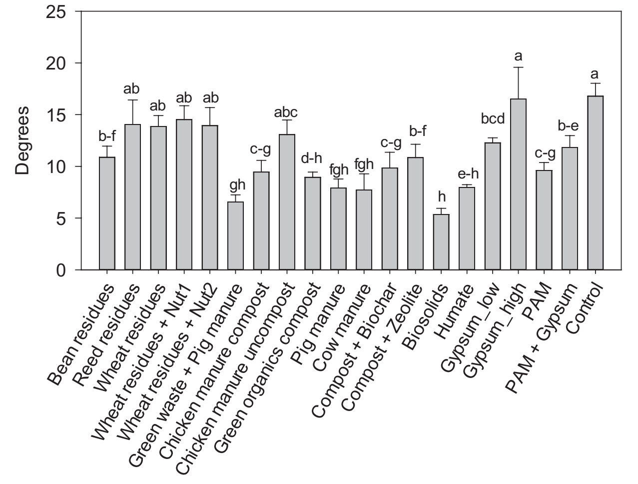


FIGURE 2 | Enzyme stoichiometry of the C-to-N acquisition (BG/(BG + NAG + LAP)) versus C-to-P acquisition (BG/(BG + PHOS)) (a), vector length (b) and vector angle (c). The small angle (i.e., on the bottom side of the 1:1 line) represents more N limitation versus P limitation. Significant differences (at 5% level, $LSD_{0.05}$) between treatments are indicated by the lowercase letters.

4.1 | Composition of the OAs Directly and Indirectly Determines Soil Aggregate Stability

The compositions of the OAs directly affected soil aggregate formation and stability, with deep placement of plant-derived residues resulting in the highest WMWD after 3 months. This was partly attributed to the higher levels of labile C in the plant-derived OAs (Table 3), which can act as binding agents to facilitate the formation of larger soil aggregates in this dispersive clay subsoil. These findings align with previous research indicating that labile organic matter can help to 'glue' soil particles together across contrasting soil textures (i.e., loam, sandy clay loam, sandy loam) (Smucker et al. 2007; Sarker, Incerti, et al. 2018). For example, water-soluble carbohydrates from O-alkyl C and di-O-alkyl C in the OA bands may have been transported to the adjacent area through water diffusion, interacting with soil minerals to form micro- and macro-aggregates (Chenu et al. 2002; Abiven et al. 2009; Liu et al. 2018; Sarker, Incerti, et al. 2018). The path model further confirmed this proposition by showing a positive correlation between WMWD and aliphatic C in the OAs, specifically O-alkyl C and di-O-alkyl C (Figure 3). These findings suggest that labile OM may have an intense and transient effect on aggregate stability (Abiven et al. 2009). In contrast to plant-based OAs, animal manure-based OAs had inconsistent effects on soil aggregation (positive or no effect) in the short term. This contrasts with a long-term field study in a subtropical region of China, where pig manure accelerated soil aggregation more than plant residues (peanut and rice straw) in a clay loam soil (Lin et al. 2019). Such inconsistencies may be due to the transient effects of aggregation by labile OM and the turnover of aggregates (Abiven et al. 2009), as well as differences in soil properties and climate (Clark et al. 2009; Lin et al. 2019).

Our results indicated that short-term (3 months) increases in MBC were associated with increased aggregate stability (Figure 3a), consistent with the bottom-up model of soil aggregate formation (Clark et al. 2009; Lehmann et al. 2017). This could be due to the stimulation of fungal growth by the OAs (particularly plant residues), leading to greater production of fungal mycelia that effectively promote soil aggregation, particularly macro-aggregates in sandy clay loam cropping soils (Beare et al. 1997). The increased MBC (Table 4) may have resulted in greater production of microbial polysaccharides (which can account for 80%–90% of the cell wall dry mass for most fungal species) and extracellular polysaccharides, which can rapidly adsorb to clay minerals and 'glue' soil particles together to form aggregates (Walia and Dick 2018; Cania et al. 2020). Our findings also suggest that nutrient addition had no significant effect on aggregate stability, despite resulting in increased MBC (particularly in the wheat residues with nutrient inputs). This could be because nutrient inputs promoted more bacterial than fungal growth (Uddin et al. 2025), given the lower C:N ratio of bacteria (De Vries et al. 2006), whereas fungi—less constrained by nutrient stoichiometry—are recognised as the key biotic agent driving soil aggregation (de Goede et al. 2025). Furthermore, nutrient inputs with wheat residues decreased soil pH (by 0.17–0.22 units; Table 5) and increased the positive charge on the surface of kaolinite, which could have had a detrimental effect on the sorption of SOC to clay minerals through cation bridging (Kleber et al. 2021), decreasing soil aggregate formation.

Over time (17 months), there was a decrease in both WMWD and MBC in the soils adjacent to the amendment band due to biotic processes, which led to increased mineralisation/loss of SOC. This, in turn, resulted in low WMWD across the amended and non-amended soils. The correlation between WMWD and MBC was not detected at 17 months, as most microbial polysaccharides would have been recycled and/or lost via respiration (Kiem and Kögel-Knabner 2003; Fang et al. 2018; López-Mondéjar et al. 2020). The cumulative CO_2-C mineralised over the 17 months positively affected WMWD, indicating that C flux and biological functions can be critical indicators for stable soil structure formation (Annabi et al. 2007; Neal et al. 2020).

TABLE 5 | Soil chemical and physical properties after 17 months incubation of incubation.

Treatments	Mineral N (mg kg^{-1} soil)	CEC (cmol$_{(+)}$ kg^{-1} soil)	Dispersible clay (g kg^{-1} soil)	ESP	pH (1:5 H_2O)	EC (µS cm^{-1})
Bean residues	32.8	28.9	9.5	14.6	8.87	423
Reed residues	25.3	28.6	9.4*	13.7	8.95	382
Wheat residues	16.6	29.3	9.8	12.5*	9.07	322
Wheat residues + Nut1	42.5	29.1	10.1	12.5*	8.93	372
Wheat residues + Nut2	70.8*	30.3*	9.6	12.7	8.73	424*
Green waste + Pig manure	36.8	28.5	9.2*	13.9	8.95	384
Chicken manure composted	67.4*	29.4	9.3*	13.7	8.60*	425*
Chicken manure uncomposted	109.1*	29.8	10.0	13.2	8.35*	497*
Green organics compost	43.4	29.2	9.6	13.3	8.85	390
Pig manure	82.9*	29.3	9.6	13.5	8.48*	492*
Cow manure	50.2*	29.2	9.5*	13.2	8.70	450*
Compost + biochar	53.6*	28.9	9.9	13.8	8.85	443*
Compost + zeolite	29.2	28.5	9.6	13.1	8.95	355
Biosolids	94.4*	29.2	9.3*	13.5	8.08*	532*
Humate	38.3	29.4	11.0*	15.8*	8.93	471*
Gypsum_low	32.3	29.0	8.3*	12.3*	8.35*	493*
Gypsum_high	27	29.3	7.9*	11.8*	8.20*	505*
PAM	30.2	29.4	10.4	12.7	8.87*	383
PAM + Gypsum	34.2	31.2*	8.5*	12.7	8.05*	697*
Control	29.3	28.9	10.1	13.3	9.05	354
LSD$_{0.05}$	17.2	0.7	0.6	1.10	0.26	65

Note: LSD$_{0.05}$ indicates the least significant difference at 5% level. The asterisk indicates the significant difference between treatment and control. Data (ESP, pH and EC) were adapted from Uddin et al. (2025).
Abbreviations: CEC, cation exchange capacity; EC, electrical conductivity; ESP, exchangeable sodium percentage.

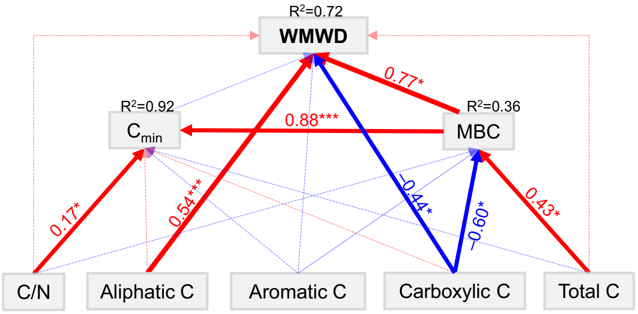
The C/N ratio of the OAs became an important indicator of soil structure response, as a higher C/N ratio of OAs increased WMWD indirectly, as shown by the path model (Figure 3).

4.2 | The Effect of Deep Placement Gypsum on Soil Aggregation Was Limited

This study found that gypsum applications significantly decreased clay dispersion (Table 5) and lowered soil pH. The role of pH in controlling the surface charge of clay is well established: higher pH increases repulsive forces in the electrical double layer at the surface of charged colloids, thereby increasing soil dispersion (Tavakkoli et al. 2015; Rengasamy et al. 2016). The decrease in soil pH resulting from gypsum application can be linked to the formation of $CaCO_3$ and the consequent reduction in zeta potential, which promotes clay flocculation (Bronick and Lal 2005).

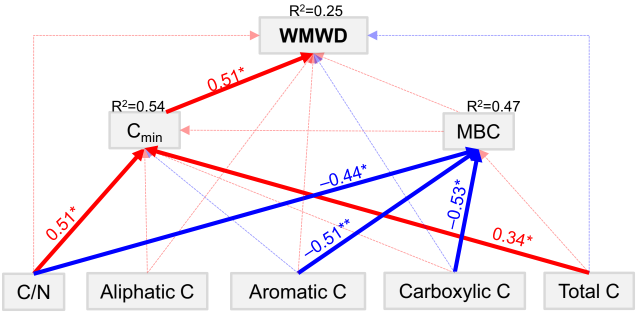
However, the deep placement of high or low gypsum rates did not affect soil aggregation in areas adjacent to banded amendments (Table 4). This contrasts with a previous soil incubation study where gypsum improved soil aggregation over 9 months when uniformly mixed with the same sodic dispersive subsoil (Fang et al. 2021). The inconsistency between these results may be due to the low solubility and mobility of gypsum in soil, resulting in limited diffusion to areas outside the banded amendment zone (ripping line). Therefore, deep placement of gypsum (compared with uniform mixing) along the ripping line may not effectively improve the physical structure of subsoil beyond the immediate vicinity of the amendment. However, combined applications of gypsum and OAs have been shown to be effective

(a) 3 months



χ^2=0.012, df=1, p=0.914; RMSEA<0.001, p=0.917

(b) 17 months



χ^2=0.161, df=1, p=0.688; RMSEA<0.001, p=0.699

FIGURE 3 | Partial least squares path modelling (PLS-PM) examining the direct and indirect effects of different factors on soil aggregate stability (i.e., wet mean weight diameter; WMWD) at 3 months (a) and 17 months (b). The factors include microbial biomass C (MBC), cumulative total C mineralised (C_{min}), C functional groups of the organic amendments (OAs), total C (TC) and total N (TN) contents in the OAs, dissolved organic C (DOC) and dissolved N (DN) contents in the OAs. Each box represents an observed variable. The blue and red solid arrows indicate positive and negative path coefficients, respectively. The values next to the arrows are the path coefficients calculated after 1000 bootstraps, and significance levels are indicated by *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. Dashed arrows show that path coefficients did not differ significantly ($p > 0.05$). The Goodness of Fit (GoF) statistic, a measure of the overall prediction performance.

and may serve as an alternative to conventional gypsum applications (Fang et al. 2021).

5 | Conclusions

This study provides a comprehensive understanding of how organic amendments of contrasting compositions (i.e., total carbon and nitrogen, C/N ratio and carbon functional groups) and inorganic amendments affect aggregate formation over a 17-month period. Among the organic amendments tested, plant-derived residues such as those from reed, bean and wheat residues were the most effective in enhancing soil aggregation, while the addition of exogenous nutrients to wheat residues had no additional impact. (Un)composted chicken manure also improved soil aggregation over the short-term (3 months) and maintained high N availability over a longer period of 17 months, indicating their potential use in addressing subsoil constraints and providing a source of nutrients. The disappearance of long-term effects on soil aggregation in our 17-month incubation suggests that repeated applications of suitable organic amendments may be required to maintain improved soil structure under field conditions. Caution is needed when extrapolating these laboratory findings to subsoil field conditions. In conclusion, our study provides new knowledge on how organic amendment composition affects soil structure via soil biotic and abiotic processes, with important implications for managing alkaline sodic subsoils.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

Abiven, S., S. Menasseri, and C. Chenu. 2009. "The Effects of Organic Inputs Over Time on Soil Aggregate Stability—A Literature Analysis." *Soil Biology and Biochemistry* 41: 1–12. https://doi.org/10.1016/j.soilbio.2008.09.015.

Annabi, M., S. Houot, C. Francou, M. Poitrenaud, and Y. L. Bissonnais. 2007. "Soil Aggregate Stability Improvement With Urban Composts of Different Maturities." *Soil Science Society of America Journal* 71: 413–423. https://doi.org/10.2136/sssaj2006.0161.

Bai, T., P. Wang, S. J. Hall, et al. 2020. "Interactive Global Change Factors Mitigate Soil Aggregation and Carbon Change in a Semi-Arid Grassland." *Global Change Biology* 26: 5320–5332. https://doi.org/10.1111/gcb.15220.

Baldock, J., J. Oades, P. Nelson, T. Skene, A. Golchin, and P. Clarke. 1997. "Assessing the Extent of Decomposition of Natural Organic Materials Using Solid-State 13C NMR Spectroscopy." *Soil Research* 35: 1061–1084. https://doi.org/10.1071/S97004.

Baldock, J. A., C. Creamer, S. Szarvas, J. McGowan, T. Carter, and M. Farrell. 2021. "Linking Decomposition Rates of Soil Organic Amendments to Their Chemical Composition." *Soil Research* 59: 630–643. https://doi.org/10.1071/sr20269.

Beare, M., S. Hu, D. Coleman, and P. Hendrix. 1997. "Influences of Mycelial Fungi on Soil Aggregation and Organic Matter Storage in Conventional and No-Tillage Soils." *Applied Soil Ecology* 5: 211–219. https://doi.org/10.1016/S0929-1393(96)00142-4.

Bell, C. W., B. E. Fricks, J. D. Rocca, J. M. Steinweg, S. K. McMahon, and M. D. Wallenstein. 2013. "High-Throughput Fluorometric Measurement of Potential Soil Extracellular Enzyme Activities." *Journal of Visualized Experiments: JoVE* 81: e50961. https://doi.org/10.3791/50961.

Bronick, C. J., and R. Lal. 2005. "Soil Structure and Management: A Review." *Geoderma* 124: 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005.

Butler, D. G. 2021. "ASReml: Fits the Linear Mixed Model. R Package Version 4.1.0.160." www.vsni.co.uk.

Cania, B., G. Vestergaard, M. Suhadolc, et al. 2020. "Site-Specific Conditions Change the Response of Bacterial Producers of Soil Structure-Stabilizing Agents Such as Exopolysaccharides and Lipopolysaccharides to Tillage Intensity." *Frontiers in Microbiology* 11: 568. https://doi.org/10.3389/fmicb.2020.00568.

Chan, K., D. Heenan, and A. Oates. 2002. "Soil Carbon Fractions and Relationship to Soil Quality Under Different Tillage and Stubble Management." *Soil and Tillage Research* 63: 133–139. https://doi.org/10.1016/S0167-1987(01)00239-2.

Chen, C., Z. Xu, and N. Mathers. 2004. "Soil Carbon Pools in Adjacent Natural and Plantation Forests of Subtropical Australia." *Soil Science Society of America Journal* 68: 282–291. https://doi.org/10.2136/sssaj2004.2820.

Chenu, C., G. Stotzky, P. Huang, and J. Bollag. 2002. "Interactions Between Microorganisms and Soil Particles: An Overview." *Interactions Between Soil Particles and Microorganisms: Impact on the Terrestrial Ecosystem* 1: 1–40.

Clark, G., N. Dodgshun, P. Sale, and C. Tang. 2007. "Changes in Chemical and Biological Properties of a Sodic Clay Subsoil With Addition of Organic Amendments." *Soil Biology & Biochemistry* 39: 2806–2817. https://doi.org/10.1016/j.soilbio.2007.06.003.

Clark, G., P. Sale, and C. Tang. 2009. "Organic Amendments Initiate the Formation and Stabilisation of Macroaggregates in a High Clay Sodic Soil." *Soil Research* 47: 770–780. https://doi.org/10.1071/SR09119.

de Goede, S. P., S. E. Hannula, B. Jansen, and E. Morriën. 2025. "Fungal-Mediated Soil Aggregation as a Mechanism for Carbon Stabilization." *ISME Journal* 19: ewraf074. https://doi.org/10.1093/ismejo/wraf074.

Deng, S., H. Kang, and C. Freeman. 2011. "Microplate Fluorimetric Assay of Soil Enzymes." *Methods of Soil Enzymology* 9: 311–318. https://doi.org/10.2136/sssabookser9.c14.

De Vries, F. T., E. Hoffland, N. Van Eekeren, L. Brussaard, and J. Bloem. 2006. "Fungal/Bacterial Ratios in Grasslands With Contrasting Nitrogen Management." *Soil Biology and Biochemistry* 38: 2092–2103. https://doi.org/10.1016/j.soilbio.2006.01.008.

Fang, Y., B. P. Singh, D. Collins, B. Li, J. Zhu, and E. Tavakkoli. 2018. "Nutrient Supply Enhanced Wheat Residue-Carbon Mineralization, Microbial Growth, and Microbial Carbon-Use Efficiency When Residues Were Supplied at High Rate in Contrasting Soils." *Soil Biology and Biochemistry* 126: 168–178. https://doi.org/10.1016/j.soilbio.2018.09.003.

Fang, Y., B. P. Singh, M. Farrell, et al. 2020. "Balanced Nutrient Stoichiometry of Organic Amendments Enhances Carbon Priming in a Poorly Structured Sodic Subsoil." *Soil Biology and Biochemistry* 145: 107800. https://doi.org/10.1016/j.soilbio.2020.107800.

Fang, Y., B. P. Singh, L. Van Zwieten, et al. 2021. "Additive Effects of Organic and Inorganic Amendments Can Significantly Improve Structural Stability of a Sodic Dispersive Subsoil." *Geoderma* 404: 115281. https://doi.org/10.1016/j.geoderma.2021.115281.

Fang, Y., E. Tavakkoli, Z. Weng, et al. 2022. "Disentangling Carbon Stabilization in a Calcisol Subsoil Amended With Iron Oxyhydroxides: A Dual-^{13}C Isotope Approach." *Soil Biology and Biochemistry* 170: 108711. https://doi.org/10.1016/j.soilbio.2022.108711.

Fanin, N., D. Moorhead, and I. Bertrand. 2016. "Eco-Enzymatic Stoichiometry and Enzymatic Vectors Reveal Differential C, N, P Dynamics in Decaying Litter Along a Land-Use Gradient." *Biogeochemistry* 129: 21–36. https://doi.org/10.1007/s10533-016-0217-5.

FAO. 2024. *Global Status of Salt-Affected Soils*. FAO. https://doi.org/10.4060/cd3044en.

Fernandez, C. W., J. A. Langley, S. Chapman, M. L. McCormack, and R. T. Koide. 2016. "The Decomposition of Ectomycorrhizal Fungal Necromass." *Soil Biology and Biochemistry* 93: 38–49. https://doi.org/10.1016/j.soilbio.2015.10.017.

Gillman, G. P., and E. A. Sumpter. 1986. "Modification to the Compulsive Exchange Method for Measuring Exchange Characteristics of Soils." *Soil Research* 24: 61–66. https://doi.org/10.1071/sr9860061.

Golchin, A., J. Baldock, and J. Oades. 1997. "A Model Linking Organic Matter Decomposition, Chemistry, and Aggregate Dynamics." In *Soil Processes and the Carbon Cycle*, 245–266. CRC Press.

Green, H., P. Larsen, J. Koci, W. Edwards, and P. N. Nelson. 2023. "Long-Term Effects of Gypsum on the Chemistry of Sodic Soils." *Soil and Tillage Research* 233: 105780. https://doi.org/10.1016/j.still.2023.105780.

Gupta, V. V. S. R., and J. J. Germida. 2015. "Soil Aggregation: Influence on Microbial Biomass and Implications for Biological Processes." *Soil Biology and Biochemistry* 80: A3–A9. https://doi.org/10.1016/j.soilbio.2014.09.002.

Hair, J. F., Jr., M. Sarstedt, L. Hopkins, and V. G. Kuppelwieser. 2014. "Partial Least Squares Structural Equation Modeling (PLS-SEM)." *European Business Review* 26: 106–121. https://doi.org/10.1108/EBR-10-2013-0128.

Hartmann, M., and J. Six. 2023. "Soil Structure and Microbiome Functions in Agroecosystems." *Nature Reviews Earth & Environment* 4: 4–18. https://doi.org/10.1038/s43017-022-00366-w.

Isbell, R. F. 2002. *The Australian Soil Classification (Revised Edition)*. CSIRO Publishing.

IUSS Working Group WRB. 2006. "World Reference Base for Soil Resources." In *World Soil Resources Report*, 103. IUSS Working Group WRB.

Jin, J., M. Hart, R. Armstrong, P. Sale, G. J. Clark, and C. Tang. 2025. "Subsoil Manuring Improves the Soil Physical Properties and Crop Rooting Behaviour in Soil Profiles in Dryland Cropping Systems Over Three Seasons." *Soil and Tillage Research* 252: 106588. https://doi.org/10.1016/j.still.2025.106588.

Kiem, R., and I. Kögel-Knabner. 2003. "Contribution of Lignin and Polysaccharides to the Refractory Carbon Pool in C-Depleted Arable Soils." *Soil Biology and Biochemistry* 35: 101–118. https://doi.org/10.1016/S0038-0717(02)00242-0.

Kjeldahl, C. 1883. "A New Method for the Determination of Nitrogen in Organic Matter." *Fresenius' Journal of Analytical Chemistry* 22: 366.

Kleber, M., I. C. Bourg, E. K. Coward, C. M. Hansel, S. C. B. Myneni, and N. Nunan. 2021. "Dynamic Interactions at the Mineral–Organic Matter Interface." *Nature Reviews Earth & Environment* 2: 402–421. https://doi.org/10.1038/s43017-021-00162-y.

Lebron, I., D. L. Suarez, and T. Yoshida. 2002. "Gypsum Effect on the Aggregate Size and Geometry of Three Sodic Soils Under Reclamation." *Soil Science Society of America Journal* 66: 92–98. https://doi.org/10.2136/sssaj2002.9200.

Lehmann, A., W. Zheng, and M. C. Rillig. 2017. "Soil Biota Contributions to Soil Aggregation." *Nature Ecology & Evolution* 1: 1828–1835. https://doi.org/10.1038/s41559-017-0344-y.

Lehmann, J., C. M. Hansel, C. Kaiser, et al. 2020. "Persistence of Soil Organic Carbon Caused by Functional Complexity." *Nature Geoscience* 13: 529–534. https://doi.org/10.1038/s41561-020-0612-3.

Leogrande, R., and C. Vitti. 2019. "Use of Organic Amendments to Reclaim Saline and Sodic Soils: A Review." *Arid Land Research and Management* 33: 1–21. https://doi.org/10.1080/15324982.2018.1498038.

Liang, B., J. Wei, Y. Gao, Z. Ma, and X. Xue. 2025. "A Review of Classification, Causes, and Amelioration of Global Salt-Affected Soil Based on the Meta-Analysis." *Land Degradation & Development* 36: 5485–5504. https://doi.org/10.1002/ldr.5619.

Lin, Y., G. Ye, Y. Kuzyakov, D. Liu, J. Fan, and W. Ding. 2019. "Long-Term Manure Application Increases Soil Organic Matter and Aggregation, and Alters Microbial Community Structure and Keystone Taxa." *Soil Biology and Biochemistry* 134: 187–196. https://doi.org/10.1016/j.soilbio.2019.03.030.

Liu, X., M. R. Rashti, A. Dougall, M. Esfandbod, L. Van Zwieten, and C. Chen. 2018. "Subsoil Application of Compost Improved Sugarcane Yield Through Enhanced Supply and Cycling of Soil Labile Organic Carbon and Nitrogen in an Acidic Soil at Tropical Australia." *Soil & Tillage Research* 180: 73–81. https://doi.org/10.1016/j.still.2018.02.013.

López-Mondéjar, R., V. Tláskal, T. Větrovský, et al. 2020. "Metagenomics and Stable Isotope Probing Reveal the Complementary Contribution of Fungal and Bacterial Communities in the Recycling of Dead Biomass in Forest Soil." *Soil Biology and Biochemistry* 148: 107875. https://doi.org/10.1016/j.soilbio.2020.107875.

Midwood, A. J., and T. W. Boutton. 1998. "Soil Carbonate Decomposition by Acid Has Little Effect on $\delta^{13}C$ of Organic Matter." *Soil Biology and Biochemistry* 30: 1301–1307. https://doi.org/10.1016/S0038-0717(98)00030-3.

Moorhead, D. L., R. L. Sinsabaugh, B. H. Hill, and M. N. Weintraub. 2016. "Vector Analysis of Ecoenzyme Activities Reveal Constraints on Coupled C, N and P Dynamics." *Soil Biology and Biochemistry* 93: 1–7. https://doi.org/10.1016/j.soilbio.2015.10.019.

Neal, A. L., A. Bacq-Labreuil, X. Zhang, et al. 2020. "Soil as an Extended Composite Phenotype of the Microbial Metagenome." *Scientific Reports* 10: 10649. https://doi.org/10.1038/s41598-020-67631-0.

Oades, J. M. 1984. "Soil Organic-Matter and Structural Stability - Mechanisms and Implications for Management." *Plant and Soil* 76: 319–337. https://doi.org/10.1007/Bf02205590.

Pagliai, M., N. Vignozzi, and S. Pellegrini. 2004. "Soil Structure and the Effect of Management Practices." *Soil and Tillage Research* 79: 131–143. https://doi.org/10.1016/j.still.2004.07.002.

R Core Team. 2024. *R: A Language and Environment for Statistical Computing: A Graduate Course in Probability*. R Foundation for Statistical Computing.

Rabbi, S. M. F., B. Minasny, A. B. McBratney, and I. M. Young. 2020. "Microbial Processing of Organic Matter Drives Stability and Pore Geometry of Soil Aggregates." *Geoderma* 360: 114033. https://doi.org/10.1016/j.geoderma.2019.114033.

Rabbi, S. M. F., C. R. Warren, B. Swarbrick, B. Minasny, A. B. McBratney, and I. M. Young. 2024. "Microbial Decomposition of Organic Matter and Wetting–Drying Promotes Aggregation in Artificial Soil but Porosity Increases Only in Wet-Dry Condition." *Geoderma* 447: 116924. https://doi.org/10.1016/j.geoderma.2024.116924.

Rabot, E., M. Wiesmeier, S. Schlüter, and H. J. Vogel. 2018. "Soil Structure as an Indicator of Soil Functions: A Review." *Geoderma* 314: 122–137. https://doi.org/10.1016/j.geoderma.2017.11.009.

Rengasamy, P. 2002. "Transient Salinity and Subsoil Constraints to Dryland Farming in Australian Sodic Soils: An Overview." *Australian Journal of Experimental Agriculture* 42: 351–361. https://doi.org/10.1071/EA01111.

Rengasamy, P., and K. A. Olsson. 1991. "Sodicity and Soil Structure." *Australian Journal of Soil Research* 29: 935–952. https://doi.org/10.1071/SR9910935.

Rengasamy, P., E. Tavakkoli, and G. K. McDonald. 2016. "Exchangeable Cations and Clay Dispersion: Net Dispersive Charge, a New Concept for Dispersive Soil." *European Journal of Soil Science* 67: 659–665. https://doi.org/10.1111/ejss.12369.

Sale, P. W., J. S. Gill, R. R. Peries, and C. Tang. 2019. "Crop Responses to Subsoil Manuring. I. Results in South-Western Victoria From 2009 to 2012." *Crop and Pasture Science* 70: 44–54. https://doi.org/10.1071/CP18115.

Sanchez, G., L. Trinchera, M. G. Sanchez, and FactoMineR, S. 2013. *Package 'Plspm'*. Citeseer.

Sarker, J. R., B. P. Singh, A. L. Cowie, et al. 2018. "Carbon and Nutrient Mineralisation Dynamics in Aggregate-Size Classes From Different Tillage Systems After Input of Canola and Wheat Residues." *Soil Biology and Biochemistry* 116: 22–38. https://doi.org/10.1016/j.soilbio.2017.09.030.

Sarker, T. C., G. Incerti, R. Spaccini, A. Piccolo, S. Mazzoleni, and G. Bonanomi. 2018. "Linking Organic Matter Chemistry With Soil Aggregate Stability: Insight From ^{13}C NMR Spectroscopy." *Soil Biology and Biochemistry* 117: 175–184. https://doi.org/10.1016/j.soilbio.2017.11.011.

Sarker, T. C., M. Zotti, Y. Fang, et al. 2022. "Soil Aggregation in Relation to Organic Amendment: A Synthesis." *Journal of Soil Science and Plant Nutrition* 22: 2481–2502. https://doi.org/10.1007/s42729-022-00822-y.

Singh, B. P., Y. Fang, M. Boersma, D. Collins, L. Van Zwieten, and L. M. Macdonald. 2015. "In Situ Persistence and Migration of Biochar Carbon and Its Impact on Native Carbon Emission in Contrasting Soils Under Managed Temperate Pastures." *PLoS One* 10: e0141560. https://doi.org/10.1371/journal.pone.0141560.

Six, J., E. Elliott, K. Paustian, and J. Doran. 1998. "Aggregation and Soil Organic Matter Accumulation in Cultivated and Native Grassland Soils." *Soil Science Society of America Journal* 62: 1367–1377. https://doi.org/10.2136/sssaj1998.03615995006200050032x.

Smucker, A. J., E.-J. Park, J. Dorner, and R. Horn. 2007. "Soil Micropore Development and Contributions to Soluble Carbon Transport Within Macroaggregates." *Vadose Zone Journal* 6: 282–290. https://doi.org/10.2136/vzj2007.0031.

Tavakkoli, E., P. Rengasamy, E. Smith, and G. K. McDonald. 2015. "The Effect of Cation–Anion Interactions on Soil pH and Solubility of Organic Carbon." *European Journal of Soil Science* 66: 1054–1062. https://doi.org/10.1111/ejss.12294.

Tavakkoli, E., Z. Weng, I. Tahmasbian, et al. 2019. *Understanding the Amelioration Processes of the Subsoil Application of Amendments*, 18–19. GRDC Grains Research Update.

Uddin, S., M. Rohan, Z. H. Weng, et al. 2025. "Enhancing Root Proliferation in an Alkaline Dispersive Subsoil: A Comparative Study of Organic and Inorganic Amendments With Different Amelioration Mechanisms." *Journal of Soil Science and Plant Nutrition* 25: 7381–7396. https://doi.org/10.1007/s42729-025-02602-w.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson. 1987. "An Extraction Method for Measuring Soil Microbial Biomass-C." *Soil Biology and Biochemistry* 19: 703–707. https://doi.org/10.1016/0038-0717(87)90052-6.

Verchot, L. V., L. Dutaur, K. D. Shepherd, and A. Albrecht. 2011. "Organic Matter Stabilization in Soil Aggregates: Understanding the Biogeochemical Mechanisms That Determine the Fate of Carbon Inputs in Soils." *Geoderma* 161: 182–193. https://doi.org/10.1016/j.geoderma.2010.12.017.

Vidal, A., J. Hirte, S. F. Bender, et al. 2018. "Linking 3D Soil Structure and Plant-Microbe-Soil Carbon Transfer in the Rhizosphere." *Frontiers in Environmental Science* 6: e9. https://doi.org/10.3389/fenvs.2018.00009.

Walia, M. K., and W. A. Dick. 2018. "Selected Soil Physical Properties and Aggregate-Associated Carbon and Nitrogen as Influenced by

Gypsum, Crop Residue, and Glucose." *Geoderma* 320: 67–73. https://doi.org/10.1016/j.geoderma.2018.01.022.

Wang, X., P. Sale, H. Hayden, C. Tang, G. Clark, and R. Armstrong. 2020. "Plant Roots and Deep-Banded Nutrient-Rich Amendments Influence Aggregation and Dispersion in a Dispersive Clay Subsoil." *Soil Biology and Biochemistry* 141: 107664. https://doi.org/10.1016/j.soilbio.2019.107664.

Yoder, R. E. 1936. "A Direct Method of Aggregate Analysis of Soils and a Study of the Physical Nature of Erosion Losses." *Agronomy Journal* 28: 337–351.

Zhu, Y., A. Marchuk, and J. McLean Bennett. 2016. "Rapid Method for Assessment of Soil Structural Stability by Turbidimeter." *Soil Science Society of America Journal* 80: 1629–1637. https://doi.org/10.2136/sssaj2016.07.0222.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Soil mass proportion distribution in aggregate-size classes in the control Solonetz (20–40 cm soil depth) and the amendment-treated soils at 3 and 17 months. The aggregate-size classes include macro-aggregates (> 0.25 mm), micro-aggregates (0.05–0.25 mm) and silt-clay fraction (< 0.05 mm). The numbers after '±' represent the standard errors of the mean ($n = 3$). **Figure S1:** Cumulative total C mineralised in the treatments (g CO_2-C m^{-2}) over the 17 months incubation period. Error bars represent standard errors of the means ($n = 4$).