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Enhancing Root Proliferation in an Alkaline Dispersive Subsoil: a Comparative Study of Organic and Inorganic Amendments with Different Amelioration Mechanisms

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Abstract

Purpose: Alkaline dispersive subsoils contain a range of physicochemical constraints that restrict root proliferation and limit water and nutrient extraction, leading to yield penalties. We investigated the effectiveness of organic, inorganic and a combination of organic and inorganic (combined) amendments with contrasting chemical compositions in mitigating constraints on crops grown in these subsoils. Methods: An alkaline dispersive subsoil (20 – 40 cm depth) with pH_w 8.9 and an exchangeable sodium percentage (ESP) of 12.9% was incubated for 14.5 months with 19 different organic (crop residues, animal manures and composted materials), inorganic (gypsum, polyacrylamide (PAM) and their combinations (PAM+Gypsum)) and combined (combination of wheat stubble and chemical fertilisers) amendments or control. The amendments were applied as a band within a soil core. Following incubation, the incubated subsoil was mounted on a custom-built sand core and wheat (Triticum aestivum cv. Lancer) was grown. Water use was monitored weekly. Plant biomass, root biomass, root length density, and soil physicochemical properties were determined at harvest. Results: Organic amendments with a low C:N ratio (i.e., high N content) enhanced root proliferation (up to 63%) through the nutrient patches from the mineralising organic matter and improved porosity by macro-aggregate formation (84%), and promoting fungal (500%) and bacterial (47%) abundance. In contrast, inorganic amendments such as gypsum, alone or in combination with PAM, improved micro-aggregate formation (14%) by reducing soil pH (11%) and ESP (14%) and increasing electrical conductivity (EC; 97%). Wheat stubble, alone or combined with chemical fertilisers, significantly increased macro-aggregate formation (67%), root proliferation (61%) and water use (21%). Plant biomass and water use were associated with increased root proliferation through the amended soil layer. Conclusions: The co-application of organic and inorganic amendments with contrasting modes of action might have additive effects on ameliorating alkaline dispersive subsoils with multiple physicochemical constraints.

 $\textbf{Keywords} \ \ Subsoil \ amelioration \cdot Aggregate \ formation \cdot Exchangeable \ sodium \ percentage \cdot pH \cdot Root \ length \ density \cdot Root \ biomass \cdot Plant \ biomass \cdot Water \ use$

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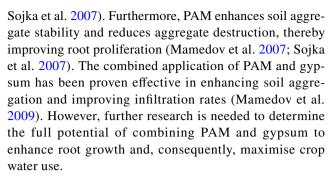


1 Introduction

A substantial portion of rainfed cropland is afflicted by various soil physicochemical constraints that can significantly reduce grain yield in Australia (Adcock et al. 2007; Orton et al. 2018; Rengasamy 2002) and globally (Basak et al. 2022). Alkaline dispersive subsoil is a primary contributor to the most significant yield penalties in many grain-cropping regions across Australia, resulting in an estimated annual loss of \$1.3 billion AUD (Orton et al. 2018). Alkaline dispersive subsoils impose a range of physicochemical constraints, including slaking and dispersion, leading to poorly structured subsoil, reduced rainfall infiltration, impeded drainage, waterlogging, high pH and soil strength (Rengasamy and Olsson 1991). These constraints limit root proliferation and subsequent water and nutrient extraction, resulting in significant yield penalties and poor water use efficiency (WUE) (Jayawardane and Chan 1994; Passioura and Angus 2010).

Enhancing grain yield and WUE in the poorly structured alkaline dispersive subsoils can be achieved by improving the storage of rainwater within the soil's root zone and optimising its accessibility to crops (Sale et al. 2021). In such scenarios, a pivotal factor in harnessing rainwater effectively lies in improving soil structure, which fosters greater infiltration rates and promotes root growth within the subsoil (Gill et al. 2009). Previous studies have provided compelling evidence of a substantial improvement in crop productivity with the amelioration of alkaline dispersive subsoils under rainfed conditions (Gill et al. 2008; Sale et al. 2021). Despite the reported benefits of ameliorating alkaline dispersive subsoils under rainfed conditions, the underlying mechanisms remain largely unknown. Therefore, appropriate management practices cannot be selected.

There are several approaches to ameliorate alkaline dispersive soils using both inorganic and organic amendments. Traditionally, inorganic amendments such as gypsum have been the most widely adopted approach, with significant impacts on both soil physical and chemical properties (Clark et al. 2007; Walia and Dick 2018) as well as grain yield (Tavakkoli et al. 2022). The gypsum-derived Ca²⁺ displaces Na⁺ from the cation exchange sites of the clay particles (Qadir et al. 2001), reducing exchangeable sodium percentage (ESP) and improving soil aggregation (Clark et al. 2007; Wang et al. 2020). However, its efficacy in ameliorating alkaline dispersive subsoil can be constrained due to its limited solubility and mobility (Shainberg et al. 1989), especially in dryland conditions. Anionic polyacrylamide (PAM) has also been used as a soil conditioner to minimise soil sealing, runoff and erosion and improve infiltration rates (Mamedov et al. 2009;



In addition to inorganic amendments, the deep placement of organic amendments has shown potential to directly re-engineer subsoil properties (Celestina et al. 2018; Gill et al. 2009; Sale et al. 2021). Some recent studies reporting detailed soil-plant interactions demonstrated an increase in grain yield following the amelioration of alkaline dispersive subsoils with deep placement of organic and inorganic amendments, which have been attributed to improvement in soil physical, chemical and biological properties thereby leading to improved root growth within the subsoil layers (Gill et al. 2009; Li et al. 2023, 2025; Uddin et al. 2022b; Wang et al. 2020). Further, the utilisation of organic amendments (i.e., crop residues, compost and animal manure) has demonstrated the ability to promote aggregate formation, both in controlled incubation experiments (Clark et al. 2009; Fang et al. 2021; Howell et al. 2024; Niaz et al. 2022; Wang et al. 2020) and in the field conditions (Gill et al. 2009; Li et al. 2023; Zhang et al. 2017).

The formation of soil aggregates following the application of organic amendments is mostly due to the presence or production of various organic binding agents, including soil organic carbon (SOC), glomalin-related soil proteins and decomposed carbohydrate polymers (Abiven et al. 2009; Fang et al. 2020; Xie et al. 2015; Zhang et al. 2016). However, the abundance and characteristics of these binding agents are largely influenced by microbial activity and the C:N ratio of organic amendments incorporated, which in turn affect the formation of soil aggregates (Abiven et al. 2009; Baldock et al. 2021; De Gryze et al. 2005; Sonnleitner et al. 2003).

The formation of larger soil aggregates increases soil porosity and permeability, which potentially can improve root growth in the amended subsoil layer (Wang et al. 2020). Previous research has focused only on a limited number of amendments. Thus, it is unclear how the chemical properties of amendments, such as C:N ratio and C functional groups, may influence the microbial populations and subsequent aggregate formation. Therefore, a controlled environment experiment was conducted to investigate the microbial processes, aggregate formation, root growth and water use of wheat following the amelioration of an alkaline dispersive subsoil with organic, inorganic and combined amendments



of contrasting C:N ratios and C functional groups. We hypothesised that:

- Nutrient-balanced organic amendments can stimulate biodegradation, thereby improving the aggregation of alkaline dispersive subsoils.
- The formation of soil aggregates following the amelioration of alkaline dispersive subsoil will lead to better root proliferation.
- Crop water use will correlate with the improved root proliferation facilitated by the improved soil structure in the amended subsoil.

2 Materials and Methods

2.1 Soil Physicochemical Properties

A highly alkaline dispersive subsoil (20–40 cm depth) was collected from a cropping paddock near Grogan in Southern New South Wales, Australia (34°14′29"S 147°45′50"E). The soil was classified as a Solonetz as per the WRB classification (IUSS Working Group WRB 2015) and a Sodosol as per the Australian Soil Classification (Isbell 2016). Following collection, the soil was air-dried, ground and sieved to < 2 mm. Mineral N in the soil (NH₄⁺-N and NO₃⁻-N) was measured following extraction with 2 M KCl (1:5 w/v) after shaking for 1 h (Blackmore et al. 1987) and measured by a SEAL AQ2 Analyzer (SEAL Analytical, Maguon, WI, USA). A soil-water release curve was determined using the pressure plate method (Klute 1986) and the soil moisture content at field capacity (-10 kPa, equivalent to 35% g/g) and wilting point (20% g/g) was estimated. The soil was highly alkaline (pH_w ~ 8.9), non-saline (EC1:5 $\sim 426 \,\mu\text{S/cm}$) and sodic (ESP~12.9%). Based on the Emerson test the soil is Type 3 of Class 2, showing a high level of dispersion and slaking (Emerson 1967). Selected physicochemical properties of the soil are shown in Table 1.

2.2 Properties of Amendments

The experiment consisted of a total of 20 treatments including 19 amendments and a Control (Table S1). The selection of different organic amendments covered a range of C:N (7~109; Table S2), being plant residues, animal manures, compost and biochar/zeolite mixtures, humate (Sigma-Aldrich, USA) and biosolids (as detailed in Table S1) used at 15 t dry weight ha⁻¹ (equivalent to 12 g kg⁻¹ dry soil). The inorganic amendments included gypsum at 5 and 15 t dry weight ha⁻¹ (4 and 12 g kg⁻¹ soil), and anionic polyacrylamide (PAM; HydraBond® HB-4305, Hydroflux Utilities Pty Ltd, Sydney, Australia) at 5 kg ha⁻¹ (equivalent to 4 mg kg⁻¹ soil) without or with gypsum (5 t ha⁻¹

Table 1 The basic physicochemical properties of the alkaline dispersive subsoil (20–40 cm soil depth) used in the study. Data are mean \pm standard error (n=4)

| Soil properties | Values |
|--|-----------------|
| pH (1:5 H ₂ O) | 8.9±0.01 |
| Electrical conductivity (μS cm ⁻¹) | 426 ± 49 |
| Exchangeable sodium percentage (%) | 12.9 ± 0.6 |
| Nitrate nitrogen (mg kg ⁻¹) | 9.9 ± 1.8 |
| Ammonium nitrogen (mg kg ⁻¹) | 1.8 ± 0.1 |
| Phosphorus—Colwell (mg kg ⁻¹) | 1.9 ± 0.3 |
| Sand (%) | 40.3 ± 0.5 |
| Silt (%) | 8.5 ± 0.8 |
| Clay (%) | 51.3 ± 0.9 |
| Field capacity θ (g/g) | 0.35 ± 0.01 |
| Permanent wilting point θ (g/g) | 0.20 ± 0.01 |

equivalent to 4 g kg⁻¹ soil). Supplementary nutrients were added with wheat residues in the treatments Wheat + Nut1 and Wheat + Nut2 (Table S1), to facilitate the transformation of the wheat residue C into stabilized SOC with the theoretical stoichiometry of the stabilized SOM (i.e., C: N: P: S ratio of 100: 8.33: 2: 1.4) (Fang et al. 2019; Kirkby et al. 2013). There were two nutrient rates with wheat residues: (1) wheat residues receiving 65 mg (NH₄)₂SO₄/NH₄NO₃-N kg⁻¹ dry soil, 15 mg KH₂PO₄-P kg⁻¹ dry soil and 11 mg $(NH_4)_2SO_4$ -S kg⁻¹ dry soil (Table S1; Wheat + Nut1); and (2) wheat residues receiving 130 mg N kg⁻¹ dry soil, 30 mg P kg⁻¹ dry soil and 22 mg S kg⁻¹ dry soil (Wheat + Nut2). Based on the source of origin, amendments were grouped into organic (crop residues, animal manures and composted materials), inorganic (Gypsum, PAM and PAM + Gypsum) and combined (combination of wheat stubble and chemical fertilisers) (Tables S1 and S2).

2.3 Experiment 1: Incubation Trial

2.3.1 Experimental Design and Preparation of PVC Cores for Incubation

An incubation experiment was designed to quantify the ameliorative capacity of different amendments and their effects on the physicochemical and biological properties of alkaline dispersive subsoil. A randomised complete block design comprising 20 amendments and 4 replicates was used. An additional subset of 80 cores was prepared for growing plants at a later stage. Amendments were mixed with 300 g subsoil and buried as a vertical band (to mimic the band application of amendments in the field) in the middle of the bulk subsoils (700 g) and packed to soil bulk density of 1.3 g cm⁻³ in an opened cylindrical Polyvinyl chloride (PVC) core (*i.e.*, 150 mm diameter×200 mm height) (Fig. 1a, b).



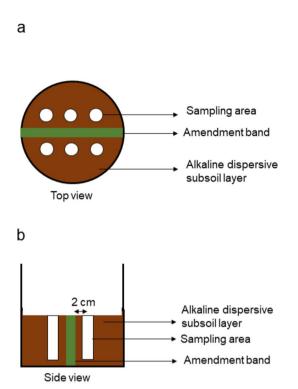
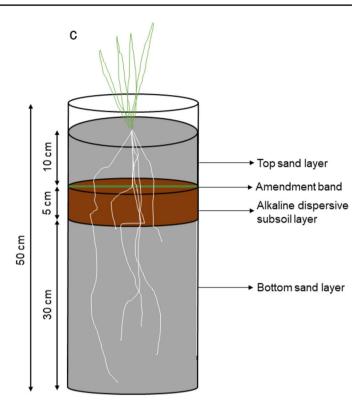


Fig. 1 Schematic diagram of the top (a) and side (b) view of the PVC core used for incubation of an alkaline dispersive subsoil with different organic, inorganic and combined amendments. The centre of the soil cores collected for different physicochemical analyses was spaced

The rate of organic amendments was equivalent to 15 t ha⁻¹ (Table S1), which was selected based on earlier research findings from dryland cropping systems (Celestina et al. 2018; Gill et al. 2019; 2008; Sale et al. 2021; 2019). The soil water content was adjusted to 70% field capacity (FC) and maintained weekly by adding reverse osmosis (RO) water to the original weights during the incubation period. The PVC cores were incubated at room temperature (20 ± 0.5 °C) in the dark and arbitrarily rotated weekly within each replicate to minimise any edge effect and temperature fluctuations. Following 14.5 months of incubation, one set of 80 cores was transferred to the glasshouse for growing plants using a custom-built sand core (Fig. 1c) and incubation continued for the remaining 80 cores. The pre-incubation aimed to minimise confounding factors, such as disturbance caused by soil handling. It also facilitates mineralisation of nutrients from the amendments. Under dryland field conditions, subsoil amelioration using large quantities (10 to 20 t/ha) of organic matter generally shows a limited effect during the first season but results in a significant increase in grain yield from the second season onwards (Hall and Edwards 2025; Sale et al. 2021). The soil was sampled 2.5 months later (total of 17 months of incubation to analyse different physicochemical and biological properties.



2 cm from the amendment band. Reconstituted PVC core (c) after adding sand layers at the top and bottom of the incubated alkaline dispersive subsoil for growing wheat

2.3.2 Soil Sampling

A total of six soil cores (three from each side of the amendment band) of 20 mm diameter were collected from adjacent to the amendment band (centre of the cores was 20 mm away from the middle of the amendment band; Fig. 1a, b). The top 10 mm of soil was discarded. Samples from three cores were used for soil aggregate stability analysis, and the other three cores were gently massaged to break into smaller pieces, mixed and subsampled for subsequent soil physical (turbidity), chemical (pH, EC and ESP) and biological (microbial biomass carbon, quantitative polymerase chain reaction (qPCR) of bacteria and fungi) properties analyses.

2.3.3 Analysis of Soil Chemical Properties

Soil subsamples were air-dried, ground (<2 mm) and measured for soil EC and pH (both in 1:5 H₂O). Exchangeable cations (Ca²⁺, Mg²⁺, K⁺ and Na⁺) were extracted using 0.1 M BaCl₂ and 0.1 M NH₄Cl solution (Gillman and Sumpter 1986) and the filtered extract was analysed using an Agilent 280FS Atomic Absorption Spectrophotometer (CA, USA). ESP was then calculated as the exchangeable



 Na^+ divided by the sum of soil exchangeable Ca^{2+} , Mg^{2+} , K^+ and Na^+ and multiplied by 100.

2.3.4 Aggregate Stability and Turbidity Analysis

Soil samples were air-dried and gently crushed by hand along planes of weakness and sieved through a 6.5 mm sieve prior to wet sieving. The soil fractions were separated into macro-aggregates (> 0.25 mm), micro-aggregates (0.05–0.25 mm), and the silt–clay fraction (< 0.05 mm) following the method described by Chan et al. (2002). About 20 g of the air-dried soil was wet-sieved using a wet sieve shaker (McAuliffe Engineering, Wagga Wagga, Australia) and submerged in RO water in a 2 L cylindrical container (170 mm diameter × 135 mm height) for 5 min before 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 macroaggregates (>0.25 mm) due to the high dispersion of the clay particles. After wet sieving, the < 0.05 mm fraction in suspension was determined using the pipette sampling technique (Chan et al. 2002; Yoder 1936), and the micro-aggregate (0.05–0.25 mm) fraction was calculated by subtracting the combined fractions from the bulk soil weight.

Turbidity of the soil samples was determined using a modified method described by Zhu et al. (2016). Briefly, 1 g air-dried soil subsample was placed into a turbidimeter cell, and 25 ml of distilled water was then pipetted slowly to avoid disturbance of the soil. The turbidimeter cell was then slowly inverted 20 times to simulate mechanical dispersion, and the soil turbidity was measured after four hours of sedimentation using a Hach Turbidimeter 2100 N (HACH, Colorado, America) (Barzegar et al. 1994).

2.3.5 Microbial Biomass Carbon

Soil microbial biomass C (MBC) was analysed using the chloroform fumigation-extraction method (Vance et al. 1987). Fresh soil was extracted by of 0.5 M K_2SO_4 at 1: 4 w/v ratio (1 h shake). A paired fresh-soil sample was fumigated with alcohol-free chloroform in a desiccator for 24 h in the dark at 22 °C and then extracted by 0.5 M K_2SO_4 at 1: 4 w/v ratio (1 h shake). The soil extracts were filtered through a glass-fibre filter paper (Whatman GF/C). The dissolved organic C (DOC) in the K_2SO_4 extracts was analysed using the TOC Analyser. Microbial biomass C was calculated as the difference between the DOC values in the fumigated and non-fumigated soils. The conversion factor (k_{ec}) of 0.45 was applied to determine MBC of the soil (Ocio and Brookes 1990; Vance et al. 1987).

2.3.6 Fungal and Bacterial Abundance Measurements

DNA was extracted from 0.25 g of frozen soil (samples were stored at -80 °C) using a DNeasy PowerSoil Pro kit (Qiagen, Chadstone, Victoria, Australia) according to manufacturer's instructions. The concentration and quality of extracted DNA was assessed using a NanoDrop ND2000c Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). The abundance of fungal and bacterial communities was determined for each sample using qPCR (Hayden et al. 2012). Gene abundance was measured in duplicate in a 96-well format (Applied Biosystems QuantStudio 3) using the primer sets nu-SSU-1196F/nu-SSU-1536R (Borneman and Hartin 2000) and Eub338/Eub518 (Fierer et al. 2005; Lane 1991) for the fungal 18S rRNA gene and bacterial 16S rRNA gene respectively. The fungal 18S rRNA assays were carried out in 10 µl reactions containing 5 µl of 2X Sensi-FAST SYBR Lo-Rox (Bioline, Alexandra, NSW, Australia), $0.4 \mu l$ of each primer (10 μM) and 25 ng of template DNA. The bacterial 16S rRNA assays were carried out in 10 µl reactions containing 5 µl of 2X SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Gladesville, NSW, Australia) 0.5 µl of each primer (10 µM) and 25 ng of template DNA. Thermal cycling conditions for both assays were as described previously by Wang et al. (2020). Gene copy numbers were calculated from the average cycle threshold (CT) for each sample by comparison with a standard curve generated using a plasmid standard (in duplicate) for each assay and converted to gene copy number per gram of dried soil. The fungal standard plasmid contained Aspergillus nidulans DNA and was used at a tenfold dilution (linear range of 1×10^3 to 1×10^8). The bacterial standard plasmid contained Pseudomonas aeruginosa 16S rRNA DNA was used at a fivefold dilution (linear range of 6.4×10^4 to 2.0×10^8). For all assays, qPCR efficiency was 83.35-99.9% and R² was 0.99.

2.4 Experiment 2: Glasshouse Trial

2.4.1 Preparation of Soil Cores

An experiment was conducted in a climate-controlled glasshouse (with day and night temperatures adjusted to 20 and 14 0 C, respectively) using the second set of the already incubated soil cores to assess the effect of various amendments on growth, root proliferation and water use of wheat (*Triticum aestivum* cv. Lancer). To achieve this, we built a reconstituted soil core (Fig. 1c) where an incubated soil core was mounted on the top of PVC core of 150 mm wide and 300 mm length (prefilled with washed sand, basal nutrients solution and adjusted to 70% field capacity) using a heavyduty tape. Basal nutrient solution was applied at the rates of (mg kg $^{-1}$ of sand) 43.0 CaSO₄.2H₂O, 41.1 MgSO₄.7H₂O,



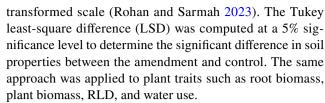
169 KNO₃, 27.5 (NH₄)₂SO₄, 16.7 NH₄NO₃, 659.1 KH₂PO₄ and (µg kg⁻¹ of sand) 119 H₃BO₃, 759 MnCl₂.4H₂O, 359 ZnSO₄.7H₂O, 33.3 CuSO₄.5H₂O, 72.1 (NH₄)₂MoO₄, 19.8 CoCl₂.6H₂O, 1530 Fe-EDTA to prevent nutrient limitation influencing plant growth. While filling the bottom cores, the sand level was raised by 10–15 mm, creating a convex shape to avoid cavity formation and to ensure good contact between the soil and the sand layer. On the surface of the incubated alkaline dispersive subsoil, an additional 100 mm in height was filled with washed sand containing basal nutrient solution and water content of 70% field capacity. Six pregerminated seeds were sown into each core at 30 mm depth and seedlings were thinned to three per core two weeks after sowing. The cores were maintained to 70% field capacity by weighing each core every week and replacing the amount of water lost through evapotranspiration. To avoid excessive fluctuations of soil water content, half of the amount of water lost to evapotranspiration in the previous week was added mid-week. The amount of added water was recorded to estimate the weekly water use and was summed to calculate cumulative water use till harvesting. Plants were harvested nine weeks after sowing at the tillering growth stage (Decimal code 28; according to Zadoks et al. 1974).

2.4.2 Roots and Shoot Measurements

After harvesting the plants, the PVC cores were disassembled and roots in the bottom sand cores were collected by repeated washing and sieving over a 2 mm meshed sieve. A soil core of 32.9 mm diameter and 45 mm length was collected from the amended alkaline dispersive subsoil comprising the amendment band and roots were collected by washing. Roots from this core were scanned at 800 dpi using an EPSON EU-35 scanner (Seiko Epson Corp, Suwa, Japan) and the images were analysed with a WinRHIZO STD 1600⁺ image analysis system (Regent Instruments, Quebec City, Canada) for estimating the root length. Root length density (RLD) was calculated by dividing the root length by the core volume. Root and shoot samples were oven dried at 70 °C for 72 h and weighed.

2.5 Statistical Analysis

Data were analysed with the statistical software R 4.2.1 (R Core Team 2023). To assess the impact of amendments on soil physicochemical and biological properties over the incubation period each soil parameter was separately analysed. To achieve this, the one-way ANOVA was used to determine whether there was a significant difference between the amendments and controls (no amendment). A Shapiro–Wilk test was used to examine the data for normality. In the case of non-normal, the data was transformed into a natural logarithm scale and the results were presented in the original and



To understand the association between the soil properties (attributes) and amendments including control, a principal components analysis was performed and presented as a biplot (Fig. 2). Nineteen soil amendments (amendment PAM was removed due to the missing measurements of soil properties) by fifteen key soil properties matrices, whose elements were previously computed predicted means, were used to conduct the principal components analysis. Before performing the principal components analysis, the data were standardised to manage the various units and to facilitate the interpretation of the results, ensuring that each soil property had a zero mean with unit variance across the amendments. The scores and loadings including cumulative variance were extracted from the principal components analysis. These results were graphically presented as a biplot with soil properties represented by arrows and amendments represented by points.

Plant traits and soil properties were incorporated to investigate the changes in plant traits resulting from the effects of amendments on soil physicochemical properties. The above-mentioned analysis was repeated to produce the biplot (Fig. 4), where a 19 by 16 (12 soil properties plus four plant traits) matrix was constructed.

3 Results

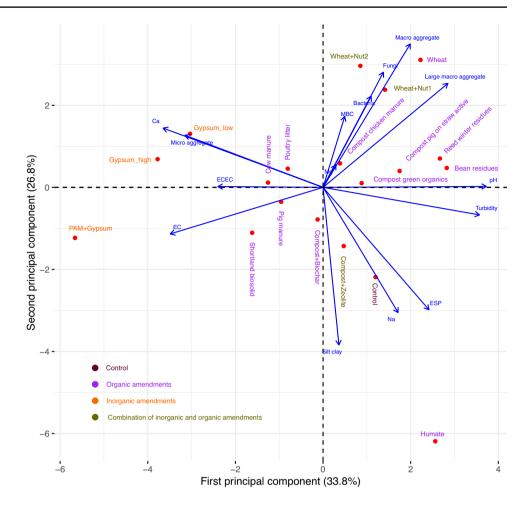
3.1 Incubation Trial

3.1.1 Effect of Amendments on Soil Chemical Properties

Following 17 months of incubation, soil pH was significantly reduced (P < 0.001) by a range of amendments, where it ranged from 9.1 (Control) to 8.1 (PAM+Gypsum; Table 2). Inorganic amendments had the greatest effects on soil pH. Applying gypsum alone at different rates reduced the soil pH by 0.7 (Gypsum_low) to 0.9 units (Gypsum_high), while combined application with PAM (PAM+Gypsum) resulted in a greater reduction (1 unit). Applying PAM without gypsum did not significantly (P > 0.05) affect the soil pH compared to the Control. Among the organic amendments, Compost chicken manure, Cow manure, Pig manure, Poultry litter and Shortland biosolid resulted in a significant reduction of pH compared to the Control. Among the combined amendments, Wheat+Nut2 reduced the soil pH by 0.32 units.



Fig. 2 Principal component analysis (PCA) biplot for different physicochemical properties of an alkaline dispersive subsoil following 17 months of incubation with different organic, inorganic and combined amendments. Colours represent different amendment groups. Ca: Calcium; EC: Electrical conductivity; ECEC: Effective cation exchange capacity; ESP: Exchangeable sodium percentage; Mg: Magnesium; MBC: Microbial biomass carbon; Na: Sodium; PAM: Polyacrylamide



Soil EC was also significantly affected by the amendments (P < 0.001), ranging from 321.5 (Wheat) to 697.3 μ S cm⁻¹ (PAM + Gypsum; Table 2). Applying either rate of gypsum increased the EC by about 40% compared to the Control. The co-application of PAM and gypsum (PAM + Gypsum) doubled the EC value; however, the sole application of PAM had no significant effect than the Control (P > 0.05). Most of the organic amendments, such as Compost + Biochar, Compost chicken manure, Cow manure, Humate, Pig manure, Poultry litter and Shortland biosolid, significantly increased the EC value compared to the Control. Among the combined amendments, only Wheat + Nut2 resulted in a significant increase in EC.

Applying gypsum at low or high rates significantly reduced the ESP by approximately 10 to 14% compared to the Control. Among the combined amendments, Wheat + Nut1 had the greatest effect on reducing soil ESP by 9% (12.5%) compared to the Control (13.7%). While some amendments led to increases in soil ESP values, only Humate caused a significant (P < 0.05) increase, raising the soil ESP value from 13.7% (Control) to 15.8%. This increase in soil ESP by Humate is attributed to the increase

in sodium (Na) ions (by 16%, data not shown) in the soil cation exchange complex (Fig. 2).

3.1.2 Effect of Amendments on Soil Physical Properties

The various aggregate fractions of the soil were significantly (P < 0.001) affected by the amendments examined (Fig. 3). Compared to the Control, the greatest increase in soil macroaggregates was observed by Bean residues (84%) followed by Reed winter residues (79%). The application of wheat alone (Wheat) or in combination with different rates of nutrients (i.e., Wheat + Nut1 and Wheat + Nut2) increased the soil macro-aggregate formation to a similar extent (67%). Organic amendments such as Poultry litter, Pig manure, Cow manure, and Composted chicken manure, green organic and pig on straw active also significantly increased the macroaggregate fractions by 27 to 57% compared to the Control.

Among the various organic amendments Compost + Biochar and Cow manure increased the soil micro-aggregate fractions by 9% compared to the Control (Fig. 3). However, Bean residues and Reed winter residues, despite leading to the highest increase in macro-aggregate fractions, resulted



Table 2 Effect of different organic, inorganic and combined amendments on pH, electrical conductivity (EC), exchangeable sodium percentage (ESP), microbial biomass carbon (MBC), fungal & bacterial abundance and turbidity of an alkaline dispersive subsoil following

incubation for 17 months. LSD indicates the significant difference between amendments means at P < 0.05. The asterisk indicates the significant difference between the amendment and the control. PAM: Polyacrylamide

| Amendments | Groups | pH (1:5 H ₂ O) | EC (μS cm ⁻¹) | ESP (%) | Large macro- aggregate (% log scale) | Turbidity (NTU) | MBC (mg C kg ⁻¹ soil log scale) | Fungal copies (g ⁻¹ soil log scale) | Bacterial copies (×10 ⁹ g ⁻¹ soil) |
|-----------------------------|-----------|------------------------------|------------------------------|------------|--|--------------------|--|--|--|
| Control | Control | 9.05 | 354.3 | 13.7 | -0.56 | 453.8 | 4.47 | 16.0 | 3.45 |
| Bean residues | Organic | 8.87 | 423.0 | 14.6 | 2.03* | 344.3* | 4.52 | 16.6 | 4.86* |
| Compost + Biochar | Organic | 8.85 | 442.3* | 13.9 | 0.02 | 330.8* | 4.37* | 16.0 | 4.87* |
| Compost chicken manure | Organic | 8.60* | 424.5* | 13.7 | 1.07* | 348.0* | 4.52 | 16.7 | 4.45 |
| Compost green organics | Organic | 8.85 | 390.3 | 13.3 | 1.39* | 395.3 | 4.41 | 15.6 | 4.19 |
| Compost pig on straw active | Organic | 8.95 | 383.5 | 13.9 | 1.69* | 381.5 | 4.32* | 16.0 | 4.84* |
| Cow manure | Organic | 8.70* | 449.5* | 13.2 | -0.31 | 264.3* | 4.40* | 15.6 | 4.75* |
| Humate | Organic | 8.93 | 470.8* | 15.8* | -0.50 | 425.0 | 4.33* | 15.0* | 4.34 |
| Pig manure | Organic | 8.48* | 491.5* | 13.5 | 0.01 | 316.5* | 4.46 | 15.7 | 4.78* |
| Poultry litter | Organic | 8.35* | 496.8* | 13.2 | 1.06* | 335.5* | 4.35* | 16.8* | 4.62* |
| Reed winter residues | Organic | 8.95 | 382.0 | 13.7 | 1.93* | 378.5 | 4.39* | 16.5 | 4.89* |
| Shortland biosolid | Organic | 8.08* | 532.0* | 13.5 | -0.15 | 318.8* | 4.30* | 16.4 | 4.73* |
| Wheat | Organic | 9.08 | 321.5 | 12.5* | 1.85* | 375.8 | 4.52 | 17.4* | 5.06* |
| Gypsum_high | Inorganic | 8.20* | 504.8* | 11.8* | -0.76 | 272.3* | 4.37* | 15.6 | 4.51* |
| Gypsum_low | Inorganic | 8.35* | 493.3* | 12.3* | -0.34 | 284.5* | 4.48 | 15.7 | 4.38 |
| PAM | Inorganic | 8.87 | 382.5 | 13.4 | - | 477.5 | 4.36* | 15.4 | 3.56 |
| PAM+Gypsum | Inorganic | 8.05* | 697.3* | 12.7 | -1.06 | 204.2* | 4.41 | 15.4 | 4.05 |
| Compost+Zeolite | Combined | 8.95 | 354.8 | 13.1 | 0.01 | 388.7 | 4.24 * | 15.9 | 4.06 |
| Wheat + Nut1 | Combined | 8.93 | 371.8 | 12.5* | 1.76* | 358.3* | 4.36* | 17.5* | 4.86* |
| Wheat + Nut2 | Combined | 8.73* | 424.0* | 12.7 | 1.76* | 408.0 | 4.46 | 17.9* | 4.78* |
| SE | | 0.09 | 22.80 | 0.39 | 0.24 | 28.08 | 0.03 | 0.26 | 0.27 |
| $LSD_{0.05}$ | | 0.26 | 64.6 | 1.10 | 0.68 | 79.10 | 0.07 | 0.74 | 0.76 |

in a 10% reduction in the micro-aggregate fraction compared to the Control. The application of gypsum at either rate significantly increased the micro-aggregate fractions, but combined application with PAM (PAM+Gypsum) did not result in a significant difference compared to the Control (P>0.05). The silt-clay fraction showed an opposite trend to both macro- and micro-aggregate fractions (Fig. 2). Therefore, amendments that increased either of the macro- and/or micro-aggregate fractions resulted in a significant reduction in silt-clay fraction than the Control (Fig. 3). Among the organic amendments only Humate resulted in a significant reduction in macro- (44%) and micro-aggregate (14%) fractions while increasing the silt-clay fraction by 30% more than the Control.

Applying different rates of gypsum alone or in combination with the PAM (PAM+Gypsum) resulted in the most significant reduction in soil turbidity compared to other amendments (Table 2). Some organic (e.g., Bean residues, Compost+Biochar and Poultry litter) and combined (Wheat+Nut1) amendments also significantly reduced

turbidity compared to the Control. Turbidity positively correlated with the soil pH, ESP, Na⁻ ions and silt–clay fraction and negatively correlated with the micro-aggregate fraction, EC, effective cation exchange capacity (ECEC) and Ca²⁺ ions (Fig. 2).

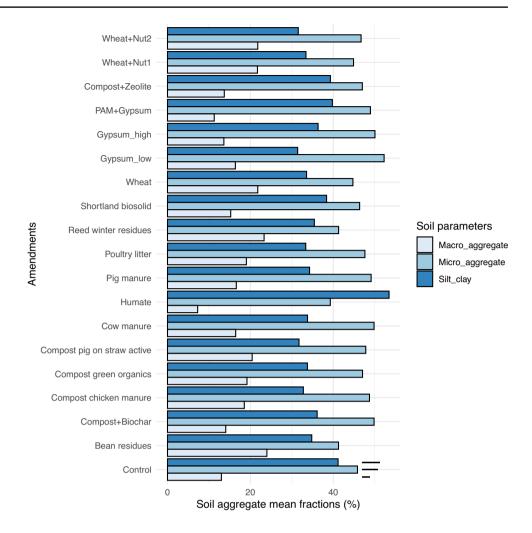
3.1.3 Effect of Amendments on Soil Biological Properties

By the end of the 17-month incubation, some amendments showed a significant reduction in MBC, but none increased MBC compared to the Control (Table 2). MBC was positively associated with the larger aggregate fractions (i.e., large- and macro-aggregates) and fungal and bacterial abundance and was negatively associated with the silt-clay fractions (Fig. 2).

Wheat stubble alone or combined with the chemical fertilisers (i.e., Wheat + Nut1 and Wheat + Nut2) and the Poultry litter increased fungal abundance compared to the Control (Table 2). Among different amendments, only Humate reduced fungal growth compared to the Control. Most



Fig. 3 Effect of different amendments on aggregate fractions of an alkaline dispersive subsoil following incubation for 17 months. Each bar segment represents the amendment mean of four replications (n=4). Horizontal bars next to each aggregate fraction represent significant differences (P < 0.05) among the amendment means for their respective aggregate fractions. See Table 3 for amendment groups. PAM: Polyacrylamide



organic and combined amendments significantly increased bacterial abundance compared to the Control (Table 2). Among different inorganic amendments, only the higher rates of gypsum (Gypsum_high) increased bacterial growth. Both fungal and bacterial abundance were strongly associated with the larger size aggregate fractions and negatively related to the dispersed silt–clay fractions (Fig. 2).

3.2 Glasshouse Trial

3.2.1 Root Parameters

Incubating with various amendments significantly (P < 0.001) enhanced root proliferation through the amended alkaline dispersive subsoil layer (Table 3). Root biomass ranged from 3.4 g core⁻¹ for Humate to 5.8 g core⁻¹ for Wheat + Nut1. Among the organic amendments, Cow manure, Poultry litter, Bean residues and Wheat increased the root biomass by 33, 34, 50 and 53%, respectively, compared to the Control (3.6 g core⁻¹). All the combined amendments showed a significant increase in root biomass (Table 3).

Root length density within the amended alkaline dispersive subsoil was significantly (P < 0.001) increased by the different amendments, with values ranging from 18.7 cm cm⁻³ (Control) to 43.9 cm cm⁻³ (Wheat; Table 3). Half of the organic amendments (6 out of 12) significantly increased the RLD, with some (Bean residues, Compost green organics, Poultry litter and Wheat) doubling it compared to the Control. Except for Gypsum_high, the rest of the inorganic amendments significantly increased the RLD, with Gypsum_low doubling it compared to the Control. All the combined amendments significantly increased the RLD in the amended layer (Table 3).

3.2.2 Plant Biomass and Water Use

Amendments significantly increased plant biomass (P < 0.001; Table 3). Different organic amendments (Bean residues, Cow manure, Poultry litter and Wheat) increased plant biomass by 30–40% compared to the Control (10.3 g core⁻¹). The application of low rates of gypsum (at 5 t ha⁻¹) resulted in a significant (28%) increase in plant biomass, while applying higher rates of gypsum (at 15 t ha⁻¹) had no significant



Table 3 Root growth, plant biomass and water use of wheat (Triticum aestivum L cv. Lancer) grown on an alkaline dispersive subsoil following 14.5 months of incubation with different organic, inorganic and combined amendments. LSD indicates the significant difference between amendments means at P < 0.05. The asterisk indicates the significant difference between the amendment and the control. RLD: Root length density; PAM: Polyacrylamide

| Amendments | Groups | Root biomass (g core ⁻¹) | RLD in the amendment layer (cm cm ⁻³) | Plant biomass (g core ⁻¹) | Water use (l) |
|-----------------------------|-----------|---|--|--|---------------|
| Control | Control | 3.6 | 18.7 | 10.3 | 2.8 |
| Bean residues | Organic | 5.4* | 42.0* | 14.4* | 3.5* |
| Compost + Biochar | Organic | 4.3 | 29.2 | 12.4 | 3.1 |
| Compost chicken manure | Organic | 3.5 | 25.3 | 11.8 | 3.0 |
| Compost green organics | Organic | 3.9 | 37.4 | 13.3 | 3.3 |
| Compost pig on straw active | Organic | 3.7 | 23.5 | 11.3 | 2.9 |
| Cow manure | Organic | 4.8* | 32.0* | 13.5* | 3.3 |
| Humate | Organic | 3.4 | 25.0 | 10.6 | 2.9 |
| Pig manure | Organic | 4.2 | 32.2* | 12.7* | 3.1 |
| Poultry litter | Organic | 4.8* | 37.9* | 14.4* | 3.5* |
| Reed winter residues | Organic | 3.9 | 28.1* | 11.1 | 2.7 |
| Shortland biosolid | Organic | 3.6 | 24.7 | 10.3 | 2.6 |
| Wheat | Organic | 5.5* | 43.9* | 13.2* | 3.2 |
| Gypsum_high | Inorganic | 3.6 | 21.6 | 11.3 | 2.9 |
| Gypsum_low | Inorganic | 4.7 | 37.6* | 13.3* | 3.5* |
| PAM | Inorganic | 4.1 | 26.4* | 11.6 | 2.9 |
| PAM + Gypsum | Inorganic | 4.7 | 27.8* | 13.0* | 3.4* |
| Compost+Zeolite | Combined | 5.0* | 40.2* | 13.9* | 3.3 |
| Wheat + Nut1 | Combined | 5.8* | 38.4* | 14.6* | 3.3 |
| Wheat + Nut2 | Combined | 4.9* | 34.1* | 13.5* | 3.4* |
| SE | | 0.4 | 2.6 | 0.7 | 0.2 |
| $\mathrm{LSD}_{0.05}$ | | 1.1 | 7.4 | 1.9 | 0.5 |

effect (P > 0.05). Application of PAM alone did not produce an increase in plant biomass, whereas PAM + Gypsum showed a significant 26% increase (P < 0.05). All the combined amendments significantly increased plant biomass compared to the Control (Table 3).

Amendments significantly increased the water use of wheat (*P*=0.014). Wheat grown in cores amended with Bean residues, Poultry litter, Gypsum_low, PAM+Gypsum and Wheat+Nut2 used about 20 to 25% more water compared to wheat grown under Control conditions. All the plant parameters showed a strong association with the large macro- and macro-aggregate fractions, aligning with the amendments that showed similar response profiles on the aggregate formation (Figs. 2, 3 and 4). Conversely, the silt-clay fraction, ESP, and Na⁻ ions concentration in the cation exchange complex showed an opposite trend, indicating a negative association with various plant parameters, including root biomass, RLD, plant biomass, and water use (Fig. 4).

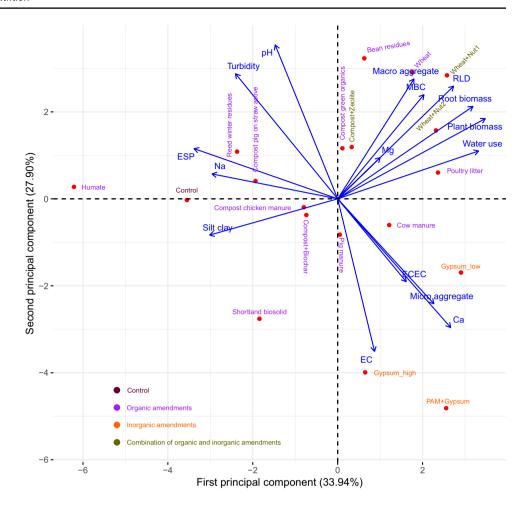
4 Discussion

We demonstrated that the legacy effect of incubating a range of organic and inorganic amendments for 17 months resulted in significant improvements in physicochemical properties and microbial activities of an alkaline dispersive subsoil (Table 2, Fig. 3), leading to increased root proliferation and crop growth (Table 3). One of the most significant findings is the improvement in soil structural stability as represented by soil aggregate fractions and the turbidity of the soil suspension. Across all amendments, Bean residues has the greatest increase in large macro- and maco-aggregates and Gypsum_low has the greatest increase in micro-aggregates. This improvement in soil structural stability following the application of different amendments was directly linked to the changes in physicochemical properties of soil, including pH, ESP, EC and fungal and bacterial abundance (Fig. 2).

The amendments differed in their impact on the formation of large macro-aggregates (> 2 mm) during the incubation period. Organic (such as Bean residue, Compost pig on straw active, Reed winter residues and Wheat) and combined (such as Wheat+Nut1 and Wheat+Nut2) amendments increased the proportion of large macro-aggregates several fold compared to the Control, and a similar trend was observed for total macro-aggregates (> 0.25 mm), but to a lesser extent. The compositions of the amendment, particularly the C substrate and N concentration, have direct effects on soil microbial abundance (Baldock et al. 2021; Ji et al. 2024; Li et al. 2018, 2023). Therefore, the stimulation of soil aggregation following the application of organic and combined



Fig. 4 Principal component analysis (PCA) biplot for different growth parameters of wheat (Triticum aestivum L cv. Lancer) and physicochemical properties of an alkaline dispersive subsoil following 17 months of incubation with different organic, inorganic and combined amendments. Colours represent different amendment groups. Ca: Calcium; EC: Electrical conductivity; ECEC: Effective cation exchange capacity; ESP: Exchangeable sodium percentage; Mg: Magnesium; MBC: Microbial biomass carbon; Na: Sodium; PAM: Polyacrylamide; RLD: Root length density



amendments was likely driven by microbial activity (Abiven et al. 2009; Chenu et al. 2011; Sonnleitner et al. 2003; Tardy et al. 2015). The same organic and combined amendments also significantly increased bacterial and fungal abundance, demonstrating a strong association between fungal and bacterial copies with the large macro- and micro-aggregate fractions. However, the contribution of fungi to macro-aggregation was more pronounced than that from bacteria, which is in line with earlier findings by Husain et al. (2024) and Vido et al. (2024). This is primarily due to the detection of greater fungal than bacterial abundance for the same amendments, resulting in increased formation of large macro-aggregates (Wang et al. 2020).

Increasing bacterial and fungal abundance produces more extracellular polysaccharides, lipids or glomalin, which act as binding substances, leading to the stabilisation of aggregates and adhesion of microbial cells to solid particles (Ali et al. 2024; Chaney and Swift 1986; Chenu 1993; Fang et al. 2020). Furthermore, extensive hyphal networks of fungi are extremely effective in improving the aggregation of poorly structured dispersive soil following amelioration with organic amendments (Sale et al. 2021; Tang et al. 2011; Wang et al. 2020). The association of arbuscular mycorrhizal

fungi with plant roots can also increase aggregate stability via their physical enmeshment (Rillig and Mummey 2006). Despite the increase in microbial abundance by organic and combined amendments, we found that MBC was largely unaffected or slightly decreased compared to the Control (Table 2). This can be partly explained by the technical limitation of fumigation extraction of MBC from living microorganisms, which may underestimate the C contribution from microbial necromass (nonliving microorganisms; Liang et al. 2019). Further studies are required to differentiate sources of microbial C contribution using approaches such as stable isotopic techniques (Buckeridge et al. 2022).

Among the inorganic amendments, the application of gypsum (Clark et al. 2007; Walia and Dick 2018; Zoca and Penn 2017) and PAM (Mamedov et al. 2009; Sojka et al. 2007) has been used to improve soil aggregation and root proliferation by changing the physicochemical properties of highly dispersive soils. Gypsum alone or in combination with PAM did not stimulate biological activity, as measured by microbial biomass carbon and soil fungal and bacterial abundance and therefore was not able to increase the formation of large macro- and macro-aggregates. The application of gypsum alone or in combination with PAM however



did reduce dispersion of the silt–clay fraction (Fig. 2). This might be attributed to replacing the dispersing agent Na⁺ on the soil colloidal complex with binding agent Ca²⁺, which is released from the dissolution of gypsum (Shainberg et al. 1989). Furthermore, increased effective cation exchange capacity, EC and decreased ESP values following gypsum (Table 2) might have also contributed to the formation of micro-aggregate fractions and thereby reducing the turbidity of alkaline (Uddin et al. 2022b) and non-alkaline (Wang et al. 2020) dispersive subsoil.

Our results also demonstrated a strong association between pH and the turbidity of the highly alkaline dispersive soil used in the study (Fig. 2). Soil pH is a key determinant controlling the surface charge of clays. High pH leads to increased repulsive forces in the electrical double layer at the surface of charged colloids which in turn leads to increased dispersion (Rengasamy et al. 2016; Tavakkoli et al. 2015), as indicated by the higher turbidity of the soil suspension. In the present study, the application of different rates of gypsum alone or in combination with PAM (PAM+Gypsum) significantly reduced soil pH with the greatest reduction of up to 1 unit for PAM + Gypsum. This also corresponded to the highest reduction (by 55%) of turbidity and improved soil aggregation. This may be attributed to the indirect effect of soil pH on the net negative charge of clay particles (Bronick and Lal 2005; Chorom et al. 1994), which has been reported earlier for the same alkaline dispersive subsoil used in the current study (Uddin et al. 2022b).

Under dryland conditions, a range of unfavourable physicochemical properties commonly present in alkaline dispersive subsoils restricts rooting depth, which can lead to reduced grain yield and poor WUE (Jayawardane and Chan 1994; Passioura and Angus 2010). Under such conditions, a key strategy for improving crop productivity in an alkaline dispersive subsoil is to enhance root growth and functions, thereby increasing accessibility to subsoil water (Azam et al. 2024; Gill et al. 2008). In this study, we observed increased root proliferation in and through the amended alkaline dispersive subsoil following incubation with a range of amendments, resulting in significantly higher root biomass in the bottom sand layer.

The increased root biomass found in the bottom sand layer of the cores reflected the improved root proliferation through the amended subsoil layer (Gill et al. 2009; Wang et al. 2020) and could be attributed to sevaral causes. Most likely however was the continuous supply of nutrients, such as nitrogen and phosphorus, derived from the large quantities of mineralising organic amendments in the amendment band, benefitting both soil biology and plants (Gill et al. 2008). These localised patches of nitrogen and phosphorus released from the mineralisation of organic amendments result in proliferation of cereal roots around the amendment bands (Hodge 2003; Robinson 1994), which may explain

the 136% increase in RLD observed in the current study (Table 3). The root biomass in the bottom sand layer was significantly correlated with the RLD in the amended alkaline dispersive subsoil layer. However, the stimulation of RLD in the amended alkaline dispersive subsoil layer was substantially higher than stimulating root biomass, indicating that root proliferation through the amendment band has a major contribution to overall root biomass in the bottom sand layer. The frequent wetting and drying cycles may have increased the formation of cracks and macropores (Ma et al. 2015), which may have also enhanced root proliferation through the amended alkaline dispersive subsoil layer.

Another possible explanation for the improved root growth into the subsoil layer is due to changes in soil structure. Application of organic amendments has been shown to improve aggregation in soils with a range of texture grades and mineral suites (Bronick and Lal 2005; Liu et al. 2020) including dispersive subsoils (Clark et al. 2009; Gill et al. 2009; Wang et al. 2020). A previous incubation study using a range of organic amendments with contrasting C:N ratios demonstrated a rapid increase in the formation of large macro-aggregates in the clay matrix (Clark et al. 2009) in line with our data. The formation of larger aggregates increases the porosity and permeability of the plant roots through the amended alkaline dispersive subsoil layer within a crop growing season, resulting in a significant and positive correlation between large macro-aggregates (> 2 mm) and RLD in the subsoil layer (Wang et al. 2020). In the current study, the formation of aggregates during the incubation period may have contributed significantly to improving root proliferation in the subsoil. Therefore, root biomass in the bottom sand layer and the RLD in the amended band of the alkaline dispersive subsoil were positively associated with the larger aggregate fractions and negatively associated with the silt-clay fractions, as well as the soil properties leading to soil dispersion, such as pH and ESP (Fig. 4).

Increases in root growth in subsoils corresponded to increased water use in this soil layer. One of the most significant agricultural outcomes of ameliorating alkaline dispersive subsoil is improving the extraction of subsoil water (Gill et al. 2008), thereby leading to greater crop water use (Uddin et al. 2022b; Wang et al. 2020) and increased grain yield. In support of this observation, our results also demonstrated a significant increase in crop water use following the amelioration of an alkaline dispersive subsoil with different organic, inorganic and combined amendments (Table 3). Amendments such as Bean residues, Gypsum, PAM + Gypsum, Poultry litter and Wheat + Nut2, which showed significant improvement in the physicochemical and biological properties of the alkaline dispersive subsoil also contributed to a greater crop water use.

Stimulation of root growth following the application of amendments resulted in increased drying of the deep profile



layers during the grain filling period (Gill et al. 2009; Wang et al. 2020). Given the narrow range of plant available water content in the studied sand core, greater cumulative water use following the application of amendments indicates better water extraction throughout the sand core. Despite the crop being harvested at the tillering stage, the root proliferation data through the amended alkaline dispersive subsoil layer provide a clear indication of the potential yield advantage of accessing stored subsoil water during grain filling under dryland conditions (Azam et al. 2024; Gill et al. 2009). Under such conditions, water use during the grain filling period has a high conversion efficiency into grain (Kirkegaard et al. 2007), resulting in a greater improvement in water use efficiency for grain yield than for biomass (Uddin et al. 2022b).

Our study demonstrated increased plant biomass with different amendments during stem elongation. Early plant biomass growth is considered a crucial trait for achieving greater grain yield under dryland conditions, particularly due to reducing evaporative water loss and better weed competition through faster canopy development (Zhao et al. 2019). However, with limited accessibility of subsoil water, greater early growth and water use may also reduce available soil water later in the season leading to 'haying-off' (premature cessation of grain filling associated with post-anthesis drought; van Herwaarden et al. 2003). Under water-limited dryland conditions, water savings early in the season in conjunction with the improved accessibility of subsoil water (through deeper root proliferation) can lead to higher grain yields following amelioration of alkaline dispersive subsoils with different amendments.

Despite the potential for long-term yield improvement (Celestina et al. 2018; Gill et al. 2012; 2019; 2008; Sale et al. 2021; 2019), the adoption of subsoil amelioration by farmers has been limited by high upfront costs of implementation and lack of sufficient quantities of low-cost ameliorants such as animal manures (Henty et al. 2022; Sale and Malcolm 2015). Among the wide range of amendments used in the current study, cereal stubble in the Australian farming systems currently provides little direct economic value and results in negative environmental impacts when disposed of or burned (Abdurrahman et al. 2020). Wheat stubble alone or combined with chemical fertiliser improved the physical, chemical, and biological properties of the studied alkaline dispersive subsoil and enhanced root proliferation and crop water use. Our findings showed great potential for the practical adoption of this low-cost ameliorant for managing alkaline dispersive subsoil as the combination of wheat stubble with chemical fertiliser has proven to be as effective as manures and legume stubble of high N content (Uddin et al. 2022a). It is important to note that the duration of the crop growth cycle in our experiment was relatively short, which prevented the investigation of grain yield and long-term benefits associated with ameliorating alkaline dispersive

subsoil. Therefore, further research is necessary to validate our findings in larger-scale field settings, exploring the longterm cumulative effects and economic returns before considering broadacre implementation.

5 Conclusions

Application of a range of amendments with different chemical compositions has shown a differential response in improving root proliferation through the highly alkaline dispersive subsoil. However, the mechanism of such root growth improvement differed for different amendments. Plant-based (such as Bean residue, Compost green organics, Wheat), animal waste-based (such as Poultry litter and Cow manure) and combined (wheat stubble and chemical fertilisers) amendments improved root growth by improving soil aggregation through promoting fungal and bacterial abundance. Whereas inorganic amendments (such as Gypsum and polyacrylamide + Gypsum) contributed to increased root proliferation by improving soil chemical properties, including pH, exchangeable sodium percentage and electrical conductivity. Wheat stubble alone or combined with chemical fertiliser demonstrated similar responses to animal manures and legume stubble of high nitrogen content, indicating a high adoption potential as a cost-effective ameliorant for alkaline dispersive subsoils. However, further research is needed to evaluate the long-term cumulative effects of different organic (particularly low-cost on-farm resources) and inorganic amendments and their combinations on crop productivity and farming profitability.

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Author Contributions SU, ET, IT, HW, and RA contributed to the conception and design of the study. SU, IT, HW, and HH prepared materials, set up the trial, and collected data. SU and MR analysed and interpreted the results. SU wrote the first draft of the manuscript and all authors commented on previous versions. All authors read and approved the final manuscript.

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Data Availability The dataset generated during and/or analysed during the current study is available from the corresponding author on reasonable request.

Declarations

Competing interests On behalf of all authors, the corresponding author states that there is no conflict of interest.

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