Ameliorating alkaline dispersive subsoils with organic amendments: Are productivity responses due to nutrition or improved soil structure?

Shihab Uddin · Stephanie Watts Williams · Naveed Aslam · Yunying Fang · Shahnaj Parvin · Josh Rust · Lukas Van Zwieten · Roger Armstrong · Ehsan Tavakkoli

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Abstract

Background and aims Alkaline dispersive subsoils are characterised by multiple physicochemical constraints that limit plant water and nutrient acquisition. Subsoil amelioration through organic amendments (OAs) requires significant financial investment. Whereas large yield responses can result following amelioration, sometimes small or even negative yield responses can occur, resulting in a significant net financial loss for the farmer. For farmers to feel confident in investing in subsoil amelioration better prediction of the likely yield improvement is required and to achieve this an understanding of the underlying mechanisms such as nutritional and non-nutritional drivers, and the longevity of benefits are required. Our study aimed to ascertain the drivers of yield improvements from subsoil amelioration with OAs.

Methods In a controlled environment, wheat (*Triticum aestivum* L. cv. Scepter) plants were grown until maturity in a Solonetz amended with (i) poultry litter (PL; 20 t ha⁻¹), and (ii) NPKS nutrients and (iii) model organic carbon (MOC) with equivalent amounts of nutrients and total carbon contained in the PL, and (iv) NPKS+MOC. Control (no amendments)
and gypsum (5 t ha\(^{-1}\)) were included as district practices. Before planting, amendments were applied as a vertical band at 20 – 40 cm depth and pre-incubated. Plant biomass, grain yield, root biomass, and physicochemical properties of the soil associated with the amendment band were quantified at harvest.

Results Compared to the control, wheat grain yield was increased by 30% for PL, 43% for NPKS + MOC, and 61% for NPKS, but no differences in yield were detected for MOC or gypsum. The lower yield increase by PL than NPKS with or without MOC was likely due to the readily available form of plant nutrients in the inorganic fertilisers vs slower mineralisation of nutrients from the OAs. Improvement in soil physicochemical properties following amelioration of alkaline dispersive subsoil resulted in better root proliferation and subsoil water use. Grain yield showed a positive correlation with root biomass in the subsoil layers.

Conclusions In the short-term (one crop cycle), organic amendments improved soil’s non-nutritional physical and chemical properties but had no additional nutritional effect on wheat grain yield compared to inorganic fertiliser application. Longer-term studies are needed to determine the legacy effect of the nutritional contribution in conjunction with the improvement of soil structure from the OAs in alkaline dispersive subsoils.

Keywords Sodicity · Soil structural stability · Root proliferation · Zeta potential · Water use efficiency

Introduction

In many Australian grain growing regions soil constraints cause reduced water use efficiency (WUE) of grain production (Jayawardane and Chan 1994), resulting in crops not achieving their water limited yield potential. Among the various soil physicochemical constraints, alkaline dispersive subsoils are associated with the greatest yield gap (difference between the constrained and water limited potential yield), with an estimated yield loss of $1.3 billion AUD per annum (Orton et al. 2018). Alkaline dispersive subsoils occur naturally and are common throughout large areas of the dryland Australian grain belt (Orton et al. 2018; Rengasamy 2016). These soils are characterised by high pH (>9.0) and poor soil structure (Rengasamy and Olsson 1991). This in turn restricts rainwater infiltration and water movement – water entering the subsoil is tightly held by clay particles and remains unavailable to plants (MacEwan et al. 2010), resulting in shallow rooting depth and poor WUE, further restricting the ability of the crop to fully achieve their yield potential (Adcock et al. 2007). Under dryland conditions, significant improvement in crop WUE can be achieved by improving access to the subsoil water (Kirkegaard et al. 2007) through the implementation of appropriate agronomic management strategies (Gill et al. 2008).

A key to increasing crop productivity in alkaline dispersive subsoils is improving soil structure. A range of approaches including both physical and/or chemical intervention (e.g., deep ripping and applying gypsum) have been tested on dispersive subsoils (Adcock et al. 2007; Gill et al. 2008; Schneider et al. 2017). Recent studies have shown that applying large amounts (15 to 20 t ha\(^{-1}\)) of organic amendments (OAs) to poorly structured subsoils can improve the grain yield by up to 70% (Celestina et al. 2018; Sale et al. 2019). Improvements in crop productivity following amelioration of alkaline dispersive subsoils in dryland has been attributed to a range of mechanisms including improvements in soil physical (structural stability, water retention) (Gill et al. 2009), biological (microbial diversity and function) (Wang et al. 2020) and chemical (pH, nutrient availability) (Chen et al. 2018; Clark et al. 2007) properties. Some studies attributed this increased grain yield is mainly due to increased nutrient (particularly N) supply in subsoils (Celestina et al. 2019) as high rates of OAs (20 t ha\(^{-1}\) as animal manure), can contain up-to 600 to 800 kg N ha\(^{-1}\) (Celestina et al. 2018; Gill et al. 2008). Yet, Armstrong et al. (pers. comm.) showed that when N was sufficient, subsoil application of OAs also increased the yield of legumes (inoculated with appropriate rhizobia), possibly due to the improvements in soil structure.

Several studies have attempted to separate the nutritional and non-nutritional contribution of OAs to grain yield in a wide range of soil nutritional conditions in temperate (Hijbeek et al. 2017) and dryland (Adeleke et al. 2021) environments. A recent review article outlined different strategies for successful attribution of
nutritional and non-nutritional contribution of grain yield improvement following amelioration of an alkaline dispersive subsoil with OAs (Celestina et al. 2019), but this study did not account for the large proportion of C contained in animal manures that can promote microbial activities and thereby impact soil physicochemical properties. However, despite the importance of understanding the mechanisms of the soil–plant-amendments interactions, the relative contribution of nutritional vs non-nutritional benefits of subsoil amelioration with OAs remains poorly understood, as much of the work on amelioration of subsoil constraints has focused on crop productivity (e.g. Celestina et al. 2018) with a little focus on the soil–plant interactions and how the OAs address the specific soil constraints.

To fill the knowledge gap, we conducted a controlled environment experiment to investigate: (a) the chemical, physical and biological changes of a dispersive Solonetz in response to various organic and inorganic amendments, (b) the physiological responses of wheat to manipulation of subsoils and (c) whether the crop response to nutrient-rich organic amendments is different to the nutrient matched inorganic fertiliser. We hypothesised that OAs will have both a nutritional contribution and non-nutritional benefit to soil, including lowering of soil pH and dispersion, which will lead to better root growth, access to resources and thereby higher grain yield than matched inorganic fertiliser and C input.

**Materials and methods**

**Collection and processing of soil**

An alkaline dispersive soil was collected at three layers (0 – 10, 10 – 20 and 20 – 40 cm) from a farm near Grogan in southern New South Wales, Australia (34°14’29″S 147°45’50″E). The site has a history of winter wheat-canaola-legume crop rotation with a summer fallow. The soil was classified as a Solonetz as per the WRB classification (IUSS Working Group WRB 2015), or Sodosol as per the Australian Soil Classification (Isbell 2016), and is a major soil type in rainfed crop production regions of south-eastern Australia. Soil was air-dried at 40 °C for 72 h, ground using a jaw crusher (BB400, Retsch GmbH, Haan, Germany), and passed through a 4 mm sieve. Gravimetric water content of the air-dried soil was determined by drying the soil at 105 °C for 72 h. The soil pH (1:5 water) was 5.5 in the 0 – 10 cm layer, 7.3 in the 10 – 20 cm layer and 9.3 in the 20 – 40 cm layer. The exchangeable sodium percentage (ESP) was 6.5, 9.5 and 13% in the 0 – 10, 10 – 20 and 20 – 40 cm layers, respectively. Field capacity and permanent wilting point were determined using the tension table and pressure plate (1600F1, 5 bar extractor; SOILMOISTURE Equipment Corp. Santa Barbara, CA) method (McKenzie et al. 2002), respectively. The soil from 20 – 40 cm depth has 51% clay content, which was dominated by kaolinite (Fang et al. 2020). Based on the Emerson test, the 20 – 40 cm layer is Type 3 of Class 2 showing a high level of dispersion and slaking (Emerson 1967). Different chemical properties at three layers of the soil are presented in Table S1.

**Subsoil amendments**

Treatments included a control (no amendment), gypsum (CaSO4·2H2O), poultry litter (PL), inorganic fertiliser (NPKS), model organic carbon (MOC), and a combination of NPKS and MOC (NPKS + MOC) (Table S2), with 4 replications. Poultry litter was applied at an equivalent to 20 t dry matter ha−1 on a surface area basis, containing the equivalent of 6694, 663, 400, 540 and 136 kg ha−1 C, N, P, K and S, respectively. This rate is selected based on earlier research findings from field trials in the medium and high rainfall zones of Australia, where deep banding of PL at 15 to 20 t ha−1 produced large (up to 70%) increase in grain yield (Celestina et al. 2018; Gill et al. 2008, 2019; Sale et al., 2019, 2021). In the NPKS treatment, inorganic fertilisers were used to match the amount of N, P, K, and S derived from the applied PL (Table S2). For the MOC treatment, a combination of synthetic products including D-glucose, mannose, cellulose, and guaiacol was used to match the equivalent amount of C from the applied PL. Gypsum was included as a traditional treatment to separate effects of nutrition and soil structural benefits on grain yield of wheat grown on alkaline dispersive subsoil. The rate of gypsum was determined based on an initial calibration curve where 5 t ha−1 resulted in flocculation of dispersed clay in the subsoil.

**Preparation of cores**

To examine the effects of subsoil amendments on physicochemical and biological properties of Solonetz as well as grain yield of wheat, we designed soil cores
using a polyvinyl chloride (PVC) tube of 15 cm diameter and 65 cm height (Fig. 1). The bottom 20 cm (40 – 60 cm core depth) of the PVC tube was first filled with 20 – 40 cm of the subsoil. Amendments were strategically placed in the 20 – 40 cm soil layer. The amendment for each core was mixed with 440 g of air-dried subsoil and placed at the centre of the core as a vertical band of 4 cm diameter and 20 cm height (20 – 40 cm core depth) in the centre of the core. The rest of the 20 – 40 cm core layer was filled with unamended subsoil from the same layer around the amendment band prior to being packed to the target bulk density. The 0 – 10 and 10 – 20 cm layers in the cores were then filled with the field soil collected from the respective soil layers. The total depth of the soil core profile was 60 cm with 5 cm empty space at the surface. To achieve target bulk density of 1.3 g cm$^{-3}$, the core was tapped against the floor for five to seven times after adding each soil layer.

To account for the very poor infiltration rate of subsoil, a single, flexible U-shaped irrigation tube of 4 mm diameter (drilled with 1.5 mm bit at 2 cm interval from a depth of 20 cm) was placed in each core (Fig. 1) to ensure uniform wetting of the soil throughout the profile. This tube also facilitated the watering of subsoil layers (20 – 60 cm core depth) independent of the surface soil layers (0 – 20 cm core depth). The irrigation tube was curved upwards on either side of the amendment band. The irrigation tube was extended above the core to apply adequate water head pressure. Plastic bottles with the bottoms cut out, were placed upside down and secured at 1.7 m height to act as a water reservoir for the irrigation tubes (Figs. 1 and S1). To achieve the target 70% plant available water content (PAWC), the 20 – 60 cm soil layer was watered via the irrigation tube, and the 0 – 20 cm layer was watered from the soil surface.

The cores were laid out in a row-column design (with four columns and six rows to total 24 cores), and incubated in a climate-controlled glasshouse with mean day/night temperatures of 20/14 °C. The soils and amendments were pre-incubated for 13 months prior to sowing. The purpose the pre-incubation was to minimise the confounding factors such as disturbance caused by soil handling, as the field experiment showed that deep ripping in the first year often had no effect or even detrimental effect on crop yield, likely due to the great disturbance (Tavakkoli et al. 2019). The soil moisture content during this period was maintained to the original target PAWC by monthly weighing of the cores and supplementing the water lost.

Plant growth conditions

At the completion of the pre-incubation period, six pre-germinated wheat (Triticum aestivum L. cv. Scepter) seeds were sown at approximately 2 cm depth in each core. The plants were thinned to two plants core$^{-1}$ at the two-leaf stage. Before sowing, basal fertiliser was surface applied (Table S3). Soil water was maintained at 70% PAWC by watering to weight on a weekly basis. During the first four weeks, water was added to the surface only. Thereafter, 60% and 40% of the water was added to the surface and through the irrigation tube (targeting subsoil), respectively, until plants reached tillering stage. From tillering until anthesis, 70% of the water was added through irrigation tube and 30% through the surface. The final irrigation event was undertaken at anthesis when 100% of the water was added (in two equal splits) to the subsoil to create the scenario of a dry surface soil with a wet subsoil
during the grain filling period. The volume of water added at each irrigation event was recorded for each core and totalled for cumulative water use (CWU).

Plant physiological measurements

Proxy measurements of chlorophyll content in flag leaves were collected non-destructively using a SPAD meter (SPAD-502, Konica Minolta, Sensing, Inc. Japan), at approximately weekly to fortnightly intervals. Chlorophyll content estimates were taken on one side of the leaf’s midrib and around the midpoint of the flag leaf following Xiong et al. (2015). For each core, chlorophyll content was averaged from the measurements of six leaves.

At maturity, plant stems were cut at soil level. Plant height, number of tillers and number of spikes were recorded. Plant material was then separated into spikes and straw. After threshing the spikes, grain yield, number of total grains, thousand kernels weight (TKW) and grain protein (using NIR; Foss NIR systems Model 6500) were determined. Straw samples were oven dried at 70 °C for 72 h and dry weights were recorded. Water use efficiency of biomass (WUEb) and grain yield (WUEy) were calculated by dividing the values of plant biomass (straw + grain) or grain yield by the CWU.

Following harvesting of the plants, the cores were disassembled, the soil core separated into four layers (0 – 10, 10 – 20, 20 – 40, and 40 – 60 cm depth) and gravimetric soil moisture content was determined. Subsampling was done from the amendment band for analysing different soil physicochemical and biological properties. The root biomass in each soil layer was collected by repeated washing and sieving (2 mm sieve) of the soil. Roots were oven dried at 70 °C for 72 h and weight. The total root biomass (TR) was estimated by summing root biomass from individual depths.

Soil physicochemical properties

The soil subsamples were air-dried, ground (<2 mm) before EC and pH (1:5 water) was assessed (Thermo Scientific Orion Star A215, Tewksbury, MA, USA). Soil mineral N (NH$_4^+$–N and NO$_3^-$–N) was extracted using 2 M KCl (1:5 w/v) following shaking for 1 h (Blackmore et al. 1987) and analysed using a Quick-Chem 8500 flow injection analyser (Lachat Instruments, Milwaukee, WI, USA). Exchangeable cations (Ca$^{2+}$, Mg$^{2+}$, K$^+$ and Na$^+$) were extracted using 0.1 M BaCl$_2$ and 0.1 M NH$_4$Cl solution (Gillman and Sumpter 1986) and then analysed using an Agilent 280FS Atomic Absorption Spectrophotometer (CA, USA). ESP was calculated using the following equation:

\[ \text{Exchangeable} \left[ \frac{(\text{Na}^+)/((\text{Ca}^{2+} + \text{Mg}^{2+} + \text{K}^+ + \text{Na}^+))}{100} \right] \]

To quantify soil structural stability, turbidity of the soil samples was determined using a modified method described by Zhu et al. (2016). Briefly, a 1 g subsample of air-dried soil was placed into the turbidimeter cell, and 25 mL of distilled water slowly added to avoid disturbance of the soil. The turbidimeter cell was then gently inverted 20 times to simulate mechanical dispersion. The sedimentation time was 4 h, and the turbidity quantified using a Hach Turbidimeter 2100 N (HACH, Colorado, America) (Barzegar et al. 1994).

To quantify the stability of the colloidal dispersion, zeta potential of the soil colloidal solution was measured following Asadi et al. (2009) with some modifications. Briefly, 50 mL distilled water added to 0.5 g of finely ground soil and shaken for 1 h. After settling the suspension for 1 h, the supernatant was filtered (0.45 μm Minisart® PES 16,555, Sartorius Stedim Biotech Gmbh, Göttingen, Germany) and the zeta potential was measured using a Malvern Zetasizer Nano ZS.

Soil biological properties

Soil microbial biomass C (MBC) was analysed using the chloroform fumigation-extraction method (Vance et al. 1987). Fresh soil (~10 g) from the amendment band was extracted in 0.5 M K$_2$SO$_4$ at 1: 4 w/v ratio by shaking for 1 h. The second subsample of fresh soil (~10 g) was fumigated with alcohol-free chloroform in a desiccator for 24 h in the dark at 22 °C and then extracted as above. The soil extracts were filtered through a glass-fibre filter paper (Whatman GF/C). The dissolved organic C (DOC) in the K$_2$SO$_4$ extracts was analysed using a TOC Analyser (Aurora 1030 W,
O. I. Analytical Corp. Texas, USA). Microbial biomass C was calculated as the difference between the DOC values from the fumigated and the non-fumigated soils. The conversion factor (\(k_{ec}\)) of 0.45 was applied to determine MBC (Ocio and Brookes 1990; Vance et al. 1987). Soil hot water-extractable organic C (HWEOC) and water-soluble C (WSC) were determined using the methods described in Ghani et al. (2003).

Statistical analyses

All experimental parameters were evaluated with considering amendment as the fixed effect row and column as the random effects by a linear mixed-effects model with R package ‘nlme’ (Pinheiro et al. 2019). Homogeneity of variance was evaluated visually using Q-Q plots, and the model was adjusted by dropping random effects when stepwise evaluation of the model indicated an improvement after correction. When the effect of amendments was significant (\(P<0.05\)), the means were compared using the Least Significant Difference (LSD) test at 5% significance under the “predictmeans” package (Luo et al. 2020).

Principal components analysis (PCA) was undertaken using the “PCA” function in the “FactoMineR” package, one included all of the measured plant biomass (including root biomass in different layers), cumulative water use and soil moisture data, and the other included soil physical, chemical and biological data, and grain yield. The PCA biplots were drawn using the “factoextra” package and the scores coloured by the levels of amendments; the group mean was also computed for each level, and a 95% confidence ellipse drawn around the mean to determine significant differences between groups for each biplot. Data analysis and PCA was performed in R version 4.1.0 (R Core Team 2021).

Results

Plant biomass and yield

The deep placement of organic and inorganic amendments led to a significant (\(P < 0.001\)) increase in the grain yield of wheat (Table 1). The greatest mean wheat grain yield was observed with the application of NPKS (35.8 g core\(^{-1}\)), which was 61% higher than the control (22.2 g core\(^{-1}\)). The NPKS + MOC treatment increased wheat grain yield by 43% compared to the control but was significantly (\(P < 0.05\)) lower than NPKS alone. The PL treatment increased

| Plant response variable | Control | NPKS | MOC | NPKS + MOC | PL | Gypsum | \(P\) value | LSD\(_{0.05}\) |
|------------------------|---------|------|-----|------------|----|--------|-------------|-------------|
| Plant height (cm)      | 98.5    | 95.8 | 100.8 | 101.5      | 99.5 | 100.5  | 0.412       | -           |
| Tiller number (core\(^{-1}\)) | 9.3     | 17.3 | 9.3  | 18.8       | 12.3 | 9.5    | <0.001      | 2.25        |
| Spike number (core\(^{-1}\)) | 9.3     | 17.3 | 9.3  | 18.8       | 12.3 | 9.5    | <0.001      | 2.25        |
| Plant biomass (g core\(^{-1}\)) | 49.5    | 70.7 | 46.9 | 65.3       | 58.8 | 54.8   | <0.001      | 5.69        |
| Grain yield (g core\(^{-1}\)) | 22.2    | 35.8 | 21.3 | 31.7       | 28.8 | 25.3   | <0.001      | 3.22        |
| HI                     | 0.45    | 0.51 | 0.45 | 0.49       | 0.49 | 0.46   | <0.001      | 0.02        |
| TKW (g)                | 47.3    | 44.7 | 47.2 | 44.0       | 47.8 | 48.3   | 0.005       | 2.33        |
| Number of grains (spike\(^{-1}\)) | 51.2    | 45.2 | 48.9 | 40.6       | 46.6 | 55.9   | <0.001      | 5.49        |
| Total grains (core\(^{-1}\)) | 469.3  | 802.4 | 451.8 | 721.3      | 607.2 | 525.5 | <0.001      | 73.87       |
| Grain protein (%)      | 11.1    | 14.0 | 10.0 | 14.0       | 11.1 | 10.3   | <0.001      | 1.13        |
| CWU (t core\(^{-1}\))  | 9.6     | 12.4 | 9.7  | 12.2       | 11.2 | 10.5   | <0.001      | 0.70        |
| WUEb (g l\(^{-1}\) core\(^{-1}\)) | 5.2     | 5.7  | 4.8  | 5.4        | 5.3  | 5.2    | 0.005       | 0.36        |
| WUEy (g l\(^{-1}\) core\(^{-1}\)) | 2.3     | 2.9  | 2.2  | 2.6        | 2.6  | 2.4    | <0.001      | 0.21        |

Statistically significant effects are shown in bold numbers. Abbreviations: HI Harvest index; TKW Thousand kernels/grains weight; CWU Cumulative water use; WUEb Water use efficiency for biomass and WUEy Water use efficiency for yield.
grain yield by 30% \((P<0.05)\). Plant biomass (straw + grain) followed a similar trend to grain yield, with the greatest mean biomass recorded for plants amended with NPKS followed by NPKS + MOC and PL. Neither MOC nor gypsum amendment were significantly different \((P>0.05)\) to the control. There was also a significant \((P<0.001)\) effect of amendment on harvest index (HI), with 8 – 13% higher in the nutrient rich amendments NPKS, NPKS + MOC and PL than the control (Table 1). The mean spike number was doubled following the combined application of inorganic fertilisers and model organic carbon (NPKS + MOC), compared to the control \((9.3 \text{ core}^{-1})\). This was followed by the amendments of inorganic fertiliser alone (NPKS) and PL that increased the spike number by about 86 and 32%, respectively, compared to the control. Significant \((P<0.001)\) increases (26%) in grain protein were observed in the NPKS and NPKS + MOC, compared to the control (Table 1).

Plant water use and residual soil moisture content at harvest

Cumulative water use by wheat plants was significantly \((P<0.001)\) affected by the amendments, and this was particularly evident following cessation of watering \((\text{Fig. 2})\). Compared to the control, a significant \((P<0.05)\) increase in plant water use first appeared for soils amended with NPKS and NPKS + MOC at 57 days after sowing (DAS), and these amendments continued to exhibit greater water use for the remainder of the experiment, ranging between 12 and 35%. A slow but significant relative increase in water use occurred in the soil amendment with PL \((\text{after 79 DAS})\) and gypsum \((\text{after 114 DAS})\). As a consequence, by the end of the experiment, the final CWU was increased by all amendments except the MOC \((\text{Fig. 2}; \text{Table 1})\).

The effect of amendments was significant for both WUEb \((P=0.005)\) and WUEy \((P<0.001)\).
Only soils amended with NPKS increased WUEb (by 10%), compared to the control. The highest value of WUEy was observed in the soils amended with NPKS, i.e., 24% greater than the control whereas both the NPKS + MOC and PL had 11% greater WUEy than the control.

The soil moisture content of the different layers at harvest was significantly affected by the amendments (Table 2). At 10 – 20 cm depth, there was no significant difference in soil moisture content between any of the amendments and the control but the moisture content of the soils amended with NPKS + MOC and PL were respectively, 11 and 7% lower (P < 0.05) than both gypsum and MOC.

At 20 – 40 cm depth, compared to the control, soil moisture was significantly (P < 0.05) lower by 23% in the NPKS, 21% in the NPKS + MOC and 18% in the PL (Table 2). Soil moisture content at the 40 – 60 cm depth followed a similar trend to the 20 – 40 cm depth, with lower values in the NPKS and NPKS + MOC, PL and gypsum than the control (Table 2).

Leaf chlorophyll content

Chlorophyll content of flag leaves during the tillering and anthesis stages were not significantly (P > 0.05) affected by the amendments (Fig. 3). However, flag leaf chlorophyll content was significantly higher in the plants with amendments during the grain filling period (140 DAS, P < 0.001; 147 DAS, P = 0.004; 154 DAS, P < 0.001). Wheat plants that were grown without amendment, or with nutrient-poor amendments (i.e., MOC or gypsum) senesced faster than plants grown with one of the nutrient-rich amendments (i.e., NPKS, NPKS + MOC or PL). Leaf chlorophyll content during the grain filling period was strongly and positively correlated (r = 0.92, P < 0.001) with CWU (Fig. S2).

Root biomass

Total root biomass was significantly (P < 0.001) affected by the amendments (Fig. 4). The total root biomass was observed to increase by 58% in the NPKS amended soil and by 37% in the PL amended soil, compared to the control. There was an interactive effect of the amendments and soil depth on root biomass (P < 0.001). Root biomass showed stronger responses to amendments at the subsoil layers (20 – 40 and 40 – 60 cm depths) than the surface layers (0 – 10 and 10 – 20 cm depths; Fig. 4). At 20 – 40 cm depth, root biomass was doubled by the NPKS, and was increased by 63% in the PL, compared to the control. At the 40 – 60 cm soil layer, root biomass was increased by about 144%, 125%, and 83% by the application of NPKS, NPKS + MOC, and PL. Root biomass was negatively correlated with soil moisture content in the 20 – 40 cm (r = 0.72, P < 0.001) and the 40 – 60 cm (r = 0.63, P < 0.001) soil layers at harvest. There was a strong positive correlation between grain yield and root biomass in the subsoil layers (20 – 40 cm r = 0.87, P < 0.001 and 40 – 60 cm depth r = 0.82, P < 0.001) (Fig. 5). Both grain yield and biomass were also negatively correlated with soil moisture content in the 20 – 40 (r = 0.84, P < 0.001) and 40 - 60 cm (r = 0.87, P < 0.001) subsoil layers (Fig. 5).

| Soil depth (cm) | Gravimetric soil moisture content (g g⁻¹) |
|-----------------|-----------------------------------------|
|                 | Control | NPKS | MOC | NPKS + MOC | PL | Gypsum | P value | LSD₀.₀₅ |
| 0 – 10           | 0.062   | 0.066 | 0.063 | 0.065 | 0.065 | 0.068 | 0.252 | - |
| 10 – 20          | 0.124   | 0.121 | 0.129 | 0.116 | 0.122 | 0.131 | <0.001 | 0.006 |
| 20 – 40          | 0.173   | 0.133 | 0.162 | 0.137 | 0.143 | 0.160 | <0.001 | 0.009 |
| 40 – 60          | 0.186   | 0.138 | 0.179 | 0.138 | 0.152 | 0.164 | <0.001 | 0.011 |

Statistically significant effects are shown in bold numbers.

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Table 2 Mean gravimetric soil moisture content at different depths at the time of harvest of wheat (Triticum aestivum cv. Scepter) plants grown in an alkaline dispersive subsoil (20 – 40 cm depth) incubated with five different amendment or un-amended control. Poultry litter (PL) was applied at 20 t ha⁻¹, and its equivalent amount of major macronutrients (N, P, K and S) and total carbon were supplemented in the amendments inorganic fertilisers (NPKS) and model organic carbon (MOC), respectively. The rate of gypsum was 5 t ha⁻¹. LSD indicates where there are significant differences between amendment means at the P < 0.05 level.
Fig. 3 Leaf chlorophyll content of wheat (*Triticum aestivum* cv. Scepter) plants being grown in an alkaline dispersive subsoil after incubation with 5 different amendments or un-amended control. Poultry litter (PL) was applied at 20 t ha\(^{-1}\), and its equivalent amount of major macronutrients (N, P, K and S) and total carbon were supplemented in the amendments inorganic fertilisers (NPKS) and model organic carbon (MOC), respectively. The rate of gypsum was 5 t ha\(^{-1}\). Each point represents the mean value of \(n=4\) amendment replicates. Vertical bars represent the significant (\(P<0.05\)) differences between the amendment means at each date of measurement, respectively. The arrow indicates the timing of anthesis in the wheat plants.

Fig. 4 Root biomass of wheat (*Triticum aestivum* cv. Scepter) plants at 4 different soil depths: 0–10, 10–20, 20–40, 40–60 cm at harvest, after being grown in an alkaline dispersive subsoil following incubation with 5 different amendments or un-amended control. Poultry litter (PL) was applied at 20 t ha\(^{-1}\), and its equivalent amount of major macronutrients (N, P, K and S) and total carbon were supplemented in the amendments inorganic fertilisers (NPKS) and model organic carbon (MOC), respectively. The rate of gypsum was 5 t ha\(^{-1}\). Each segment of a bar represents the mean value of that amendment and depth (\(n=4\)). At each depth respectively, amendment means that share no common letters (lower case) are significantly different from each other at the \(P<0.05\) level; significant differences among the amendment means for total root biomass are indicated by the upper case letters above each bar.
Effects of amendments on soil physicochemical properties

The application of gypsum, NPKS+MOC or NPKS significantly ($P<0.001$) reduced the soil pH (1:5 water) in the amendment band by approximately 0.6 units compared to the control (9.35) (Table 3). Gypsum was the only amendment that significantly increased soil EC, by 33%, compared to the control (258.0 µS cm⁻¹). Application of gypsum and NPKS+MOC significantly ($P<0.05$) reduced soil ESP by 22 and 15%, respectively compared to the control (12.6%).

Soil NO$_3$−N concentrations were increased by applications of amendments, ranging between 1.2 and 4.4 times, in the order NPKS>NPKS+MOC>PL>gypsum>MOC (Table 3). Soil NH$_4$+−N remaining in the amendment band at maturity was significantly ($P<0.001$) increased by 72–165% by different amendments, in the order of PL>NPKS>NPKS+MOC (Table 3). In contrast, soil
NH₄⁺–N was decreased by 56% with the application of gypsum. Soil turbidity in the amendment band was significantly ($P < 0.001$) reduced by all amendments, with the most prominent effects in the NPKS + MOC and gypsum (Table 3). Soil turbidity was positively correlated with both soil pH ($r = 0.85$, $P < 0.001$) and ESP ($r = 0.82$, $P < 0.001$) (Fig. 6).

The application of amendments significantly ($P < 0.001$) increased surface charge (measured as zeta potential) of the amended subsoil (Table 3). Specifically, NPKS + MOC and gypsum increased the zeta potential by 23–24%, compared to the control (-53.1 mV). Principal component analysis (PCA) showed a negative correlation between zeta potential and soil pH ($r = 0.70$, $P < 0.001$), ESP ($r = 0.66$, $P < 0.001$), and turbidity ($r = 0.79$, $P < 0.001$) (Fig. 6).

Effects of amendments on soil biological properties

Amendments significantly ($P = 0.003$) affected the water-soluble C (WSC; Table 3). The WSC was increased by 100 – 140% with the application of MOC and PL. Similarly, the hot water-extractable organic C (HWEOC) was increased significantly ($P < 0.05$) with the application of the organic amendments PL, MOC and NPKS + MOC (Table 3), but not the inorganic amendments (NPKS and gypsum). Microbial biomass C at the harvest was significantly ($P < 0.05$) affected by PL (about 246% increase), but there were no differences between other amendments and the control.

Discussion

Application of amendments resulted in improved subsoil structural stability

The dispersive Solonetz used in this study severely restricts crop production through a range of soil constraints including high pH (> 9.0), poor structure, impeded drainage, waterlogging, and high soil strength and bulk density. To understand how subsoil application of organic amendments can overcome these constraints through nutritional and non-nutritional drivers, we used organic and inorganic amendments with matching total macronutrient content. The impacts of these amendments on key soil properties and their subsequent influence on crop physiological responses within one crop cycle under controlled environment conditions were investigated. In our study, the deep (20 – 40 cm) placement of nutrient-rich OAs resulted in significant changes in soil physicochemical properties and microbial activity capable of reducing hostile conditions, for root growth and function, resulting in the positive response in crop growth and grain yield.

Table 3 Mean soil physical, chemical and biological properties at the amendment band following incubation of an alkaline dispersive subsoil with five different amendments or un-amended control and had wheat (Triticum aestivum cv. Scepter) plants grown until maturity in it. Poultry litter (PL) was applied at 20 t ha⁻¹, and its equivalent amount of major macronutrients (N, P, K and S) and total carbon were supplemented in the amendments inorganic fertilisers (NPKS) and model organic carbon (MOC), respectively. The rate of gypsum was 5 t ha⁻¹. LSD indicates where there are significant differences between amendment means at the $P < 0.05$ level.

| Soil properties | Control | NPKS | MOC | NPKS + MOC | PL | Gypsum | $P$ value | LSD₀.₀₅ |
|-----------------|---------|------|-----|------------|----|--------|-----------|---------|
| pH (1:5 water)  | 9.35    | 8.79 | 9.15| 8.76       | 9.02 | 8.71   | $<0.001$  | 0.14    |
| EC ($µS$ cm⁻¹)  | 258.0   | 238.8| 203.0| 242.0      | 248.7| 344.0  | $<0.001$  | 51.1    |
| ESP (%)         | 12.6    | 11.43| 11.5 | 10.8       | 11.5 | 9.9    | $0.003$   | 1.1     |
| NO₃-N (mg kg⁻¹ soil) | 0.25   | 1.35 | 0.55 | 1.08       | 1.08 | 0.78   | $<0.001$  | 0.27    |
| NH₄-N (mg kg⁻¹ soil) | 1.95   | 3.98 | 1.78 | 3.35       | 5.18 | 0.85   | $<0.001$  | 0.96    |
| Turbidity (NTU) | 1270.3  | 666.5| 995.3| 381.8      | 847.8| 446.0  | $<0.001$  | 228.9   |
| Zeta Potential (mV) | -53.1  | -51.3| -53.2| -40.4      | -52.2| -41.0  | $<0.001$  | 2.6     |
| WSC (mg g⁻¹)    | 0.23    | 0.19 | 0.56 | 0.35       | 0.46 | 0.12   | $0.003$   | 0.21    |
| HWEOC (mg g⁻¹)  | 0.21    | 0.20 | 0.42 | 0.47       | 0.47 | 0.19   | $0.007$   | 0.19    |
| MBC (mg g⁻¹)    | 0.09    | 0.10 | 0.13 | 0.15       | 0.30 | 0.13   | $0.001$   | 0.09    |

Statistically significant effects are shown in bold numbers. Abbreviations: EC Electrical conductivity, ESP Exchangeable sodium percentage, WSC Water soluble carbon, HWEOC Hot water extractable organic carbon and MBC Microbial biomass carbon.
A significant improvement in soil structural stability (indicated by reduced turbidity of a soil suspension) was observed across all treatments, with the greatest (up to 70%) reduction in turbidity observed for NPKS + MOC and gypsum (Table 3). These improvements in soil structural stability following application of different amendments were directly linked to the changes in different physicochemical properties of soil including pH, ESP, EC, and zeta potential (Fig. 6). pH is well recognized as critical variable controlling surface charge of the clay with high pH leading to increased repulsive forces in the electrical double layer at the surface of charged colloids, resulting in soil dispersion (Rengasamy et al. 2016; Tavakkoli et al. 2015). In the present study, a decrease in pH of between 0.59–0.64 units were associated with a 70% reduction in clay dispersion (Table 3). The clay flocculation resulting from reduced pH can be linked to the formation of CaCO3 and reduction in zeta potential (Bronick and Lal 2005; Chorom and Rengasamy 1997).
The lowered pH of alkaline soils following application of gypsum is well documented and is attributed to the formation of calcium carbonate which lowers the concentration of soluble carbonates (Chorom and Rengasamy 1997; Wong et al. 2009). However, the observed reduction of pH in soil amended with PL and MOC can be attributed to the increase in MBC (Table 3), which generates CO$_2$ and thereby reduces pH by increasing partial pressure of CO$_2$ (Sposito 2016). It was observed that the soluble C associated with MOC initially stimulated microbial activity. This resulted in increased immobilization of mineral N (Clark et al. 2007), leading to low mineral N and MBC but higher WSC at the end of the crop growing season (Table 3). Conversely, a continuous supply of N via the treatments of NPKS + MOC or PL was able to maintain the higher microbial activity, therefore increasing partial pressure of CO$_2$ that resulted in greater reductions in soil pH compared to when MOC alone was applied. Application of inorganic fertilisers alone or in combination with the MOC also resulted in a significant improvement in soil pH, and ESP, which might improve structural stability (Table 3), therefore increase root growth (Figs. 4 and 6). Root induced reduction in soil dispersion can be attributed to their ability to form soil aggregates (Reid and Goss 1981; Tisdall and Oades 1979).

The OA did not change the net surface charge (zeta potential), which showed a negative effect on soil structural stability (Fig. 6). Only gypsum and NPKS + MOC increased the surface charge of soil. The changes in zeta potential following gypsum application might be attributed to the effect of gypsum on underlying factors such as EC, pH, and dispersion (Chorom and Rengasamy 1995). The dissolution of gypsum releases Ca$^{2+}$, which can increase the flocculation of soil colloids via (i) replacing the dispersing agent, mainly Na$^+$, from the clay surfaces (Lebron et al. 2002; Rengasamy et al. 2016; Shainberg et al. 1989) and (ii) forming cation bridges between clay and soil organic matter particles (Mthimkhulu et al. 2016; Tisdall and Oades 1982). These results suggested that through improving the relevant soil physicochemical properties, gypsum can effectively lower soil dispersion and thereby potentially improve soil aggregation (Clark et al. 2009; Fang et al. 2021). Despite changes in soil physicochemical properties, gypsum application did not result in an increase in grain yield. This lack of impact on grain yield was also observed in a field trial on alkaline dispersive subsoils during the first growing season (Sale et al. 2021). It is likely that this was due to the poor solubility of gypsum (Shainberg et al. 1989). However, gypsum application significantly increased grain yield in subsequent seasons (Sale et al. 2021). Therefore, we suggest long-term observations of subsoil amelioration are required to fully understand the benefits to soil properties and crop productivity.

Improved soil physicochemical properties lead to better root proliferation and water extraction from the subsoil

The key to improving crop productivity and minimizing the yield gap on hostile subsoils, particularly in dryland cropping systems, is the improvement of root access to stored subsoil water during the grain filling period (Sadras et al. 2002). In this study we observed increased root biomass in the subsoil layers (20 – 60 cm) following the application of the nutrient-rich amendments (PL, NPKS and NPKS + MOC) (Fig. 4). This increased root growth into the amended subsoil layers is attributed to the preferential proliferation of wheat roots around the amendment bands due to readily-available N (Table 3) and P (Hodge 2003; Robinson 1994). In addition, the increased root proliferation in the subsoil layers in the current study (Fig. 4) could be due to changes in soil physicochemical properties such as improved soil aggregation, which leads to an increased soil porosity, aeration and reduced soil bulk density (Gill et al. 2009).

One of the most significant agricultural outcomes from the amelioration of alkaline dispersive subsoil was the increase in the extraction of subsoil water (Gill et al. 2008). Despite being in a controlled environment, an important feature of this study was that the subsoil was exclusively irrigated during anthesis, resulting in a scenario of dry surface soil with stored subsoil water, which mirrored rainfed dryland farming systems in southern Australia (Connor et al. 1992; Robertson et al. 2016). Wheat plants grown with nutrient rich amendments (PL, NPKS and NPKS + MOC) were able to extract more water and led to a drier subsoil profile at harvest compared to those from the control (Table 2). Increased root growth and root activity in the subsoil layers (Fig. 4) appeared to be responsible for accessing the subsoil water. Interestingly, there were different patterns in cumulative water use between the ameliorants during the later crop developmental stages (Fig. 2), although by the time these had occurred they
had minimal effects on plant biomass than grain yield. Water captured by roots in the subsoil layers is particularly critical if it coincides with the grain filling period as it may lead to a three times higher conversion efficiency into grain than that of the total seasonal water use (Kirkegaard et al. 2007). This may explain the improved WUEy of the current study (Table 1).

Nutritional contribution of subsoil amelioration outperformed the non-nutritional effects

In our study, the deep (20 – 40 cm) placement of nutrient-rich OAs resulted in wheat grain yield increases of 30%, inorganic fertiliser of 61%, and the combination of inorganic fertiliser and MOC of 43% (Table 1). Although the extent of observed grain yield increase is similar to some earlier subsoil amelioration studies (Celestina et al. 2018; Wang et al. 2020), the greater yield effect of OAs compared to inorganic fertiliser reported in the high-rainfall zone of south-eastern Australia (Gill et al. 2008, 2012) was not shown in the present study, and the data do not support our hypothesis.

Despite having matched inputs of total N, P, K and S, the greater increase in relative grain yield by NPKS and MOC+NPKS, compared with PL treatment, may be due to the differential timing of availability of nutrients from the inorganic fertiliser, compared to the OAs. OAs applied to soils undergo decomposition which subsequently results in mineralisation of N, P and S (Eghball et al. 2002; Quilty and Cattle 2011). Mineralisation of OAs varies widely depending on their chemical composition and the environmental conditions (Diacono and Montemurro 2010), which makes matching rates of application of plant nutrients challenging. For example, N mineralisation from PL can range from 19 (Hartz et al. 2000) to 67% of organic N over 6 to 7 months of incubation (Chae and Tabatabai 1986). Adegbidi and Briggs (2003) demonstrated that around 32% of the total N in PL was mineralized following 9 months of incubation. Using these values, we estimate that the total plant available N in the current study arising from the application of PL was approximately 212 kg N ha⁻¹, which is only one-third of the N supplied 663 kg ha⁻¹ in the NPKS treatment. In contrast, Gill et al. (2008) reported greater yield responses for organic amendments over inorganic fertiliser but applied only 11 kg N ha⁻¹ as inorganic fertiliser, which is likely to have been significantly lower than the available N following mineralisation of the organic amendments containing up to an estimated 600 to 800 kg N ha⁻¹. Moreover, although soil nitrate N was increased by PL and NPKS irrespective of MOC, which contributed directly to wheat growth, threefold increase in ammonium N by PL may have not been preferred by the wheat cultivar in the current study (Wang et al. 2015).

In the current study, the high level of N supply by the inorganic fertiliser was associated with high grain protein concentration (Table 3). Given yield and grain protein concentration are often negatively correlated (Halloran 1981), it was interesting that the NPKS treatment led to 26% higher grain protein despite the 61% increase in grain yield. The reported grain protein for the control treatment in previous studies (Gill et al., 2008, 2012) were below 10% indicating moderate to severe N deficiency (Holford et al. 1992). Grain protein in the control treatment in the current study was >11%, which is likely to be attributed to the supply of about 37 kg N ha⁻¹ at sowing as basal nutrient. Although the effect of additional N supply from incorporated OAs on yield responses tend to diminish when the grain protein exceeds 10.6% (Celestina et al. 2018), this was not the case with the PL in this study. Instead, it resulted in increased grain yield without any reduction in grain protein, which might be attributed to the continuing mineralisation of organic N and the maintenance of high rates of N supply to the plants over extended periods (Diacono and Montemurro 2010). Combination of mineralisation of nutrients (particularly N) from the applied PL along with the application of basal nutrients at sowing were more than the existing fertiliser recommended to meet the nutrient demand for the rainfed wheat in Australia (Angus and Grace 2017; GRDC 2016). The application of PL resulted in the highest mineral N in the amendment band during harvest (Table 1), providing a potential legacy effect of OAs on the subsequent crops through the slow-release of nutrient (Chen et al. 2018).

The combined application of NPKS and MOC (with very high C:N ratio) demonstrated intermittent responses for mineral N than their sole application, which can be attributed to temporal N unavailability due to immobilisation (Kuzyakov et al. 2000). However, mineralisation is an ongoing process and from the current study it is difficult to
articulate the rate of change in availability of mineral N for different amendments during the growing season. Therefore, it is difficult to compare the overall effect of subsoil amelioration between inorganic fertiliser and OAs over one crop cycle and we recommend considering slow-release fertilisers (as per (Sistani et al. 2014) or split applications (as per (Mantovi et al. 2005) of inorganic fertiliser to ensure matching nutrient supply with equivalent rates of release from the organic amendments throughout the growing season.

In addition to the increase in grain yield the demonstrated improvement in soil functionality following the amelioration of alkaline dispersive subsoil with OAs has the potential for long-term environmental benefits such as lower the risk of nutrient losses and load into the natural ecosystems (Heathwaite et al. 1998). This soil management strategy may help to achieve sustainable development goals which aims to strive towards land degradation neutrality in the alkaline dispersive soils in dryland rainfed systems. The organic matter content in such soil is frequently low, which has important consequences for the retention of nutrients in the soil and microbial activity. Moreover, the stability of the organic C is low as the dissolution of the organic matter (measured as the concentration of dissolved organic C (DOC)) increases with increasing soil pH (Bolan et al. 2011; McDonald et al. 2017; Tavakkoli et al. 2015). Therefore, the observed reduction in pH may contribute to soil C sequestration, which is considered as an effective strategy to help mitigate the effects of climate change (Lal 2004) by both increasing productivity of crops and pastures and reducing dissolution of the organic matter (Tavakkoli et al. 2022).

Conclusions

The results from this study help partially fill the knowledge gap in distinguishing the nutritional and non-nutritional (soil physical and biological improvements) effects of subsoil amendments on crop yield. In the short-term (one crop cycle), organic amendments had no additional effect on wheat growth compared to fertiliser application alone, suggesting a primarily nutritional effect. However, it must be acknowledged that while the total mass of nutrients contained in the poultry litter were matched with inorganic fertiliser, the timing of release and plant availability could not be controlled. Therefore, longer-term controlled-environment and field studies are needed to investigate the potential legacy effects (e.g., slow-release of nutrients, improvements in soil aggregation and water infiltration) of subsoil amelioration with organic amendments on grain yield beyond the first growing season. Given the worldwide distribution of alkaline dispersive subsoil and the observed increased in grain yield in the current study, the amelioration of this soil with organic amendments may be an effective strategy to meet the world food demand as well as to mitigate the effects of climate change through soil C sequestration.

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Author contributions SU, ET and RA contributed to the study conception and design. Material preparation, setting up the trial and data collection was performed by SU, NA and JR. SU, SWW and SP did the data analysis and interpretation of results. The first draft of the manuscript was written by SU and all authors commended on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interest to disclose.

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