**Plant roots and deep-banded nutrient-rich amendments influence aggregation and dispersion in a dispersive clay subsoil**

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**Abstract**

The ameliorating effect of deep banding of nutrient-rich organic amendments, termed subsoil manuring, on improving physical structure of sodic high-clay subsoils, has been often attributed to organic amendments *per se*. However, this cannot explain the transformation of soil physical properties between the rip-lines, away from the amendments. This study assessed the effect of deep-banding nutrient-rich amendments on aggregation and dispersion of a clay subsoil in the presence and absence of wheat (*Triticum aestivum*) roots under controlled environment conditions. A specially-designed dual-column was set up to simulate a soil profile where a well-structured topsoil overlaid a sodic clay subsoil with an exchangeable sodium percentage (ESP) of 21%. The five amendments include a control (zero amendments), fertilizer nutrients (NPKS), wheat straw + fertilizer nutrients (straw/NPKS), poultry litter (PL) and poultry litter + controlled-release fertilizer (PL/mac). All amendments were added to the centre of the subsoil, 6 cm below the base of the topsoil. Our results showed that the presence of deep-placed nutrient-rich amendments, such as straw/NPKS and PL/mac, greatly enhanced deep root proliferation in this sodic clay subsoil, and resulted in the rapid build-up of large (>2,000 μm) water-stable macroaggregates. There was a significant ($P<0.05$) positive linear relationship between the root length density and the formation of large macroaggregates in the subsoil adjacent to and below the amendment. The stimulation of microbial growth by root exudates or by mucilage, as indicated by a significantly higher bacterial and fungal abundance ($P<0.05$) in the planted than unplanted soils, is likely to have contributed to the formation of these macroaggregates. The effectiveness of wheat straw/NPKS in promoting the formation of macroaggregates in the unplanted soil could be attributed to the 'straw effect' which induced a marked increase in fungal growth ($P<0.05$). Soil electrical conductivity (EC) and aggregate size were the key determinants of clay dispersion in the aggregated subsoil. Plant roots showed a contrasting effect on clay dispersion: increasing clay dispersion by reducing soil EC while suppressing clay dispersion via root-induced increases in large macroaggregates. We argue that the degree of slaking or disaggregation is likely to determine the net effect of roots on clay dispersion, and that root effect on increasing dispersion of macroaggregates in wet subsoil is limited. The major finding of the study is that increased aggregation in a dispersive clay subsoil can occur when wheat roots grow actively in these layers, in response to deep-placed nutrient-rich amendments.

**Key words:** clay dispersion, deep-root proliferation, fungal growth, nutrient-rich amendments, soil electrical conductivity, water-stable macroaggregates, wheat straw

# 1. Introduction

Dense dispersive clay subsoils constrain crop yields across the high-rainfall cropping zone of south eastern Australia (MacEwan et al., 2010). Root growth and root function in these subsoils are frequently limited by high bulk density (1.4-1.6 g cm$^{-3}$), high sodicity (exchangeable sodium percentage (ESP) >15%), periodic waterlogging or limited plant-available water (MacEwan et al., 2010). Traditional practices such as deep ripping and application of gypsum have achieved limited success in ameliorating these clay subsoils (Clark, 2004; Gardner and McDonald, 1988). However, deep banding of nutrient-rich organic amendments, termed subsoil manuring, has produced large crop yield responses in soils with sodic subsoils (Gill et al., 2008; Leskiw et al., 2012). The causes of these yield benefits from subsoil manuring remain unclear. Celestina et al. (2019) consider the benefits arise mainly from increased nutrient availability from the amendments, especially N availability, while Gill et al., (2008) consider that both extra water extraction from subsoils and extra nutrients are responsible. An important finding reported by Gill et al. (2009) was that increased air-filled porosity at -10 kPa, and saturated hydraulic conductivity occurred rapidly during the growth of the first wheat crop following the subsoil manuring intervention.

Organic amendments, once mixed with soil, have been shown to enhance the formation of soil aggregates via the presence or the production of various organic binding agents. These binding agents include humic acids (Gu and Doner, 1993), decomposing products such as carbohydrate polymers (Kinsbursky et al., 1989) and microbial-derived extracellular polysaccharides (Abiven et al., 2009; Schlecht-Pietsch et al., 1994). The production of microbial-derived binding agents is largely affected by microbial activity (De Gryze et al., 2005), and the quality of organic amendments has a significant impact on aggregate formation (Martens and Frankenberger, 1992; Sonnleitner et al., 2003). For

example, crop residues with higher decomposability and higher soluble-C were able to form aggregates over a shorter period, compared to residues with higher C/N ratio and greater resistance to decomposition (Bossuyt et al., 2001; Clark et al., 2010). The effects of animal manures on the formation and stability of soil aggregates are mixed, showing enhanced effects due to increases in biological binding agents (Celik et al., 2010; Tripathi et al., 2014), or detrimental effects because of increases in dispersing agents such as $Na^+$ (Guo et al., 2018b; Guo et al., 2019). The rapid transformation of physical properties by subsoil manuring in the field might also be attributed to the positive and direct effect of organic amendments on soil aggregation. However, compared to the surface application, subsoil manuring involves incorporation of organic amendments at the base of rip-lines with minimal mixing with soil.

Plant roots have also been shown to play an important role in improving soil aggregation via physical, biochemical, and/or biological processes. For instance, growing roots, root hairs and root-associated mycorrhizal hyphae are able to physically rearrange and mechanically enmesh soil particles, promoting the formation of aggregates (Koebernick et al., 2017; Moreno-Espíndola et al., 2007). Living roots also provide mucilage and exudates which can have a gluing effect on soil particles (Czarnes et al., 2000; Totsche et al., 2018). Dead and sloughed-off root cells or root rhizodeposits stimulate intensive microbial activity (van Hees et al., 2005) or promote a shift in the composition of the microbial community towards fungi (Baumert et al., 2018). Both bacteria and fungi can produce various binding agents, such as extra-cellular polysaccharides, lipids or glomalin, binding soil particles into larger aggregates (De Gryze et al., 2005; De Gryze et al., 2006; Rillig and Mummey, 2006). The extensive hyphal networks of fungi enable the fungi to be extremely effective in aggregating soil at large spatial scales (Chenu and Cosentino, 2011). The arbuscular mycorrhizal fungi (AMF) association, if it occurs, can contribute further to root-induced increase in aggregate stability via physical enmeshment (Rillig and Mummey, 2006). Finally, root-induced wetting and drying cycles enhance the formation and stability of aggregates, possibly by the reorientation of clay particles, or by improving the effectiveness of organic binding agents with soil drying (Denef et al., 2002; Graf and Frei, 2013; Rillig et al., 2015). However, most of these findings have focused on the role of plant roots in forming aggregates in topsoils, which are characterized by high biological activity and high root densities. Direct evidence that roots can ameliorate dense clay subsoil, in response to deep-banded nutrient-rich amendments, is lacking.

In dispersive sodic soils, the stability of soil structure is also assessed by the extent of clay dispersion. Increased clay dispersion results in a poor soil structure due to the blockage of soil pores and reductions in pore volume and continuity, and reductions in soil infiltration rates (Bronick and Lal, 2005; Oster and Shainberg, 2001). Research on the effects of plant roots and organic amendments on clay dispersion is controversial. Root growth stimulated clay dispersion by increasing the negative charges on clay surfaces via releasing organic anions (Reid and Goss, 1982; Reid et al., 1982). Alternatively, roots were able to prevent dispersion by forming macroaggregates and reducing the exposure of clay particles to water (Baumert et al., 2018; Tisdall, 1996). In considering the effects of amendments, the addition of animal manure has been reported to increase clay dispersion by introducing dispersing agents such as $Na^+$, organic anions or $NH_4^+$ (Guo et al., 2018b; Guo et al., 2019), or to inhibit clay dispersion by the releasing flocculating agents such as $Ca^{2+}$ or $Mg^{2+}$ ions (Gu and Doner, 1993). The degree of clay dispersion is also affected by other soil properties such as organic carbon content, electrolyte concentration and soil pH (Chorom et al., 1994; Rengasamy and Olsson, 1991). The effects of subsoil manuring on clay dispersion in a dispersive clay subsoil, are largely not unknown. There is a need to understand the separate and the combined effect of root growth and organic amendments on clay dispersion in a dispersive clay subsoil.

This study reports on a large soil column experiment set up with a simulated soil profile where a well-structured topsoil overlaid a dispersive clay subsoil with an exchangeable sodium percentage (ESP) of 21%. The effects of deep-banded amendments on subsoil aggregation and dispersion were assessed in the presence and absence of wheat roots. The study tested three key hypotheses: (i) the presence of deep-banded nutrient-rich amendments will increase root growth in the subsoil; (ii) the enhanced

growth of roots and associated microbial communities will increase the aggregation in the subsoil; and (iii) the amendments and plant roots will reduce clay dispersion in a sodic subsoil.

## 2. Materials and Methods

### 2.1. Soils

Soils were collected from the topsoil (0-10 cm) and subsoil (20-50 cm) layers from a cropping paddock (37.88 $^{\circ}$S, 144.23$^{\circ}$E) on Yaloak Estate, a farm near Fiskville in south-west Victoria. The soil was classified as Sodosol (Isbell, 2016) or Solonetz (IUSS Working Group 2015), and is a major soil type used for rain-fed crop production in south-western Victoria. The subsoil, collected from a low-yielding area of the paddock, was highly sodic with an ESP of 21%. The soils were air-dried, and the subsoil was broken down to pass through a 4-mm sieve while the topsoil was passed through a 2-mm sieve. Selected properties of the topsoil and subsoil are presented in Table S1.

### 2.2. Experimental design and treatments

The experiment was laid out as a randomized complete block design in a controlled environment room. The treatments involved a factorial combination of five amendments, two plant treatments (± wheat plants) and two harvest times (at stem elongation and at maturity). All treatments were replicated three times. The five amendments include a control (zero amendments), fertilizer nutrients (NPKS), wheat straw + fertilizer nutrients (straw/NPKS), poultry litter (PL), and poultry litter + macracote (PL/mac).

The fertilizer nutrient treatment (NPKS) involved mixing three fertilizers in 10 g of dry soil and placing the mixture in the subsoil. The fertilizer mixture comprised 0.94 g diammonium phosphate (DAP), 0.72 g urea and 1.18 g $K_2SO_4$ column$^{-1}$, which were equivalent to 300 kg N, 125 kg P, 300 kg K, and 123 kg S ha$^{-1}$ on a surface-area basis, respectively. The straw/NPKS treatment involved the addition of wheat straw at the rate of 17.7 g dry matter (DM) column$^{-1}$, equivalent to 10 t ha$^{-1}$ on a surface-area basis. The wheat straw was chopped to pass through a 6-mm sieve and had a C:N ratio of 165. The NPKS fertilizer nutrients were mixed through the straw pieces in liquid form, at the same rates as the NPKS treatment above. The poultry litter, collected from a broiler shed, was added to columns at 35.3 g column$^{-1}$, equivalent to 20 t DM ha$^{-1}$ on a surface area basis. The poultry litter had a C:N ratio of 7.6, and contained 45, 17, 27 and 4 mg g$^{-1}$ of N, P, K and S, respectively. The PL/mac treatment involved the same amount of poultry litter as the PL treatment, as well as added Macracote Orange, a controlled-release inorganic fertilizer (Langely Fertilisers, Australia). The rationale of this treatment was based on earlier findings (Wang et al., 2019) that the release of N from poultry litter was too slow to meet the requirements of wheat plants, in a column experiment, after 6 weeks of growth in glasshouse. Macracote was added to the poultry litter at 7.5 g column$^{-1}$ to rectify the problem. The N, P and K concentrations in Macracote were 165, 35, and 100 mg g$^{-1}$, respectively. Total N released at wheat maturity, from the poultry litter (estimated at 15% of total N) and from the Macracote (estimated at 25% of total N over 120 days at 20 °C, Langely Fertilisers), was calculated to be equivalent to the N supply in the NPKS treatment.

### 2.3. Column set-up

A specially-designed dual-column system was constructed to ensure that wheat roots would grow directly into the subsoil and subsoil amendments and minimize root growth down the sides of the PVC column (Fig. 1). The soil profile consisted of 24 cm of topsoil in a small PVC cylinder (30 cm high, 10 cm in diameter), overlying 32 cm of subsoil in a larger PVC cylinder (45 cm high, 15 cm in diameter). The subsoil was packed layer by layer with consistent tapping and watering to reach the final bulk density of 1.30 g cm$^{-3}$. During the packing, a watering tube was inserted into the subsoil at the side of the lower column for adding water to the 38-58 cm lower subsoil. In addition, soil moisture probes (WaterScout SM100, Thermoline Scientific) were placed in two replicate columns for each amendment treatment with plants, that were harvested at wheat maturity. The probes were positioned beside and below the subsoil amendments (Fig. 1). The amendments were added at the center of the column into a small plastic cylinder (8 cm high, 6 cm in diameter), 6 cm below the base of the topsoil, to ensure that plant roots would reach all amendments at a similar time. The plastic cylinders for packing the organic amendments were removed once soils had been packed outside the cylinder. The small PVC column was inserted into the subsoil of the larger PVC column to a depth of 4 cm, before the topsoil was added. Basal nutrients were added to the topsoil at the following rates (mg kg$^{-1}$): $CO(NH_2)_2$, 86; $KH_2PO_4$, 180;

K$_2$SO$_4$, 180; CaCl$_2$.2H$_2$O, 180; MgSO$_4$.7H$_2$O, 50; MnSO$_4$.H$_2$O, 15; ZnSO$_4$.7H$_2$O, 9; CuSO$_4$.5H$_2$O, 6; Na$_2$MoO$_4$.2H$_2$O, 0.4; FeEDTA, 5.5. The soil surfaces in both PVC columns were covered with a 3-cm layer of plastic beads to minimize surface evaporation.

## 2.4. Growing conditions

The experiment was conducted in a controlled-environment room with conditions set to a 14-h day at 25 °C, a 10-h night at 18 °C and light intensity of 450 µmol m$^{-2}$ s$^{-1}$. Eight pre-germinated seeds of wheat (*Triticum aestivum* cv. Yipti) were sown in each column. After emergence, the plants were thinned to three uniform seedlings per column. Water supply in this study was non-limiting for the wheat plants, to reflect the field situation where rainfall patterns in the Victorian high-rainfall zone generally result in a full supply of soil water in the subsoil of the Sodosol at the beginning of spring.

So, all columns were watered to weight, either from the top of the small PVC column, or from the watering tube, every 2 days with deionized water to maintain 80% field capacity. Water had been added to the topsoil only during the early growth stages, and then also to the subsoil via the watering tubing when the moisture content in the subsoil, as indicated by the moisture probes, started to drop. The amount and proportion of water allocated to the two PVC columns was estimated from the total water loss and from the soil moisture probe readings.

Supplemental N was added as urea at 40 mg N kg$^{-1}$ to the topsoil for all columns 32 days after sowing to minimize N depletion from the topsoil. Columns were destructively harvested 42 and 120 days after sowing, when the wheat plants had reached the stem elongation and maturity stages of growth, respectively. Columns in which the wheat plants grew on to maturity received a further N addition of 40 mg N kg$^{-1}$ to the topsoil at Day 42.

## 2.5. Root and soil sampling

At each harvest, the gravimetric soil moisture content was adjusted to ~60% field capacity to facilitate the soil sampling. All columns were sectioned into three layers to collect roots for root length measurements. These were the topsoil layer (0-28 cm deep), the amendment layer containing the amendment band (28-36 cm deep), and the subsoil layer below the amendment (36-56 cm deep). Soil samples used for chemical, physical, microbiological and dispersion measurements were subsampled from two sampling locations. The first was from 'beside the amendment', where soil was collected 1-2 cm away from the amendment band and the wall of the PVC column (Fig. 1). The second sampling location was from 'below the amendment' (8 cm below the NPKS and 3-4 cm below other amendment bands), consisting of a central core of subsoil, 5 cm high and 10 cm in diameter (Fig. 1). The soil sampled from these locations was broken up into small pieces and mixed gently before collecting a 150-g sample for physical and chemical measurements and a 50-g sample for microbial measurements. Samples for microbial measurements were stored frozen at -20 °C. After soil sampling, roots were recovered by soaking the remaining soil from each layer in water in buckets for several hours, and then gently washing the soil from the roots over a 1-mm sieve.

## 2.6. Root and shoot measurements

All washed roots were cut into 3-4 cm segments, evenly mixed, and subsampled for root scanning. The subsamples were scanned for root length and root diameter measurements using an EPSON EU-35 scanner (Seiko Epson Corp, Suwa, Japan) and the WinRHIZO STD 1600+image analysis system (Regent Instruments, Quebec City, Canada). Root, shoots and threshed grains were oven-dried at 70 °C and weighed. Shoots and grains were ground, ball-milled and analyzed for N using a CHNS Analyzer (PerkinElmer EA2400, Shelton, CT, USA). The concentrations of P, K, Mg, Ca, and Na in shoots and grain were measured using ICP-OES (Inductively Coupled Plasma Optical 154 Emission Spectrometry) after being digested with concentrated nitric and perchloric acid (4:1).

## 2.7. Fungal and bacterial abundance measurements

DNA was extracted from 0.25 g of soil from both amendment and subsoil layers (120 samples) using a DNeasy PowerSoil Pro (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 quantitative PCR (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 SensiFAST SYBR Lo-Rox (Bioline, Alexandra, NSW, Australia), 0.4 µl of each primer (10 µM) and 20 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 20 ng of template DNA. Thermal cycling conditions for the fungal assay were 3 min at 95 °C, 40 cycles of 5 s at 95 °C, 30 s at 60 °C. Thermal cycling conditions for the bacterial assay were 3 min at 98 °C, 40 cycles of 15 sec at at 95 °C, 60 s at 60 °C. Gene copy numbers were calculated from the average cycle threshold (CT) of two replicates for each sample by comparison with a standard curve (10-fold dilution with a linear range of $10^{-3}$ to $10^{-8}$), generated using the relevant plasmid for that assay (Hayden et al., 2012) and converted to gene copy number per gram of dried soil and copy number per ng of extracted DNA (see supplementary data). For all assays QPCR efficiency was 81.4–92.9% and $R^2$ was 0.995–0.999.

*2.8. Soil chemical and physical measurements*
The soluble organic C in soil was extracted by 0.5 M $K_2SO_4$ (1:5, w/v) from moist soil, and measured using a TOC analyzer (GE Sievers InnoyOx Boulder, 160 USA). Soil inorganic N ($NH_4^+ + NO_3^-$) in the same extracts was measured using a QuickChem 8500 flow-injection analyzer (Lachat Instruments, Loveland, CO, USA). The remaining subsamples were then air-dried, ground (<1 mm) and measured for soil EC (1:5 soil: water) and pH (1:5 soil: 0.01 M $CaCl_2$). Exchangeable cations were extracted in 1 M $NH_4Cl$ at a soil solution ratio of 1:20 at pH 7 (Rayment and Lyons 2010). Soluble cations were measured in a 1:20 soil:water extract, after being centrifuged and filtered through a 0.1-µm filter. The cation composition of all extracts was measured by the ICP-OES. The exchangeable Na percentage (ESP) and the cation ratio for soil structural stability (CROSS) (Rengasamy and Marchuk, 2011) was calculated as following:
ESP = Exchangeable [(Na)/(Ca + Mg + K + Na)] × 100
CROSS = Soluble [(Na + 0.56 K)/(Ca + 0.6 Mg)$^{0.5}$]
The structural stability of moist soil was determined using a standard wet-sieving apparatus (Clark et al., 2010). Moist soil samples were expected to give a more accurate assessment of the subsoil behavior under field conditions. Briefly, 16-18 moist aggregates with sizes of 4-6 mm were added to the top of a nest of three pre-weighed sieves (with apertures of 2000 µm, 250 µm, and 50 µm). The sieves were immersed and shaken in 2.8 l of distilled water through a 1.3-cm vertical distance for 5 min, at 35 cycles per minute. After the wet-sieving, the soil remaining on each sieve was dried at 105 °C and weighed to give the dried aggregate mass with diameters exceeding the aperture of the sieve above which they were retained. Each sample was divided in four size distributions: the fine particle sizes (< 50 µm), microaggregates (50-250 µm), small macroaggregates (250-2000 µm), and large macroaggregates (> 2,000 µm). The small and the large macroaggregate percentages in the total sample mass were corrected for the sand mass in their respective size ranges.

To determine the air-filled porosity at field capacity, two intact core samples were taken from the subsoil layer, 2 cm below the amendments, using brass rings (3 cm long, 3.8 cm in diameter). The rings were pushed into subsoil by hand, with a spacer on the top of ring, to avoid any compaction on soil samples. All intact core samples were saturated slowly in a water bath for 3-4 days. After saturation, core samples were weighed, before being placed on a ceramic suction plate with a 1-m hanging water column (-10 kPa). The core samples were weighed again after 7 days when there was no further decrease in their weight. The amount of water contained in the saturated macropores was determined by the differences between weights of the saturated cores and cores equilibrated on the 1-bar ceramic plates. The air-filled porosity at field capacity was calculated by dividing this weight by the volume of the brass ring. The bulk density was calculated by dividing the weight of the oven-dried soil by the volume of the brass ring.

Spontaneous clay dispersion in soil samples was determined using a modified method described by Zhu et al. (2016). Five grams of either air-dried aggregates (4-6 mm in diameter) or ground air-dried subsoil samples (<1 mm) were weighed into 30-ml plastic tubes, and 25 ml of distilled water was then pipetted slowly down the side of the tube. The tubes were then gently inverted three times to immerse all soil samples completely. The samples were allowed to settle for 5 h. Then 10 ml of the clay suspension in the solution above the clay was pipetted carefully and consistently, without any disturbance to the top of the loose clay at the base of the tube, into pre-weighed aluminum trays. All suspensions were oven-dried at 105 °C and weighed to calculate the percentage of clay dispersed. The final clay dispersion was corrected for the salt contributions by oven-drying 5 ml suspension after filtering through 0.1-µm filter.

### 2.10. Statistical analysis

A one-way analysis of variance (ANOVA) was conducted to assess the effects of amendments on shoot and root biomass, grain yield, root length density, shoot nutrient concentration and uptake. A two-way ANOVA was used to determine the significance of the subsoil amendment treatments, the presence/absence of plants, and their interactions on soil macroaggregate formation and stability, clay dispersion, soil chemical properties, and microbial rRNA gene abundance for soil collected from beside and below the amendments, at each harvest (42 and 120 days). The log transformation was used on data that failed to meet the assumptions of normal distribution for ANOVA, including the fungal and bacterial data. Significant differences between means were identified using Tukey's HSD test. All statistical analyses were performed using Genstat (11th version; VSNI Hertfordshire, UK).

The linear relationships between the percentage of macroaggregates (> 2,000 µm) in soil collected beside and below the amendments, and the root length densities in the amendment and subsoil layers, were determined using a simple linear regression model. Similarly, the linear relationships between the percentage of dispersed clay and soil EC, pH, ESP and CROSS were determined using linear regression models. Pearson's correlation coefficients ($r$) between clay dispersion and soil EC, soil pH, ESP and CROSS, and between soil EC and pH, ESP and CROSS were also determined at each harvest in the presence or absence of plants.

## 3. Results

### 3.1. Shoot and root growth and grain yield

Wheat shoots responded to all subsoil amendments with significant ($P<0.05$) increases in shoot biomass above the control, at stem elongation and at wheat maturity (Table S2). The PL/mac amendment produced the largest shoot biomass at maturity, which was double that of the control, followed by the straw/NPKS, NPKS and PL amendments. Grain yields generally reflected the total shoot biomass, and increased by 102% and 50% in response to the PL/mac and straw/NPKS amendments, respectively. Root biomass also responded significantly ($P<0.05$) to the subsoil amendments, with the largest root biomass occurring with the straw/NPKS amendment at stem elongation, and with straw/NPKS and PL/mac amendments at maturity (Table S2). Among all amendments, the NPKS amendment resulted in the least root biomass at both growth stages. The averaged root diameter (0.33 ± 0.01 mm) in the subsoil was much higher than that of top and amendment layer (0.28 ± 0.02 mm) (data not presented).

### 3.2. Nutrient concentrations and uptake

Subsoil amendments significantly affected the concentrations of N, P and K in wheat shoots and grains (Table S2). The PL/mac and NPKS amendments produced the highest shoot and grain N concentrations. In contrast, the shoot and grain N concentrations did not differ between the straw/NPKS and PL amendments, and the control. The shoot P and K concentrations were significantly higher with the PL/mac than other amendments at stem elongation (Table S2). The highest nutrient uptake in shoots occurred invariably with the PL/mac amendment at both harvests (Table S2). Another notable finding was the consistently low N uptake with the PL amendment at stem elongation and at maturity.

### 3.3. Subsoil moisture content prior to watering

The gravimetric soil water content in the subsoil, 2-8 cm below the amendments, remained relatively unchanged in the control throughout the experiment. In contrast, it decreased progressively from Day 15 to Day 49 in all amended soils prior to watering events, especially for the PL/mac treatment (Fig. 2).

After Day 49, soil moisture content remained low between 28-30% in the PL/mac-amended soil while increased gradually for other treatments. The wilting point (-1.5 MPa) for this Sodosol subsoil occurred at a gravimetric water content of 24%.

### 3.4. Root length densities, soil macroaggregation and air-filled porosity at field capacity

Root length density in the topsoil layer did not differ between treatments, except that the straw/NPKS amendment produced a significantly ($P<0.05$) higher root density than the control at maturity (Fig. 3). However, subsoil amendments generally increased root length density above the control in both the amendment and subsoil layers (Fig. 3). At stem elongation, the straw/NPKS amendment resulted in the highest root length density in the amendment layer, with NPKS being the only amendment not to increase root length density in the subsoil. At maturity, root length density did not differ between amendments in the amendment layer, but the PL/mac and straw/NPKS amendments produced a 3.4-fold, and 3-fold increase in root densities, respectively, relative to the control in the subsoil.

The presence of wheat roots corresponded with the increased formation of large macroaggregates (> 2,000 μm) (Fig. 4). Although this root effect was less noticeable in the soil below the amendment at stem elongation, it had increased markedly at maturity. Overall, the formation of large macroaggregates, from the combined effects of amendments and plants at maturity, ranged from 50 to 75%, compared with 18 to 37% for the equivalent amendment treatments without plants.

Subsoil amendments differed in their effectiveness in promoting the formation of large macro-aggregates (Fig. 4). The straw/NPKS amendment was particularly effective in soil beside the amendment, which resulted in 57% and 75% of the soil forming into large macroaggregates at stem elongation and crop maturity, respectively, compared to 30 and 42% for the control in the plus-plant treatment. Noticeably, the straw/NPKS was the only amendment which increased ($P<0.05$) the formation of large macroaggregates above the control at maturity in soil beside the amendment, in the absence of plants. In the subsoil layer, the straw/NPKS and PL/mac amendments produced the largest increase in large macroaggregates at maturity, followed by PL and NPKS.

The formation of large macroaggregates was positively correlated with root length density, although the relationship was stronger in soil below the amendment ($R^2=0.86$; $P<0.001$) than in soil beside the amendment ($R^2=0.53$; $P<0.05$) (Fig. 5).

Small macroaggregates (250-2,000 μm) and fine soil particles (< 50 μm) were the two dominant size fractions in the control soil without plants (Fig. 6). However, at maturity, the size fractions in soils with plants were dominated by large macroaggregates (>2,000 μm), and to a lesser extent by fine soil particles (< 50 μm), with a decline in microaggregates (50-250 μm) and small macroaggregates (250-2,000 μm). A greater reduction occurred with the small macroaggregates (250-2,000 μm). The finest fraction (< 50 μm) was less affected by plant roots, although there was a small reduction in this fraction with the straw/NPKS and PL/mac amendment.

At the stem elongation, the air-filled porosity values at the field capacity below the amendment were lower for plus-plant treatments, with an average value of 6.5%, than the minus-plant soils, with an average of 10.6%. The averaged subsoil bulk density did not vary between amendment treatments, but was slightly higher in the plus-plant (1.37 g cm$^{-3}$) than in the minus-plant soils (1.27 g cm$^{-3}$) (Table 1).

### 3.5. Fungal and bacterial abundance

Soil fungal and bacterial abundance showed different response to all amendments in the presence or absence of plants. Both the amendment and plus plant treatments significantly affected fungal abundance at both harvest times ($P<0.001$) in soil beside the amendment (Table 2). Noticeably, fungal abundance was invariably higher in the plus-plant than minus-plant treatments. When compared to the control, the straw/NPKS treatment increased fungal abundance by nearly 6-fold in soil beside the amendment, regardless of the presence of plants roots. The PL/mac treatment showed consistent increases in fungal abundance relative to the control in the plus-plant soils in soil beside and below the amendment at both harvests. Amendments such as NPKS showed little or no impact on fungal growth.

Compared to fungi, bacterial abundance was less affected by amendment types, regardless of soil depth and growth stage. However, bacterial abundance increased in plus-plant samples compared to minus-plant samples, in soil below the amendment at stem elongation ($P<0.001$) and in soil beside and below the amendment at maturity ($P<0.001$) (Table 2).

### 3.5. Treatment effects on the soil chemical properties

All amendments reduced soil pH in soil beside the amendment by 0.17 to 0.40 units ($P<0.05$) at stem elongation and at maturity (Tables S3 and S4). Soil pH in the soil below the amendment was less affected by amendments, except for a significant decrease ($P<0.05$) with the PL/mac amendment at maturity. All amendments increased soil EC ($P<0.05$), above the control, in soil beside the amendment at both harvests (Tables S3 and S4). The PL/mac amendment consistently resulted in the largest increase in EC at both harvests. In soil below the amendment, increased EC only occurred with the PL/mac amendment. In contrast, wheat roots decreased soil EC substantially, with greater reductions in soil beside the amendment. When plants were absent, adding amendments substantially increased the inorganic N concentrations in soil beside the amendment (Tables S3 and S4). The PL/mac amendment resulted in the largest increase in inorganic N, followed by NPKS, straw/NPKS and PL. Plant roots however, resulted in the depletion of inorganic N in soil beside the amendment at both harvests. The PL and PL/mac amendments were the only amendments to increase the concentration of extractable organic C (EOC) in soil beside the amendment at both harvests (Tables S3 and S4).

Exchangeable sodium percentage (ESP) values were less affected by amendment types, but were higher in plus-plants than minus-plant treatments (Tables S3 and S4). Only the PL/mac amendment reduced soil ESP in soil beside the amendment at maturity in the minus-plant treatments. In contrast, some amendments decreased CROSS values in soil from beside and below the amendment at both harvests, but the reductions were inconsistent and varied between amendments at different harvests. The presence of plants had no impact on the CROSS values.

### 3.6. Clay dispersion

The propensity for the air-dried aggregates to disperse in water was markedly affected by the amendments and the presence of plants (Fig. 7). All amendments decreased clay dispersion in the minus-plant treatments, with the PL/mac resulting in the lowest dispersion. However, the presence of plant roots significantly increased clay dispersion in water, especially in the case of the PL/mac amendment.

### Relationships between chemical properties and clay dispersion

Soil EC was the most consistent predictor of the clay dispersion when air-dried aggregates were immersed in water. Clay dispersion was negatively correlated with soil EC ($R^2 \geq 0.70$, $P<0.001$)., using either pooled (Fig. 8a) or separate data (Table 3) for ± plants. In contrast, soil pH, ESP and CROSS were poor predictors of clay dispersion. There was no relationship between dispersion and soil pH in samples collected at stem elongation, although a significant relationship was detected at maturity (Fig. 8b, Table 3).

Similarly, ESP and CROSS were not related to clay dispersion in the plus-plant soils, but were positively related to clay dispersion in the minus-plant soils (Fig. 8c and 8d, Table 3). Soil pH, ESP and CROSS were however significantly related to soil EC in these soil samples.

## 4. Discussion

In agreement with our first hypothesis, the presence of deep-banded nutrient-rich amendments increased root growth in the sodic clay subsoil. The increase in root growth into the subsoil around the amendment bands was substantial, in line with findings that patches of nutrients encouraged localized root proliferation to capture immobile nutrients such as P, or mobile nutrients such as N as it is released from mineralising organic sources (Hodge, 2003; Robinson, 1994). The extension of roots into the subsoil below the amendments is believed to be driven by the need to extract water from the clay matrix, as indicated by the apparent decreases in soil water in the amended subsoil, relative to the control. Roots of wheat plants in the control were barely able to extract water from the subsoil layer. The plasticity of

root systems in producing deep roots to exploit either water or nutrients in the subsoil has been well documented (Hodge, 2003; Koevoets et al., 2016). Our study demonstrated that plant roots were capable of exploring these resources even in this highly dispersive (ESP 21%), high clay (50%) and poorly-aerated subsoil (macroporosity <12%), following deep incorporation of nutrient-rich amendments. The issue here is how wheat roots coped with the physical constraints and initiated their penetration into the subsoils below the amendments.

Several mechanisms were likely to have assisted wheat roots in growing into the subsoil layer, in response to the presence of nutrient-rich amendments. Firstly, given the pronounced shrink-swell capacity of the clay in this study, rapid depletion of water in soil below the amendments (Fig. 2) possibly led to a shrinkage of clay, creating entry points for roots to extend into the clay matrix (Dexter 1988). There is ample evidence suggesting that the repetitions of drying and wetting cycles could increase the formation of macropores (Ma et al., 2015; Bodner et al., 2013). Gao et al. (2016) suggested that the presence of deep roots in clay soils primarily resulted from exploitation of cracks formed during desiccation of clay rather than root-induced soil deformation. Nevertheless, increased root diameter, in response to increased soil strength in the subsoil, might enable roots in cracks or macropores grow into bulk subsoil with increased mechanical impedance (Bengough et al., 2011; Clark et al., 2003). Secondly, amendment-induced root proliferation might have enhanced the concentration of root exudates or mucilage immediately below the amendments, which decreased the interparticle friction and penetration resistance via their lubricating effect (Bengough et al., 2011; Oleghe et al., 2017). Finally, it is likely that the physical environment in the subsoil had been improved over time, in the presence of the amendments or plant roots. For instance, organic amendments *per se* could has a significant impact on aggregate formation and clay dispersion (Guo et al., 2019; Sonnleitner et al., 2003), resulting in improved soil structure close to the amendments. The elongation of roots into the pre-existing or newly-formed macropores contributed to the transformation of subsoil away from the amendments (see below). Loss of air-filled porosity under field capacity at the stem elongation in the plus- relative to the minus-plant soil (Table 1) might indicate a temporal macropore clogging due to roots growing into pre-excising pores, as also reported by other studies (Gish and Jury 1983; Scanlan 2009).

In line with our second hypothesis, active root growth in the subsoils in response to deep-banded amendments promoted a rapid increase in the proportion of water-stable large macroaggregates (>2,000 µm) in this sodic clay subsoil. This is confirmed by a highly significant ($P<0.05$) positive linear relationship between root length density and the formation of large macroaggregates. Moreover, the average percentage of large macroaggregates for the plus-plant treatments was more than double that of the minus-plant treatments. Previous studies found plant roots contributed significantly to the formation of macroaggregates (>250 µm) in surface soil (Helfrich et al., 2008; Tisdall and Oades, 1982). This study provides direct evidence that deep root growth can also contribute to the formation of macroaggregates in deeper soil layers. The stabilizing effect of living roots can be attributed to physical entanglement and containment by roots, root hairs and root-associated mycorrhizal hyphae (Koebernick et al., 2017; Moreno-Espíndola et al., 2007). The stimulation of microbial growth by root exudates and mucilage in the plus-, compared to minus-plant soil (Table 2), should also contribute to the enhanced soil aggregation by plant roots. Both bacteria and fungi could enhance soil aggregation via release of binding agents such as extra-cellular polysaccharides, lipids or glomalin (De Gryze et al., 2005; De Gryze et al., 2006; Rillig and Mummey, 2006). In this study, the contribution of fungi to macroaggregation was more pronounced than that from bacteria, given the fact that the highest fungal abundance was detected with the straw/NPKS and PL/mac treatments which also resulted in the highest proportion of macroaggregate formation. Enhanced fungal growth in the planted soil could result from mycorrhizal infection of wheat roots or a response by fungal communities to the root exudates or rhizodepositions. Future studies will be focus on determining which fungal taxa responded to the presence of plant roots and their roles in the formation of macroaggregates.

The formation of large water-stable macroaggregates was more pronounced at plant maturity than at the stem elongation. This did not appear to result simply from the extra root biomass formed during this period, as there were more macroaggregates at maturity than at stem elongation, for a given root length

density. Previous research suggests that root-derived binding agents such as root exudates, dead roots or root hairs or cellular root materials, tend to peak at plant maturity due to senescence of roots (Lucas García et al., 2001; Wang et al., 2016). The nature of binding agents could also change over time, and microbial-derived extracellular polysaccharides with high affinity and low reversibility, generally dominate at later growth stages (De Gryze et al., 2005; Malik and Letey, 1991). Moreover, root-induced biological effects as well as wetting and drying cycles, were likely to have a cumulative effect, producing a strong and prolonged impact on stabilizing the macroaggregates. With time, more stable microaggregates with considerable longevity might have formed within macroaggregates with shorter turnover times (De Gryze et al., 2006; Six et al., 2002). It is clear that the formation and the stability of macroaggregates was time-dependent, and increased with continuing root growth of the wheat plants.

Root-induced increases in the formation of large macroaggregates (> 2,000 μm) were associated with a marked decline in the percentage of small macroaggregates (250-2,000 μm). This suggests that the smaller macroaggregates acted as building blocks for the formation of large macroaggregates by plant roots in this dispersive clay subsoils. Other studies also found that larger macroaggregates (> 1,000 or 2,000 μm) were formed from smaller macroaggregate (250-1,000 or 250-2,000 μm) in the presence of plant roots (Blankinship et al., 2016) or following the addition of crop residue (Angers, 1998; Grosbellet et al., 2011; Poirier et al., 2014). The latter studies attributed the formation of larger macroaggregates to a fine film of the decomposing residues, surrounding the existing smaller macroaggregates. We also propose a process by which smaller aggregates are bound into large macroaggregates via living roots. This process begins with roots, with or without fungal hyphae, growing around existing smaller macroaggregates in order to access oxygen and water in the voids between these smaller macroaggregates. There is evidence that roots grow preferentially around smaller macroaggregates rather than penetrate through them (Kavdır and Smucker, 2005; Whiteley and Dexter, 1983). Root mucilage or rhizodeposition would then accumulate and form a coating on the exterior surfaces of the smaller macroaggregates, as demonstrated in earlier studies (Santos, 1998; Smucker, 2003). Finally, root-induced soil drying would draw small macroaggregates closer, resulting in enhanced adherence and formation of large macroaggregates. Further studies will be required to verify this proposed mechanism.

The amendments differed in their impact on the formation of large macroaggregates in the subsoil. Among all amendments, PL/mac and the straw/NPKS amendments resulted in much higher root length density, fungal abundance and therefore a greater percentage of large macroaggregates. Noticeably, the straw/NPKS amendment was surprisingly more effective than the NPKS amendment, despite both amendments having the same levels of fertiliser nutrients. Even in the absence of plant roots, the straw/NPKS amendment produced a greater percentage of large macroaggregates relative to the control beside the amendments at the maturity (Fig. 4). The effectiveness of straw/NPKS can be partly attributed to a 'straw effect' which resulted in a marked increase in fungal abundance (Table 2). The result is consistent with earlier findings where the incorporation of wheat straw, low in N and rich in cellulose or lignin, promoted fungal development (Abiven et al., 2009; Chenu and Cosentino, 2011; Sonnleitner et al., 2003; Tardy et al., 2015) and this pattern was hardly affected by the addition of fertilizer N (Allison and Killham, 1988; Guo et al., 2018a). The effectiveness of the PL/mac might lie in the highest nutrient availability or the combined effect of manure/fertiliser, which warrants the further evaluation.

Plant roots increased clay dispersion by reducing the electrolyte concentration in the soil solution, which is inconsistent with our third hypothesis. It is possible that dispersion could have been increased by roots vis release of organic acids (Reid and Goss, 1982; Reid et al., 1982). However, the circumstances where organic acids promote dispersion might not be applied to the bulk subsoil, away from the rhizosphere. In this study, soil EC acted as the key determinant of clay dispersion regardless of the presence of plant roots, as indicated by the consistent negative linear regressions between clay dispersion and soil EC (Table 3). The increases in EC in the amendment layer resulted from the direct input of ions from amendments, while root activity and the uptake of nutrient ions from the soil solution reduced soil EC. The suppression of clay dispersion by high EC was in line with many other studies (Nelson et al., 1998; Rengasamy and Olsson, 1991). The mechanisms underpinning the effect of EC on

clay dispersion could be explained by the compression/expansion of the diffuse double-layer between clay particles (Van Olphen and Hsu, 1978) and/or by changes in osmotic pressure in the soil solution (Rengasamy, 1998). The critical electrolyte concentration that results in the complete suppression of clay dispersion, is generally referred to as the threshold electrolyte concentration (Nelson et al., 1998; Rengasamy and Olsson, 1991). In this study, clay dispersion decreased sharply when soil EC exceeded 400 µS cm$^{-1}$, and there was virtually no dispersion with the PL/mac amendment without plants.

Changes in soil pH, ESP or CROSS were not directly related to differences in clay dispersion. Earlier research found that increasing soil pH could increase clay dispersion due to increased negative charges on clay particles (Bronick and Lal, 2005; Chorom et al., 1994). In this study, soil pH was strongly related to dispersion at maturity ($P<0.001$, Fig. 8b). For instance, the higher soil pH in the planted soil, attributed to the excess uptake of anions, mainly as $NO_3^-$, over cations (Tang et al., 1999; Wang and Tang, 2017), occurred along with higher clay dispersion. However, there was no relationship between dispersion and soil pH at stem elongation. The close relationship between soil pH and dispersion at maturity was more likely to have resulted from the close negative correlation between soil pH and EC ($P<0.001$, Table 3), and not due to any causal effect of pH on dispersion *per se*. Similarly, cation ratios such as ESP and CROSS, which have been used as indicators of clay dispersion (Smiles and Smith, 2004; Rengasamy and Marchuk, 2011), were poor predictors of clay dispersion in this study. ESP and CROSS values were positively related to clay dispersion, but only at maturity in the absence of plants (Table 3), when both cation ratios were closely correlated with soil EC (Table 3). Thus, soil EC remains the one consistent predictor of clay dispersion in this column experiment, and this is most likely due to its wide range from 138 to 754 µS cm$^{-1}$ including values below and above the threshold electrolyte concentration.

Thus, there were contrasting effects of plant roots on clay dispersion in this study. On the one hand, plant root growth increased the formation of large water-stable macroaggregates (>2,000 µm), which would lower clay dispersion by reducing the exposure of clay particles to water (Baumert et al., 2018; Tisdall, 1996). On the other hand, root growth reduced soil EC, which in turn increased clay dispersion from aggregates. The more likely explanation for this apparent aggregation-dispersion paradox in the subsoil results from the method used to measure clay dispersion. This method involved several steps which increased the breaking down of macroaggregates and exposure of clay surfaces to water and hence exacerbated the dispersion. For example, the use of air-dried aggregates, the sudden immersion of the aggregates in water, and the two end-over-end inversions of the immersed aggregates had led to the rapid disaggregation of the macroaggregates via either slaking or, to a lesser extent, mechanical disturbance. Hence, the final dispersibility of clay was independent of the size of aggregates and mainly reflect the predominant effect of soil chemical properties such as EC. Earlier research has shown how the stability of aggregates to slaking declines as the aggregates become wetter and as their rate of wetting decreases (Harris et al., 1966). We would expect that the newly-formed macroaggregates by plant roots in the wet subsoil would be less dispersive due to their higher water-stability or less slaking upon slow wetting or lack of any mechanical disturbance. The above complications also suggest that a new methodology needs to be developed to measure the propensity of newly-formed macroaggregates to disperse *in situ* in a dispersive subsoil.

## 5. Conclusions

The benefit of subsoil manuring in improving physical structure of sodic high-clay subsoils has been often attributed to the ameliorating effect of organic amendments *per se*. This study, for the first time, has shown that crop roots contribute to the formation of large macroaggregates in a dispersive simulated sodic-clay subsoil, in response to deep-banded, nutrient-rich amendment. The amendments varied in their effectiveness in stimulating root growth and formation of large macroaggregates in the subsoil. Wheat straw impregnated with inorganic nutrients shows promise as an amendment to replace the animal-manure-based amendments, given its low cost and 'straw effect" in promoting fungal growth and soil macroaggregation. An important question is whether root-induced water or nutrient depletion will increase slaking and clay dispersion of root-induced macroaggregates in the subsoil. Further research is needed to determine the effect of slaking on soil dispersion under field conditions, following the infiltration of water from rain or irrigation water. Also, caution is required in extrapolating results

from this column experiment to the field where root growth might be more impeded by high bulk density ($>1.4$ g cm$^{-3}$).

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**Table 1.** Air-filled porosity at field capacity and bulk density for soils amended with different amendments, with and without plants, in soil collected beside and below the amendments at stem elongation. Standard errors are shown in parentheses.

| | Air-filled porosity at field capacity (%) | | Bulk density (g cm$^{-3}$) | |
| --- | --- | --- | --- | --- |
| | + plant | - plant | + plant | - plant |
| Control | 7.5 *(0.5)* | 9.4 *(0.3)* | 1.33 *(0.05)* | 1.28 *(0.02)* |
| NPKS | 6.4 *(0.5)* | 10.3 *(0.2)* | 1.36 *(0.03)* | 1.26 *(0.01)* |
| Straw/NPKS | 5.8 *(0.3)* | 10.8 *(0.6)* | 1.38 *(0.05)* | 1.25 *(0.03)* |
| Poultry litter | 6.4 *(0.5)* | 11.0 *(0.9)* | 1.37 *(0.01)* | 1.29 *(0.04)* |
| Poultry litter/macracote | 6.3 *(0.6)* | 11.5 *(0.7)* | 1.39 *(0.06)* | 1.27 *(0.03)* |

**Table 2**. Effect of different amendments, with and without plants, on the abundance of bacteria and fungi in soil collected beside and below the amendment, at stem elongation and maturity. Different lower-case letters indicate significant differences between interaction means, while different upper-case letters indicate significant differences between main effects means, using Tukey's HSD test. n.s, *, ** and *** represent $P$ values of >0.05, <0.05, <0.01 and <0.001, respectively.

| Amendments | ± plant | Fungal ITS copies (×10$^7$) g$^{-1}$ soil | | Bacterial 16S copies (×10$^8$) g$^{-1}$ soil | |
|---|---|---|---|---|---|
| | | Beside | Below | Beside | Below |
| ***Stem elongation*** | | | | | |
| *Interaction means* | | | | | |
| Control | + | 7.9 | 6.5 abc | 16.2 | 13.4 |
| | - | 5.5 | 3.4 a | 14.6 | 12.2 |
| NPKS | + | 10.3 | 11.7 bcd | 11.6 | 15.0 |
| | - | 6.1 | 4.2 a | 13.3 | 11.8 |
| Straw/NPKS | + | 55.3 | 11.4 bcd | 29.5 | 12.6 |
| | - | 15.7 | 4.4 ab | 13.2 | 12.1 |
| Poultry litter | + | 13.9 | 14.4 cd | 14.7 | 16.0 |
| | - | 6.8 | 4.4 ab | 12.8 | 10.4 |
| Poultry litter | + | 18.3 | 28.2 d | 13.2 | 16.3 |
| /macracote | - | 9.0 | 3.1 a | 12.2 | 9.4 |
| *Main effect means* | | | | | |
| Control | | 6.7 a | 5.0 | 15.4 | 12.8 |
| NPKS | | 8.2 ab | 8.0 | 12.5 | 13.4 |
| Straw/NPKS | | 35.5 c | 7.9 | 21.3 | 12.4 |
| Poultry litter | | 10.4 b | 9.4 | 13.8 | 13.2 |
| Poultry litter/macracote | | 13.7 b | 15.7 | 12.7 | 12.9 |
| +plant | | 21.1 B | 14.4 | 17.0 | 14.7 B |
| -plant | | 8.6 A | 3.9 | 13.2 | 11.2 A |
| *Two-way ANOVA* | | | | | |
| Amendments | | *** | * | n.s | n.s |
| ±plant | | *** | *** | n.s | *** |
| Amendments × ±plant | | n.s | * | n.s | n.s |
| ***Maturity*** | | | | | |
| *Interaction means* | | | | | |
| Control | + | 5.2 | 5.3 a | 16.5 | 14.8 |
| | - | 2.9 | 4.1 a | 11.9 | 13.9 |
| NPKS | + | 9.1 | 6.0 a | 22.8 | 18.7 |
| | - | 2.7 | 3.8 a | 10.6 | 11.7 |
| Straw/NPKS | + | 16.6 | 17.0 b | 22.2 | 18.0 |
| | - | 8.0 | 3.6 a | 11.4 | 12.5 |
| Poultry litter | + | 8.2 | 5.6 a | 19.3 | 15.3 |
| | - | 4.5 | 6.3 a | 11.5 | 12.1 |
| Poultry litter | + | 14.3 | 13.2 b | 24.8 | 19.1 |
| /macracote | - | 5.7 | 3.7 a | 13.0 | 11.5 |
| *Main effect means* | | | | | |
| Control | | 2.0 a | 4.7 | 14.2 | 14.4 |
| NPKS | | 5.9 ab | 4.9 | 16.7 | 15.2 |
| Straw/NPKS | | 12.3 d | 10.3 | 16.8 | 15.3 |
| Poultry litter | | 6.4 bc | 6.0 | 15.4 | 13.7 |
| Poultry litter/macracote | | 10.0 cd | 8.5 | 18.9 | 15.3 |
| +plant | | 10.7 B | 9.4 | 21.1 B | 17.2 B |
| -plant | | 4.8 A | 4.3 | 11.7 A | 12.3 A |
| *Two-way ANOVA* | | | | | |
| Amendments | | *** | n.s | n.s | n.s |
| ±plant | | *** | *** | *** | *** |
| Amendments × ±plant | | n.s | ** | n.s | n.s |

**Table 3**. Pearson's correlation coefficient ($r$) of clay dispersion against soil electrical conductivity (EC), soil pH, exchangeable sodium percentage (ESP) and cation ratio for soil structural stability (CROSS), or of EC against pH, ESP and CROSS, for soils collected beside and below the amendments at stem elongation, and at maturity, for the plus (+) and minus plant (-) treatments. *, ** and *** represent $P$ values of >0.05, <0.05, <0.01 and <0.001, respectively.

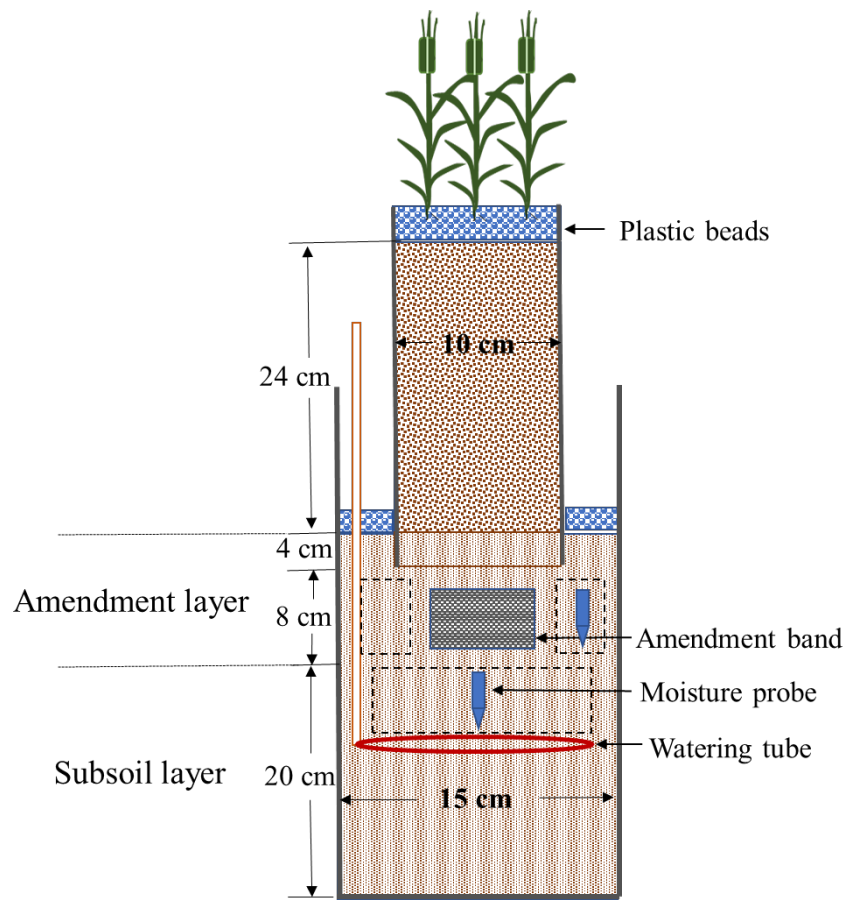| Parameter | % dispersive clay | | | | EC | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Stem elongation | | Maturity | | Stem elongation | | Maturity | |
| | + | - | + | - | + | - | + | - |
| EC | -0.93*** | -0.91*** | -0.83*** | -0.87*** | | | | |
| pH | 0.37 | 0.02 | 0.67*** | 0.82*** | -0.37 | -0.17 | -0.82*** | -0.95*** |
| ESP | 0.03 | 0.56*** | 0.05 | 0.82*** | -0.10 | -0.40* | -0.32 | -0.81*** |
| CROSS | 0.35 | 0.59*** | 0.31 | 0.89*** | -0.36 | -0.79*** | -0.08 | -0.71*** |

**Figure 1.** Diagram of the specially-designed soil column. The dashed rectangles indicate where soil samples were collected from the amendment and subsoil layer.
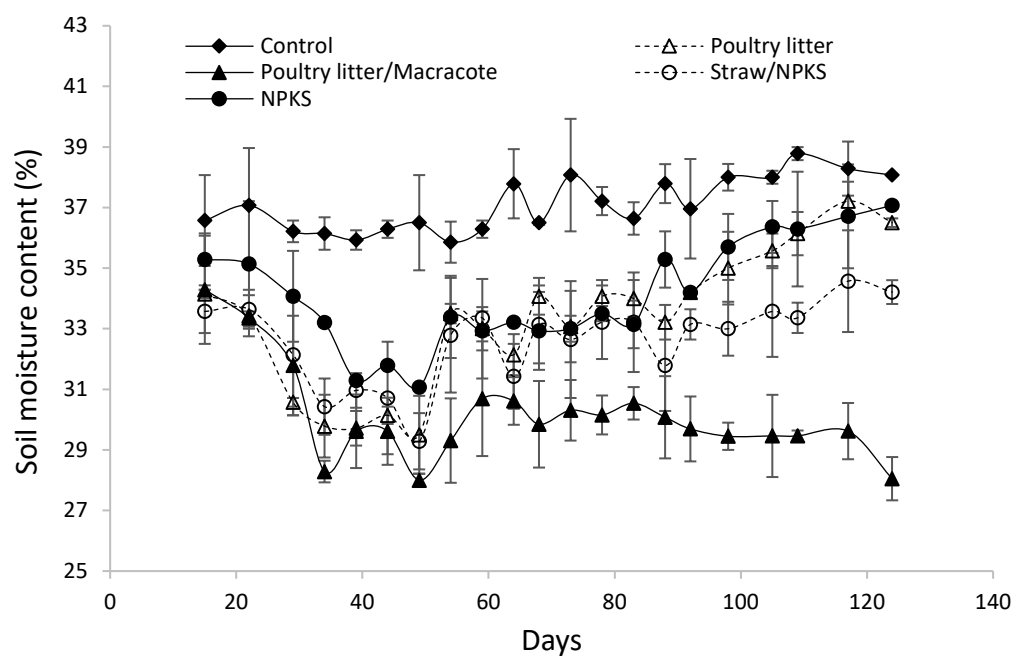
**Figure 2**. Effect of amendments on the changes in subsoil gravimetric water content over time. Measurements were made using the soil moisture probe positioned beneath the amendment bands, just prior to each watering event. Error bars represent ± the standard error of means of two replicates.
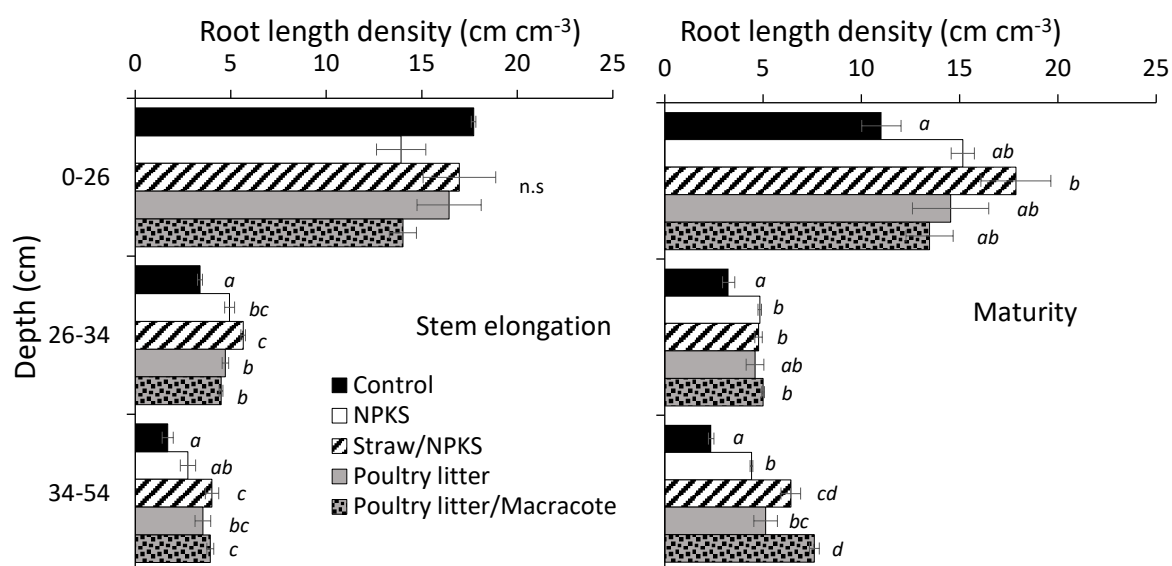
**Figure 3.** Effect of different amendments on the root length density of wheat plants in the topsoil layer (0-24 cm), the amendment layer (24-36 cm) and the subsoil (36-56 cm) at stem elongation and maturity. Different letters indicate significant differences between treatments at each depth (Tukey's test, $P<0.05$). Error bars represent means ± the standard error of means of three replicates.
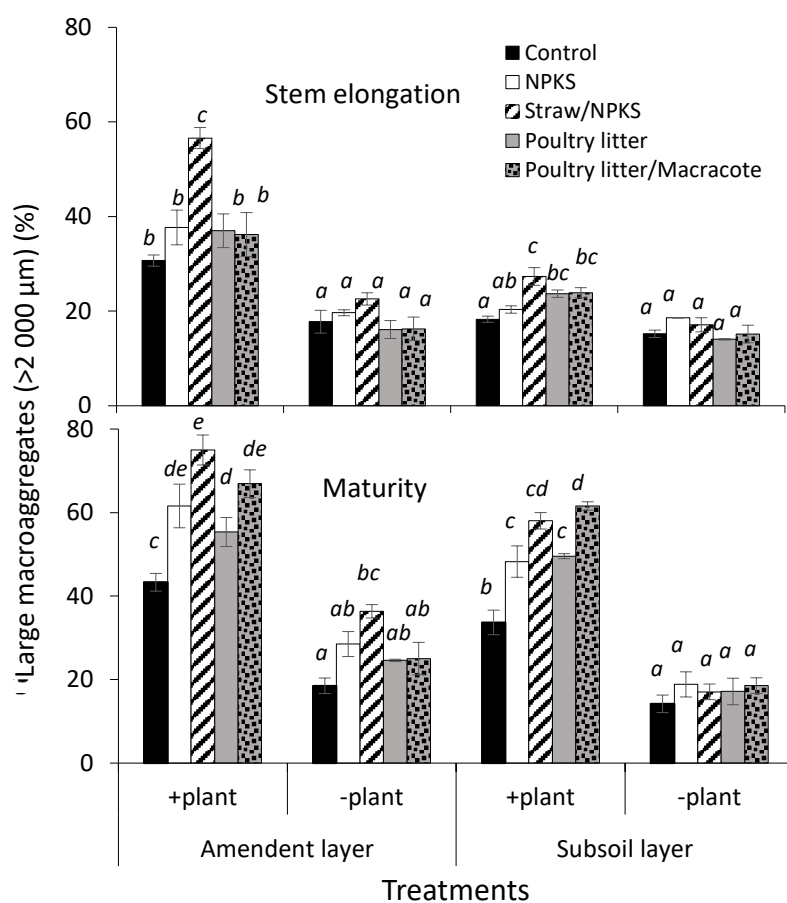
**Figure 4.** Effect of different amendments, with and without plants, on the formation of macroaggregates (>2 000 μm diameter) beside and below the amendment band, at stem elongation and maturity. Different letters indicate significant differences between different treatments at each layer (Tukey' test, $P<0.05$). Error bars represent means ± the standard error of three replicates.
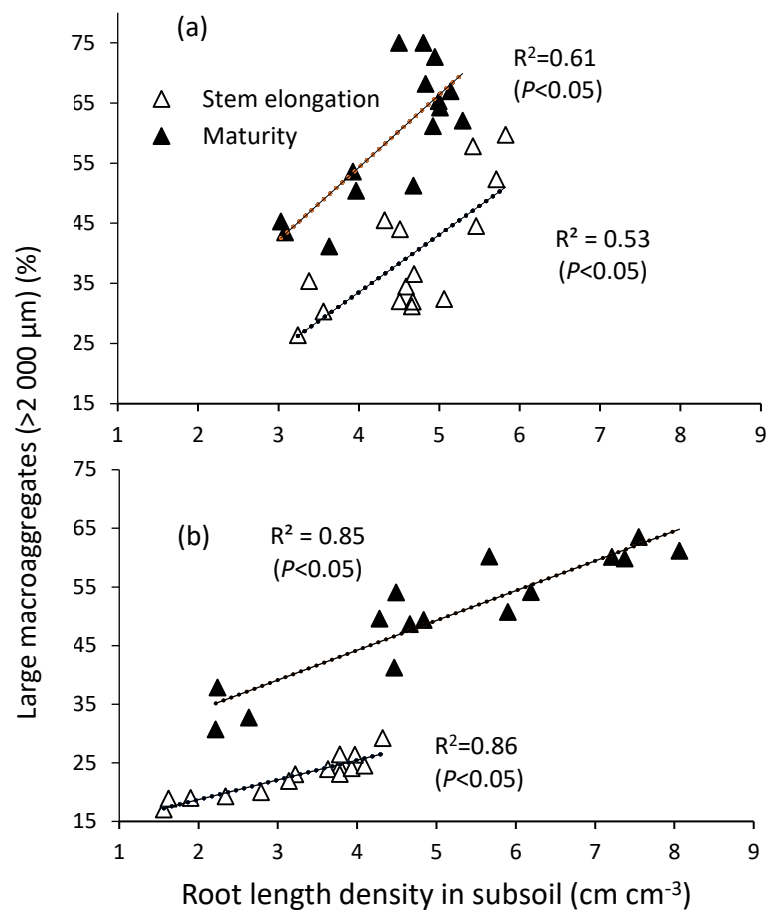
**Figure 5**. The linear relationships between the percentage of large macroaggregates (>2 mm) and root length density for soils collected from beside the amendment (a) and below the amendment (b), at stem elongation and at wheat maturity.

**Fig. 6**
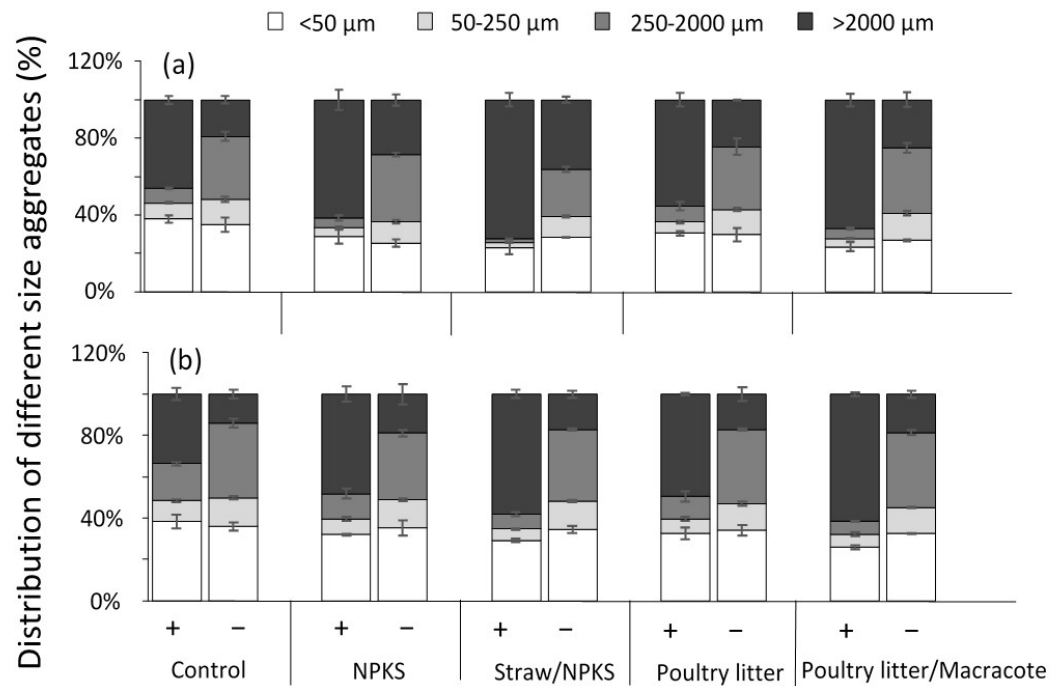
**Figure 6.** Effect of different amendments, with (+) and without (-) plants, on the distribution of aggregates among size <53 µm, 50-250 µm, 250-2000 µm and >2000 µm in soil collected from beside the amendment (a) and below the amendment (b) at the maturity. Error bars represent means ± the standard error of three replicates.

**Fig. 7**
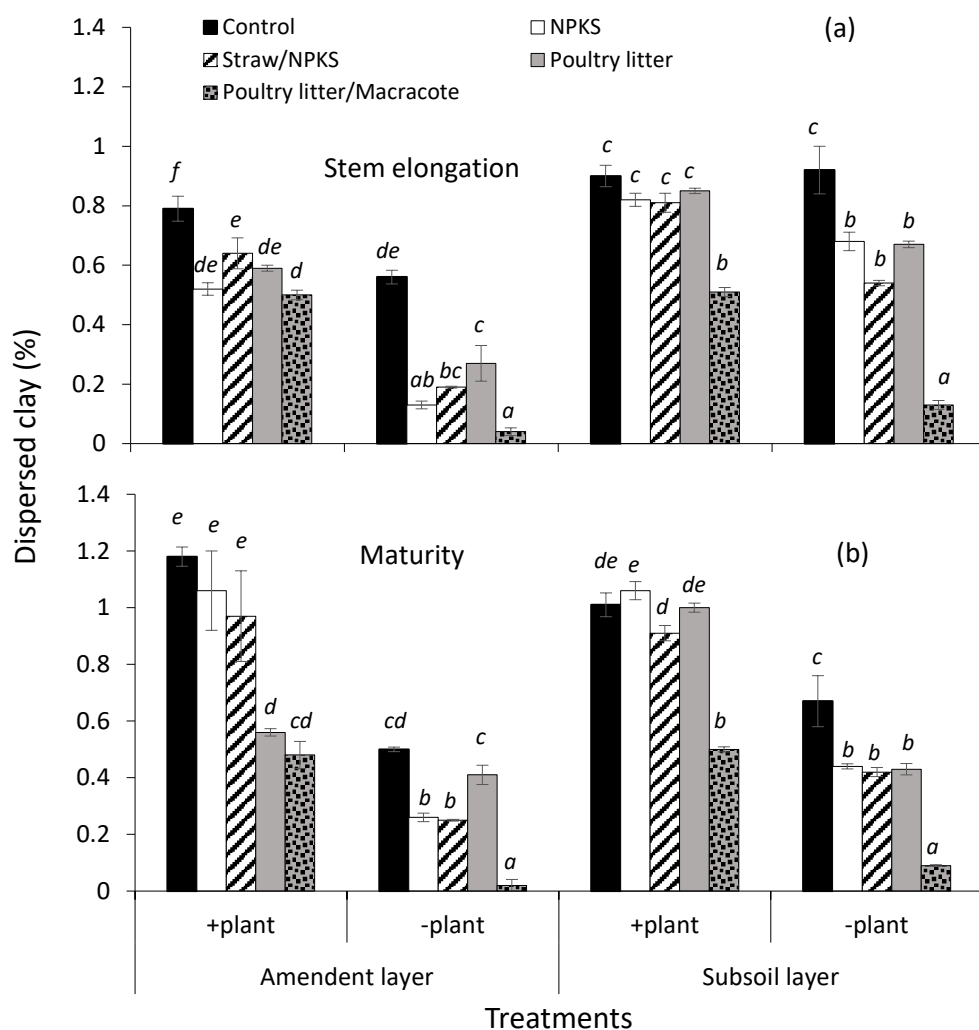
**Figure 7.** Effect of different amendments, with and without plants, on the percentage of clay dispersion of air-dried aggregates (4-6 mm) beside and below the amendment, at stem elongation (a) and crop maturity (b). Different letters indicate significant differences between different treatments at each layer (Tukey's test, $P<0.05$). Error bars represent means $\pm$ the standard error of three replicates.

**Figure 8**. The relationship between dispersive clay (%) and soil electrical conductivity (EC) (a), pH (b), exchangeable sodium percentage (ESP) (%) (c) and cation ratio for soil structural stability (CROSS) values (d), for soils collected at stem elongation and maturity from beside and below the amendments, for the plus plant (+plant) and minus plant (-plant) treatments

**Table S1.** Selected basic properties of the topsoil and subsoil used to construct soil columns.

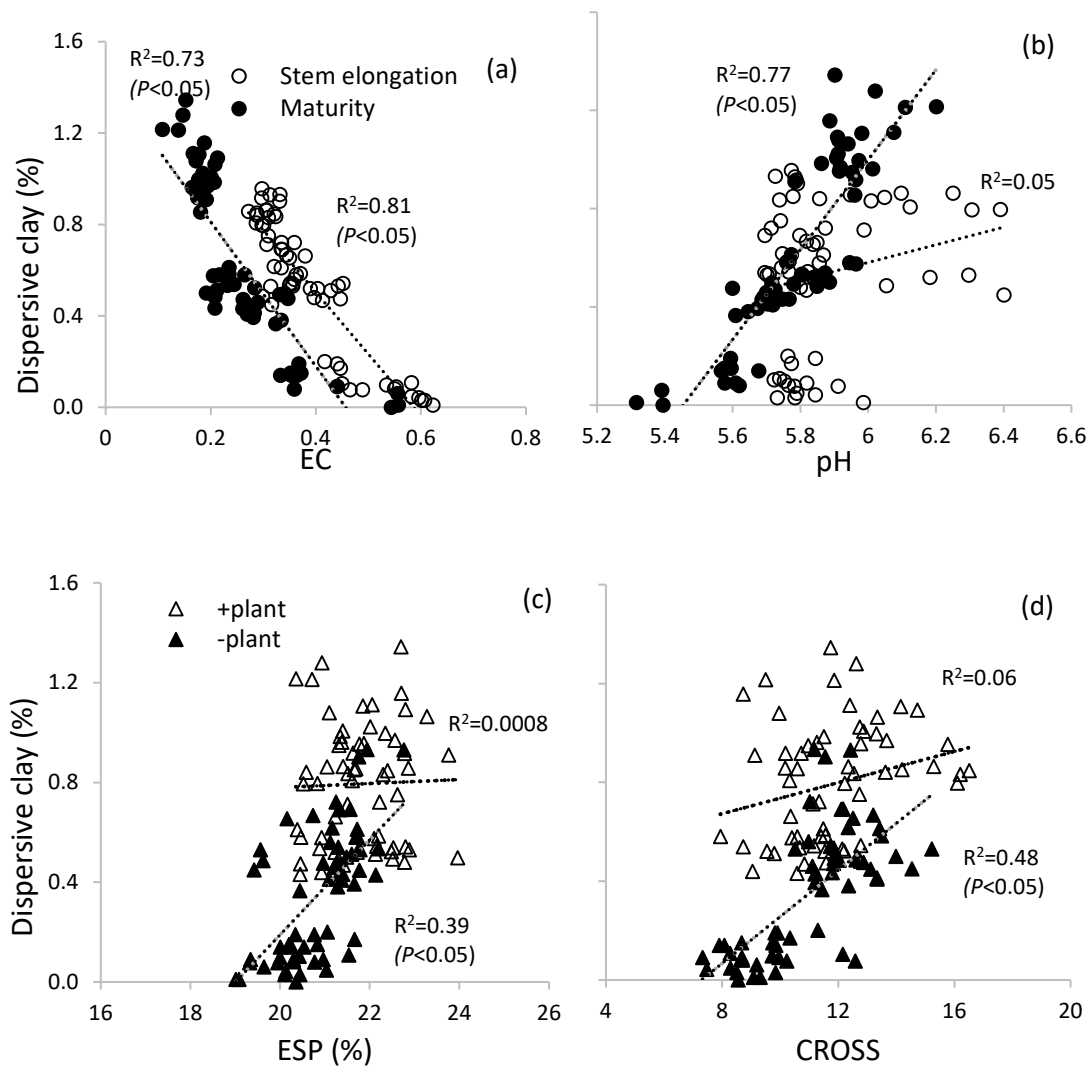| Measurement | Topsoil | Subsoil |
|---|---|---|
| Organic carbon (g kg$^{-1}$) | 44 | 8 |
| pH - 1:5 water | 5.3 | 5.8 |
| Electrical conductivity (EC) -1:5 water (dS m$^{-1}$) | 0.10 | 0.31 |
| Olsen P (mg kg$^{-1}$) | 29.9 | < 2.0 |
| Phosphate buffer index | 120 | 320 |
| Inorganic N (mg kg$^{-1}$) | 20.2 | 17.3 |
| | | |
| Exchangeable Ca (cmol$_{(+)}$ kg$^{-1}$) | 4.3 | 4.0 |
| Exchangeable Mg (cmol$_{(+)}$ kg$^{-1}$) | 1.8 | 11.0 |
| Exchangeable K (cmol$_{(+)}$ kg$^{-1}$) | 0.17 | 0.24 |
| Exchangeable Na (cmol$_{(+)}$ kg$^{-1}$) | 0.67 | 4.1 |
| Exchangeable Al (cmol$_{(+)}$ kg$^{-1}$) | 0.38 | 0.47 |
| Cation Exchange Capacity (cmol$_{(+)}$ kg$^{-1}$) | 7.3 | 19.3 |
| Exchangeable sodium percentage (ESP) | 9.2 | 21.0 |
| Clay (%) | 20.6 | 53.7 |

**Table S2.** Effect of different amendments on shoot and root biomass, grain yield and N concentration, shoot N, P and K concentration of wheat plants, and the nutrient uptake of N, P, K, Na, Ca and Mg at stem elongation (7 weeks) and at maturity (16 weeks). Different letters indicate significant differences between treatments at each growth stage (Tukey' test, $P<0.05$).

| Amendment treatment | Dry biomass (g column$^{-1}$) | | Grain yield (g column$^{-1}$) | Grain N con. (mg g$^{-1}$) | Shoot nutrient concentration (mg g$^{-1}$) | | | Above-ground nutrient uptake (mg column$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | | N | N | P | K | N | P | K | Na | Ca | Mg |
| *Stem elongation* | | | | | | | | | | | | | |
| Control | 9.9 a | 2.89 a | | | 30.0 ab | 3.02 a | 31.0 ab | 298 a | 29.8 a | 305 a | 5.7 a | 26.5 a | 22.9 a |
| NPKS | 15.4 b | 3.22 a | | | 42.4 c | 4.10 b | 31.8 b | 654 d | 63.1 b | 491 c | 8.9 c | 34.8 b | 37.5 bc |
| Straw/NPKS | 19.0 c | 4.05 c | | | 31.8 b | 4.48 b | 30.2 ab | 602 c | 85.1 c | 589 d | 7.4 b | 41.3 c | 42.4 c |
| Poultry litter | 15.2 b | 3.79 b | | | 28.4 a | 4.69 b | 27.4 a | 430 b | 71.0 bc | 415 b | 7.6 b | 31.0 b | 31.8 b |
| Poultry litter/macracote | 20.0 c | 3.87 b | | | 44.1 c | 5.72 c | 34.2 c | 838 e | 108.9 d | 652 e | 9.5 c | 34.9 b | 46.2 d |
| | | | | | | | | | | | | | |
| *Maturity* | | | | | | | | | | | | | |
| Control | 44.7 a | 2.71 a | 19.2 a | 17.7 a | 2.6 a | 0.25 a | 14.6 a | 393 a | 66.6 a | 417 a | 15.2 a | 57.4 a | 62.5 a |
| NPKS | 68.0 c | 3.46 b | 27.1 bc | 21.6 b | 3.7 b | 0.31 b | 15.2 ab | 752 d | 121.1 b | 670 b | 23.8 b | 79.5 bc | 110.9 c |
| Straw/NPKS | 75.5 d | 4.81 c | 28.8 c | 18.4 a | 2.8 a | 0.34 b | 15.9 ab | 657 c | 117.1 b | 805 c | 79.6 c | 89.4 c | 106.4 c |
| Poultry litter | 60.2 b | 3.69 b | 24.9 b | 18.4 a | 2.6 a | 0.84 c | 20.7 c | 551 b | 120.3 b | 788 bc | 33.9 b | 75.7 b | 90.3 b |
| Poultry litter/macracote | 96.4 e | 4.52 c | 38.9 d | 21.2 b | 3.8 b | 0.87 c | 18.0 bc | 1045 e | 195.0 c | 1123 d | 32.5 b | 105.5 d | 151.8 d |

**Table S3.** Effect of different amendments, with (+) and without (-) plants, on soil pH, electrical conductivity (EC), inorganic N, extractable organic C (EOC), exchangeable sodium percentage (ESP) and cation ratio for soil structural stability (CROSS) of soils collected beside and below the amendments at stem elongation. Different lower-case letters indicate significant differences between interaction means, while different upper-case letters indicate significant differences between main effects means, using Tukey's HSD test. n.s, *, ** and *** represent P values of >0.05, <0.05, <0.01 and <0.001, respectively.

| Amendments | Plant | pH | | EC (1:5 water) ($\mu s\ cm^{-1}$) | | Inorganic N ($mg\ kg^{-1}$) | EOC ($mg\ kg^{-1}$) | ESP (%) | | CROSS | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Beside | Below | Beside | Below | Beside | Beside | Beside | Below | Beside | Below |
| *Interaction means* | | | | | | | | | | | |
| Control | + | 6.02 | 5.73 *ab* | 292 *ab* | 216 | 1.9 *a* | 152 *ab* | 21.9 | 22.5 | 12.5 | 13.2 |
| | - | 6.25 | 5.67 *a* | 229 *a* | 245 | 36.0 *b* | 137 *a* | 21.2 | 22.1 | 12.4 | 12.1 |
| NPKS | + | 5.73 | 5.97 *c* | 416 *cd* | 222 | 17.8 *ab* | 160 *ab* | 21.4 | 21.8 | 9.9 | 12.5 |
| | - | 5.79 | 5.72 *ab* | 587 e | 251 | 227.3 *e* | 142 *ab* | 20.9 | 21.3 | 10.6 | 10.7 |
| Straw/NPKS | + | 5.85 | 5.79 *ab* | 364 *bc* | 215 | 2.9 *a* | 168 *bc* | 21.9 | 21.6 | 8.8 | 11.7 |
| | - | 5.78 | 5.68 *a* | 546 e | 277 | 177.3 *d* | 148 *ab* | 20.1 | 21.1 | 10.3 | 11.4 |
| Poultry litter | + | 5.83 | 5.89 *bc* | 356 *bc* | 232 | 2.0 *a* | 186 *c* | 21.2 | 22.0 | 10.7 | 12.8 |
| | - | 5.81 | 5.78 *ab* | 468 de | 262 | 100.4 *c* | 162 *bc* | 19.9 | 21.6 | 11.5 | 11.3 |
| Poultry litter | + | 5.83 | 5.80 *ab* | 411 *cd* | 415 | 9.0 *ab* | 185 *c* | 21.8 | 22.3 | 10.9 | 10.4 |
| /macracote | - | 5.83 | 5.79 *ab* | 754 f | 435 | 212.6 *e* | 156 *ab* | 19.9 | 21.2 | 10.5 | 9.9 |
| | | | | | | | | | | | |
| *Main effects means* | | | | | | | | | | | |
| Control | | 6.14 *B* | 5.70 | 261 | 231 *A* | 19.0 | 145 | 21.6 | 22.3 | 12.5 *B* | 12.7 *B* |
| NPKS | | 5.76 *A* | 5.85 | 502 | 237 *A* | 122.6 | 151 | 21.2 | 21.6 | 10.3 *A* | 11.6 *B* |
| Straw/NPKS | | 5.82 *A* | 5.74 | 455 | 246 *A* | 90.1 | 158 | 21.0 | 21.4 | 9.6 *A* | 11.6 *B* |
| Poultry litter | | 5.82 *A* | 5.84 | 412 | 248 *A* | 51.2 | 174 | 20.6 | 21.8 | 11.1 *AB* | 12.1 *B* |
| Poultry litter/Macracote | | 5.83 *A* | 5.80 | 583 | 425 *B* | 110.8 | 171 | 20.9 | 21.8 | 10.7 *A* | 10.2 *A* |
| | | | | | | | | | | | |
| +plants | | 5.85 | 5.84 | 368 | 260 *A* | 6.7 | 170 | 21.6 *B* | 22.0 | 10.6 | 12.1 |
| -plants | | 5.89 | 5.73 | 517 | 294 *B* | 150.7 | 149 | 20.4 *A* | 21.5 | 11.1 | 11.1 |
| | | | | | | | | | | | |
| *Two-way ANOVA* | | | | | | | | | | | |
| Amendments | | *** | *** | *** | *** | *** | *** | n.s | n.s | ** | *** |
| ±plant | | n.s | ** | *** | *** | *** | *** | *** | n.s | n.s | n.s |
| Amendments × ±plant | | n.s | * | *** | n.s | *** | * | n.s | n.s | n.s | n.s |

**Table S4**. Effect of different amendments, with (+) and without (-) plants, on soil pH, electrical conductivity (EC), inorganic N, extractable organic C (EOC), exchangeable sodium percentage (ESP) and cation ratio for soil structural stability (CROSS) of soils collected from beside and below the amendments at maturity. Different lower-case letters indicate significant differences between interaction means, while different upper-case letters indicate significant differences between main effects means, using Tukey's HSD test. n.s, *, ** and *** represent P values of >0.05, <0.05, <0.01 and <0.001, respectively.

| Amendments | ± plant | pH Beside | pH Below | EC ($\mu$s cm$^{-1}$) Beside | EC Below | Inorganic N (mg kg$^{-1}$) Beside | EOC (mg kg$^{-1}$) Beside | ESP (%) Beside | ESP Below | CROSS Beside | CROSS Below |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Interaction means* | | | | | | | | | | | |
| Control | + | 6.13 *f* | 5.90 *d* | 138 *a* | 202 | 0.4 *a* | 171 *a* | 21.0 *abc* | 22.0 *ab* | 12.4 | 12.6 |
| | - | 5.77 *cd* | 5.78 *c* | 204 *bc* | 234 | 31.4 *b* | 166 *a* | 21.5 *bc* | 21.9 *ab* | 12.6 | 12.4 |
| NPKS | + | 5.86 *de* | 5.97 *d* | 224 *c* | 188 | 1.6 *a* | 178 *a* | 23.1 *d* | 22.5 *ab* | 10.4 | 10.6 |
| | - | 5.62 *bc* | 5.73 *bc* | 358 *d* | 253 | 96.9 *c* | 181 *a* | 20.6 *ab* | 21.3 *a* | 9.8 | 11.6 |
| Straw/NPKS | + | 5.98 *e* | 5.96 *d* | 161 *ab* | 176 | 2.9 *a* | 190 *ab* | 21.1 *bc* | 21.5 *ab* | 9.3 | 10.0 |
| | - | 5.58 *b* | 5.69 *b* | 357 *d* | 273 | 87.9 *c* | 194 *ab* | 20.2 *ab* | 21.9 *ab* | 9.8 | 11.2 |
| Poultry litter | + | 5.93 *e* | 5.91 *d* | 222 *c* | 194 | 0.7 *a* | 202 *bc* | 22.1 *cd* | 22.0 *ab* | 10.1 | 12.1 |
| | - | 5.62 *bc* | 5.72 *bc* | 335 *d* | 272 | 77.4 *c* | 224 *cd* | 20.6 *ab* | 21.2 *a* | 10.7 | 12.4 |
| Poultry litter | + | 5.73 *cd* | 5.78 *c* | 257 *c* | 319 | 4.0 *a* | 286 *e* | 20.6 *ab* | 23.0 *b* | 10.9 | 11.2 |
| /macracote | - | 5.37 *a* | 5.60 *a* | 552 *e* | 414 | 211.1 *d* | 238 *d* | 19.7 *a* | 21.0 *a* | 8.9 | 9.6 |
| | | | | | | | | | | | |
| *Main effects means* | | | | | | | | | | | |
| Control | | 5.95 | 5.84 | 171 | 218 *A* | 15.9 | 169 | 21.3 | 22.0 | 12.5 *B* | 12.5 *B* |
| NPKS | | 5.74 | 5.85 | 291 | 221 *A* | 49.3 | 180 | 21.9 | 21.9 | 10.1 *A* | 11.1 *A* |
| Straw/NPKS | | 5.78 | 5.83 | 259 | 225 *A* | 45.4 | 192 | 20.7 | 21.7 | 9.6 *A* | 10.6 *A* |
| Poultry litter | | 5.78 | 5.82 | 279 | 233 *A* | 39.1 | 213 | 21.4 | 21.6 | 10.4 *A* | 12.3 *B* |
| Poultry litter/macracote | | 5.55 | 5.69 | 405 | 367 *B* | 107.6 | 262 | 20.2 | 22.0 | 9.9 *A* | 10.4 *A* |
| | | | | | | | | | | | |
| +plants | | 5.93 | 5.90 | 200 | 216 *A* | 1.9 | 205 | 21.6 | 22.2 | 10.6 | 11.3 |
| -plants | | 5.59 | 5.70 | 361 | 289 *B* | 100.9 | 201 | 20.5 | 21.5 | 10.4 | 11.4 |
| | | | | | | | | | | | |
| *Two-way ANOVA* | | | | | | | | | | | |
| Amendments | | *** | *** | *** | *** | *** | *** | *** | n.s | ** | *** |
| ±plant | | *** | *** | *** | *** | *** | * | *** | ** | n.s | n.s |
| Amendments × ±plant | | * | ** | *** | n.s | *** | *** | ** | * | n.s | n.s |