https://doi.org/10.5194/egusphere-2022-469
Preprint. Discussion started: 27 September 2022

Wetting and drying cycles, organic matter and gypsum play a key role in structure formation and stability of sodic Vertisols

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Abstract

In the natural environment, soils undergo wetting and drying (WD) cycles due to precipitation and evapotranspiration. The WD cycles have a profound impact on soil physical, chemical, and biological properties and drive the development of structure in soils. Degraded soils are often lacking structure and the effect of organic amendments and WD cycles on structure formation of these soils is poorly understood. The aim of this study was to evaluate the role of biotic and abiotic factors on aggregate formation and stabilisation of sodic soils after the addition of gypsum and organic amendments (feedlot manure, chicken manure, lucerne pallets, and anionic poly acrylamide). Amended soils were incubated at 25°C over four WD cycles, with assessment of soil microbial respiration, electrical conductivity, pH, sodium adsorption ratio (SAR), aggregate stability in water (ASWAT), aggregate size distribution, and mean weight diameter. Our results demonstrate that WD cycles can improve aggregate stability after the addition of amendments in sodic Vertisols, but this process depends on the type of organic amendment. Lucerne pellets resulted in highest soil microbial respiration, proportions of large macroaggregates, and mean weight diameter. In contrast, dispersion was significantly reduced when soils were treated with chicken manure, whilst PAM only had a transient effect on aggregate stability. When these organic amendments were applied together with gypsum, the stability of aggregates was further enhanced, and dispersion became negligible after the second WD cycle. The formation and stability of small macroaggregates was less dependent on the type of organic amendments and more dependent on WD cycles as the proportion of small macroaggregates also increased in control soils after four WD cycles, highlighting the role of WD cycles as one of the key factors that improves aggregation and stability of sodic Vertisols.

Key words: Sodic soils, organic amendments, aggregate stability, mean weight diameter, wetting and drying cycles

1 Introduction

Soils are subjected to seasonal and daily variations of water and temperature. These variations effect the physical, biological, and chemical properties of soils. Natural variations in soil water content can lead to wetting and drying (WD) cycles, which are affected by rainfall, solar radiation, capillarity, wind and condensation (Utomo and Dexter 1982) and evapotranspiration. In most terrestrial ecosystems, surface soils experience rapid changes in soil water content with a longer dry period followed by a relatively rapid re-wetting (Borken and Matzner 2009). Southern Queensland (Australia) usually has hot wet summers with cool dry winters, and soils of this region and other semi-arid areas are particularly susceptible to drying and re-wetting stresses due to the infrequency of rainfall. In the field, most soils experience more than one WD cycle throughout the year. These WD cycles have a substantial influence on soil aggregation and structure stabilisation because of its direct effect on hydration of minerals, and indirect effect on soil microbial activity (Denef *et al.* 2001; Cosentino *et al.* 2006).

Soil aggregation is an important mechanism for stabilisation of soil organic matter (Six *et al.* 2000a). Furthermore, it also supports soil fertility as it reduces soil erosion and controls soil aeration, water infiltration, hydraulic conductivity and nutrient cycling (Oades 1984; Six *et al.*

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2000b). Soil aggregation is caused by various aggregate stabilising compounds, which work together at different spatial scales (Tisdall and Oades 1982). Aggregate formation also depends on soil microbial activity as it influences the production of binding materials such as microbial exudates and hyphae (Rahman *et al.* 2017). Aggregate stability often exhibits seasonal and inter-annual variability that is controlled mainly by rainfall, temperature, humidity, insolation, and organic matter (Perfect *et al.* 1990).

Aggregation is important to rehabilitate sodic soils. Sodic soils are generally characterised as soils with poor structure which makes those soils difficult to work when wet or dry (Rengasamy and Olsson 1991). Poor structural stability of sodic soils restricts seedling emergence and root growth which directly limits crop growth and development, and indirectly affects plant nutrition by limiting water infiltration, nutrient uptake, and gaseous exchange (Curtin and Naidu 1998). Rehabilitation of sodic soils requires an understanding of the complex interactions between biological and physico-chemical factors that contributes to soil structure formation and stability (Oades 1993; Nelson and Oades 1998).

In soils containing shrink-swell (smectitic) clays WD cycles can lead to more intensive changes in soil structure as they can affect aggregation process directly through physical processes (Utomo and Dexter 1982; Denef *et al.* 2001). However, there is no consensus in the literature regarding the role of WD cycles in improving soil aggregation and its stability. For example, Rahman *et al.* (2018) reported that WD cycles improved (increased) the mean weight diameter (MWD, a proxy of aggregate stability measurement) of Vertisols when treated with maize straw after four WD cycles. In a similar manner, WD cycles also increased the proportion of large macro aggregates of smectite Vertisols (Bravo-Garza *et al.* 2009). However, in contrast, Peng *et al.* (2011), while comparing swelling and non-swelling soils, reported that WD cycles decreased the MWD of swelling soils but not of non-swelling soils. Due to the physical disturbance caused by WD cycles, Six *et al.* (2000b) found that repeated WD cycles decreased

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the MWD, with this being related to the loss of soil organic matter. These apparently contradictory results can potentially be explained on the basis of the initial conditions of the soil, such as the physical conditions of aggregates, organic matter content and quality, and the intensities and duration of the WD cycles (Cosentino *et al.* 2006).

It is well established that the addition of organic matter effects aggregate stability within a period of days to weeks due to the stimulation of microbial activity (Six *et al.* 2004), depending upon the quality and quantity of organic matter (Monnier, 1965 cited in Abiven *et al.* (2009)). Studies investigating the effects of WD cycles on microbial activity were inconclusive, with results differing due to varying experimental designs, incubation period and temperatures, soil properties, and treatments applied. Xiang *et al.* (2008) found that multiple WD cycles increased the microbial respiration of grassland soils up to 6-fold when compared to the soil that remained wet. Drying and rewetting of these soils gave a new pulse of respiration with each WD cycle, but when these soils were kept at constant water content, microbial respiration decreased to almost zero. In contrast, however, Rahman *et al.* (2018) reported that repeated WD cycles in Vertisols decreased soil respiration significantly but that the magnitude of this decrease became smaller over the time.

Although the interaction of biotic and abiotic factors and its effect on aggregate stability and formation is complex and inconsistent, little effort has been put into studying the underlying mechanisms, particularly in sodic Vertisols. Furthermore, there is little information available on the relationship between aggregate formation and stability following repeated WD cycles after addition of gypsum and organic amendments. Thus, in the present study we used two sodic Vertisols, with the aim to: i) investigate the effect of WD cycles on microbial respiration, ii) assess the effects of WD cycles on aggregate formation and stability, and iii) determine how many WD cycles are needed to improve aggregate stability. We hypothesised that i) organic amendments enhance the formation of large aggregates and their stability increase with WD cycles, and ii) the addition of gypsum reduces dispersion and aggregate stability is enhanced with the introduction of WD cycles compared with a continuous wet regime.

2 Materials and methods

2.1 Soils

The two soils used in this experiment were collected from a farm located in southern Queensland near Goondiwindi (28.54° S, 150.30° E), Australia. The soils were being cropped using a maize (*Zea mays*) and wheat (*Triticum aestivum*) rotation system. Both soils were classified as sodic Vertisols according to the FAO-World Reference Base (2015) (Vertosols in the Australian Soil Classification). Soils were collected to a depth of 10 cm at two locations from the cropped land. These sites were selected as they were in close proximity to each other but were slightly different in regard to their physical behaviour (Table 1) with Soil 1 being more dispersive than Soil 2. Soil cores for bulk density determinations were also collected separately from each site and dried at 105°C for several days. After collection, soils were air dried, and the larger clods gently broken into smaller aggregates by hand. The soils were then passed through a 10 mm sieve to remove stones, visible roots, and plant litter. For chemical analysis, a portion of each soil was sieved to <2 mm. Soil pH (ISO, 2005) and electrical conductivity (EC) (ISO, 1994) were measured in 1:5 soil: water suspensions. Particle size analysis was performed using the pipette method (Day, 1965), and field water capacity (-10 kPa) was measured using a pressure plate apparatus (Cassel and Nielsen, 1986). Exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}), and effective cation exchange capacity were determined after pre-washing with 60% ethanol, and then leaching with non-alcoholic 1 M NH_4Cl solution at pH 7 (Tucker, 1985). The exchangeable sodium percentage was calculated as exchangeable Na concentration divided by the effective cation exchange capacity. Total organic C, and total N analyses were conducted using a LECO TruMac instrument using the Dumas method (Nelson and Sommers, 1996). The electrochemical stability index was used as an indication of the potential for soil dispersion. The threshold electrochemical stability index below which structural breakdown occurs is 0.05 (McKenzie, 1998). The dispersion index (DI), another measure of soil structural stability, is defined as the amount of dispersed silt+clay expressed as a percentage of the total silt+clay of the soil (see section 2.3) (Mustafa and Letey, 1969). Soil dispersion assessed by aggregate stability in water (ASWAT) is described in detail in Section 2.3.

Table 1 *Physico-chemical properties of soil samples*

	Soil 1	Soil 2
pH (1:5 water)	7.07	7.03
EC (1:5 dS m^{-1})	0.26	0.22
Sand (%)	34	37
Silt (%)	17	17
Clay (%)	49	46
Bulk density (g cm^{-3})	1.29	1.29
Field capacity (%)	33	29
Total organic C (g kg^{-1})	8.8	9.8
Total N (g kg^{-1})	0.90	0.90
Effective cation exchange capacity (cmol$^{(+)}$ kg^{-1})	23	24
Exchangeable sodium percentage	15	16
Electrochemical stability index	0.02	0.01
ASWAT	15	11
DI (%)	48	28

2.2 Collection of different organic materials used as amendments

Four different organic amendments were used for this study, viz. feedlot manure (FLM), chicken manure (CM), lucerne pellets (LP), and polyacrylamide (PAM). The FLM, and CM were collected from cattle, and chicken sheds, respectively, located at The University of Queensland (Gatton, Australia) before being air dried, whilst the LPs were a commercial animal feed product. All organic amendments were ground and sieved through a 0.5 mm sieve in order to minimise the effect of different size particles and to create a homogenised sample. Anionic PAM Flobond L33 liquid (SNF Australia) had a charge density of 30% and the molecular weight was 12-15 million Dalton (as per product specifications). Gypsum ($CaSO_4.2H_2O$) was laboratory grade obtained from Sigma Aldrich.

A chemical analysis of the organic amendments was performed before mixing with the soils (Table 2). Total C and N analysis were performed using the LECO TruMac instrument with the Dumas method (Nelson and Sommers, 1996). The major cations, including Na, Ca, Mg, and K, were quantified by inductively coupled plasma-optical emission spectrometry (ICP-OES) after nitric acid microwave digestion (Kovács et al., 1996). The pH and EC of organic amendments were measured in (1:5) water suspensions after shaking for 1 h.

***Table 2** Chemical properties of organic amendments*

	pH (1:5)	EC (1:5) (dS m^{-1})	TC (%)	TN (%)	C:N	Ca (g kg^{-1})	Na (g kg^{-1})	Mg (g kg^{-1})	K (g kg^{-1})
FLM	8.4	3.0	14.9	1.4	10:1	18.1	4.2	12.8	12.5
CM	8.2	10.7	26.0	2.6	10:1	179.0	4.7	9.7	29.7
LP	5.6	5.7	43.8	3.1	14:1	13.8	2.9	3.7	12.8
PAM			49.7	0.9	56:1				

2.3 Incubation experiment

An incubation experiment was conducted using a complete randomised design. The treatments consisted of the five amendments (gypsum (G), PAM, FLM, CM, and LP) plus an unamended control. In addition, the FLM, CM, LP, and PAM were also applied in combination with G in order to check for synergistic effects between gypsum and organic amendments. This yielded a total of 10 treatments, each with three replicates, being a total of 60 experimental units. Soil samples (300 g) were mixed with the respective amendments (Table 3) added at rates that were commercially feasible (10 Mg ha^{-1}). The gypsum requirement of both soil samples was

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calculated based on the formula given by Oster and Jayawardane (1998). The soil water content at field capacity (-10 kPa) was ~ 0.30 g water g^{-1} soil. The WD cycles were imposed as follows. First deionised water was added to the soils (0.30 g g^{-1} for Soil 1 and 0.31 g g^{-1} for Soil 2) at 25°C, and after 1 d, the lids were removed and soils were allowed to dry at 25°C during which the soil water content decreased to air-dry conditions (~0.1 g water g^{-1} soil). The dry-down typically completed in 14 d. The WD regime was applied four times with the total duration of the experiment being 60 d. Although the WD regime might not entirely represent the field conditions due to lack of plant growth, it is representative of the mean temperature and the field water content.

Table 3 *Treatments and their application rates*

	Treatments	Application rates
1	Control	No amendment
2	G	2.5 Mg ha^{-1}
3	FLM	10 Mg ha^{-1}
4	CM	10 Mg ha^{-1}
5	LP	10 Mg ha^{-1}
6	PAM	1 kg ha^{-1}
7	G + FLM	2.5 Mg ha^{-1}+ 10 Mg ha^{-1}
8	G + CM	2.5 Mg ha^{-1} + 10 Mg ha^{-1}
9	G + LP	2.5 Mg ha^{-1} + 10 Mg ha^{-1}
10	G + PAM	2.5 Mg ha^{-1} + 1 Mg ha^{-1}

Soil solutions (~3-5 ml) were extracted using polyacrylonitrile hollow fibre samplers (Menzies and Guppy, 2000) embedded in the jars containing the treated soils. Soil solution was collected four times at the start of each WD cycle after the water was added to soils. Soil solution pH, EC, NH_4^+-N, NO_3^--N, dissolved organic carbon (DOC), Na, Ca, Mg, and K were measured as follows: NH_4^+-N by the phenate method (Rice et al., 2017), NO_3^--N by cadmium reduction (Rice et al., 2017) using a segmented flow analyser (SEAL AA3), DOC using a total organic carbon liquid analyser (Shimadzu, Japan), and the cations using ICP-OES. The sodium adsorption ratio (SAR) was calculated from the soil solution concentrations of Na, Ca, and Mg.

Soil dispersion was determined using the ASWAT test (Field *et al.* 1997) and DI (Mustafa and Letey 1969). Air dried aggregates from each sample after completion of each cycle were placed in a Petri dish filled with deionised water. A visual assessment of aggregate dispersion was made after 10 min and 2 h, assessing dispersion on a scale of 0-4. The scores were as follows: 0: no dispersion, 1: slight dispersion (slight milkiness adjacent to aggregates in water), 2: moderate dispersion (obvious milkiness), 3: strong dispersion (considerable milkiness with about half of the material dispersed in water), and 4: complete dispersion (leaving sand particles in a cloud of dispersed clay). Dispersion scores that were determined after 10 min and 2 h were added together and then added to 8, thus giving a range of values from 9-16. Aggregates which were not dispersed after 2 h were remolded, submerged in water and dispersion reassessed again after 10 min and 2 h. Scores were given from 0-4 for both times and added (giving values from 0-8). Thus, aggregates were considered stable if they had a low ASWAT score, and the higher the ASWAT score, the more unstable the aggregate.

For the measurement of DI, 15 g of air-dry soil (< 2 mm) was placed in a sedimentation cylinder with an approximate volume of 1.2 L. Deionised water was added to the cylinder to bring the volume to a 1 L. The suspension was then shaken for 30 min on an end-over-end shaker. The suspension was then stirred with 10 strokes of a plunger. The temperature was noted, the suspension was allowed to settle, and after an appropriate time (8 h) the percentage of clay was measured with hydrometer. The measurements were done after the first, second, and fourth WD cycles. Results were expressed as DI (Mustafa and Letey 1969):

$$DI(\%) = 100 \times \frac{silt+clay\ (easily\ dispersed,\%)}{silt+clay(particle\ size\ analysis,\%)} \qquad (1)$$

Aggregate size distribution was also determined to quantify changes in large and small

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aggregates with WD cycles. Air dried soil samples (25 g) were broken gently to pass through a 10 mm sieve and then slowly wetted up in water for 5 min (Hernandez *et al.* 2017). They were then wet sieved for 5 min at 33 oscillations per minute (Cook *et al.* 1992) using a set of sieves: 2000 µm, 250 µm and 53 µm (Kemper and Rosenau 1986). Three replications were made of each sample. The water level was adjusted so that the aggregates on the upper sieve were submerged in water at the highest point of the oscillation. The material retained on each sieve and the fraction passing through after wet sieving was collected, dried at 40 °C, and weighed. (Kemper and Rosenau 1986). Measurements were made after the first, second, and fourth WD cycles. The >2000 µm aggregates hereafter are referred to as large macroaggregates, 2000-250 µm as small macroaggregates, 250-53 µm as micro-aggregates (MIC), and <53 µm as silt+clay fraction. The MWD was calculated from the aggregate fractions obtained after wet sieving on each sieve size, as follows:

$$MWD = \sum_{i=1}^{n} x^i w^i \qquad (2)$$

Where x^i is the mean diameter of each fraction and w^i is the proportion of the total sample weight in the corresponding size fraction (Kemper and Rosenau 1986).

2.4 Soil microbial respiration

The biolability (susceptibility to microbial decomposition) of the organic amendments was determined by measuring CO_2 release over a 60 d incubation period comprising four WD cycles. Briefly, 50 g of each soil sample was added to 250 mL jars with the relevant amendments (Table 3) and covered with lids having two 5 mm holes. All the treatments were replicated three times and randomised. The soils were wetted to field capacity on the first day of incubation using deionised water and incubated at 25 °C. After the first day of soil incubation, the jars were left open to allow the soil to dry at 25 °C. For the microbial respiration measurements, rubber tubes with Luer locks were inserted into the holes and connected to a CO_2 analyser (WMA-4 CO_2 analyzer, John Morris USA). The lids were kept closed for 1 h

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prior to the measurement being taken. The readings were then converted in g C-CO_2 kg^{-1} of soil d^{-1}. Cumulative CO_2 produced was calculated as the sum of the daily rate and the interval days between the two measurements (by linear extrapolation) for the incubation period. The measurements were taken daily for the first 7 d. Thereafter, there was no significant cahnge in microbial respiration, so the measurements were performed after 10 d and 15 d of each WD cycle.

2.5 Statistical analysis

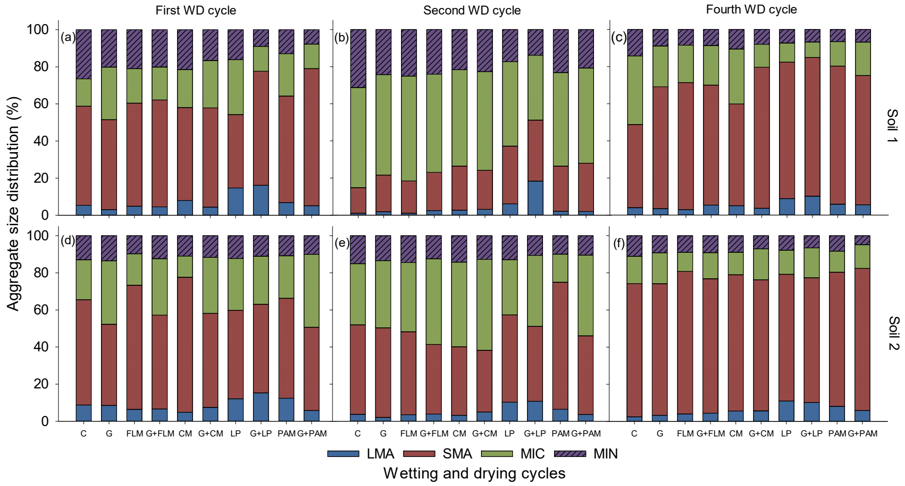
Statistical analyses were performed using R x 64 3.3.3 statistical software (R Core Team, 2020). Histograms were plotted to check the normality of each parameter separately and found that transformation of data was not necessary. Soil microbial respiration was recorded on a daily basis hence respiration data were subjected to analysis of variance with days as the repeated measures (mixed effect model). All the remaining parameters including EC, pH, NH^+_4-N and NO^-_3-N, SAR, ASWAT, DI, aggregate size distribution, and MWD were measured after each WD cycles. Hence, these data were analysed using a two-way analysis of variance (ANOVA) taking treatments and WD cycles as two factors. The ANOVA for each soil was conducted separately using general linear model. Tukey's honest significant difference was used for pairwise comparisons between treatment means. After checking the significance of data, mean data were graphed using Sigma Plot v14.0 (Systat Software Inc., 2017). Principal component analyses (PCA) were performed to identify the roles of microbial, chemical and physical properties after the addition of different treatments in each WD cycle.

3 Results

3.1 Soil aggregation dynamics measured by aggregate size distribution and mean weight diameter

Aggregate size distribution was significantly ($p<0.001$) affected by amendments and WD cycles in both soils except for the proportion of large macroaggregates and silt+clay fraction in Soil 2 (Fig. 1), although the main effects of treatments and WD cycles were highly significant ($p<0.001$) in Soil 2. The finding of a significant interaction between amendments and WD cycles indicates that although aggregate size distribution changed with the WD cycles, the pattern of change depended on the amendment. For example, we observed significant changes in the proportion of large macroaggregates in both soils. Overall, the proportion of large macroaggregates decreased after the first WD cycle and increased by the end of fourth WD cycle. Among the various amendments, the LP and G+LP treatments had the highest proportion of large macroaggregates, whereas PAM, FLM, and CM had little or no effect on large macroaggregates for either soil. We also observed changes in the small macroaggregate fraction, with this markedly decreasing by the second WD cycle but greatly increasing by the fourth WD cycle. In contrast to the changes in small macroaggregates, the proportion of microaggregates increased considerably (ca. two-fold) by the second WD cycle and greatly decreased by the fourth WD cycle in both soils. The silt+clay fraction slightly increased by the end of second WD cycle and again decreased slightly by the fourth WD cycle in both soils.

The MWD of both soils was calculated from the aggregate size distribution. The main effects of amendments and WD cycles were found highly significant ($p<0.001$) in both soils, however, the interaction between amendments and WD cycles was significant only for Soil 1. It was observed that MWD decreased after the second WD cycle (Fig. 2) and increased after the fourth WD cycle, irrespective of amendments or soil, reflecting the changes in actual aggregate sizes (see Fig.1). Expressing changes in MWD relative to the control or gypsum treated soil showed that LP increased the MWD in both soils over the four WD cycles (Fig. S1), with PAM being less effective than LP, whereas the other amendments had no significant effect.



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290 *Figure 1. Distribution of the aggregate sizes in the two soils after addition of amendments after first,*
291 *second, and fourth WD cycles: First WD cycle (a; Soil 1, and d; Soil 2), Second WD cycle (b; Soil 1,*
292 *and e; Soil 2), and fourth WD cycle (c; Soil 1, and f; Soil 2) (C: control, G: gypsum, PAM: anionic*
293 *polyacrylamide, FLM: feedlot manure, CM: chicken manure, LP: lucerne pellets). Large*
294 *macroaggregates (>2000 µm); small macroaggregates (2000-250 µm); microaggregates (250-53 µm),*
295 *and silt+clay fraction (<53 µm). Error bars have been omitted to improve readability.*

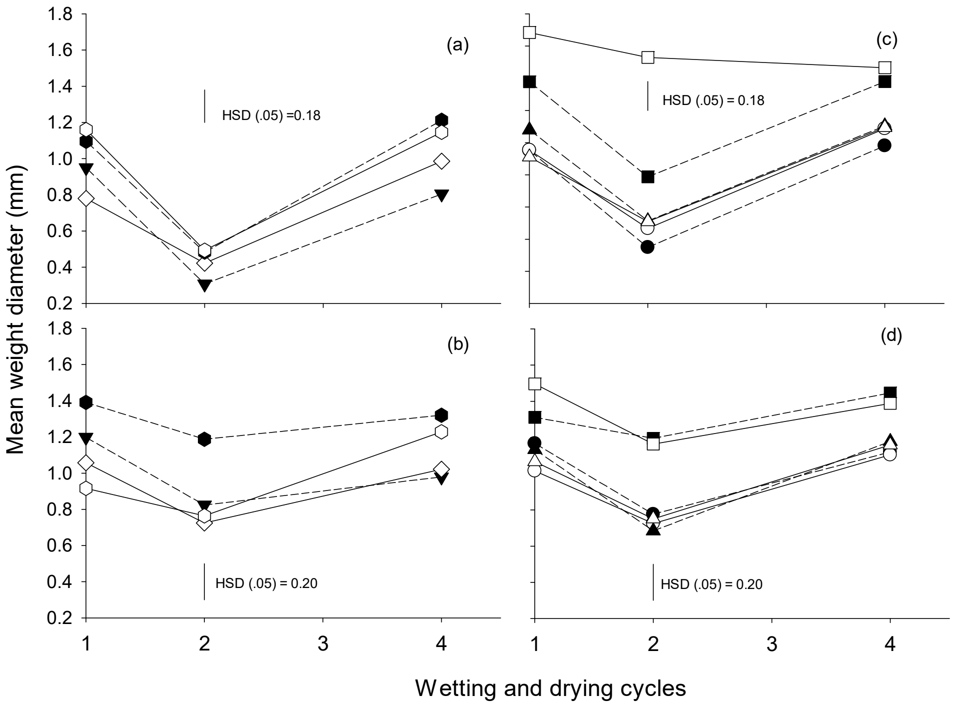

Figure 2. The mean weight diameter (MWD) of the soils after addition of amendments during four wetting and drying (WD) cycles. Soil 1 (a, c), and Soil 2 (b, d). The treatments are C: control, G: gypsum, PAM: anionic polyacrylamide, FLM: feedlot manure, CM: chicken manure, LP: lucerne pellets. Vertical bars represent Tukey's honest significant difference (HSD) values at P=0.05 for pairwise comparisons among cycles.

3.2 Soil dispersion dynamics as measured by ASWAT and DI

We assessed soil dispersion using both the ASWAT test (Fig. 3) and DI (see Supplementary Data Fig. S2), with good agreement between the DI results and ASWAT scores (Fig. S3). Overall, we observed that soil dispersion decreased with WD cycles in both soils. Although, Soil 1 was more dispersive than Soil 2, both soils showed a similar decrease in dispersion with WD cycles. Addition of gypsum significantly decreased dispersion in all WD cycles, and this effect of gypsum was greater than the effect of organic amendments in decreasing soil dispersion. PAM had only a short-term effect, with PAM decreasing dispersion for the first WD cycle but not thereafter. Of the organic amendments, only CM decreased the dispersion in both soils, but FLM and LP did not decrease dispersion in both soils in the first and second WD cycles. Given that both EC (Fig. S4) and SAR (Fig. S5) are known to affect soil dispersion, it was not surprising that the ASWAT scores were negatively correlated with EC (Fig. S6), and positively correlated with SAR (Fig. S7). However, the ASWAT scores became less affected by SAR after second WD cycle, with this highlighting the importance of WD cycles on structure improvement.

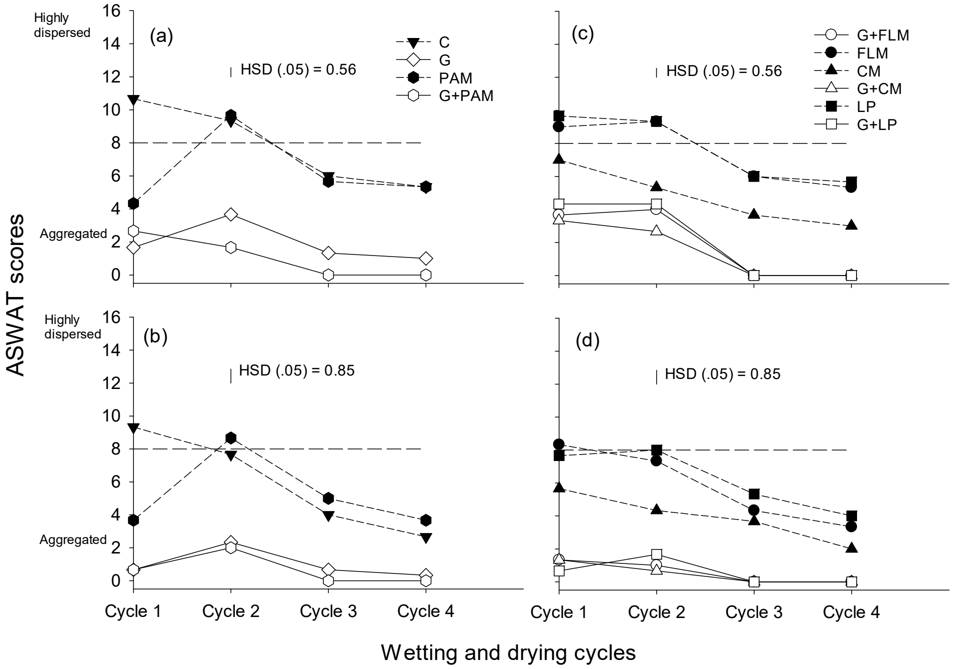

Figure 3. The ASWAT scores of soils after addition of amendments during four wetting and drying (WD) cycles; Soil 1 (a, c), and Soil 2 (b, d). The amendments are C: control, G: gypsum, PAM: anionic polyacrylamide, G+PAM: gypsum+anionic polyacrylamide, FLM: feedlot manure, G+FLM: gypsum+feedlot manure, CM: chicken manure, G+CM: gypsum+chicken manure, LP: lucerne pellets,

G+LP: gypsum+lucerne pellets). Vertical bars represent Tukey's honest significant difference (HSD) values at P=0.05 for pairwise comparisons among cycles.

3.3 Soil microbial respiration

Soil WD cycles impose a significant stress on the soil microbial community. The addition of treatments resulted in a significant increase ($p<0.001$) in soil microbial respiration for both the soils (Fig. 4). Rewetting of the dried soils caused a transient increase in microbial respiration (Fig. 4), with respiration being highest during the first WD cycle and decreasing during subsequent WD cycles. The lowest microbial respiration rates were observed in the fourth WD cycle in both soils (Fig. 4). The highest microbial respiration rates were observed in soils amended with LP or CM in the first WD cycle, whereas FLM had little effect on microbial respiration, whilst PAM and gypsum had no effect on microbial respiration.

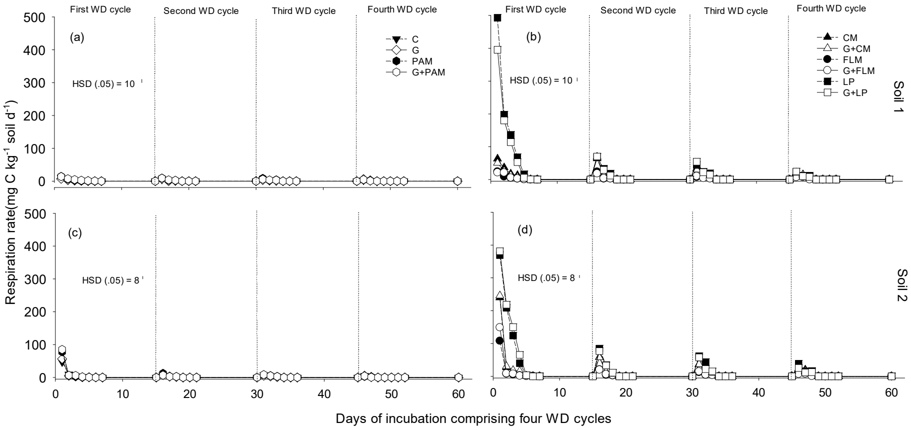

Figure 4. Changes in soil respiration rate after addition of amendments during four alternate WD cycles; Soil 1 (a, b) Soil 2 (c, d) The treatments were C: control, G: gypsum, PAM: anionic polyacrylamide, G+PAM: gypsum+anionic polyacrylamide, FLM: feedlot manure, G+FLM: gypsum+feedlot manure, CM: chicken manure, G+CM: gypsum+chicken manure, LP: lucerne pallets, G+LP: gypsum+lucerne pallets. Vertical bars represent Tukey's honest significant difference (HSD) values at P=0.05 for pairwise comparisons among the four WD cycles. The vertical dotted lines are showing the days when deionised water was added to start the next WD cycle.

3.4 Relationship between soil physical, chemical, and microbial properties

Soil properties determined for both soils after the first, second, and fourth WD cycles (Fig. 5) were used to examine inter-relationships by PCA. Overall, the PCA biplots indicated that soil microbial respiration, MWD, and the proportion of large macroaggregates were positively correlated with each other. A positive correlation between ASWAT, DI with SAR was observed in both soils, whereas EC had a negative correlation with ASWAT and DI. Surprisingly, there was not a strong correlation between silt+clay and DI or ASWAT scores. The effect of SAR and EC on soil dispersion decreased after the second WD cycle (Fig. 5), underlining the importance of WD cycles on structure formation and stability, which offsets the detrimental effects of SAR on stability. Obvious treatment effects on different soil properties could also be seen in each WD cycle (Fig. 5). The G+LP and LP treatments increased

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Preprint. Discussion started: 27 September 2022

microbial respiration, the proportion of large macroaggregates and MWD in all the cycles. The gypsum added with and without organic amendments produced the highest EC in all the cycles. The control soils had highest SAR, ASWAT scores and DI followed by FLM and LP. It was also observed that the proportion of large macroaggregates and the MWD were negatively correlated with the silt+clay fraction in each WD cycle.

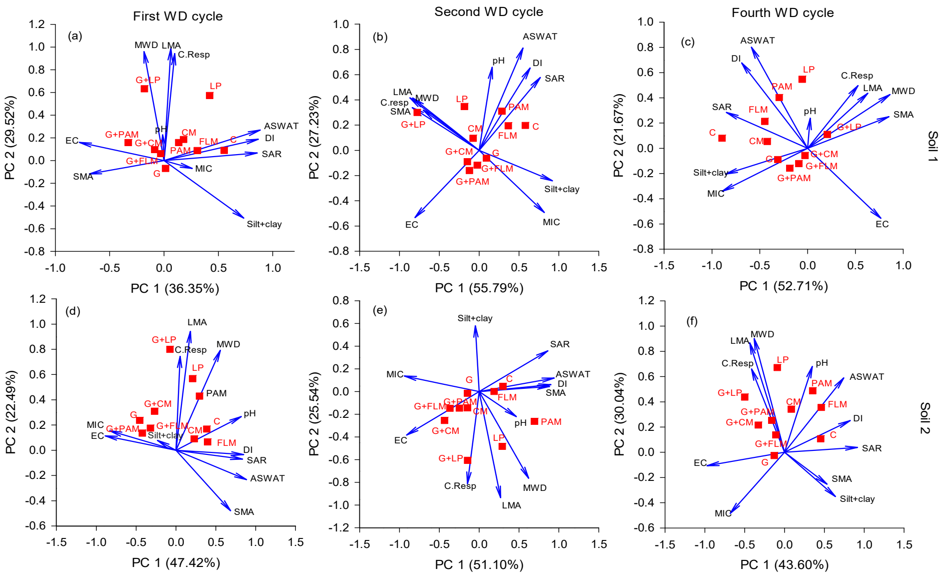

Figure 5. Principal component analysis (PCA) biplot of the physicochemical and microbial properties effected by different treatments in two soils after first, second, and fourth WD cycle: Soil 1(a, b, and c) and Soil 2 (d, e, and f). The treatments are C: control, G: gypsum, PAM: anionic polyacrylamide, FLM: feedlot manure, CM: chicken manure, LP: lucerne pellets. The data presented in these PCA biplots are the mean data of all the days for each parameter (blue arrows) and factorial scores of all the treatments (red squares). The parameters are ASWAT scores (aggregate stability in water test), DI (Dispersion index), C. Resp (cumulative microbial respiration), SAR (sodium adsorption ratio), EC (electrical conductivity 1:5), LMA (large macroaggregates), SMA (small macroaggregates), MIC (microaggregates), Silt+Clay, and MWD (mean weight diameter).

https://doi.org/10.5194/egusphere-2022-469
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4 Discussion

The results of this experiment showed clear differences in the role of organic amendments, gypsum (Ca), and WD cycles on the formation and stabilisation of soil aggregates. We showed that the formation and stability of large macroaggregates was controlled by the type of organic amendments, whereas the WD cycles increased the formation and stability of small macroaggregates. Formation of microaggregates is beneficial in that it decreases dispersion, but microaggregates may not be sufficient to improve soil water infiltration (Collis-George and Greene 1979; Nemati *et al.* 2002). Regardless, even a small increase either in large macroaggregates and small macroaggregates may increase infiltration, with this facilitating the leaching of excess Na. Here, we discuss the importance of each factor (WD cycles, organic amendments, and Ca) individually.

4.1 The relative role of WD cycles on aggregation

The WD cycles result in rearrangement of pores and soil particles and may lead to increased rigidity and stability of soil aggregates (Horn *et al.* 2014). We observed a marked change in aggregate size distribution with repeated WD cycles (Fig. 1), from macroaggregates (large macroaggregates and small macroaggregates) after first WD cycle, to microaggregates after second WD cycle, and back to macroaggregates (large macroaggregates and small macroaggregates) after the fourth WD cycle. We suggest that extracellular polysaccharides formed by microbial activity are responsible for the formation of large macroaggregates after the first WD cycle. After the second WD cycle, the microbial activity greatly decreased (Fig. 3) and macroaggregates (large macroaggregates and small macroaggregates) were broken down into microaggregates and silt+clay. This allowed the soil particles to settle into tightly packed configurations, resulting in stronger interconnections upon WD cycles (Kemper and Rosenau 1984). The decrease in large macroaggregates (Fig.1) after the first WD cycle is also consistent with the findings of Rahman *et al.* (2018). Macroaggregates are expected to be more susceptible to disintegration due to water content changes, because of the large number of planes of weakness and greater angular momentum (Kay 1990). As a result, macroaggregates break more easily during WD cycles compared to microaggregates.

The changes observed in the proportion of small macroaggregates followed the same pattern as large macroaggregates, with an initial increase in the proportion of small macroaggregates when the soils were treated with organic amendments. In contrast to these results, no significant differences were observed when the same soils were treated with organic amendments under continuous wet conditions (Niaz *et al.,* unpublished). Also, the increase in proportion of larger aggregates (large macroaggregates and small macroaggregates) is 2x greater when the same soils were exposed to WD cycles as compared to constant wet regime (Niaz *et al.,* unpublished). In this study, the proportion of small macroaggregates did not increase after the second WD cycle, but the proportion of microaggregates and silt+clay fraction increased, suggesting that upon repeated WD cycles, large macroaggregates break down into microaggregates and silt+clay fractions. These observations support the finding that macroaggregates are composed of microaggregates (Six *et al.,* 2000a). In addition, we observed that the control soils (where no amendments were added) also showed an increase in the proportion of small macroaggregates with WD cycles. This highlights the importance of WD cycles and suggests that the formation of small macroaggregates is less dependent on the organic matter content, in agreement with the PCA biplots (Fig. 5). The significant increase in the proportion of small macroaggregates in control soils after the fourth WD cycle also indicates that there could be an increase in interparticle bond strength with ageing or time (Utomo and Dexter 1982; Dexter 1988; Kong *et al.* 2005; Bravo-Garza *et al.* 2009).

4.2 The role of organic amendments on soil respiration and aggregation

The addition of organic amendments provided an energy and nutrient source for microorganisms and resulted in increased respiration rates, although we observed that respiration rate decreased with repeated WD cycles (Fig. 4), presumably due to depletion of easily metabolisable organic compounds (Harrison-Kirk *et al.* 2013; Rahman *et al.* 2018). In contrast to LP, CM had a modest effect on microbial respiration. Whereas, FLM had no significant effect on microbial respiration as it was already decomposed. Rewetting of dry soil led to immediate flush of microbial respiration (Fig. 4). This could be attributed to the slaking of larger aggregates (Fig. 1), thereby exposing some occluded organic matter, or due to the utilization of substrates that become available upon rewetting (Wu and Brookes 2005; Borken and Matzner 2009). These available (labile) substrates after rewetting include remnants of the added organic matter and microbial biomass (Wu and Brookes 2005). Over time, with successive WD cycles, it is likely that the labile organic C pool was either exhausted or became physically protected within aggregates and hence became less accessible to microbial degradation.

For the LP and G+LP treatments, where the increase in respiration rate was ~4-fold greater in Soil 1 and ~2 fold greater in Soil 2 than for any other treatment (Fig. 4), we observed that the soils also had a significant increase in the proportion of large macroaggregates (Fig. 1) and MWD (Fig. 2), suggesting that labile organic compounds are important for structure formation (also confirmed by PCA biplots Fig. 5). The formation of large macroaggregates after incorporation of organic amendments, as observed here for LP, has also been reported by Denef *et al.* 2001; Bravo-Garza *et al.* 2009; Rahman *et al.* 2018. Although the maximum respiration rate was observed during the first WD cycle for LP and G+LP, the proportion of large macroaggregates and MWD increased at the end of fourth WD cycle. During later WD cycles, it is likely conversion of readily-metabolisable organic compounds (e.g., microbial polymers) to more resistant forms resulted in the formation of macroaggregates (large macroaggregates and small macroaggregates), but in this case, aggregation may have been caused by more hydrophobic compounds. However, the proportion of large macroaggregates did not increase much as compared to the first WD cycle, likely because microbial activity was lower. One of the possible reasons of increased MWD could be the accumulation of microbial binding agents over time that are released continuously from microbial activity due to organic matter decomposition (Rahman *et al.* 2018).

When the soils were treated with organic amendments, a decrease in dispersion was observed but not significantly different from control except in CM treated soils. The possible reason for increased stability in CM treated soils was its higher Ca content (Table 2), increased EC (Fig. S4) and decreased SAR (Fig. S5). For the remaining organic amendments viz; FLM, LP, and PAM, an improvement in aggreagte stability was observed after the second WD cycle. Although, LP had the highest MWD, it still did not reduce dispersion in the first WD cycle. We suggest that high MWD in itself is a misleading measure of soil stability since only a few large aggregates can result in a high MWD (Niaz *et al.,* unpublished). Some dispersion can occur in parallel which is sensitively detected by the ASWAT test, but not by the MWD. Both assays detect different physical processes - hence a high MWD (Fig. 1) and dispersion (Fig. 2) are not mutually exclusive (Niaz *et al.,* unpublished). Organic amendments rich in aliphatic compounds or waxes can lead to hydrophobicity of aggregate surfaces during the drying step which slows down the wetting of aggregate surfaces reducing the disruption caused during rewetting (Piccolo and Mbagwu 1999; Borken and Matzner 2009). Monnier (1965) proposed that fresh organic amendments can increase aggregate stability in the time frame of weeks and months as compared to already decomposed stable organic amendments. But in our study, we found no significant differences in dispersion after the addition of LP (fresh) and FLM (partially decomposed). These findings suggest that there might be some other mechanisms which are responsible for the binding of organic matter with clay particles to control dispersion, such as the size of the organic matter molecule and charge density. This requires further investigation.

https://doi.org/10.5194/egusphere-2022-469
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4.3 The relative role of Ca (gypsum) in improving aggregate stability

In this experiment we found that gypsum (Ca) flocculated soil particles and reduced soil dispersion (Fig. 3, Fig. S2), because the addition of Ca led to a significant increase in the EC (Fig. S4) and a decrease in SAR (Fig. S5), with this presumably resulting in the flocculation of soil particles (van Olphen 1977; Muneer and Oades 1989; Chorom and Rengasamy 1997; Bennett *et al.* 2015). Improved aggregation and stability were also observed when the soils were treated with gypsum and organic amendments. Gypsum reduces dispersion by increasing flocculation, which is not same as improving aggregation. For the latter, organic amendments are required. It is noteworthy, however, that there are also other mechanisms by which Ca can interact with organic substances to form Ca-bridging through which clay particles are attached to organic matter, with polyvalent cations forming micro-and macroaggregates (Edwards and Bremner 1967; Muneer and Oades 1989; Tisdall 1996; Chenu *et al.* 1998).

Conclusion

Aggregate formation in two sodic Vertisols was significantly affected by WD cycles, organic matter amendments and gypsum. However, we observed that not all organic amendments were beneficial for increasing the formation of macroaggregates. Organic amendments LP and CM increased the proportion of larger aggregates, whereas FLM was ineffective which can be attributed to the effects of amendments on microbial activity. While G helped in reducing soil dispersion, it had no significant role in the formation of larger aggregates. Implementation of these findings in the field would favour the use of organic amendments with gypsum to improve the physicochemical properties of sodic soils, which is further enhanced by WD cycles. The aim should be initially to prevent soil dispersion which can be achieved by the application of Ca (through application of gypsum) and then to build larger aggregates which can be achieved by the application of organic amendments, and imposing wetting and drying cycles. The large aggregates then improve water and air entry in soil.

Author contribution

Conceptualization: Niaz. S., J. Wehr, B. J., Kopittke, P.M., Dalal, R.C., and Menzies, N.W.; Formal analysis, investigation, and writing original draft: Niaz. S; Review and editing: J. Wehr, B. J., Kopittke, P.M., Dalal, R.C., and Menzies, N.W.; Supervision: Wehr, B. J., and Menzies, N.W.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. We thank David Appleton and Stephen Appleton (analytical services, UQ) for the soil solution analyses. We express our gratitude to Dr Brigid McKenna (UQ) for general help with the laboratory work.

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