

Dear Editor

We thank you and the reviewers for their generous feedback to our paper:... and we thank you for accepting our paper with minor revisions. Below please find our comments to the reviewer suggestions; the line numbers refer to the paper with highlighted revisions.

### **Reviewer 1**

The concerns and questions raised by reviewer 1 have been answered in response to reviewers report. To recap, we agree with the reviewer that wetting and drying cycles may cause anaerobic or anoxic conditions. However, in this experiment, the soils were kept wet at field capacity only for 24 hr and then left in open air for moisture loss (to initiate drying conditions) and the soils were packed to a height of 3-5 cm in jars with 12 cm width (now added at L246-247). Hence, we do not believe our experimental conditions have caused anoxia in the soil. Indeed, we have not measured a decrease in nitrate in our soil solution samples (which would happen if conditions were anoxic) and we can include graphs if considered necessary to show this. The organic amendments except anionic polyacrylamide (PAM) were used in dried form to overcome differences in moisture content and particle sizes. While there may have been some losses of nutrients in the organic amendments due to drying, the nutrient content was measured on the dry material. Hence, we do not believe this would affect the interpretation of our results.

We also agree that different microbial communities may be added (or favoured) by adding organic amendments. However, the focus of our paper was not on different microbial populations but on aggregation (by whatever means the aggregates are formed, be it different microbes or iron oxides). While we measured the soluble chemical fractions (soil solution composition) in our samples (these data are reported in the supplementary section of our paper), we have not analysed if there are changes in the mineral phases during the trial since this was outside the scope of our experiment.

The focus of this experiment was to understand the effect of wetting and drying cycles on improving the structure of sodic soils under the application of different organic amendments. Therefore, to correlate the changes in aggregate stability and aggregation with microbial activity, daily microbial respiration was measured. In our earlier study (Niaz et al 2022) we measured Dissolved Organic Carbon (DOC) using the same soils and amendments under continuous wet conditions and found that DOC and soil microbial respiration were strongly

positively correlated with each other, hence we are confident relating microbial respiration to DOC in this paper.

## Reviewer 2

As suggested by the reviewer, we reworded the aims and hypothesis section (L142-159) as follows: “Thus, in the present study we used two sodic Vertisols, with the aim to: i) determine the role of gypsum and different organic amendments on aggregate formation and stability, ii) explore the combined effect of gypsum and organic amendments on soil physico-chemical and microbial properties, iii) investigate the effect of WD cycles on microbial respiration, iv) assess the effects of WD cycles on aggregate formation and stability, and v) determine how many WD cycles are needed to improve aggregate stability. We hypothesised that i) organic amendments will increase the microbial respiration and improves the formation of large macroaggregates and MWD, (ii) gypsum will improve aggregate stability due to increases Ca concentration and ionic strength, iii) organic amendments act synergistically with gypsum on aggregation, and iv) repeated WD cycles will increase the process of aggregate formation and stability”

Other sections of the paper, e.g. 3.1 (L343-L344), 4.1 (L469-477) and 4.3 (L586-599) had minor changes in wording and grammar to improve readability.

Some specific comments raised by the reviewer were addresses as follows:

1. Organic matter in title has been replaced with organic amendments at line 1. The title has been updated as “Wetting and drying cycles, organic amendments and gypsum play a key role in structure formation and stability of sodic Vertisols”
2. The full name of PAM has now been incorporated in L29-30 as “In contrast, dispersion was significantly reduced when soils were treated with chicken manure, whilst anionic polyacrylamide only had a transient effect on aggregate stability”.
3. The choice of amendments has been included (L211-216) “The four organic amendments were chosen because of three reasons: 1) they were easily available and are being used by farmers, 2) LP is used as green manure and studies have shown it is effective in ameliorating sodic soils, and 3) PAM is used in mining and construction to treat sodic dispersive soils. Furthermore, these amendments were different in terms of their chemical properties (Table 2) and C functional groups (Niaz et al., 2022) and may give a good contrast between the amendments.”

4. The 0-10 cm soil layer was chosen because topsoil has the greatest OM content and microbial activity. In any case, Vertisols are relatively uniform in texture and structure throughout the soil profile; using subsoil would not add much information to the study.
5. The soil was sieved to 10 mm as we wanted minimise physical disturbance of the natural soil structure. The soil contained no stones or coarse fragments, and the visible plant litter was manually removed.
6. The experiment was performed jars with 12 cm diameter and 15 cm height, and the soil was packed to a height of 3-5 cm. This information has been incorporated in L246-247.
7. As there were 10 treatments, individual error bars or asterisks would have cluttered the graphs. Therefore, we preferred to insert the Tukey HSD bar in graphs.
8. The discussion has been revised in L586-599 as follows “*The results of this experiment showed that addition of gypsum (Ca) significantly reduced soil dispersion and increased aggregate stability. This improvement in aggregate stability is because of the increased EC (ionic strength, Fig. S4) and decreased SAR (Fig. S5) after the addition of gypsum. The increased EC likely resulted in the flocculation of soil particles by reducing the diffuse double layer (van Olphen 1977, Ghosh et al., 2010, Bennett et al. 2015). Improved stability was also observed when organic amendments were applied with gypsum especially in G+PAM, G+LP, and G+FLM treated soils, which when applied alone were not able to improve aggregate stability. The PAM had an initial positive effect but led to decreased stability at completion of the second WD cycle. Although, the addition of gypsum increased aggregate stability, it was observed that addition of gypsum did not affect the proportion of large macroaggregates and MWD. However, when organic amendments were added with gypsum an improvement in proportion of large macroaggregates and MWD was observed in G+LP treated soils. This can be explained as Ca-bridging effect through which clay particles are attached to organic matter and polyvalent cations resulting in the formation of macro and micro aggregates (Wuddivira and Camps-Roach 2007).*”
9. More references (highlighted in blue italic colour) have been included (L557): “However, the proportion of large macroaggregates did not increase much as compared to the first WD cycle, likely because microbial activity was lower (Cosentino et al. 2006; Zhang et al. 2022). “

10. The conclusion has be re-worded (L601-654) as “The stability of dispersive sodic Vertisols was improved by the application of organic amendments and gypsum, which was further enhanced by WD cycles. Gypsum reduced soil dispersion but did not affect the proportion of large macroaggregates and MWD. We observed that not all organic amendments were equally beneficial in improving soil aggregation and aggregate stability. LP significantly increased the proportion of large macroaggregates compared to FLM and PAM. In contrast, CM significantly reduced soil dispersion as it had higher calcium content. It was also found that PAM only had a transient effect in controlling dispersion. In the absence of organic amendments, repeated WD cycles reduced the dispersion of sodic soils, but when organic amendments were added (with or without gypsum) soil aggregation and soil stability was improved even more. It is likely that soil microbial activity contributed to the aggregate formation. 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”

### Reviewer 3

1. We agree that a correlation does not prove the existence of a mechanism or cause. We represented the correlations among different soil physical, chemical, and microbial characteristics as PCA biplots after each WD cycle to show how soil properties change with repeated WD cycles. We have reworded the section 3.4 to make it clearer that we refer to correlations only (L440-441).
2. We have added more references to place our findings in a broader context and relating our results to other work done with Vertisols, e.g. work by Ghosh et al., 2010, Rahman et al., 2017, Rahman et al., 2018, Bennett et al., 2015, and Nachimuthu et al., 2022.
3. The hypotheses (L143-159) have been updated as already suggested by reviewer 2.
4. The introduction has been expanded by adding information about soil microbial respiration and aggregate formation in L 79-91: “Addition of organic matter effect 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)). While organic matter increase soil microbial respiration resulting in the formation of extracellular polysaccharides which help in the formation of soil aggregates

(Bossuyt et al., 2001), studies investigating the effect of organic amendments in improving the soil structure are inconclusive. For instance, the extracellular polysaccharides and large polyanions can bind clay particles into stable macroaggregates. On the other hand, organic anions can enhance dispersion by increasing the negative charge on clay particles and by complexing calcium and other polyvalent cations (such as those of aluminium), hence reducing their activity in soil solution (Ghosh *et al.* 2010)”

5. We have revised sections 3.1 and 4.1 to make our statements clearer. For example, the reviewer has highlighted sentences which was changed to “at the completion of the first WD cycle” etc (L472-474). (Samples were collected for aggregate size analysis after completion of either 1, 2 or 4 WD cycles). In our revision we also expand on the explanation of the results (L475-488); “We suggest that extracellular polysaccharides formed by microbial activity (indicated by soil microbial respiration, Fig. 3) are responsible for the formation of large macroaggregates at the completion of 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). Macroaggregates were more susceptible to disintegration during wet sieving compared to microaggregates at the completion of second WD cycle. By the fourth WD cycle, some rearrangements of soil particles likely occurred, facilitated by soil drying, thereby rebuilding macroaggregates.”
6. The reviewer showed concern about the statement we made about the breakdown of large macroaggregates compared to microaggregates during WD cycles. We rephrased that sentence in L481-L488 as “Macroaggregates were more susceptible to disintegration during wet sieving compared to microaggregates at the completion of second WD cycle. By the fourth WD cycle, some rearrangements of soil particles likely occurred, facilitated by soil drying, thereby rebuilding macroaggregates.” We could also insert a schematic to illustrate the process.
7. The conclusions have been revised as already suggested by reviewer 2 (L601-654).
8. The citations have now been included at various points in manuscript, e.g. Brangari et al., 2022, Fraser et al., 2016, Niaz et al. 2022, Zhang et al., 2022.

9. The previously unpublished data has now been published and available online as Niaz et al., 2022. (<https://doi.org/10.1016/j.geoderma.2022.116047>)
10. All the small corrections have been incorporated in the manuscript and the legend to Fig 2 has been inserted.

#### **Reviewer 4**

1. The introduction has been revised as was already suggested by other reviewers. In addition, the reviewer asked to add a brief information about how gypsum and organic amendments can affect aggregation and aggregate stability, this has now been added at L72-91: “Traditionally the management practices used to improve the structure of sodic soils involves the displacement of Na ions from the soil exchange complex with the help of divalent cations such as Ca or increasing the ionic strength of soil solution (Ghosh *et al.* 2010), both of which can be achieved by the application of gypsum to these soils. The effect of gypsum on increasing ionic strength is immediate, but short lived. In contrast, the effect of gypsum for providing the counter ion (Ca to replace Na) is permanent unless additional Na is added to system (e.g., using poor quality irrigation water). Another frequently used management practice to ameliorate sodic soils is the use of organic amendments. Addition of organic matter effect 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)). While organic matter increase soil microbial respiration resulting in the formation of extracellular polysaccharides which help in the formation of soil aggregates (Bossuyt et al., 2001), studies investigating the effect of organic amendments in improving the soil structure are inconclusive. For instance, the extracellular polysaccharides and large polyanions can bind clay particles into stable macroaggregates. On the other hand, organic anions can enhance dispersion by increasing the negative charge on clay particles and by complexing calcium and other polyvalent cations (such as those of aluminium), hence reducing their activity in soil solution (Ghosh *et al.* 2010)”.

2. The aims and hypotheses have been rewritten in L142-L159 as already suggested by reviewers 2 and 3.
3. We have use two-way ANOVA to analyse the results which makes the description of results rather complex and repetitive. That's why the discussion was written as the relative role of each factor (WD cycles and organic amendments or gypsum). The interaction of these factors was clearly demonstrated by presenting PCA biplots after first, second and fourth WD cycle in Fig. 5 and section 3.4 (lines 431-445). The two-way ANOVA table could be included in the supplementary data if required.
4. The dose of PAM in Table 3 has been corrected (L237, L259).
5. The discussion have been revised as already suggested by other reviewers (see reviewer 2 and 3).

#### **Reviewer 5**

1. The aims and research hypothesis have been revised as recommended by reviewers 2, 3, and 4 (L143-159).
2. Several sentences have been rephrased to highlight changes driven by different amendments or WD cycles (e.g. in sections 4.1, 4.2 and 4.3).
3. The information regarding Vertisols has been added to the Introduction from L92-101: “Apart from the changes in soil structure due to the addition of different ameliorants, WD cycles can lead to more intensive changes in structure of soils dominated by smectitic clays (Vertisols), through physical processes (Utomo and Dexter 1982; Deneff *et al.* 2001). These soils are generally characterised as self-mulching soils as they exhibit shrink-swell properties imposed by the WD cycles (Pal *et al.* 2012). Vertisols cover a total of an estimated 340 million ha in the world (Australia, Asia, Africa, and America), out of which approximately 150 million ha is potential crop land. However, the physical properties and moisture regime of Vertisols represents serious management constraints (Pal *et al.* 2012). Sodic Vertisols are common in arid parts of the world. The effect of sodicity on the physical properties of Vertisols is still a subject of debate.”
4. We are not sure we correctly understand the question about preincubation; soils were not pre-incubated in the study, but they were collected in the field and would be pre-incubated there. We are not sure why pre-incubation would be useful – in the field,

treatments are applied without pre-incubation. Our apologies if we misunderstood the question.

5. The effect of rapid slaking was not checked as the soils were subjected to end over end shaking for the measurement of easily dispersible silt+clay.
6. The conclusion was reworded as suggested by other reviewers.

### **Minor corrections**

7. All minor corrections have been addressed
8. The formula used for gypsum requirement has now been incorporated in the main text from line 238-245: “The gypsum requirement of both soil samples was calculated based on the formula given by Oster and Jayawardane (1998) as follows:

Gypsum requirement (GR)=  $0.00086 \times F \times D \times \rho_b \times (CEC) \times (ESP_i - ESP_f)$

Where F is exchanged efficiency of Ca-Na and for this case considered equal to 1.

$D_s$  is the depth of soil to be reclaimed (cm)

$\rho_b$  is soil bulk density ( $\text{g/cm}^3$ )

CEC is cation exchange capacity ( $\text{cmol}^+/\text{kg}$ )

$ESP_i$  is initial soil exchangeable sodium percentage

$ESP_f$  is final or desired exchangeable sodium percentage.

Hence for soil 1  $GR = 0.00086 \times 1 \times 10 \times 1.29 (23) (14.9 - 6) = 2.27 \text{ Mg/ha}$

And for soil 2  $GR = 0.00086 \times 1 \times 10 \times 1.29 (24) (15.9 - 6) = 2.52 \text{ Mg/ha}$

For simplicity, a single gypsum rate of 2.5 Mg/ha was selected for both soils “

### **Reviewer 6**

1. The full name of PAM has now been added (L29-30).
2. Abbreviations have now been spelled out in the Introduction and Materials and methods
3. The hypotheses have been revised as already recommended by other reviewers (L143-159).

4. We presented Table 1 and 2 after the description of soils and organic amendments in the Materials and methods section, but we don't mind to move the tables to the Results section if the editor recommends.
5. As explained for reviewer 2, we prefer to use the Tukey's HSD bar to avoid cluttering the graphs.
6. The unpublished data at that time was under review and now has been published as Niaz et al., 2022 (<https://doi.org/10.1016/j.geoderma.2022.116047>).

### **Specific comments**

7. As explained for reviewer 2, topsoil layer has high OM and microbial activity and Vertisols are relatively uniform in texture and structure to depth
8. Three core samples were collected for the measurement of bulk density for each site (Soil1 and Soil 2) while the bulk soil samples were collected with a shovel to 10 cm depth. This has now been added (L166, L169-170)
9. The formula used for gypsum requirement has now been added, as already advised by reviewer 5 (L238-245).
10. Our apologies but we do not understand what is meant by "Amend "change"". We have corrected a spelling mistake at L321, which this statement may refer to?
11. The significance for each parameter is indicated in the text and figures by using the p (<0.05) value and Tukey's HSD bar in the figures. The error bars and letters to show significance were purposely removed as the line graphs got clumsier and were difficult to distinguish.
12. In Fig. 1, the legends LMA, SMA, MIC and MIN have now been clearly explained in figure caption. The four different aggregate classes have been abbreviated as LMA, SMA, MIC and MIN which have been updated as "Large macro aggregates, small macroaggregates, microaggregates and silt+clay".