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Effects of long-term inorganic and organic fertilization on the soil micro and macro structure of rice paddies

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Abstract

The soil structure of paddy soil is very dynamic from the aggregate to the pedon scale because of intensive anthropogenic management strategies. In this study, we tested the hypothesis that long-term inorganic and organic fertilization can affect soil structure at different scales. Microstructure assessed by soil aggregates (3 - 5 mm in diameter) and macrostructure assessed by small soil cores (CoreS) (5 cm in diameter, 5 cm in height) and large soil cores (CoreL) (10 cm in diameter, 10 cm in height) were sampled from three long-term fertilization treatments, including no fertilizer (CK), application of inorganic fertilizer (NPK), and a combination of inorganic fertilizer and organic manure (NPKOM), established in 1982. They were scanned at two scales with two types of micro-Computed Tomography (micro-CT) and quantified using image analysis. Results showed that relative to CK treatment, long-term NPKOM fertilization increased soil organic C (SOC) by 28% and available water content (AWC) by 20%, but decreased soil bulk density by 0.2 g cm^{-3} whereas NPK showed no difference. Soils under CK and NPK treatments exhibited an identical dense structure at both aggregate and core scales in which pores were mainly cracks resulting from shrink/swell processes, and showed no significant difference in porosity and size distribution of the CT-identified pores (> 3.7 μm). As compared with the CK treatment, the soil in the NPKOM treatment had greater intra- and inter-aggregate pores, and increased porosity by 58.3%, 144.9%, and 65.9% at aggregate, CoreS, and CoreL scales, respectively. These were attributed to the biopores formed from decayed roots, stubble, and organic manures as a result of increased yields and direct amendment of organic manure. Overall, this study demonstrates that organic fertilization can improve the physical qualities of paddy soils across different scales but inorganic fertilization in isolation does not.

Key Words

Paddy soil; Soil structure; Aggregate; Pore; Micro-CT; Fertilization

1 Introduction

Soil structure is a fundamental property of soil health because it impacts the storage and movement of water, gas and nutrients, root growth, and microbe activity (Bronick and Lal, 2005). Soil structure can be assessed over several order of scales from mineral-organic complexes, aggregates, typically referred as soil microstructure, to peds and clods in the soil profile, usually considered soil macrostructure (Carter, 2004). And the size of the corresponding pores range from μm to mm or even larger. Tisdall and Oades (1982) proposed that the factors and processes controlling the formation of soil structure are different at contrasting scales in an aggregate hierarchy concept model. Management practices, e.g. tillage and fertilization, have been proven to impact each level of soil structure either directly or indirectly (Bronick and Lal, 2005). Kravchenko et al. (2011) showed large intra-aggregate pores in no tillage and native succession vegetation treatments are more heterogeneous than those in conventional tillage treatment. Macropores (> 0.75 mm) were more abundant in pastureland than under arable crops and provided pathways for preferential flow at the core scale (Luo et al., 2008). Despite the numerous evaluations of land use and management effects on soil structure, most studies have been limited to a specific scale and knowledge of the responses of soil structure at different scales is lacking.

Information about a soil’s inner structure has usually been inferred from soil properties (e.g., hydraulic properties, gas permeability) (Hill et al., 1985; Marshall, 1958; Moldrup et al., 2001). These calculations were based on assumptions of ideal pore shapes and typically could not provide information regarding the architecture of soil pore system. Therefore, a direct study of soil structure is necessary. Direct observation and quantification of the structure of soil was typically conducted on soil thin sections (Pagliai et al., 2004; Mooney et al. 2007). However, soil thin section can only provide two-dimensional (2D) information of soil structure. And the preparation of thin sections is time consuming (Murphy, 1986). Computed Tomography (CT) offers a rapid and non-destructive way to study soil structure over a range of scales (Taina et al., 2008; Wildenschild et al., 2002, Helliwell et al., 2014). High-resolution CT can show the detailed organization of soil aggregates and has been used to study aggregation processes (Atkinson et al. 2009; Zhou et al., 2013), soil microstructure (Peth et al., 2008), and soil biophysical interactions (Martin et al. 2012; Vos et al., 2013) at the aggregate scale. CT with a low resolution, on the other hand, can scan large samples and is frequently used to study macropores and their relationship with soil hydraulic properties (Luo et al., 2008) at the soil core scale. The study of the micro and macro scale soil structure is possible by using a combination of CT systems with different resolution capabilities. Schlüter et al. (2011) studied soil structure development at two different scales and proposed a method to combine soil pore size distribution (PSD) acquired at the different scales. Dal Ferro et al. (2013) found that both the micro- and macro-scales soil structure were affected by fertilization from the scanning of soil aggregates and soil cores using micro-CT.

Rice is the most important staple food in China and the cultivation area of rice is 25 million ha, accounting for 25% of the national arable land area (Li, 1992). Long-term traditional cultivation of rice, specifically flooding during most of the growing season, drastically changed soil physical, chemical, and biological properties and resulted in a special anthropogenic paddy soil (Gong, 1986). The structure of paddy soil is more dynamic at the aggregate to soil core scales compared with those of upland soils. The plough layer of the paddy soil is homogenized before each growing season to prepare the seedbed, which destroys surface soil structure considerably (Eickhorst and Tippkötter, 2009; Kirchhof et al., 2000; Sharma and Datta, 1986). Moreover, paddy soil experiences frequent swell-shrink cycles caused by periodic flooding and drying management (Zhang et al., 2013). These processes are accompanied by the creation and closing of cracks which has critical importance for the evolution of the structure of paddy soil (Liu et al., 2003; Sander and Gerke, 2007). At the micro-scale, aggregation of paddy soil is greatly influenced by the oxidation-reduction conditions caused by flooding and drainage cycles (Kögel-Knabner et al., 2010). For example, Fe oxides are important binding agents of soil aggregates, but their effects vary among different Fe species (Duiker et al., 2003). Poorly crystalline Fe has a larger and more reactive surface area and therefore is more effective in soil aggregation than crystalline Fe (Duiker et al., 2003; Yan et al., 2013). Repeated flooding and drainage cycles have been shown to increase Fe_o oxides while reducing Fe_d oxides; therefore, these processes are beneficial to soil aggregation (Zhang et al., 2003).

The application of organic or inorganic fertilizers can directly or indirectly introduce different ions and organic matter to the soil, which may cause soil disaggregation or aggregation (Haynes and Naidu, 1998). In the past few years, research regarding the effects of fertilization on paddy soil has mostly focused on SOC sequestration (Anders et al., 2012; Brar et al., 2013; Das et al., 2014), greenhouse gas emissions (Yagi and Minami, 1990), and microbial and geochemical processes (Zhong and Cai, 2007) due to environmental and ecological concerns. These processes are closely linked with soil structure, which determines the transport of water, gas and solutes and provides a habitat for soil microorganisms (Young and Crawford, 2004). Although the change in aggregate stability under fertilization in paddy soils has been evaluated (Li and Zhang. 2007; Huang et al., 2010; Yan et al., 2013), the effect of fertilization on the formation and dynamics of the structure of paddy soil is still unclear.

To better understand the sustainability of paddy soil to continuously received inorganic and organic fertilizers, this study aimed to evaluate the soil micro and macro structure of a long-term fertilization experiment. The specific objectives were: (1) to evaluate the effects of fertilization on aggregate- and core- scale structure using synchrotron based micro-CT and industrial micro-CT and (2) to investigate the mechanisms of the structure evolution of paddy soil.

2 Materials and methods

2.1 Experimental site

Soil was taken from long-term experiment established in 1982 at the Jiangxi Institute of Red Soil, Jinxian County, Jiangxi Province, China (116°10′ E, 28°21′ N). The experiment site lies in a flat area of the hilly region of Southern China. The experiment site has a subtropical climate and a mean annual temperature and precipitation of 17.7 °C and 1706 mm, respectively. The paddy soil (Typic Stagnic Anthrosols, Chinese Soil Taxonomic Classification, 2002) is clay loam (20% sand, 48% silt, and 32% clay) for the plough layer (0- to 15 cm). Before the long-term experiment, the paddy soil had organic C 16.3 g kg^{-1}, total N 1.49 g kg^{-1}, and pH 6.9 in the plough layer. The site had been cultivated with rice for more than 100 years prior to the experiment. The cropping system is early rice – late rice from April to

October and fallow in the winter.

The experiment was designed as a randomized complete block with three replicates. Three fertilization treatments were studied: (1) no fertilization as a control (CK), (2) a combination of inorganic fertilizers (NPK), including 90 kg N ha^{-1}, 20 kg P ha^{-1}, and 62 kg K ha^{-1} for each season; and (3) organic manure and the inorganic fertilizers (NPKOM) together, including the same amount of inorganic fertilizer as the NPK treatment plus 22.5 t ha^{-1} pig manure. Each plot had an area of 46.67 m^2. A detailed description of the management of the field experiment can be found in Yan et al. (2013).

2.2 Sampling

Sampling was conducted in September 2012 just before the harvest of late rice. Two sizes of undisturbed soil cores, a large size (diameter 10 cm, height 10 cm, CoreL) and a small size (diameter 5 cm, height 5 cm, CoreS), were randomly collected using PVC tubes with triplicates in each plot. The tubes were gently pushed into the topsoil and were excavated using a spade. Soil cores were wrapped with plastic film to prevent water evaporation and stored in the refrigerator at 4 °C. A total of 27 CoreL and 27 CoreS were sampled. Bulk soil was also sampled with a spade from the 0 – 10 cm depth. In each plot five samples were randomly collected and they were then mixed together to form one bulk sample. The bulk soil samples were manually broken to small parts (<8 mm) and air-dried at room temperature. Care was taken to prevent compression during sampling and breaking.

2.3 CT scanning and image reconstruction

Both CoreL (n = 27) and CoreS (n = 27) were scanned at field moisture (0.30 - 0.35 cm^3 cm^{-3}) using an industrial Phoenix Nanotom X-ray µ-CT (GE, Sensing and Inspection

Technologies, GmbH, Wunstorf, Germany). Detailed scan parameters are listed in Table 1. The voltage and current were higher for CoreL than for CoreS because more energy was needed to penetrate larger samples. A 0.2 mm Cu filter was used to reduce the beam hardening effect. The distance between the source and the sample and between the sample and the detector was 30 cm and 20 cm, respectively. At this distance, the detector could fully capture the signal of CoreS but detector shift was needed to acquire the full image of the CoreL samples. Reconstruction was performed using the Datos|x 2.0 software using the filtered back-projection algorithm. This generated slices of 4000 × 4000 and 2000 × 2000 voxels for CoreL and CoreS, respectively, with each voxel representing a volume of 30 x 30 x 30 μm^3. The slices were stored in 8-bit format, which means that each voxel had a value between 0 and 255 representing the attenuation coefficient of the corresponding material.

The scanning of aggregates from the bulk samples was conducted with a synchrotron-based μ-CT at beam line BL13W1 of the Shanghai Synchrotron Radiation facility (SSRF). Air-dried aggregates (3 – 5 mm) were first collected by sieving the bulk samples and then randomly selected for CT scanning. A total of 27 aggregates were scanned. Details of the experimental setup, scanning, and reconstruction can be found in Zhou et al. (2012) and main scan parameters were listed in Table 1. The final slices were in 8-bit type, with each voxel representing a volume of 3.7×3.7×3.7 μm^3.

2.4 Image processing and analysis

Image processing and visualization were conducted with the open-source software ImageJ ver. 1.47 (Rasband, 1997-2011). The size of the image stacks of the soil cores was beyond the RAM and computation capacity of the available computer, so the image stacks were resized by binning the voxels by a factor of 3 and 2 for the CoreL and CoreS, respectively to facilitate further image processing and analysis. The resulting images had a resolution of 90 μm and 60 μm for the CoreL and CoreS, respectively. A region of interest (ROI) was selected from the central part of soil cores to avoid artifacts at the boundary region caused by sampling (Table 1). Images of the soil aggregates were first preprocessed to remove ring artifacts (Zhou et al., 2011). The image stacks were cropped to a ROI of 500×500×500 voxels, representing a volume of 1.85×1.85×1.85 mm^3.

Procedures of image processing and subsequent analysis were identical for the ROIs of both soil cores and soil aggregates. A three dimensional median filter was used to reduce noise before image segmentation. Segmentation is critical to the quantitative characterization of the pore system (Iassonov et al. 2009). Comparisons of different methods in previous studies have showed the disadvantages of the global threshold method and indicated the advantages of using the local threshold method for soil samples (Iassonov et al., 2009; Wang et al., 2011). In this study, a bi-level method (Vogel and Kretzschmar, 1996) was used to segment soil pores and solids. Briefly, two threshold values (T_0 and T_1) were selected based on the histogram, voxels with a grayscale value smaller than T_0 or larger than T_1 were classified as pores or solids, respectively. The unclassified voxels with grayscale values between T_0 and T_1 were attributed to pores if one of the 26 neighbours was a pore. This process iterated until there were no more changes, and the remaining unclassified voxels were attributed to solids.

2.5 Pore system analysis

Although there are many descriptors (e.g., shape and topology parameters) of the pore system, we chose porosity and PSD because they are most comprehensive and are easy to compare across scales. Porosity was determined as the percentage of pore volume that was quantified with image analysis to the total volume of the ROI. PSD was obtained by morphological "opening" operations. Briefly, pores smaller than a certain size were removed by erosion followed by dilation using a spherical structuring element, which is called "opening". By changing the size of the structuring element, the PSD could be derived. A detailed description to this method is reported in Schlüter et al. (2011).

2.6 Soil properties analysis

The soil water retention curve (SWRC) and bulk density were measured on the same CoreS after CT scanning. The SWRC was determined using a sandbox at the range 0 - 100 cm water head and using a pressure plate method at the range 0.1 - 15 bar water potential. Plant available water capacity (PAWC) was the difference between water content at field capacity (0.33 bar) and permanent wilting point (15 bar). Bulk density was determined based on the mass of the oven-dried sample (105 °C) and the volume of the soil core.

Soil organic carbon (SOC) was measured by oxidation with potassium dichromate in a heated oil bath. Oxalate-soluble Fe oxides (Fe_o) were extracted by oxalate and then were determined by ICP-OES (PerkinEimer's, Optima 8000, USA). Soil pH was measured using a glass electrode with a 1:2.5 soil: water ratio. Aggregate stability was examined following Le Bissonnais (1996) using fast wetting, slow wetting, and mechanical stirring methods. The bulk samples were passed through a 5 mm sieve, and aggregates with the size 3 – 5 mm were used for the stability test. The aggregate stability was expressed as the mean weight diameter (MWD) following equation (1):

$$MWD = \sum_{1}^{n+1} \frac{r_{i-1} + r_i}{2} \times m_i \quad (1)$$

where r_i is aperture of the i^{th} sieve (mm), m_i is mass fraction of aggregates remaining on i^{th} sieve; n is number of the sieves. All the soil properties were measured in three replicates.

2.7 Statistical analysis

To compare the differences in soil properties and the parameters of pore space among the treatments, analysis of variance (ANOVA) was conducted using the GLM procedure in the SAS software program (SAS institute, 2011). Mean values were tested using the Fisher's least significant difference (LSD) at the p = 0.05 level of statistical significance.

3 Results

3.1 Soil properties

Table 2 shows some selected properties of the paddy soil and yields of different fertilization treatments. No significant difference was found for the clay, silt, and sand content among the treatments. SOC content of NPKOM treatment was 28% higher than those of CK and NPK treatments while the latter two treatments did not differ. Similar to SOC, total porosity (calculated from bulk density and soil density, 2.65 g cm^{-3}), saturated hydraulic conductivity (Ks), and PAWC significantly increased after receiving long-term NPKOM relative to the CK treatment, whereas no change was found between NPK and CK treatments. Fe_o significantly increased in the order of NPKOM > NPK > CK. SSA was similar among the treatments. Grain yields significantly increased in the order of NPKOM > NPK > CK. The MWD was lowest when determined using the fast wetting method than using the mechanical stirring and slow wetting methods (Fig. 1), indicating that slaking was the main breakdown mechanism for the studied paddy soil. The MWD values from the mechanical stirring and slow wetting methods were close to 3 mm, showing that the aggregates of paddy soil were resistant to mechanical disturbance and partial swelling. The difference in aggregate water stability was only found using the slow wetting method and soils receiving long-term inorganic fertilizer showed significant lower stability compared with the CK and NPKOM treatments.

3.2 Microstructure of soil aggregates

Representative 2-D and 3-D images of the aggregates from different treatments are presented in Fig. 2. Aggregates from the CK treatments had a dense microstructure and few intra-aggregate pores as shown in the 2-D image. The 3-D images clearly showed that the intra-aggregate pores were mostly isolated pores. The microstructure of aggregates from the NPK treatment is similar to that of the CK, except that several continuous pores were presented. In contrast, the aggregates from the NPKOM treatment possessed a more porous microstructure with many larger and more connected intra-aggregate pores originating from the decay of roots or other organic debris. It is worth noting that all the aggregates exhibited a relatively homogeneous microstructure without any evidence of hierarchy (Fig. 2).

The intra-aggregate pores that could be distinguished at the image resolution (i.e., 3.7 μm) were quantified. Aggregates from the NPKOM treatment had higher intra-aggregate porosity (5.62%) than aggregates from the CK (3.61%) and NPK (3.33%) treatments (Table 3). The PSD had a similar trend as the porosity, porosities was highest in the NPKOM treatment for different sizes although the differences were not statistically significant (Fig. 3).

Application of NPK fertilizer did not affect intra-aggregate porosity or PSD compared with the CK treatment (Fig. 3). Both visual observation and quantitative analysis revealed that application of NPKOM, but not NPK, increased the intra-aggregate porosity.

3.3 Structure of small soil cores (CoreS)

Figure 4 showed the representative vertical slice from the center of the CoreS and the corresponding 3-D images. The structure of CoreS was more porous than aggregates (Fig.2). Two types of pores were identified in the CK and NPK treatments: cracks and channels. Cracks appeared as planar shaped pores resulted from shrinkage while channels were mainly root channels in this study. Cracks dominated in the CoreS in both CK and NPK treatments while few cracks were observed in the CoreS from NPKOM treatment. The pores in the CoreS from NPKOM treatment were mostly composed of smooth channels with round or tubular shapes. And the CoreS from NPKOM treatment showed a more porous structure than those from CK and NPK treatment.

Quantitative analysis indicated CoreS from NPKOM treatment had the highest porosity followed by NPK treatment and CK treatment, while the differences between the latter two were not statistically different (Table 3). Pore size distribution of the CK and NPK treatments showed pores peaked at 238 μm, which is in accordance with the width of the cracks that mostly lied in the range 150 - 300 μm. The peak of pores of NPKOM treatment was at 473 μm (Fig. 5), which was similar to the size of the small channels (Fig. 4). For pores ≤ 370 μm no significant difference was found among the treatments. For pores > 370μm, however, porosity was significantly higher in NPKOM treatment than in CK and NPK treatments ($P<0.05$). Porosity for the whole pore size range was higher in NPK treatment than in CK treatment but the difference was not statistically significant (Fig. 5).

3.4 Structure of large soil cores (CoreL)

Several vertically oriented cracks that penetrated the whole sample were identified from the vertical 2-D and 3-D images of the CoreLs as shown in Fig. 6. From the 2-D observation, CoreLs from the CK and NPK treatments had a similar pattern of pore space, which was dominated by cracks. The 3-D pore structure revealed elongated channels in the samples from the CK and NPK treatments. Long-term application of NPKOM, however, increased the complexity of the pore system. More complex pores, which were connected or disconnected with cracks, were observed in the NPKOM treatment compared with the CK and NPK treatments. Visual observation showed the presence of half-decayed rice straw and roots inside the pores, most likely from incorporation into the soil. Porosity for the CK and NPK treatments were 10.0% and 9.9%, respectively, significantly lower than that of the NPKOM treatment (16.2%). PSD (Fig. 7) showed similar trend as CoreS (Fig. 5). NPKOM had a higher porosity for all the pore size classes than the CK and NPK treatments, while the latter two showed little difference. The highest porosity was found for the pores with diameter 2790 μm, confirming the existence of large pores in the CoreLs (Fig. 6).

4 Discussion

4.1 Effects of inorganic and organic fertilization on soil properties

Long-term application of NPKOM increased SOC due to the addition of organic manure and increased input of stubble and roots as a result of increased yields (Yan et al., 2013). As SOC increased, bulk density decreased and total porosity increased. Similar positive effects of using NPKOM on SOC, bulk density, and AWC have been reported in previous studies

(Edmeades, 2003; Haynes and Naidu, 1998; Rasool et al., 2007; Naveed et al., 2014). The long-term application of inorganic fertilizer, however, showed no difference in SOC, bulk density, total porosity, and AWC relative to CK treatment. The aggregate water stability test indicated long-term use of inorganic fertilizer decreased soil stability, which is consistent with a previous study at this experimental site (Yan et al., 2013). Blanco-Canqui and Schlegel (2013) also reported that aggregate stability of a Ulysses silt loam decreased after 50-years of inorganic fertilization, although SOC concentration increased. For the paddy soil, the main binding agents of aggregation are attributed to SOC and iron oxides (specially oxalate-soluble Fe, Fe_o) (Kögel-Knabner et al., 2010), both of which however were not decreased in the NPK treatment (Table 2). The decline in aggregate water stability in NPK treatment could be attributed to the addition of dispersing ions included in the fertilizers. However, further studies are needed to investigate the mechanisms.

4.2 Structure of paddy soil across scales

The structure of paddy soil showed distinct morphological characteristics at different scales. Soil aggregates showed a dense, massive structure with only discrete small pores (Fig. 2). This was because puddling at the beginning of the growing season destroyed the soil structure, particularly macro-aggregates, and therefore the intra-aggregate pore system was less developed. Root penetration and swell/shrink are the main factors of structure evolution in a paddy soil. Root penetration resulted in elongated channels with round-shaped cross sections, while shrinkage generated cracks (Sander et al., 2008). These two types of pores were both frequently observed from the 2-D and 3-D images of soil cores (Fig. 4, 6). CoreS images showed abundant small secondary cracks that were randomly distributed in the samples, while the CoreL showed only a few vertical primary cracks that penetrated the whole sample.

Soil aggregates, CoreS and CoreL exhibited different morphology and therefore had different pore characteristics. Considering the CK treatment, the porosities for aggregate, CoreS and CoreL were 3.55%, 5.21% and 9.35% respectively (Table 3). When scanning a smaller sample with a higher resolution by CT, the finer pores are detectable and the porosity is expected to increase. However, the paddy soils are very heterogeneous, with the presence of cracks. Samplings of small cores are normally conducted avoiding big primary cracks and usually on an apparently homogeneous area as shown in Fig. 8a. In this case, although some finer secondary or tertiary cracks were detected, information concerning the primary cracks was lost (Fig. 8b). This was confirmed by the PSD as shown in Fig. 9. CoreL had more >500 μm pores than CoreS while the latter had more <500 μm pores. Schlüter et al. (2011) scanned soil cores of different size (diameter 77mm and 46 mm, respectively) with different resolution (75 μm and 50 μm, respectively) and also found the large cores were preferable for capturing large pores. At the aggregate scale, the porosity only reflected the intra-aggregate structure and the pore sizes were small within the range between 3.7 and 115 μm (Fig. 9). The aggregates of paddy soil are less well developed due to puddling as mentioned above, which leads to a lower porosity in soil aggregates than larger samples with cracks and inter-aggregate pores. Dal Ferro et al. (2013) found the porosity of soil aggregates was always greater than that of soil cores because of the increased resolution. This discrepancy might be caused by the homogeneous structure and more developed intra-aggregate microstructure of the silty loam soil in their study.

4.3 Effects of long-term inorganic and organic fertilization on the micro and macro scale pore structure

Effects of inorganic fertilizer on soil aggregation have been under discussion for a long time but without a unanimous conclusion due to the difference in soil type, fertilizer type, fertilizer amount, crop type, and tillage etc. (Blanco-Canqui and Schlegel, 2013). On one hand, inorganic fertilizer improves crop yields and thereafter increases the return of biomass to the soil in the form of roots and residues. This leads to the accumulation of SOC which is a major binding agent of soil aggregation (Blanco-Canqui and Lal, 2004). From the other perspective the use of inorganic fertilizer introduces ions that disperse soil colloids and secondary particles and then reduce aggregation (Haynes and Naidu, 1998). Previously both positive (Rasool et al., 2007) and negative (Blanco-Canqui et al., 2014) effects of inorganic fertilization on soil aggregation have been reported. Most of those findings were based on the test of soil aggregate stability (Darusman et al., 1991; Blanco-Canqui and Schlegel, 2013) while very few have been investigated using CT (Zhou et al., 2013; Naveed et al., 2014). In this study, the stability tests showed negative or no effect of inorganic fertilization on soil aggregation (Fig. 1) although yields increased (Bi, et al., 2009). Visual observation of pore morphology and quantification of the pore system showed that the long-term application of NPK did not change intra-aggregate structure as compared with unfertilized soil. These findings are in agreement with our previous study on an upland Ultisol which was close to the experiment field (Zhou et al., 2013). In contrast, long-term application of NPKOM helped the development of intra-aggregate pores system through increasing soil organic matter, which helped the binding of soil particles and micro-aggregates and also formed intra-aggregate pores through the decay of the manure. The shape of the pores in the 2-D and 3-D aggregate images also revealed more root channels in the NPKOM treatment, suggesting it offered a more beneficial environment for root to penetrate.

At the soil core scale the difference in pore structure as affected by inorganic and organic fertilization was more pronounced than at the aggregate scale. Cracks were the main constitution of the pore system in CK and NPK treatment (Fig.4, 6), suggesting shrinkage/swell process was the main mechanism of the formation of pores in the two treatments. Cracks in CK and NPK treatment were planar in shape (Fig. 4) and could potentially impede the elongation of roots (Dexter, 1988). They were also less connected compared to the pores in NPKOM treatment (Fig. 4), which leads to a less aerated environment for rice cultivation. The pores in the NPKOM treatment were mostly of biological origin and cracks were seldom observed. This indicated that long-term application of NPKOM altered the mechanical properties and the formation mechanisms of the soil pore system.

5 Conclusions

The intra-aggregate and inter-aggregate structure of paddy soil was assessed by scanning multi-scale soil samples at different resolutions. The porosity of the paddy soil increased with the increasing samples size due to the incorporation of more cracks in the larger samples. However, the trends in soil porosity between the different treatments were similar at the aggregate, small core, and large core scales, respectively.

Long-term fertilization affected soil structure at all scales. Soil in CK and NPK treatment had a similar structure, with a dense intra-aggregate structure and a low porosity at aggregate scale, and a low porosity in the form of cracks at both small and large core scales.

Application of NPKOM improved the intra-aggregate and inter-aggregate pore system mainly due to the development of biopores. Relative to CK, application of NPKOM increased soil porosity by 58.3%, 144.9%, and 65.9% at aggregate, CoreS, and CoreL scales, respectively. Saturated hydraulic conductivity, plant available water capacity, and SOC were also significantly improved in NPKOM treatment but not in NPK treatment. This study suggested inorganic fertilizer did not improve soil structure and highlighted the importance of using organic manure to improve the physical quality of paddy soil.

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References

Anders, M.M., Brye, K.R., Olk, D.C., Schmid, B.T., 2012. Rice rotation and tillage effects on soil aggregation and aggregate carbon and nitrogen dynamics. Soil Sci. Soc. Am. J. 76, 994-1004.

Atkinson, B.S., Sparkes, D.L. & Mooney, S.J. 2009. Effect of seedbed cultivation and soil macrostructure on establishment of winter wheat (Triticum aestivum). Soil Till. Res, 103, 291-301.

Blanco-Canqui, H., and R. Lal. 2004. Mechanisms of carbon sequestration in soil aggregates. Crit. Rev. Plant Sci. 23:481–504.

Blanco-Canqui, H., Schlegel, A.J., 2013. Implications of inorganic fertilization of irrigated corn on soil properties: lessons learned after 50 years. J. Environ. Qual. 42, 861-871.

Brar, B., Singh, K., Dheri, G., 2013. Carbon sequestration and soil carbon pools in a rice–wheat cropping system: Effect of long-term use of inorganic fertilizers and organic manure. Soil Till. Res. 128, 30-36.

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma 124, 3-22.

Carter, M.R., 2004. Researching structural complexity in agricultural soils. Soil Till. Res. 79, 1-6.

Dal Ferro, N., Charrier, P., Morari, F., 2013. Dual-scale micro-CT assessment of soil structure in a long-term fertilization experiment. Geoderma 204–205, 84–93.

Darusman, L.R. Stone, D.A. Whitney, K.A. Janssen, and J.H. Long. 1991. Soil properties after twenty years of fertilization with different nitrogen sources. Soil Sci. Soc. Am. J. 55:1097–1100.

Das, B., Chakraborty, D., Singh, V.K., Aggarwal, P., Singh, R., Dwivedi, B.S., Mishra, R.P., 2014. Effect of integrated nutrient management practice on soil aggregate properties, its stability and aggregate-associated carbon content in an intensive rice–wheat system. Soil Till. Res. 136, 9-18.

Dexter, A.R., 1988. Advances in characterization of soil structure. Soil Till. Res. 11, 199-238.

Duiker, S.W., Rhoton, F.E., Torrent, J., Smeck, N.E., Lal, R., 2003. Iron (Hydr)oxide crystallinity effects on soil aggregation. Soil Sci. Soc. Am. J. 67, 606-611.

Edmeades, D., 2003. The long-term effects of manures and fertilisers on soil productivity and quality: a review. Nutr. Cycl. Agroecosys. 66, 165-180.

Eickhorst T., Tippkötter R., 2009. Management-induced structural dynamics in paddy soils of south east China simulated in microcosms. Soil Till. Res 102(2): 168-178.

Gong Z., 1986. Origin, evolution, and classification of paddy soils in China, In: Stewart, B.A. (Ed.), Advances in Soil Science. Springer New York, pp. 179-200.

Haynes, R.J., Naidu, R., 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutr. Cycl. Agroecosys. 51, 123-137.

Helliwell, J.R., Miller, A.J., Whalley, W.R., Mooney, S.J., Sturrock, C.J., 2014. Quantifying the impact of microbes on soil structural development and behaviour in wet soils. Soil Biol. Biochem. 74, 138-147.

Hill, R., Horton, R., Cruse, R., 1985. Tillage effects on soil water retention and pore size distribution of two Mollisols. Soil Sci. Soc. Am. J. 49, 1264-1270.

Huang, S., Peng, X., Huang, Q., Zhang, W., 2010. Soil aggregation and organic carbon fractions affected by long-term fertilization in a red soil of subtropical China. Geoderma 154, 364-369.

Iassonov, P., Gebrenegus, T., Tuller, M., 2009. Segmentation of X- ray computed tomography images of porous materials: A crucial step for characterization and quantitative analysis of pore structures. Water Resour. Res. 45.

Kirchhof, G., Priyono, S., Utomo, W., Adisarwanto, T., Dacanay, E., So, H., 2000. The effect of soil puddling on the soil physical properties and the growth of rice and post-rice crops. Soil Till. Res. 56, 37-50.

Kögel-Knabner, I., Amelung, W., Cao, Z., Fiedler, S., Frenzel, P., Jahn, R., Kalbitz, K., Kölbl, A., Schloter, M., 2010. Biogeochemistry of paddy soils. Geoderma 157, 1-14.

Li Q, 1992. Paddy soils of China. Beijing: Chinese Science Press.

Li, J., Zhang, B., 2007. Paddy soil stability and mechanical properties as affected by long-term application of chemical fertilizer and animal manure in subtropical China. Pedosphere 17, 568-579.

Liu, C., Cheng, S., Yu, W., Chen, S., 2003. Water infiltration rate in cracked paddy soil. Geoderma 117, 169-181.

Luo, L., Lin, H., Halleck, P., 2008. Quantifying soil structure and preferential flow in intact soil using X-ray computed tomography. Soil Sci. Soc. Am. J. 72, 1058-1069.

Martin, S.L., Mooney, S.J., Dickinson, M.J. & West, H.M. 2012. The effects of simultaneous root colonisation by three G*lomus* species on soil pore characteristics. *Soil Biol. Biochem.* 49, 167-173.

Marshall, T., 1958. A relation between permeability and size distribution of pores. J. Soil Sci. 9, 1-8.

Moldrup, P., Olesen, T., Komatsu, T., Schjønning, P., Rolston, D., 2001. Tortuosity, diffusivity, and permeability in the soil liquid and gaseous phases. Soil Sci. Soc. Am. J. 65, 613-623.

Mooney, S.J., Morris, C., Craigon, J. & Berry, P.M. 2007. Quantification of soil structural changes induced by cereal anchorage failure: Image analysis of thin sections. J. Plant Nutr。 Soil Sci. 170, 1-10.

Murphy, C., 1986. Thin section preparation of soils and sediments. AB Academic.

Pagliai, M., Vignozzi, N., Pellegrini, S., 2004. Soil structure and the effect of management practices. Soil Till. Res. 79, 131-143.

Peth, S., Horn, R., Beckmann, F., Donath, T., Fischer, J., Smucker, A.J.M., 2008. Three-dimensional quantification of intra-aggregate pore-space features using synchrotron-radiation-based microtomography. Soil Sci. Soc. Am. J. 72, 897-907.

Rachman A, Anderson S H, Gantzer C J., 2005. Computed-tomographic measurement of soil macroporosity parameters as affected by stiff-stemmed grass hedges. Soil Sci. Soc. Am. J. 69(5): 1609-1616.

Rasool, R., Kukal, S., Hira, G., 2007. Soil physical fertility and crop performance as affected by long term application of FYM and inorganic fertilizers in rice–wheat system. Soil Till. Res. 96, 64-72.

Sander, T., Gerke, H. H., Rogasik, H., 2008. Assessment of Chinese paddy-soil structure using X-ray computed tomography. Geoderma 145:303-314

Sander, T., Gerke, H.H., 2007. Preferential flow patterns in paddy fields using a dye tracer. Vadose Zone J. 6, 105-115.

SAS Institute, 2011. SAS Users Guide. SAS Institute, Cary, NC.

Schlüter, S., Weller, U., Vogel, H.J., 2011. Soil- structure development including seasonal dynamics in a long- term fertilization experiment. J. Plant Nutr. Soil Sci. 174, 395-403.

Sharma, P., Datta, S., 1986. Physical properties and processes of puddled rice soils, In: Stewart, B.A. (Ed.), Advances in Soil Science. Springer New York, pp. 139-178.

Taina, I.A., Heck, R.J., Elliot, T.R., 2008. Application of X-ray computed tomography to soil science: A literature review. Can. J. Soil Sci. 88, 1-19.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water - stable aggregates in soils. Euro. J. Soil Sci. 33, 141–163.

Vogel, H., Kretzschmar, A., 1996. Topological characterization of pore space in soil—sample preparation and digital image-processing. Geoderma 73, 23-38.

Vos, M., Wolf, A.B., Jennings, S.J., Kowalchuk, G.A., 2013. Micro-scale determinants of bacterial diversity in soil. FEMS Microbiol. Rev. 37, 936-954.

Wang, W., Kravchenko, A., Smucker, A., Rivers, M., 2011. Comparison of image segmentation methods in simulated 2D and 3D microtomographic images of soil aggregates. Geoderma 162, 231-241.

Wildenschild, D., Vaz, C., Rivers, M., Rikard, D., Christensen, B., 2002. Using X-ray computed tomography in hydrology: systems, resolutions, and limitations. J. Hydrol. 267, 285-297.

Yagi, K., Minami, K., 1990. Effect of organic matter application on methane emission from some Japanese paddy fields. Soil Sci. Plant Nutr. 36, 599-610.

Yan, X., Zhou, H., Zhu, Q., Wang, X., Zhang, Y., Yu, X., Peng, X., 2013. Carbon sequestration efficiency in paddy soil and upland soil under long-term fertilization in southern China. Soil Till. Res. 130, 42-51.

Young, I.M., Crawford, J.W., 2004. Interactions and self-organization in the soil-microbe complex. Science 304, 1634-1637.

Zhang, Y., Lin, X., Werner, W., 2003. The effect of soil flooding on the transformation of Fe oxides and the adsorption/desorption behavior of phosphate. J. Plant Nutr. Soil Sci. 166, 68-75.

Zhang, Z., Peng, X., Wang, L., Zhao, Q., Lin, H., 2013. Temporal changes in shrinkage behavior of two paddy soils under alternative flooding and drying cycles and its consequence on percolation. Geoderma, 192, 12-20.

Zhong, W., Cai, Z., 2007. Long-term effects of inorganic fertilizers on microbial biomass and community functional diversity in a paddy soil derived from quaternary red clay. Appl. Soil Ecol. 36, 84-91.

Zhou, H., Peng, X., Perfect, E., Xiao, T., Peng, G., 2013. Effects of organic and inorganic fertilization on soil aggregation in an Ultisol as characterized by synchrotron based X-ray micro-computed tomography. Geoderma 195, 23-30.

Zhou, H., Peng, X., Peth, S., Xiao, T., 2012. Effects of vegetation restoration on soil aggregate microstructure quantified with synchrotron-based micro-computed tomography. Soil Till. Res. 124, 17-23.