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

Hu Zhou^{*1}, Huan Fang^{1,2}, Sacha Jon Mooney³, Xinhua Peng¹

¹ State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil
Sciences, Chinese Academy of Sciences. 71 East Beijing Road, Nanjing 210008,
P.R. China

² University of Chinese Academy of Sciences, Beijing 100049, China

³ Division of Agricultural and Environmental Sciences, School of Biosciences,
University of Nottingham, Sutton Bonington Campus, Loughborough,
Leicestershire LE12 5RD, UK

* Corresponding author

Phone: 86(25) 86881221

Fax: 86(25) 86881000

E-mail: zhouhu@issas.ac.cn

26 **Abstract**

27 The soil structure of paddy soil is very dynamic from the aggregate to the pedon scale
28 because of intensive anthropogenic management strategies. In this study, we tested the
29 hypothesis that long-term inorganic and organic fertilization can affect soil structure at
30 different scales. Microstructure assessed by soil aggregates (3 - 5 mm in diameter) and
31 macrostructure assessed by small soil cores (CoreS) (5 cm in diameter, 5 cm in height) and
32 large soil cores (CoreL) (10 cm in diameter, 10 cm in height) were sampled from three
33 long-term fertilization treatments, including no fertilizer (CK), application of inorganic
34 fertilizer (NPK), and a combination of inorganic fertilizer and organic manure (NPKOM),
35 established in 1982. They were scanned at two scales with two types of micro-Computed
36 Tomography (micro-CT) and quantified using image analysis. Results showed that relative to
37 CK treatment, long-term NPKOM fertilization increased soil organic C (SOC) by 28% and
38 available water content (AWC) by 20%, but decreased soil bulk density by 0.2 g cm^{-3}
39 whereas NPK showed no difference. Soils under CK and NPK treatments exhibited an
40 identical dense structure at both aggregate and core scales in which pores were mainly
41 cracks resulting from shrink/swell processes, and showed no significant difference in porosity
42 and size distribution of the CT-identified pores ($> 3.7 \text{ }\mu\text{m}$). As compared with the CK
43 treatment, the soil in the NPKOM treatment had greater intra- and inter-aggregate pores,
44 and increased porosity by 58.3%, 144.9%, and 65.9% at aggregate, CoreS, and CoreL
45 scales, respectively. These were attributed to the biopores formed from decayed roots,
46 stubble, and organic manures as a result of increased yields and direct amendment of
47 organic manure. Overall, this study demonstrates that organic fertilization can improve the

48 physical qualities of paddy soils across different scales but inorganic fertilization in isolation
49 does not.

50 **Key Words**

51 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 ($> 0.75 \text{ mm}$) 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

74 structure of soil was typically conducted on soil thin sections (Pagliai et al., 2004; Mooney et
75 al. 2007). However, soil thin section can only provide two-dimensional (2D) information of
76 soil structure. And the preparation of thin sections is time consuming (Murphy, 1986).
77 Computed Tomography (CT) offers a rapid and non-destructive way to study soil structure
78 over a range of scales (Taina et al., 2008; Wildenschild et al., 2002, Helliwell et al., 2014).
79 High-resolution CT can show the detailed organization of soil aggregates and has been used
80 to study aggregation processes (Atkinson et al. 2009; Zhou et al., 2013), soil microstructure
81 (Peth et al., 2008), and soil biophysical interactions (Martin et al. 2012; Vos et al., 2013) at
82 the aggregate scale. CT with a low resolution, on the other hand, can scan large samples and
83 is frequently used to study macropores and their relationship with soil hydraulic properties
84 (Luo et al., 2008) at the soil core scale. The study of the micro and macro scale soil structure
85 is possible by using a combination of CT systems with different resolution capabilities.
86 Schlüter et al. (2011) studied soil structure development at two different scales and
87 proposed a method to combine soil pore size distribution (PSD) acquired at the different
88 scales. Dal Ferro et al. (2013) found that both the micro- and macro-scales soil structure
89 were affected by fertilization from the scanning of soil aggregates and soil cores using
90 micro-CT.

91 Rice is the most important staple food in China and the cultivation area of rice is 25
92 million ha, accounting for 25% of the national arable land area (Li, 1992). Long-term
93 traditional cultivation of rice, specifically flooding during most of the growing season,
94 drastically changed soil physical, chemical, and biological properties and resulted in a special
95 anthropogenic paddy soil (Gong, 1986). The structure of paddy soil is more dynamic at the

96 aggregate to soil core scales compared with those of upland soils. The plough layer of the
97 paddy soil is homogenized before each growing season to prepare the seedbed, which
98 destroys surface soil structure considerably (Eickhorst and Tippkötter, 2009; Kirchhof et al.,
99 2000; Sharma and Datta, 1986). Moreover, paddy soil experiences frequent swell-shrink
100 cycles caused by periodic flooding and drying management (Zhang et al., 2013). These
101 processes are accompanied by the creation and closing of cracks which has critical
102 importance for the evolution of the structure of paddy soil (Liu et al., 2003; Sander and
103 Gerke, 2007). At the micro-scale, aggregation of paddy soil is greatly influenced by the
104 oxidation-reduction conditions caused by flooding and drainage cycles (Kögel-Knabner et al.,
105 2010). For example, Fe oxides are important binding agents of soil aggregates, but their
106 effects vary among different Fe species (Duiker et al., 2003). Poorly crystalline Fe has a
107 larger and more reactive surface area and therefore is more effective in soil aggregation than
108 crystalline Fe (Duiker et al., 2003; Yan et al., 2013). Repeated flooding and drainage cycles
109 have been shown to increase Fe_o oxides while reducing Fe_d oxides; therefore, these
110 processes are beneficial to soil aggregation (Zhang et al., 2003).

111 The application of organic or inorganic fertilizers can directly or indirectly introduce
112 different ions and organic matter to the soil, which may cause soil disaggregation or
113 aggregation (Haynes and Naidu, 1998). In the past few years, research regarding the effects
114 of fertilization on paddy soil has mostly focused on SOC sequestration (Anders et al., 2012;
115 Brar et al., 2013; Das et al., 2014), greenhouse gas emissions (Yagi and Minami, 1990), and
116 microbial and geochemical processes (Zhong and Cai, 2007) due to environmental and
117 ecological concerns. These processes are closely linked with soil structure, which determines

118 the transport of water, gas and solutes and provides a habitat for soil microorganisms (Young
119 and Crawford, 2004). Although the change in aggregate stability under fertilization in paddy
120 soils has been evaluated (Li and Zhang. 2007; Huang et al., 2010; Yan et al., 2013), the
121 effect of fertilization on the formation and dynamics of the structure of paddy soil is still
122 unclear.

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

129 **2 Materials and methods**

130 ***2.1 Experimental site***

131 Soil was taken from long-term experiment established in 1982 at the Jiangxi Institute of
132 Red Soil, Jinxian County, Jiangxi Province, China (116°10' E, 28°21' N). The experiment site
133 lies in a flat area of the hilly region of Southern China. The experiment site has a subtropical
134 climate and a mean annual temperature and precipitation of 17.7 °C and 1706 mm,
135 respectively. The paddy soil (Typic Stagnic Anthrosols, Chinese Soil Taxonomic Classification,
136 2002) is clay loam (20% sand, 48% silt, and 32% clay) for the plough layer (0- to 15 cm).
137 Before the long-term experiment, the paddy soil had organic C 16.3 g kg⁻¹, total N 1.49 g kg⁻¹,
138 and pH 6.9 in the plough layer. The site had been cultivated with rice for more than 100
139 years prior to the experiment. The cropping system is early rice – late rice from April to

140 October and fallow in the winter.

141 The experiment was designed as a randomized complete block with three replicates.
142 Three fertilization treatments were studied: (1) no fertilization as a control (CK), (2) a
143 combination of inorganic fertilizers (NPK), including 90 kg N ha⁻¹, 20 kg P ha⁻¹, and 62 kg K
144 ha⁻¹ for each season; and (3) organic manure and the inorganic fertilizers (NPKOM) together,
145 including the same amount of inorganic fertilizer as the NPK treatment plus 22.5 t ha⁻¹ pig
146 manure. Each plot had an area of 46.67 m². A detailed description of the management of the
147 field experiment can be found in Yan et al. (2013).

148 **2.2 Sampling**

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

159 **2.3 CT scanning and image reconstruction**

160 Both CoreL (n = 27) and CoreS (n = 27) were scanned at field moisture (0.30 - 0.35 cm³
161 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 $30 \times 30 \times 30 \mu\text{m}^3$. 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 $3.7 \times 3.7 \times 3.7 \mu\text{m}^3$.

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

184 were resized by binning the voxels by a factor of 3 and 2 for the CoreL and CoreS,
185 respectively to facilitate further image processing and analysis. The resulting images had a
186 resolution of 90 μm and 60 μm for the CoreL and CoreS, respectively. A region of interest
187 (ROI) was selected from the central part of soil cores to avoid artifacts at the boundary
188 region caused by sampling (Table 1). Images of the soil aggregates were first preprocessed
189 to remove ring artifacts (Zhou et al., 2011). The image stacks were cropped to a ROI of
190 $500 \times 500 \times 500$ voxels, representing a volume of $1.85 \times 1.85 \times 1.85 \text{ mm}^3$.

191 Procedures of image processing and subsequent analysis were identical for the ROIs of
192 both soil cores and soil aggregates. A three dimensional median filter was used to reduce
193 noise before image segmentation. Segmentation is critical to the quantitative
194 characterization of the pore system (Iassonov et al. 2009). Comparisons of different
195 methods in previous studies have showed the disadvantages of the global threshold method
196 and indicated the advantages of using the local threshold method for soil samples (Iassonov
197 et al., 2009; Wang et al., 2011). In this study, a bi-level method (Vogel and Kretzschmar,
198 1996) was used to segment soil pores and solids. Briefly, two threshold values (T_0 and T_1)
199 were selected based on the histogram, voxels with a grayscale value smaller than T_0 or larger
200 than T_1 were classified as pores or solids, respectively. The unclassified voxels with grayscale
201 values between T_0 and T_1 were attributed to pores if one of the 26 neighbours was a pore.
202 This process iterated until there were no more changes, and the remaining unclassified
203 voxels were attributed to solids.

204 **2.5 Pore system analysis**

205 Although there are many descriptors (e.g., shape and topology parameters) of the pore

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

213 **2.6 Soil properties analysis**

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

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

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

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 (K_s), 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 $\text{NPKOM} > \text{NPK} > \text{CK}$. SSA was similar among the treatments. Grain yields significantly increased in the order of $\text{NPKOM} > \text{NPK} > \text{CK}$. The MWD was lowest when determined using the fast wetting method than using the mechanical

248 stirring and slow wetting methods (Fig. 1), indicating that slaking was the main breakdown
249 mechanism for the studied paddy soil. The MWD values from the mechanical stirring and slow
250 wetting methods were close to 3 mm, showing that the aggregates of paddy soil were
251 resistant to mechanical disturbance and partial swelling. The difference in aggregate water
252 stability was only found using the slow wetting method and soils receiving long-term
253 inorganic fertilizer showed significant lower stability compared with the CK and NPKOM
254 treatments.

255 **3.2 Microstructure of soil aggregates**

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

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

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

273 **3.3 Structure of small soil cores (CoreS)**

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

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

292 treatment but the difference was not statistically significant (Fig. 5).

293 **3.4 Structure of large soil cores (CoreL)**

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

308 **4 Discussion**

309 **4.1 Effects of inorganic and organic fertilization on soil properties**

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

314 (Edmeades, 2003; Haynes and Naidu, 1998; Rasool et al., 2007; Naveed et al., 2014). The
315 long-term application of inorganic fertilizer, however, showed no difference in SOC, bulk
316 density, total porosity, and AWC relative to CK treatment. The aggregate water stability test
317 indicated long-term use of inorganic fertilizer decreased soil stability, which is consistent with
318 a previous study at this experimental site (Yan et al., 2013). Blanco-Canqui and Schlegel
319 (2013) also reported that aggregate stability of a Ulysses silt loam decreased after 50-years
320 of inorganic fertilization, although SOC concentration increased. For the paddy soil, the main
321 binding agents of aggregation are attributed to SOC and iron oxides (specially
322 oxalate-soluble Fe, Fe_o) (Kögel-Knabner et al., 2010), both of which however were not
323 decreased in the NPK treatment (Table 2). The decline in aggregate water stability in NPK
324 treatment could be attributed to the addition of dispersing ions included in the fertilizers.
325 However, further studies are needed to investigate the mechanisms.

326 ***4.2 Structure of paddy soil across scales***

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

336 the samples, while the CoreL showed only a few vertical primary cracks that penetrated the
337 whole sample.

338 Soil aggregates, CoreS and CoreL exhibited different morphology and therefore had
339 different pore characteristics. Considering the CK treatment, the porosities for aggregate,
340 CoreS and CoreL were 3.55%, 5.21% and 9.35% respectively (Table 3). When scanning a
341 smaller sample with a higher resolution by CT, the finer pores are detectable and the porosity
342 is expected to increase. However, the paddy soils are very heterogeneous, with the presence
343 of cracks. Samplings of small cores are normally conducted avoiding big primary cracks and
344 usually on an apparently homogeneous area as shown in Fig. 8a. In this case, although some
345 finer secondary or tertiary cracks were detected, information concerning the primary cracks
346 was lost (Fig. 8b). This was confirmed by the PSD as shown in Fig. 9. CoreL had more >500
347 μm pores than CoreS while the latter had more <500 μm pores. Schlüter et al. (2011)
348 scanned soil cores of different size (diameter 77mm and 46 mm, respectively) with different
349 resolution (75 μm and 50 μm , respectively) and also found the large cores were preferable
350 for capturing large pores. At the aggregate scale, the porosity only reflected the
351 intra-aggregate structure and the pore sizes were small within the range between 3.7 and
352 115 μm (Fig. 9). The aggregates of paddy soil are less well developed due to puddling as
353 mentioned above, which leads to a lower porosity in soil aggregates than larger samples with
354 cracks and inter-aggregate pores. Dal Ferro et al. (2013) found the porosity of soil
355 aggregates was always greater than that of soil cores because of the increased resolution.
356 This discrepancy might be caused by the homogeneous structure and more developed
357 intra-aggregate microstructure of the silty loam soil in their study.

358 **4.3 Effects of long-term inorganic and organic fertilization on the micro and macro** 359 **scale pore structure**

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

380 pores through the decay of the manure. The shape of the pores in the 2-D and 3-D aggregate
381 images also revealed more root channels in the NPKOM treatment, suggesting it offered a
382 more beneficial environment for root to penetrate.

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

393 **5 Conclusions**

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

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

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

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