Accumulation of physically protected organic carbon promoted biological activity in macro-aggregates of rice soils under long term rice cultivation

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Running title: carbon and microbial activity in aggregates of rice soil

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23 **Abstract:**

24 While soil organic carbon (OC) accumulation and stabilization had been increasingly
25 concerned as ecosystem properties, the link between carbon stabilization and soil
26 biological activity had been poorly assessed. In this study, topsoil samples were
27 collected from soils shifted from salt marsh to rice cultivation for different lengths up
28 to 700 years from a coastal area of eastern China. Particle size fractions of soil
29 aggregates were separated using a low energy dispersion protocol. OC chemical groups
30 in the fractions were analyzed with Fourier transform infrared (FTIR) spectroscopy
31 while OC pools using chemical procedures. Soil microbial community of bacterial,
32 fungal and archaeal were analyzed with molecular fingerprinting using specific gene
33 primers. Soil respiration and enzyme activities were respectively measured, using lab
34 incubation protocols. While the aggregate size fractions were dominated by fine sand
35 (200-20μm) and silt (20-2μm) fractions, the mass proportion both of coarse sand (2000-
36 200μm) and clay (<2μm) fraction increased with prolonged rice cultivation. SOC was
37 enriched highly in coarse sand fraction (40-60 g kg^{-1}), moderately in clay fraction (20-
38 25 g kg^{-1}), but depleted in silt fraction (~10 g kg^{-1}). Recalcitrant OC pool was higher
39 (0.9-3.7%) in both coarse sand and clay fractions than in fine sand and silt fractions
40 (0.6-2.3%). Total soil DNA content in the size fractions followed a similar trend to that
41 of OC. Gene abundance of bacteria and of archaeal were concentrated in both sand and
42 clay fractions, but their diversity generally consistent between the fractions. However,
43 gene abundance and diversity of fungi generally peaked in coarse sand fraction only,
44 decreasing respectively sharply and gently with decreasing size of the aggregate

fractions. Soil respiration quotient (ratio of respired CO_2-C to total OC) was highest in silt fraction, followed by the fine sand fraction but lowest in coarse sand and clay fractions in the rice soils cultivated over 100 years. Whereas, microbial metabolic quotient was lower in sand sized fraction than in other fractions. Scaled by total DNA concentration, respiration was higher in silt fraction than in other fractions for the rice soils. For the size fractions other than clay fraction, OC scaled DNA concentration, archaeal gene abundance and normalized enzyme activity were seen increased but SOC- and DNA- content scaled soil respiration decreased, more or less with prolonged rice cultivation. Finally, both microbial gene abundance and normalized enzyme activity were well correlated to SOC and labile OC content in coarse sand fraction only though chemical stability and respiratory of OC were similar between coarse sand and clay fractions. Thus, biological activity was generally promoted with accumulation of physically protected organic carbon in coarse sand sized macro-aggregates of the rice soils, being in a positive response to prolonged rice cultivation management. However, the mechanism underspin this trend and the effects on soil functions deserve further studies under field conditions.

Key words: rice soil, carbon stabilization, soil bio-activity, soil aggregates, size fractions, rice cultivation, microbial community, chronosequence

64 **1 Introduction**

65 Soil organic matter (SOM), as a continuum of organic substances with different degree
66 of decomposition (Lehmann and Kleber, 2015), provided a key driver for soil
67 aggregation, mediating soil ecosystem functions and services (Banwart et al., 2014).
68 Soil aggregates had been considered as fundamental soil particle units where organic
69 matter, minerals and microbes interacted to store carbon and nutrient as well as moisture
70 (Tisdall and Oades, 1982; Lützow et al., 2006; Marschner et al., 2008; Schmidt et al.,
71 2011), and mediated their cycling in soil-plant systems (Six et al., 2004). It had been
72 increasingly considered as a primary mechiansm for soil carbon sequestration that
73 organic carbon (OC) tended physically protected against microbial access and
74 decomposition (Blanco-Canqui and Lal, 2004; Six et al., 2004; Kong et al., 2005; Six
75 and Paustian, 2014), with separate allocation of mineral associated OM fractions
76 (Lehmann et al., 2008; Dungait et al., 2012; Vogel et al., 2014) between micro-
77 aggregates within macro-aggregates. Soil aggregation shaped the micro-habitats for soil
78 microbial communities (Six et al., 2000; Ettema and Wardle, 2002; Balser et al., 2006;
79 Kögel-Knabner et al., 2008), with changes in OC substrate availability, chemical
80 recalcitrance and redox potential with or within aggregates (Rillig et al., 2001; Six et
81 al., 2006; Strickland and Rousk, 2010). Consequently, changes in composition of soil
82 agregate fractions could lead to changes in bio-activity as a whole determined by size,
83 diversity and biochemical activity of soil microbes (Six et al., 2006; Lagomarsino et al.,
84 2012; Bardgett and van der Putten, 2014). Particularly, particulate OC had been
85 increasingly considered as an indicator of soil quality and health under different stresses

86 or human disturbance (Cambardella and Elliot 1992; Marriott and Wander, 2006).
87 Particulate organic matter (POM), as a labile OC pool, had been suggested as a
88 measurement of OM accumulation and stabilization with co-existing microbial activity
89 in different ecosystems (Gajda 2010; Six and Paustian 2014). Soil aggregation, affected
90 by land use and management practices, could lead to changes in allocation of particulate
91 OC inter- and/or intra- microaggregates in size fractions of soil (Yang et al., 2009;
92 Lagomarsino et al., 2012; Six and Paustian 2014; Smith et al., 2014). Unfortunately,
93 the links between changes in OC pools in OC stabilization and in microbial biological
94 activity in soil aggregates had not yet been well understood and quantitatively assessed
95 (Six and Paustian 2014; Smith et al., 2014).
96 Soil aggregation could be characterized by distributions of particle size fractions
97 (PSFs), which could differ in soil microbial biomass and the activity among them, in
98 response to the accumulation and stabilization of soil organic matter in agro-ecosystems
99 (Salinas-Garcia et al., 1997; Kandeler et al., 1999; Smith et al. 2014). Such difference
100 could mimic the micro-scale interactions driving SOC stabilization and nutrient cycling
101 in soils (Kandeler et al., 2006; Lagomarsino et al., 2012; Six and Paustian, 2014). For
102 this, separation should be required with least low energy dispersion of bulk soil into
103 particle size fractions of aggregates (Kandeler et al., 2000), but without any chemical
104 dispersion (Smith et al. 2014). Stemmer et al. (1998) developed such a low energy
105 ultrasonic dispersion, which could allow least disturbed size fraction separation for
106 analyzing microbial community and enzyme activity in soil aggregates (Kandeler et al.,
107 2000). This approach was followed in later studies (Sessitsch et al., 2001; Poll et al.,

108 2003; Matocha et al., 2004; Marx et al., 2005; Zhang et al., 2013), addressing the
109 impacts of different management practices or environmental disturbance on organic
110 matter persistence, microbial communities and enzyme activity in aggregates
111 agricultural soils. However, the interactions of organic matter, microbial and enzyme
112 activities in aggregate size fractions with carbon stabilization and their trend with
113 continuing management impacts in long term cultivated soils had been not yet well
114 characterized.

115 Soil matrix or microsite properties had been well known playing an important role
116 in the spatial allocation of SOM and microbial community and thus the link between
117 OC pools and microbial bio-activity among different fractions of soil aggregates (Smith
118 et al. 2014). Rice soils from China, classified as hydroagric Anthrosols in the new
119 Chinese Soil Taxonomy, is s a particular soil type with dynamic redox regime and neo-
120 formation of iron/manganese oxyhydrates due to hydromorphic pedogenesis under long
121 term hydroagric paddy management (Li, 1992; Gong et al., 1999). Recently, these soils
122 had been known of high SOC storage and sequestration potential compared to dry-land
123 croplands (Pan et al., 2004; Pan et al., 2010; Wissing et al., 2013). This had been often
124 attributed to enhanced aggregation and thus the aggregate stability (Lu et al., 1998;
125 Yang et al., 2005) as well as to increased humification of SOC (Olk et al., 2000), in rice
126 soils. OC accumulation and stabilization in paddy soils with management practices had
127 been found related to increased OC bound to free oxyhydrates (Zhou et al., 2009; Cui
128 et al., 2014), to enhanced physical protection with increased aggregate stability (Li et
129 al., 2007; Zhou et al. 2008), or to their interactions (Song et al., 2012; Song et al., 2013)

130 as well as to enhanced chemical recalcitrance of OC pools (Zhou et al., 2009a, 2011;
131 Song et al., 2012). Furthermore, OC could be continuously accumulated with increasing
132 rice cultivation intensity, a process being promoted following the desalinization and
133 decalcification in the initial stage after the salt marsh shifted to rice paddy, in a rice soil
134 chronosequence (Kalbitz et al., 2013). Wherein, the accumulated SOC was increasingly
135 stabilized with neoformed iron-oxyhydrates (Cheng et al., 2009; Wissing et al., 2011),
136 accumulated in the rice soils with prolonged rice cultivation in the long run. Whereas,
137 an increase in proportion of water-stable macro-aggregates (>250μm) and the
138 associated particulate OC pool was indicative of total OC accumualtion in a study of a
139 rice paddy with well managed fertilization from Southeastern China (Zhou et al., 2007).
140 This could further supported the later finding of a potential contribution of physically
141 protected OC in the coarse sand size fraction of soil aggregates to bulk soil OC
142 accumualtion and stabilziationin rice paddies under l;ong--term fertilization trials from
143 South China (Zhou et al., 2008).

144 Furthermore, co-evolution of soil microbial community and diversity was
145 observed with SOC accumulation and stabilization in rice paddies (Zhang et al., 2007;
146 Zheng et al., 2007; Liu et al., 2011). In line with the trend of OC accumulation,
147 microbial biomass and community diversity was found enhanced in paddy soils across
148 the chornosequence under prolonged rice cultivation (Bannert et al., 2011; Jiang et al.,
149 2013). Using a similar chronosequence, the enhanced biological activity could be well
150 portraied with an increasing trend of mean weight diameter of soi aggregates and of
151 particlate OC pool across the soils with prolonged rice cultiavtion (Wang et al., 2015),

152 indicating a potential role of physically protected labile OC pool in enhancing
153 biological activity with OC accumualtion in rice soils (Zou et al., 2015). Recently,
154 changes in mcirobial gene abundance and community compsoition had been reported
155 for the bulk soils (Liu et al., 2016a; Liu et al., 2016b) and aggregate size fractions of
156 soils from a rice soil chronosequence (Wang et al., 2015). Thus, physical protection
157 may involve the change in the spatial distribution of OC pools but not mainly the
158 chemical recalcitrance among aggregate size fractions. Accordingly, changed allocation
159 of both OC pools and microbial community could contribute to OC stabilization with
160 increased microbial abundance and microbial carbon use efficiency as a result of
161 enhanced aggregation. However, the link of microbial activity to OC accumualtion and
162 stabilziation among different aggregate fractions and the evolution with increasing
163 length of rice cultivation had been unknwon. Such information would be of key
164 importance for understanding carbon sequestration in relation to sustainable
165 management of rice paddy soils as carbon biogeochemical cycling had driven
166 ecosystem functions and services provided by soils (Smith et al., 2015).

167 In this study, two hypotheses are tested. First, microbial bioactivity and carbon
168 stability in soil aggregates could differ among their size fractions, leading to changes
169 in spatial allocation of OC pools among aggregate size fractions in rice paddies.
170 Physically protection of OC may improve microbial micro-habitat conditions and thus
171 microbial carbon use efficiency, through enhanced aggregation, enabling existence of
172 labile OC pool within micro-aggregates in macro-aggregates or between micro-
173 aggregates (Six and Paustian 2014; Smith et al., 2014). Then biological activity could

174 be enhanced with physically protected carbon in macro-aggregates, relatively to in
175 micro (clay sized) aggregates with chemically stabilized organic carbon; Second, a
176 strong link of microbial activity to labile OC pool would be promoted with increasingly
177 enhancement of physically stabilized SOC in macro-aggregates, due to continuing
178 hydroagric paddy management under long-term rice cultivation. In a series of soils
179 formed on similar paleo-deposits rich in silt, the changes due to the pedogenetic process
180 under continuous rice cultivation could result in a directional changes in soil
181 aggregation, and thus in microhabitat conditions as well as nutrients. This directional
182 pedogenetic development would in turn affect a more or less directional change in OC
183 stabilization (with increasing mineral bound OC, accumulation of recalcitrance OC
184 pool as well as physically protected OC pools such as POM) (Wang et al., 2015). This
185 study aims to help understand that carbon stabilization would not confront but improve
186 biological activity in soils under rice cultivation over centuries.
187

188 **2 Materials and methods**

189 **2.1 Methodology rational**

190 Using a recommend sonification separation procedure, we looked into the changes in
191 aggregate size fraction composition for aggregate stability, OC functional group
192 composition for chemical recalcitrance, soil respiration for microbial energy use, in
193 order to characterize the OC accumulation and stabilization in rice soils. Moreover,
194 changes are explored with OC accumulation/stabilization in microbial activity for soil
195 functioning. We analyzed total microbial gene abundance and estimated overall enzyme
196 activity in aggregate size fractions. The potential link between OC stabilization and
197 bioactivity among the aggregate fractions are quantitatively assessed using the
198 parameters of carbon- or gene abundance- scaled respiration and enzyme activity.
199 Furthermore, the evolution of such interlink is traced by comparing the soils of
200 sequential length of rice cultivation in a soil chronosequence up to 700 years.

201 **2.2 Site and soils**

202 In this study are investigated a series of soils of a soil chronosequence, shifted from
203 tidal marsh to rice cultivation under different lengths in a coast land located in Cixi
204 Municipality, Zhejiang Province, China (Fig.1). Lying in the south bank of Hangzhou
205 Bay, the area is within the typical northern subtropical monsoon climate for Eastern
206 China, with a mean annual temperature of 17.7 °C and precipitation of 1,367 mm during
207 2004-2014 (http://cdc.nmic.cn/home.do). In the area, coastal tidal marsh had been
208 increasingly reclaimed for rice production, with dyke establishments at different
209 historical stages for the last 2000 years. These soils allowed chronosequence studies for

210 rice soil development, including a pedological characterization by Cheng et al. (2009)
211 and a morphological, mineralogical and microbiological investigation by Kölbl et al.,
212 (2014).

Fig. 1

214 In this study, individual soils of the chronosequence were identified based on dyke
215 establishment history recorded in Cixi County Annals (with brief information in
216 Chinese available at www.cixi.gov.cn), including an initial tidal marsh soil before rice
217 cultivation (P0), and rice soils of P50, P100, P300 and P700 shifted for rice cultivation
218 on dyke establishment respectively 50, 100, 300 and 700 years before present (Fig.1).
219 These soils were apart from each other in a distance no more than 40-km in nearly the
220 same topography. All the soils developed on comparable parent materials of paleo-
221 deposit from Yangtze River, with a particle composition of silt (75%-84%), followed
222 by clay but low in sand content (Chen and Zhang, 2009). Soil texture ranged from silty
223 loam to silty clay-loam. The clay mineral assemblage consisted of illite (40-50%),
224 chlorite (20-30%) and kaolinite (10-20%) with a minor amount of smectite and quartz
225 (Zhang et al., 2010b).

226 As situated in a relatively small area with a traditional summer rice-winter rape
227 rotation, rice production management on the soils of the chronosequence could be
228 considered relatively consistent across sites, with similar cultivars and management
229 practices including crop protection, irrigation and fertilization (Cheng et al., 2009). Of
230 course, influence of salt on rice production could occur in the early stage of rice
231 cultivation on the tidal marsh derived soils while the ground water table had been

232 enough low without restricting rice growth (Kölbl et al., 2014). The directional
233 evolution of soil properties (Cheng et al., 2009; Chen et al., 2011), neo-formation of
234 clay minerals particularly of iron/manganese oxyhydrates (Wissing et al., 2013;
235 Wissing et al., 2011; Kölbl et al., 2014), interaction of organic matter with minerals
236 (Wissing et al., 2011; 2014) as well as organic carbon pools (Wissing et al., 2011; Wang
237 et al., 2015) have been well characterized.

238 **2.3 Soil sampling**

239 Topsoil (0-15 cm in depth) samples of the five individual soils of the chronosequence
240 were used in the study. To avoid influence of fresh straw material on soil aggregates
241 and OC substrates in soil samples, the sampling was done at the harvest stage of the
242 growing rice in early November 2011. While sampling in field, an undisturbed soil core
243 was collected using an Eijkelkamp soil core sampler (Agrisearch Equipment, Giesbeek,
244 The Netherlands) while a bulk soil sample using a stainless steel shovel. A topsoil was
245 collected in triplicates respectively from three adjacent individual fields. All soil
246 samples were shipped to the lab within two days after sampling, and stored at 4 °C
247 before soil analysis in the following 2 weeks. The basic properties of the studied soils
248 are listed in Table 1. Changes of OC stability and microbial activity of bulk soil along
249 the chronosequence had been assessed in our previous study by Wang et al. (2015) and
250 Liu et al. (2016a and 2016b).

Table 1

252

253 **2.4 Particle size fractionation of soil aggregates**

254 In this study, the undisturbed soil cores were used for dispersion in water with low
255 energy sonication procedure, without chemical dispersing agents. Particle size fractions
256 (PSFs) of water stable aggregates were separated with a modified procedure described
257 by Stemmer et al. (1998) and later on followed by Stemmer et al (1999), Sessitsch et
258 al., (2001), Kandeler, et al (1999, 2000 and 2006). A portion of field moist soil core (50
259 g equivalent d.w.), removed of discernible straw material if any, was placed into a glass
260 beaker and dispersed in 100 ml of distilled water using a low-energy ultrasonic
261 disaggregator (Zhixin, JVD-650, Shanghai, China) with an output energy of 170 J g^{-1}
262 for 5 min. A coarse sand sized fraction of aggregates in diameter of 2000-200-μm was
263 separated by wet sieving and the fine sand sized fraction of 200-20–μm was
264 subsequently obtained by sedimentation after siphonage. The remainder was
265 centrifuged to collect the silt sized fraction of 20-2–μm and the supernatant was
266 centrifuged to collect the clay sized fraction of ≤< 2-μm. The samples of the obtained
267 size fractions were freeze-dried with a frozen dryer (Thermo, Modulyo D-230, NY, US)
268 and then stored at -70 °C. –Here, water stable macro--aggregates larger than 2000μm
269 were not taken into consideration as they were insignificant in rice soils under
270 prevailing water submergence and pudding activities under long-term hydroagric
271 management (Deng and Xu, 1965). The classes of the size fractions were kept basically
272 consistent with our previous studies (Li et al., 2007a, b; Zheng et al., 2007; Pan et al.,
273 2008 and Chen et al., 2014).

274 **2.5 Organic carbon pool and FTIR spectroscopy analysis**

275 Total soil organic carbon (SOC) and total nitrogen (TN) of the separated PSFs were

276 determined with a CNS elemental analyzer (Elementar Vario-max CNS Analyser,
277 Germany Elementar Company). Labile organic carbon (LOC) content was measured by
278 0.33 M potassium permanganate oxidation ($KMnO_4$), following a procedure described
279 by Blair et al. (1995). Microbial biomass carbon (MBC) was measured using the
280 chloroform fumigation-extraction method. The MBC content was estimated as the
281 difference of OC between the unfumigated and fumigated samples using the conversion
282 factor of 0.45, following Joergensen (1996). Herein, MBC of coarse sand fraction of P0
283 soil was not provided due to the very small sample obtained from the sonification and
284 separation procedure.

285 Chemical composition of organic carbon in the PSFs were characterized with
286 Fourier transform infrared (FTIR) spectroscopy using a Bruker FTIR
287 spectrophotometer (Bruker TENSOR 27 Spectrometer, Ettlingen, Germany). Briefly, a
288 portion of frozen-dried aggregate sample was powdered in an agate mill, and 1 mg of
289 the homogenized sample powder was mixed thoroughly with 100 mg KBr. The pellet
290 prepared with a press was placed in a sample holder and FTIR spectra were recorded.
291 FTIR scanning was conducted in ambient conditions at 22±1°C. The resolution was set
292 to 4 cm^{-1} and the operating range was 400 to 4000 cm^{-1}. In all cases, 20 scans per sample
293 were recorded, averaged for each spectrum and corrected against the spectrum with
294 ambient air as background. Following Ellerbrock et al. (1999) and Cocozza et al. (2003),
295 the charcteristic vibration peak at 1088 cm^{-1} was alogned to polysccharides, those at
296 1633 cm^{-1} to aromatic compounds and those at 2931 cm^{-1} to aliphatic compiounds as
297 well as those at 3424 cm^{-1} to O-H of phenols. Subsequently, .a general semi-

298 quantification of three major functional OC groups of polysaccharides, aliphatic and
299 aromatic compounds was done following Tivet et al., 2013. Nevertheless, it was not
300 able to quantify potential contributions from organic Si or P compounds to the intensity
301 of the band assigned to polysaccharides (Mao et al., 2008; Tivet et al., 2013). All the
302 obtained FTIR spectra are given in Fig. S1.

303 **2.6 SEM observation of soil aggregates**

304 The aggregate assembly of a portion of an undisturbed soil core was examined under a
305 scanning electron microscope (SEM, Model Hitachi S-3000N) at an electron
306 acceleration voltage of 20 kV. Prior to scanning, a sample was mounted on a stub using
307 double sticky stickers and coated with gold using Hummer sputter coating equipment
308 (Anatech Ltd., Union City, CA). Pictures were captured by automatic image capturing
309 software (Hitachi Science Systems LTD., Schaumburg, IL). Magnifications and linear
310 scale are indicated in the micrographs.

311 **2.7 DNA extraction, microbial gene abundance and diversity analysis**

312 A portion (0.45 g) of a PSF sample stored at -70 °C was used for DNA extraction with
313 PowerSoil™ DNA Isolation Kit (MoBio, USA), following the manufacturer guide. The
314 concentration of the DNA extracts was checked with a spectrophotometer (Eppendorf,
315 Germany), and its integrity and size were checked by using 1.0% agarose gel
316 electrophoresis. Extracted DNA was stored at -70 °C prior to molecular microbiological
317 assay.

318 Quantitative real-time PCR assay was performed on a 7500 real-time PCR system
319 (Applied Biosystems, USA) using SYBR green as a fluorescent dye. Primer

320 combinations of 338F/518R (Øvreås and Torsvik, 1998), ITS1F/ITS4 (Gardes and
321 Bruns, 1993) and Ar109F/Ar915R (Lueders and Friedrich, 2000) were used for
322 bacterial 16S rRNA, fungal Internal Transcribed Spacer (ITS) region and archaeal 16S
323 rRNA genes respectively in the Real-time PCR assay.

324 PCRs were carried out on all PSF's DNA samples with specific primers to amplify
325 the 16S rRNA genes from bacteria (27F and 1492R) and archaea (Ar109F and Ar915R)
326 and the ITS regions from fungi (ITS1F and ITS4). The forward primer from each pair
327 had a fluorescent label (6-FAM) attached to the 5' end. Amplification of the 16S rRNA
328 gene and ITS regions, purification, digestion and amplicon separation for T-RFLP
329 analysis are described in the supplementary materials and methods.

330 From the T-RFLP profiles, the Shannon diversity index (*H'*) of the individual T-
331 RFs was calculated following Blackwood et al., (2007), using an equation:

332 $$H' = \Sigma Pi\,(\ln Pi) \quad (1)$$

333 where, *Pi* is the proportion of each T-RF in a single sample.

334 **2.8 Soil enzyme activity**

335 In this study were analyzed soil enzyme activities involved mainly in cycling of C, N
336 and P in soils. In detail, activities of invertase, urease and acid phosphatase were
337 determined using the methods described by Guan et al., (1986) while β-glucosidase, β-
338 cellobiosidase and peroxidase were measured using 96 micro-plates colorimetric
339 methods described by Saiya-Cork et al., (2002). For an integrated assessment of
340 microbial biochemical activity, the six different enzyme activities analyzed were
341 normalized to give a single value as normalized enzyme activity (NEA) of an individual

342 fraction, which was estimated with the following equation:

343 $$x_i' = \frac{x_i}{\sum_{i=1}^{n} x_i} (i=1,2,\ldots,5), \quad (2)$$

344 where, i was the number of each soil sample (P0, P50, P100, P300, P700), x was the
345 enzyme activity and x' was the normalized enzyme activity of each soil sample.
346 Subsequently, an arithmetic mean value of enzyme activity of each sample was obtained
347 for the NEA.

348 **2.9 Soil respiration**

349 For assessing microbial use of carbon in different PFSs, soil respiration as measured by
350 CO_2 production was determined using an anaerobic laboratory incubation protocol,
351 following Zheng et al., (2007). A PSF sample (20g d.w. equivalent) was placed into a
352 125ml glass jar and submerged with 40ml distilled water before being gently mixed.
353 The jar was then sealed with a butyl rubber stopper and two Teflon tubes for gas
354 sampling and N_2 circulation were inserted into the stopper. The headspace was
355 repeatedly evacuated and flushed with N_2 gas into the jar at a rate of 300ml min^{-1} for
356 30min, creating an anaerobic condition. The jars with soil slurry were incubated in an
357 incubator, as described in Section 2.8, at 25 ± 1 °C for 37 days. During incubation, a
358 0.25 ml sample of the headspace gas was collected by a pressure syringe every 5 days
359 since the third day after incubation was initiated. After each gas sampling, N_2 gas was
360 again flushed into the jar at a rate of 300ml min^{-1} for 30 min to removing all the emitted
361 gas in the jar. CO_2 concentration in a gas sample was determined with a gas
362 chromatograph (Agilent 4890D) equipped with a stainless steel column (Porapak Q)
363 (80/100 mesh) and flame-ionization detector (FID). Following the procedures described

364 by Zhang et al., (2010a), the determination was done with an oven temperature of 80ºC
365 and a FID temperature of 200ºC, with N_2 as the carrier gas at a flow rate of 40ml min^{-1}
366 and a make-up gas mixture of H_2 and air at a flow rate of 35 ml min^{-1}. A blank of 40 ml
367 distilled water was used as the control for the gas concentration in the bottle. The total
368 CO_2 evolved was estimated from the cumulative sum of the gas evolved in all
369 monitoring intervals and was used to calculate the anaerobic soil respiration expressed
370 in terms of soil mass.

371 **2.10 Data treatment and statistical analysis**

372 All data was treated with EXCEL 2013 and expressed as mean plus/minus standard
373 deviation of triplicate samples. The significant differences in carbon fractions and in
374 microbial parameters between particle size fractions in a single soil and between soil
375 samples of a single particle size fraction were respectively statistically analyzed by one-
376 way ANOVA with Tukey's test using a SPSS software package 20.0. Statistical
377 significance was defined at 95% confidence level.

378 **3 Results**

379 **3.1 Organic carbon characterization in size fractions of aggregates**

380 As shown in Table 2, the fine sand (200-20μm) and silt (20-2μm) sized fractions
381 together accounted for up to 80% of a bulk soil across soils. However,; the proportion
382 of coarse sand macro-aggregates (2000-200μm) and clay (< 2μm) sized fine aggregates
383 increased with prolonged rice cultivation over the chronosequence. As indicated in
384 Fig.2, soil aggregates from P0, the initial marsh soils were sharp edged single individual
385 minerals, and mostly uncovered with clear surfaces; However, in the rice soils with
386 increasing rice cultivation lengths, soil aggregates became increasingly round shaped,
387 loosely assembled of fine minerals but covered with more or less amorphous materials.
388 Particularly in P700, soil aggregates were seen in larger size, very loosely assembled
389 of non-clear shaped mineral particles with amorphous materials, of which some
390 particulate OM including some fungal hyphae on the aggregate surface (magnified
391 P700 image in Fig. 2).

392

Table 2

394 Soil properties of SOC, total N and LOC were extensively different among the
395 size fractions and between uncultivated and rice soils (Table 3). SOC, LOC and total N
396 pools were all generally in an order of sand size fraction > clay sized fraction > fine
397 sand fraction > silt sized fraction in a single soil. And these pools of all the particle size
398 fractions except fine sand fraction, were greater in rice soils than the uncultivated marsh
399 soil. Particularly for rice soils, OC was enriched mostly in coarse sand sized macro

400 aggregates, moderately in clay sized fraction, fairly in fine sand sized fraction but

401 depleted in silt sized fraction, respectively in a range of 41-61 g kg^{-1}, 20-24 g kg^{-1}, 8.5-

402 20 g $kg^{-1}$and 10-11 g kg^{-1}. However, C/N ratio was in a significantly decreasing trend

403 with the decreasing size of the aggregate fractions across the chronosequence. The ratio

404 of LOC to SOC, an indicator of C lability in soils, was in a significantly decreasing

405 order of coarse sand fraction>fine sand fraction>silt and clay sized fractions.

406 The FTIR spectra showed generally sharp peaks at vibration of 1088cm^{-1} (assigned

407 to polysaccharides) but broad shoulders at vibration of 1633cm^{-1} assigned to– aromatic

408 carbon across the aggregates fractions (Fig.S1). There was a clear trend of decreasing

409 intensity the polysaccharide peaks but increasing shoulder intensity of aromatic carbon

410 in a single fraction, with increasing rice cultivation. The semi-quantitative data –of

411 carbon chemical groups obtained with FTIR analysis is presented in Table 4. -Herein,

412 carbon groups in aggregates were dominated by polysaccharides (60-70%), followed

413 by aromatic carbon (0.6-3.7%) with small contribution of aliphatic carbon in a single

414 fraction. Relative proportion of aromatic carbon was lower but of polysaccharide

415 carbon higher in silt fraction than in other fractions, without a significant difference in-

416 between the latter. Consequently, the estimated OC chemical recalcitrance (ratio of

417 aromatic to polysaccharide C) was lowest in silt fraction, followed by fine sand fraction

418 but highest in coarse sand and clay fractions.

419 Recalcitrance of OC of in a single fraction was generally lower in uncultivated

420 marsh soil than in the shifted rice soils, but tended to increase with increasing length of

421 rice cultivation. While the fine sand fraction, bearing the majority of SOC for the

422 soil (Table 2 and Table 3), had a moderate OC recalcitrance, the coarse sand fraction
423 had similar OC recalcitrance but higher carbon lability and higher C/N ratio, indicating
424 greater existence of potentially available carbon pool (for example, particular
425 particulate OC).

426 Table 3

427 Table 4

428 Fig. 2

429 **3.2 Microbial biomass carbon, microbial gene abundance and diversity**

430 The measured microbial biomass carbon (MBC) was highest in the coarse sand fraction
431 of macro-aggregates while lowest in the clay sized fraction of fine micro-aggregates
432 over the sequence (Table 3). Generally, MQ, the microbial quotient, was not
433 significantly different between the coarse sand-, fine sand- and silt- sized fractions but
434 significantly higher than in the clay sized fractions.

435 The microbial DNA content (equivalent to biomass) and gene abundance of
436 microbial communities in the fractions over the chronosequence are shown in Table 5.
437 Total DNA ranged from 1.57 μg g^{-1} in silt fraction to 4.00 μg g^{-1} in clay fraction of the
438 tidal marsh and from 4.35 μg g^{-1} in fine sand fraction to 35.33 μg g^{-1} in coarse sand size
439 in the rice soils. Fungal ITS gene copies were generally higher in coarse sand fractions,
440 decreasing with the size of other fractions. Whereas, generally in a bimodal pattern
441 among the particle size fractions, total DNA, bacterial and archaeal 16S rRNA gene
442 copy numbers were higher in both coarse sand and clay fractions compared to other
443 fractions across the chronosequence. Clearly, microbial gene abundance was dominated

444 by bacterial, with archaeal and fungal gene abundance one and two order lower than
445 bacterial respectively across the fractions, Whereas, the ratio of fungal to bacterial gene
446 abundance generally decreased but that of archaeal to bacterial increased with
447 decreasing size of the aggregate fractions.

448 Over the studied chronosequence, DNA contents of a PSF were several folds
449 higher in the rice soils over the initial tidal marsh. Accordingly, gene copy numbers of
450 microbial communities from a PSF were greatly higher in rice soils than in the initial
451 tidal marsh. Bacterial and fungal abundance in coarse sand, fine sand, silt and clay
452 fraction in P50 was increased by 688%, 72%, 498% and 622 %, and 74%, 149%, 7%
453 and 152 %, respectively over P0. A mean increase over P0 in bacterial gene copy
454 numbers in the rice soils cultivated for over 100 years was seen significant, by 73% to
455 437 %, 0.4% to 67 %, 225% to 246 % and 147% to 201 %, respectively in coarse sand
456 fraction, fine sand fraction, silt fraction and clay fraction. However, such change were
457 much smaller of fungal gene abundance across all the soils, particularly in silt and clay
458 sized fractions. In contrast, archaeal abundance in a single fraction was found increased
459 over P0 consistently across the soils with the prolonged rice cultivation, though smaller
460 in fine sand and silt sized fractions. For the coarse sand fraction only, both of fungal to
461 bacterial ratio and archaeal to bacterial ratio tended to increase with increasing rice
462 cultivation lengths.

463 Microbial Shannon diversity index of the four PSFs of the chronosequence soils
464 are presented in Table S1. In detail, Shannon's index of bacterial community was much
465 higher in coarse sand fraction and, to a lesser extent, in clay size fraction than in fine
466 sand and silt fractions across the chronosequence. Fungal community Shannon's index

467 was shown generally decreased with the size of the fractions, being highest in coarse
468 sand fraction among all the fractions. However, there were no significant changes in
469 archaeal Shannon's index among the PSFs across the sequence. Generally, Shannon
470 diversity index of the microbial communities in a single PSF were greatly higher in the
471 rice soils than in the uncultivated tidal marsh.

472 **3.3 Enzyme activity and basal respiration**

473 All analyzed enzyme activities (Table S2) were seen increased in the rice soils over the
474 initial tidal marsh. Furthermore, NEA (normalized enzyme activity) was 0.07 in the
475 coarse sand and 0.10 in the fine sand fraction, and 0.07 and 0.14 in the silt and clay
476 fractions in P0. In contrast, NEA was 0.18-0.30 in coarse sand and 0.12-0.30 in fine
477 sand fraction, but 0.17-0.30 in silt fraction and 0.19-0.24 in clay fraction of the rice
478 soils. Moreover, NEA in a single size fraction showed a significantly increasing trend
479 with prolonged rice cultivation (Table 6).

480 Soil respiration was much higher in a single fraction from the rice soils than from
481 the marsh soil, and in sand sized macro-aggregate fraction than in silt and fine sand
482 fraction over the soils (Table 6). In detail, soil respiration was 662 mgCO_2 kg^{-1} and 565
483 mgCO_2 kg^{-1} in coarse and fine sand fraction, and 298 mgCO_2 kg^{-1} and 496 mgCO_2 kg^{-}
484 ^{1} in silt and clay fraction, respectively in P0. While in rice soils, soil respiration was in
485 a range of 1588-2914 mg CO_2 kg^{-1} in coarse sand, and of 1076-1256 mgCO_2 kg^{-1} in
486 fine sand fraction, and of 740-1354 mgCO_2 kg^{-1} in silt and of 1028-1434 mgCO_2 kg^{-1}
487 in clay fraction, of the rice soils. Basal respiration in a single size fraction generally
488 increased with rice cultivation length (Table 6).

489 Using the data from Table 3, the estimated RQ (the ratio of respired OC to total

490 OC) and qCO_2 (the ratio of respired OC to total MBC) were seen variable across the
491 size fractions and among the soils (Table S3). Generally, RQ was lower both in sand-
492 and clay- sized fractions than in fine sand- and silt- sized fractions. Whereas, mean
493 qCO_2 was lowest in the coarse sand sized fraction but highest in the clay sized fraction.
494 While there was no overall trend of RQ and qCO_2 in a single fraction between the marsh
495 soil and rice soils, both RQ and qCO_2 in a single fraction followed more or less a
496 decreasing trend with increasing length of rice paddy management.
497

498 **4 Discussions**

499 **4.1 Carbon accumulation versus stabilization in soil aggregates**

500 In this study, level of OC, soil respiration and microbial gene abundance/diversity
501 differed significantly among different size fractions of water stable aggregates from the
502 chronosequence. Similar to the findings by Li et al. (2007b) and Zheng et al. (2007),
503 OC was seen accumulated highly in sand sized and moderately in clay sized fractions
504 but depleted in silt sized aggregate fractions (Table 3). As shown in Fig. 3, soil organic
505 carbon content (level of OC accumulation) in a fraction was found very significantly
506 positively linearly correlated to OC recalcitrance from the FTIR analysis (Table 4).
507 Whereas, respiration quotient as a rate indicate of carbon turnover for microbial energy
508 use (Kennedy and Papendick, 1995), was in a very significant negative logarithm
509 function of OC level (Fig. 3b). The divergence of the uncultivated marsh soil to the rice
510 soils could be attributed to the land use impact as a determinant factor for OC turnover
511 (Qian et al., 2013). The correlations hereby could suggest the accumulation of OC in
512 soil aggregates related to chemical stabilization against biological use for their energy
513 supply, which had been traditionally considered as an inherent carbon sequestration
514 with selective persistence of non-degradable or residue OM in soils (Lützow et al., 2006;
515 Mikutta et al., 2006).

516 Fig. 3

517 However, calculated using the OC contents (Table 3) and the fraction mass
518 percentage (Table 2) of a single fraction, the amount of OC allocated only in sand and
519 clay sized fractions were closely correlated to the bulk OC contents (Table 1) of the

520 soils (Fig. S2). This was in general agreement with the finding for similar rice paddy
521 soils from an adjacent area (Pan et al., 2008). The increased allocation of OC to clay
522 sized fraction could be attributed to the accelerated formation of clay and hydroxyl
523 Fe/Mn minerals (Wissing et al., 2013) due to long term paddy management (Kölbl et
524 al., 2014).

525 Furthermore, the enrichment index (EI) of OC, calculated with OC content in a
526 fraction divided by that in the bulk soil, was higher than 1 in both sand and clay sized
527 fraction but much lower than 1 in silt fractions. When plotting the EI values against
528 LOC content (Table 3) for all the fractions (Fig. 4), enrichment of OC was seen relevant
529 to labile OC pool in the fractions. Moreover, the EI value was seen significantly but
530 weakly positively correlated to both F/B ratio of gene abundance (Table 5) and the OC
531 recalcitrance (Table 4). These evidenced that accumulation of labile OC, mostly
532 particular OM, contributed significantly to OC pool in sand sized macro-aggregates
533 (Zhou et al., 2008) though hereby the apparent recalcitrance was in a similar range to
534 that in clay fractions (Table 4). It had been well understood, light fraction or macro-
535 aggregates in soil were rich in new or more labile carbon substrates, more or less related
536 to root fungal activities, which were largely physically protected in micro-aggregates
537 within macro-aggregates (Elliott et al., 1986; Jastrow et al., 1998; Six et al., 2000). As
538 shown by Wang et al. (2015) for the bulk soil of the studied chronosequence, OC
539 accumulation in bulk soil could be well accounted for by the changes in particulate OM.

Fig. 4

541 Synthesizing data from Tables 2 and 3, OC protected in the sand and fine sand

542 fractions constituted 51%-62% while chemically protected or mineral bound OC in the
543 clay sized fractions 11%-19%, to the total OC pool of soils over the studied sequence.
544 In a study of a river bed sediments from a Californian river basin (Wakeham and Canuel,
545 2016), light fractions contributed largely to the total OC pool but the heavy (clay)
546 fraction contained smaller amount but old OC. Six et al. (2002a) addressed that OM
547 accumulated mainly as unprotected POM in micro-aggregates in size lager than 53μm
548 though intimately associated with silt and clay with high chemical recalcitrance. The
549 higher enrichment of OC related to LOC in macro-aggregates of sand size fraction and
550 smaller enrichment attributable to clay sized fraction in this study supported the general
551 understanding of relatively unprotected labile carbon in macro-aggregates but relatively
552 recalcitrant carbon in micro-aggregates in clay complexes (Six et al., 2002a). Micro-
553 aggregates and other primary particles could be bound into macro-aggregates with close
554 association of fungal hyphae and organic matter/materials (Oades, 1984; Tisdall, 1994;
555 Miller and Jastrow, 2000).

556 Physical protection of labile carbon in macro-aggregates rather than inherent
557 chemical stability of OC (a minor mass fraction of the clay sized micro-aggregates,
558 Table 2) had been increasingly concerned for soil carbon sequestration (Six et al., 2004;
559 Kong et al., 2005; Six and Paustian, 2014). For the rice soils under long term rice
560 cultivation here, OC accumulated and stabilized mainly through physical protection of
561 new or more labile carbon in macro-aggregated though old or mineral bound OC
562 preserved in fine aggregates of clay size (Marschner et al., 2008). This study also
563 confirmed our previous understanding that sand-sized fraction of aggregates could play

564 a prevalent role in soil carbon sequestration (Zhou et al 2008).

565 **4.2 Bio-activities versus OC stabilization between sand and clay sized fractions**

566 Biological activity of soil microbes including soil respiration and soil enzyme activity
567 had been well known varying across size fractions of soil aggregates (Kandeler et al.,
568 1999; Sessitsch et al., 2001; Poll et al., 2003; Allison and Jastrow, 2006). In this study,
569 total DNA content was found significantly positively but linearly correlated with
570 content either of organic carbon and nitrogen, or of labile organic carbon, across the
571 size fractions of the studied sequence (Fig. S3). However, gene abundance of bacterial,
572 fungal and archaeal communities could be correlated neither to total pool of organic
573 carbon and labile organic carbon nor to carbon recalcitrance and lability (LOC/SOC),
574 across the sequence. Likewise, OC level did not necessarily affect microbial
575 populations along a soil reclamation gradient with exotic carbon amendments (Yin et
576 al., 2000; Torsvik and Øvreås, 2002). Indeed, different carbon lability and accessibility
577 could shape microbial communities within and between size fractions of aggregates
578 (Six et al., 2000; Ettema and Wardle, 2002; Balser et al., 2006; Kögel-Knabner et al.,
579 2008).

580 Soil matrix and micro-habitat conditions (aggregation and associated nutrients
581 and C substrate as well as redox) play a critical role in changes in soil microbial
582 abundance and structure (Lehmann et al, 2011; Smith et al., 2014). Here, a clear marked
583 difference in microbial abundance and community could be found between the rice soils
584 and the initial marsh soil before shift to rice cultivation, either for bulk soils or for
585 aggregates fractions (Wang et al., 2015; Liu et al., 2016a). This is coincident with the

shift in soil physical and chemical conditions between the rice soils and the initial marsh soil, with the latter is alkaline in reaction, poor aggregation due to depleted OC and high salinity (Data in Table 1).

Among the soils studied, both the coarse sand and clay sized fractions showed higher enrichment of OC, which was relevant to different association of carbon pools and interaction to minerals. There was a difference in the ratio of LOC/SOC, as a negative indicator of chemical stability, and in OC recalcitrance measured with FTIR, between the coarse sand and clay sized fractions. The trends of carbon stability with microbial respiration were similar between the sand and clay sized fractions (Fig. 5). ~~Herein, the similar carbon stabilization measured with microbial respiration between the sand sized and clay sized fractions could not be explained by the difference in the trend of LOC/SOC, and of carbon recalcitrance (Table 3).~~

Fig. 5

We further compare the bio-activity versus OC accumulation between sand and clay sized fractions of aggregates. –Here, a correlation of DNA content as microbial biomass to OC content was very significant for coarse sand fraction but not valid for clay fraction (Fig. 6a). Meanwhile, normalized enzyme activity was in a positively linear function with SOC accumulation for coarse sand fraction but failed for clay fractions (Fig. 6b). In contrast, DNA content scaled soil basal respiration was in a negatively power function with total DNA content, being higher for the coarse sand than for the clay sized fractions (Fig. 6c), showing an increased carbon use efficiency with the SOM accumulation both in sand and clay sized fractions. Whereas, DNA

608 content was linearly correlated positively both to the content of LOC (Fig. 6d) and to
609 carbon recalcitrance (Fig. 6e), for sand sized aggregate fractions only but for clay sized
610 fractions.

Fig. 6

612 The failure of improvement of bio-activity with OC accumulation in clay sized
613 fractions indicated an insignificant potential to support biological activities in fine
614 aggregates rich in stabilized OC with high recalcitrance. In clay sized fractions of
615 aggregates, DNA content was independent of OC, which could be either inaccessible
616 to microbes or non-degradable due to binding to minerals or as inert OC (Lützow et al.,
617 2006; Kögel-Knabner et al., 2008). On contrary, the DNA of microbes, mainly as
618 bacterial or archaeal, could be mostly adsorbed on clay minerals or hidden in minute
619 pores within the fine aggregates (Poll et al., 2003; Chiu et al., 2006). Soil enzyme
620 activities could represent an overall microbial activity for soil functioning (Allison et al.,
621 2010), which was no response to accumulation of OC in the clay fractions though
622 extracellular enzymes could be also adsorbed on to clay particles (Allison and Jastrow,
623 2006).

624 On contrast, high microbial biomass and enzyme activities were in line with
625 carbon accumulation and stabilization in coarse sand sized macro-aggregates. The high
626 response of total mcirobial DNA and carbon use efficiency to OC accumualtion in the
627 coarse sand size fraction could suggest an improvement of either carbon substrate
628 supply or of habitat environemnt through increases in mass proportion of macro
629 aggregates with enhanced aggregation in soils (Lehmann et al., 2011). While containing

630 a recalcitrant OC pool similar to clay sized fractions, the macro-aggregates in coarse
631 sand sized fraction preserved also a significant amount of labile carbon (Table 3), which
632 could become easily decomposed and potentially used by microbes (Cleveland et al.,
633 2007). For the bulk soil of this chronosequence, improved microbial activity was found
634 linked to the increase in particulate OC content which was enhanced via physical
635 protection, in line with increasing aggregate stability (Wang et al., 2015). Although
636 habits within macro-aggregates offered protection of the young and labile carbon
637 against microbial decomposition (Gupta and Germida, 2015), enhanced aggregation
638 could lead to increased population and activities of specific microbial groups in
639 between micro-aggregates within macro-aggregates (Six et al., 2002b).

640 With soil aggregation improved, macro-aggregates could provide more diverse
641 soil microhabitats with varying types of OC accessible to microbes under sustainable
642 agricultural management (Six and Paustian, 2014). Improvement of spatial allocation
643 within and between micro-aggregates of carbon resource, microbial communities and
644 extracellular enzymes could favor growth of microbiota and their functional
645 performance in well aggregated soils (Caldwell, 2005; Burns et al., 2013). Many studies
646 on bulk soils showed correlation of enzyme activity with microbial biomass in
647 agricultural soils including rice paddies under proper management practices (Marx et
648 al., 2005; Allison and Jastrow, 2006; Shi et al., 2006; Yu et al., 2012). Thus, carbon
649 stabilization (indicated of carbon recalcitrance or respiration quotient) could not
650 confront microbial activity (Janzen, 2006) in macro-aggregates, where highly enriched
651 OM with labile OC pool was physically protected, in rice soils under long term paddy

652 management. This could explain a potential co-evolution of improved bio-activity with
653 enhanced carbon sequestration in agricultural soils (Rabbi et al., 2010). Of course, the
654 relation between carbon pools and specific microbial communities and biogeochemical
655 activities seemed still unclear (Smith et al., 2014).

656 **4.3 Trend of bioactivity against OC stabilization with prolonged rice cultivation**

657 While being developed on a similar matrix of paleo deposits rich in silt, the rice soils
658 have been subject to a directional development through an initial desalinization when
659 shortly shifted, followed by decalcification and finally a long existing semi-
660 hydromorphic pedogenesis characterized by mobilization of iron and manganese to for
661 minerals of metal oxyhydrates over several centuries (Wissing et al., 2013). The
662 directional changes of clay minerals particularly those of oxyhydrates, of OC pool and
663 the association with the minerals as well as archaeal and methanogenic archaeal
664 community abundance have been well characterized in the works by Cheng et al., 2009;
665 Chen et al., 2011; Wissing et al., 2011, 2014 and 2014; Kölbl et al., 2014 as well as in
666 our work by Wang et al., 2015.

667 The above mentioned changes could result in a directional changes in soil
668 aggregation, and thus in microhabitat conditions as well as nutrients (Table 1). SEM
669 observation (Fig. 2) evidenced a clear change in size of the randomly sampled
670 aggregates of the soils studied. This supported the change in mean weight diameter
671 (MWD), an indicator of soil aggregate stability, with increasing rice cultivation length
672 over the chronosequence (Wang et al. 2015). There were dispersed distinct, sharp edged
673 but less OM covered mineral particles in the uncultivated tidal marsh (P0). However,

674 aggregates became larger in size and softer and more porous with minute mineral
675 particles bound together by OM in rice soils cultivated over 100 years. This is particular
676 the case for P700, where the sand sized macro-aggregates were highly porous and soft,
677 containing smaller sized micro-aggregates and with some string-like particulate OM on
678 the surface. The increased aggregate size and thus the mean weight diameter (MWD)
679 could suggest increasing OM in-between micro-aggregates in macro-aggregates in rice
680 soils cultivated over hundreds of years., an indicator of energy use by live soil microbial
681 organisms (Schlesinger & Andrews, 2000). This change, through the improvement of
682 micro-habitat conditions and nutrient storage, could lead to some directional change in
683 the association of microbial community abundance/activity over the long run of rice
684 paddy management. The higher MBC and lower RQ and qCO_2 in coarse sand sized
685 macro-aggregates and the decreasing trend of RQ and qCO_2 with increasing length of
686 rice paddy management (Table S3) could suggest some adaptive change in microbial
687 community and improve their carbon use efficiency (Chen et al.2016). Particularly,
688 methanogenic community as particular microbial community of rice soils (Conrad,
689 2009), had been shown in a directional changes towards prolonged rice paddy
690 management (Liu et al., 2016b).

691 In a previous study (Wang et al., 2015), the bulk soil OC accumulation was found
692 concurrent with carbon stabilization and promotion of biological activity through
693 particular carbon accumulation in line with aggregate stability with long-term rice
694 cultivation. Here we synthesize all the analysis data in terms of aggregate size fraction
695 partitioning over the sequence, presented in Fig. 7. After salt marsh soil (P0) shifted to

696 rice cultivation (P50), total SOC, enzyme activity and soil respiration showed a more
697 or less consistent increase in both sand and clay sized fractions. The changes in relative
698 portion by sand sized (coarse and fine sand fractions together) aggregates against silt
699 and clay sized ones exerted different patterns between of carbon pools and of microbial
700 activities, across the soils of the chronosequence.

701 Over the sequence, the prevalence of physically protected portion in sand fractions
702 over unprotected portion in silt and clay fractions (Six et al., 2002a) was in a range of
703 1.5-3.2 and of 1.1-2.6 for SOC and total N, of 0.9-2.2 for total DNA, of 1.2-3.3 for
704 fungal gene copy numbers and of 0.8-1.5 for NEA, respectively. In contrast, the
705 prevalence of archaeal copy numbers and soil respiration was in a range of 2.6-1.0 and
706 2.0-1.3, decreasing with rice cultivation lengths. Therefore, most of analyzed carbon
707 pools and bioactivities were dominated by the macro- and large micro-aggregates in
708 sand sized fractions, which was generally in a consistent directional change with
709 prolonged paddy management under long term rice cultivation though clay particles
710 were consistently increased (Kölbl et al., 2014).

Fig. 7

712 Long term SOC sequestration in agricultural soils had been questioned (Powlson
713 et al., 2011) and OC enriched in coarse sand fractions of aggregates could indeed be
714 subject to fast decomposition in dry condition, for example, after shifting to maize land
715 (Li et al., 2007a). In this study, however, hydroagric paddy management was kept
716 continuing with ever prolonged rice cultivation, which could have driven the ever
717 increasing trend of OC accumulation up to millennium (Wissing et al., 2011; 2013).

718 Consequently, OC accumulation and stabilization could ever take place in sand sized
719 aggregates with physical protection of labile OC pool intra micro-aggregates, with
720 prolonged rice cultivation (Wang et al., 2015). POM, as a pool of relative fast turnover
721 (Cambardella and Elliott, 1992), had been also kept increasing in paddies cultivated for
722 centuries (Wang et al., 2015). Allison and Jastrow (2006) suggested that microbial
723 biochemical activity and carbon turnover was stronger in POM-enriched size fractions,
724 but weaker in mineral-dominated fractions where enzymes and their carbon substrates
725 were immobilized on mineral surfaces. Long term hydroagric paddy management
726 (Zhang and Gong, 2003), through reduced decomposition of root-, crop- or microbial-
727 residue input under reduced conditions (Roth et al., 2011). Moreover, the changes in
728 relative proportion of carbon pools and microbial activities (NEA and soil respiration)
729 by sand sized aggregates further demonstrated that physically protected and stabilized
730 carbon supported high soil bioactivities, which had been increasingly prevailed over
731 the smaller sized fractions of soil aggregates.

732 The changes in OC pools and the accessibility to microbes could lead to changes
733 in the relative abundance and activity of microbes, potentially affecting C cycling and
734 storage, in different size aggregates (Six et al., 2006). Unlike the finding by Allison and
735 Jastrow (2006), this study proposed enhanced microbial activity but improved carbon
736 use through reduced respiration quotient for microbial energy in coarse sand sized
737 macro-aggregates compared to clay fraction over centuries of rice cultivation. This is
738 supported by the recent finding that qCO_2 was seen reduced but mciorbial biomass
739 carbon increased in biochar amended agricultural soils, in a case study by Zheng et al.,

740 (2016) and in a meta-analysis by Zhou et al (2016). The strong inter-link found in this

741 study between physical protection of OC and microbiological activity in large sized

742 aggregates and the evolution with prolonged rice paddy management could help

743 enhance ecosystem functioning and services provided by rice soils (Six and Paustian

744 2014; Smith et al., 2015).

745 However, the methodology used here could not allow to characterize the spatial

746 allocation of carbon substrate, specific microbial communities and extracellular

747 enzyme activities among the aggregate fractions. Specially, labile OC pools particularly

748 those intra- aggregates or inter micro-aggregates within macro aggregates could not be

749 further explored. While such data has been considered critical –to unravel the micro-

750 scale process mediating bio-activities at aggregate level (Six and Paustian 2014).

751 Therefore, more studies are deserved on the effects on soil functions deserve further

752 studies under field conditions.

753 **5 Conclusions**

754 This study, using a rice soil chronosequence derived from salt marsh, revealed that soil

755 organic carbon could be accumulated and stabilized both in coarse sand- and clay- sized

756 fractions of soil aggregates. However, microbial abundance and enzyme activity were

757 high but metabolic quotient low in sand sized fractions –rather than in silt and clay

758 sized fractions of soil aggregates, possibly through the enhanced spatial allocation of

759 labile OC pool for improved microhabitat condition. Thus, carbon stabilization with

760 reduced turnover was not confronting soil bioactivities in a way that carbon and

761 microbial communities increasingly physically protected in macro-aggregates other

762 than in silt and clay sized aggregates. This study further supported our previous finding
763 for bulk soils that long term rice cultivation led to accumulation and stabilization of
764 SOC and promoted soil biological activities through physical protection of labile
765 carbon in line with enhanced soil aggregation. Thus, labile organic carbons
766 accumulated in macro-aggregates could help enhancing microbial C use efficiency and
767 improving their biogeochemical activity related to ecosystem functioning. More studies
768 are deserved on interaction of soil organic matter, minerals and soil microbial
769 communities to unravel the micro-scale process mediating bio-activities at aggregate
770 level.

771 **Acknowledgements:**

772 This study was partially funded by China Natural Science Foundation under a grant
773 number 40830528 and 41601305. The Ph D fellowships for the two first authors were
774 awarded with the Priority Academic Program Development of Jiangsu Higher
775 Education Institutions, China. The international cooperation was partially supported by
776 State Foreig\n Expert Agency with a "111"project under a grant number B12009.
777

778 **Reference**

779 Allison, S.D. and Jastrow, J.D.: Activities of extracellular enzymes in physically
780 isolated fractions of restored grassland soils. Soil Biol. Biochem., 38, 3245-
781 3256, 2006.
782 Allison, S. D., Wallenstein, M. D. and Bradford, M. A.: Soil-carbon response to
783 warming dependent on microbial physiology, Nat. Geosci., 3, 336-340, 2010.
784 Balser, T.C., McMahon, K., Bart, D., Bronson, D., Coyle, D., Craig, N., Flores-
785 Mangual, M., Forshay, K., Jones, S. and Kent, A.: Bridging the gap between
786 micro-and macro-scale perspectives on the role of microbial communities in
787 global change ecology, Plant Soil, 289, 59-70, 2006.
788 Bannert, A., Kleineidam, K., Wissing, L., Mueller-Niggemann, C., Vogelsang, V.,
789 Welzl, G., Cao, Z. and Schloter, M.: Changes in diversity and functional gene
790 abundances of microbial communities involved in nitrogen fixation, nitrification,
791 and denitrification in a tidal wetland versus paddy soils cultivated for different
792 time periods. Appl. Environ. Microbiol., 77, 6109-6116, 2011..
793 Banwart, S., Black, H., Cai, Z., Gicherud, P., Joosten, H., Victoria, R., Milne, E.,
794 Noellemeyer, E., Pascual, U., Nziguheba, G., Vargas, R., Bationo, A.,
795 Buschiazzo, D., De-Brogniez, D., Melillo, J., Richter,D., Termansen, M., Van
796 Noordwijk, M., Goverse, T., Ballabio, C., Bhattacharyya, T., Goldhaber, M.,
797 Nikolaidis, N., Zhao, Y., Funk, R., Chris, D., Pan, G., La Scala, N., Gottschalk,
798 P., Batjes, N., Six, J., Van Wesemael, B., Stocking, M., Bampa, F., Bernoux, M.,
799 Feller, C., Lemanceau, P. and Montanarell, L.: Benefits of soil carbon: report on
800 the outcomes of an international scientific committee on problems of the
801 environment rapid assessment workshop, Carb. Manag., 5, 185-192,2014.
802 Bardgett, R. D. and Van Der Putten, W. H.: Belowground biodiversity and ecosystem

803 functioning, Nature, 515, 505-511, 2014

804 Blackwood, C.B., Hudleston, D., Zak, D.R. and Buyer, J.S.: Interpreting ecological
805 diversity indices applied to terminal restriction fragment length polymorphism
806 data: insights from simulated microbial communities. Appl. Environ. Microbiol.,
807 73, 5276-5283, 2007.

808 Blair, G.J., Lefroy, R.D. and Lisle, L.: Soil carbon fractions based on their degree of
809 oxidation, and the development of a carbon management index for agricultural
810 systems, Crop Past. Sci., 46, 1459-1466,1995.

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

813 Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E.,
814 Wallenstein, M. D., Weintraub, M. N. and Zoppini, A.: Soil enzymes in a
815 changing environment: current knowledge and future directions, Soil Biol.
816 Biochem., 58, 216-234, 2013.

817 Caldwell, B. A.: Enzyme activities as a component of soil biodiversity: a review,
818 Pedobiologia, 49, 637-644, 2005.

819 Cambardella, C. A. and Elliott, E. T.: Particulate soil organic-matter changes across a
820 grassland cultivation sequence, Soil Sci. Soc. Am. J., 56, 777-783, 1992.

821 Chen, J., Sun, X., Li, L., Liu, X., Zhang, B., Zheng, J., Pan, G.: Change in active
822 microbial community structure, abundance and carbon cycling in an acid rice
823 paddy soil with the addition of biochar. Euro J., Soil Sci.,
824 doi;10.1111/ejss.12388, 2016.

825 Chen, L. and Zhang, G.: Parent material uniformity and evolution of soil
826 characteristics of a paddy soil chronosequence derived from marine sediments,
827 Act Pedol. Sin., 46, 753-763, 2009.

828 Chen, L., Zhang, G. and Jin, Z.: Rare earth elements of a 1000-year paddy soil
829 chronosequence: Implications for sediment provenances, parent material
830 uniformity and pedological changes, Geoderma, 230, 274-279, 2014.
831 Cheng, Y.Q., Yang, L.Z., Cao, Z.H., Ci, E. and Yin, S.: Chronosequential changes of
832 selected pedogenic properties in paddy soils as compared with non-paddy soils.
833 Geoderma, 151, 31-41, 2009.
834 Chui, C. Y., Chen, T.H., Imberger, K. and Tian, G.: Particle size fractionation of
835 fungal and bacterial biomass in subalpine grassland and forest soils. Geoderma,
836 130, 265-271, 2006.
837 Cleveland, C.C., Nemergut, D.R., Schmidt, S.K. and Townsend, A.R.: Increases in
838 soil respiration following labile carbon additions linked to rapid shifts in soil
839 microbial community composition, Biogeochem., 82, 229-240,2007..
840 Cocozza, C., D'orazio, V., Miano, T. and Shotyk, W.: Characterization of solid and
841 aqueous phases of a peat bog profile using molecular fluorescence spectroscopy,
842 ESR and FT-IR, and comparison with physical properties, Org. Geochem., 34,
843 49-60, 2003.
844 Conrad, R.: The global methane cycle: recent advances in understanding the microbial
845 processes involved, Env. Microbiol. Rep., 1, 285-292, 2009.
846 Cui, J., Li, Z., Liu, Z., Ge, B., Fang, C., Zhou, C. and Tang, B.: Physical and chemical
847 stabilization of soil organic carbon along a 500-year cultived soil
848 chronosequence originating from estuarine wetlands: Temporal patterns and land
849 use effects, Agric., Ecosyst. Environ., 196, 10-20, 2014.
850 Deng, S.Q. and Xu, M.X.: A protocol of separating water stable aggregates from rice
851 soils and its application, Acta Pedo. Sin., 13(2), 229-234,1965. In Chinese.
852 Dungait, J.A., Hopkins, D.W., Gregory, A.S. and Whitmore, A.P.: Soil organic matter

853 turnover is governed by accessibility not recalcitrance, Glob. Chang. Biol., 18,
854 1781-1796, 2012.
855 Ellerbrock, R., Höhn, A. and Rogasik, J.: Functional analysis of soil organic matter as
856 affected by longterm manurial treatment, Euro. J. Soil Sci., 50, 65-71, 1999.
857 Elliott, E. T.: Aggregate structure and carbon, nitrogen, and phosphorus in native and
858 cultivated soils. Soil Sci. Soc. Am. J., 50, 627-633, 1986.
859 Ettema, C.H. and Wardle, D.A.: Spatial soil ecology, Trends Ecol. Evol., 17, 177-183,
860 2002.
861 Gajda, A.M.: Microbial activity and particulate organic matetr content in soils with
862 diffrerent tillage system use, Int. Agrophys., 24, 129-137, 2010.
863 Gardes, M. and Bruns, T.D.: ITS primers with enhanced specificity for
864 basidiomycetes - application to the identification of mycorrhizae and rusts,
865 Molec. Eecol., 2, 113-118, 1993.
866 Gong, Z., Zhang, G., Wang, J.Z., Jia, H.Y., Shen, H. and Lu, J.C.: Anthorosols. In:
867 Gong, Z. (editor-in-chief), Chinese Soil Taxonomy: Priciples, Methodology and
868 Application. Science Press, Beijing (China), pp109-131, 1999.
869 Guan, S., Zhang, D. & and Zhang, Z.: Soil enzyme and its research methods. China
870 Agriculture Press, Beijing, 274-297, 1986.
871 Gupta, V. V. and Germida, J. J.: Soil aggregation: Influence on microbial biomass and
872 implications for biological processes, Soil Biol. Biochem., 80, A3-A9, 2015.
873 Janzen, H. H.: The soil carbon dilemma: shall we hoard it or use it? Soil Biol.
874 Biochem., 38, 419-424, 2006.
875 Jastrow, J. D., Miller, R. M. and Lussenhop, J.: Contributions of interacting biological
876 mechanisms to soil aggregate stabilization in restored prairie. Soil Biol.
877 Biochem., 30, 905-916, 1998.

878 Jiang, X., Liu, W., Liu, Q., Jia, Z., Wright, A.L. and Cao, Z.: Soil N mineralization,
879 nitrification and dynamic changes in abundance of ammonia-oxidizing bacteria
880 and archaea along a 2000 year chronosequence of rice cultivation, Plant Soil,
881 365, 59-68, 2013.
882 Joergensen, R.G. : The fumigation-extraction method to estimate soil microbial
883 biomass: calibration of the k EC value. Soil Biol. Biochem., 28, 25–31,1996..
884 Kalbitz, K., Kaiser, K., Fiedler, S., Kölbl, A., Amelung, W., Bräuer, T., Cao, Z., Don,
885 A., Grootes, P. and Jahn, R.: The carbon count of 2000 years of rice cultivation,
886 Glob. Chang. Biol., 19, 1107-1113, 2013.
887 Kandeler, E., Mosier, A.R., Morgan, J. A., Milchunas, D. G., King, J. Y., Rudolph, S.
888 and Tscherko, D.: Response of soil microbial biomass and enzyme activities to
889 the transient elevation of carbon dioxide in a semi-arid grassland, Soil Biol.
890 Biochem., 38, 2448-2460, 2006.
891 Kandeler, E., Stemmer, M. and Klimanek, E.-M.: Response of soil microbial biomass,
892 urease and xylanase within particle size fractions to long-term soil management,
893 Soil Biol. Biochem., 31, 261-273, 1999.
894 Kandeler, E., Tscherko, D., Bruce, K., Stemmer, M., Hobbs, P.J., Bardgett, R.D. and
895 Amelung, W.: Structure and function of the soil microbial community in
896 microhabitats of a heavy metal polluted soil, Biol. Fert. Soils, 32, 390-400, 2000.
897 Kennedy, A. C. and Papendick, R. I.: Microbial characteristics of soil quality. J. Soil
898 Water Conserv., 50, 243-248, 1995.
899 Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu,
900 S., Eusterhues, K. and Leinweber, P.: Organo-mineral associations in temperate
901 soils: integrating biology, mineralogy, and organic matter chemistry, Zeitsch.
902 Pflanzenern. Bodenk., 171, 61, 2008.

903 Kölbl, A., Schad, P., Jahn, R., Amelung, W., Bannert, A., Cao, Z., Fiedler, S., Kalbitz,
904 K., Lehndorff, E. and Müller-Niggemann, C.: Accelerated soil formation due to
905 paddy management on marshlands (Zhejiang Province, China), Geoderma, 228,
906 67-89, 2014.
907 Kong, A.Y., Six, J., Bryant, D.C., Denison, R.F. and van Kessel, C.: The relationship
908 between carbon input, aggregation, and soil organic carbon stabilization in
909 sustainable cropping systems, Soil Sci. Soc. Amer. J., 69, 1078-1085, 2005.
910 Lagomarsino, A., Grego, S. and Kandeler, E.: Soil organic carbon distribution drives
911 microbial activity and functional diversity in particle and aggregate-size
912 fractions, Pedobiol., 55, 101-110, 2012.
913 Lehmann, J. and Kleber, M.: The contentious nature of soil organic matter, Nat., 258,
914 60-68, doi:10.1038/nature16069, 2015.
915 Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C. and Crowley,
916 D.: Biochar effects on soil biota–a review. Soil Biol. Biochem., 43, 1812-1836,
917 2011.
918 Lehmann, J., Solomon, D., Kinyangi, J., Dathe, L., Wirick, S. and Jacobsen, C.:
919 Spatial complexity of soil organic matter forms at nanometre scales, Nat.
920 Geosci., 1, 238-242,2008.
921 Li, Z.P., Pan, G., and Zhang, X.H.: Topsoil organic carbon pool and ^{13}C natural
922 abundance changes from a paddy after 3 years corn cultivation, Acta Ped. Sin.,
923 44(2), 244-251, 2007a. In Chinese with English summary.
924 Li, L., Zhang, X., Zhang, P., Zheng, J. and Pan, G.: Variation of organic carbon and
925 nitrogen in aggregate size fractions of a paddy soil under fertilisation practices
926 from Tai Lake Region, China, J Sci. Food Agri., 87, 1052-1058, 2007b.
927 Li, Q.: Paddy Soils of China, Science Presss, Beijing, China. 1992. In Chinese.

928 Liu, D., Liu, X., Liu, Y., Li, L., Pan, G., Crowley, D. and Tippkötter, R.: Soil organic
929 carbon (SOC) accumulation in rice paddies under long-term agro-ecosystem
930 experiments in southern China–VI. Changes in microbial community structure
931 and respiratory activity,. Biogeosci. Disc., 8, 1529-1554, 2011.
932 Liu, Y., Wang, P., Crowley, D., Liu, X., Chen, J., Li, L., Zheng, J., Zhang, X., Zheng,
933 J. and Pan, G.: Methanogenic abundance and changes in community structure
934 along a rice soil chronosequence from east China, Euro. J. Soil Sci., doi:
935 10.1111/ejss.12348, 2016a.
936 Liu, Y., Wang, P., Pan, G., Crowley, D., Li, L., Zheng, J., Zhang, X. and Zheng, J.:
937 Functional and structural responses of bacterial and fungal communities from
938 paddy fields following long-term rice cultivation, J. Soils Sediments, 16, 1460-
939 1471, 2016b.
940 Lu, G., Sakagami, K.-i., Tanaka, H. and Hamada, R.: Role of soil organic matter in
941 stabilization of water-stable aggregates in soils under different types of land use,
942 Soil Sci Plant Nut., 44, 147-155,1998.
943 Lueders, T. and Friedrich, M.: Archaeal population dynamics during sequential
944 reduction processes in rice field soil, Appl. Environ. Microbiol., 66, 2732-2742,
945 2000.
946 Lützow, M., Kögel−Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G.,
947 Marschner, B. and Flessa, H.: Stabilization of organic matter in temperate soils:
948 mechanisms and their relevance under different soil conditions–a review, Euro. J
949 Soil Sci., 57, 426-445, 2006.
950 Marriott, E.E. and Wander, M.: Qualitative and quantitative differences in particulate
951 organic matter fractions in organic and conventional farming systems, Soil Biol.
952 Biochem., 38, 1527-1536, 2006.

953 Marschner, B., Brodowski, S., Dreves, A., Gleixner, G., Gude, A., Grootes, P.M.,
954 Hamer, U., Heim, A., Jandl, G. and Ji, R.: How relevant is recalcitrance for the
955 stabilization of organic matter in soils? J Plant Nutr. Soil Sci., 171, 91-110, 2008.
956 Marx, M.C., Kandeler, E., Wood, M., Wermbter, N. and Jarvis, S.: Exploring the
957 enzymatic landscape: distribution and kinetics of hydrolytic enzymes in soil
958 particle-size fractions, Soil Biol. Biochem., 37, 35-48, 2005.
959 Matocha, C.J., Haszler, G.R. and Grove, J.H.: Nitrogen fertilization suppresses soil
960 phenol oxidase enzyme activity in no-tillage systems, Soil Sci, 169, 708-714,
961 2004.
962 Mikutta, R., Kleber, M., Torn, M. S. and Jahn, R.: Stabilization of soil organic matter:
963 association with minerals or chemical recalcitrance? Biogeochem., 77, 25-56,
964 2006.
965 Miller, R. and Jastrow, J.: Mycorrhizal fungi influence soil structure. In: Arbuscular
966 Mycorrhizas: Physiology And Function, Springer, 3-18, 2000.
967 Nelson, P., Dictor, M. and Soulas, G.: Availability of organic carbon in soluble and
968 particle-size fractions from a soil profile, Soil Biol. Biochem., 26, 1549-
969 1555,1994.
970 Oades, J.M.: Soil organic matter and structural stability: mechanisms and implications
971 for management, Plant Soil, 76, 319-337,1984.
972 Olk, D., Brunetti, G. and Senesi, N.: Decrease in Humification of Organic Matter with
973 Intensified Lowland Rice Cropping A Wet Chemical and Spectroscopic
974 Investigation, Soil Sci. Soc. Amer. J., 64, 1337-1347, 2000.
975 Øvreås, L. and Torsvik, V.: Microbial diversity and community structure in two
976 different agricultural soil communities, Microb. Ecol., 36, 303-315. 1998.
977 Pan, G., Li, L., Wu, L. and Zhang, X.: Storage and sequestration potential of topsoil

978 organic carbon in China's paddy soils, Glob. Chang. Biol.,10, 79-92, 2004.
979 Pan, G., Wu, L., Li, L., Zhang, X., Gong, W. and Wood, Y.: Organic carbon
980 stratification and size distribution of three typical paddy soils from Taihu Lake
981 region, China, J. Environ. Sci., 20, 456-463,2008.
982 Pan, G., Xu, X., Smith, P., Pan, W. and Lal, R.: An increase in topsoil SOC stock of
983 China's croplands between 1985 and 2006 revealed by soil monitoring. Agr.
984 Ecosyst. Environ., 136, 133-138, 2010.
985 Poll, C., Thiede, A., Wermbter, N., Sessitsch, A. and Kandeler, E.: Microscale
986 distribution of microorganisms and microbial enzyme activities in a soil with
987 longterm organic amendment, Euro. J Soil Sci., 54, 715-724, 2003.
988 Pozo, C., Diaz-Visurraga, J., Contreras, D., Freer, J., Rodriguez, J. Characterization of
989 temporal biodegradation of Radiata pine by Gloeophyllum trabeaum through
990 principal component analysis-based two dimensional correlation FTIR
991 spectroscopy. J. Chil. Chem. Soc., 61,2878-2883, 2016.
992 Powlson, D. S., Whitmore, A. P. and Goulding, K. W. T.: Soil carbon sequestration to
993 mitigate climate change: a critical re-examination to identify the true and the
994 false. Euro. J. Soil Sci., 62, 42-55, 2011.
995 Qian, H. Y., Pan, J. J. and Sun, B.: The relative impact of land use and soil properties
996 on sizes and turnover rates of soil organic carbon pools in Subtropical China.
997 Soil Use Manage., 29, 510-518, 2013.
998 Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W.F. and Torn, M.S. : Large
999 contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical
1000 forest soils, Plant Soil, 233, 167-177,2001.
1001 Roth, P. J., Lehndorff, E., Zhuang, S., Bannert, A., Wissing, L., Schloter, M., Kogel-
1002 Knabner, I. and Amelung, W.: Accumulation of nitrogen and microbial residues

1003 during 2000 years of rice paddy and non-paddy soil development in the Yangtze
1004 River Delta, China, Global Change Biol., 17, 3405-3417, 2011.
1005 Saiya-Cork, K., Sinsabaugh, R. and Zak, D.: The effects of long term nitrogen
1006 deposition on extracellular enzyme activity in an Acer saccharum forest soil, Soil
1007 Biol. Biochem.,34, 1309-1315,2002.
1008 Salinas-Garcia, J., Hons, F., Matocha, J. and Zuberer, D. : Soil carbon and nitrogen
1009 dynamics as affected by long-term tillage and nitrogen fertilization, Biol. Fert.
1010 Soils, 25, 182-188,1997.
1011 Schlesinger, W.H. and Andrews, J.A.: Soil respiration and the global carbon cycle,
1012 Biogeochem.,48, 7-20,2000.
1013 Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A.,
1014 Kleber, M., Kögel-Knabner, I., Lehmann, J. and Manning, D.A.: Persistence of
1015 soil organic matter as an ecosystem property, Nature, 478, 49-56,2011.
1016 Sessitsch, A., Weilharter, A., Gerzabek, M.H., Kirchmann, H. and Kandeler, E.:
1017 Microbial population structures in soil particle size fractions of a long-term
1018 fertilizer field experiment, Appl. Environ. Microbiol., 67, 4215-4224,2001.
1019 Shi, W., Dell, E., Bowman, D. and Iyyemperumal, K.: Soil enzyme activities and
1020 organic matter composition in a turfgrass chronosequence, Plant Soil, 288, 285-
1021 296, 2006.
1022 Six, J., Bossuyt, H., Degryze, S. and Denef, K.:A history of research on the link
1023 between (micro) aggregates, soil biota, and soil organic matter dynamics. Soil
1024 Till. Res., 79, 7-31, 2004.
1025 Six, J., Conant, R., Paul, E.A. and Paustian, K.: Stabilization mechanisms of soil
1026 organic matter: implications for C-saturation of soils, Plant Soil, 241, 155-176 ,
1027 2002a.

1028 Six, J., Feller, C., Denef, K., Ogle, S., Sa, J.C.D.M. and Albrecht, A.: Soil organic
1029 matter, biota and aggregation in temperate and tropical soils-Effects of no-tillage,
1030 Agron, 22, 755-775, 2002b..
1031 Six, J., Frey, S., Thiet, R. and Batten, K.: Bacterial and fungal contributions to carbon
1032 sequestration in agroecosystems, Soil Sci. Soc. Amer. J., 70, 555-569, 2006.
1033 Six, J. and Paustian, K. :Aggregate-associated soil organic matter as an ecosystem
1034 property and a measurement tool, Soil Biol. Biochem., 68, A4-A9,2014.
1035 Six, J., Paustian, K., Elliott, E. and Combrink, C.: Soil structure and organic matter I.
1036 Distribution of aggregate-size classes and aggregate-associated carbon, Soil Sci.
1037 Soc. Amer. J., 64, 681-689, 2000.
1038 Smith, A., Marín-Spiotta, E., de Graaff, M. and Balser, T.: Microbial community
1039 structure varies across soil organic matter aggregate pools during tropical land
1040 cover change, Soil Biol. Biochem., 77, 292-303, 2014.
1041 Smith, P., Cotrufo, M. F., Rumpel, C., Paustian, K., Kuikman, P. J., Elliott, J. A.,
1042 Mcdowell, R., Griffiths, R. I., Asakawa, S., Bustamante, M., House, J. I.,
1043 Sobocka, J., Harper, R., Pan, G., West, P. C., Gerber, J. S., Clark, J. M., Adhya,
1044 T., Scholes, R. J. and Scholes, M. C.: Biogeochemical cycles and biodiversity as
1045 key drivers of ecosystem services provided by soils. Soil Discussions, 2, 537-
1046 586, 2015.
1047 Song, X., Li, L., Zheng, J., Pan, G., Zhang, X., Zheng, J., Hussain, Q., Han, X. and
1048 Yu, X.: Sequestration of maize crop straw C in different soils: role of
1049 oxyhydrates in chemical binding and stabilization as recalcitrance, Chem., 87,
1050 649-654, 2012.
1051 Song, X., Spaccini, R., Pan, G. and Piccolo, A.: Stabilization by hydrophobic
1052 protection as a molecular mechanism for organic carbon sequestration in maize-

1053 amended rice paddy soils, Sci Tot. Environ., 458, 319-330, 2013.
1054 Stemmer, M., Gerzabek, M.H. and Kandeler, E.:Organic matter and enzyme activity
1055 in particle-size fractions of soils obtained after low-energy sonication, Soil Biol.
1056 Biochem., 30, 9-17,1998.
1057 Stemmer, M., von Lützow, M., Kandeler, E., Pichlmayer, F. and Gerzabek, M.H.: The
1058 effect of maize straw placement on mineralization of C and N in soil particle size
1059 fractions. Euro. J. Soil Sci., 50, 73-85,1999.
1060 Strickland, M.S. and Rousk, J.:Considering fungal: bacterial dominance in soils–
1061 methods, controls, and ecosystem implications, Soil Biol. Biochem., 42, 1385-
1062 1395, 2010.
1063 Tisdall, J. and Oades, J.M.: Organic matter and water - stable aggregates in soils, J.
1064 Soil Sci., 33, 141-163, 1982.
1065 Tisdall, J.: Possible role of soil microorganisms in aggregation in soils, Plant Soil,
1066 159, 115-121, 1994.
1067 Tivet, F., de Moraes Sa, J. C., Lal, R., Milori, D.M.B.P., Briedis, C.,Letourmy, C.,
1068 Pinheiro, L. A., Borszowskei, P. R., da Cruz Hartman, D.: Assessing
1069 humification and organic C compunds by laser-induced fluorescence and FTIR
1070 spectroscopies under conventional and no-tillage management in Brazilian
1071 Oxisols. Geoderma, 27-28, 71-81, 2013.
1072 Torsvik, V. and Øvreås, L.: Microbial diversity and function in soil: from genes to
1073 ecosystems, Curr. Opin. Microb., 5, 240-245, 2002.
1074 Vogel, C., Mueller, C.W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schloter,
1075 M. and Kögel-Knabner, I.: Submicron structures provide preferential spots for
1076 carbon and nitrogen sequestration in soils, Nature Comm., 5, 2947. doi:
1077 10.1038/ncomms3947, 2014.

1078 Wakeham, S. G. and Canuel, E. A.: The nature of organic carbon in density-
1079 fractionated sediments in the Sacramento-San Joaquin River Delta (California),
1080 Biogeosci., 13, 567-582, doi:10.5194/bg-13-567-2016, 2016.
1081 Wang, P., Liu, Y., Li, L., Cheng, K., Zheng, J., Zhang, X., Zheng, J., Joseph, S. and
1082 Pan, G.: Long-term rice cultivation stabilizes soil organic carbon and promotes
1083 soil microbial activity in a salt marsh derived soil chronosequence, Sci Rep, 5,
1084 15704,2015.
1085 Wissing, L., Kölbl, A., Häusler, W., Schad, P., Cao, Z.-H. and Kögel-Knabner, I.:
1086 Management-induced organic carbon accumulation in paddy soils: the role of
1087 organo-mineral associations, Soil Till. Res., 126, 60-71,2013.
1088 Wissing, L., Kölbl, A., Vogelsang, V., Fu, J.-R., Cao, Z.-H. and Kögel-Knabner, I.:
1089 Organic carbon accumulation in a 2000-year chronosequence of paddy soil
1090 evolution, Catena, 87, 376-385, 2011.
1091 Yang, C., Yang, L. and Ouyang, Z.: Organic carbon and its fractions in paddy soil as
1092 affected by different nutrient and water regimes, Geoderma, 124, 133-142, 2005.
1093 Yang, Y., Guo, J., Chen, G., Yin, Y., Gao, R., Lin, C.: Effect of forest conversion on
1094 labile organic carbon fractions and aggregate stability in subtropical China, Plant
1095 Soil, 323,153-162, 2009.
1096 Yin, B., Crowley, D., Sparovek, G., De Melo, W.J. and Borneman, J.: Bacterial
1097 functional redundancy along a soil reclamation gradient, Appl. Environ. Microb.,
1098 66, 4361-4365, 2000.
1099 Yu, H., Ding, W., Luo, J., Donnison, A. and Zhang, J.: Lonterm effect of compost and
1100 inorganic fertilizer on activities of carbon cycle enzymes in aggregates of an
1101 intensively cultivated sandy loam, Soil Use Manag., 28, 347-360, 2012.
1102 Zhang, A., Cui, L., Pan, G., Li, L., Hussain, Q., Zhang, X., Zheng, J. and Crowley,

1103 D.:. Effect of biochar amendment on yield and methane and nitrous oxide
1104 emissions from a rice paddy from Tai Lake plain, China, Agric., Ecosyst.
1105 Environ., 139, 469-475, 2010a.
1106 Zhang, G., Chen, L.and Gilkes, R.: Soil genesis along a paddy soil chronosequence in
1107 a millennium scale. In: Proceedings of the 19th World Congress of Soil Science:
1108 Soil solutions for a changing world, Brisbane, Australia, 1-6 August 2010.
1109 Symposium 1.3. 1 Pedogenesis: ratio and ranges of influence, pp. 88-91.
1110 International Union of Soil Sciences (IUSS), c/o Institut für Bodenforschung,
1111 Universität für Bodenkultur. 2010b.
1112 Zhang, G. and Gong, Z.: Pedogenic evolution of paddy soils in different soil
1113 landscapes, Geoderma, 115, 15-29, 2003.
1114 Zhang, P., Zheng, J., Pan, G., Zhang, X., Li, L. and Rolf, T.: Changes in microbial
1115 community structure and function within particle size fractions of a paddy soil
1116 under different long-term fertilization treatments from the Tai Lake region,
1117 China, Coll. Surf. B: Biointerfaces, 58, 264-270, 2007.
1118 Zhang, S., Li, Q., Lü, Y., Zhang, X. and Liang, W.: Contributions of soil biota to C
1119 sequestration varied with aggregate fractions under different tillage systems, Soil
1120 Biol. Biochem., 62, 147-156, 2013.
1121 Zheng, J., Zhang, X., Li, L., Zhang, P. and Pan, G.: Effect of long-term fertilization on
1122 C mineralization and production of CH_4 and CO_2 under anaerobic incubation
1123 from bulk samples and particle size fractions of a typical paddy soil, Agric.
1124 Ecosyst. Environ., 120, 129-138, 2007.
1125 Zheng, J.F., Zhang, X., Chen, J. Pan, G.: Biochar decreased microbial metabolic
1126 quotient and shifted community composition four years after a single
1127 incorporation in a slightly acid rice paddy from southwest China. Sci. Tot.

1128 Environ., 571:206-217, 2016.
1129 Zhou, P. and Pan, G.: Effect of different long-term fertilization treatments on
1130 particulate organic carbon in water-stable aggregates of a paddy soil, Chin. J Soil
1131 Sci, 38, 256-261, 2007. In Chinese with English sumamry.
1132 Zhou, P., Song, G., Pan, G., Li, L., Zhang, X. and Wu, L.:SOC accumulation in three
1133 major types of paddy soils under long-term agro-ecosystem experiments from
1134 South China I. Physical protection in soil micro-aggregates, Act. Pedol. Sin., 45,
1135 1063-1071, 2008.
1136 Zhou, P., Piccolo, A., Pan, G. and Smejkalova D.: SOC enhancement in three major
1137 types of paddy soils in a long-term agro-ecosystem experiment in South China.
1138 III. structural variation of particulate organic matter of two paddy soils, Act.
1139 Pedol. Sin., 46, 398-405, 2009a.
1140 Zhou, P., Song, G., Pan, G., Li, L. and Zhang, X.: Role of chemical protection by
1141 binding to oxyhydrates in SOC sequestration in three typical paddy soils under
1142 long-term agro-ecosystem experiments from South China, Geoderma, 153, 52-
1143 60, 2009b.
1144 Zhou, P., Piccolo, A., Pan, G. and Spaccini R.: SOC enhancement in three major types
1145 of paddy soils in a long-term agro-ecosystem experiment in South China. IV.
1146 Molecular characterization of particulate organic carbon by TMAH
1147 thermochemolysis GC/MS, Act. Pedol. Sin., 48, 112-124, 2011.
1148 Zhou, H.M., Zhang, D.X., Wang, P., Liu, X., Cheng, K., Li, L.Q., Zheng, J.W., Zhang,
1149 X.H., Zheng, J.F., Crowley, D., van Zwieten, L., Pan, G.: Changes in microbial
1150 biomass and the metabolic quotient under biochar amendment in agricultural soils:
1151 A Meta-analysis. Agriculture, Ecosystem and Environment, under review, 2016
1152 Zou, P., Fu, J., Cao, Z., Ye, J. and Yu, Q.: Aggregate dynamics and associated soil

1153 organic matter in topsoils of two 2,000-year paddy soil chronosequences, J Soils
1154 Sedim., 15, 510-522, 2015.

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1157 **Figure captions**

1158 **Fig. 1** Sampling sites for the individual soils constituting the rice soil chronosequence
1159 from Cixi County, Zhejiang province, China. The suffix number following P
1160 (paddy soil) designates the years under rice cultivation after shifting from salt
1161 marsh since dyke establishment.

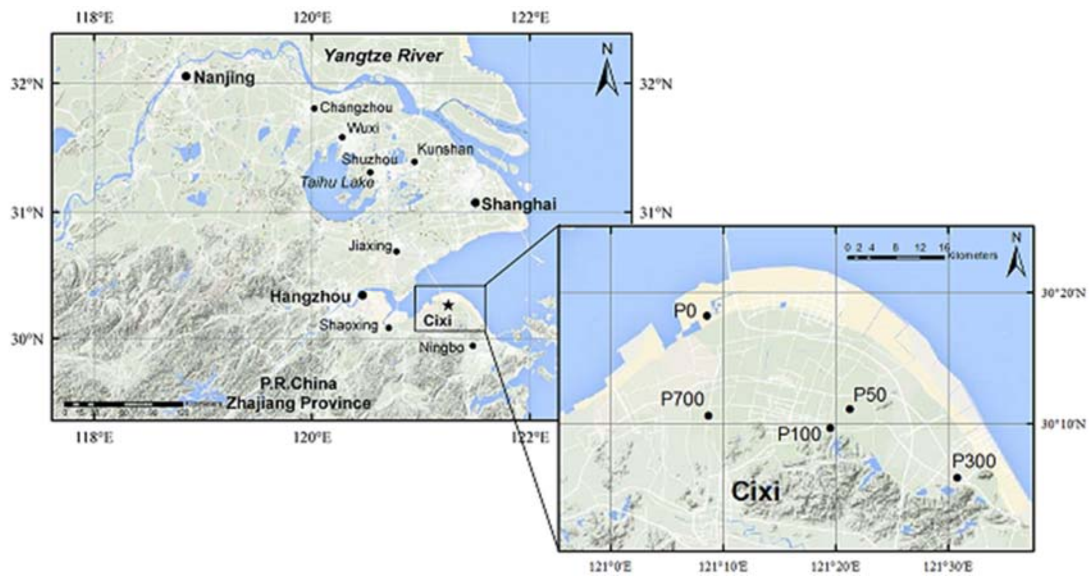
1162 **Fig. 2** Scanning electron microscopy images of aggregates separated with sonification
1163 dispersion in water from topsoil sample of the studied chronosequence. P0, P50,
1164 P100, P300 and P700 represents respectively the uncultivated mash soil and the
1165 shifted rice soils cultivated for 50, 100, 300 and 700 years.

1166 **Fig. 3** Correlation of carbon recalcitrance (the ratio of aromatic to polysaccharide and
1167 aliphatic carbon) (a) and respiration quotient (b) to organic carbon level with of
1168 the particle size fractions of topsoil of the chronosequence soils.

1169 **Fig. 4** Correlation of organic carbon enrichment index (SOC content in a fraction
1170 divided by SOC content of the bulk soil) to content of labile carbon of size
1171 fractions of soil aggregates of the chronosequence soils**.** The open circle are those
1172 fractions from the uncultivated marsh soil (P0). Above or below the black long
1173 dashed line representing OC enrichment or depletion in a fraction.

1174 **Fig. 5** Inter-correlation between carbon pools and microbial biomass to address the
1175 differences of soil carbon stability and microbial functioning between coarse sand
1176 (left) and clay (right) sized aggregates fractions (Soil organic carbon accumulation
1177 as a function of relative recalcitrant C (aromatic) (a) and negatively of relative
1178 labile C (aliphatic and polysaccharide) (b); CO_2 production as a plateau function

1179 of soil microbial biomass (c) and bacterial abundance (d)). Data was the mean
1180 value of triplicates.
1181 **Fig. 6** Inter-correlation between particulate organic carbon and soil microbial activity
1182 to compare the biological activity versus carbon between coarse sand (left) and
1183 clay (right) sized aggregate fractions (Soil microbial biomass was as an
1184 exponential function of total soil organic carbon (a) and a linear function of labile
1185 organic carbon (d). Normalized enzyme activity (b) and DNA content scaled CO_2
1186 production (c) as a linear and negative power function of soil microbial biomass.
1187 Soil microbial biomass was as a linear function of relative recalcitrant C (aromatic)
1188 (e)). Data was the mean value of triplicates.
1189 **Fig. 7** Change in partitioning of soil organic carbon (a, g/kg), total DNA (b, μg/g) ,
1190 normalized enzyme activity (c, relative enzyme activity index) and soil respiration
1191 (d, mgCO_2/g) among coarse and fine sand fraction (blue base), silt fraction (brown
1192 base) and clay fraction (gray base) of soil aggregates, over the chronosequence of
1193 rice soils (P50-P700) shifted from a salt marsh (P0) under long term rice
1194 cultivation. The size of a circle in a row is relevant to that of an analyzed parameter
1195 among the soils.
1196
1197



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1199

1200 **Fig. 1**

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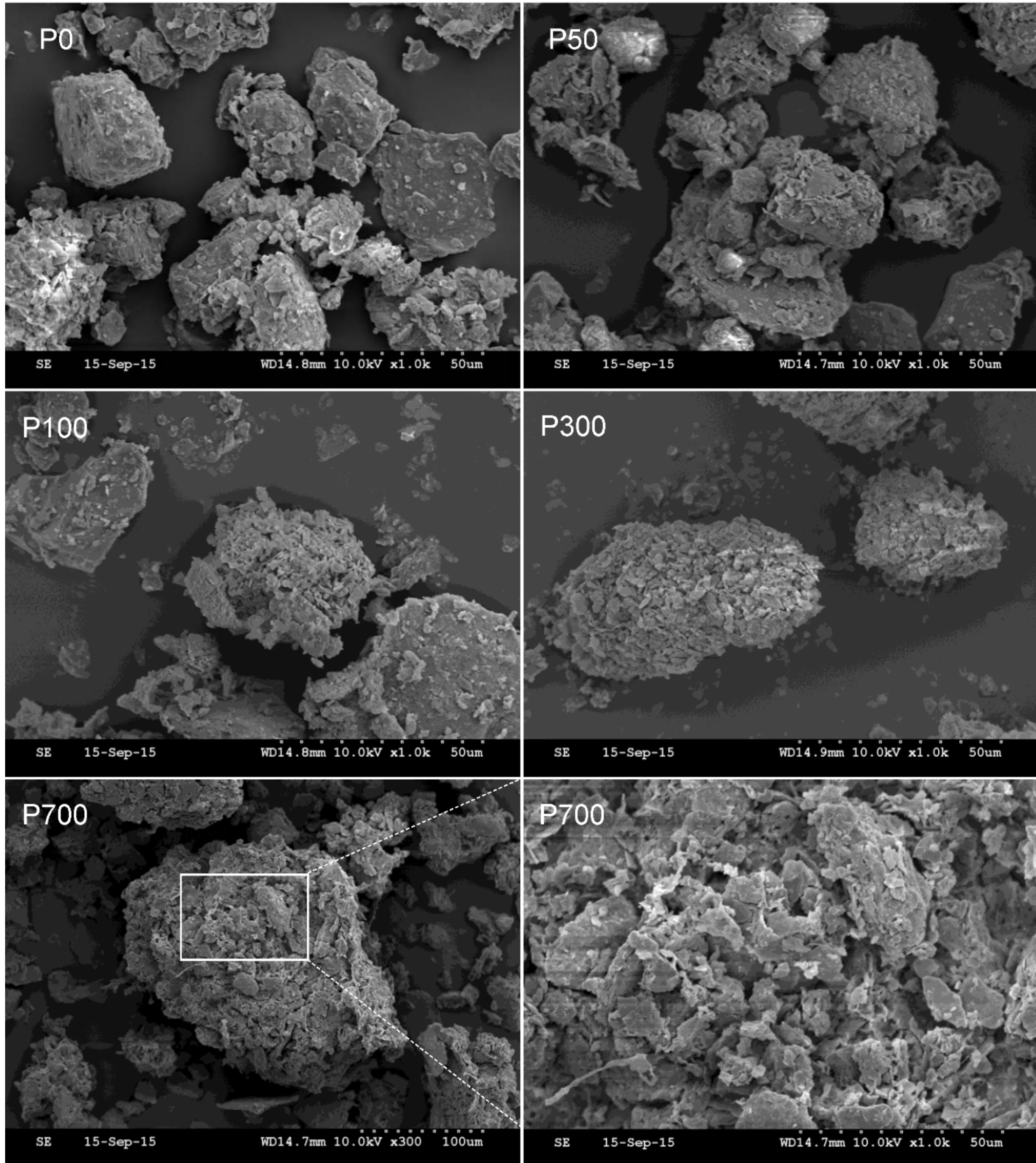

1203 **Fig. 2**

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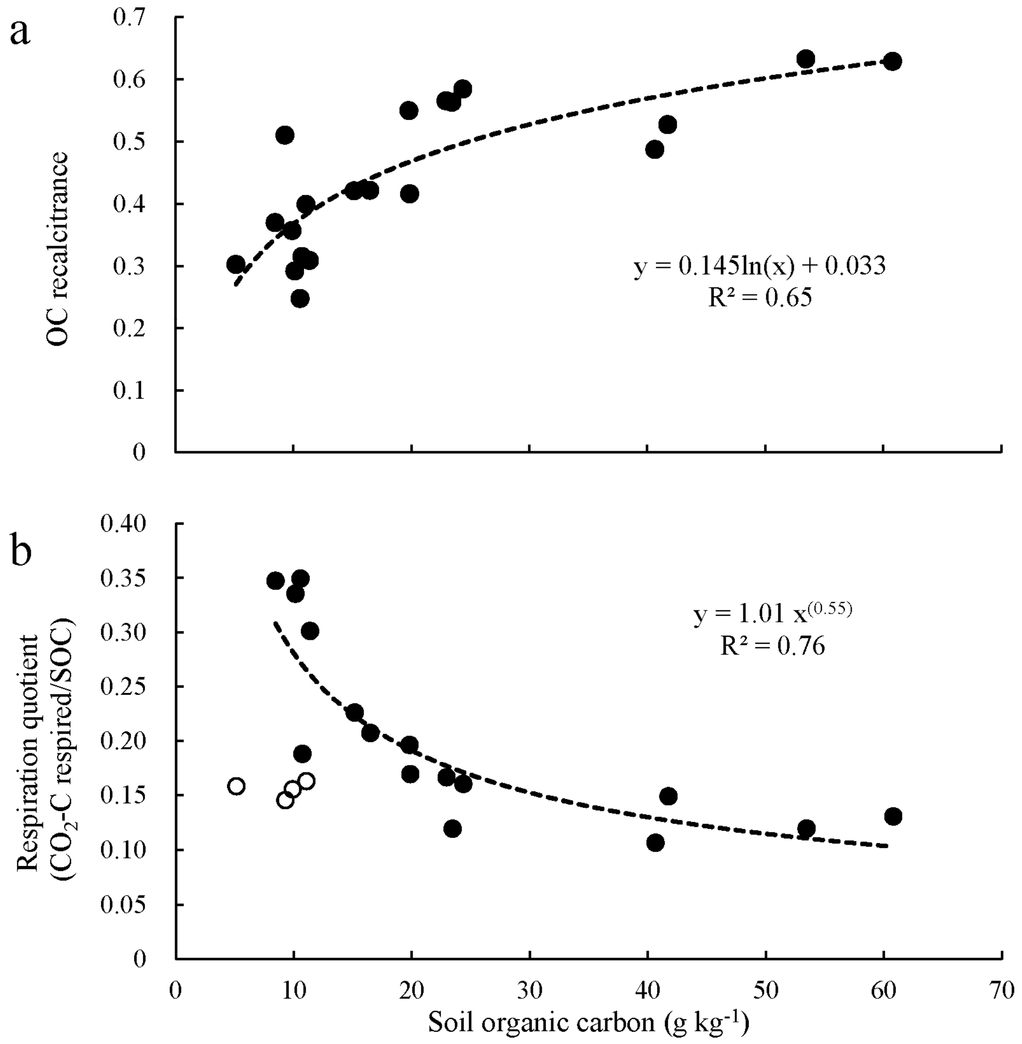
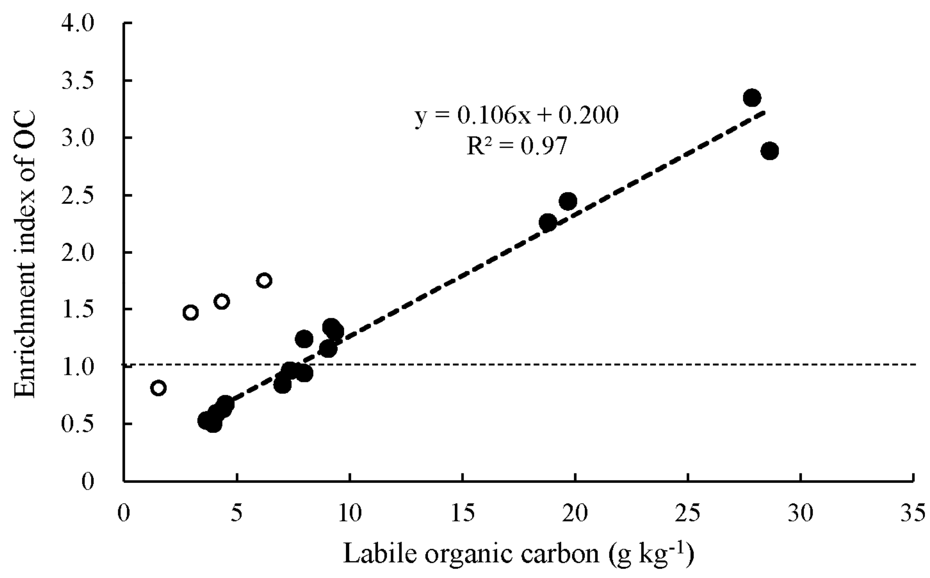


1206

1207 **Fig. 3**



1208

1209 **Fig. 4**

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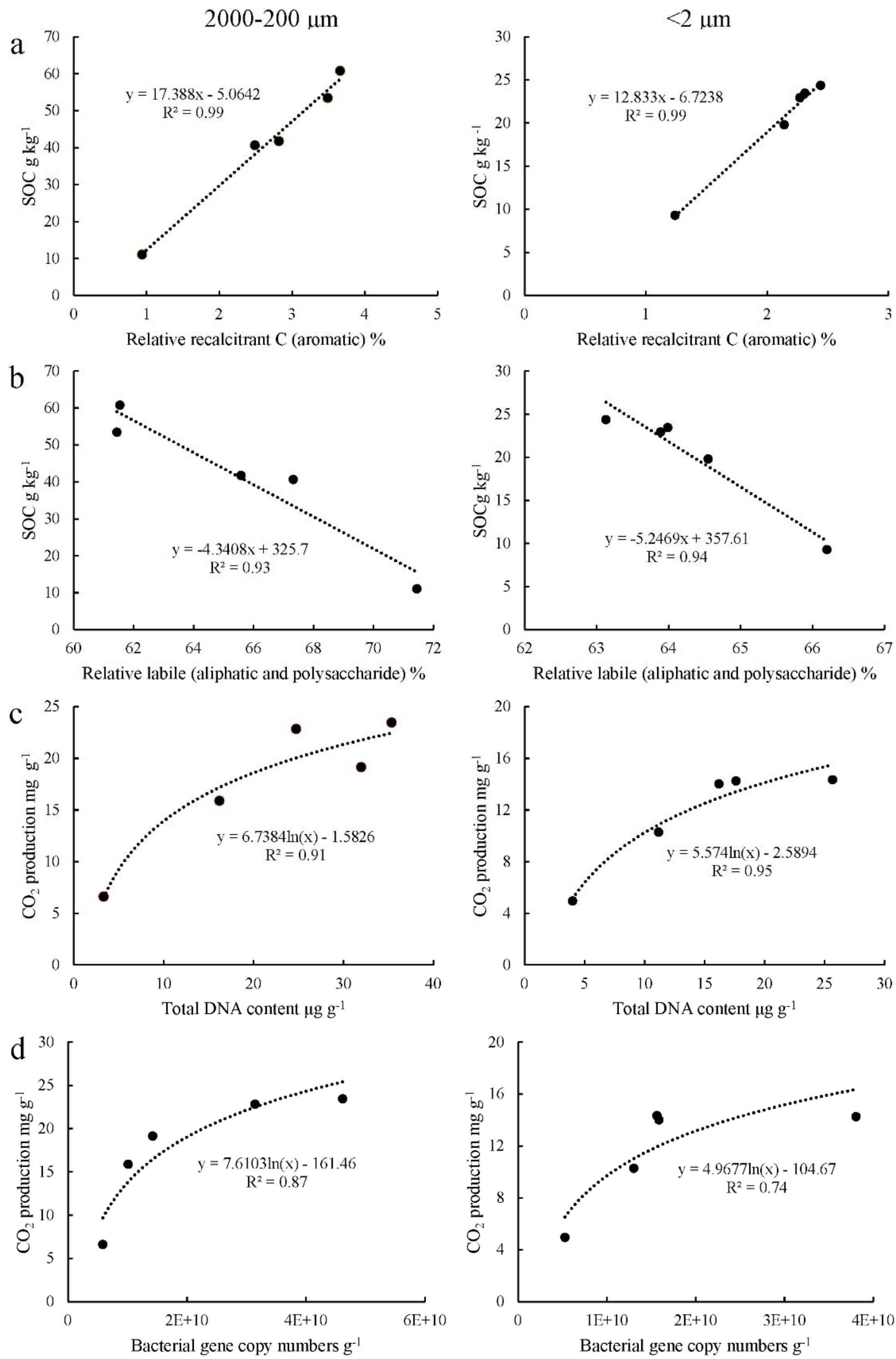

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1212 **Fig. 5**

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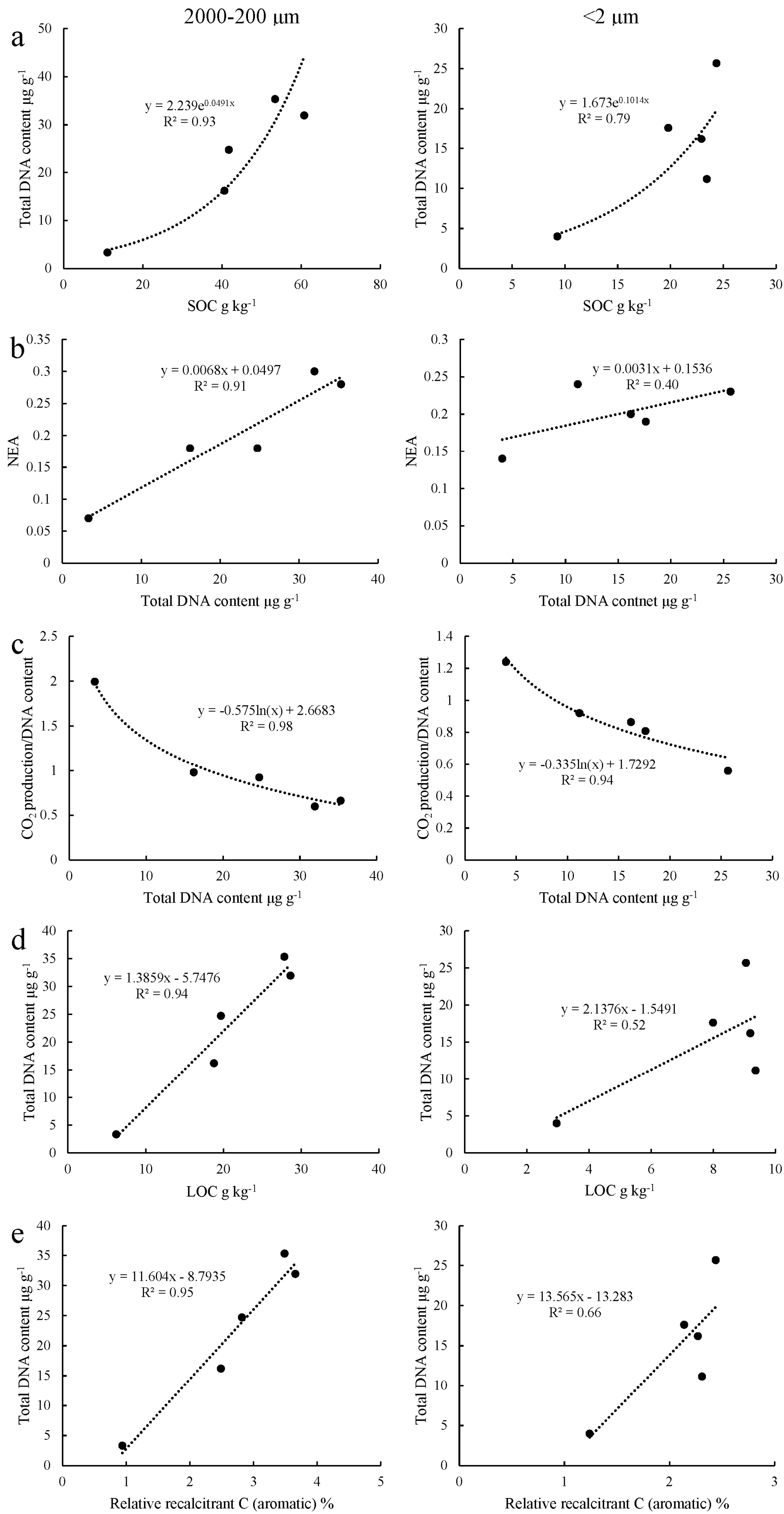
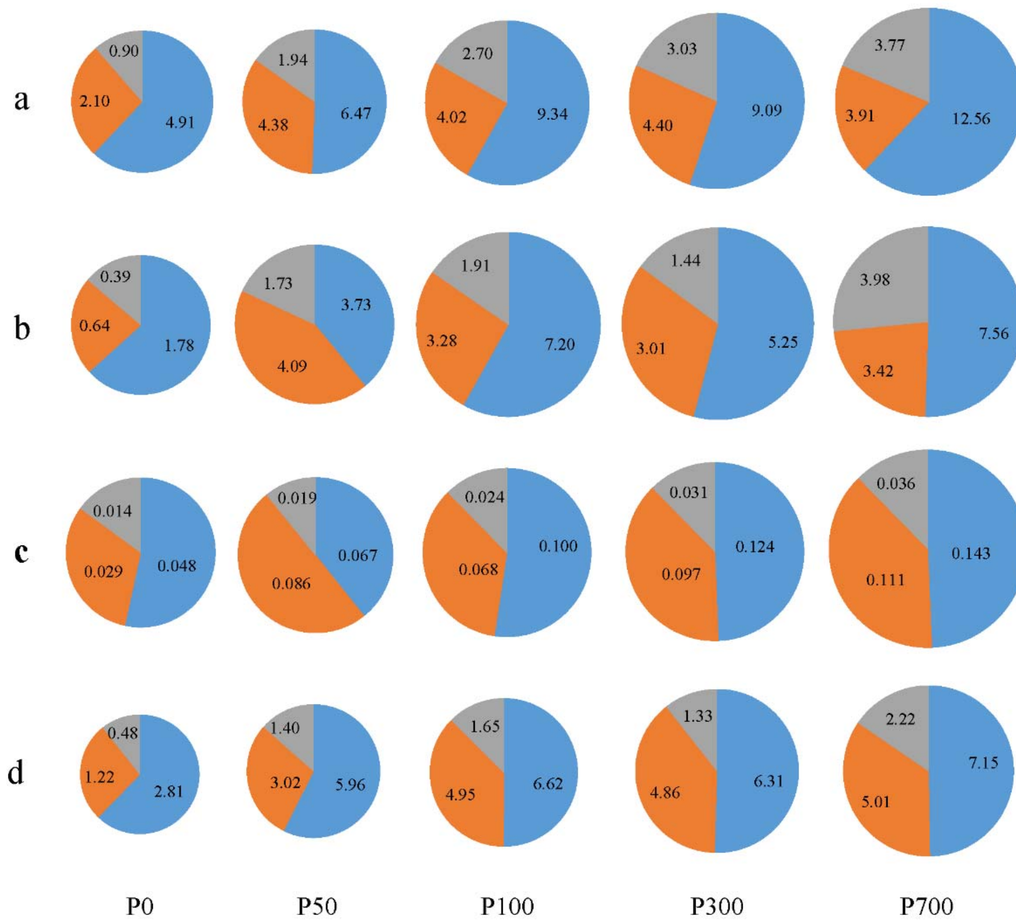


1214

1215 **Fig. 6**



1216

1217 **Fig. 7**

1218

1219 **Table 1** Basic properties of the studied soils of the chronosequence (Mean ± SD,

1220 $n = 3$)

Soil	pH (H_2O)	SOC ($g\ kg^{-1}$)	TN ($g\ kg^{-1}$)	BD ($g\ cm^{-3}$)	CEC ($cmol\ kg^{-1}$)	Fed ($g\ kg^{-1}$)
P0	8.62±0.07a	6.32±0.58	0.79±0.02c	1.31±0.05	6.32±0.34	1.76±0.02
P50	7.84±0.04b	15.96±0.66c	1.81±0.06b	1.13±0.03	12.82±0.06b	1.96±0.01b
P100	6.39±0.05d	17.07±0.49b	2.06±0.09a	1.06±0.04	12.54±0.12b	2.04±0.04a
P300	6.40±0.03d	17.97±0.81b	2.09±0.08a	1.07±0.07b	13.78±0.26a	2.08±0.05a
P700	6.65±0.08c	21.07±1.21a	2.14±0.06a	1.06±0.05b	12.97±0.27b	1.71±0.02c

1221 Note: BD, bulk density; TN: total nitrogen; CEC, cation exchange capacity; Fed:

1222 dithionate extractable iron oxyhydrates.

1223

1224 **Table 2** Particle-size distribution (%) of aggregates of the studied soils of the

1225 chronosequence. Low case letters indicate a significant (p <0.05) difference between

1226 soils for a single fraction, in a column.

Soil	2000-200µm	200-20µm	20-2µm	<2µm	MWD(µm)
P0	2.78±0.59c	46.53±1.30a	41.00±2.46a	9.69±0.57d	86.5± 6.2c
P50	5.10±0.25b	44.31±0.02b	40.79±0.41a	9.8±0.14d	109.5±2.1b
P100	5.34±0.10b	43.17±0.53c	39.72±0.72a	11.78±0.09c	110.8±1.3b
P300	6.87±1.04a	41.53±1.64d	38.67±0.33a	12.92±0.27b	125.8±7.8a
P700	7.63±1.40a	39.91±5.16d	36.97±3.59a	15.49±0.16a	132.2±8.5a

1227

1228

1229 **Table 3** –SOC, total N and LOC in g kg^{-1} and MBC in mg kg^{-1} of the size fractions
1230 of the size fractions (PSFs) of the chronosequence soils. Different capital and low
1231 case letters indicate a significant (p <0.05) difference respectively between fractions
1232 of a single soil, and between soils for a single fraction, in a single column.

PSF	Soil	SOC	Total N	LOC	MBC
Coarse sand	P0	11.07±1.20Ad	1.04±0.11Ad	6.22±0.18Ac	not determined
	P50	53.44±1.09Ab	4.15±0.49Aa	27.85±1.61Aa	794.7±47.0Ac
	P100	41.74±1.31Ac	3.37±0.38Ab	19.69±1.16Ab	1051.8±73.7Ab
	P300	40.64±1.57Ac	2.72±0.12Ac	18.80±1.45Ab	1385.5±88.1Aa
	P700	60.79±1.88Aa	4.43±0.22Aa	28.64±1.90Aa	1479.9±166.2Aa
Fine sand	P0	9.90±0.43Ac	1.01±0.14Ac	4.34±0.14Bb	188.0±8.0Ac
	P50	8.45±0.27Cc	0.73±0.11Dd	3.66±0.57Cb	309.2±16.5Bb
	P100	16.48±0.41Cb	1.57±0.14Cb	7.36±0.32Ca	441.1±13.4Ba
	P300	15.16±1.45Cb	1.51±0.13Bb	7.03±0.30Ca	445.9±28.2Ba
	P700	19.86±1.11Ca	1.81±0.12Ca	7.99±0.65Ba	449.9±25.9Ba
Silt	P0	5.13±0.19Bb	0.52±0.14Bd	1.53±0.13Db	166.7±4.5Ad
	P50	10.73±0.55Ba	1.20±0.11Cb	4.50±0.13Ca	296.2±15.0Bc
	P100	10.13±0.44Da	1.15±0.09Cc	4.10±0.26Da	287.0±2.7Cc
	P300	11.37±0.58Da	1.33±0.11Ba	4.39±0.29Da	392.1±15.0Ba
	P700	10.57±0.43Da	1.11±0.08Dc	3.95±0.69Ca	348.3±10.5Cb
Clay	P0	9.29±0.29Ac	1.17±0.15Ad	2.96±0.27Cc	155.6±18.1Ac
	P50	19.80±1.47Bb	2.27±0.14Bc	7.99±0.28Bb	284.9±19.7Bb
	P100	22.94±1.43Ba	2.70±0.12Bb	9.19±0.35Ba	279.4±5.0Cb
	P300	23.45±1.46Ba	2.92±0.12Aa	9.36±0.40Ba	324.8±13.1Ca
	P700	24.36±1.65Ba	2.73±0.16Bb	9.05±0.47Ba	325.7±8.1Ca

1233 Table 4 Relative proportion (%) of carbon chemical groups in size fractions by FTIR

1234 analysis. Different capital and low case letters indicate a significant (p <0.05) difference

1235 respectively between fractions of a single soil, and between soils for a single fraction**.**

Size fraction	Soil	Aromatic	Aliphatic	Polysaccharide
Coarse sand	P0	0.94±0.03Bc	0.03±0.00Ac	71.41±5.76ABa
	P50	3.49±0.47Aab	0.50±0.09Aa	60.94±2.54Cb
	P100	2.82±0.34Ab	0.27±0.03Ab	65.31±4.72Bab
	P300	2.49±0.12Ab	0.28±0.04Ab	67.04±4.66BCab
	P700	3.66±0.14Aa	0.37±0.03Ab	61.17±4.30Cb
Fine sand	P0	0.98±0.05Bb	0.05±0.01Ab	73.64±4.83ABa
	P50	1.08±0.06Cb	0.04±0.00Bb	72.98±4.43ABa
	P100	2.10±0.18Ba	0.13±0.03Ba	70.24±3.47ABa
	P300	2.08±0.05Ba	0.07±0.02Bb	70.32±4.60ABa
	P700	2.30±0.10Ba	0.17±0.02Ba	70.51±4.09Ba
Silt	P0	0.60±0.03Cb	0.01±0.00Ba	76.76±3.81Aa
	P50	1.01±0.03Ca	0.01±0.00Ca	76.02±4.29Aa
	P100	0.95±0.06Ca	0.00±0.00Db	77.37±4.73Aa
	P300	1.02±0.10Ca	0.00±0.00Db	76.39±4.21Aa
	P700	0.89±0.02Ca	0.00±0.00Db	80.14±3.87Aa
Clay	P0	1.24±0.06Ab	0.00±0.00Bb	66.20±3.2B2a
	P50	2.14±0.15Ba	0.03±0.00Ba	64.52±4.23Ba
	P100	2.27±0.12Ba	0.04±0.01Ca	63.85±4.57Ba
	P300	2.31±0.08Aa	0.03±0.01Ca	63.96±4.65Ca
	P700	2.44±0.17Ba	0.05±0.01Ca	63.08±3.73Ca

1236

1237 **Table 5** DNA content (μg g^{-1}), copy numbers of bacterial (BA, copies×$10^9 g^{-1}$), fungi
1238 (FA, copies×$10^7 g^{-1}$) and archaeal (ArA, copies×$10^8 g^{-1}$) of the size fractions. Different
1239 capital and low case letters in a single column indicate a significant (p <0.05) difference
1240 respectively between fractions of a single soil, and between soils for a single fraction.

Fraction	Soil	DNA	BA	FA	ArA
Coarse sand	P0	3.32±0.07Ae	5.86±0.75Ad	8.92±1.50Ab	0.81±0.03Ce
	P50	35.33±0.42Aa	46.18±9.21Aa	15.50±2.60Aa	6.37±0.81Bd
	P100	24.72±2.14Ac	31.45±5.79Ab	10.49±0.87Ab	13.54±0.73Bc
	P300	16.20±0.05Ad	10.12±2.39Ac	8.12±0.32Ab	16.01±1.06Ab
	P700	31.95±0.64Ab	14.25±1.03Ac	9.40±0.71Ab	21.17±0.48Ba
Fine sand	P0	3.63±0.28Ab	4.90±0.45Ab	3.23±0.27Bc	2.83±0.18Ac
	P50	4.35±0.40Db	8.42±1.75Ba	8.04±0.25Ba	5.27±1.12Bd
	P100	13.63±3.30Ba	7.75±1.18Ca	8.37±0.67Aa	8.16±2.27Cab
	P300	9.97±0.33Ba	4.92±1.10Bb	6.23±0.23Bb	3.57±0.24Cb
	P700	12.83±0.33Ca	8.16±1.64Ba	2.43±0.19Cd	7.68±0.66Ca
Silt	P0	1.57±0.28Bc	1.78±0.15Bc	3.98±0.57Ba	0.29±0.02Dd
	P50	10.02±1.58Ca	10.64±2.95Ba	4.25±0.30Ca	2.48±0.44Cc
	P100	8.25±0.12Cab	5.78±0.36Cb	2.17±0.20Bb	8.65±0.09Ca
	P300	7.78±0.31Cb	5.91±0.81Bb	2.47±0.45Bb	6.60±0.27Bb
	P700	9.25±0.64Da	6.16±0.29Bb	3.68±0.19Ba	9.44±1.41Ca
Clay	P0	4.00±1.89Ad	5.27±0.61Ac	0.52±0.03Cd	1.83±0.10Bc
	P50	17.62±0.26Bb	38.05±4.92Aa	1.31±0.07Dc	14.08±2.13Ab
	P100	16.20±0.38Bb	15.86±3.31Bb	1.94±0.30Bb	44.66±13.68Aa
	P300	11.17±0.90Bc	13.03±2.58Ab	1.39±0.40Cb	22.16±6.17Aa
	P700	25.67±0.57Ba	15.63±2.24Ab	2.48±0.31Ca	36.00±3.82Aa

1241 **Table 6** Normalized enzyme activity (NEA) and soil respiration (mg CO_2 kg^{-1}) of the
1242 chronosequence soils. Different capital and low case letters in a single column indicate
1243 a significant (*p* <0.05) difference respectively between fractions of a single soil, and
1244 between soils for a single fraction.

Size fraction	Soil	NEA	Basal respiration
Coarse sand	P0	0.07±0.01Bc	662±66Ac
	P50	0.28±0.03Aa	2345±805Aab
	P100	0.18±0.01Ab	2283±506Aab
	P300	0.18±0.01Bb	1588±309Ab
	P700	0.30±0.05Aa	2914±190Aa
Fine sand	P0	0.10±0.01Bc	565±153ABb
	P50	0.12±0.03Cc	1076±139Ba
	P100	0.21±0.03Ab	1252±103Ba
	P300	0.27±0.03Aa	1256±096Aa
	P700	0.30±0.02Aa	1234±143Ba
Silt	P0	0.07±0.01Bd	298±053Cc
	P50	0.21±0.02Bb	740±258Bb
	P100	0.17±0.01Ac	1246±063Ba
	P300	0.25±0.02Ab	1256±071Aa
	P700	0.30±0.02Aa	1354±095Ba
Clay	P0	0.14±0.01Ac	496±053Bb
	P50	0.19±0.02Bb	1425±430Aa
	P100	0.20±0.02Aab	1401±289Aa
	P300	0.24±0.02Aa	1028±226Aa
	P700	0.23±0.01Ba	1434±196Ba