Microbial activity promoted with organic carbon accumulation in macro-aggregates of paddy soils under long term rice cultivation

Yalong Liu[1,2*], Ping Wang[1,2*], Yuanjun Ding[1], Haifei Lu[1], Lianqing Li[1], Kun Cheng[1], Jufeng Zheng[1], Timothy Filley[3], Xuhui Zhang[1], Jinwei Zheng[1], Genxing Pan[1,4#]

[1] Institute of Resource, Ecosystem and Environment of Agriculture, and Department of Soil Science, Nanjing Agricultural University, 1 Weigang, Nanjing 210095, China;

[2] Department of Soil Sciences, Land and Environment College, Shenyang Agricultural University, Shenyang 110866, China;

[3] Department of Earth, Atmospheric, and Planetary Sciences, Purdue University, West Lafayette, IN 47907, USA;

[4] Center of Terrestrial Ecosystem Carbon Sink and Land remediation, School of Environmental and Resource Sciences, Zhejiang A & F University, Lin'an, Hangzhou 311300, China

 # Corresponding author: Genxing Pan

Address: Institute of Resource, Ecosystem and Environment of Agriculture, Nanjing Agricultural University, 1 Weigang, Nanjing 210095, China

Tel/Fax: +86 25 8439 6027

Email: pangenxing@aliyun.com

Running title: carbon and microbial activity in rice soil aggregates

*These authors contributed equally to this work, with PW on soil aggregate separation and carbon pool analysis, and YL on soil biological activity.

Abstract:

While soil organic carbon (SOC) accumulation and stabilization had been increasingly concerned as ecosystem properties, how this could be linked to soil biological activity enhancement had been poorly assessed. In this study, topsoil samples were collected from a series of rice soils shifted from salt marsh respectively for 0, 50, 100, 300 and 700 years from a coastal area of eastern China. Soil aggregates were fractioned into different sizes of coarse sand (200-2000 μm), fine sand (20-200 μm), silt (2-20 μm) and clay (<2μm), using separation with a low energy dispersion protocol. Soil properties were determined to investigate niche specialization of different soil particle fractions in response to long-term rice cultivation, including recalcitrant and labile organic carbon, microbial diversity of bacterial, archaeal and fungal communities, soil respiration and enzyme activity. The results showed that the mass proportion both of coarse sand (2000-200μm) and clay (<2μm) fraction increased with prolonged rice cultivation but the aggregate size fractions were dominated by fine sand (200-20μm) and silt (20-2μm) fractions across the chronosequence. SOC was enriched highly in coarse sand fractions (40-60 g kg^{-1}), moderately in clay fractions (20-25 g kg^{-1}), but was depleted in silt fractions (~10 g kg^{-1}). The recalcitrant carbon pool was higher (33-40% of SOC) in both coarse sand and clay fractions than in fine sand and silt fractions (20-29% of SOC). However, the ratio of labile organic carbon (LOC) to SOC showed a weakly decreasing trend with decreasing size of aggregate fractions. Total soil DNA content in the size fractions followed a similar trend to that of SOC. Despite of the largely similarly diversity between the fractions, 16S ribosomal gene abundance of bacteria and of

archaeal were concentrated in both coarse sand and clay fractions. Being highest generally in coarse sand fractions, 18S rRNA gene abundance of fungi decreased sharply but the diversity gently, with decreasing size of the aggregate fractions. Soil respiration quotient (ratio of respired CO_2-C to SOC) 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 coarse sand sized fraction than in other fractions. Soil respiration was higher in silt fraction than in other fractions for the rice soils. For the size fractions other than clay fraction, enzyme activity was increased with prolonged rice cultivation, whereas soil respiration appeared to have a decreasing trend. Only in the coarse sand fractions, both microbial gene abundance and enzyme activity were well correlated to SOC and to LOC content though chemical stability and respiratory of SOC were similar between coarse sand and clay fractions. Thus, biological activity was generally promoted with LOC accumulation in the coarse sand sized macro-aggregates of the rice soils, positively responding to prolonged rice cultivation management. The finding here provides a mechanistic understanding of soil organic carbon turnover and microbial community succession at fine scale of soil aggregates that have evolved along with anthropogenic activity of rice cultivation in the field.

Key words: rice soil, carbon stabilization, soil bioactivity, soil aggregates, size fractions, rice cultivation, microbial community, chronosequence

1 Introduction

Soil organic matter (SOM), as a continuum of organic substances that accumulated over time from decomposition of plants and microorganisms (Lehmann and Kleber, 2015), provided a key driver for soil aggregation and thus soil ecosystem functions and services (Banwart et al., 2014). Soil aggregates had been considered as fundamental soil particle units where organic matter, minerals and microbes interacted to store carbon and nutrient as well as moisture (Tisdall and Oades, 1982; Lützow et al., 2006; Marschner et al., 2008; Schmidt et al., 2011), and mediated their cycling in soil-plant systems (Six et al., 2004). One of the primary mechiansms for soil carbon sequestration could be the increased physical protection of SOC within aggregates which decreased decomposition rates (Blanco-Canqui and Lal, 2004; Six et al., 2004; Kong et al., 2005; Six and Paustian, 2014). This could be concerned with separated allocation of mineral associated SOC fractions between micro-aggregates within macro-aggregates (Lehmann et al., 2008; Dungait et al., 2012; Vogel et al., 2014). Soil aggregation further shaped the micro-habitats for soil microbial communities (Six et al., 2000; Ettema and Wardle, 2002; Balser et al., 2006; Kögel-Knabner et al., 2008), with changes in SOC substrate availability, chemical recalcitrance and redox potential with or within aggregates (Rillig et al., 2001; Six et al., 2006; Strickland and Rousk, 2010). Consequently, changes in composition of soil aggregate fractions could lead to changes in bio-activity reflected by size, diversity and biochemical activity of the microbial community (Six et al., 2006; Lagomarsino et al., 2012; Bardgett and van der Putten, 2014). Particulate organic carbon (POC) had been increasingly considered as an

87 indicator of soil quality and health under different stresses or human disturbance
88 (Cambardella and Elliot 1992; Marriott and Wander, 2006). As a labile carbon pool,
89 POC had also been suggested as a measurement of SOC accumulation and stabilization
90 with co-existing microbial activity of soils in different ecosystems (Gajda 2010; Six
91 and Paustian 2014). Soil aggregation, affected by land use and management practices,
92 could result in changes in allocation of POC inter- and/or intra- microaggregates in size
93 fractions of soil (Yang et al., 2009; Lagomarsino et al., 2012; Six and Paustian 2014;
94 Smith et al., 2014). Unfortunately, the link between changes in carbon pools and those
95 in microbial biological activity with SOC stabilization in soil aggregates had not yet
96 been well understood and quantitatively assessed (Six and Paustian 2014; Smith et al.,
97 2014).
98 Soil aggregation could be characterized by measuring distributions of defined particle
99 size fractions, which could differ in soil microbial biomass and activity, in response to
100 SOC accumulation and stabilization of soil (Salinas-Garcia et al., 1997; Kandeler et al.,
101 1999; Smith et al. 2014). Such difference could mimic the micro-scale interactions
102 driving SOC stabilization and nutrient cycling in soils (Kandeler et al., 2006;
103 Lagomarsino et al., 2012; Six and Paustian, 2014). To examine these interactions,
104 aggregate separation methods should use least low energy dispersion of bulk soil into
105 particle size fractions (Kandeler et al., 2000), and avoid use of any chemical dispersion
106 methods (Smith et al. 2014). Stemmer et al. (1998) developed a low energy ultrasonic
107 dispersion protocol, which allowed the least disturbed separation for analyzing
108 microbial community composition and enzyme activity in the obtained size fractions of

soil aggregates (Kandeler et al., 2000). This approach was followed in later studies (Sessitsch et al., 2001; Poll et al., 2003; Matocha et al., 2004; Marx et al., 2005; Zhang et al., 2013), addressing the impacts of different management practices or environmental disturbances on SOC persistence, microbial communities and enzyme activity in aggregates agricultural soils. However, the interactions between these attributes in aggregate size fractions with carbon stabilization and their trend with continuing management in long term cultivated soils had been not yet well characterized.

Soil matrix or microsite properties had been well known to have an important role in the spatial allocation of organic matter and microbial community and thus the link between SOC pools and microbial bioactivity among different fractions of soil aggregates (Smith et al. 2014). Rice paddy soils were developed with dynamic redox regime and neo-formation of iron/manganese oxyhydrates due to hydromorphic pedogenesis under long term hydroagric paddy management (Li 1992). These soils were thus classified as a particular soil group of hydroagric Anthrosols in the new Chinese Soil Taxonomy (Gong et al., 1999). Recently, these soils had been shown to have high SOC storage and sequestration potential compared to dry-land croplands (Pan et al., 2004; Pan et al., 2010; Wissing et al., 2013). This had previously been attributed to enhanced aggregation and aggregate stability (Lu et al., 1998; Yang et al., 2005) as well as to increased humification of SOC (Olk et al., 2000). However, SOC accumulation and stabilization in paddy soils with management practices had been addressed with a number of processes. These processes were understood with either

131 increased binding to free oxyhydrates (Zhou et al., 2009; Cui et al., 2014) and enhanced
132 chemical recalcitrance (Zhou et al., 2009a, 2011; Song et al., 2012), or enhanced
133 physical protection with increased aggregate stability (Li et al., 2007; Zhou et al. 2008)
134 or their interactions (Song et al., 2012; Song et al., 2013).
135 Moreover, SOC could be continuously accumulated in rice soils with prolonged rice
136 cultivation in the long run. In a rice soil chronosequence, SOC accumulation was
137 promoted shortly following the desalinization and decalcifiation in the initial stage after
138 the salt marsh shifted to rice paddy (Kalbitz et al., 2013). Across this chronosequence,
139 the accumulated SOC was increasingly stabilized with neoformed iron-oxyhydrates
140 (Cheng et al., 2009; Wissing et al., 2011), as rice cultivation prolonged. Whereas, in a
141 rice paddy with well managed fertilization from Southeastern China, SOC
142 accumualtion was well represented by an increase in proportion of water-stable macro-
143 aggregates (>250μm) and the associated POC pool (Zhou et al., 2007). In rice paddies
144 under long term fertilization trials from South China, physically protected SOC in the
145 coarse sand size fraction of soil aggregates contributed to bulk soil SOC accumualtion
146 and stabilziationin (Zhou et al., 2008).
147 Importantly, co-evolution of soil microbial community and diversity had been observed
148 with SOC accumulation and stabilization in rice paddies (Zhang et al., 2007; Zheng et
149 al., 2007; Liu et al., 2011). In line with the trend of SOC accumulation in paddy soils,
150 microbial biomass and community diversity was shown to be enhanced across a
151 chornosequence under prolonged rice cultivation (Bannert et al., 2011; Jiang et al.,
152 2013). Using a similar chronosequence, the enhanced biological activity could be well

153 portraied with an increase in mean weight diameter of soi aggregates and in POC pool
154 across the soils with prolonged rice cultiavtion (Wang et al., 2015). This indicated a
155 potential role of physically protected labile carbon pool in enhancing biological activity
156 with bulk SOC accumualtion in rice soils (Zou et al., 2015). Rcently, changes in
157 mcirobial gene abundance and community compsoition had been reported for bulk soils
158 (Liu et al., 2016a) and for aggregate size fractions of soils (Liu et al., 2016b), from such
159 a rice soil chronosequence. It could be speculated that physical protection could involve
160 a change in the spatial distribution of pools rather than in the chemical recalcitrance, of
161 organic carbon located among aggregate size fractions. The changed allocation of both
162 carbon pools and microbial community could contribute to SOC stabilization with
163 increased microbial abundance and the carbon use efficiency, qCO_2 (Schlesinger &
164 Andrews, 2000), as a result of enhanced aggregation (Lehmann 2011). Such
165 information would be of key importance for understanding carbon stabilization in
166 relation to sustainable management of rice paddy soils with respect to carbon
167 biogeochemical cycling and ecosystem functions provided by soils (Smith et al., 2015).
168 In this study, two hypotheses were tested. First, we sought to examine whether
169 microbial bioactivity and carbon stability in soil aggregates could differ among their
170 size fractions, leading to changes in spatial allocation of SOC pools among aggregate
171 size fractions in rice paddies. In this case, physical protection of SOC could improve
172 microbial microhabitat conditions and thus microbial carbon use efficiency through
173 enhanced aggregation. And it could enable an existence of labile carbon pool within
174 micro-aggregates that comprised macro-aggregates or between micro-aggregates

within macro-aggregates (Six and Paustian 2014; Smith et al., 2014). Thus biological activity could be enhanced with physically protected carbon in macro-aggregates, as compared to micro (clay sized) aggregates with chemically stabilized organic carbon; Second, we sought to examine whether the strong link between microbial activity and the size of labile carbon pool in macro-aggregates could be promoted with enhancement of physically stabilized SOC through continuing hydroagric paddy management under long term rice cultivation. In a series of soils formed on similar paleo-deposits rich in silt, continuous rice cultivation could result in a directional change in soil aggregation, and thus in microhabitat conditions as well as nutrients. This directional pedogenetic development would in turn affect a more or less directional change in SOC stabilization (with increasing POC pool and accumulation of recalcitrant carbon and mineral bound carbon). This study aimed to understand that carbon stabilization could not confront but improve biological activity in soils under rice cultivation over centuries.

2 Materials and methods

2.1 Methodology rational

Using a recommended sonification separation procedure, we examined changes in aggregate size fraction composition for aggregate stability, in functional group composition for chemical recalcitrance of SOC, and in soil respiration for microbial energy use, in order to characterize SOC accumulation and stabilization in rice soils. Meanwhile, changes with SOC accumulation/stabilization were explored in microbial activity for soil functioning. For this, we analyzed total microbial gene abundance and estimated overall enzyme activity in aggregate size fractions. Furthermore, the potential link between OC stabilization and bioactivity among the aggregate fractions were quantitatively assessed using the parameters of carbon- or gene abundance- scaled respiration and enzyme activity. Finally, the evolution of such interlink was traced by comparing the soils of sequential lengths of rice cultivation up to 700 years in a soil chronosequence.

2.2 Site and soils

The study reported here examined a series of soils along a paddy chronosequence, shifted from tidal marsh to rice cultivation for different lengths of time in a coast land area located in Cixi Municipality, Zhejiang Province, China (Fig.1). Lying in the south bank of Hangzhou Bay, the area was within the typical northern subtropical monsoon climate for Eastern China, with a mean annual temperature of 17.7 °C and precipitation of 1,367 mm during 2004-2014 (http://cdc.nmic.cn/home.do). In this area, coastal tidal marsh had been increasingly reclaimed for rice production, with dyke establishments at

211 different historical stages for the last 2000 years. These soils allowed chronosequence
212 studies for rice soil development, including a pedological characterization by Cheng et
213 al. (2009) and a morphological, mineralogical and microbiological investigation by
214 Kölbl et al. (2014).

Fig. 1

216 Individual soils of the chronosequence were identified based on dyke establishment
217 history recorded in Cixi County Annals (with brief information in Chinese available at
218 www.cixi.gov.cn), including an initial tidal marsh soil before rice cultivation (P0), and
219 rice soils of P50, P100, P300 and P700 shifted for rice cultivation respectively 50, 100,
220 300 and 700 years before present (Fig.1). These soils were apart from each other in a
221 distance no more than 40-km in nearly the same topography. All of the soils developed
222 on comparable parent materials of paleo-deposit from Yangtze River, with a particle
223 composition of silt (75%-84%), followed by clay but low in sand content (Chen and
224 Zhang, 2009). Soil texture ranged from silty loam to silty clay-loam. The clay mineral
225 assemblage consisted of illite (40-50%), chlorite (20-30%) and kaolinite (10-20%) with
226 a minor amount of smectite and quartz (Zhang et al., 2010b).
227 The cropping system in this area followed a traditional summer rice-winter rape rotation.
228 Rice production management on the chronosequence was relatively consistent across
229 sites, with similar cultivars and management practices including crop protection,
230 irrigation and fertilization (Cheng et al., 2009).The influence of soil salinity on rice
231 production could occur in the early stage of rice cultivation on the reclaimed tidal marsh
232 though the ground water table had been enough low without restricting rice growth

(Kölbl et al., 2014). The directional evolution of soil properties (Cheng et al., 2009; Chen et al., 2011), neo-formation of clay minerals particularly of iron/manganese oxyhydrates (Wissing et al., 2013; Wissing et al., 2011; Kölbl et al., 2014), interaction of organic matter with minerals (Wissing et al., 2011; 2014) as well as organic carbon pools (Wissing et al., 2011; Wang et al., 2015) had been already characterized.

2.3 Soil sampling

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

Table 1

2.4 Particle size fractionation of soil aggregates

Soil aggregates were obtained from the undisturbed soil cores by dispersion in water

with low energy sonication, without chemical dispersing agents. Particle size fractions of water stable aggregates were separated with a modified procedure described by Stemmer et al. (1998) and later on followed by Stemmer et al (1999), Sessitsch et al., (2001), Kandeler, et al (1999, 2000 and 2006). A portion of field moist soil core (50 g equivalent d.w.), cleaned of discernible straw material if any, was placed into a glass beaker in 100 ml of distilled water. The soil mass was dispersed using a low-energy ultrasonic disaggregator (Zhixin, JVD-650, Shanghai, China) with an output energy of 170 J g^{-1} for 5 min. A coarse sand sized fraction of aggregates having a diameter range of 2000-200 μm was separated by wet sieving and the fine sand sized fraction of 200-20 μm was subsequently obtained by sedimentation after siphoning. The remainder was centrifuged to collect the silt sized fraction of 20-2 μm and the supernatant was centrifuged again to collect the clay sized fraction of ≤ 2 μm. The samples of the obtained size fractions were freeze-dried (Thermo, Modulyo D-230, NY, US) and then stored at -70 ºC. Here, water stable macro-aggregates larger than 2000μm were not taken into consideration as they were insignificant in rice soils under prevailing water submergence with long term hydroagric management (Deng and Xu, 1965). The classes of the size fractions were kept basically consistent with our previous studies (Li et al., 2007a, b; Zheng et al., 2007; Pan et al., 2008 and Chen et al., 2014).

2.5 Organic carbon pool and FTIR spectroscopy analysis

Soil organic carbon (SOC) and total nitrogen (TN) of the separated fractions were determined with a CNS elemental analyzer (Elementar Vario-max CNS Analyser, Germany Elementar Company). Labile organic carbon (LOC) content was measured by

277 0.33 M potassium permanganate oxidation ($KMnO_4$), following a procedure described
278 by Blair et al. (1995). Microbial biomass carbon (MBC) was measured using the
279 chloroform fumigation-extraction method. The MBC content was estimated as the
280 difference of OC between the unfumigated and fumigated samples using the conversion
281 factor of 0.45, following Joergensen (1996). Herein, MBC of coarse sand fraction of P0
282 soil was not provided due to the very small sample obtained via the sonification and
283 separation procedure.
284 Chemical composition of the organic carbon in the particle size fractions were
285 characterized with FTIR spectroscopy using a Bruker FTIR spectrophotometer (Bruker
286 TENSOR 27 Spectrometer, Ettlingen, Germany). Briefly, a portion of frozen-dried
287 aggregate sample was powdered in an agate mill, and 1 mg of the homogenized sample
288 powder was mixed thoroughly with 100 mg KBr. The pellet prepared with a pressure
289 was placed in a sample holder and FTIR spectra were recorded. FTIR scanning was
290 conducted in ambient conditions at 22±1°C. The resolution was set to 4 cm^{-1} and the
291 operating range was 400 to 4000 cm^{-1}. In all cases, 20 scans per sample were recorded,
292 averaged for each spectrum and corrected against the spectrum with ambient air as
293 background. Following Ellerbrock et al. (1999) and Cocozza et al. (2003), the
294 characteristic vibration peak at 1050 cm^{-1} was assigned to polysccharides, those at 1630
295 cm^{-1} to aromatic compounds and those at 2927 cm^{-1} to aliphatic compounds as well as
296 those at 3405 cm^{-1} to phenols. Subsequently, a general semi-quantification of three
297 major functional OC groups of polysaccharides, aliphatic and aromatic compounds was
298 performed following Tivet et al. (2013). Nevertheless, it was not able to quantify

299 potential contributions from organic Si or P compounds to the intensity of the band
300 assigned to polysaccharides (Mao et al., 2008; Tivet et al., 2013). All the obtained FTIR
301 spectra are given in Supplement Fig. 1.

302 **2.6 SEM observation of soil aggregates**

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

310 **2.7 DNA extraction, microbial gene abundance and diversity analysis**

311 A portion (0.45 g) of a PSF sample stored at -70 ºC was used for DNA extraction with
312 PowerSoil™ DNA Isolation Kit (MoBio, USA), following the manufacturer guide. The
313 concentration of the DNA extracts was checked with a spectrophotometer (Eppendorf,
314 Germany), and its integrity and size were checked by using 1.0% agarose gel
315 electrophoresis. Extracted DNA was stored at -70 ºC prior to molecular bioassay.
316 Quantitative real-time PCR assay was performed on a 7500 real-time PCR system
317 (Applied Biosystems, USA) using SYBR green as a fluorescent dye. Primer
318 combinations of 338F/518R (Øvreås and Torsvik, 1998), ITS1F/ITS4 (Gardes and
319 Bruns, 1993) and Ar109F/Ar915R (Lueders and Friedrich, 2000) were used for
320 bacterial 16S rRNA, fungal Internal Transcribed Spacer (ITS) region and archaeal 16S

rRNA genes respectively in the Real-time PCR assay.

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

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

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

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

2.8 Soil enzyme activity

Soil enzyme activities relevant to cycling of C, N and P in soils were measured. In detail, activities of invertase, urease and acid phosphatase were determined using the methods described by Guan et al., (1986) while β-glucosidase, β-cellobiosidase and peroxidase were measured using 96 micro-plates colorimetric methods described by Saiya-Cork et al. (2002). For an integrated assessment of microbial biochemical activity, the six different enzyme activities analyzed were normalized to give a single value as normalized enzyme activity (NEA) of an individual fraction, which was estimated with the following equation:

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

where, *i* was the number of each soil sample (P0, P50, P100, P300, P700), *x* was the

343 enzyme activity and x' was the normalized enzyme activity of each soil sample.
344 Subsequently, an arithmetic mean of enzyme activity of each sample was obtained for
345 the NEA.

346 **2.9 Soil respiration**

347 For assessing microbial use of carbon in aggregates of different size fractions, soil
348 respiration was determined by measuring CO_2 production using an anaerobic laboratory
349 incubation protocol, following Zheng et al. (2007). A size fraction sample (20g d.w.
350 equivalent) was placed into a 125ml glass jar and submerged with 40ml distilled water
351 before being gently mixed. The jar was then sealed with a butyl rubber stopper and two
352 Teflon tubes for gas sampling and N_2 circulation were inserted into the stopper. The
353 headspace was repeatedly evacuated and flushed with N_2 gas into the jar at a rate of
354 300ml min^{-1} for 30min, creating an anaerobic condition. The jars with soil slurry were
355 incubated in an incubator, as described in Section 2.8, at 25 ± 1 ^oC for 37 days. During
356 incubation, a 0.25 ml sample of the headspace gas was collected by a syringe every 5
357 days starting the third day after incubation was initiated. After each gas sampling, N_2
358 gas was again flushed into the jar at a rate of 300ml min^{-1} for 30 min to remove all the
359 emitted gas in the jar. CO_2 concentration in a gas sample was determined with a gas
360 chromatograph (Agilent 4890D) equipped with a stainless steel column (Porapak Q)
361 (80/100 mesh) and flame-ionization detector (FID). Following the procedures described
362 by Zhang et al. (2010a), the determination was done with an oven temperature of 80^oC
363 and a FID temperature of 200^oC, with N_2 as the carrier gas at a flow rate of 40ml min^{-1}
364 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

365 distilled water was used as the control for the gas concentration in the bottle. The total
366 CO_2 evolved was estimated from the cumulative sum of the gas evolved in all
367 monitoring intervals and was used to calculate the anaerobic soil respiration expressed
368 in terms of soil mass.

369 **2.10 Data treatment and statistical analysis**

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

3 Results

3.1 Organic carbon characterization in aggregate size fractions

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

Table 2

Soil properties of SOC, total N and LOC were significantly different among the size fractions and between the uncultivated and rice soils (Table 3). SOC, LOC and total N pools all generally followed the order: coarse sand size fraction > clay sized fraction> fine sand fraction >silt sized fraction in a single soil. With the exception of the fine sand fraction, all these pools were greater in rice soils than in the uncultivated marsh soil. Particularly, SOC of rice soils was enriched mostly in the coarse sand sized macro-aggregates, moderately in the clay sized fractions, fairly in the fine sand sized fractions

but were depleted in the silt sized fraction, respectively in a range of 41-61 g kg^{-1}, of 20-24 g kg^{-1}, of 8.5-20 g $kg^{-1}$and of 10-11 g kg^{-1}. However, C/N ratio showed a significantly decreasing trend with the decreasing size of the aggregate fractions across the chronosequence. The ratio of LOC to SOC, an indicator of C lability in soils, followed a decreasing order: coarse sand fractions >fine sand fractions >silt and clay sized fractions.

The FTIR spectra showed sharp peaks generally at vibration of 1050cm^{-1} (assigned to polysaccharides) but broad shoulders at vibration of 3405cm^{-1} assigned to aromatic carbon across the aggregates fractions (Supplement Fig.1). There was a clear trend of decreasing intensity in the polysaccharide peaks but increasing shoulder intensity of aromatic carbon in a single fraction, with increasing rice cultivation. The semi-quantitative data of carbon chemical groups obtained with FTIR analysis is presented in Table 4. Herein, carbon groups in aggregates were dominated by polysaccharides (60-70%), followed by aromatic carbon (20-39%) with small contribution (0.6-3.7%) of aliphatic carbon in a single fraction. The relative proportion of aromatic carbon was lower but polysaccharide carbon higher in the silt fractions as compared to the other fractions. Consequently, the estimated SOC chemical recalcitrance (ratio of aromatic to polysaccharide C) was lowest in the silt fractions, followed by the fine sand fractions but highest in the coarse sand and clay fractions.

Recalcitrance of SOC of in a single fraction was generally lower in uncultivated marsh soil than in the shifted rice soils, but tended to increase with increasing length of rice cultivation. The fine sand fraction, bearing the majority of total SOC for the soil (Table

2 and Table 3), had a moderate carbon recalcitrance but the coarse sand fraction had similar carbon recalcitrance but higher carbon lability and higher C/N ratio. This indicated a greater existence of potentially available carbon pool (POC, for example) in the coarse sand fraction, compared to other fractions.

Table 3

Table 4

Fig. 2

3.2 Microbial biomass carbon, microbial gene abundance and diversity

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

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

441 abundance was dominated by bacterial, with archaeal and fungal gene abundance
442 respectively one and two orders of magnitude lower than bacterial copy numbers across
443 the fractions. Whereas, the ratio of fungal to bacterial gene abundance generally
444 decreased but that of archaeal to bacterial gene abundance increased with decreasing
445 size of the aggregate fractions.
446 Over the studied chronosequence, DNA contents of a fraction were several folds higher
447 in the rice soils as compared to that of the initial tidal marsh. Accordingly, gene copy
448 numbers of microbial communities from a fraction were much higher in rice soils than
449 in the initial tidal marsh. Bacterial and fungal abundance in coarse sand, fine sand, silt
450 and clay fraction in P50 was increased by 688%, 72%, 498% and 622 %, and 74%,
451 149%, 7% and 152 %, respectively over P0. A mean increase in the rice soils cultivated
452 for over 100 years over P0 in bacterial gene copy numbers was seen statistically
453 significant, with percentages ranging from 73% to 437 %, 0.4% to 67 %, 225% to 246 %
454 and 147% to 201 %, respectively in the coarse sand, fine sand, silt and clay fractions.
455 Comparatively, changes in fungal gene abundance of aggregates were much smaller
456 across the soils, particularly in the silt and clay sized fractions. In contrast, archaeal
457 gene abundance in a single fraction across the soils was increased over P0 consistently
458 with the prolonged rice cultivation, though smaller in fine sand and silt sized fractions.
459 For the coarse sand fraction only, both the fungal to bacterial ratio and the archaeal to
460 bacterial ratio tended to increase with increasing rice cultivation lengths.
461 Data of microbial Shannon diversity index of the four size fractions of the
462 chronosequence soils are presented in Table S1. In detail, Shannon index of bacterial

community was much higher in the coarse sand fractions and, to a lesser extent, in the clay size fraction than in the fine sand and silt fractions across the chronosequence. Fungal community Shannon indice generally decreased with the size of aggregate fractions. In contrast, there were no significant changes in archaeal Shannon index among the size fractions across the sequence. Generally, Shannon diversity index of the microbial communities in a single fraction was greatly higher in the rice soils than in the uncultivated tidal marsh.

3.3 Enzyme activity and basal respiration

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

Soil respiration of a single fraction was much higher for the rice soils than for the marsh soil, and in the sand sized macro-aggregate fraction than in the silt and fine sand fractions over the chronosequence (Table 6). In detail, soil respiration was 662 $mgCO_2$ kg^{-1} and 565 $mgCO_2$ kg^{-1} in the coarse and fine sand fractions, and 298 $mgCO_2$ kg^{-1} and 496 $mgCO_2$ kg^{-1} in the silt and clay fractions, respectively in P0. While in rice soils, soil respiration ranged between 1588-2914 mg CO_2 kg^{-1} in the coarse sand, and 1076-1256 $mgCO_2$ kg^{-1} in the fine sand, and 740-1354 $mgCO_2$ kg^{-1} in the silt and 1028-1434 $mgCO_2$ kg^{-1} in the clay fractions of the rice soils. Basal respiration in a single size

fraction generally increased with rice cultivation length (Table 6).

Using the data in Table 3, the estimated RQ (the ratio of respired carbon to total SOC) and qCO_2 (the ratio of respired carbon to MBC) were seen variable across the size fractions and among the soils (Supplement Table S3). Generally, RQ was lower both in sand- and clay- sized fractions than in fine sand- and silt- sized fractions. Value of qCO_2 was lowest in the coarse sand sized fraction but highest in the clay sized fraction. While there was no overall trend of RQ and qCO_2 in a single fraction between the marsh soil and rice soils, both RQ and qCO_2 in a single fraction followed more or less a decreasing trend with increasing length of rice paddy management.

4 Discussions

4.1 Carbon accumulation versus stabilization in soil aggregates

In this study, the level of SOC, soil respiration and microbial gene abundance/diversity differed significantly among different size fractions of water stable aggregates from the chronosequence. Similar to the findings by Li et al. (2007b) and Zheng et al. (2007), SOC accumulated predominantly in the coarse sand sized and moderately in the clay sized but was depleted in the silt sized aggregate fractions (Table 3). As shown in Fig. 3a, SOC content in a fraction was positively linearly correlated to organic carbon recalcitrance as determined by the FTIR analysis (Fig. S1; Table 4). Whereas, respiration quotient as a rate indicator of carbon turnover for microbial energy use (Kennedy and Papendick, 1995), indicated a significant negative logarithm function of SOC level (Fig. 3b). The divergence of the uncultivated marsh soil to the rice soils could be attributed to the land use impact as a determinant factor for SOC turnover (Qian et al., 2013). The correlations hereby could suggest the accumulation of SOC in soil aggregates related to chemical stabilization against biological use for their energy supply, which had been traditionally considered as an inherent carbon sequestration with selective persistence of non-degradable or residue organic carbon in soils (Lützow et al., 2006; Mikutta et al., 2006).

Fig. 3

Calculations using the SOC contents (Table 3) and the fraction mass percentage (Table 2) of a single fraction showed that the amounts of SOC allocated only in the sand and clay sized fractions were closely correlated to the bulk SOC contents (Table 1) of the

518 soils (Fig. S2). This was in general agreement with the finding for similar rice paddy
519 soils from an adjacent area (Pan et al., 2008). The increased allocation of SOC to clay-
520 sized fraction could be attributed to the accelerated formation of clay and hydroxyl
521 Fe/Mn minerals (Wissing et al., 2013) due to long term paddy management (Kölbl et
522 al., 2014).
523 Furthermore, the enrichment index (EI) of SOC, calculated with SOC content in a
524 fraction divided by that in the bulk soil, was higher than 1 in both sand and clay sized
525 fraction but much lower than 1 in silt fractions. When plotting the EI values against
526 LOC content (Table 3) for all the fractions (Fig. 4), enrichment of SOC was seen
527 relevant to LOC pool in the fractions. Moreover, the EI values were significantly but
528 weakly positively correlated both to F/B ratio of gene abundance (Table 5) and to SOC
529 recalcitrance (Table 4). These data indicated that accumulation of labile carbon, mostly
530 POC, contributed significantly to the SOC pool in sand sized macro-aggregates (Zhou
531 et al., 2008) although the apparent recalcitrance was in a similar range to that in clay
532 fractions (Table 4). Light fraction or macro-aggregates in soil were considered to be
533 relatively rich in new or labile carbon substrates, more or less related to root fungal
534 activities, and were largely physically protected in micro-aggregates within macro-
535 aggregates (Elliott et al., 1986; Jastrow et al., 1998; Six et al., 2000). As shown by Wang
536 et al. (2015), SOC accumulation in bulk soil could be well accounted for by the changes
537 in POC of the studied chronosequence.

Fig. 4

539 Based on the data in Tables 2 and 3, organic carbon protected in the sand and fine sand

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

Physical protection of labile carbon in macro-aggregates rather than inherent chemical stability of SOC (a minor mass fraction of the clay-sized micro-aggregates, Table 2) had been increasingly considered as a mechanism for soil carbon sequestration (Six et al., 2004; Kong et al., 2005; Six and Paustian, 2014). For the rice soils under long term rice cultivation that were studied here, SOC accumulated and stabilized mainly through physical protection of new or relatively labile carbon in macro-aggregated though old or mineral bound SOC preserved in fine aggregates of clay size (Marschner et al., 2008).

562 This study also confirmed our previous understanding that coarse sand-sized fraction
563 of aggregates could play a prevalent role in soil carbon sequestration (Zhou et al 2008).
564 **4.2 Bio-activities versus carbon stabilization between sand and clay sized fractions**
565 Biological activity of soil microbes including soil respiration and soil enzyme activity
566 were known to vary across size fractions of soil aggregates (Kandeler et al., 1999;
567 Sessitsch et al., 2001; Poll et al., 2003; Allison and Jastrow, 2006). In this study, total
568 DNA content was linearly correlated with content either of SOC and total nitrogen, or
569 of LOC, across the size fractions of the studied sequence (Fig. S3). However, gene
570 abundance of bacterial, fungal and archaeal communities was correlated neither to the
571 size of SOC and LOC nor to measures of carbon recalcitrance and lability (LOC/ SOC),
572 across the sequence. Likewise, SOC level did not necessarily affect microbial
573 populations along soil reclamation gradients with exotic carbon amendments (Yin et al.,
574 2000; Torsvik and Øvreås, 2002). Indeed, different carbon lability and accessibility
575 could shape microbial communities within and between size fractions of aggregates
576 (Six et al., 2000; Ettema and Wardle, 2002; Balser et al., 2006; Kögel-Knabner et al.,
577 2008).
578 Soil matrix and micro-habitat conditions (aggregation and associated nutrients and C
579 substrate as well as redox potential) played a critical role in changes in soil microbial
580 abundance and community composition (Lehmann et al, 2011; Smith et al., 2014). Here,
581 a clearly marked difference in microbial abundance and community could be found
582 between the rice soils and the initial marsh soil before shift to rice cultivation, either for
583 bulk soils (Wang et al., 2015) or for aggregates fractions (Liu et al., 2016b). This could

584 be coincident with the shift in soil physical and chemical conditions between the rice
585 soils and the initial marsh soil, with the latter was alkaline in reaction, poor aggregation
586 due to depleted SOC and high salinity (Data in Table 1).
587 Among the soils studied, both the coarse sand and clay sized fractions showed higher
588 enrichment of SOC, which was relevant to different association of carbon pools and
589 interaction to minerals. There was a difference in the ratio of LOC to total SOC, as a
590 negative indicator of chemical stability, and in carbon recalcitrance measured with
591 FTIR, between the coarse sand and clay sized fractions. The trends of carbon stability
592 with microbial respiratory (RQ) were similar between the sand and clay sized fractions
593 (Fig. 5). Clearly, this similarity could not be explained by the difference in the trend of
594 LOC to SOC ratio, and of carbon recalcitrance (Table 3).
595 Fig. 5
596 We further compared the bio-activity versus SOC accumulation between sand and clay
597 sized fractions of aggregates. Here, a correlation of DNA content, as an indicator of
598 microbial biomass, to SOC content was highly significant for the coarse sand fraction
599 but not for the clay fraction (Fig. 6a). Meanwhile, normalized enzyme activity followed
600 a positive linear function with total SOC content for coarse sand fraction but again not
601 for clay fractions (Fig. 6b). In contrast, soil basal respiration scaled with DNA content
602 reflected a negative power function with total DNA content, being more highly for the
603 coarse sand than for the clay sized fractions (Fig. 6c). This could suggest a higher
604 increase with SOC accumulation in carbon use efficiency in sand sized fractions,
605 compared to clay sized fractions. Furthermore, a positively linear correlation of DNA

606 content to the content of LOC (Fig. 6d) was found for the sand sized aggregate fractions
607 but not for clay sized fractions.

Fig. 6

609 The lack of improvement in bio-activity with SOC accumulation in the clay sized
610 fractions indicated an insignificant potential to support biological activities in fine
611 aggregates rich in stabilized SOC with high recalcitrance. In the clay- sized fractions of
612 aggregates, DNA content was independent of SOC, which could be either inaccessible
613 to microbes or non-degradable due to binding to minerals or as inert carbon (Lützow et
614 al., 2006; Kögel-Knabner et al., 2008). In contrast, the DNA of microbes, mainly as
615 bacterial or archaeal in the soils here, could be mostly adsorbed on clay minerals or
616 hidden in small micropores within the fine aggregates (Poll et al., 2003; Chiu et al.,
617 2006). Soil enzyme activities could represent an overall microbial activity for soil
618 functioning (Allison et al., 2010), which was no response to accumulation of SOC in
619 the clay fractions although extracellular enzymes also could be adsorbed on to clay
620 particles (Allison and Jastrow, 2006).
621 High microbial biomass and enzyme activities were in line with carbon accumulation
622 and stabilization in the coarse sand sized macro-aggregates. The large response of total
623 microbial DNA and carbon use efficiency to SOC accumualtion in the coarse sand size
624 fraction could suggest an improvement of either carbon substrate supply or of habitat
625 environemnt through increases in mass proportion of macro aggregates with enhanced
626 aggregation in soils (Lehmann et al., 2011). While containing a recalcitrant carbon pool
627 similar to that in the clay sized fractions, the macro-aggregates in the coarse sand sized

628 fractions also preserved a significant amount of labile carbon (Table 3), which could
629 become easily decomposable and potentially used by microbes (Cleveland et al., 2007).
630 For the bulk soil of this chronosequence, improved microbial activity was linked to the
631 increase in POC content, which was enhanced via physical protection with increasing
632 aggregate stability (Wang et al., 2015). Although habitats within macro-aggregates
633 offered protection of the young and labile carbon against microbial decomposition
634 (Gupta and Germida, 2015), enhanced aggregation could lead to increased population
635 and activities of specific microbial groups in between micro-aggregates within macro-
636 aggregates (Six et al., 2002b).
637 The metabolic quotient qCO_2 was proposed as an indicator of energy use by live soil
638 microbial organisms (Schlesinger & Andrews, 2000). The data in Table 3 and
639 Supplement Table S3 clearly demonstrated the lowest qCO_2 in the coarse sand sized
640 fractions but the highest qCO_2 in the clay sized fractions, among the size fractions of
641 aggregates. Again, qCO_2 of the coarse sand sized fractions was in a generally
642 decreasing trend with SOC accumulation under prolonged rice paddy management.
643 With soil aggregation improved, macro-aggregates could provide increasingly diverse
644 soil microhabitats with varying types of carbon substrates accessible to microbes under
645 sustainable agricultural management (Six and Paustian, 2014). Improvement of spatial
646 allocation within and between micro-aggregates of carbon resource, microbial
647 communities and extracellular enzymes could favor growth of microbiota and their
648 functional performance in well-aggregated soils (Caldwell, 2005; Burns et al., 2013).
649 Many studies on bulk soils showed correlation of enzyme activity with microbial

650 biomass in agricultural soils including rice paddies under proper management practices
651 (Marx et al., 2005; Allison and Jastrow, 2006; Shi et al., 2006; Yu et al., 2012). Thus,
652 carbon stabilization (indicative of carbon recalcitrance or respiration quotient) could
653 not restrict microbial activity (Janzen, 2006) in macro-aggregates, where highly
654 enriched SOC (particularly of LOC pool) was physically protected, in rice soils under
655 long term paddy management. This could explain a potential co-evolution of improved
656 bio-activity with enhanced carbon sequestration in agricultural soils (Rabbi et al., 2010).
657 As noted by Smith et al. (2014), the relationship between carbon pools and specific
658 microbial communities and biogeochemical activities are still unclear.

659 **4.3 Trend of bioactivity with carbon stabilization after prolonged rice cultivation**

660 Being developed on a similar matrix of paleo deposits rich in silt, the rice soils had been
661 subject to a directional development with long term paddy management (Cheng et al.,
662 2009; Wissing et al., 2013). Desalinization initiated after drainage and conversion and
663 decalcification proceeded as paddy rice cultivation prolonged. Finally, there was a long
664 existing semi-hydromorphic pedogenesis over several centuries, characterized by
665 mobilization of iron and manganese to form minerals of metal oxyhydrates (Wissing et
666 al., 2013). The resultant directional changes of clay minerals, particularly those of
667 oxyhydrates, the size and nature of SOC pools and the difference in archaeal and
668 methanogenic archaeal community abundance had been well characterized by Cheng
669 et al. (2009), Chen et al. (2011), Wissing et al. (2011, 2014 and 2014) and Kölbl et al.
670 (2014) as well as by Wang et al. (2015).

671 The above mentioned directional changes were also seen in soil aggregation, and thus

672 in microhabitat conditions as well as in nutrients (Table 1). SEM observation (Fig. 2)
673 evidenced a clear change in size of the randomly sampled aggregates of the soils studied.
674 This was in an agreement with the change in mean weight diameter (MWD), an
675 indicator of soil aggregate stability, with increasing rice cultivation length over the
676 chronosequence (Wang et al. 2015). There were dispersedly distinct, sharply-edged but
677 less organic matter-covered mineral particles in the uncultivated tidal marsh (P0).
678 However, aggregates became larger in size and softer, and more porous with minute
679 mineral particles bound together by organic matter in rice soils cultivated over 100
680 years. This is particular the case for P700, where the coarse sand sized macro-
681 aggregates were highly porous and soft, containing smaller sized micro-aggregates and
682 with some string-like particulate organic matter on the surface. The increased aggregate
683 size and thus the mean weight diameter (MWD) could suggest increasing organic
684 matter in-between micro-aggregates in macro-aggregates in rice soils cultivated over
685 centuries. This change, through the improvement of micro-habitat conditions and
686 nutrient storage, could lead to some directional change in the association of microbial
687 community abundance/activity over the long run of rice paddy management. The higher
688 MBC and lower RQ and qCO_2 in coarse sand sized macro-aggregates and the
689 decreasing trend of RQ and qCO_2 with increasing length of rice paddy management
690 (Supplement Table S3) could suggest some adaptive change in the microbial
691 community and improvements in their carbon use efficiency (Chen et al., 2016).
692 Particularly, the methanogenic community as particular microbial community in rice
693 soils (Conrad, 2009), had been shown to undergo a directional change towards

694 prolonged rice paddy management (Liu et al., 2016b).

695 In a previous study (Wang et al.,2015), bulk soil carbon accumulation and promotion
696 of biological activity was concomitant with carbon stabilization through POC
697 accumulation, in line with aggregate stability with long-term rice cultivation. Here we
698 synthesize all the analysis data with respect to aggregate size fraction partitioning over
699 the sequence (Fig. 7). After salt marsh soil (P0) was converted to rice cultivation (P50),
700 SOC, enzyme activity and soil respiration showed a more or less consistent increase in
701 both coarse sand and clay sized fractions. The changes in relative portion by sand sized
702 (coarse and fine sand fractions together) aggregates against silt and clay sized ones
703 exerted different patterns between of carbon pools and of microbial activities, across
704 the soils of the chronosequence.

705 Over the sequence, the prevalence of physically protected organic carbon in coarse and
706 fine sand fractions as compared to the percentage of unprotected organic carbon in the
707 silt and clay fractions (Six et al., 2002a) ranged between 1.5-3.2 and 1.1-2.6 for SOC
708 and total N, 0.9-2.2 for total DNA, 1.2-3.3 for fungal gene copy numbers and 0.8-1.5
709 for NEA, respectively. In contrast, the prevalence of archaeal copy numbers and soil
710 respiration was in a range of 2.6-1.0 and 2.0-1.3, decreasing with rice cultivation
711 lengths. Therefore, most of analyzed carbon pools and bioactivities were dominated by
712 the macro- and large micro-aggregates in size fractions of coarse and fine sand, which
713 was in general a consistent directional change with prolonged paddy management under
714 long term rice cultivation although abundance of clay particles was consistently
715 increased (Kölbl et al., 2014).

Fig. 7

Long term SOC sequestration in agricultural soils had been questioned (Powlson et al., 2011) and SOC enriched in coarse sand fractions of aggregates could indeed be subject to fast decomposition in dry condition, for example, after shifting to maize cropping (Li et al., 2007a). In this study, however, hydroagric paddy management was kept continuous with ever prolonged rice cultivation, which could have driven the ever increasing trend of SOC accumulation up to millennium (Wissing et al., 2011; 2013). Consequently, SOC accumulation and stabilization could take place in coarse sand sized aggregates with physical protection of labile carbon pool intra micro-aggregates, with prolonged rice cultivation (Wang et al., 2015). POC, as a pool of relatively fast turnover (Cambardella and Elliott, 1992), also had been shown to keep increasing in paddies cultivated for centuries (Wang et al., 2015). Allison and Jastrow (2006) suggested that microbial biochemical activity and carbon turnover was stronger in POC-enriched size fractions, but weaker in mineral-dominated fractions where enzymes and their carbon substrates were immobilized on mineral surfaces. Long term hydroagric paddy management (Zhang and Gong, 2003) reduced decomposition of root-, crop- or microbial- residue input under low-oxygen conditions (Roth et al., 2011). Moreover, the changes in relative proportion of carbon pools and microbial activities (NEA and soil respiration) by aggregates in the size of coarse and fine sand further demonstrated that physically protected and stabilized carbon supported high soil bioactivities in macro-aggregates, which had been increasingly prevalent over the smaller sized fractions of soil aggregates.

738 The changes in organic carbon pools and the accessibility to microbes could lead to
739 changes in the relative abundance and activity of microbes, potentially affecting C
740 cycling and storage, in different size aggregates (Six et al., 2006). Unlike the finding
741 by Allison and Jastrow (2006), this study proposed enhanced microbial activity but
742 improved carbon use efficiency with reduced respiration quotient for microbial energy
743 in coarse sand sized macro-aggregates, compared to clay fraction over centuries of rice
744 cultivation. This could be supported by the recent finding that qCO_2 was reduced but
745 that the microbial biomass carbon increased in biochar amended agricultural soils, in a
746 case study by Zheng et al., (2016) and in a meta-analysis by Zhou et al (2016). This
747 study indicated a strong inter-link between microbiological activity and labile carbon
748 in large sized aggregates of paddy soils, though the later had been generally considered
749 as physically protected carbon. As strengthened with prolonged rice paddy management,
750 such a link could help enhance ecosystem functioning and services provided by rice
751 soils (Six and Paustian 2014; Smith et al., 2015).
752 Unfortunately, the methodology used here did not allow us to characterize the spatial
753 allocation of carbon substrate, specific microbial communities and extracellular
754 enzyme activities among the aggregate fractions. Specially, labile OC pools,
755 particularly those intra- aggregates or inter micro-aggregates within macro-aggregates,
756 could not be further explored. Such data are considered to be critical to unravel the
757 micro-scale process mediating bio-activities at the aggregate level (Six and Paustian
758 2014).

759 **5 Conclusions**

760 Study of soils collected from a rice soil chronosequence derived from salt marsh,
761 revealed that soil organic carbon could be accumulated and stabilized both in coarse
762 sand- and clay- sized fractions of soil aggregates. However, microbial abundance and
763 enzyme activity were high but the metabolic quotient was low in the aggregates in size
764 larger than 20 μm as compared to those of silt and clay sized fractions, possibly through
765 the enhanced spatial allocation of labile carbon pool for improved microhabitat
766 condition in the larger sized aggregates. Thus, carbon stabilization with reduced
767 turnover was not limiting soil bioactivities in macro-aggregates other than in silt and
768 clay sized micro-aggregates. This study further supported our previous finding for bulk
769 soils that long term rice cultivation led to accumulation and stabilization of SOC and
770 promoted soil biological activities through physical protection of labile carbon in line
771 with enhanced soil aggregation. Thus, labile organic carbons accumulated in macro-
772 aggregates could help enhancing microbial carbon use efficiency and improve their
773 biogeochemical activity related to ecosystem functioning. More studies are deserved
774 on interaction of soil organic matter, minerals and microbial communities to unravel
775 the micro-scale process mediating bio-activities at the aggregate level.

776 **Acknowledgements:**

777 This study was partially funded by China Natural Science Foundation under a grant
778 number 40830528. The Ph D fellowships for the two first authors were awarded with
779 the Priority Academic Program Development of Jiangsu Higher Education Institutions,
780 China. The international cooperation was partially supported by State Foreign Expert
781 Agency with a “111”project under a grant number B12009. The authors are grateful to

782 Dr David Crowley from University of California Riverside for smoothing the
783 manuscript.
784

Reference

Allison, S.D. and Jastrow, J.D.: Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biol. Biochem., 38, 3245-3256, 2006.

Allison, S. D., Wallenstein, M. D. and Bradford, M. A.: Soil-carbon response to warming dependent on microbial physiology, Nat. Geosci., 3, 336-340, 2010.

Balser, T.C., McMahon, K., Bart, D., Bronson, D., Coyle, D., Craig, N., Flores-Mangual, M., Forshay, K., Jones, S. and Kent, A.: Bridging the gap between micro-and macro-scale perspectives on the role of microbial communities in global change ecology, Plant Soil, 289, 59-70, 2006.

Bannert, A., Kleineidam, K., Wissing, L., Mueller-Niggemann, C., Vogelsang, V., Welzl, G., Cao, Z. and Schloter, M.: Changes in diversity and functional gene abundances of microbial communities involved in nitrogen fixation, nitrification, and denitrification in a tidal wetland versus paddy soils cultivated for different time periods. Appl. Environ. Microbiol., 77, 6109-6116, 2011..

Banwart, S., Black, H., Cai, Z., Gicherud, P., Joosten, H., Victoria, R., Milne, E., Noellemeyer, E., Pascual, U., Nziguheba, G., Vargas, R., Bationo, A., Buschiazzo, D., De-Brogniez, D., Melillo, J., Richter,D., Termansen, M., Van Noordwijk, M., Goverse, T., Ballabio, C., Bhattacharyya, T., Goldhaber, M., Nikolaidis, N., Zhao, Y., Funk, R., Chris, D., Pan, G., La Scala, N., Gottschalk, P., Batjes, N., Six, J., Van Wesemael, B., Stocking, M., Bampa, F., Bernoux, M., Feller, C., Lemanceau, P. and Montanarell, L.: Benefits of soil carbon: report on the outcomes of an international scientific committee on problems of the environment rapid assessment workshop, Carb. Manag., 5, 185-192,2014.

Bardgett, R. D. and Van Der Putten, W. H.: Belowground biodiversity and ecosystem

functioning, Nature, 515, 505-511, 2014

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

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

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

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

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

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

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

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

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

turnover is governed by accessibility not recalcitrance, Glob. Chang. Biol., 18, 1781-1796, 2012.

Ellerbrock, R., Höhn, A. and Rogasik, J.: Functional analysis of soil organic matter as affected by longterm manurial treatment, Euro. J. Soil Sci., 50, 65-71, 1999.

Elliott, E. T.: Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J., 50, 627-633, 1986.

Ettema, C.H. and Wardle, D.A.: Spatial soil ecology, Trends Ecol. Evol., 17, 177-183, 2002.

Gajda, A.M.: Microbial activity and particulate organic matetr content in soils with diffrerent tillage system use, Int. Agrophys., 24, 129-137, 2010.

Gardes, M. and Bruns, T.D.: ITS primers with enhanced specificity for basidiomycetes‐application to the identification of mycorrhizae and rusts, Molec. Eecol., 2, 113-118, 1993.

Gong, Z., Zhang, G., Wang, J.Z., Jia, H.Y., Shen, H. and Lu, J.C.: Anthorosols. In: Gong, Z. (editor-in-chief), Chinese Soil Taxonomy: Priciples, Methodology and Application. Science Press, Beijing (China), pp109-131, 1999.

Guan, S., Zhang, D. and Zhang, Z.: Soil enzyme and its research methods. China Agriculture Press, Beijing, 274-297, 1986.

Gupta, V. V. and Germida, J. J.: Soil aggregation: Influence on microbial biomass and implications for biological processes, Soil Biol. Biochem., 80, A3-A9, 2015.

Janzen, H. H.: The soil carbon dilemma: shall we hoard it or use it? Soil Biol. Biochem., 38, 419-424, 2006.

Jastrow, J. D., Miller, R. M. and Lussenhop, J.: Contributions of interacting biological mechanisms to soil aggregate stabilization in restored prairie. Soil Biol. Biochem., 30, 905-916, 1998.

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

910 Kölbl, A., Schad, P., Jahn, R., Amelung, W., Bannert, A., Cao, Z., Fiedler, S., Kalbitz,
911 K., Lehndorff, E. and Müller-Niggemann, C.: Accelerated soil formation due to
912 paddy management on marshlands (Zhejiang Province, China), Geoderma, 228,
913 67-89, 2014.
914 Kong, A.Y., Six, J., Bryant, D.C., Denison, R.F. and van Kessel, C.: The relationship
915 between carbon input, aggregation, and soil organic carbon stabilization in
916 sustainable cropping systems, Soil Sci. Soc. Amer. J., 69, 1078-1085, 2005.
917 Lagomarsino, A., Grego, S. and Kandeler, E.: Soil organic carbon distribution drives
918 microbial activity and functional diversity in particle and aggregate-size
919 fractions, Pedobiol., 55, 101-110, 2012.
920 Lehmann, J. and Kleber, M.: The contentious nature of soil organic matter, Nat., 258,
921 60-68, doi:10.1038/nature16069, 2015.
922 Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C. and Crowley,
923 D.: Biochar effects on soil biota–a review. Soil Biol. Biochem., 43, 1812-1836,
924 2011.
925 Lehmann, J., Solomon, D., Kinyangi, J., Dathe, L., Wirick, S. and Jacobsen, C.:
926 Spatial complexity of soil organic matter forms at nanometre scales, Nat.
927 Geosci., 1, 238-242,2008.
928 Li, Z.P., Pan, G., and Zhang, X.H.: Topsoil organic carbon pool and ^{13}C natural
929 abundance changes from a paddy after 3 years corn cultivation, Acta Ped. Sin.,
930 44(2), 244-251, 2007a. In Chinese with English sumamry.
931 Li, L., Zhang, X., Zhang, P., Zheng, J. and Pan, G.: Variation of organic carbon and
932 nitrogen in aggregate size fractions of a paddy soil under fertilisation practices
933 from Tai Lake Region, China, J Sci. Food Agri., 87, 1052-1058, 2007b.
934 Li, Q.: Paddy Soils of China, Science Presss, Beijing, China. 1992. In Chinese.

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

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

985 organic carbon in China's paddy soils, Glob. Chang. Biol.,10, 79-92, 2004.
986 Pan, G., Wu, L., Li, L., Zhang, X., Gong, W. and Wood, Y.: Organic carbon
987 stratification and size distribution of three typical paddy soils from Taihu Lake
988 region, China, J. Environ. Sci., 20, 456-463,2008.
989 Pan, G., Xu, X., Smith, P., Pan, W. and Lal, R.: An increase in topsoil SOC stock of
990 China's croplands between 1985 and 2006 revealed by soil monitoring. Agr.
991 Ecosyst. Environ., 136, 133-138, 2010.
992 Poll, C., Thiede, A., Wermbter, N., Sessitsch, A. and Kandeler, E.: Microscale
993 distribution of microorganisms and microbial enzyme activities in a soil with
994 longterm organic amendment, Euro. J Soil Sci., 54, 715-724, 2003.
995 Pozo, C., Diaz-Visurraga, J., Contreras, D., Freer, J., Rodriguez, J. Characterization of
996 temporal biodegradation of Radiata pine by Gloeophyllum trabeaum through
997 principal component analysis-based two dimensional correlation FTIR
998 spectroscopy. J. Chil. Chem. Soc., 61,2878-2883, 2016.
999 Powlson, D. S., Whitmore, A. P. and Goulding, K. W. T.: Soil carbon sequestration to
1000 mitigate climate change: a critical re-examination to identify the true and the
1001 false. Euro. J. Soil Sci., 62, 42-55, 2011.
1002 Qian, H. Y., Pan, J. J. and Sun, B.: The relative impact of land use and soil properties
1003 on sizes and turnover rates of soil organic carbon pools in Subtropical China.
1004 Soil Use Manage., 29, 510-518, 2013.
1005 Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W.F. and Torn, M.S. : Large
1006 contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical
1007 forest soils, Plant Soil, 233, 167-177,2001.
1008 Roth, P. J., Lehndorff, E., Zhuang, S., Bannert, A., Wissing, L., Schloter, M., Kogel-
1009 Knabner, I. and Amelung, W.: Accumulation of nitrogen and microbial residues

during 2000 years of rice paddy and non-paddy soil development in the Yangtze River Delta, China, Global Change Biol., 17, 3405-3417, 2011.

Saiya-Cork, K., Sinsabaugh, R. and Zak, D.: The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil, Soil Biol. Biochem.,34, 1309-1315,2002.

Salinas-Garcia, J., Hons, F., Matocha, J. and Zuberer, D. : Soil carbon and nitrogen dynamics as affected by long-term tillage and nitrogen fertilization, Biol. Fert. Soils, 25, 182-188,1997.

Schlesinger, W.H. and Andrews, J.A.: Soil respiration and the global carbon cycle, Biogeochem.,48, 7-20,2000.

Schmidt, M.W., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J. and Manning, D.A.: Persistence of soil organic matter as an ecosystem property, Nature, 478, 49-56,2011.

Sessitsch, A., Weilharter, A., Gerzabek, M.H., Kirchmann, H. and Kandeler, E.: Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment, Appl. Environ. Microbiol., 67, 4215-4224,2001.

Shi, W., Dell, E., Bowman, D. and Iyyemperumal, K.: Soil enzyme activities and organic matter composition in a turfgrass chronosequence, Plant Soil, 288, 285-296, 2006.

Six, J., Bossuyt, H., Degryze, S. and Denef, K.:A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. Soil Till. Res., 79, 7-31, 2004.

Six, J., Conant, R., Paul, E.A. and Paustian, K.: Stabilization mechanisms of soil organic matter: implications for C-saturation of soils, Plant Soil, 241, 155-176 , 2002a.

1035 Six, J., Feller, C., Denef, K., Ogle, S., Sa, J.C.D.M. and Albrecht, A.: Soil organic
1036 matter, biota and aggregation in temperate and tropical soils-Effects of no-tillage,
1037 Agron, 22, 755-775, 2002b..
1038 Six, J., Frey, S., Thiet, R. and Batten, K.: Bacterial and fungal contributions to carbon
1039 sequestration in agroecosystems, Soil Sci. Soc. Amer. J., 70, 555-569, 2006.
1040 Six, J. and Paustian, K. :Aggregate-associated soil organic matter as an ecosystem
1041 property and a measurement tool, Soil Biol. Biochem., 68, A4-A9,2014.
1042 Six, J., Paustian, K., Elliott, E. and Combrink, C.: Soil structure and organic matter I.
1043 Distribution of aggregate-size classes and aggregate-associated carbon, Soil Sci.
1044 Soc. Amer. J., 64, 681-689, 2000.
1045 Smith, A., Marín-Spiotta, E., de Graaff, M. and Balser, T.: Microbial community
1046 structure varies across soil organic matter aggregate pools during tropical land
1047 cover change, Soil Biol. Biochem., 77, 292-303, 2014.
1048 Smith, P., Cotrufo, M. F., Rumpel, C., Paustian, K., Kuikman, P. J., Elliott, J. A.,
1049 Mcdowell, R., Griffiths, R. I., Asakawa, S., Bustamante, M., House, J. I.,
1050 Sobocka, J., Harper, R., Pan, G., West, P. C., Gerber, J. S., Clark, J. M., Adhya,
1051 T., Scholes, R. J. and Scholes, M. C.: Biogeochemical cycles and biodiversity as
1052 key drivers of ecosystem services provided by soils. Soil Discussions, 2, 537-
1053 586, 2015.
1054 Song, X., Li, L., Zheng, J., Pan, G., Zhang, X., Zheng, J., Hussain, Q., Han, X. and
1055 Yu, X.: Sequestration of maize crop straw C in different soils: role of
1056 oxyhydrates in chemical binding and stabilization as recalcitrance, Chem., 87,
1057 649-654, 2012.
1058 Song, X., Spaccini, R., Pan, G. and Piccolo, A.: Stabilization by hydrophobic
1059 protection as a molecular mechanism for organic carbon sequestration in maize-

amended rice paddy soils, Sci Tot. Environ., 458, 319-330, 2013.

Stemmer, M., Gerzabek, M.H. and Kandeler, E.:Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication, Soil Biol. Biochem., 30, 9-17,1998.

Stemmer, M., von Lützow, M., Kandeler, E., Pichlmayer, F. and Gerzabek, M.H.: The effect of maize straw placement on mineralization of C and N in soil particle size fractions. Euro. J. Soil Sci., 50, 73-85,1999.

Strickland, M.S. and Rousk, J.:Considering fungal: bacterial dominance in soils– methods, controls, and ecosystem implications, Soil Biol. Biochem., 42, 1385-1395, 2010.

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

Tisdall, J.: Possible role of soil microorganisms in aggregation in soils, Plant Soil, 159**,** 115-121, 1994.

Tivet, F., de Moraes Sa, J. C., Lal, R., Milori, D.M.B.P., Briedis, C.,Letourmy, C., Pinheiro, L. A., Borszowskei, P. R., da Cruz Hartman, D.: Assessing humification and organic C compunds by laser-induced fluorescence and FTIR spectroscopies under conventional and no-tillage management in Brazilian Oxisols. Geoderma, 27-28, 71-81, 2013.

Torsvik, V. and Øvreås, L.: Microbial diversity and function in soil: from genes to ecosystems, Curr. Opin. Microb., 5, 240-245, 2002.

Vogel, C., Mueller, C.W., Höschen, C., Buegger, F., Heister, K., Schulz, S., Schloter, M. and Kögel-Knabner, I.: Submicron structures provide preferential spots for carbon and nitrogen sequestration in soils, Nature Comm., 5, 2947. doi: 10.1038/ncomms3947, 2014.

1085 Wakeham, S. G. and Canuel, E. A.: The nature of organic carbon in density-
1086 fractionated sediments in the Sacramento-San Joaquin River Delta (California),
1087 Biogeosci., 13, 567-582, doi:10.5194/bg-13-567-2016, 2016.
1088 Wang, P., Liu, Y., Li, L., Cheng, K., Zheng, J., Zhang, X., Zheng, J., Joseph, S. and
1089 Pan, G.: Long-term rice cultivation stabilizes soil organic carbon and promotes
1090 soil microbial activity in a salt marsh derived soil chronosequence, Sci Rep, 5,
1091 15704,2015.
1092 Wissing, L., Kölbl, A., Häusler, W., Schad, P., Cao, Z.-H. and Kögel-Knabner, I.:
1093 Management-induced organic carbon accumulation in paddy soils: the role of
1094 organo-mineral associations, Soil Till. Res., 126, 60-71,2013.
1095 Wissing, L., Kölbl, A., Vogelsang, V., Fu, J.-R., Cao, Z.-H. and Kögel-Knabner, I.:
1096 Organic carbon accumulation in a 2000-year chronosequence of paddy soil
1097 evolution, Catena, 87, 376-385, 2011.
1098 Yang, C., Yang, L. and Ouyang, Z.: Organic carbon and its fractions in paddy soil as
1099 affected by different nutrient and water regimes, Geoderma, 124, 133-142, 2005.
1100 Yang, Y., Guo, J., Chen, G., Yin, Y., Gao, R., Lin, C.: Effect of forest conversion on
1101 labile organic carbon fractions and aggregate stability in subtropical China, Plant
1102 Soil, 323,153-162, 2009.
1103 Yin, B., Crowley, D., Sparovek, G., De Melo, W.J. and Borneman, J.: Bacterial
1104 functional redundancy along a soil reclamation gradient, Appl. Environ. Microb.,
1105 66, 4361-4365, 2000.
1106 Yu, H., Ding, W., Luo, J., Donnison, A. and Zhang, J.: Lonterm effect of compost and
1107 inorganic fertilizer on activities of carbon cycle enzymes in aggregates of an
1108 intensively cultivated sandy loam, Soil Use Manag., 28, 347-360, 2012.
1109 Zhang, A., Cui, L., Pan, G., Li, L., Hussain, Q., Zhang, X., Zheng, J. and Crowley,

1110 D.:. Effect of biochar amendment on yield and methane and nitrous oxide
1111 emissions from a rice paddy from Tai Lake plain, China, Agric., Ecosyst.
1112 Environ., 139, 469-475, 2010a.
1113 Zhang, G., Chen, L.and Gilkes, R.: Soil genesis along a paddy soil chronosequence in
1114 a millennium scale. In: Proceedings of the 19th World Congress of Soil Science:
1115 Soil solutions for a changing world, Brisbane, Australia, 1-6 August 2010.
1116 Symposium 1.3. 1 Pedogenesis: ratio and ranges of influence, pp. 88-91.
1117 International Union of Soil Sciences (IUSS), c/o Institut für Bodenforschung,
1118 Universität für Bodenkultur. 2010b.
1119 Zhang, G. and Gong, Z.: Pedogenic evolution of paddy soils in different soil
1120 landscapes, Geoderma, 115, 15-29, 2003.
1121 Zhang, P., Zheng, J., Pan, G., Zhang, X., Li, L. and Rolf, T.: Changes in microbial
1122 community structure and function within particle size fractions of a paddy soil
1123 under different long-term fertilization treatments from the Tai Lake region,
1124 China, Coll. Surf. B: Biointerfaces, 58, 264-270, 2007.
1125 Zhang, S., Li, Q., Lü, Y., Zhang, X. and Liang, W.: Contributions of soil biota to C
1126 sequestration varied with aggregate fractions under different tillage systems, Soil
1127 Biol. Biochem., 62, 147-156, 2013.
1128 Zheng, J., Zhang, X., Li, L., Zhang, P. and Pan, G.: Effect of long-term fertilization on
1129 C mineralization and production of CH_4 and CO_2 under anaerobic incubation
1130 from bulk samples and particle size fractions of a typical paddy soil, Agric.
1131 Ecosyst. Environ., 120, 129-138, 2007.
1132 Zheng, J.F., Zhang, X., Chen, J. Pan, G.: Biochar decreased microbial metabolic
1133 quotient and shifted community composition four years after a single
1134 incorporation in a slightly acid rice paddy from southwest China. Sci. Tot.

1135 Environ., 571:206-217, 2016.
1136 Zhou, P. and Pan, G.: Effect of different long-term fertilization treatments on
1137 particulate organic carbon in water-stable aggregates of a paddy soil, Chin. J Soil
1138 Sci, 38, 256-261, 2007. In Chinese with English sumamry.
1139 Zhou, P., Song, G., Pan, G., Li, L., Zhang, X. and Wu, L.:SOC accumulation in three
1140 major types of paddy soils under long-term agro-ecosystem experiments from
1141 South China I. Physical protection in soil micro-aggregates, Act. Pedol. Sin., 45,
1142 1063-1071, 2008.
1143 Zhou, P., Piccolo, A., Pan, G. and Smejkalova D.: SOC enhancement in three major
1144 types of paddy soils in a long-term agro-ecosystem experiment in South China.
1145 III. structural variation of particulate organic matter of two paddy soils, Act.
1146 Pedol. Sin., 46, 398-405, 2009a.
1147 Zhou, P., Song, G., Pan, G., Li, L. and Zhang, X.: Role of chemical protection by
1148 binding to oxyhydrates in SOC sequestration in three typical paddy soils under
1149 long-term agro-ecosystem experiments from South China, Geoderma, 153, 52-
1150 60, 2009b.
1151 Zhou, P., Piccolo, A., Pan, G. and Spaccini R.: SOC enhancement in three major types
1152 of paddy soils in a long-term agro-ecosystem experiment in South China. IV.
1153 Molecular characterization of particulate organic carbon by TMAH
1154 thermochemolysis GC/MS, Act. Pedol. Sin., 48, 112-124, 2011.
1155 Zhou, H.M., Zhang, D.X., Wang, P., Liu, X., Cheng, K., Li, L.Q., Zheng, J.W., Zhang,
1156 X.H., Zheng, J.F., Crowley, D., van Zwieten, L., Pan, G.: Changes in microbial
1157 biomass and the metabolic quotient under biochar amendment in agricultural soils:
1158 A Meta-analysis. Agriculture, Ecosystem and Environment, under review, 2016
1159 Zou, P., Fu, J., Cao, Z., Ye, J. and Yu, Q.: Aggregate dynamics and associated soil

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

Figure captions

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

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

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

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

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

function of soil microbial biomass (c) and bacterial abundance (d)). Data was the mean value of triplicates.

Fig. 6 Inter-correlation between particulate organic carbon and soil microbial activity to compare the biological activity versus carbon between coarse sand (left) and clay (right) sized aggregate fractions (Soil microbial biomass was as an exponential function of total soil organic carbon (a) and a linear function of labile organic carbon (d). Normalized enzyme activity (b) and DNA content scaled CO_2 production (c) as a linear and negative power function of soil microbial biomass. Soil microbial biomass was as a linear function of relative recalcitrant C (aromatic and phenol) (e)). Data was the mean value of triplicates.

Fig. 7 Change in partitioning of soil organic carbon (a, g/kg), total DNA (b, μg/g) , normalized enzyme activity (c, relative enzyme activity index) and soil respiration (d, mgCO_2/g) among coarse and fine sand fraction (blue base), silt fraction (brown base) and clay fraction (gray base) of soil aggregates, over the chronosequence of rice soils (P50-P700) shifted from a salt marsh (P0) under long term rice cultivation. The size of a circle in a row is relevant to that of an analyzed parameter among the soils.

Supplement material

Supplement Figure S1. FTIR spectrum of aggregate size fractions of the paddy soil chronosequence (a: 2000-200μm; b: 200-20μm; c: 20-2μm; d: <2μm). The code of P0 and P50-P700 denotes respectively the uncultivated marsh soil, and soils shifted under rice cultivation for 50-700 years.

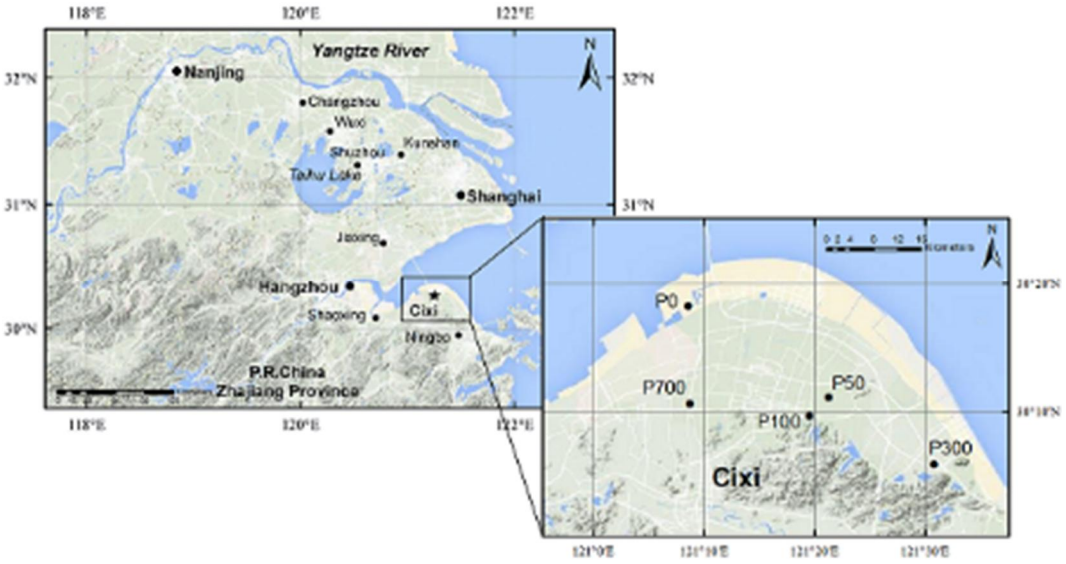
Supplement Figure S2. Correlation of bulk SOC with amount of OC in coarse sand (a) and clay (b) size fractions of soil aggregates.

Supplement Figure S3. Correlation of total DNA content to organic carbon (a), total N (b) and labile carbon (c) of the size fractions of soil aggregates.

Supplement Table S1. Shannon diversity index of bacterial (BD), fungal (FD) and archaeal (ArD) of soil size fraction of the studied chronosequence. Different capital and low case letters in a single column indicate a significant (p<0.05) difference respectively between fractions of a single soil, and between soils for a single fraction.

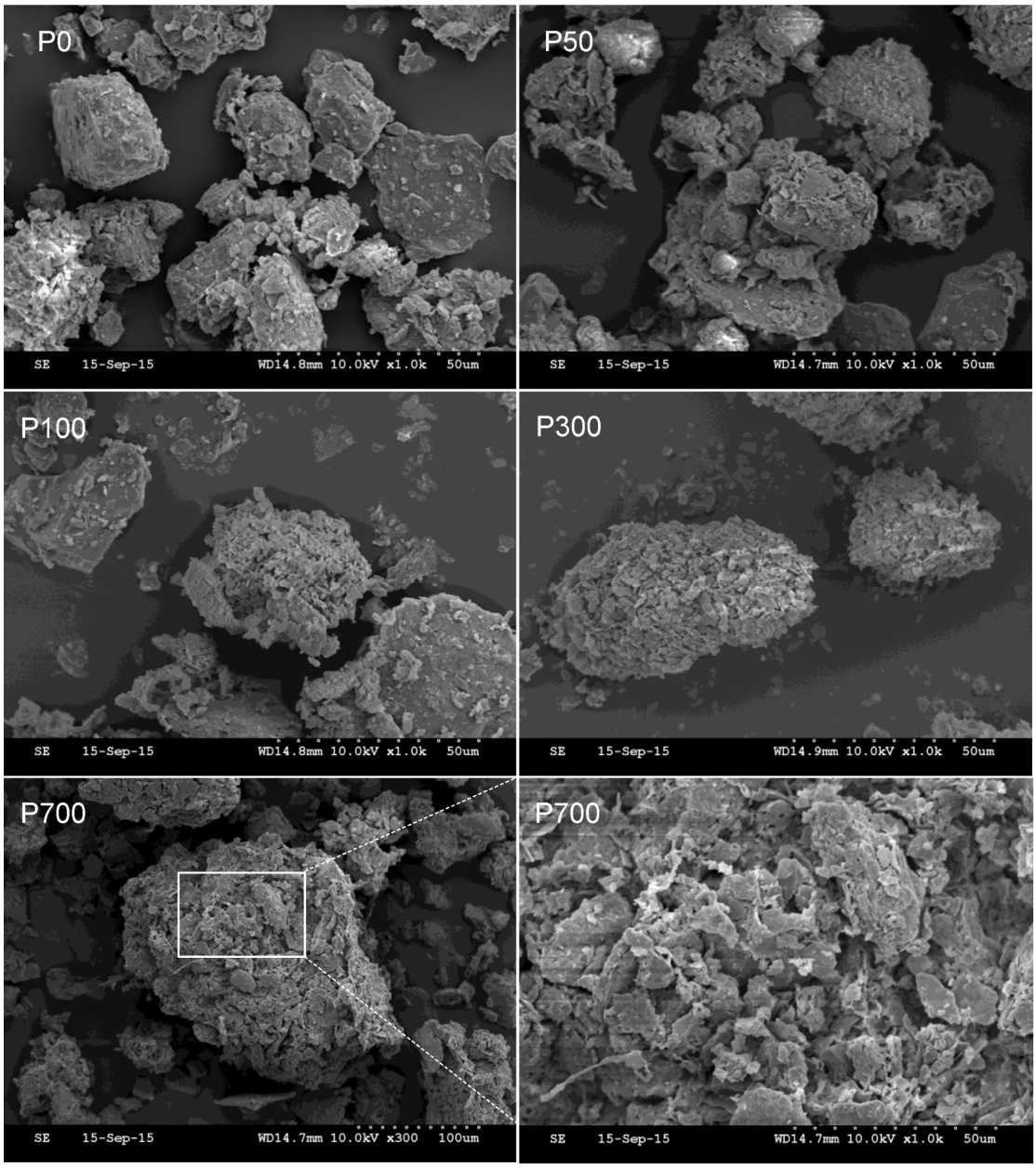
Supplement Table S2. Activity of invertase, urease, acid phosphatase, β-glucosidase, β-cellobiosidase and peroxidase in particle size fractions of soils over the chronosequence.

Supplement Table S3. Mean soil respiration quotient (portion of respired CO_2-C to SOC) and soil metabolic quotient (ratio of respired CO_2-C to MBC) of the soil aggregate size fractions estimated using the data in Table 3 in the text. N.d., not determined due to the very small amount of the fraction.



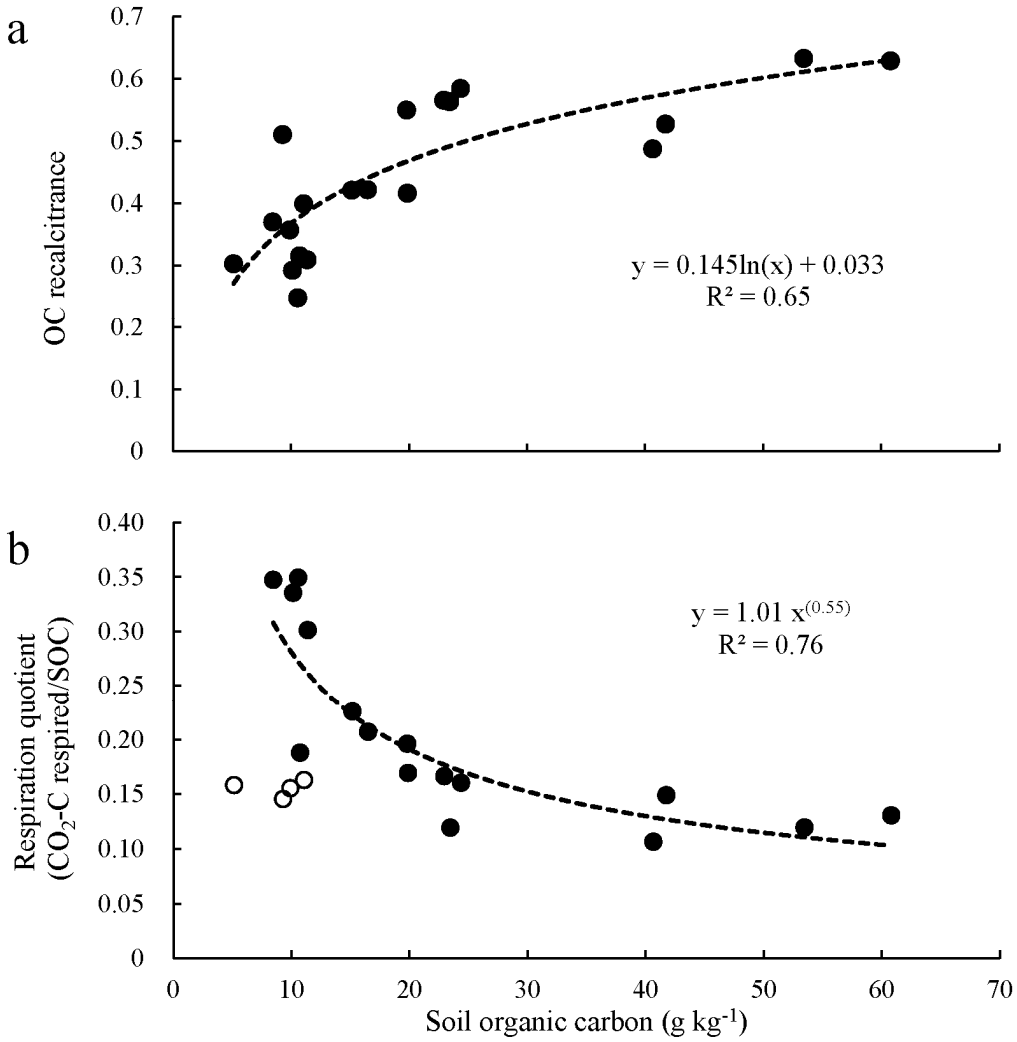
1226

1227 **Fig.1**



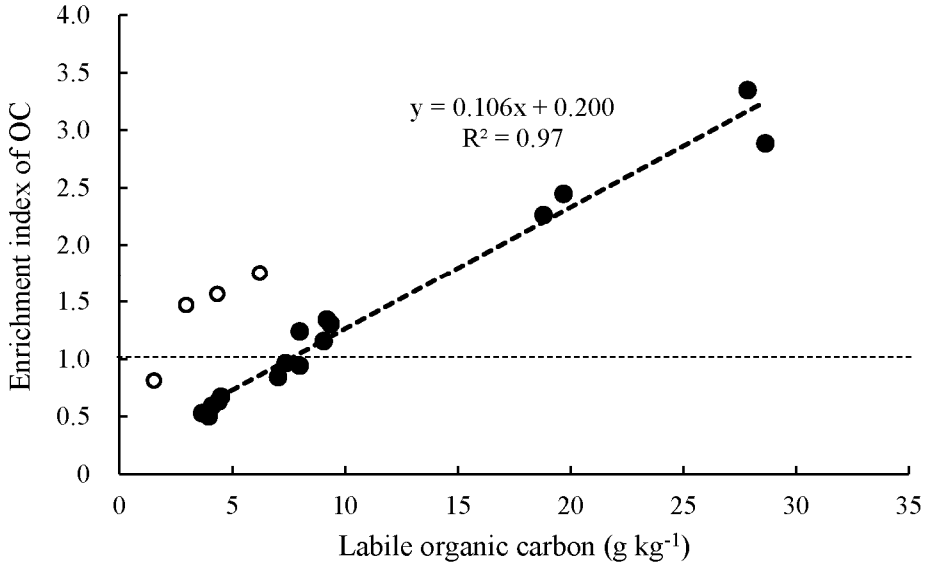
1228

1229 **Fig.2**



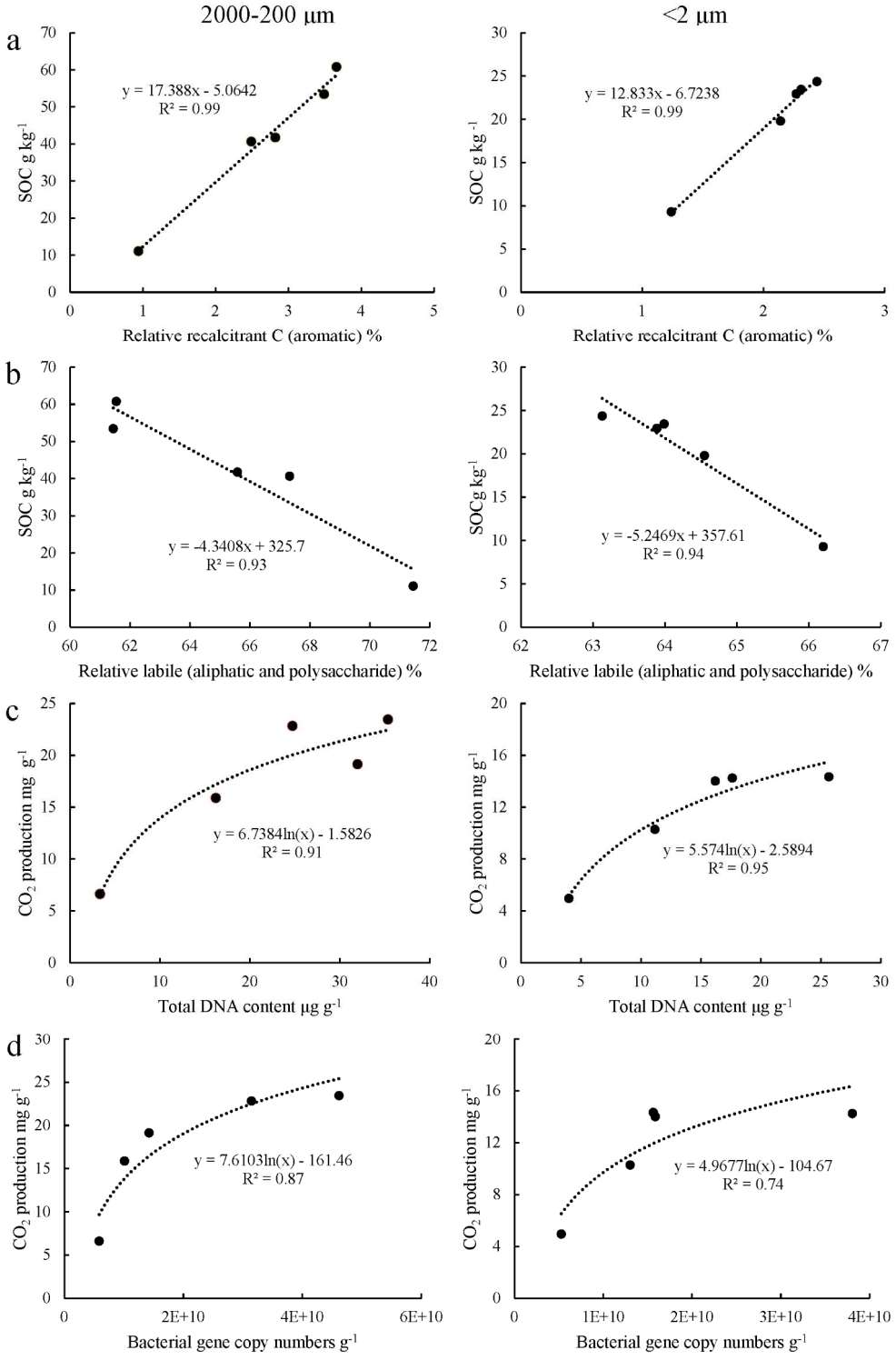
1230

1231 **Fig.3**



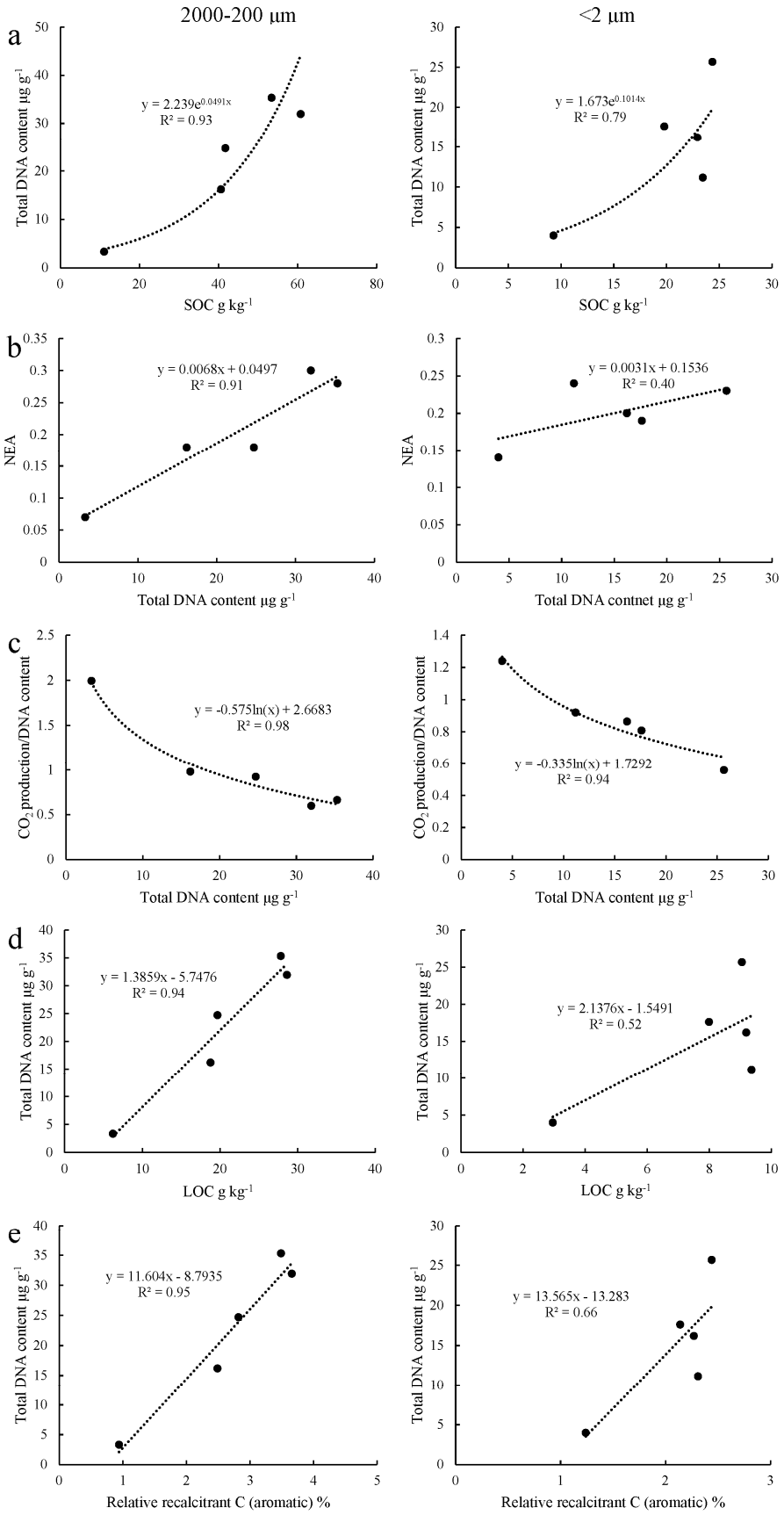
1232

1233 **Fig.4**



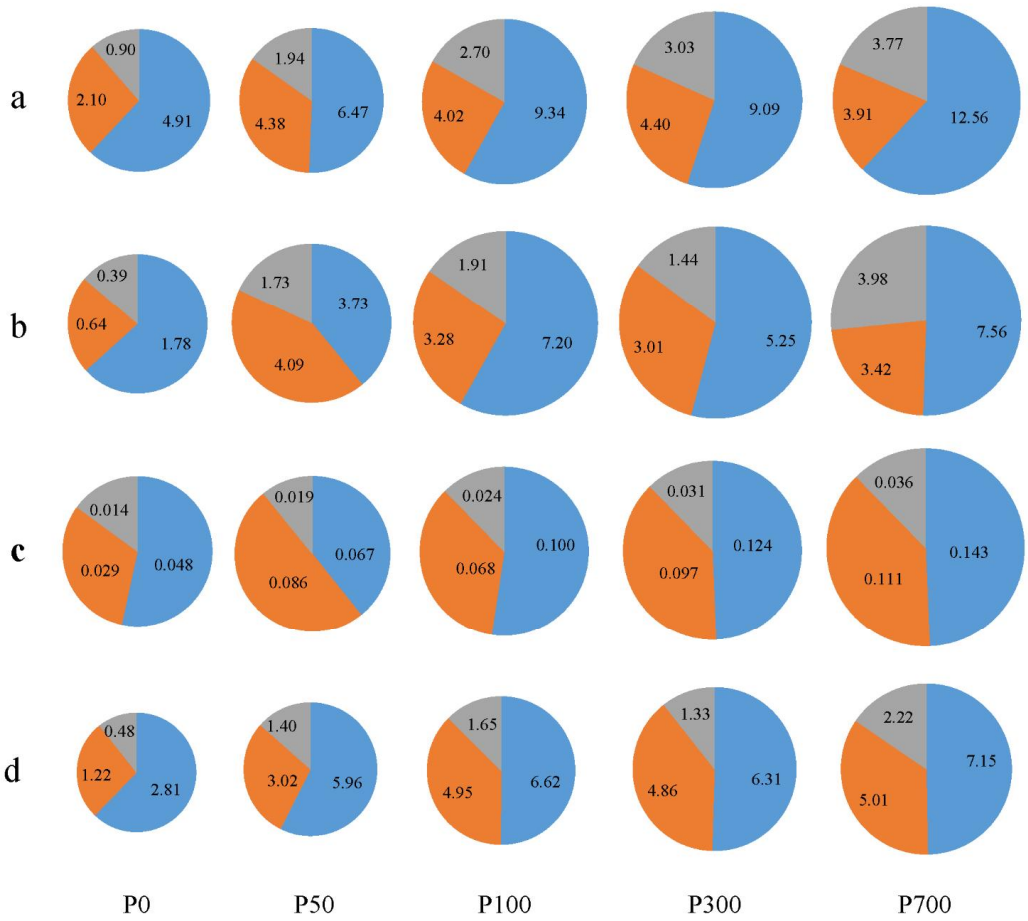
1234

1235 **Fig.5**



1236

1237 **Fig.6**



1238

1239 **Fig.7**

1240 **Table 1** Basic properties of the soils in the chronosequence (Mean ± SD, $n = 3$)

Soil	pH (H_2O)	SOC ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	BD ($g\ cm^{-3}$)	CEC ($cmol\ kg^{-1}$)	Fed ($g\ kg^{-1}$)
P0	8.62±0.07	6.32±0.58	0.79±0.02	1.31±0.05	6.32±0.34	1.76±0.02
P50	7.84±0.04	15.96±0.66	1.81±0.06	1.13±0.03	12.82±0.06	1.96±0.01
P10	6.39±0.05	17.07±0.49	2.06±0.09	1.06±0.04	12.54±0.12	2.04±0.04
P30	6.40±0.03	17.97±0.81	2.09±0.08	1.07±0.07	13.78±0.26	2.08±0.05
P70	6.65±0.08	21.07±1.21	2.14±0.06	1.06±0.05	12.97±0.27	1.71±0.02

1241 Note: SOC, soil organic carbon; BD, bulk density; CEC, cation exchange capacity;

1242 Fed: dithionate extractable iron oxyhydrates.

1243

1244 **Table 2** Particle-size distribution (%) of aggregates of the studied chronosequence soils.
1245 Lower case letters indicate a significant (p<0.05) difference between soils for a single
1246 fraction, in a column.

Soil	Coarse sand (2000-200 μm)	Fine sand (200-20 μm)	Silt (20-2 μm)	Caly (<2 μm)	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

1247

1248

1249 **Table 3** SOC, total N and LOC in g kg^{-1} and SMBC in mg kg^{-1} of the size fractions
1250 (PSFs) of the soil chronosequence. Different capital and lower case letters indicate a
1251 significant (p<0.05) difference respectively between fractions of a single soil, and
1252 between soils for a single fraction, in a single column.

PSF	Soil	SOC	Total N	LOC	SMBC
Coarse sand (2000-200 µm)	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	1052±73.7Ab
	P300	40.64±1.57Ac	2.72±0.12Ac	18.80±1.45Ab	1385±88.1Aa
	P700	60.79±1.88Aa	4.43±0.22Aa	28.64±1.90Aa	1480±166.2Aa
Fine Sand (200-20 µm)	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 (20-2 µm)	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 (<2µm)	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

1253

1254 **Table 4** Relative proportion (%) of carbon chemical groups and carbon recalcitrance
1255 (ratio of aromatic to polysaccharide carbon) in size fractions by FTIR analysis.
1256 Different capital and lower case letters indicate a significant (p <0.05) difference
1257 respectively between fractions of a single soil, and between soils for a single fraction**.**

Size fraction	Soil	Total aromatic	Aliphatic	Polysaccharide
Coarse sand (2000-200 μm)	P0	28.58±1.41Bc	0.03±0.00Ac	71.41±5.76ABa
	P50	38.55±5.73Aab	0.50±0.09Aa	60.94±2.54Cb
	P100	34.43±3.78ABab	0.27±0.03Ab	65.31±4.72Bab
	P300	32.67±0.78ABb	0.28±0.04Ab	67.04±4.66BCab
	P700	38.47±1.59Aa	0.37±0.03Ab	61.17±4.30Cb
Fine sand (200-20 μm)	P0	26.30±1.57Ba	0.05±0.01Ab	73.64±4.83ABa
	P50	26.98±1.15Ba	0.04±0.00Bb	72.98±4.43ABa
	P100	29.62±1.07Ba	0.13±0.03Ba	70.24±3.47ABa
	P300	29.60±1.42Ba	0.07±0.02Bb	70.32±4.60ABa
	P700	29.33±1.28Ba	0.17±0.02Ba	70.51±4.09Ba
Silt (20-2 μm)	P0	23.22±1.27Ca	0.01±0.00Ba	76.76±3.81Aa
	P50	23.98±1.50Ca	0.01±0.00Ca	76.02±4.29Aa
	P100	22.61±1.32Ca	0.00±0.00Db	77.37±4.73Aa
	P300	23.61±1.14Ca	0.00±0.00Db	76.39±4.21Aa
	P700	19.87±0.83Cb	0.00±0.00Db	80.14±3.87Aa
Clay (<2μm)	P0	33.78±1.69Aa	0.00±0.00Bb	66.20±3.2B2a
	P50	35.46±1.36Aa	0.03±0.00Ba	64.52±4.23Ba
	P100	36.10±1.74Aa	0.04±0.01Ca	63.85±4.57Ba
	P300	36.02±1.72Aa	0.03±0.01Ca	63.96±4.65Ca
	P700	36.86±1.88Aa	0.05±0.01Ca	63.08±3.73Ca

1258

1259 **Table 5** DNA content (μg g^{-1}), copy numbers of bacterial (BA, copies×$10^9 g^{-1}$), fungi
1260 (FA, copies×$10^7 g^{-1}$) and archaeal (ArA, copies×$10^8 g^{-1}$) of the size fractions. Different
1261 capital and lower case letters in a single column indicate a significant ($p<0.05$)
1262 difference respectively between fractions of a single soil, and soils for a single fraction.

Fraction	Soil	DNA	BA	FA	ArA
Coarse sand (2000-200 μm)	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 (200-20 μm)	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 (20-2 μm)	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 (<2μm)	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

1263 **Table 6** Normalized enzyme activity (NEA) and soil respiration (mg CO_2 kg^{-1}) of the
1264 chronosequence soils. Different capital and low case letters in a single column indicate
1265 a significant ($p < 0.05$) difference respectively between fractions of a single soil, and
1266 between soils for a single fraction.

Size fraction	Soil	NEA	Basal respiration
Coarse sand (2000-200μm)	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 (200-20μm)	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 (20-2μm)	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 (<2μm)	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

1267