Resuscitation of soil microbiota after > 70-years of desiccation

Jun Zhao[1‡], Dongfeng Chen[1‡*], Wei Gao[1,2], Zhiying Guo[1,2], Zhongjun Jia[1], Marcela Hernández[3*]

[1]State Key Laboratory of Soil and Sustainable Agriculture. Institute of Soil Science, Chinese Academy of Sciences. Nanjing, 210008, Jiangsu Province, China

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

[3]School of Environmental Sciences, Norwich Research Park, University of East Anglia, Norwich, UK

[‡]These authors contribute equally to this work

*** Correspondence:**

Marcela Hernández: marcela.hernandez@uea.ac.uk

Dongfeng Chen: dfchen@issas.ac.cn

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Highlights

- Soil contained measurable abundance of bacteria after >70-years desiccation
- Bacterial composition was still distinct in soils after long desiccation
- Rewetting resuscitated bacterial growth in desiccated soils
- *Firmicutes* were the main responders to rewetting
- Many *Actinobacteria* and *Proteobacteria* were also resuscitated

24 **Abstract**

25 The response pattern of soil bacterial communities to drought events has been
26 characterized from short-term laboratory desiccation and in naturally arid soils, but the
27 resistance of soil bacteria to persistently long periods of desiccation remains largely
28 unknown. This study assessed the abundance and composition of bacteria in 24
29 historical soils air-dried for more than 70 years, by quantification and high-throughput
30 sequencing analyses of 16S rRNA genes. All soils contained measurable abundances of
31 bacteria varying from 10^3 to 10^8 gene copies g^{-1} soil and contrasting community
32 compositions were observed in soils with different land histories, suggesting that the
33 detected bacteria were indigenous to the field. A 28-day soil rewetting incubation
34 significantly increased the bacterial gene abundance in soils, indicating some bacteria
35 in these soils were still alive and quickly resuscitated. Among all identified taxa,
36 *Paenibacillus, Cohnella* and two unclassified *Bacillales* genera within the phylum
37 *Firmicutes* showed growth in the highest number of soils (≥12 soils), whereas
38 *Bacillales* genera *Tumebacillus, Alicyclobacillus* and *Brevibacillus* displayed the
39 strongest growth activity (>1000-fold increase in gene abundance) following rewet.
40 Some *Actinobacteria* and *Proteobacteria* genera also showed relatively high activity
41 following the rewet, suggesting that the tolerance to prolonged desiccation is a common
42 trait across phylogenetically divergent microbes. The present study thus confirmed the
43 value of desiccated soils for detecting the composition difference of bacteria, and
44 provided evidence for strong adaptation of some bacteria to long-term desiccation and
45 extreme osmotic change.

Keywords: Long-term soil desiccation; Dry-rewet; 16S rRNA gene; Quantitative PCR; Amplicon sequencing; soil archives.

1 Introduction

Although frozen storage is widely accepted as the optimal method for preserving the chemical and biological components of soils, desiccation (i.e. air-drying) is more commonly used for large scale or cross-generational soil archives [1]. Desiccated soils have been frequently used for analyzing the chemical composition even after decades of storage [2-6], but the persistence of the biological components, especially the microbes in these soils remain largely uncharted. Microbes are famously known for tolerance to extreme environmental stresses, exemplified by growth of a few isolated strains after millions of years of dormancy under extremely low thermal conditions [7-10]. With respect to water stress, microbes have demonstrated strong resistance to long periods of drought based on both the cultivation of pure strains and the ecological investigation of samples from arid ecosystems [11, 12]. For instance, taxonomically diverse groups of bacteria are abundantly present and detectable in drylands and deserts, including members from phyla of *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* and *Firmicutes* [13-15], suggesting that the ability of adaptation to long periods of drought is not restricted to a few particular taxa [16]. Compared to natural arid soils, desiccated soils systematically archived in laboratory have endured persistent water depletion without intermittent rain precipitation as occurs in the field. The careful curation of the soils in the laboratory prevents the occurrence of other major environmental perturbations, such as vegetation and animal activities. Additionally, the air-dried soil

68 samples can provide information on drought tolerance of microbial communities that
69 originated from non-arid ecosystems. Therefore, the desiccated soils in the laboratory
70 might provide unique samples for studying microbial tolerance and adaptation to
71 persistent drought. However, due to the inaccessibility of such samples to most research
72 groups, the information on soil microbial tolerance to long-term laboratory desiccation
73 is limited.

74 A few studies on historical soil samples identified a diverse guild of soil microbes,
75 including bacteria, archaea and eukaryotes [17-22], and detected largely distinguished
76 patterns of soil bacterial composition in different soils. For instance, previous studies
77 revealed bacterial composition in manure-amended soil distinct from that in the soil
78 receiving only mineral fertilizer after laboratory air-drying and storage for 30-84 years,
79 and thus proposed that soil desiccation can protect some bacterial DNA and it is
80 possible to detect systematic differences in bacterial community composition between
81 soils even following decades of desiccation [18, 19]. However, less is known about the
82 viability of the bacteria under such adverse condition, because the DNA detected in
83 these desiccated soils might derive from already dead cells rather than still alive bacteria.
84 A medium growth study by counting colony forming units (CFU) after resuspending
85 soil sample in PBS solution reactivated the growth of several cultivable bacteria, mostly
86 from the phylum *Firmicutes* [19], but the vitality and reactivation potential of the
87 majority of uncultivable bacteria were not explored. A rewet event after a short-term
88 air-drying process usually relieves cells from osmotic pressure and allow regaining of
89 diffusive soluble nutrient substrates, leading to resuscitated growth of soil bacteria [23-

90 26]. We suspected that some of the bacteria with high tolerance to persistent desiccation
91 will be reactivated by the rewet after longer period of desiccation.

92 In the present study, we obtained 24 historical soil samples collected from across
93 Chinese mainland during the years of 1934-1939 from the Soil Archives at the Institute
94 of Soil Science, Chinese Academy of Sciences. By combined analyses of real-time
95 quantitative PCR (qPCR) and high-throughput sequencing targeting bacterial 16S
96 rRNA genes from desiccated soils and following a rewetting incubation, we aimed to
97 generate high resolution profiles of bacterial abundance, diversity and activity in these
98 soils, with predicted observations that (i) bacterial compositions in these desiccated
99 soils are still distinct and possibly site-specific, and (ii) some soil bacteria will be
100 resuscitated after the rewet, especially from the phylum of *Firmicutes*.

101 **2 Material and methods**

102 **2.1 Soil description and preparation**

103 Samples were collected from seven provinces across Chinese mainland during the
104 years of 1934-1939 (Fig. S1), and stored in air-dried conditions. The available records
105 by the collectors indicate widely spread field origins and/or properties for most of the
106 soils, including different locations, soil taxonomies and vegetation (Table 1). Soon after
107 each collection, a small part of the soil was air-dried and kept in a reservation box in
108 dry atmospheric condition as part of the soil archives at the Institute of Soil Science,
109 Chinese Academy of Sciences. The weight of the archive soil was about 15-50 g for
110 each sample. Due to the scarcity in quantity and historic values of these samples, we

111 obtained no more than 10 g of each soil for molecular analysis and rewetting incubation
112 and did not perform tests on the soil chemical and physical properties. Most soil
113 samples were granular and completely dried out by visual observation. The
114 measurement of moisture content from three of the soils (with adequate quantity after
115 molecular use) estimated < 1% gravimetric water content.

116 **2.2 Rewetting microcosm incubation**

117 All soils were subjected to rewetting microcosm incubations in triplicate, except
118 for S01, S05, S23 and S24, which were incubated in duplicate due to a low soil quantity
119 obtained. For each microcosm, 1 g of soils were added into sterilized 5-ml centrifuge
120 tubes and rewetted up to 30% soil weight with sterile water. The tubes were sealed and
121 incubated at 28°C for 28 days. Soil samples were destructively collected and frozen at
122 -80°C for molecular analyses.

123 **2.3 DNA extraction**

124 Both dry soils (with 2 replicates) and all rewetted soils (with 2-3 replicates) were
125 used for DNA extraction, resulting in a total of 116 DNA extract samples. Each DNA
126 sample was obtained from 1 g of soil, using FastDNA spin kit for DNA extraction (MP
127 Biomedicals, Cleveland, OH, USA), according to the manufacturer's instructions, and
128 dissolved in a final volume of 50 μl sterile water. The quantity and quality of DNA
129 extracts were measured using a NanoDrop ND-1000 UV-visible light
130 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

131 **2.4 Real-time quantitative PCR**

132 Real-time quantitative PCR (qPCR) was conducted on a CFX96 Optical Real-Time
133 detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) to determine the
134 abundance of 16S rRNA genes in the 116 DNA extracts, using universal PCR primers
135 515F (GTGCCAGCMGCCGCGG) and 907R (CCGTCAATTCMTTTRAGTTT) [27].
136 The thermal process started with 3 min of incubation at 95°C, followed by 40 cycles of
137 95°C for 30s, 55°C for 30s, and 72°C for 30s with plate read, and ended with a melt
138 curve from 65 to 95°C with increments of 0.5°C per 5s. The real-time quantitative PCR
139 standards were generated using plasmid DNA from one representative clone containing
140 16S rRNA genes, and a dilution series of standard template over eight orders of
141 magnitude per assay was used. Amplification efficiencies ranged from 96% to 105%,
142 with R^2 values of 0.992 to 0.996.

143 **2.5 High-throughput sequencing and analysis**

144 All 116 DNA extracts were subject to amplicon-based high-throughput sequencing
145 to investigate the bacterial community composition in soils. 16S rRNA gene fragments
146 were amplified using the same universal 515F-907R primer pair but with an adaptor, a
147 key sequence, and a unique tag sequence for each sample. The thermal conditions were
148 as follows: 5 min of incubation at 94°C, followed by 30 cycles of 94°C for 30s, 54°C
149 for 30s, and 72°C for 45s, with a 10 min extension at 72°C. The resulting PCR products
150 were gel purified and combined in equimolar ratios into single tubes before
151 pyrosequencing. The pyrosequencing was conducted on a Roche 454 GS FLX Titanium
152 sequencer (Roche Diagnostics Corporation, Branford, CT, USA).

153 All sequencing data were processed using multiple software packages. Reads from
154 each sample were first sorted by specific tag sequences using Mothur software [28].
155 Sequences were further trimmed, and only reads of > 350 bp in length with an average
156 quality score > 25 and no ambiguous base calls were included for subsequent analyses.
157 The operational taxonomic units (OTUs) were clustered at 97% similarity cutoff by
158 UPARSE algorithm after the removal of chimeras using QIIME version 1 [29]. A
159 representative sequence of each OTU was classified using the SILVA-132 16S rRNA
160 gene database (bootstrap confidence threshold of 80 %) in Mothur [28].

161 The raw sequencing data of all samples have been deposited in the European
162 Nucleotide Archive (ENA) with accession numbers ERR1520684 and ERR1520685.

163 **2.6 Statistical analyses**

164 The 16S rRNA amplicon sequencing data contained a small proportion of archaea
165 genes, therefore we calculated the total bacterial abundance as following: Bacterial 16S
166 rRNA gene copy number = (Total 16S rRNA gene copy number) × (Proportion of
167 Bacteria). The total 16S rRNA gene copy number was the quantification result from
168 qPCR as described above, and the proportion of *Bacteria* was estimated by high-
169 throughput sequencing analysis. Similarly, the 16S rRNA gene copy number of one
170 specific genus in a soil sample was calculated as following: 16S rRNA gene copy
171 number of genus X = (Total 16S rRNA gene copy number) × (Proportion of genus X).
172 The total 16S rRNA gene copy number was quantified by qPCR and the proportion of
173 genus X was generated by high-throughput sequencing analysis.

174 The bacterial 16S rRNA gene abundances were transformed to $\log_{10}$ values. The
175 effect of rewet on bacterial 16S rRNA gene copy number was then assessed by paired
176 samples t-test comparing $\log_{10}$-transformed gene copy numbers in the 24 soils
177 (calculated as mean value of replicates) before and after the rewet. The 16S rRNA gene
178 copy number of each genus before and after rewetting incubation was compared by t-
179 test. A genus is deemed as actively growing in a soil if the fold change of 16S rRNA
180 gene copy number was significantly larger than 1 ($p < 0.05$) after rewetting. The
181 statistical analyses were performed on Statistics 18.0 (SPSS, IL, USA).

182 Heatmaps were constructed as previously described in Hernández et al., [30] using
183 the vegan package in R. The samples and OTUs were clustered according to Euclidean
184 distances between all Hellinger transformed data.

185 **3 Results**

186 **3.1 Change in bacterial abundance**

187 The 28-day rewetting incubation led to a significant increase in bacterial abundance
188 in most soils based on paired samples t-test (n=24) (Fig. 1a). The total bacterial
189 abundances in soils were 1.9×10^{3} - 1.7×10^{8} gene copies g^{-1} dry soil in the desiccated
190 soils and reached 2.6×10^{3} - 4.1×10^{8} gene copies g^{-1} dry soil following the rewetting
191 incubation (Fig. S2). Specifically, bacterial abundance showed a significant increase in
192 18 soils ($P < 0.05$), remained unchanged in 5 soils and only in 1 soil (S21) declined
193 following the rewet. Notably, total bacterial abundance in two soils (S03 and S05)
194 increased by > 100-fold and in another seven soils (S04, S06, S08, S09, S12, S14 and

195 S16) increased by > 10-fold following the rewetting incubation (Fig. 1b).

196 **3.2 Change in bacterial composition**

197 Taxonomic classification of 16S rRNA genes revealed a broad spectrum of
198 bacterial taxa in soils. A total of 32 bacterial phyla were identified in soils. In the
199 desiccated soils, the most abundant bacterial phyla identified were *Firmicutes* (55.4%
200 of total bacteria), *Actinobacteria* (19.9%), *Proteobacteria* (9.7%), *Chloroflexi* (6.3%),
201 *Acidobacteria* (1.7%) and *Planctomycetes* (1.1%) (Fig. 2 and S3a). Following the
202 rewetting incubation, *Firmicutes* represented the most abundant phylum (80.4%),
203 followed by *Proteobacteria* (9.4%), *Actinobacteria* (3.5%), *Acidobacteria* (1.6%) and
204 *Chloroflexi* (1.0%) (Fig. 2 and S3b). The bacterial composition was distinct in different
205 soils (Fig. S4). Archaea were also detected in some of the soils but with much lower
206 proportions than bacteria, and *Nitrososphaeria*, *Halobacteria* and *Methanomicrobia*
207 represented the most abundant archaeal classes identified in both desiccated and
208 rewetted soils (Fig. S5).

209 **3.3 Identification of active bacteria**

210 At the genus level, a total of 759 taxa were identified in all soils, and about 24.1%
211 (183) of them showed a significant increase in 16S rRNA gene copy number in at least
212 one soil following the rewet. Fig. 3 shows the top 21 genera with significant increase
213 of 16S rRNA gene copy number in more than three of the soils. Among all the detected
214 genera, *Paenibacillus*, *Cohnella* and two other unclassified *Bacillales* genera showed
215 increases in at least half of the soils following rewetting incubations (Fig. 3),

216 representing the most frequently activated genera in these historical soils. On the other
217 hand, *Bacillales* genera *Tumebacillus*, *Alicyclobacillus* and *Brevibacillus* showed the
218 highest fold-changes following rewetting, with an average >1,000-fold increase in
219 abundance in soils (Fig. 3). Although *Bacillales* comprised the most active bacterial
220 communities in our soils, other bacterial taxa, represented by one unclassified
221 *Actinobacteria* genus, two *Gammaproteobacteria* genera (*Halomonas* and *Ralstonia*)
222 and four genera belonging to *Firmicutes* order *Clostridia*, also showed marked growth
223 after rewetting (Fig. 3). No archaeal genera were identified to be actively growing in
224 soils.

225 **4 Discussion**

226 Our results demonstrated that the soils still contained measurable 16S rRNA gene
227 abundance after decades of desiccation, ranging between 10^3 to 10^8 gene copies g^{-1} dry
228 soil (Fig. 1a). As expected, this range was lower than that observed in fresh soils usually
229 containing 10^7-10^9 bacterial cells g^{-1} soil [31]. It is worth mentioning that the standard
230 template used for gene quantification were generated from a reference organism, which
231 potentially induced a biased estimation due to different amplification efficiencies of
232 target and the standard DNA templates. Therefore, the 16S rRNA gene copy numbers
233 in soils could be over- or underestimated in this study. Nevertheless, the magnitude of
234 difference in gene copy numbers (up to 5 orders of magnitude) across different soils
235 should still exist irrespective of the standard used. We cannot rule out the possibility
236 that a portion of the detected 16S rRNA genes in the dry soils were residues from dead
237 cells, i.e. extracellular DNA [32, 33]. The extracellular DNA could dominate the gene

238 abundance in some soils, become degraded and accessible for the living cells following
239 rewetting [34], which might explain the decreased total bacterial abundance in one of
240 the soils (S21) after the incubation.

241 Phylogenetically diverse guilds of bacteria were identified in the dry soils,
242 demonstrating high resistance of soil bacteria to long-term desiccation. Driven by the
243 stress from both water and nutrient deprivation for more than seven decades, the
244 bacteria detected in the dry soils were most likely in dormant/resting state [35]. This
245 was supported by the highest proportion of *Firmicutes* in most of the desiccated soils
246 (Fig. 2), which consisted of *Paenibacillus*, *Cohnella, Bacillus* and *Clostridium* species
247 (Fig. S3a) that are well known for producing resting bodies of endospores. In addition,
248 in many soils, the frequent presence of taxa from *Actinobacteria*, *Proteobacteria* and
249 *Chloroflexi* (Fig. S3a), which are less known for tolerance to extreme drought stress,
250 implies other cell mechanisms to cope with long-term desiccation across non-
251 *Firmicutes* microbes as previously proposed [16, 36]. This emphasizes the importance
252 of future study on the physiological adaptations to environment adversity of the vast
253 uncultured diversity of soil microbes.

254 Our study predicted different bacterial compositions in the desiccated soils
255 collected from geographically distant locations across China. Although all soil samples
256 in the present study have been processed by the same laboratory treatment (long-term
257 air-dry and storage in the same room conditions since 1939), the bacterial community
258 of each sample was distinct (Fig. S4). No apparent association of bacterial community
259 composition with soil type or land use were observed in these soils at the national scale,

260 but a Euclidean distance clustering indeed revealed bacterial compositions highly
261 resembled in some local soils with similar background. Markedly, soil samples #13-16
262 were collected from Fujian province and the sampling sites were in proximity to each
263 other. These four soils also had the same soil type (all classified as red Fe stagnic
264 anthrosols) and a history of rice cultivation (Table 1). The bacterial compositions in
265 these four soils closely clustered with each other even after decades of desiccation, and
266 were different from those in other soils (Fig. S3A). This showed a long-lasting impact
267 of field history on shaping soil bacterial composition. Additionally, some niche specific
268 microbes were identified and tightly linked to the land type. For example, although
269 archaea were only detected at low frequency in soils, the class *Halobacteria* known for
270 salt tolerance was exclusively detected in the two coastal saline soils (S22 and S23) in
271 our study, accounting for >99.4% of the archaeal gene sequences (Fig. S5). Collectively,
272 our results support a previous proposal that the desiccated soil can preserve genetic
273 information of indigenous microbes (either as intact cell component or dead cell
274 residue), and can be used to detect systematic bacterial composition difference in these
275 historical soils [18].

276 Some of the detected genes belonged to living bacterial cells, based on the
277 increased 16S rRNA gene copy numbers of some bacterial taxa after the rewetting
278 incubation of the soils. The increased gene copy numbers of several genera, including
279 *Paenibacillus* and *Cohnella*, were observed in ≥12 of the soils after rewetting (Fig. 3).
280 Genera *Tumebacillus*, *Alicyclobacillus* and *Brevibacillus* showed the highest activity
281 (with gene copy number >1000-fold increase) after rewetting. All these genera

282 belonged to order *Bacillales* within the phylum of *Firmicutes*. These endospore-
283 forming bacteria thus represented the majority of the living microbes and showed the
284 highest resuscitation potentials in these soils. Culture-based studies on a desert soil
285 revealed growth of *Firmicutes* cells in cultivation medium, despite their low abundance
286 in the soil [13]. Similarly, *Firmicutes* in many of our soils (S01, S03, S06, S09, S12 and
287 S23) were not the most abundant phylum under desiccated conditions, but only became
288 dominant after the rewetting incubation (Fig. 2). These results suggested that the
289 formation of endospores is the most efficient mechanism for population recovery from
290 resting state when the environmental stress is relieved. Additionally, two genera within
291 the *Gammaproteobacteria*, i.e. *Halomonas* and *Ralstonia*, also showed marked growth
292 after the rewet (Fig. 3). Although members of *Halomonas* have not been reported to
293 have strong resilience to extreme drought-rewet occurrences, they are halotolerant and
294 capable of producing ectoine as osmolytes to maintain cell-osmotic balance [37]. The
295 ectoine can protect the cells against salinity, desiccation and high temperature [38],
296 which might facilitate cell survival in our soils following extreme moisture changes.
297 Similarly, the disaccharide trehalose contributes to osmotic stress tolerance of *Ralstonia*
298 species [39], which might have implications for resilience to water stress. However, we
299 cannot rule out other mechanisms for such high tolerance to osmotic changes in these
300 two Gammaproteobacterial genera, and future investigation is encouraged to test our
301 speculations.

302 It is noteworthy that *Actinobacteria* represent the second-most abundant group in
303 desiccated soils (Fig. 2), suggesting high resistance to long-term desiccation as

304 previously documented [13-15]. However, the increase in *Actinobacteria* 16S rRNA
305 gene copy number appears much lower than *Firmicutes* and *Proteobacteria*, leading to
306 sharp declines in average proportion in many of the soils after rewetting (Fig. 2).
307 Similar trends were also found in a short period of a dry-rewet experiment, where the
308 proportion of *Actinobacteria* increased after desiccation but then decreased following
309 a rewet [24, 40]. It is also possible that the activity of *Actinobacteria* species might be
310 stimulated at the early stage of the 28-day incubation and outcompeted later by the
311 stronger growth of *Firmicutes* and *Proteobacteria.* A previous study defined
312 *Actinobacteria* as rapid responders with fast reactivation within one hour following
313 rewetting, whereas *Firmicutes* and many *Proteobacteria* classes (*Alpha-*, *Beta-* and
314 *Gamma-proteobacteria*) were consistently delayed responders [41]. This can be
315 assessed by monitoring temporal changes in relative abundance of different phyla or
316 classes in soils following rewetting in future studies.

317 **5 Conclusions**

318 The present study revealed a diversified guild of bacteria resistant to seven decades
319 of desiccation in 24 historical soils and 24.1% of the taxa (at the genus level) still show
320 the ability to grow. A dissimilar responses of different phylotypes to the rewetting were
321 observed, confirming a previous study suggesting the resuscitation strategy of soil
322 microbes may be a phylogenetically conserved ecological trait [41]. Considering long-
323 term desiccation of microbes and the fact that only water was added for incubation in
324 our study, we expect growth of more microbes with the additional supply of nutrients,
325 such as carbon and nitrogen. Moreover, the activity of microbes was assessed by only

326 DNA-based molecular analyses, which cannot capture all the active microbes in the
327 soils, especially for those that did not quickly propagate within our incubation time. In
328 addition, in future studies microbial viability would be more accurately depicted using
329 more sensitive molecular tools, e.g. rRNA molecules rather than genes as a better
330 indicator for cell activity [42]. Other methods would also be of great potential in
331 distinguishing growing, dormant and dead cells, e. g. isotope tracing [25, 43-45] and
332 single-cell based imaging [46, 47]. The removal of extracellular nucleic acids should
333 also be considered in the future study to validate our conclusions [32]. Finally, future
334 studies targeting specific microbial groups could enhance our understanding of some
335 important functional microbes and their response to extreme stress [48]. By adopting
336 more sensitive techniques, the desiccated historical soils could serve as precious
337 "fossils" for soil microbiologists to discover tenacious species and retrieve
338 undocumented site-specific historical events.

339 **Declaration of competing interest**

340 The authors declare that they have no known competing financial interests or personal
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493 **Figure captions:**

494 **Figure 1.** (a) Box-plot of bacterial gene copy numbers (log_{10} transformed) in desiccated
495 soils (Dry) and after a rewetting soil incubation (Rewet). Centre lines show median
496 values and boxes show upper and lower quartiles. Paired samples t-tests were used to
497 test for differences in bacterial gene abundance in soils and *p* values are shown between
498 paired dry and rewetted soils. Each data point (dot) represents the mean value of gene
499 copy number in either dry or rewetted soils (n=24 for both conditions). (b) The fold
500 change of bacterial gene copy numbers following the rewetting incubation compared to
501 the desiccated soils. Open black circles indicates a fold change significantly greater
502 than 1 ($p < 0.05$), solid green circles represent no significant difference from 1 ($p >$
503 0.05), and solid red circles indicate a change significantly lower than 1 ($P < 0.05$).

504 **Figure 2.** Composition of the bacterial communities in soils under desiccated
505 conditions (Dry) and after the rewetting incubation (Rewet). The bacterial composition
506 was plotted at the phylum level and the most abundant phyla (with relative
507 abundance >1% on average) are shown. Taxa not seen more than three times in at least
508 20% of the samples were removed using the phyloseq package in *R*. The relative
509 abundances of minor phyla or classes were summed and shown as “others”, which do
510 not exceed 7.5% of total bacterial reads in any given soil.

511 **Figure 3.** Heatmap showing significant fold change ($p < 0.05$) in gene abundance of
512 bacterial taxa in different soils following the 28-day rewetting incubation. A total of 21
513 taxa at the genus level are listed that display growth in > 3 soils. Each taxon designation

514 contains the phylum (p), class (c) and genus (g) affiliation. Whenever a genus
515 designation is not defined (as unclassified genus), a defined family (f) or order (o)
516 affiliation is specified instead. The values in the column "Average" indicate the average
517 fold change in abundance of each taxon in soils following rewetting. Blank (white)
518 squares indicate no significant increase in abundance in a soil after rewetting ($p > 0.05$).
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520 **Table 1. The documented information of archive soils**

Sample No.	Series No.	Collecting date	Collector(s)	Province	City/ County	Land use	Vegetation	Soil genetic classification	Soil parent materials	Soil taxonomy
S01	5151	1934/10/9	Po Suo, Guangjiong Hou	Qinghai	Dulan	livestock farming	-	calcareous clay	-	uap-ustic isohumisols
S02	5213	1935/12/24	Lianqing Zhu et. al	Sichuan	Huayang	dry land	-	brown soil	granite	hapli-udic argosols
S03	5009	1939/11/15	Lianqing Zhu, Fanqi Zeng	Yunnan	Mengzi	farmland	rice	red soil	-	Fe-leachi-stagnic anthrosols
S04	5157	1934/11/13	Lianjie Li et. al	Guangxi	Yongning	wasteland	miscellaneous tree e.g. Chinese fir, pine	red soil	sandstone	hap-udic ferrisols
S05	HN	1935/1/1	Changyun Zhou	Hunan	Changsha	-	-	red soil	-	-
S06	5001	1934/11/13	Guangjiong Hou	Jiangxi	Nanchang	-	-	red soil	-	hap-udic ferrisols
S07	5289	1938/5/24	Daquan Song et. al	Fujian	Jianyang	vegetable garden	vegetables	fluvisols	river alluvium	acidic humid alluvial entisols
S08	5143	1938/10/1	Daquan Song et. al	Fujian	Longyan	wasteland	-	lime soil	limestone	black lithologic isohumisols
S09	5277	1938/5/24	Daquan Song et. al	Fujian	Chongan	tea orchard	tea	purple soil	calcareous purple shale	calcareous purpli-udic cambosols
S10	5061	1938/12/1	Daquan Song et. al	Fujian	Jianyang	wasteland	-	podzol	-	typic spodosol
S11	5062	1938/12/1	Daquan Song et. al	Fujian	Dehua	wasteland	grass	podzol	volcanic rock	typic spodosol
S12	5275	1938/5/3	Daquan Song et. al	Fujian	Chongan	wasteland	weeds	purple soil	purple sandshale	purpli-udic cambosols
S13	5030	1938/10/23	Daquan Song, Zhenyu Yu	Fujian	Longxi	farmland	rice	red soil	river alluvium	typic Fe-accumuli-stagnic anthrosols
S14	5031	1938/10/23	Daquan Song et. al	Fujian	Jianou	farmland	rice	red soil	river alluvium	typic Fe-accumuli-stagnic anthrosols
S15	5032	1938/12/23	Daquan Song et. al	Fujian	Longxi	farmland	rice	red soil	-	typic Fe-accumuli-stagnic anthrosols
S16	5033	1938/ 12/23	Daquan Song et. al	Fujian	Longxi	farmland	rice	red soil	-	Fe-accumuli-stagnic anthrosols
S17	5171	1938/8/5	Daquan Song et. al	Fujian	Yongchun	wasteland	shrubs and weeds	red soil	white effusive rock	hap-udic ferrisols
S18	5172	1938/5/5	Daquan Song et. al	Fujian	Yongchun	wasteland	evergreen broad-leaved trees	red soil	quartzite and sandstone	humic udic ferrisols
S19	5174	1938/5/5	Daquan Song et. al	Fujian	Yongchun	wasteland	evergreen broad-leaved	red soil	quartzite	hap-udic ferrisols
S20	5274	1938/10/3	Changyun Zhou et. al	Fujian	Yongchun	wasteland	grass	acidic residual soil	granite	acidic ochri-aquic cambosols
S21	5278	1938/5/24	Changyun Zhou et. al	Fujian	Yongan	wasteland	-	red soil	sandy lake sediment	semi-humic organic soil
S22	5119	1938/10/22	Chengfan Xi et. al	Fujian	Putian	wasteland	natural vegetation	coastal saline soil	sea mudalluviu m	halogenic soil
S23	5120	1938/10/22	Chengfan Xi et. al	Fujian	Putian	wasteland	natural vegetation	coastal saline soil	-	halogenic soil
S24	5173	1938/5/5	Daquan Song et. al	Fujian	Zhangping	wasteland	weeds	yellow soil	granite	humic udic ferrisols

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