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Deciphering the biotic and abiotic drivers of coalescence asymmetry between soil and manure microbiomes

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1 **Deciphering the biotic and abiotic drivers of coalescence asymmetry between soil**
2 **and manure microbiomes**

3

4 **Authors:**

5 Chunkai Li ^{a,b}, Xianping Li ^a, Sana Romdhane ^c, Yanhong Cheng ^d, Gen Li ^a, Rui Cao
6 ^a, Peng Li ^e, Jingjing Xu ^a, Yexin Zhao ^a, Yang Yang ^a, Jiaguo Jiao ^a, Feng Hu ^a, Jun Wu
7 ^a, Huixin Li ^{a,f,*}, Laurent Philippot ^c

8

9 **Affiliations:**

10 ^a College of Resources and Environmental Sciences, Nanjing Agricultural University,
11 Weigang, Nanjing, Jiangsu, China, 210095.

12 ^b College of Chemical Engineering, Nanjing Forestry University, No. 159, Longpan
13 Road, Nanjing, Jiangsu, China, 210037.

14 ^c Université Bourgogne Franche-Comté, INRAE, Institut Agro Dijon, Department of
15 Agroécologie, Dijon, France, 21000.

16 ^d Key Laboratory of Red Soil Cultivated Land Conservation, Jiangxi Institute of Red
17 Soil and Germplasm Resource, Nanchang, Jiangxi, China, 331717.

18 ^e Key Laboratory of Vector Biology and Pathogen Control of Zhejiang Province,
19 College of Life Sciences, Huzhou University, Huzhou, China, 313000.

20 ^f Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization,
21 Weigang, Nanjing, China, 210014.

22

23 * **Corresponding author:**

24 Dr. Huixin Li

25 Tel.: +86-025-84395210; Fax: +86-025-84395210; E-mail: huixinli@njau.edu.cn

26

27 **E-mail addresses:**

28 Chunkai Li: chunkaili217@njfu.edu.cn

29 Xianping Li: xianpingli@njau.edu.cn

30 Sana Romdhane: Sana.Romdhane@inrae.fr

31 Yanhong Cheng: yanhongch007@163.com

32 Gen Li: T2021113@njau.edu.cn

33 Rui Cao: 2020103010@stu.njau.edu.cn

34 Peng Li: lp152690@163.com

35 Jingjing Xu: xujingjing@njau.edu.cn

36 Yexin Zhao: zhaoyexin@njau.edu.cn

37 Yang Yang: yyang0335@njau.edu.cn

38 Jiaguo Jiao: jiaguojiao@njau.edu.cn

39 Feng Hu: fenghu@njau.edu.cn

40 Jun Wu: wujun2013@njau.edu.cn

41 Huixin Li: huixinli@njau.edu.cn

42 Laurent Philippot: laurent.philippot@inrae.fr

43

44 **Keywords**

45 Organic fertilization; Bacteria; Fungi; Environmental filtering; Biotic interactions

46

47 **Abstract**

48 Manure application improves soil fertility, yet its implications for the invasion success
49 of manure-borne microorganisms into the soil are poorly understood. Here, we assessed
50 the importance of abiotic and biotic factors in modulating the extent to which manure-
51 borne fungal and bacterial communities can invade resident soil microbial communities.
52 For this purpose, we applied different manure treatments over 180 days and monitored
53 changes in bacterial and fungal communities. Two different amounts of manure were
54 applied at varying frequencies to nine soils differing in their physico-chemical
55 properties as well as in land use history. Variance partitioning revealed the differential
56 contributions of abiotic and biotic factors to invasion success, that together accounted
57 for up to 82% of the variance explained. We showed that the interaction effects between
58 biotic and abiotic factors increased with coalescence frequency and with manure
59 amount for the bacterial and fungal community, respectively. Both abiotic and biotic
60 factors were important in modulating coalescence asymmetry for the bacterial
61 community, while abiotic factors had a greater effect on the fungal community. Our
62 results provide new insights into the drivers of coalescence events between manure and
63 resident soil microbial communities. Moreover, our findings highlight the roles of the
64 mixing ratio and frequency of coalescence events in modulating manure-borne
65 microorganism survival.

66

67 **1. Introduction**

68 Manure is increasingly used as a substitute for mineral fertilizers as an environmentally
69 friendly alternative towards sustainable agriculture (Bender et al., 2016; Tilman et al.,
70 2002). The estimated manure production worldwide reached 127.6 Tg N yr⁻¹ in 2019,
71 with more than one-fifth of the produced manure applied to soil (FAO, 2019). Manure
72 application not only provides a valuable source of nutrients required for plant growth,
73 as it contains nitrogen, phosphorus, and potassium (Maillard and Angers, 2014; Hazra,
74 2016), but also improves soil physical properties such as soil aggregate stability and
75 soil porosity (Karami et al., 2012; Tripathi et al., 2014). Moreover, manure application
76 can reduce carbon losses and increase soil carbon sequestration, thereby mitigating the
77 impacts of climate change (Gattinger et al., 2012). As such, the application of manure
78 to agricultural soils could contribute to achieving various targets of the Sustainable
79 Development Goals of the United Nations (Bernstein, 2017).

80 The impact of manure application on soil microbial communities has been
81 well documented using both field and microcosm experiments (Chen et al., 2017;
82 Hartmann et al., 2015). For example, Hartmann et al. (2015) showed that manure
83 application altered soil microbial community composition, richness, and evenness.
84 However, these effects of manure applications are the net consequences of both shifts
85 in native soil communities and invasion by manure-borne microorganisms. Thus,
86 manure application, by supplying valuable nutrients, can significantly increase the
87 abundance of several soil copiotrophic taxa that exhibit optimal growth at high nutrient
88 concentrations, such as the Proteobacteria (Fierer et al., 2007; Zhang et al., 2017). A

89 few studies also reported that some bacterial taxa in manure can survive in the soil for
90 several months (Johansson et al., 2005; Lourenco et al., 2018). Most invasion ecology
91 studies have focused on plants and to a lesser extent on animals (Alp et al., 2016; Li et
92 al., 2022), and therefore knowledge of the factors contributing to the invasion success
93 of microorganisms in soil is scarce (Litchman, 2010). Moreover, whereas manure
94 application exposes resident soil microbial communities to multiple microbial invaders,
95 previous studies have examined soil invasion by only a single microbial species (Pettay
96 et al., 2015; Van Elsas et al., 2012).

97 There are key challenges in studying and understanding the effect of manure
98 application on soil microbial communities, as both biotic and abiotic components of
99 soil and manure can influence the invasion success of manure-borne microorganisms.
100 For example, Elton's diversity-invasibility hypothesis states that diversity of the native
101 communities confers resistance to invasion by reducing resources availability for newly
102 arriving species (Elton, 1958). Accordingly, Van Elsas et al (2012) found a negative
103 correlation between the diversity of the soil microbial community and the survival of
104 the bacterial invader. Previous studies also showed that the survival of bacterial invader
105 can also be negatively affected by its phylogenetic relatedness to the native
106 communities, because more closely related species exhibit higher niche overlap, as
107 suggested by Darwin's naturalization hypothesis (Darwin et al., 1895; Tan et al., 2015).
108 The addition of nutrients through manure fertilization also modifies soil properties,
109 which can not only affect the native soil microbial community but also the survival of
110 manure-borne microorganisms (Lourenco et al., 2018; Sun et al. 2016). Thus, resource

111 pulses due to manure application can abiotically improve invasion success by providing
112 additional niches and disrupting the resident community through habitat disturbance
113 (Ma et al., 2015; Mallon et al., 2015a). Consequently, differential responses of
114 microbial communities to manure fertilization according to the soil properties and to
115 the manure fertilization regimes have previously reported in several studies (Ren et al.
116 2019, Pérez-Valera et al. 2019; Feng et al. 2022, Sadet-Bourgeteau et al., 2019), but
117 very few have provided a clear understanding of the underlying factors.

118 Recently, the concept of coalescence has been used to describe such
119 encounter of previously separate microbial communities and their habitats to better
120 understand and predict the resulting microbial assemblages (Huet et al., 2023;
121 Ramoneda et al., 2021; Rillig et al., 2015). As proposed by Rillig et al. (2016), manure
122 application to soil results in asymmetric coalescence, with the soil microbial
123 community overrepresented compared to the manure-borne microbial community.
124 Little is known about the relative importance of biotic and abiotic factors in modulating
125 the outcome of coalescence between soil- and manure-borne microorganisms. This is
126 even more important as manure is a reservoir of both pathogenic and antibiotic-resistant
127 bacteria (Udikovic-Kolic et al., 2014).

128 Here, our main objective was to investigate the factors influencing
129 coalescence asymmetry between soil- and manure-borne microbial communities. For
130 this purpose, we used a comprehensive approach to explicitly quantify the importance
131 of the soil abiotic and biotic factors during coalescence events involving two different
132 amounts of manure added at varying frequencies and nine soils differing in their

133 physico-chemical properties as well as land use history. To avoid transient effects of
134 manure application on microbial communities, we monitored changes in bacterial and
135 fungal communities after 60 and 180 days. We hypothesized that the relative
136 contributions of biotic and abiotic soil factors to the outcome of coalescence events
137 between native soil microbial communities and invading manure microbial
138 communities would be modulated by the mixing ratio (i.e., manure amount) and by the
139 frequency of coalescence between soil and manure. Specifically, we hypothesized that
140 the importance of biotic factors should increase when the frequency of coalescence
141 events is higher as repeated manure application may generate a legacy effect that
142 influences future invasion attempts (Mallon et al., 2018). To test our hypothesis, we
143 analyzed manure and soil physico-chemical properties and sequenced both bacterial
144 and fungal communities. Our findings indicate that manure application treatment can
145 have a major impact on the outcome of coalescence events between complex microbial
146 communities and that the interaction effect between the abiotic and biotic soil properties
147 increases with the amount of manure added.

148

149 **2. Materials and methods**

150 *2.1 Soil sampling*

151 Soil samples were collected in 2018 from five agricultural fields and four adjacent
152 uncultivated grasslands across China and classified as black soil, fluvo-aquic soil,
153 desert saline soil, red soil, or coastal saline soil. At each site, samples were collected
154 from the uppermost 20 cm of the soil by the S-shaped sampling method in 50-m interval

155 and immediately sieved (< 5 mm). The details of the sampling sites and soil physico-
156 chemical properties are indicated in Table S1.

157

158 *2.2 Laboratory-controlled mesocosm experimental design*

159 Soil mesocosms were established by filling PVC containers (35 cm length × 25 cm
160 width × 25 cm depth) with 5 kg sieved soil samples following a completely randomized
161 design. The experiment was conducted in triplicate with five manure application
162 regimes: nonamended soil mesocosms used as controls (CS); 6.60 g kg⁻¹ manure added
163 in one application at day 0 (1M1) or in three equal applications at days 0, 60 and 120
164 (1M3; 2.20 g kg⁻¹ manure each time); or 19.80 g kg⁻¹ manure added in one application
165 at day 0 (3M1) or in three equal applications at days 0, 60 and 120 (3M3; 6.60 g kg⁻¹
166 manure each time). The manure was added by mixing it with soil, and the amounts of
167 manure added were based on traditional fertilization regimes in China, which ranged
168 between 15 and 45 t ha⁻¹ (Li et al., 2009). The manure was a thermal-composted cattle
169 dung, which was provided by the Nanjing Institute of Vegetable and Flower Sciences,
170 China. The manure contained 205.14 g kg⁻¹ total organic carbon (C), 14.25 g kg⁻¹ total
171 nitrogen (N), 17.02 g kg⁻¹ total phosphorus (P), 27.04 g kg⁻¹ total potassium (K), 293.28
172 mg kg⁻¹ ammonium nitrogen (NH₄⁺-N) and 31.43 mg kg⁻¹ nitrate nitrogen (NO₃⁻-N)
173 and had a pH of 7.4 and an electrical conductivity (EC) of 2.09 ms cm⁻¹. All mesocosms
174 were then incubated at 25°C under sterile conditions and maintained at 60% of their
175 water holding capacity in the dark for 180 days. All mesocosms were non-destructively
176 sampled to a depth of 20 cm using a ø 5-cm soil corer after 60 and 180 days (i.e., 60

177 days after the first and last fertilization treatment) to avoid transient effects of manure
178 application on microbial communities. Each soil sample as well as the manure sample
179 were split into two subsamples. The first subsample was stored at 4°C and used to
180 determine soil physico-chemical properties, and the second was stored at -80°C and
181 used for soil DNA extraction.

182

183 *2.3 Soil and manure physico-chemical analyses*

184 pH and EC were measured using a digital pH meter. Total C and total N were measured
185 by combustion using a Sercon SL C/N elemental analyser. Total P and total K were
186 quantified by the molybdenum blue method and by using a flame photometer,
187 respectively (Jackson, 1973). NH_4^+ -N and NO_3^- -N were measured using a continuous-
188 flow stream autoanalyzer (SEAL-AA3, Norderstedt, Germany). Soil basal respiration
189 was estimated by determining the CO_2 release using gas chromatography after soil
190 incubation (equivalent to 5 g dry soil) at 25°C for 12 h. Soil texture was assessed
191 according to the protocol provided by Gee and Bauder (1986). Soil microbial biomass
192 C, N, and P were determined using the chloroform fumigation extraction method
193 (Brookes et al., 1982; Brookes et al., 1985; Vance et al., 1987).

194

195 *2.4 DNA extraction, PCR amplification and sequencing*

196 DNA was extracted from 250 mg of soil or of manure from the 273 collected samples
197 using the E.Z.N.A.[®] Soil DNA Isolation Kit (Omega Bio-Tek, Inc., Norcross, GA, USA)
198 according to the manufacturer's instructions. Amplicon libraries of all 273 DNA

199 extracts were generated by a two-step PCR approach. The 314F-806R (Klindworth et
200 al., 2013) and ITS1F-ITS2R (Bellemain et al., 2010) primer sets were used to assess
201 the bacterial and fungal communities, respectively. In the first PCR step, targets were
202 amplified using primers modified with adaptors (forward: 5'-
203 AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTC
204 CGATCT, reverse: 5'-
205 CAAGCAGAAGACGGCATAACGAGATCGGTCTCGGCATTCCTGCTGAACCGCT
206 CTTCCGATCT). In the second PCR step, PCR amplification added multiplexing index
207 sequences to the overhang adaptors using a unique multiplex primer pair (provided by
208 Biozeron Co. Ltd., Shanghai, China) combination for each sample. The 20 μ L volume
209 PCR system contained 0.4 μ L of DNA polymerase (TransStart[®] FastPfu, Nanterre,
210 France), 0.2 μ M each primer, 10 ng of DNA extract, 0.25 mM dNTPs, and FastPfu
211 Buffer 1 \times (TransStart[®], Nanterre, France). Thermal cycling conditions consisted of a
212 denaturing step of 95°C for 5 min, followed by 27 cycles of 95°C for 30 s, 55°C for 30
213 s, and 72°C for 45 s, and a final step of 72°C for 10 min. PCR assays were carried out
214 using the GeneAmp[®] 9700 platform (ThermoFisher Scientific, Waltham, MA, USA).
215 All PCR products were purified with the E.Z.N.A.[®] Reagent (Omega Bio-Tek, Inc.,
216 Norcross, GA, USA) and pooled in equimolar concentrations. Amplicon sequencing
217 (2 \times 250 bp) was performed on an Illumina HiSeq PE 2500 platform with a sequencing
218 depth of 30,000 reads per sample. Metagenomic shotgun sequencing on the manure
219 samples was performed on an Illumina Novaseq PE150 platform with a sequencing
220 depth of 30 Gbp. Detailed bioinformatics analyses of the 16S and ITS rRNA gene

221 sequences, as well as the metagenomic analysis, are described in Supplemental
222 Methods.

223 16S rRNA gene sequences have been deposited in the NCBI SRA database
224 under the BioProject PRJNA784282. ITS rRNA gene sequences have been deposited
225 in the NCBI SRA database under the BioProject PRJNA784364. Metagenome-
226 assemble genome sequences have been deposited in the NCBI SRA database under the
227 BioProject PRJNA868803.

228 *2.5 Statistical analysis*

229 Statistical analyses were conducted using R statistical software version 3.6.1 (R Core
230 Team, 2019). Principal coordinates analysis was performed to evaluate the differences
231 in microbial taxonomic composition based on the Bray–Curtis dissimilarity matrix. The
232 effect of soil type, land use history, manure application amount and frequency were
233 tested using a multiple–way permutational multivariate analysis of variance
234 (PERMANOVA) with 999 permutations using the *vegan* R package (Anderson, 2001).
235 Differences in microbial diversity indices, proportion of manure-borne microorganisms,
236 and Mean Nearest Taxon Distance (MNTD) between microbial communities in the
237 manure treatments and those in the added manure across soils were tested by ANOVA
238 followed by Tukey's honestly significant difference (HSD) test. Spearman's rank
239 correlation was calculated to assess the relationships between the proportion of manure-
240 borne microorganisms in the different treatments after 180 days of incubation and each
241 biodiversity index estimated in the control soils. All *P* values were subsequently
242 adjusted by false-discovery rate (FDR) (Benjamini and Yekutieli, 2001). Normality and

243 homogeneity of the residuals were tested, and log-transformations were performed
244 when necessary.

245 To estimate the proportion of soil- and manure-borne bacteria and fungi in
246 the coalesced community at days 60 and 180, the Bayesian algorithm-based program
247 SourceTracker (version 0.9.1) was used with default parameters (Knights et al., 2011).
248 Using mineral fertilization treatments (performed in parallel to the manure treatments
249 on the same soils; data not shown), as negative controls for the source tracking analysis,
250 we found average false positive rates of 0.08% and 0.01% for the bacterial and fungal
251 community, respectively. Differential abundance analysis was performed between each
252 treatment and the control soil after 180 days of incubation using the negative binomial
253 generalized linear model in the *DESeq2* R package, with FDR adjusted *P* values < 0.01
254 (Love et al., 2014). As zero counts in sequencing datasets may inflate the number of
255 false positives, low-abundance operational taxonomic units (OTUs) among manure
256 treatments were filtered out before DESeq2 analysis, keeping OTUs representing over
257 0.05% (Romdhane et al., 2022; Huet et al., 2023). Maximum likelihood-based
258 phylogenetic trees of significantly increased OTUs relative to the control soils were
259 built using the GTR model with default parameters in FastTree (version 2.1.11) (Price
260 et al., 2010) and visualized using Interactive Tree of Life (iTOL) (Letunic and Bork,
261 2007). Metagenome-assemble genomes corresponding to the OTUs that were
262 significantly increased in most soils after manure addition were identified using the
263 BLASTn algorithm.

264 To determine the drivers of both bacterial and fungal community coalescence

265 after 180 days of incubation, variance partitioning was performed based on partial
266 regression analysis. We used the estimated proportion of manure-borne bacteria or fungi
267 as a proxy for the outcome of community coalescence. Before analysis, all predictors
268 were standardized to Z scores, with a mean of 0 and a standard deviation of 1 (Gelman,
269 2009). To limit the potential problems caused by multicollinearity and overfitting on
270 model performance, the numbers of abiotic (i.e., climate conditions – mean annual
271 temperature and precipitation as well as precipitation seasonality, soil textural
272 composition, differences in pH, in EC, in C:N ratio, and in nutrients between the control
273 soil and the added manure) and biotic (i.e., soil microbial basal respiration, biomass,
274 diversity indices and the phylogenetic relatedness between microbial communities in
275 the control soil and the added manure) variables were reduced by principal components
276 analysis (PCA), respectively (Jolliffe and Cadima, 2016). A fivefold cross-validated
277 elastic net regression model with 999 replicates was then conducted to assess the pure
278 and interacting effects of the abiotic and biotic factors (Hans, 2011).

279

280 **3. Results**

281 *3.1 Changes in soil microbial communities under different manure treatments*

282 We identified 77,038 OTUs assigned to 42 phyla for bacterial communities and 8,413
283 OTUs assigned to 7 phyla for fungal communities. After 180 days, the bacterial
284 communities in the manure-amended soils were dominated by Proteobacteria (35.66%)
285 and Actinobacteria (18.58%), while the dominant phylum in the fungal community was
286 Ascomycota (75.81%) (Fig. 1A and 1B). In contrast, Chloroflexi (34.28%) and

287 Actinobacteria (29.69%) were the main bacterial phyla in the manure, while the fungal
288 community was also dominated by Ascomycota (85.35%) (Figs. 1C). The amount or
289 frequency of manure application had weak or no effects on bacterial diversity but all
290 indices were significantly affected by the interaction among soil type \times amount \times
291 frequency of manure application (Table S2). Thus, most bacterial diversity indices were
292 higher in the manure treatments for the desert saline soils, the red soils, and the coastal
293 saline soils (Tukey's HSD test, $P < 0.05$). For example, bacterial richness increased
294 from 9.61% to 27.43% across soils after manure applications (Fig. S1). In contrast, a
295 considerable decrease in fungal diversity was observed in the uncultivated desert saline
296 soil for the 3M3 treatment (Tukey's HSD test, $P < 0.05$) (Figs. S2). Manure amount,
297 rather than application frequency, also affected fungal α -diversity indices, except for
298 inverse Simpson's index after 180 days (Table S2).

299 As expected, the largest differences in microbial community composition
300 after 180 days were explained by the soil type alone and by the soil type in interaction
301 with the land use (Fig. 2; Table S3). A lower but significant effect of manure amount
302 and, to a lesser extent, manure frequency, was also observed on both the bacterial and
303 the fungal community composition (Fig. 2; Table S3). However, manure applications
304 caused greater shifts in the composition of the bacterial community (PERMANOVA,
305 $R^2 = 0.04$, $P = 0.02$) than in that of the fungal community (PERMANOVA, $R^2 = 0.03$,
306 $P = 0.65$) across nine soils (Fig. 2; Table S3). MNTD calculations showed that the
307 microbial communities in the manure-amended soils were more phylogenetically
308 related to the manure-borne communities than to the control soil communities, and

309 stronger effects were observed in uncultivated soils than in cultivated soils (ANOVA,
310 $F = 785.28$, $P < 0.001$ for bacteria; $F = 175.28$, $P < 0.001$ for fungi) (Table S4). In six
311 of nine soil types, lower MNTD values were observed between bacterial communities
312 in the manure-amended soils and in the manure for the high-manure amount treatments
313 (3M1 and 3M3) compared to the low-manure amount treatments (1M1 and 1M3)
314 (ANOVA, $F = 434.61$, $P < 0.001$ for bacteria) (Fig. S3; Table S4).

315

316 *3.2 Proportion of manure-borne microorganisms in fertilized soils*

317 Large differences in the estimated proportions of bacteria and fungi originating from
318 soil or manure were observed in the manure-amended soils. Estimates of the
319 proportions of manure-borne bacterial and fungal populations were 0.35–30.94% (7.33%
320 on average) and 0.01–57.27% (2.95% on average), respectively, across soils and
321 manure applications (Fig. 3). Overall, the highest proportions of manure-borne bacteria
322 and fungi were observed in the uncultivated desert saline soil and coastal saline soil,
323 respectively, regardless of the manure treatment (Fig. 3).

324 Both manure application regimes had a significant effect on the proportion of
325 manure-borne bacteria and fungi. However, manure amount had a stronger effect than
326 application frequency on the bacterial community (ANOVA, $F = 662.36$, $P < 0.001$ and
327 $F = 38.33$, $P < 0.001$, respectively) and, to a lesser extent, on the fungal community
328 (ANOVA, $F = 63.62$, $P < 0.001$ and $F = 59.23$, $P < 0.001$, respectively) (Table S5).

329 Venn diagrams validated the strong effect of manure amount on the bacterial community,
330 with the applied manure sharing double the number of OTUs with the 3M1 and 3M3

331 treatments (234 and 253 OTUs, respectively) than with the 1M1 and 1M3 treatments
332 (119 and 113 OTUs, respectively) after 180 days (Fig. S4A). In contrast, no clear pattern
333 was observed for the fungal community (Fig. S4B).

334

335 *3.3 Identification of OTUs increasing in relative abundance after manure application*

336 Overall, a significant effect of manure amount (ANOVA, $F = 5.49$, $P = 0.03$) but not of
337 application frequency was observed with the application of a high amount of manure
338 resulting in increased relative abundances of more bacterial OTUs in the uncultivated
339 than in the cultivated soils after 180 days (Table S6; Table S7). We found a lower
340 proportion of OTUs increasing in relative abundance after manure fertilization for the
341 fungal communities (6.21% on average) than for the bacterial communities (18.71% on
342 average) in most soil types, which also reflects the larger shifts in bacterial than in
343 fungal communities after manure application (Table S7). Unlike the bacterial
344 community, the number of increasing fungal OTUs after manure application was not
345 affected by the amount and frequency of manure applications across soils (ANOVA, F
346 $= 0.75$, $P = 0.39$ for the amount, and $F = 0.05$, $P = 0.82$ for the frequency) (Table S6).

347 The iTOLs showed the phylogenetic relationships and distribution of the
348 OTUs present in the soil or in the added manure that exhibited significantly higher
349 relative abundance in manure amended soils (Fig. 4). The bacterial OTUs that
350 significant increases in relative abundance were dominated by Proteobacteria (39.28%),
351 Actinobacteria (13.78%), and Chloroflexi (9.05%) (Fig. 4A), while the fungal OTUs
352 were dominated by Ascomycota (67.02%) (Fig. 4B). The relative abundances of 11

353 bacterial and 1 fungal OTUs increased significantly in at least seven different soils after
354 application of a high amount of manure while 10 of these OTUs were not detected in
355 70% of the control soils (Fig. 4; Fig. S5).

356 For further insights into the genetic traits of these OTUs, we performed a
357 metagenomic analysis of the manure samples in which their relative abundance was up
358 to 6%. We identified 1 of the 46 metagenome-assembled genomes affiliated to the
359 *Cytophaga*, which exhibited 100% identity to OTU 128 (Fig. S6). Analysis of the
360 functional potential of this metagenome-assembled genome revealed not less than 8
361 antibiotic-resistance genes such as daptomycin, tetracycline, fluoroquinolone (Table 1).

362

363 *3.4 Drivers of coalescence asymmetry*

364 The proportion of manure-borne microorganisms in the amended soils was used as a
365 proxy for coalescence asymmetry. First, we explored the relationships between the
366 estimated proportion of manure-borne microorganisms in the different manure
367 treatments and the diversity of the resident soil microbial community. After 180 days,
368 the proportion of manure-borne bacteria was significantly negatively correlated with
369 richness, the Shannon index, the inverse Simpson index, and Faith's phylogenetic
370 diversity of the control soil regardless of the manure treatment (Fig. 5A and S7A). In
371 contrast, the proportion of manure-borne fungi was not correlated with soil fungal
372 diversity indices, except for the richness in the 1M1 and 1M3 treatments (Fig. 5B and
373 S7B). An effect of manure application frequency was also observed for the relationships
374 between the proportions of manure-borne fungi and, to a lesser extent, of manure-borne

375 bacteria and the MNTD of microbial communities in the control soils and in the added
376 manure ($P < 0.05$) (Fig. 5).

377 To evaluate the relative importance of abiotic and biotic factors in modulating
378 the proportion of manure-borne microorganisms in soil, elastic net regression-based
379 variance partitioning was performed and the selected factors explained between 56.1%
380 and 82.1% of the total variance (Fig. 6). Three composite variables corresponding to
381 the first three PCA axes explaining over 75% of the total variation for each set of abiotic
382 and biotic variables were kept for this variance partitioning (Fig. S8). Both abiotic and
383 biotic factors explained the variation in the proportion of manure-borne bacteria across
384 manure treatments after 180 days, while the abiotic factors were more important for the
385 proportion of manure-borne fungi. We also found that the importance of the abiotic
386 factors for the proportion of manure-borne bacteria was favored by application of high-
387 manure amount (Fig. 6A), and on average, abiotic factors accounted for 16.10% and
388 31.85% of the explained variance under the low- and high-manure treatments,
389 respectively. In contrast, the abiotic factors had a weaker influence on the proportion of
390 manure-borne fungi when applying high manure amount (Fig. 6B). However,
391 application of high manure amount increased the interaction effect between abiotic and
392 biotic factors for both microbial communities, but to a lower extent for the fungal
393 community. We also found an increase in the interaction effect between abiotic and
394 biotic factors for both microbial communities as manure application frequency
395 increased (Fig. 6). For instance, the interaction between abiotic and biotic factors
396 represented 9.7% and 29.0% of the variance in the proportion of manure-borne bacteria

397 in the 1M1 and 1M3 treatments, respectively (Fig. 6A).

398

399 **4. Discussion**

400 Overall, manure fertilization resulted in shifts in the diversity and composition of the
401 soil microbiome, depending on the soil type and land use history. Thus, we observed
402 that manure application had a greater effect on the coalescence outcome in uncultivated
403 soils than in the cultivated ones (Figs. 3 and S3). This difference could be due to lower
404 resource availability in uncultivated soils, given that the addition of nutrient-rich
405 manure can both facilitate the invasion success of the manure-borne microorganisms
406 and stimulate the native soil microbial community (Feng et al., 2015; Mallon et al.,
407 2015a). We also found that manure application affects the bacterial communities more
408 than the fungal communities (Fig. 2). This finding supports previous studies showing
409 that bacterial communities are more affected by organic fertilizers, whereas fungal
410 communities exhibit stronger responses to mineral fertilizers (Pan et al., 2020).

411 *4.1 The amount and frequency of manure application both influence microbial* 412 *community coalescence*

413 Supporting our hypothesis, the coalescence outcome depended on both the
414 mixing ratio and frequency of application with the bacterial communities in the manure-
415 amended soils and in the added manure being more similar under high manure amounts
416 and application frequencies (Figs. 2 and S3). This finding was consistent with the higher
417 proportions of manure-borne microorganisms in two-thirds of the soils fertilized with a
418 high amount of manure (Fig 3). These higher proportions of manure-borne

419 microorganisms were likely due to a mass effect, wherein a large number of
420 microorganisms was transferred along with the manure, their “home” habitat, which
421 favored their survival (Fukami, 2015; Svoboda et al., 2018). While most previous
422 studies addressing the impact of microbial invaders on soil communities were
423 conducted within 3 months (Mawarda et al., 2020), our findings indicate that the effect
424 of manure addition on the soil microbial community can last longer with manure-borne
425 bacteria and fungi being capable of surviving at least 6 months in soil. Furthermore, the
426 estimated proportions of manure-borne bacteria were greater than 10% in half of the
427 tested soils, suggesting that their survival might be more widespread and ecologically
428 important for the spread of pathogens and antibiotic-resistance genes than previously
429 thought (Yang et al., 2022).

430 Differential abundance analysis enabled the identification of OTUs with
431 higher relative abundances in the coalesced microbial communities compared to control
432 soils (Fig. 4). Due to limitations related to the sequencing depth and the use of partial
433 16S rRNA gene sequences to identify OTUs in the different treatments, it is difficult to
434 decipher whether these OTUs corresponded to resident soil microorganisms whose
435 relative abundances increased due to shifts in biotic and abiotic factors or to manure-
436 borne microorganisms that survived in soil. However, we identified ten OTUs detected
437 in the manure but not in most soils that were significantly increasing in relative
438 abundances after the addition of a high amount of manure in seven different soils. This
439 result suggests that these ten OTUs not only originated from the added manure but also
440 had a high invasiveness capacity allowing them to survive in disparate soils. Elucidating

441 the exact mechanisms driving higher invasiveness is difficult, as microbiologists are
442 only beginning to understand the myriad of interactions occurring between
443 microorganisms in complex environments (Hibbing et al., 2010; Romdhane et al., 2022).
444 However, analyzing the genetic potential of a metagenome-assembled genome that was
445 taxonomically related to one of these ten OTUs revealed the presence of several
446 antibiotic-resistance genes (Table 1; Fig. S6). As antibiotics are naturally produced by
447 soil bacteria as a competitive mechanism and are also present at high concentrations in
448 cattle manure (Xie et al., 2016), antibiotic resistance may confer a competitive
449 advantage to manure-borne bacteria therefore contributing to their invasiveness
450 (Hibbing et al., 2010). Moreover, the presence of several antibiotic-resistance genes in
451 the metagenome-assembled genome supports previous studies highlighting the role of
452 manure fertilizer in the spread of antibiotic-resistant bacteria in the environment
453 (Larsson and Flach, 2022).

454 *4.2 Coalescence asymmetry is driven by both biotic and abiotic factors for bacteria,*
455 *but primarily by abiotic factor for fungi*

456 Ecological theory predicts that resident microbial diversity is a key factor
457 controlling the extent to which invaders can establish (Mallon et al., 2015b; Van Elsas
458 et al., 2012). Accordingly, we found that both the richness and phylogenetic diversity
459 of soil bacteria were negatively correlated with the estimated proportions of manure-
460 borne bacteria in the different treatments (Fig. 5 and S7). Our findings therefore support
461 the diversity resistance hypothesis, which proposes that more diverse communities act
462 as a biological barrier to invasion due to higher interference and resource competition

463 (Tilman, 2004; Van Elsas et al., 2012). The phylogenetic relatedness between invaders
464 and the resident community was shown either to hamper invasion due to niche overlap
465 or to facilitate invasion due to pre-adaption (Gravuer and Scow, 2021). Here we
466 identified weak but significant positive correlations between the phylogenetic distance
467 of the added manure and resident fungal communities and the percentage of manure-
468 borne fungi only at a high manure application frequency (Fig. 5B). This finding
469 suggests that niche overlap might be important for manure-borne fungi survival only
470 when manure application occurs frequently.

471 Using the estimated proportion of manure-borne microorganisms in a
472 variance partitioning analysis, we provide insights into coalescence asymmetry. To our
473 knowledge, this is the first study to comprehensively assess the relative importance of
474 abiotic and biotic soil factors in driving the coalescence outcome after manure
475 application across different soil types, land use history and microbial domains. We
476 found that abiotic and biotic factors explained between 56% and 82% of the variability
477 in coalescence asymmetry between manure-borne and resident microorganisms (Fig.
478 6). Abiotic and biotic factors were both important in modulating bacterial community
479 coalescence, whereas abiotic factors were more important for fungal community
480 coalescence. Accordingly, recent studies have revealed the key role of biotic
481 interactions between soil bacteria, but studies focusing on fungal communities remain
482 limited (Huet et al. 2023; Kehe et al. 2021; Palmer et al. 2022). The asymmetric
483 outcome of microbial community coalescence was affected by changes in the manure
484 amount with increased importance of the interaction effects between biotic and abiotic

485 factors under application of high manure amount. However, our analysis also showed
486 that increasing the manure amount had the opposite effect on the contribution of abiotic
487 factors to the proportion of manure-borne bacteria and fungi (Fig 6). This result adds
488 support to previous studies highlighting the role of resource availability in modulating
489 the interactions between invaders and resident communities (Mallon et al., 2015a; Yang
490 et al., 2017). Moreover, manure application frequency strongly influenced the
491 proportion of manure-borne bacteria, with the interaction between biotic and abiotic
492 factors becoming increasingly important with higher mixing frequency. Accordingly, a
493 previous study showed that frequent invasion events can create a legacy in the niche
494 structure of the resident bacterial community that facilitates the survival of the
495 subsequent bacterial invaders (Mallon, et al., 2018). Therefore, our results suggest that
496 legacy effects caused by invaders and by habitat disturbances due to the repeated
497 addition of resources and antibiotics might act synergistically on the survival of
498 manure-borne microorganisms.

499

500 **5. Conclusions**

501 Our findings provide new insights into the role of abiotic and biotic factors in
502 modulating the outcome of coalescence events between manure-borne and resident soil
503 microbial communities under different manure fertilization regimes. We demonstrated
504 that the outcome of coalescence was more affected by the manure fertilization regime
505 for the bacterial than for the fungal community. Differences in the survival of manure-
506 borne microorganisms were better explained by both abiotic and biotic factors for

507 bacteria and by abiotic factors for fungi. Our study also showed that the manure
508 fertilization regime can have opposite effect on the contribution of abiotic and biotic
509 factors to the survival of manure-borne microorganisms depending on the microbial
510 community. These results are of importance for the adoption of organic agriculture due
511 to the risk of the introducing non-native species, which may be detrimental to the
512 delivery of some soil services.

513

514

515 **Authorship**

516 Conceptualization: HL and JW; Methodology: CL, XL, SR, LP, JJ and JW;

517 Investigation: YC, GL, RC, PL, JX, YZ and JW; Visualization: CL, SR, YY, and LP;

518 Supervision: FH; Writing—original draft: CL; Writing—review & editing: LP and SR.

519 All authors contributed to this work.

520

521

522 **Declaration of competing interest**

523 The authors declare no competing financial interest.

524

525

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536

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538 **Data accessibility statement**

539 All data supporting the conclusions of this article are included in Zenodo Digital
540 Repository at [https://doi.org/ 10.5281/zenodo.6950532](https://doi.org/10.5281/zenodo.6950532).

541

542

543 **Appendix A. Supplementary data**

544 Supplementary data to this article can be found online at

545

546

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784

785 **Table**

786 **Table 1** Descriptions of key antibiotic resistance gene types identified in MAG.86.

Antibiotic names	ARGs
Daptomycin	<i>CdsA</i>
	<i>liaR</i>
	<i>liaS</i>
	<i>walK</i>
	<i>vanR</i>
Glycopeptide antibiotic	<i>vanT</i>
	<i>vanH</i>
	<i>vanX</i>
Tetracycline	<i>Tet(O)</i>
Fluoroquinolone	<i>gyrA</i>
Lysocin	<i>menA</i>
Elfamycin	<i>EF-Tu</i>
Vancomycin	<i>rpoC</i>
Pyrazinamide	<i>rpsA</i>
	<i>kdpDE</i>
	<i>fusE</i>
	<i>gidB</i>
	<i>ndh</i>
Others	<i>murA</i>
	<i>murG</i>

787

788

789 **Figure legends**

790 **Figure 1** Phylum-level bacterial and fungal community composition. Mean relative
791 abundances of (A) bacterial and (B) fungal phyla for each treatment ($n = 3$) in nine
792 types of soils at day 180, and (C) the added manure ($n = 3$) at day 0. CS refers to control
793 soil; 1M1 and 1M3 refer to soils with a lower amount of manure in one application and
794 in three equal applications, respectively; 3M1 and 3M3 refer to soils with a higher
795 amount of manure in one application and in three equal applications, respectively.

796

797 **Fig. 2. Shifts in the composition of the microbial community related to manure**
798 **application.** Principal coordinates analysis of (A) bacterial and (B) fungal communities
799 based on Bray-Curtis distance matrices showing differences in the microbial
800 composition among treatments in nine types of soils after 60 (unfilled symbols) and
801 180 days (filled symbols) of incubation, as well as in the added manure. Each dot
802 represents the geometric center of the three replicates. The different treatments and soils
803 are represented by different shapes and colours, respectively, as specified in the legend.
804 (C) Bray-Curtis distance between each treatment and the added manure. Different
805 letters indicate significant differences among treatments in each panel (Tukey's HSD
806 test, $P < 0.05$). n.s. indicates no significant difference among treatments (Tukey's HSD
807 test, $P > 0.05$). The error bar in each column represents variability of corresponding
808 dataset.

809

810 **Fig. 3. Proportion of manure-borne microorganisms in the different manure-**

811 **amended soils.** Proportions of the microbial sources in the different soils after manure
812 application as predicted by SourceTracker. Stack bar plots represent the proportions of
813 each source (added manure = red, soil = brown, and unknown = grey) for (A) bacterial
814 and (B) fungal communities at days 60 and 180 of incubation. Values are represented
815 as the mean (n = 3). Different letters indicate proportions significantly different among
816 soil types (Tukey's HSD test, $P < 0.05$).

817

818 **Fig. 4. Phylogenetic relationships and distribution of the OTUs positively affected**
819 **by manure addition.** Distribution of the (A) 718 bacterial OTUs and (B) 188 fungal
820 OTUs with a significant increase in relative abundance in the manure treatments
821 compared to the control soil as calculated by DESeq2 analysis in all treatments at day
822 180 of incubation. The colours of each OTU in the inner ring represent the sources in
823 which the OTUs are detected. The affiliation of OTUs at the phylum level is indicated
824 by different colours on the second ring. The outer rings around the tree represent
825 different treatments. Node size represents the number of soils in which the OTUs are
826 exhibiting significantly higher relative abundances after manure applications. Venn
827 diagram showing the shared and unique OTUs among treatments in each iTOL are
828 included in each panel.

829

830 **Fig. 5. Relationships between manure-borne microbial proportions and soil**
831 **microbial diversity as well as Mean Nearest Taxon Distance (MNTD) of the soil**
832 **and manure communities.** The relationships between the proportions of (A) manure-

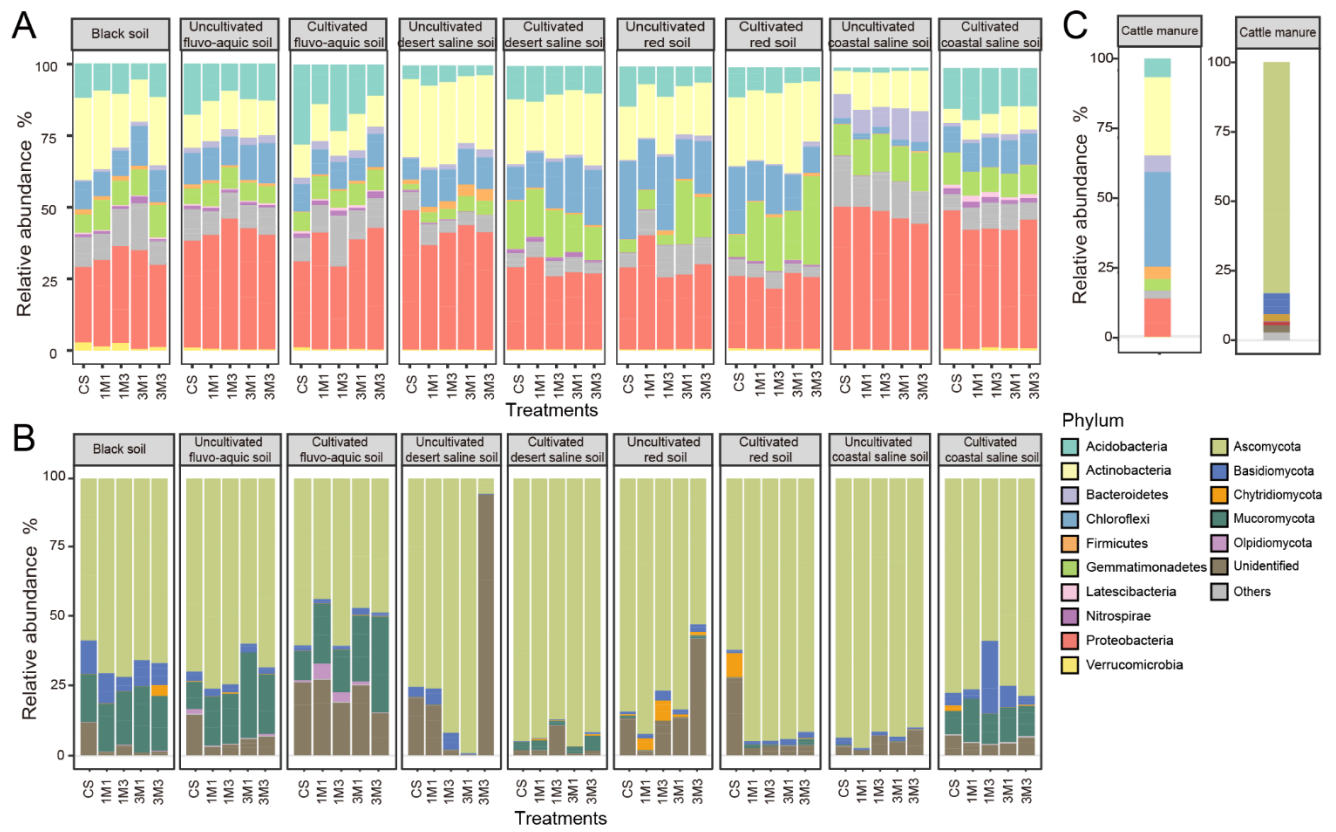
833 borne bacteria or (B) manure-borne fungi and Shannon index or the MNTD of control
834 soil (CS) and the added manure. $n = 27$ for each treatment. Statistical analysis was
835 performed using Spearman's rank correlation; the adjusted R^2 values and false-
836 discovery rate adjusted P values are also inserted in each panel.

837

838 **Fig. 6. Variance partitioning analysis showing the effects of abiotic and biotic**
839 **factors on the outcomes of microbial community coalescence.** The proportions of
840 manure-borne bacteria and fungi are used as proxies of the coalescence outcomes for
841 (A) bacteria and (B) fungal communities. The abiotic factors include three principal
842 components explaining of climate variables; soil textural composition; and the
843 differences in EC, in pH, in nutrients, and in C:N ratio between each control soil and
844 the added manure. The biotic factors include three principal components of soil
845 microbial respiration, microbial biomass C, N, and P, biodiversity indices, and the Mean
846 Nearest Taxon Distance of each control soil and the added manure. Numbers indicate
847 the percentage of variations in the community coalescence. The proportion of variance
848 of each principal component is also inserted in each panel. The relationship between
849 the coalescence outcome and each principal component is performed by ordinary least
850 squares linear regression, and the P values were adjusted by false-discovery rate. *
851 significant at 5% level of significance; ** significant at 1% level of significance; ***
852 significant at 0.1% level of significance.

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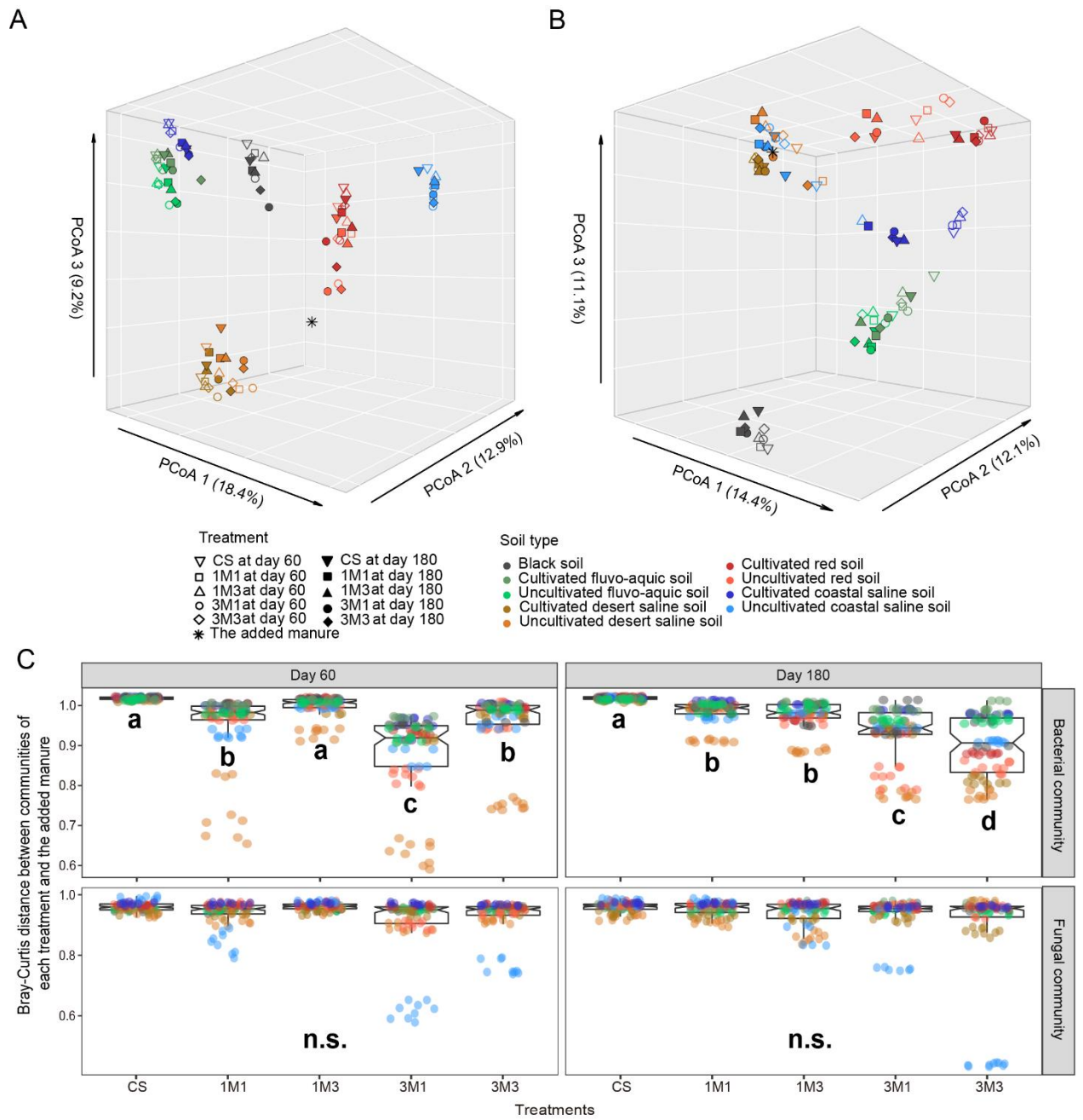


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Fig. 1

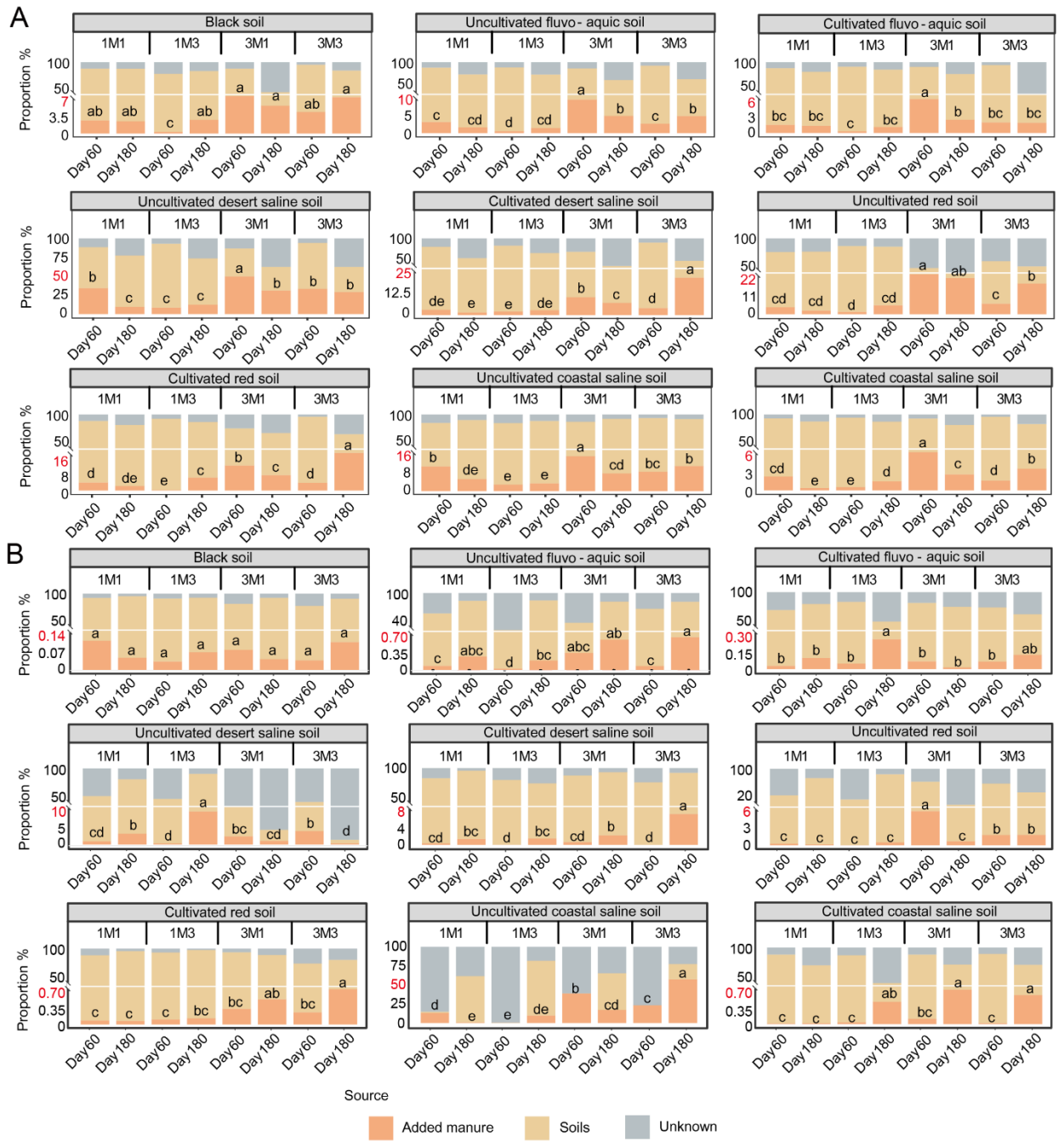


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Fig. 2

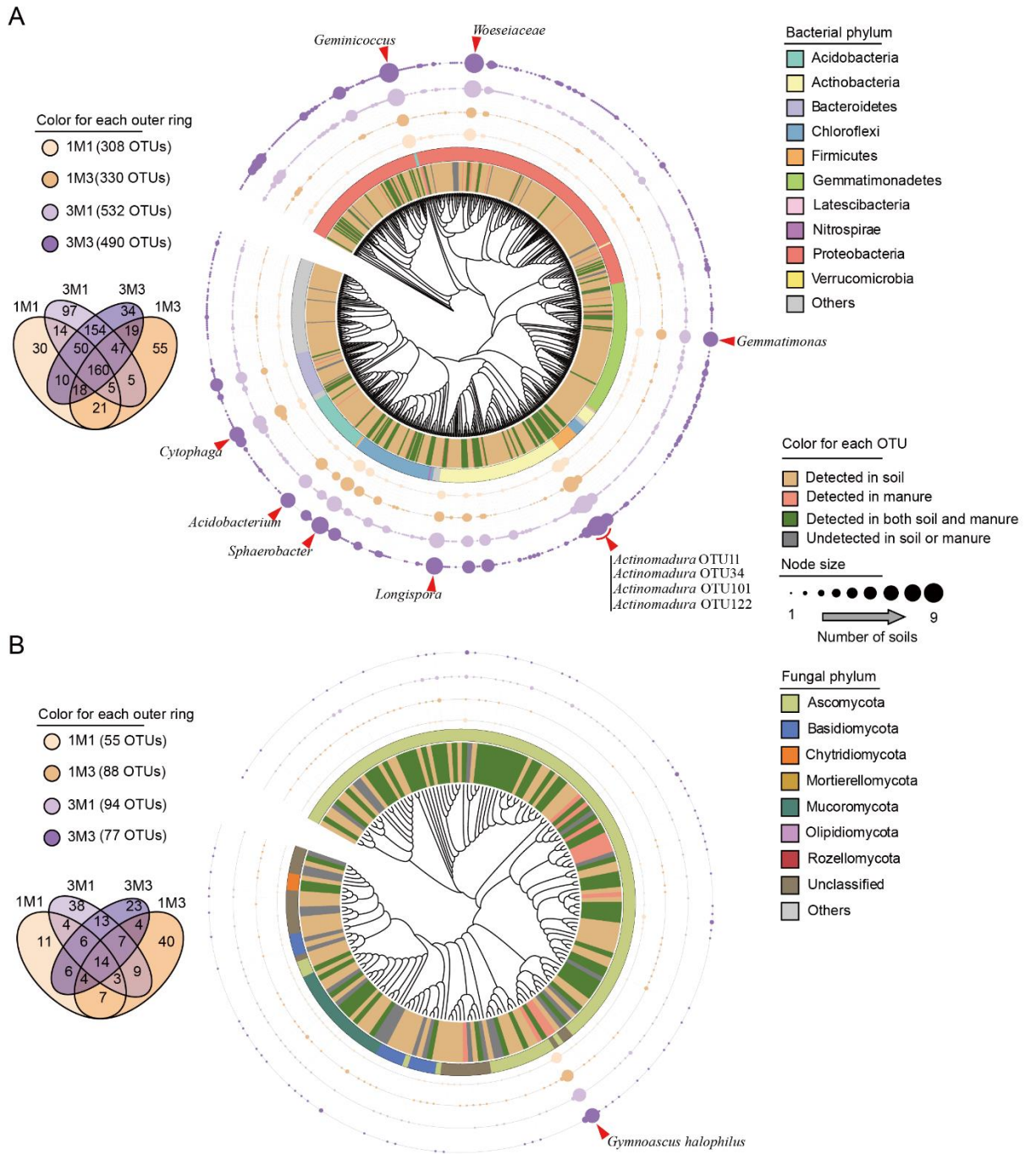


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Fig. 3

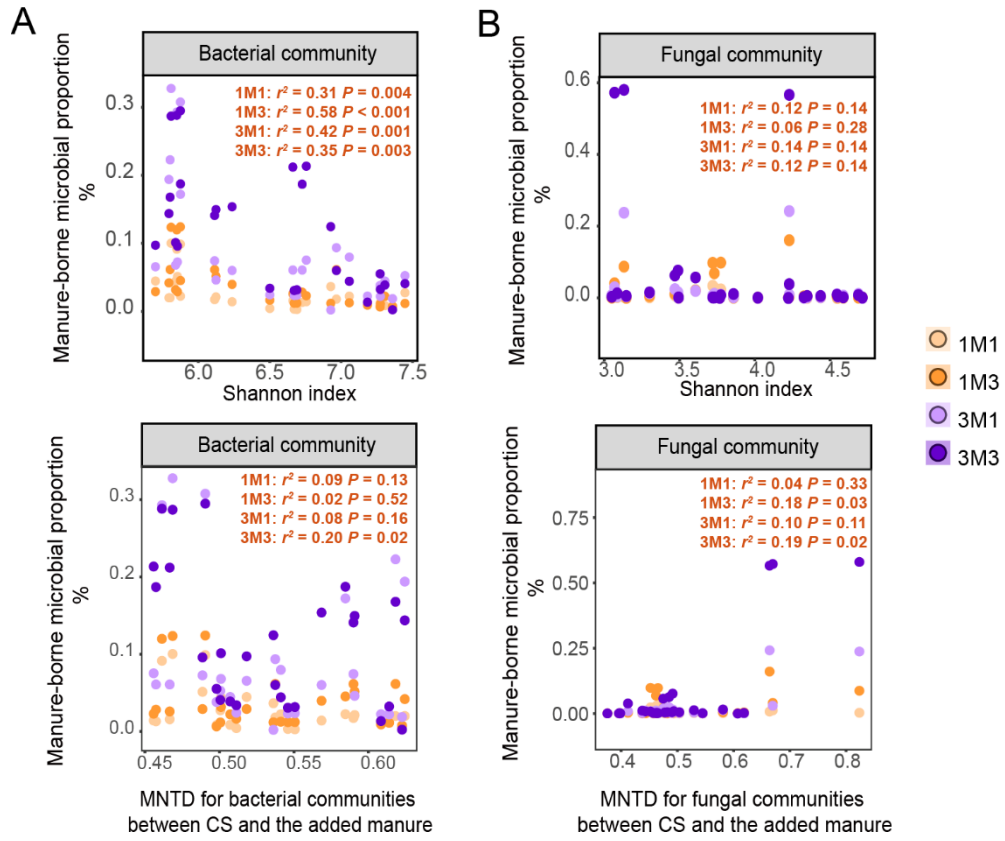


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Fig. 4

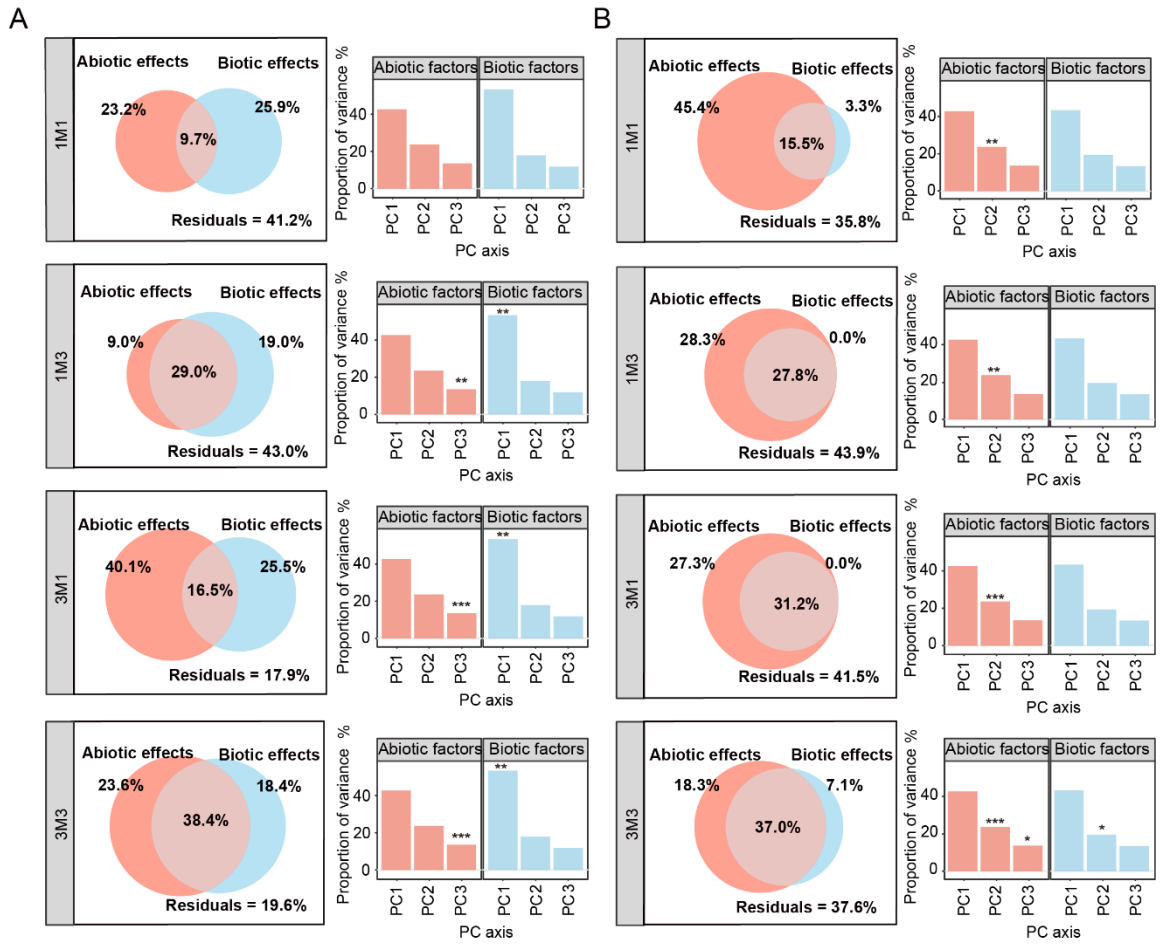


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Fig. 5



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Fig. 6