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Keywords

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

Abstract

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

1. Introduction

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

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

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

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

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

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

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

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

2. Materials and methods

2.1 Soil sampling

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

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

2.2 Laboratory-controlled mesocosm experimental design

Soil mesocosms were established by filling PVC containers (35 cm length × 25 cm width × 25 cm depth) with 5 kg sieved soil samples following a completely randomized design. The experiment was conducted in triplicate with five manure application regimes: nonamended soil mesocosms used as controls (CS); 6.60 g kg^{-1} manure added in one application at day 0 (1M1) or in three equal applications at days 0, 60 and 120 (1M3; 2.20 g kg^{-1} manure each time); or 19.80 g kg^{-1} manure added in one application at day 0 (3M1) or in three equal applications at days 0, 60 and 120 (3M3; 6.60 g kg^{-1} manure each time). The manure was added by mixing it with soil, and the amounts of manure added were based on traditional fertilization regimes in China, which ranged between 15 and 45 t ha^{-1} (Li et al., 2009). The manure was a thermal-composted cattle dung, which was provided by the Nanjing Institute of Vegetable and Flower Sciences, China. The manure contained 205.14 g kg^{-1} total organic carbon (C), 14.25 g kg^{-1} total nitrogen (N), 17.02 g kg^{-1} total phosphorus (P), 27.04 g kg^{-1} total potassium (K), 293.28 mg kg^{-1} ammonium nitrogen (NH_4^+-N) and 31.43 mg kg^{-1} nitrate nitrogen (NO_3^--N) and had a pH of 7.4 and an electrical conductivity (EC) of 2.09 ms cm^{-1}. All mesocosms were then incubated at 25°C under sterile conditions and maintained at 60% of their water holding capacity in the dark for 180 days. All mesocosms were non-destructively sampled to a depth of 20 cm using a ⌀ 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

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

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

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

2.5 Statistical analysis

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

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

3. Results

3.1 Changes in soil microbial communities under different manure treatments

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

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

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

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

3.2 Proportion of manure-borne microorganisms in fertilized soils

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

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

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

4. Discussion

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

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

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

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

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

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

Authorship

Conceptualization: HL and JW; Methodology: CL, XL, SR, LP, JJ and JW; Investigation: YC, GL, RC, PL, JX, YZ and JW; Visualization: CL, SR, YY, and LP; Supervision: FH; Writing—original draft: CL; Writing—review & editing: LP and SR. All authors contributed to this work.

Declaration of competing interest

The authors declare no competing financial interest.

Acknowledgements

Our research was kindly supported by the Joint Project of Yazhou Bay Science and Technology City (2021JJLH0093), the Modern Agro-industry Technology Research

System-Green Manure (CARS-22-G-10), Guidance Foundation, the Sanya Institute of Nanjing Agricultural University (NAUSY-ZD06), the Key R&D Program of Jiangsu Province, China (BE2021378) and the Cooperative Doctoral Program of China Scholarship Council (202006850065) for supporting the 18 month visit of Chunkai Li at the Agroecology Department of the INRAE center in Dijon. We sincerely thank Professor Youzhi Feng and the Biozeron Co. Ltd. (Shanghai, China) for the assistance in this work. We also sincerely thank Kathleen Farquharson for the language polish.

Data accessibility statement

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at

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785 **Table**

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

Antibiotic names	**ARGs**
Daptomycin	*Cds*A
	*lia*R
	*lia*S
Glycopeptide antibiotic	*wal*K
	*van*R
	*van*T
	*van*H
	*van*X
Tetracycline	*Tet*(O)
Fluoroquinolone	*gyr*A
Lysocin	*men*A
Elfamycin	*EF-Tu*
Vancomycin	*rpo*C
Pyrazinamide	*rps*A
Others	*kdp*DE
	*fus*E
	*gid*B
	ndh
	*mur*A
	*mur*G

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-**

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

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

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

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

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

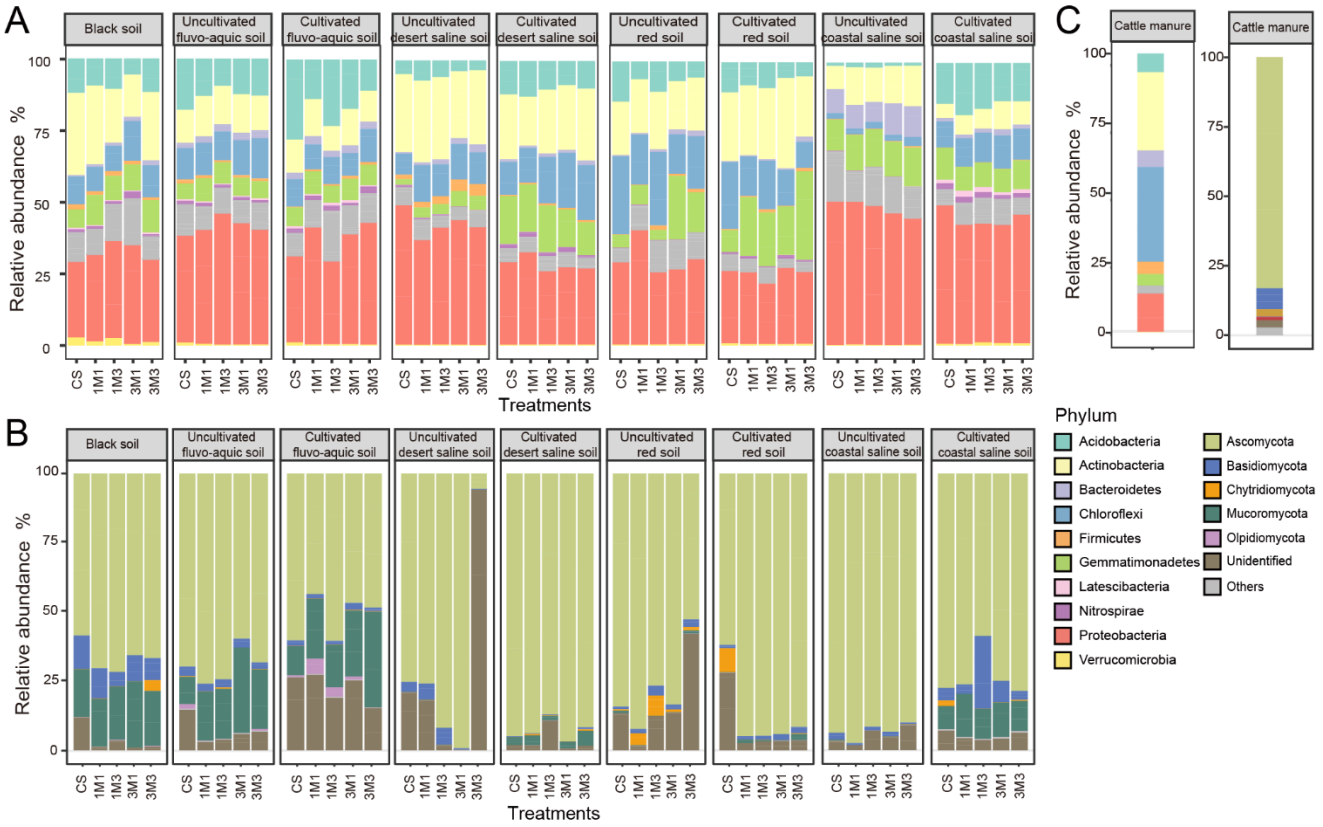
A
Black soil
Uncultivated fluvo-aquic soil
Cultivated fluvo-aquic soil
Uncultivated desert saline soil
Cultivated desert saline soil
Uncultivated red soil
Cultivated red soil
Uncultivated coastal saline soil
Cultivated coastal saline soil
Relative abundance %
Treatments
CS
1M1
1M3
3M1
3M3
B
C
Cattle manure
Phylum
Acidobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Firmicutes
Gemmatimonadetes
Latescibacteria
Nitrospirae
Proteobacteria
Verrucomicrobia
Ascomycota
Basidiomycota
Chytridiomycota
Mucoromycota
Olpidiomycota
Unidentified
Others

Fig. 1

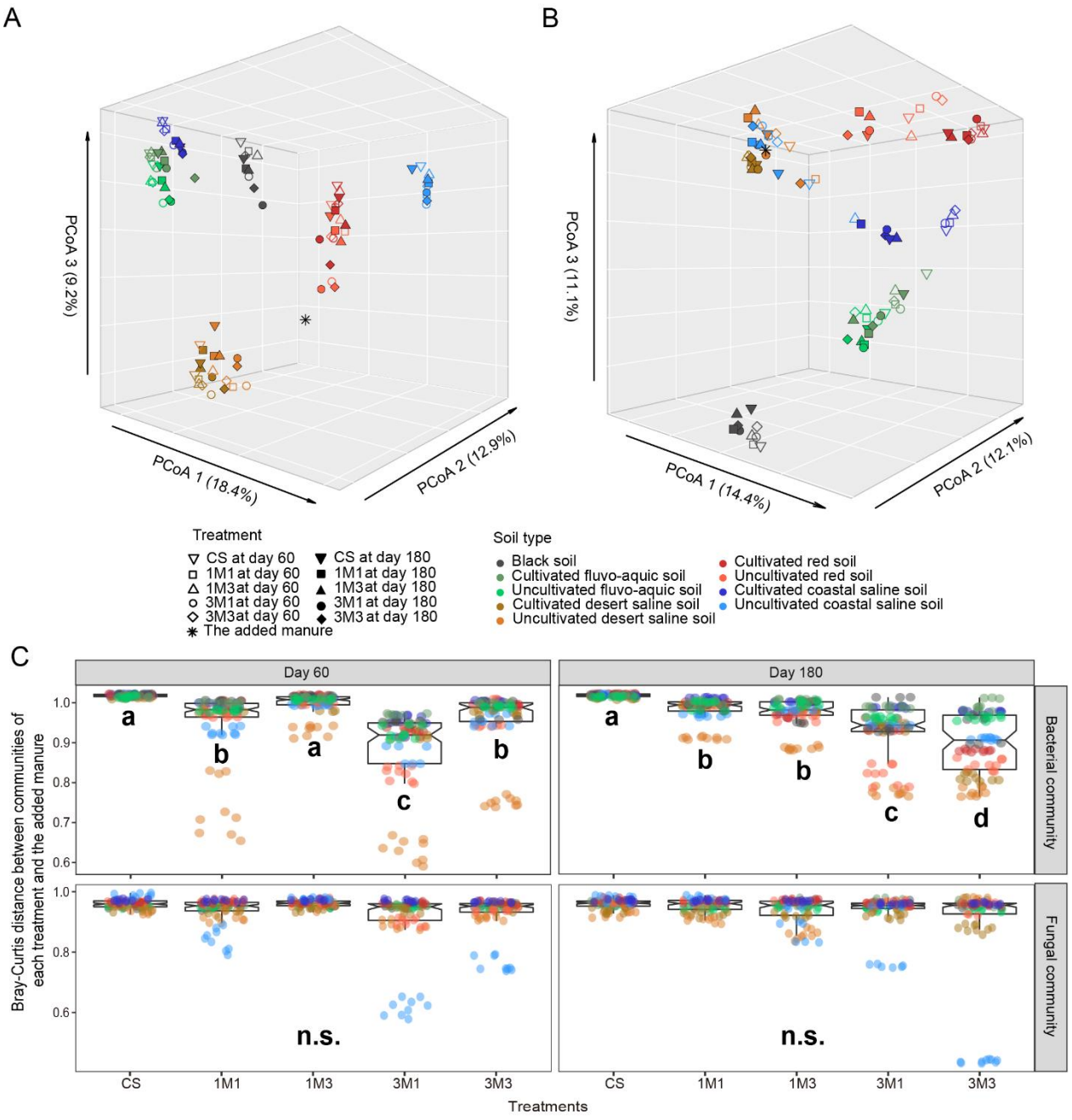
A
B
PCoA 3 (9.2%)
PCoA 1 (18.4%)
PCoA 2 (12.9%)
PCoA 3 (11.1%)
PCoA 1 (14.4%)
PCoA 2 (12.1%)
Treatment
CS at day 60
1M1 at day 60
1M3at day 60
3M1at day 60
3M3at day 60
The added manure
CS at day 180
1M1at day 180
1M3at day 180
3M1 at day 180
3M3 at day 180
Soil type
Black soil
Cultivated fluvo-aquic soil
Uncultivated fluvo-aquic soil
Cultivated desert saline soil
Uncultivated desert saline soil
Cultivated red soil
Uncultivated red soil
Cultivated coastal saline soil
Uncultivated coastal saline soil
C
Day 60
Day 180
Bacterial community
Fungal community
Bray-Curtis distance between communities of each treatment and the added manure
n.s.
CS
1M1
1M3
3M1
3M3
Treatments

Fig. 2

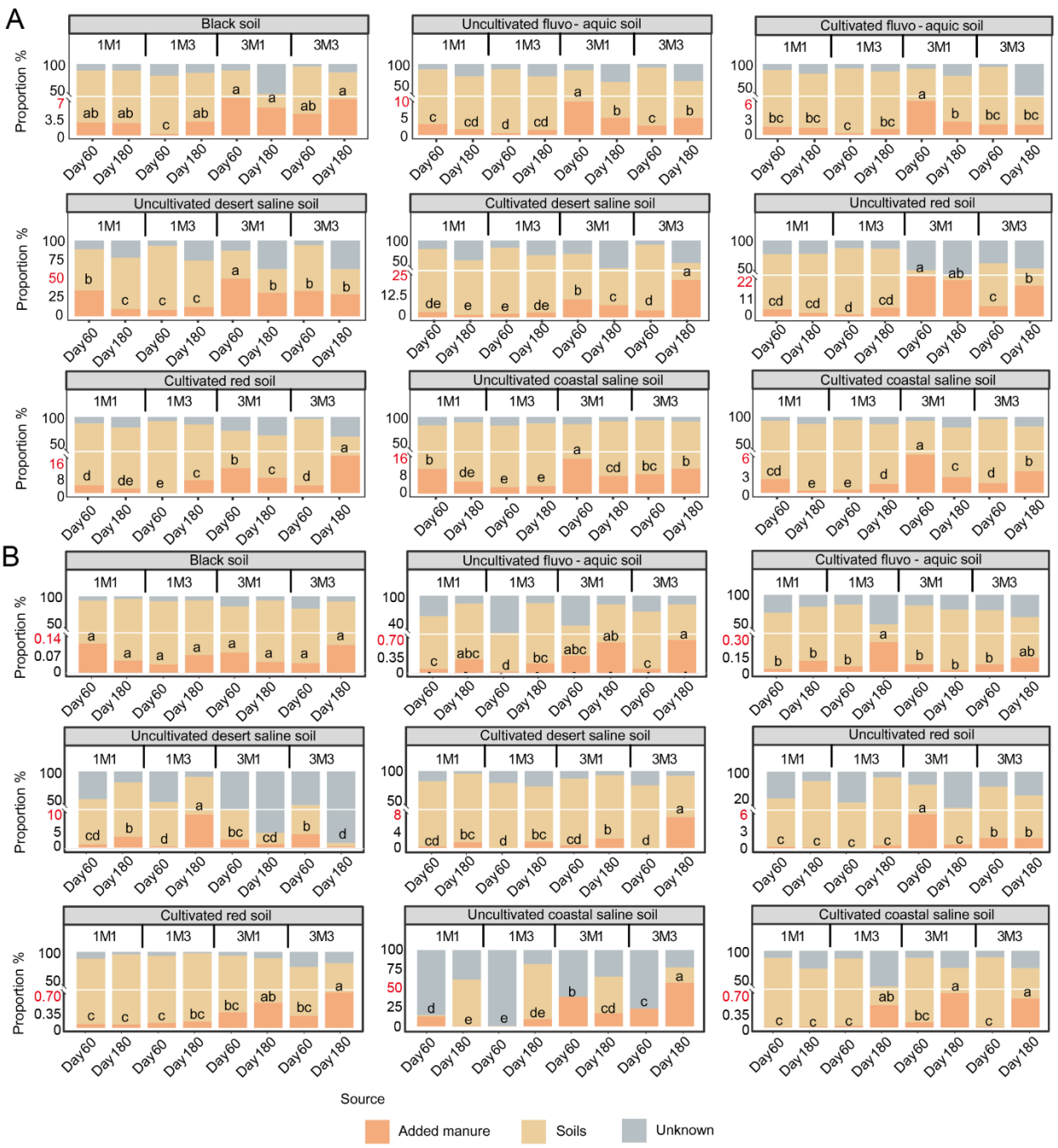
A
B
Black soil
Uncultivated fluvo - aquic soil
Cultivated fluvo - aquic soil
Uncultivated desert saline soil
Cultivated desert saline soil
Uncultivated red soil
Cultivated red soil
Uncultivated coastal saline soil
Cultivated coastal saline soil
1M1
1M3
3M1
3M3
Proportion %
Day60
Day180
Source
Added manure
Soils
Unknown

Fig. 3

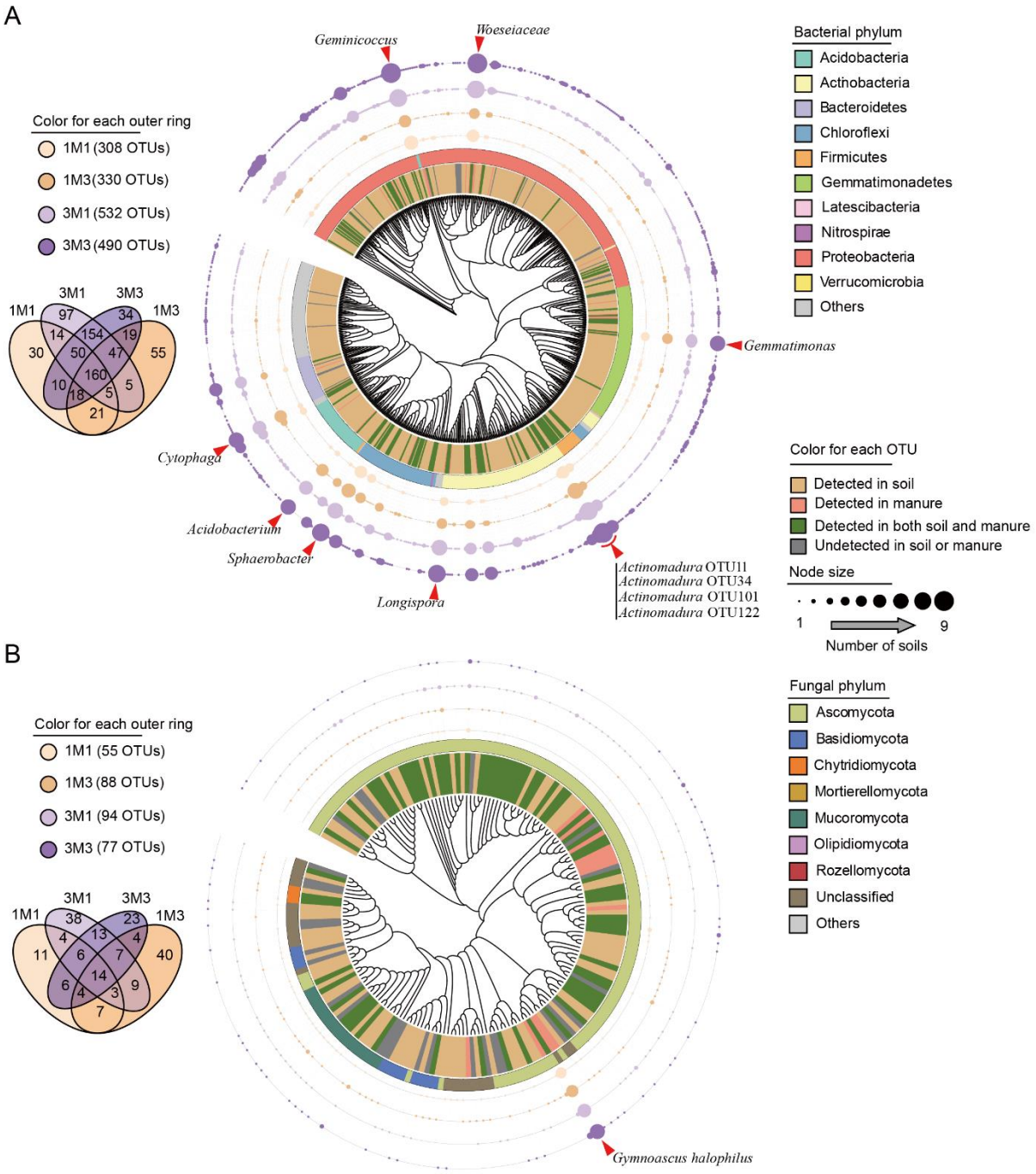
A
Color for each outer ring
1M1 (308 OTUs)
1M3(330 OTUs)
3M1 (532 OTUs)
3M3 (490 OTUs)
3M1
3M3
1M1
1M3
97
34
14
154
19
30
50
47
55
160
10
5
18
5
21
Geminicoccus
Woeseiaceae
Gemmatimonas
Cytophaga
Acidobacterium
Sphaerobacter
Longispora
Actinomadura OTU11
Actinomadura OTU34
Actinomadura OTU101
Actinomadura OTU122
Bacterial phylum
Acidobacteria
Acthobacteria
Bacteroidetes
Chloroflexi
Firmicutes
Gemmatimonadetes
Latescibacteria
Nitrospirae
Proteobacteria
Verrucomicrobia
Others
Color for each OTU
Detected in soil
Detected in manure
Detected in both soil and manure
Undetected in soil or manure
Node size
1
9
Number of soils
B
Color for each outer ring
1M1 (55 OTUs)
1M3 (88 OTUs)
3M1 (94 OTUs)
3M3 (77 OTUs)
3M1
3M3
1M1
1M3
38
23
4
13
4
11
6
7
40
14
6
4
3
9
7
Gymnoascus halophilus
Fungal phylum
Ascomycota
Basidiomycota
Chytridiomycota
Mortierellomycota
Mucoromycota
Olipidiomycota
Rozellomycota
Unclassified
Others

Fig. 4

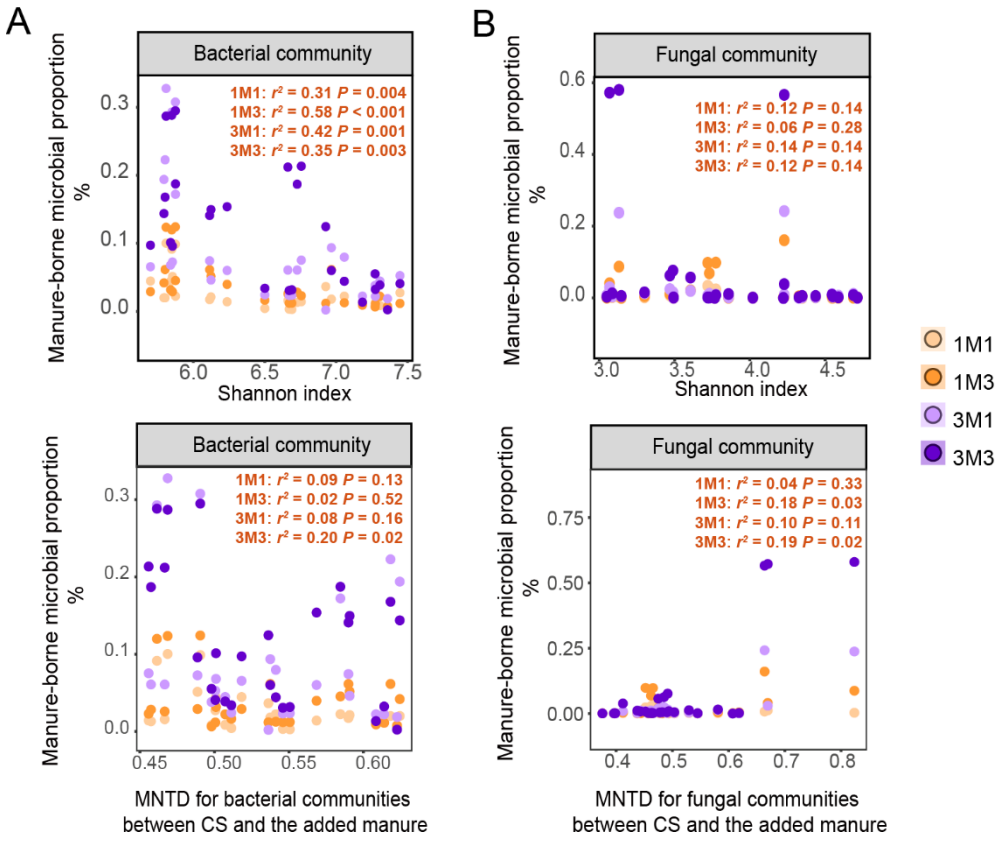
A
B
Bacterial community
Fungal community
Manure-borne microbial proportion %
Shannon index
1M1: r^2 = 0.31 P = 0.004
1M3: r^2 = 0.58 P < 0.001
3M1: r^2 = 0.42 P = 0.001
3M3: r^2 = 0.35 P = 0.003
1M1: r^2 = 0.12 P = 0.14
1M3: r^2 = 0.06 P = 0.28
3M1: r^2 = 0.14 P = 0.14
3M3: r^2 = 0.12 P = 0.14
1M1: r^2 = 0.09 P = 0.13
1M3: r^2 = 0.02 P = 0.52
3M1: r^2 = 0.08 P = 0.16
3M3: r^2 = 0.20 P = 0.02
1M1: r^2 = 0.04 P = 0.33
1M3: r^2 = 0.18 P = 0.03
3M1: r^2 = 0.10 P = 0.11
3M3: r^2 = 0.19 P = 0.02
MNTD for bacterial communities between CS and the added manure
MNTD for fungal communities between CS and the added manure
1M1
1M3
3M1
3M3

Fig. 5

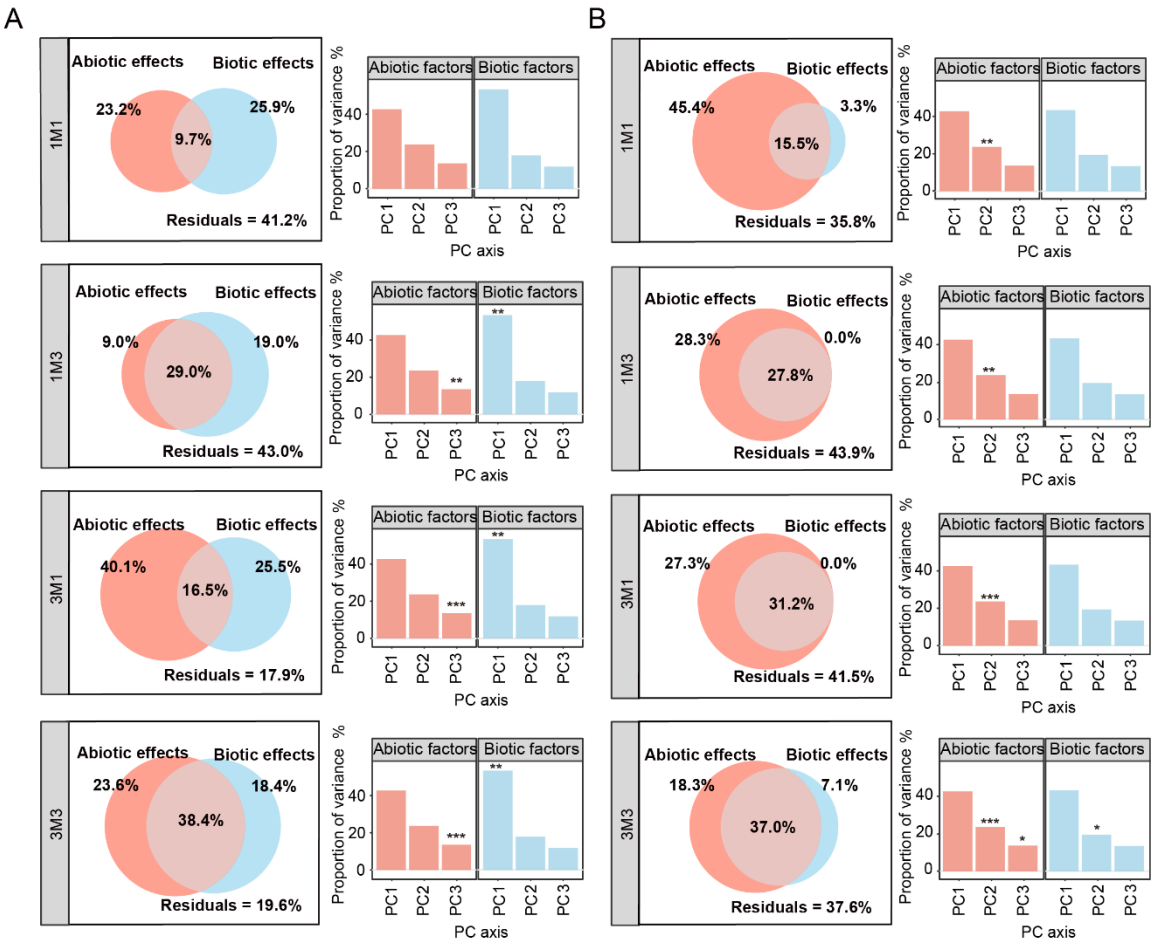
A
B
1M1
1M3
3M1
3M3
Abiotic effects
Biotic effects
23.2%
25.9%
9.7%
Residuals = 41.2%
45.4%
3.3%
15.5%
Residuals = 35.8%
9.0%
19.0%
29.0%
Residuals = 43.0%
28.3%
0.0%
27.8%
Residuals = 43.9%
40.1%
25.5%
16.5%
Residuals = 17.9%
27.3%
0.0%
31.2%
Residuals = 41.5%
23.6%
18.4%
38.4%
Residuals = 19.6%
18.3%
7.1%
37.0%
Residuals = 37.6%
Abiotic factors
Biotic factors
Proportion of variance %
PC1
PC2
PC3
PC axis

Fig. 6