The role of long-term mineral and manure fertilization on P species accumulation and phosphate solubilizing microorganisms in paddy red soils

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Abstract

21   Understanding soil P transformation and turnover under various fertilization

22   managements is important for evaluating sustainable P fertility and potential

23   bioavailability in agriculture managements. Thus, long-term fertilization experiments

24   (~38 years) with the application of different inorganic and organic fertilizers in paddy

25   red soils were conducted to determine the effect of different fertilizer applications on P

26   pool accumulation and microbial communities, especially for phosphate solubilizing

27   microorganisms (PSM). Long-term inorganic P fertilization increased the

28   concentrations of total P (~479 mg·kg^{-1}), available P (~417 mg·kg^{-1}), and inorganic P

29   (~18 mg·kg^{-1}), but manure fertilization accelerated the accumulation of organic P,

30   especially for orthophosphate monoesters (e.g. myo-IHP, ~12 mg·kg^{-1}). Long-term

31   mineral fertilization decreased bacterial richness, evenness, and complexation of

32   bacterial networks. In contrast, long-term manure fertilization and rhizosphere

33   accumulated more amounts of total carbon, total nitrogen, and organic carbon, as well

34   as regulated the soil pH, thus improving the separation of bacterial communities.

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35　Furthermore, PSM compositions were greatly influenced by fertilization managements

36　and rhizosphere. For example, inorganic P fertilization increased the abundance of

37　*Thiobacillus* (i.e. the most abundant phosphate solubilizing bacteria (PSB) in this study)

38　and shifted the community structure of PSB. Correspondingly, the concentrations of

39　inorganic and total P were the key factors for the variation of PSB community structure.

40　These findings are beneficial for understanding the variation of inorganic and organic

41　P pool, and microbial community, especially for PSM under long-term inorganic and/or

42　organic fertilization.

43　**Keywords**: long-term fertilization, P species accumulation, phosphate solubilizing

44　microorganisms, paddy red soils, P-NMR

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46　# 1. Introduction

47　Phosphorus (P) as an essential nutrient for crop growth has been widely applied to soil

48　through mineral and/or organic fertilization (Grant et al., 2005). Manures have been

49　frequently used as organic fertilizers in agriculture production (Braos et al., 2020). The

50　P from manures exists in forms of various inorganic and organic species, whereas

51　mineral fertilizers usually only contain highly soluble $Ca(H_2PO_4)_2$ (Sharpley and Moyer,

52 2000). Fertilization managements are important factors for P species transformation and

53 bioavailability. For example, mineral fertilization results in an initial high P availability

54 but follows a decrease of P concentration over time by adsorption, complexation, and

55 precipitation with soil particles. On the other side, the application of manure usually

56 leads to an accumulation in labile organic P pools with potential supply to plants

57 (Schneider et al., 2016). Additionally, the application of mineral fertilizer and manure

58 brought different changes in soil physical, chemical, and biological attributes such as

59 soil pH, organic carbon, microbial communities, and so on, which also induce different

60 P transformation processes and potential availability (Yue et al., 2016; Tao et al., 2021).

61 Soil microorganisms are usually involved in a wide range of biological processes

62 including the transformation of insoluble soil nutrients (Babalola and Glick, 2012).

63 After long-term fertilization, insoluble or soluble organic matter in soil may increase,

64 thus leading to the increases of microbial biomass and activity (Marschner et al., 2003).

65 Among them, phosphorus solubilizing microorganisms (PSM) could solubilize

66 insoluble inorganic P, mineralize organic P, and play an important role in P

67 transformation and availability (Sharma et al., 2013). The response of PSM in soil is

68 strongly related to the availability of P which is greatly different under various

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69   fertilization managements (Sánchez-Esteva et al., 2016; Gómez-Muñoz et al., 2018;

70   Raymond et al., 2021).

71   Currently, the information about how long-term various inorganic and organic

72   fertilization managements affect the evolution characteristics of different P pools

73   remains scarce. Furthermore, the responses of microbial community especially PSM

74   shift in bulk and rhizosphere soils to the different P pool evolution under various

75   fertilization managements are still unclear. This information plays a pivotal role for

76   understanding soil P transformation mechanisms and evaluating sustainable P fertility

77   and potential bioavailability in agriculture managements. The accumulation, turnover,

78   and bioavailability of soil P pool under different fertilization managements could be

79   well evaluated by long-term fertilization experiences. Currently, numerous long-term

80   fertilization experiences have been established to evaluate the impact of different

81   fertilizer amendments on crop production and at the same time provide valuable

82   information on soil fertility by investigating changes in soil process over time (Wen et

83   al., 2019). Thus, in this study, long-term fertilization experiments (~38 years) under

84   inorganic fertilizer and/or manure amendments were conducted to determine their

85   effects on P pool accumulation, soil microbial communities, and PSM in paddy red soils.

86   We hypothesized that (1) long-term input of inorganic fertilizers accumulates more

87   inorganic P but the manure application and rhizosphere may accelerate the

88   accumulation of organic P and (2) the long-term manure fertilization and rhizosphere

89   could accumulate more organic nutrients, thus driving the separation of bacterial

90   communities compared to the mineral fertilizer application.

2.Materials and methods

2.1. Field design and sampling

93   Long-term fertilization experiments were conducted since 1982 in a national

94   observation and research station of farmland ecosystem (26°45′N, 111°52′E), Qiyang,

95   Hunan Province, China. The soil was classified as Ferralic Cambisol according to World

96   Reference Base for soil resources (Wrb, 2014), and classified as red soil according to

97   Chinese soil classification (Baxter, 2007). Rice (*Oryza sativa*) is the major crop in this

98   region. The early rice was transplanted at the end of April and harvested in July, and the

99   late rice was transplanted at the end of July and harvested in October. All straw (except

100 the rice stubble) was removed from the fields after each seasonal rice harvest (Zhang et

101 al., 2017; Yang et al., 2012). The experimental field was disposed with five different

102 fertilizer treatments: CK (control without fertilizer), NPK (mineral N, P, and K

103 fertilizers), M (cattle manure), NPKM, and NKM (Qaswar et al., 2020; Gao et al., 2011).

104 Mineral fertilizers were applied in the forms of urea for N, calcium superphosphate for

105 P, and potassium chloride for K with the amounts of 145 kg ha^{-1} of N, 49 kg ha^{-1} of P,

106 and 56 kg ha^{-1} of K, respectively. Additionally, the manure was added with the average

107 nutrient contents including 18000 kg ha^{-1} of C, 145 kg ha^{-1} of N, 49 kg ha^{-1} of P, and

108 56 kg ha^{-1} of K. All the mineral fertilizers and manure were applied as basal application.

109 Bulk soil samples collection with five different fertilizer treatments were conducted

110 before the harvest of late rice in October 2020 with field replications. In each field,

111 three soil cores (0-20 cm topsoil) were collected and then pooled to form a composite

112 sample. Besides, before the rhizosphere soil collection, the bulk soil was manually

113 removed, and approximately 1 mm of soil on the rice roots was collected as rhizosphere

114 soil (Shao et al., 2021). Soil samples used for physical and chemical analyses were four

115 replications (2 field replication×2 replication of each field, n=4) and those for DNA

116 extraction were six replications (2 field replication×3 replication of each field, n=6).

117 ## 2.2. Soil physical and chemical properties

118 Soil pH was measured by pH meter in the mixed solution (the mass ratio of soil and

119 water is 1:2.5). Soil moist content was measured by drying moist soil at 105 ℃ for 16

h until it became a constant mass. Total carbon (TC), organic carbon (OC), and total nitrogen (TN) were determined by CHNS elemental analyzer (Vario EL Cube manufactured by Elementar, Germany) (Schumacher, 2002). The soil was pretreated by 1M HCl with a soil-liquid ratio of 1:1 before OC determination. 1g soil was extracted with 5mL KCl (2M) to determine for ammonia-N (NH_4^+) by indophenol blue colorimetric method (Dorich and Nelson, 1983), and for nitrate-N (NO_3^-) by dual-wavelength ultraviolet spectrophotometry (Norman et al., 1985). After potassium persulfate and H_2SO_4 pre-digestion (Bowman, 1989), soil samples were determined for total P by a colorimetric method (Murphy and Riley, 1962). The extraction of available phosphorus (AP) was referred to the method described by Olsen (1954), and the concentration was measured using a colorimetric method (Murphy and Riley, 1962).

The extracted P with 0.5 M $NaHCO_3$ before/after 24 h of $CHCl_3$ fumigation was determined using ICP-OES (PerkinElmer, Avio 500, USA). A KEC factor of 0.4 was used for the calculation of soil microbial biomass P. Soil microbial biomass P was measured using a chloroform fumigation-extraction technique (Brookes et al., 1982). Additionally, phosphatases could mediate soil P transformation and recycling. The alkaline phosphatase in soil is released by bacteria, whereas acid phosphatase can derive

137 from plants, fungi and bacteria (Nannipieri et al., 2011; Acosta-Martínez and Ali

138 Tabatabai, 2011). The activities of acid and alkaline phosphatase were indicators to

139 reflect the microbial activity and P cycling ability in soil, and were assayed by the

140 method described by Tabatabai and Bremner (1969) using *p*-nitrophenyl phosphate as

141 substrate at 37 °C.

2.3 Organic P analyses

143 Soil organic P was extracted with NaOH-EDTA solution according to the method

144 described by Jiang et al. (2017). In short, 4 g air-dried soil was extracted for 4 h using

145 40 ml solution containing 0.25 M NaOH and 0.05 M Na_2EDTA. After centrifuging at

146 $13,000 \times g$ for 20 min, 2 mL aliquot of each supernatant was used to determine Fe, Mn,

147 and P by ICP-OES. The remaining supernatants were freeze-dried and prepared for

148 solution ^{31}P-NMR spectroscopy. Each freeze-dried extract (~100 mg) was re-dissolved

149 in 0.1 mL of deuterium oxide and 0.9 mL of a solution containing 1.0 M NaOH and 0.1

150 M Na_2EDTA, then immediately determined with solution ^{31}P-NMR spectra using a

151 Bruker 500-MHz spectrometer. The NMR parameters were: 28 K data points, 0.68 s

152 acquisition time, 90° pulse width, and 8000 scans. The repetition delay time was

153 calculated based on the concentration ratio of P to (Fe+Mn) according to the research

by Mcdowell et al. (2006). Peak areas were calculated by integration on spectra processed with 2 and 7 Hz line-broadening using MestReNova software. Phosphorus species were identified based on their chemical shifts, including orthophosphate (6 ppm), pyrophosphate (~ -5 ppm), polyphosphate (-4 to -5, -5 to -50 ppm), orthophosphate monoesters (3 to 6, 6 to 7 ppm), orthophosphate diesters (3 to -4 ppm), and phosphonates (7 to 50 ppm). The orthophosphate peak was standardized to 6 ppm during processing (Cade-Menun et al., 2010; Young et al., 2013). Individual P compounds were identified based on their chemical shifts from the study by (Cade-Menun, 2015) and by spiking selected samples with myo-inositol hexakisohosphate (myo-IHP), α- and β-glycerophosphates (Fig. S1 and S2).

The concentrations of individual P species were calculated by multiplying ^{31}P-NMR proportions by the total NaOH-Na$_2$EDTA extractable P concentration. The α- and β-glycerophosphates and mononucleotides were considered as degradation of orthophosphate diesters, though they were detected in the orthophosphate monoester region (Young et al., 2013; Liu et al., 2015).

2.4. Soil DNA extraction, PCR amplification, Illumina Miseq sequencing, and bioinformatics analyses

171   The DNA was extracted from 0.25 g soil using FastDNA® Spin Kit (MP Biomedicals,

172   USA). The purity and concentration of DNA were measured by Nanodrop 2000

173   (Thermo Fisher Scientific, USA). For bacteria, the V3-V4 region of the 16S rRNA gene

174   was amplified with the primer pair 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and

175   806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2012; Dennis et al.,

176   2013). For fungi, the primer pair ITS1F (CTTGGTCATTTAGAGGAAGTAA) and

177   ITS2 (GCTGCGTTCTTCATCGATGC) were used to target the ITS1 region (Blaalid et

178   al., 2013). After sequencing, the raw sequences of each sample were assembled by

179   QIIME 2 according to the unique barcode after removing the adaptors and primer

180   sequences (Bolyen et al., 2019). Demultiplexed sequences were quality filtered,

181   trimmed, de-noised, and merged, then the QIIME2 dada2 plugin was used to identify

182   and remove chimeric sequences to obtain the feature table of amplicon sequence variant

183   (ASV) (Callahan et al., 2016). ASV sequences were aligned to the GREENGENES

184   database and UNITE database separately to generate the taxonomy table for bacteria

185   and fungi (Bokulich et al., 2018). Besides, phosphorus-solubilizing microbes were

186   collected according to the researches by RodríGuez and Fraga (1999) as well as Alori

187   et al. (2017) (see Table S1). The raw reads of bacteria and fungi were deposited in the

NCBI Sequence Read Archive (SRA) database under accession numbers

PRJNA804681 and PRJNA805018, respectively.

2.5. Statistical analyses

All statistical analyses were conducted using SPSS 25.0. All indicators between

different fertilizer treatments (i.e., CK, NPK, M, NPKM, and NKM) were tested for

significant differences (set to $p < 0.05$) by one-way ANOVA. The LSD was used to test

significant differences of all indicators between bulk and rhizosphere soils. Alpha (α)

diversity indices, such as Chao1 richness estimator and Shannon diversity index, were

calculated using the core-diversity plugin within QIIME2. Nonmetric multidimensional

scaling (NMDS) based on Bray Curtis distance was measured by R package "vegan"

and visualized via R package "ggplot 2". Co-occurrence network analysis was

performed by using R package "psych" to calculate Spearman's rank correlations for

taxa among 6 repetitions of each treatment group and then Gephi 0.9.2 software was

used to draw networks. Redundancy analysis (RDA) was performed by Monte Carlo

analysis using Canoco 5 to reveal the association of microbial communities and soil

environmental factors.

3. Results

3.1 Soil physicochemical properties

In this study, we found that TC, TN, and OC increased significantly after the long-term application of fertilizers, especially for manure fertilization (Table 1). The concentrations of microbial biomass P increased under long-term fertilization (Table 1). Additionally, the activities of acidic phosphatase (ACP) were higher than alkaline phosphatase activities (ALP) for all the treatments (Fig. 1H and I). On the other side, soil pH value, gravimetric moisture, NO_3^--N, and NH_4^+-N contents were not affected by the long-term fertilizer treatments significantly (Table 1).

3.2 Soil P species

Long-term application of inorganic P fertilizer (NPK and NPKM) could significantly increase total P (TP), available P (AP) and inorganic P (IP) concentrations in both bulk and rhizosphere soil (Fig. 1A, B, and C). The concentrations of $NaOH-Na_2EDTA$ extracted P in the soils were ~243-739 mg·kg^{-1}, accounting for ~38-66% of total P (Table 2). Orthophosphate, pyrophosphate, orthophosphate monoesters (e.g. myo-IHP, scyllo-IHP), and orthophosphate diesters (e.g. DNA) were found in the soils (Table 2). The amounts of soil organic P (i.e. sum of orthophosphate monoesters and diesters) were not much and accounted for 8-30% of total P (data not shown). Generally, the

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222 concentrations of organic P were higher with long-term manure fertilization compared

223 to those of CK and NPK (Fig. 1D). Among the OP, the amounts of orthophosphate

224 monoesters (57-96 mg·kg^{-1}) were higher than those of orthophosphate diesters (34-65

225 mg·kg^{-1}) (Table 2). The long-term manure amendments had an obvious effect on the

226 accumulation of orthophosphate monoesters: the concentrations of orthophosphate

227 monoesters and myo-IHP were higher significantly with manure fertilization (i.e. M,

228 NPKM, NKM) than those with other treatments (i.e. CK, NPK) (Fig. 1E and G). The

229 concentrations of orthophosphate diesters were also higher with manure treatments

230 compared to CK and NPK although the tendency was not significant (Fig. 1F).

231 **3.3 Long-term fertilization and rhizosphere effect on the composition**

232 **of microbial community**

233 The dominant bacteria for different treatments at the phylum level were *Proteobacteria,*

234 *Acidobacteria, Chloroflexi,* and *Nitrospirae* and the dominant fungi were *Ascomycota*

235 and *Basidiomycota* (Fig. 2). As the most abundant phylum of bacteria, *Proteobacteria*

236 were further classified into *Alphaproteobacteria, Betaproteobacteria,*

237 *Gammaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria,* and unclassed

238 groups at the class level. *Gammaproteobacteria* was significantly more abundant for

239   manure treatments than for CK and NPK treatments. The abundance of

240   *Epsilonproteobacteria* increased after mineral fertilization. Both inorganic and organic

241   fertilization could increase the abundance of *Alphaproteobacteria* (Fig. S3). On the

242   other side, certain bacteria and fungi at the phylum level affected by fertilization were

243   different in rhizosphere and non-rhizosphere soils. For example, the long-term manure

244   fertilization accumulated more *Spirochaetes* but less *Actinobacteria* and *TM7* in non-

245   rhizosphere soils (Fig. 2A and C). The relative abundance of *Ascomycota* increased

246   significantly with fertilization in non-rhizosphere soils but not the case in the

247   rhizosphere soils (Fig. 2B and D). These results suggested that both long-term

248   fertilization and rhizosphere affected the microbial community composition together.

249   The relative abundances of PSB were also greatly influenced by fertilization and

250   rhizosphere. The *Thiobacillus* was the most abundant bacterium at genus level and

251   increased with long-term input of inorganic P in both bulk and rhizosphere soils (Fig. 3

252   A and C). Additionally, the long-term manure fertilization increased the abundance of

253   *Flavobacterium* in bulk soil. On the other side, the *Fusarium* was the most abundant

254   fungus at genus level (Fig. 3 B and D). The influence of fertilization on the phosphorus-

255   solubilizing fungi (PSF) in bulk soil was not obvious. However, manure fertilization

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256 increased the abundance of *Aspergillus* and *Trichoderma* in rhizosphere soils.

257 **3.4 Microbial community diversity**

258 Soil with long-term mineral fertilization (NPK) presented a lower bacterial richness and

259 evenness (i.e. Chao 1 and Shannon index) than those with manure fertilization

260 (M/NPKM/NKM) and even lower than control soil (CK), indicating that bacterial α-

261 diversity decreased after long-term mineral fertilizer regimes, but was not changed

262 under manure fertilization (Fig. 4). On the other side, rhizosphere effect was clearly

263 observed on the bacterial diversity: the richness and evenness of bacterial community

264 in rhizosphere soil were significantly higher than those in non-rhizosphere soil

265 (P<0.001). It is worth noting that fertilization and rhizosphere effect have no obvious

266 influence on fungal richness and evenness. It suggested that long-term fertilization and

267 rhizosphere affected the richness and evenness of bacterial and fungal communities

268 differently.

269 The plot of NMDS identified the variations in microbial β-diversity between different

270 sites, with the response of bacterial β-diversity being greater than that of fungal β-

271 diversity (Fig. 5). Specially, the profiles of bacterial β-diversity with manure

272 fertilizations (M, NKPM, NKM) were clearly separated from that for CK soil (Fig. 5 A

273   and C). The analysis of similarities (ANOSIM) revealed that R values for rhizosphere

274   soils between different fertilization treatments were higher than those for bulk soils

275   (Table S2). Accordingly, the variations in bacterial β-diversity of rhizosphere soils with

276   manure fertilization were greater than that of bulk soils (Fig. 5 A and C). These results

277   indicated that manure fertilization and rhizosphere effect exacerbated the variation of

278   bacterial β-diversity.

279   **3.5 Co-occurrence networks**

280   The co-occurrence network was used to analyze the ecological relationship of both

281   bacterial and fungal communities under five fertilization treatments. After long-term

282   mineral fertilization (NPK), total edges, average degree, positive edges, and

283   positive/negative edges ratio (i.e. P/N ratio) of bacteria and fungi network decreased

284   (Fig. 6 and Table S3). Additionally, the high P input (NPKM vs NKM) brought a larger

285   and more complex but less stable bacterial network (e.g. more total nodes, edges,

286   average degree, average clustering coefficient, average path length, and less

287   modularity). However, the opposite tendency was shown for fungus network (Fig. 6

288   and Table S3), indicating that the response of bacteria and fungi to the input of inorganic

289   P was different.

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3.6 Factors correlating with microbial community diversity

Redundancy analysis (RDA) was conducted to determine the correlation of soil properties with microbial community diversity in bulk and rhizosphere soils. The results showed that TC (10.4%, F=4.4, P=0.03), soil pH value (10.3%, F=4.4, P=0.03), TN (10.1%, F=4.3, P=0.03), and OC (9.2%, F=3.9, P=0.03) were significantly correlated with bacterial community diversity (Fig. 7A, Table S4). On the other side, for the fungus, the soil properties had extremely small explanations of <4.4% for the variation for fungi community (Table S4).

The RDA was also performed to establish the linkages of soil properties with community diversity of PSM. The soil properties together explained more than 55% of the variation in PSB community structure and those correlated with PSB contained TP (27.5%, F=14.4, P=0.03) and IP (26.6%, F=13.7, P=0.03) (Fig. 7C and Table S5). The PSB was well separated by RDA1 (52.60 %) between the samples with inorganic P application (i.e. NPK, NPKM) and without inorganic P application (i.e. CK, M, NKM) (Fig. 7C). The 30.08% of the total variance in the PSF community could be explained by the first and second axes (Fig. 7D).

4. Discussion

4.1. Long-term fertilization on soil P accumulation

Long-term organic P fertilization increased the utilization of P for crops compared to inorganic P fertilization. The same amount of P was added to soil whatever inorganic or organic fertilization but the total P of soil was significantly higher with mineral fertilization compared to manure treatment, suggesting more P was retained in soil and less P was utilized by crops under long-term mineral fertilization (Fig. 1A). Several researchers have already reported that inorganic P was easily immobilized by clay minerals and was dominantly associated with amorphous Fe/Al oxides compared to crystalline Fe/Al oxides fractions in many soil types such as Sandy soils, Ultisols, Luvisol, Ferralic Cambisol, and so on (Arai et al., 2005; Rick and Arai, 2011; Jiang et al., 2015; Ahmed et al., 2019). On the other side, it has been confirmed that the application of manure usually leads to an increase in labile organic P pools, which are protected from the process of adsorption on clay minerals and are readily available to plants (Braos et al., 2020; Kashem et al., 2004). In this study, manure fertilization increased microbial biomass P concentration and alkaline phosphatase activity compared to mineral fertilization (Fig. 1I, Table 1). It was possible that the mineralization of organic P such as orthophosphate diesters from microbes by alkaline

324   phosphatase increased under organic fertilization, thus improving the P availability for

325   crops.

326   The application of inorganic P fertilizer mainly increased the concentration of inorganic

327   P but manure fertilization accelerated the accumulation of organic P in soil, which was

328   consistent with our hypotheses (Fig. 1C and D). Phosphorus speciation was usually

329   regulated by the changes in soil mineralogy, mineral and organic P inputs, biological

330   production, and the utilization of various P species (Turner et al., 2007; Jiang et al.,

331   2017). Fertilization especially for manure accelerated the accrual of organic carbon

332   (Table 1) significantly, which also co-accumulated organic P. Generally, the content of

333   organic phosphorus (OP) from manures accounts for a large proportion of total P,

334   among which inositol phosphate (IHP) was the most abundant OP (Maguire et al., 2004).

335   Therefore, long-term manure fertilization also increased the input of OP in the field.

336   The organic P could be effectively mineralized by microorganisms and thus transferred

337   into various inorganic P fractions (Song et al., 2007).

338   The application of manure increased the accumulation of orthophosphate monoesters

339   significantly, especially for myo-inositol phosphates (myo-IHP) (Fig. 1E and G).

340   Normally, phosphate monoesters were the main group of organic P compounds and

341 existed as IHP mainly in most soils (Turner et al., 2005). Those orthophosphate

342 monoesters were commonly stabilized by association with soil minerals such as Fe/Al

343 oxides (Celi and Barberis, 2007; Turner and Engelbrecht, 2011; Jiang et al., 2015).

344 Therefore, the stability and immobilization of orthophosphate monoesters promoted

345 their accumulation in soil no matter by the input of manure or by the P transformation.

346 On the other side, separate manure fertilization (M) also increased the contents of

347 orthophosphate diesters significantly (Fig. 1F). Long-term manure fertilization

348 accumulated more microbial biomass P significantly (Table 1) that were rich in

349 orthophosphate diesters (Turner et al., 2007). The accumulation of orthophosphate

350 diesters under manure fertilization was probably due to the reduced decomposition of

351 plant residues and manure or increased microbial synthesis under anaerobic paddy-rice

352 management (Jiang et al., 2017).

353 **4.2 Long-term fertilization and rhizosphere effect on soil microbial**

354 **communities**

355 Our results indicated that long-term mineral fertilization decreased bacterial richness,

356 evenness, and the decrease of complexation of bacterial networks, indicating that long-

357 term mineral fertilization increased the stability of microbial network (e.g. lower P/N

358   ratio) but decreased the complexity of network (e.g. less total edges and lower average

359   degree) (Tu et al., 2020; Olesen et al., 2007; Hernandez et al., 2021). On the other side,

360   long-term organic fertilization did not change the bacterial richness and evenness, and

361   even promoted the separation of bacterial communities. This conclusion was also

362   expected in our hypothesis. Meanwhile, long-term manure treatments (M, NPKM,

363   NKM) increased the negative connections of microorganisms, and also promoted the

364   stability of network (Zhou et al., 2020). Previous studies have reported that long-term

365   mineral fertilization changed soil properties and these perturbations may have an

366   adverse effect on soil microbes (Marschner et al., 2003; Geisseler and Scow, 2014;

367   Liang et al., 2020). In contrast, organic fertilizer contained a large amount of organic

368   matter which could be utilized by soil bacteria (Wu et al., 2020; Wu et al., 2021).

369   Additionally, there were significant increases for diversity of bacterial communities in

370   rhizosphere soil compared to bulk soil. Generally, microbes concentrated in the

371   rhizosphere where organic compounds were released by plant roots (Achat et al., 2010),

372   and plants tend to recruit bacteria as symbiotic microbes by releasing phenolic

373   compounds (Gkarmiri et al., 2017; Badri et al., 2013).

374   Accordingly, redundancy analysis showed that the key factors related to the shift of

375   bacterial communities included pH, TC, TN, and OC. The previous study showed that

376   the soil bacterial community was indirectly impacted by pH via the alteration of metals

377   and nutrient availability (Xiao et al., 2021), and directly modulated by the abundance

378   and mineralization of carbon in soil (Chen et al., 2019) as well as soil nitrogen

379   deposition (Zeng et al., 2016). In this study, the long-term organic fertilization and

380   rhizosphere soil accumulated more TC, TN, and OC, which provided more nutrients,

381   changed the soil pH, and thus drove the shift of bacterial communities (Ingwersen et al.,

382   2008; Liu et al., 2019).

383   Additionally, the application of both mineral and organic fertilizers increased the

384   stability of bacterial networks (i.e., increasing negative correlations). Compared to CK,

385   long-term fertilization provided more nutrient elements, stimulated the growth and

386   competition of bacteria, and finally facilitated the stability of ecological network (Faust

387   and Raes, 2012; Simard et al., 2012).

388   It was worth noting that fertilization and rhizosphere effect had no obvious influence

389   on fungal community structure. Redundancy analysis showed that the explanations of

390   soil properties were extremely small for the variation for fungi community. It has been

391   found that fungi were less sensitive to soil substrates and environmental conditions

392 whereas bacteria were more sensitive (Dong et al., 2014). The high TOC provided by

393 the long-term fertilization and rhizosphere soil gave an advantage for bacteria to

394 compete with fungi for resources, thus decreasing influences of long-term fertilization

395 and rhizosphere on fungi (Zelezniak et al., 2015).

396 **4.3 Response of PSM**

397 *Thiobacillus* was the most abundant PSB at genus level and increased with the input of

398 inorganic P fertilizers in bulk and rhizosphere soil (Fig. 3 a and c). It was involved in

399 sulfur oxidation, and acidity resulted from sulfur oxidation could solubilize mineral P

400 (Aria et al., 2010). Acidic and anaerobic conditions provided by paddy-rice

401 management of red soil in this study were beneficial for the growth of *Thiobacillus*

402 considering that it belongs to acidophilic bacterium (Monachon et al., 2019; Kumar et

403 al., 2020). The applied calcium superphosphate as inorganic P fertilizer in this study

404 contained a certain amount of $CaSO_4$, therefore the input of inorganic P fertilizer also

405 provided S source for the growth of *Thiobacillus*. On the other side, *Fusarium* was the

406 most abundant PSF at genus level (Fig. 3 B and D) and was proven to produce organic

407 acid to solute the mineral P (Elias et al., 2016). It was known that *Fusarium* was widely

408 distributed in soil around the world and acted as a saprophyte (Deacon, 1997), among

409   which many species were also found as phytopathogens (Suga and Hyakumachi, 2004).

410   Besides, the long-term organic fertilization increased the abundance of *Flavobacterium,*

411   *Aspergillus*, and *Trichoderma. Flavobacterium* was associated with the degradation of

412   phosphotriester (Brown, 1980) and was proven to grow in a nutrient-rich condition

413   (Kraut-Cohen et al., 2021). *Aspergillus*, as a saprophytic fungus, could produce organic

414   acid to dissolve mineral phosphorus (Li et al., 2016) and also preferred to the nutrient-

415   rich condition (Martins et al., 2014). Additionally, *Trichoderma* as a biological control

416   fungi (Zin and Badaluddin, 2020) was colonized in the root epidermis and outer cortical

417   layers (Harman, 2006). Long-term organic fertilization provided more organic matter

418   for these microbes.

419   PSM could solubilize mineral P and mineralize organic P (Sharma et al., 2013). The

420   PSB of samples with inorganic P input (i.e. NPK, NPKM) and none mineral P

421   application (i.e. CK, M, NKM) could be well separated, indicating mineral P had a

422   strong effect on community diversity of PSB. Correspondingly, TP and IP were key

423   factors driving the diversity of soil PSB community and those indicators were all higher

424   significantly with inorganic P amendments (Fig. 1A, and C). As discussed before,

425   *Thiobacillus* as the most abundant PSB at genus level in this study increased with the

426 input of mineral P. It is because that mineral P could provide additional S source for the

427 growth of *Thiobacillus*. Furthermore, the availability of P in soil was considered as a

428 key condition for PSM to express P-solubilization traits. Low availability of P in soil is

429 widely considered as a favorable condition for PSM whereas recent studies suggested

430 that a minimum P threshold is required to achieve a response by plants (Sánchez-Esteva

431 et al., 2016; Gómez-Muñoz et al., 2018; Raymond et al., 2021).

432 5. Conclusion

433 Long-term inorganic and organic fertilization managements brought different effects on

434 P accumulation, microbial community, and PSB. Long-term mineral fertilization

435 increased inorganic and available P concentrations, while manure fertilization increased

436 soil organic P concentrations, microbial biomass P contents, and potential organic P

437 mineralization. The turnover of P by bacteria seems strong under long-term organic

438 fertilization and rhizosphere soil considering that more organic nutrient was provided

439 for bacteria and the bacterial community diversity increased. Furthermore, inorganic P

440 fertilization increased the abundance of *Thiobacillus* whereas organic fertilization

441 increased the abundance of *Flavobacterium, Aspergillus*, and *Trichoderma*. The

442 concentrations of TP and IP strongly influenced by inorganic P fertilization were key

factors driving the diversity of soil PSB community. These findings provide useful

insights into P accumulation, turnover, and soil P sustainable fertility under different

fertilization strategies.

Author contribution:

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References:

Achat, D., Bakker, M., Zeller, B. o. B., Pellerin, S., Bienaimé, S., and Morel, C.: Long-term organic phosphorus mineralization in Spodosols under forests and its relation to carbon and nitrogen mineralization, SOIL BIOL BIOCHEM, 42, 1479-1490, https://doi.org/10.1016/j.soilbio.2010.05.020, 2010.

Acosta-Martínez, V. and Ali Tabatabai, M.: Phosphorus Cycle Enzymes, in: Methods of Soil Enzymology, SSSA Book Series, 161-183, https://doi.org/10.2136/sssabookser9.c8, 2011.

Ahmed, W., Liu, K., Qaswar, M., Huang, J., Huang, Q., Xu, Y., Ali, S., Mehmood, S., Ammar Asghar, R. M., Mahmood, M., and Zhang, H.: Long-term mineral fertilization improved the grain yield and phosphorus use efficiency by changing soil P fractions in ferralic cambisol, AGRONOMY, 9, 784, https://doi.org/10.3390/agronomy9120784, 2019.

Alori, E. T., Glick, B. R., and Babalola, O. O.: Microbial phosphorus solubilization and its potential for use in sustainable agriculture, FRONT MICROBIOL, 8, 971, https://doi.org/10.3389/fmicb.2017.00971, 2017.

Arai, Y., Livi, K. J. T., and Sparks, D. L.: Phosphate reactivity in long-term poultry litter-amended southern delaware sandy soils, SOIL SCI SOC AM J, 69, 616-629, https://doi.org/10.2136/sssaj2004.0218, 2005.

Aria, M. M., Lakzian, A., Haghnia, G. H., Berenji, A. R., Besharati, H., and Fotovat, A.: Effect of *Thiobacillus*, sulfur, and vermicompost on the water-soluble phosphorus of hard rock phosphate, BIORESOURCE TECHNOL, 101, 551-554, https://doi.org/10.1016/j.biortech.2009.07.093, 2010.

Babalola, O. and Glick, B.: Indigenous African agriculture and plant associated microbes: Current

487 practice and future transgenic prospects, SCI RES ESSAYS, 7, 2431-2439,
488 https://doi.org/10.5897/SRE11.1714, 2012.

489 Badri, D. V., Chaparro, J. M., Zhang, R., Shen, Q., and Vivanco, J. M.: Application of natural blends of
490 phytochemicals derived from the root exudates of *Arabidopsis* to the soil reveal that phenolic-related
491 compounds predominantly modulate the soil microbiome, J BIOL CHEM, 288, 4502-4512,
492 https://doi.org/10.1074/jbc.M112.433300, 2013.

493 Baxter, S.: World Reference Base for Soil Resources. World Soil Resources Report 103. Rome: Food and
494 Agriculture Organization of the United Nations (2006), pp. 132, US$22.00 (paperback). ISBN 92-5-
495 10511-4, Experimental Agriculture, 43, 264-264, https://doi.org/10.1017/S0014479706394902, 2007.

496 Blaalid, R., Kumar, S., Nilsson, R. H., Abarenkov, K., Kirk, P. M., and Kauserud, H.: ITS1 versus ITS2
497 as DNA metabarcodes for fungi, MOL ECOL RESOUR, 13, 218-224, https://doi.org/10.1111/1755-
498 0998.12065, 2013.

499 Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., and
500 Gregory Caporaso, J.: Optimizing taxonomic classification of marker-gene amplicon sequences with
501 QIIME 2's q2-feature-classifier plugin, MICROBIOME, 6, 90, https://doi.org/10.1186/s40168-018-
502 0470-z, 2018.

503 Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H.,
504 Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J.,
505 Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., Cope, E. K., Da Silva, R., Diener,
506 C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki,
507 M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J.,
508 Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang,
509 L., Kaehler, B. D., Kang, K. B., Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciolek,
510 T., Kreps, J., Langille, M. G. I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M.,
511 Marotz, C., Martin, B. D., McDonald, D., McIver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C.,
512 Morton, J. T., Naimey, A. T., Navas-Molina, J. A., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples,
513 S. L., Petras, D., Preuss, M. L., Pruesse, E., Rasmussen, L. B., Rivers, A., Robeson, M. S., Rosenthal, P.,
514 Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear, J. R., Swafford, A. D., Thompson, L.
515 R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., van der Hooft, J. J. J., Vargas, F.,
516 Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber,
517 K. C., Williamson, C. H. D., Willis, A. D., Xu, Z. Z., Zaneveld, J. R., Zhang, Y., Zhu, Q., Knight, R., and
518 Caporaso, J. G.: Reproducible, interactive, scalable and extensible microbiome data science using QIIME
519 2, NAT BIOTECHNOL, 37, 852-857, https://doi.org/10.1038/s41587-019-0209-9, 2019.

520 Bowman, R. A.: A Sequential Extraction Procedure with Concentrated Sulfuric Acid and Dilute Base for

Soil Organic Phosphorus, SOIL SCI SOC AM J, 53, 362-366, https://doi.org/10.2136/sssaj1989.03615995005300020008x, 1989.

Braos, L. B., Bettiol, A. C. T., Di Santo, L. G., Ferreira, M. E., and Cruz, M. C. P.: Dynamics of phosphorus fractions in soils treated with dairy manure, SOIL RES, 58, 289-298, https://doi.org/10.1071/SR18325, 2020.

Brookes, P. C., Powlson, D. S., and Jenkinson, D. S.: Measurement of microbial biomass phosphorus in soil, SOIL BIOL BIOCHEM, 14, 319-329, https://doi.org/10.1016/0038-0717(82)90001-3, 1982.

Brown, K. A.: Phosphotriesterases of *flavobacterium* sp, SOIL BIOL BIOCHEM, 12, 105-112, https://doi.org/10.1016/0038-0717(80)90044-9, 1980.

Cade-Menun, B. J.: Improved peak identification in ^{31}P-NMR spectra of environmental samples with a standardized method and peak library, GEODERMA, 257-258, 102-114, https://doi.org/10.1016/j.geoderma.2014.12.016, 2015.

Cade-Menun, B. J., Carter, M. R., James, D. C., and Liu, C. W.: Phosphorus forms and chemistry in the soil profile under long-term conservation tillage: A phosphorus-31 nuclear magnetic resonance study, J ENVIRON QUAL, 39, 1647-1656, https://doi.org/10.2134/jeq2009.0491, 2010.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P.: DADA2: High-resolution sample inference from Illumina amplicon data, NAT METHODS, 13, 581-583, https://doi.org/10.1038/nmeth.3869, 2016.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., and Knight, R.: Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms, ISME J, 6, 1621-1624, https://doi.org/10.1038/ismej.2012.8, 2012.

Celi, L. and Barberis, E.: Abiotic reactions of inositol phosphates in soil. Inositol phosphates, in: Inositol phosphates: Linking agriculture and the environment, 1st ed., edited by: Turner, B. L., Richardson, A. E., Mullaney, E. J., CABI Publishing, Wallingford, Oxfordshire, England, 207-220, https://doi.org/10.1079/9781845931520.0207, 2007.

Chen, H., Zhao, X., Lin, Q., Li, G., and Kong, W.: Using a combination of PLFA and DNA-based sequencing analyses to detect shifts in the soil microbial community composition after a simulated spring precipitation in a semi-arid grassland in China, SCI TOTAL ENVIRON, 657, 1237-1245, https://doi.org/10.1016/j.scitotenv.2018.12.126, 2019.

Deacon, J. W.: Modern mycology, 3rd, Blackwell Science Oxford, Hoboken, New Jersey, 1997.

Dennis, K. L., Wang, Y., Blatner, N. R., Wang, S., Saadalla, A., Trudeau, E., Roers, A., Weaver, C. T., Lee, J. J., Gilbert, J. A., Chang, E. B., and Khazaie, K.: Adenomatous polyps are driven by microbe-instigated focal inflammation and are controlled by IL-10–Producing T cells, CANCER RES, 73, 5905-

5913, https://doi.org/10.1158/0008-5472.CAN-13-1511, 2013.

Dong, W.-Y., Zhang, X., Dai, X.-Q., Fu, X., Yang, F.-T., Liu, X.-Y., Sun, X., Wen, X., and Schaeffer, S.: Changes in soil microbial community composition in response to fertilization of paddy soils in subtropical China, APPL SOIL ECOL, 84, 140–147, https://doi.org/10.1016/j.apsoil.2014.06.007, 2014.

Dorich, R. A. and Nelson, D. W.: Direct colorimetric measurement of ammonium in potassium chloride extracts of soils, SOIL SCI SOC AM J, 47, 833-836, https://doi.org/10.2136/sssaj1983.03615995004700040042x, 1983.

Elias, F., Woyessa, D., and Muleta, D.: Phosphate solubilization potential of rhizosphere Fungi isolated from plants in Jimma Zone, Southwest Ethiopia, INT J MICROBIOL, 2016, 5472601, https://doi.org/10.1155/2016/5472601, 2016.

Faust, K. and Raes, J.: Microbial interactions: from networks to models, NAT REV MICROBIOL, 10, 538-550, https://doi.org/10.1038/nrmicro2832, 2012.

Gao, J., Cao, W., Li, D., Xu, M., Zeng, X., Nie, J., and Zhang, W.: Effects of long-term double-rice and green manure rotation on rice yield and soil organic matter in paddy field, ACTA ECOL SIN, 31, 4542-4548, 2011.

Geisseler, D. and Scow, K. M.: Long-term effects of mineral fertilizers on soil microorganisms – A review, SOIL BIOL BIOCHEM, 75, 54-63, https://doi.org/10.1016/j.soilbio.2014.03.023, 2014.

Gkarmiri, K., Mahmood, S., Ekblad, A., Alström, S., Högberg, N., Finlay, R., and Loeffler Frank, E.: Identifying the Active Microbiome Associated with Roots and Rhizosphere Soil of Oilseed Rape, APPL ENVIRON MICROB, 83, e01938-01917, https://doi.org/10.1128/AEM.01938-17, 2017.

Gómez-Muñoz, B., Jensen, L. S., de Neergaard, A., Richardson, A. E., and Magid, J.: Effects of *Penicillium bilaii* on maize growth are mediated by available phosphorus, PLANT SOIL, 431, 159-173, https://doi.org/10.1007/s11104-018-3756-9, 2018.

Grant, C., Bittman, S., Montreal, M., Plenchette, C., and Morel, C.: Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development, CAN J PLANT SCI, 85, 3-14, https://doi.org/10.4141/p03-182, 2005.

Harman, G. E.: Overview of mechanisms and uses of *Trichoderma* spp, PHYTOPATHOLOGY®, 96, 190-194, https://doi.org/10.1094/PHYTO-96-0190, 2006.

Hernandez, D. J., David, A. S., Menges, E. S., Searcy, C. A., and Afkhami, M. E.: Environmental stress destabilizes microbial networks, ISME J, 15, 1722-1734, https://doi.org/10.1038/s41396-020-00882-x, 2021.

Ingwersen, J., Poll, C., Streck, T., and Kandeler, E.: Micro-scale modelling of carbon turnover driven by microbial succession at a biogeochemical interface, SOIL BIOL BIOCHEM, 40, 864-878, https://doi.org/10.1016/j.soilbio.2007.10.018, 2008.

589 Jiang, X., Bol, R., Willbold, S., Vereecken, H., and Klumpp, E.: Speciation and distribution of P
590 associated with Fe and Al oxides in aggregate-sized fraction of an arable soil, BIOGEOSCIENCES, 12,
591 6443-6452, https://doi.org/10.5194/bg-12-6443-2015, 2015.
592 Jiang, X., Amelung, W., Cade-Menun, B. J., Bol, R., Willbold, S., Cao, Z., and Klumpp, E.: Soil organic
593 phosphorus transformations during 2000 years of paddy-rice and non-paddy management in the Yangtze
594 River Delta, China, SCI REP-UK, 7, 10818, https://doi.org/10.1038/s41598-017-10071-0, 2017.
595 Kashem, M. A., Akinremi, O. O., and Racz, G. J.: Phosphorus fractions in soil amended with organic and
596 inorganic phosphorus sources, CAN J SOIL SCI, 84, 83-90, https://doi.org/10.4141/S03-018, 2004.
597 Kraut-Cohen, J., Shapiro, O. H., Dror, B., and Cytryn, E.: Pectin induced colony expansion of soil-
598 derived *Flavobacterium* strains, FRONT MICROBIOL, 12, 544,
599 https://doi.org/10.3389/fmicb.2021.651891, 2021.
600 Kumar, M., Zeyad, M. T., Choudhary, P., Paul, S., Chakdar, H., and Singh Rajawat, M. V.: *Thiobacillus*,
601 in: Beneficial Microbes in Agro-Ecology, 1st ed., edited by: Amaresan, N., Senthil Kumar, M.,
602 Annapurna, K., Kumar, K., and Sankaranarayanan, A., Academic Press, 545-557,
603 https://doi.org/10.1016/B978-0-12-823414-3.00026-5, 2020.
604 Li, Z., Bai, T., Dai, L., Wang, F., Tao, J., Meng, S., Hu, Y., Wang, S., and Hu, S.: A study of organic acid
605 production in contrasts between two phosphate solubilizing fungi: *Penicillium oxalicum* and *Aspergillus*
606 *niger*, SCI REP-UK, 6, 25313, https://doi.org/10.1038/srep25313, 2016.
607 Liang, R., Hou, R., Li, J., Lyu, Y., Hang, S., Gong, H., and Ouyang, Z.: Effects of different fertilizers on
608 rhizosphere bacterial communities of winter wheat in the North China plain, AGRONOMY, 10, 93,
609 https://doi.org/10.3390/agronomy10010093, 2020.
610 Liu, J., Hu, Y., Yang, J., Abdi, D., and Cade-Menun, B. J.: Investigation of soil legacy phosphorus
611 transformation in long-term agricultural fields using sequential fractionation, P K-edge XANES and
612 solution P NMR spectroscopy, ENVIRON SCI TECHNOL, 49, 168-176,
613 https://doi.org/10.1021/es504420n, 2015.
614 Liu, M., Sui, X., Hu, Y., and Feng, F.: Microbial community structure and the relationship with soil
615 carbon and nitrogen in an original Korean pine forest of Changbai Mountain, China, BMC MICROBIOL,
616 19, 218, https://doi.org/10.1186/s12866-019-1584-6, 2019.
617 Maguire, R. O., Sims, J. T., Saylor, W. W., Turner, B. L., Angel, R., and Applegate, T. J.: Influence of
618 phytase addition to poultry diets on phosphorus forms and solubility in litters and amended soils, J
619 ENVIRON QUAL, 33, 2306-2316, https://doi.org/10.2134/jeq2004.2306, 2004.
620 Marschner, P., Kandeler, E., and Marschner, B.: Structure and function of the soil microbial community
621 in a long-term fertilizer experiment, SOIL BIOL BIOCHEM, 35, 453-461,
622 https://doi.org/10.1016/S0038-0717(02)00297-3, 2003.

Martins, I., Hartmann, D. O., Alves, P. C., Martins, C., Garcia, H., Leclercq, C. C., Ferreira, R., He, J., Renaut, J., Becker, J. D., and Silva Pereira, C.: Elucidating how the saprophytic fungus *Aspergillus nidulans* uses the plant polyester suberin as carbon source, BMC GENOMICS, 15, 613, https://doi.org/10.1186/1471-2164-15-613, 2014.

McDowell, R. W., Stewart, I., and Cade-Menun, B. J.: An examination of spin–lattice relaxation times for analysis of soil and manure extracts by liquid state phosphorus-31 nuclear magnetic resonance spectroscopy, J ENVIRON QUAL, 35, 293-302, https://doi.org/10.2134/jeq2005.0285, 2006.

Monachon, M., Albelda-Berenguer, M., and Joseph, E.: Biological oxidation of iron sulfides, in: Advances in Applied Microbiology, 1st ed., edited by: Gadd, G. M., and Sariaslani, S., Academic Press, Cambridge, Massachusetts, USA, 1-27, https://doi.org/10.1016/bs.aambs.2018.12.002, 2019.

Murphy, J. and Riley, J. P.: A modified single solution method for the determination of phosphate in natural waters, ANAL CHIM ACTA, 27, 31-36, https://doi.org/10.1016/S0003-2670(00)88444-5, 1962.

Nannipieri, P., Giagnoni, L., Landi, L., and Renella, G.: Role of Phosphatase Enzymes in Soil, in, 215-243, https://doi.org/10.1007/978-3-642-15271-9_9, 2011.

Norman, R. J., Edberg, J. C., and Stucki, J. W.: Determination of nitrate in soil extracts by dual-wavelength ultraviolet spectrophotometry, SOIL SCI SOC AM J, 49, 1182-1185, https://doi.org/10.2136/sssaj1985.03615995004900050022x, 1985.

Olesen, J. M., Bascompte, J., Dupont, Y. L., and Jordano, P.: The modularity of pollination networks, PNAS, 104, 19891-19896, https://doi.org/10.1073/pnas.0706375104, 2007.

Olsen, S. R., C. V. Cole, F. S. Watanabe, and L. A. Dean.: Estimation of available phosphorus in soils by extraction with sodium bicarbonate, 1st, Miscellaneous Paper Institute for Agricultural Research, Washington, D.C.: U.S. Government Printing Office.1954.

Qaswar, M., Jing, H., Ahmed, W., Dongchu, L., Shujun, L., Lu, Z., Cai, A., Lisheng, L., Yongmei, X., Jusheng, G., and Huimin, Z.: Yield sustainability, soil organic carbon sequestration and nutrients balance under long-term combined application of manure and inorganic fertilizers in acidic paddy soil, SOIL TILL RES, 198, 104569, https://doi.org/10.1016/j.still.2019.104569, 2020.

Raymond, N. S., Gómez-Muñoz, B., van der Bom, F. J. T., Nybroe, O., Jensen, L. S., Müller-Stöver, D. S., Oberson, A., and Richardson, A. E.: Phosphate-solubilising microorganisms for improved crop productivity: a critical assessment, NEW PHYTOL, 229, 1268-1277, https://doi.org/10.1111/nph.16924, 2021.

Rick, A. R. and Arai, Y.: Role of natural nanoparticles in phosphorus transport processes in ultisols, SOIL SCI SOC AM J, 75, 335-347, https://doi.org/10.2136/sssaj2010.0124nps, 2011.

Rodríguez, H. and Fraga, R.: Phosphate solubilizing bacteria and their role in plant growth promotion, BIOTECHNOL ADV, 17, 319-339, https://doi.org/10.1016/S0734-9750(99)00014-2, 1999.

Sánchez-Esteva, S., Gómez-Muñoz, B., Jensen, L. S., de Neergaard, A., and Magid, J.: The effect of *Penicillium bilaii* on wheat growth and phosphorus uptake as affected by soil pH, soil P and application of sewage sludge, CHEM BIOCHEM TECH AGR, 3, 21, https://doi.org/10.1186/s40538-016-0075-3, 2016.

Schneider, K. D., Cade-Menun, B. J., Lynch, D. H., and Voroney, R. P.: Soil phosphorus forms from organic and conventional forage fields, SOIL SCI SOC AM J, 80, 328-340, https://doi.org/10.2136/sssaj2015.09.0340, 2016.

Schumacher, B. A.: Methods for the determination of total organic carbon (TOC) in soils and sediments, Ecological Risk Assessment Support Center Office of Research and Development, Washington, DC, 2002.

Shao, J., Miao, Y., Liu, K., Ren, Y., Xu, Z., Zhang, N., Feng, H., Shen, Q., Zhang, R., and Xun, W.: Rhizosphere microbiome assembly involves seed-borne bacteria in compensatory phosphate solubilization, SOIL BIOL BIOCHEM, 159, https://doi.org/10.1016/j.soilbio.2021.108273, 2021.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., and Gobi, T. A.: Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils, SPRINGERPLUS, 2, 587, https://doi.org/10.1186/2193-1801-2-587, 2013.

Sharpley, A. and Moyer, B.: Phosphorus forms in manure and compost and their release during simulated rainfall, J ENVIRON QUAL, 29, 1462-1469, https://doi.org/10.2134/jeq2000.00472425002900050012x, 2000.

Simard, S. W., Beiler, K. J., Bingham, M. A., Deslippe, J. R., Philip, L. J., and Teste, F. P.: Mycorrhizal networks: Mechanisms, ecology and modelling, FUNGAL BIOL REV, 26, 39-60, https://doi.org/10.1016/j.fbr.2012.01.001, 2012.

Song, C., Han, X. Z., and Tang, C.: Changes in phosphorus fractions, sorption and release in udic mollisols under different ecosystems, BIOL FERT SOILS, 44, 37-47, https://doi.org/10.1007/s00374-007-0176-z, 2007.

Suga, H. and Hyakumachi, M.: Genomics of phytopathogenic *fusarium*, in: Applied Mycology and Biotechnology, 4th ed., edited by: Arora, D. K., and Khachatourians, G. G., Elsevier, Amsterdam, 161-189, https://doi.org/10.1016/S1874-5334(04)80009-1, 2004.

Tabatabai, M. A. and Bremner, J. M.: Use of p-nitrophenyl phosphate for assay of soil phosphatase activity, SOIL BIOL BIOCHEM, 1, 301-307, https://doi.org/10.1016/0038-0717(69)90012-1, 1969.

Tao, L., Wen, X., Li, H., Huang, C., Jiang, Y., Liu, D., and Sun, B.: Influence of manure fertilization on soil phosphorous retention and clay mineral transformation: Evidence from a 16-year long-term fertilization experiment, APPL CLAY SCI, 204, 106021, https://doi.org/10.1016/j.clay.2021.106021, 2021.

691 Tu, Q., Yan, Q., Deng, Y., Michaletz, S. T., Buzzard, V., Weiser, M. D., Waide, R., Ning, D., Wu, L., He,
692 Z., and Zhou, J.: Biogeographic patterns of microbial co-occurrence ecological networks in six American
693 forests, SOIL BIOL BIOCHEM, 148, 107897, https://doi.org/10.1016/j.soilbio.2020.107897, 2020.

694 Turner, B. L. and Engelbrecht, B. M. J.: Soil organic phosphorus in lowland tropical rain forests,
695 BIOGEOCHEMISTRY, 103, 297-315, https://doi.org/10.1007/s10533-010-9466-x, 2011.

696 Turner, B. L., Cade-Menun, B. J., Condron, L. M., and Newman, S.: Extraction of soil organic
697 phosphorus, TALANTA, 66, 294-306, https://doi.org/10.1016/j.talanta.2004.11.012, 2005.

698 Turner, B. L., Condron, L. M., Richardson, S. J., Peltzer, D. A., and Allison, V. J.: Soil organic phosphorus
699 transformations during pedogenesis, ECOSYSTEMS, 10, 1166-1181, https://doi.org/10.1007/s10021-
700 007-9086-z, 2007.

701 Wen, Y., Liu, W., Deng, W., He, X., and Yu, G.: Impact of agricultural fertilization practices on organo-
702 mineral associations in four long-term field experiments: Implications for soil C sequestration, SCI
703 TOTAL ENVIRON, 651, 591-600, https://doi.org/10.1016/j.scitotenv.2018.09.233, 2019.

704 WRB: World Reference Base for soil resources 2014: international soil classification system for naming
705 soils and creating legends for soil maps, Food and Agriculture Organization of the United Nations, Rome,
706 2014.

707 Wu, J., Sha, C., Wang, M., Ye, C., Li, P., and Huang, S.: Effect of organic fertilizer on soil bacteria in
708 maize fields, LAND, 10, 328, https://doi.org/10.3390/land10030328, 2021.

709 Wu, L., Jiang, Y., Zhao, F., He, X., Liu, H., and Yu, K.: Increased organic fertilizer application and
710 reduced chemical fertilizer application affect the soil properties and bacterial communities of grape
711 rhizosphere soil, SCI REP-UK, 10, 9568, https://doi.org/10.1038/s41598-020-66648-9, 2020.

712 Xiao, E., Ning, Z., Xiao, T., Sun, W., and Jiang, S.: Soil bacterial community functions and distribution
713 after mining disturbance, SOIL BIOL BIOCHEM, 157, 108232,
714 https://doi.org/10.1016/j.soilbio.2021.108232, 2021.

715 Yang, Z.-p., Xu, M.-g., Zheng, S.-x., Nie, J., Gao, J.-s., Liao, Y.-l., and Xie, J.: Effects of Long-Term
716 Winter Planted Green Manure on Physical Properties of Reddish Paddy Soil Under a Double-Rice
717 Cropping System, J INTEGR AGR, 11, 655-664, https://doi.org/10.1016/S2095-3119(12)60053-7, 2012.

718 Young, E. O., Ross, D. S., Cade-Menun, B. J., and Liu, C. W.: Phosphorus speciation in riparian soils: a
719 phosphorus-31 nuclear magnetic resonance spectroscopy and enzyme hydrolysis study, SOIL SCI SOC
720 AM J, 77, 1636-1647, https://doi.org/10.2136/sssaj2012.0313, 2013.

721 Yue, X., Zhang, J., Shi, A., Yao, S., and Zhang, B.: Manure substitution of mineral fertilizers increased
722 functional stability through changing structure and physiology of microbial communities, EUR J SOIL
723 BIOL, 77, 34-43, https://doi.org/10.1016/j.ejsobi.2016.10.002, 2016.

724 Zelezniak, A., Andrejev, S., Ponomarova, O., Mende, D. R., Bork, P., and Patil, K. R.: Metabolic

725 dependencies drive species co-occurrence in diverse microbial communities, PNAS, 112, 6449-6454,
726 https://doi.org/10.1073/pnas.1421834112, 2015.
727 Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., and Chu, H.: Nitrogen fertilization directly
728 affects soil bacterial diversity and indirectly affects bacterial community composition, SOIL BIOL
729 BIOCHEM, 92, 41-49, https://doi.org/10.1016/j.soilbio.2015.09.018, 2016.
730 Zhang, X., Zhang, R., Gao, J., Wang, X., Fan, F., Ma, X., Yin, H., Zhang, C., Feng, K., and Deng, Y.:
731 Thirty-one years of rice-rice-green manure rotations shape the rhizosphere microbial community and
732 enrich beneficial bacteria, SOIL BIOL BIOCHEM, 104, 208-217,
733 https://doi.org/10.1016/j.soilbio.2016.10.023, 2017.
734 Zhou, H., Gao, Y., Jia, X., Wang, M., Ding, J., Cheng, L., Bao, F., and Wu, B.: Network analysis reveals
735 the strengthening of microbial interaction in biological soil crust development in the Mu Us Sandy Land,
736 Northwestern China, SOIL BIOL BIOCHEM, 144, 107782,
737 https://doi.org/10.1016/j.soilbio.2020.107782, 2020.
738 Zin, N. A. and Badaluddin, N. A.: Biological functions of *Trichoderma* spp. for agriculture applications,
739 ANN AGR SCI, 65, 168-178, https://doi.org/10.1016/j.aoas.2020.09.003, 2020.
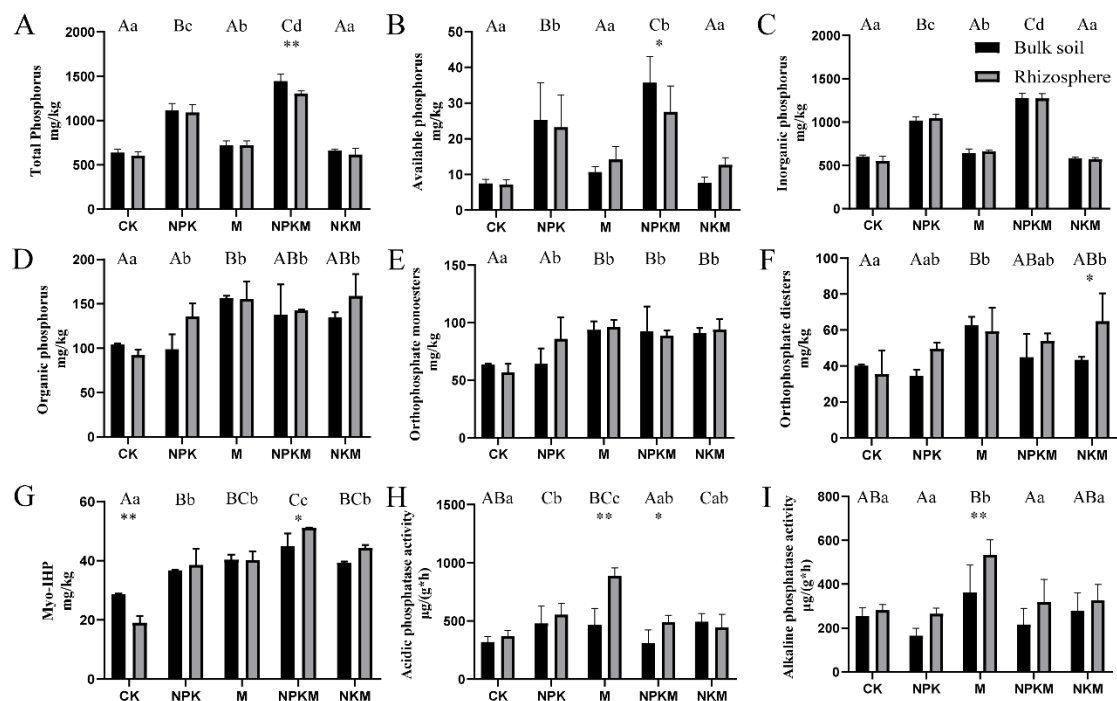
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Fig. 1 Different phosphorus forms and phosphatase activities in five treatments (CK, NPK, M, NPKM, NKM) and

two sample types (rhizosphere and bulk soil), where A: Total phosphorus, B: Available phosphorus, C: Inorganic

phosphorus, D: Organic phosphorus, E: Orthophosphate monoesters, F: Orthophosphate diesters, G: Myo-IHP, H:

Acidic phosphatase activity, I: Alkaline phosphatase activity. Significant differences between treatments in bulk soil

are indicated by capital letters (p<0.05, n = 4). Significant differences between treatments in rhizosphere are indicated

by lowercase letters (p<0.05, n = 4). Significant differences between rhizosphere and bulk soil are indicated by

asterisks, where * p < 0.05, ** p < 0.01 (Duncan's test, n=4)

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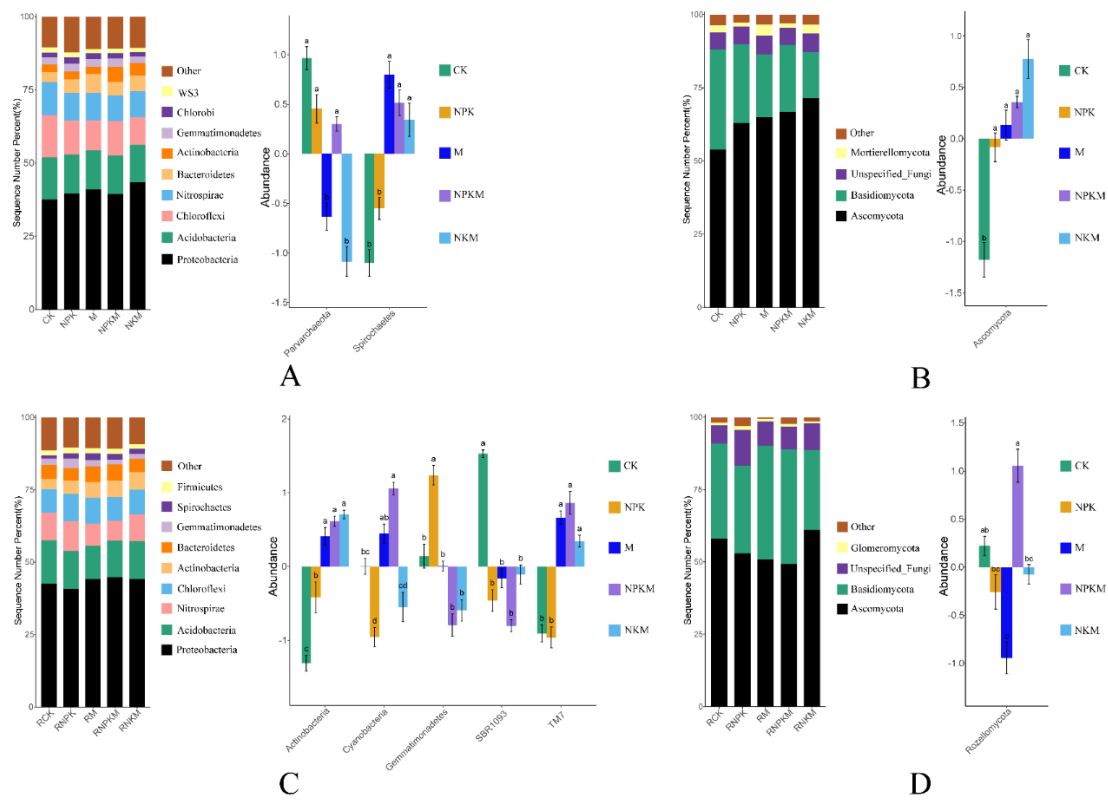
755 Fig. 2 The microbial relative abundance (left) and the features with significant differences (Anova + Duncan, p<0.05,

756 n=6) between groups (right) at the phylum level in five treatments (CK, NPK, M, NPKM, NKM). Capital letters

757 means different classification (A: bacteria in bulk soil, B: fungi in bulk soil, C: bacteria in rhizosphere soil, D: fungi
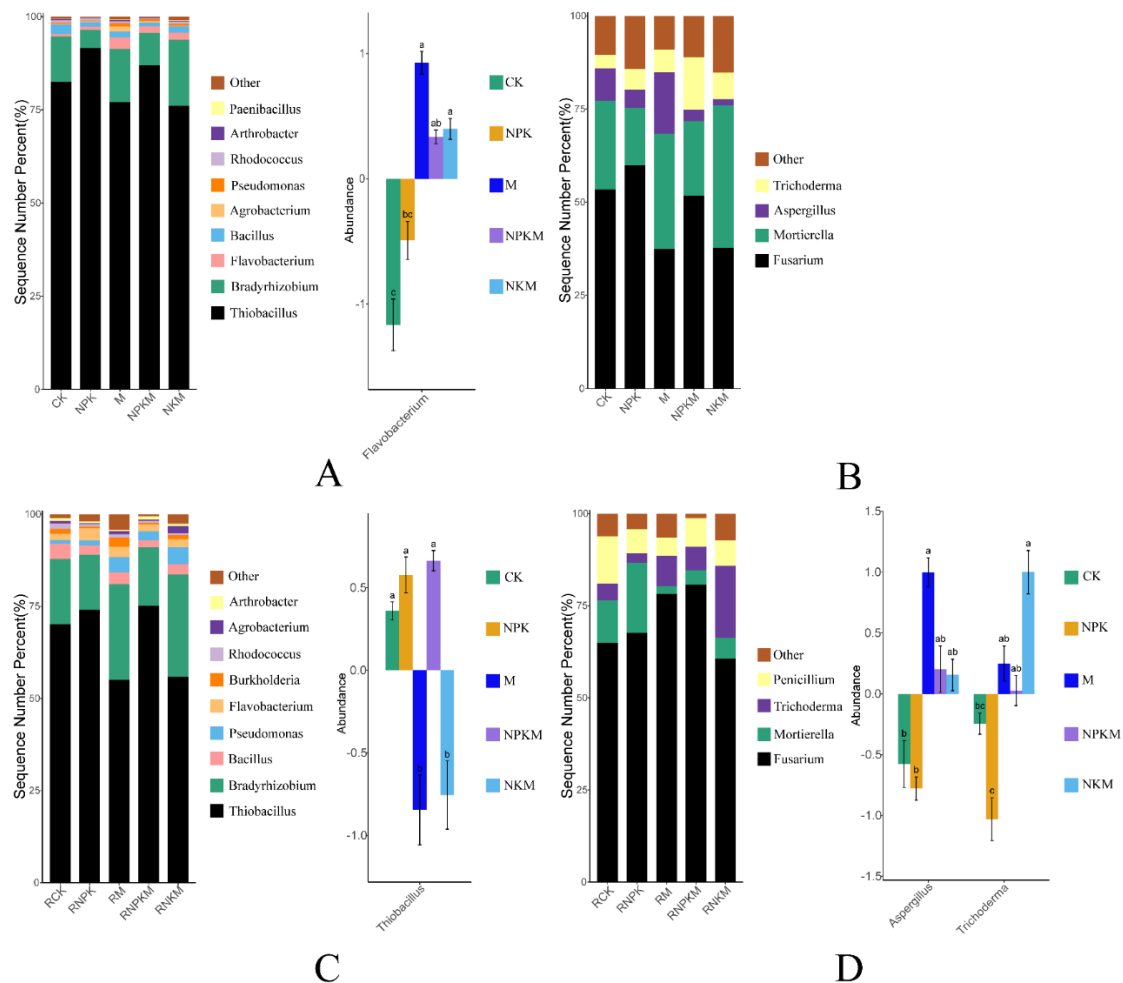
758 in rhizosphere soil)

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761 Fig. 3 The relative abundance of phosphorus-solubilizing microbe (left) and features with significant differences
762 (Anova + Duncan, p<0.05, n=6) between groups (right) at the genus level in five treatments (CK, NPK, M, NPKM,
763 NKM). Capital letters means different classification (A: bacteria in bulk soil, B: fungi in bulk soil, C: bacteria in
764 rhizosphere soil, D: fungi in rhizosphere soil)
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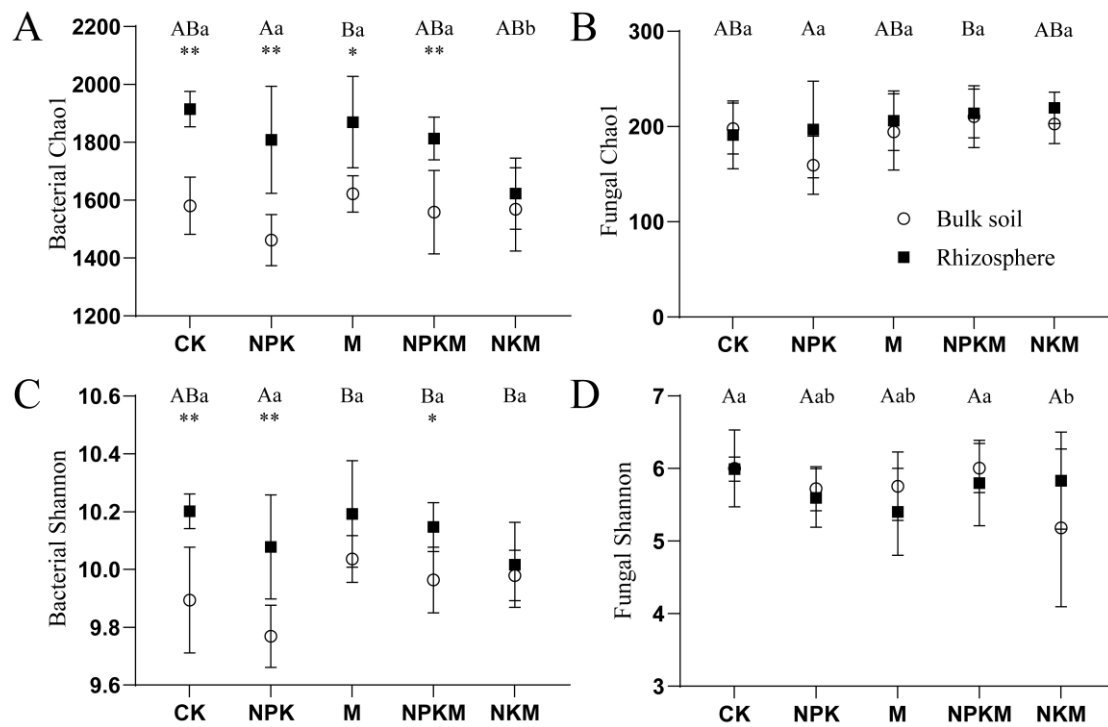
Fig. 4 Mean ± SE values for microbial α-diversity (A: Bacterial Chao1 index, B: Fungal Chao1 index, C: Bacterial Shannon index, D: Fungal Shannon index) in five treatments (CK, NPK, M, NPKM, NKM) and two sample types (rhizosphere and bulk soil). Significant differences between treatments in bulk soil are indicated by capital letters ($p<0.05$, n = 6). Significant differences between treatments in rhizosphere are indicated by lowercase letters ($p<0.05$, n = 6). Significant differences between rhizosphere and bulk soils are indicated by asterisks, where * $p < 0.05$, ** $p < 0.01$ (Duncan's test, n=6)

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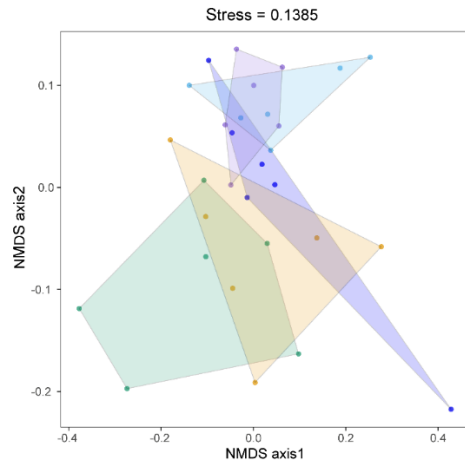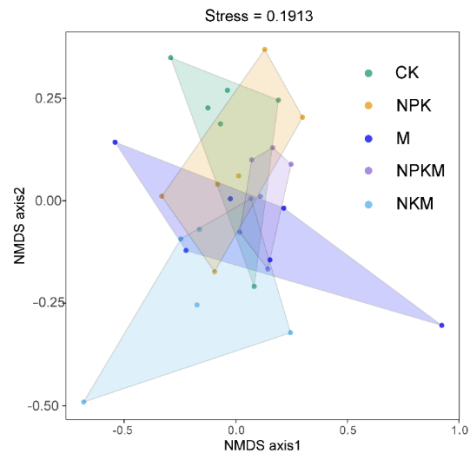
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776 Fig. 5 Nonmetric multi-dimensional scaling (NMDS) ordination of the microbial community by comparing with
777 Bray-Curtis distance similarities based on the abundance of OTUs. Capital letters means different classification (A:
778 bacteria in bulk soil, B: fungi in bulk soil, C: bacteria in rhizosphere soil, D: fungi in rhizosphere soil)
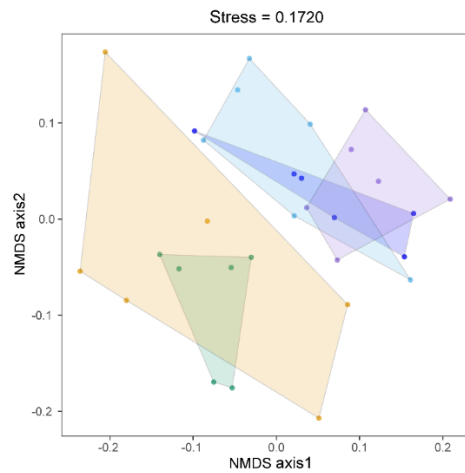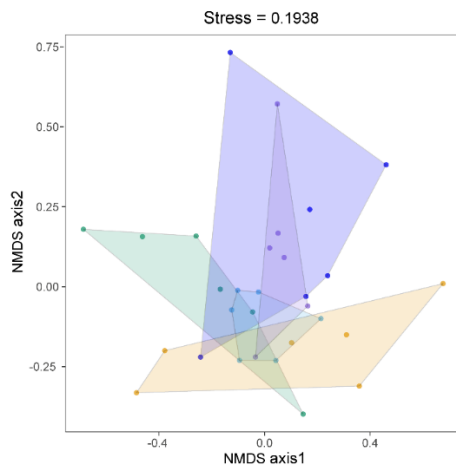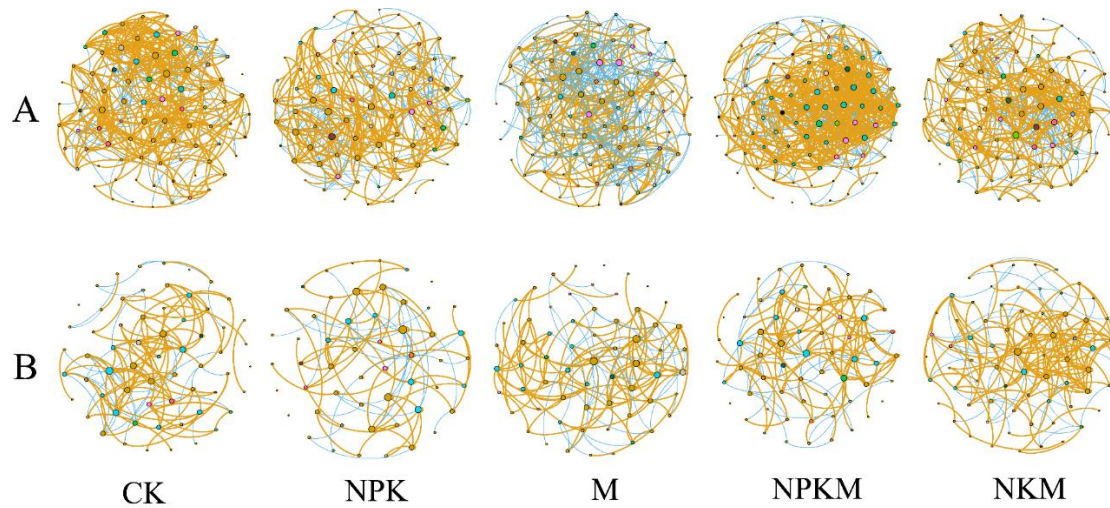779

Fig. 6 Network of co-occurring bacterial (A) and fungal (B) OTUs across five fertilizer treatments. Only Spearman's correlation coefficient r > 0.6 or r < − 0.6 significant at P < 0.01 is shown. The nodes are colored according to phylum. orange edges represent positive correlations and blue edges represent negative correlations. Node size presents the connecting numbers of each OUT.
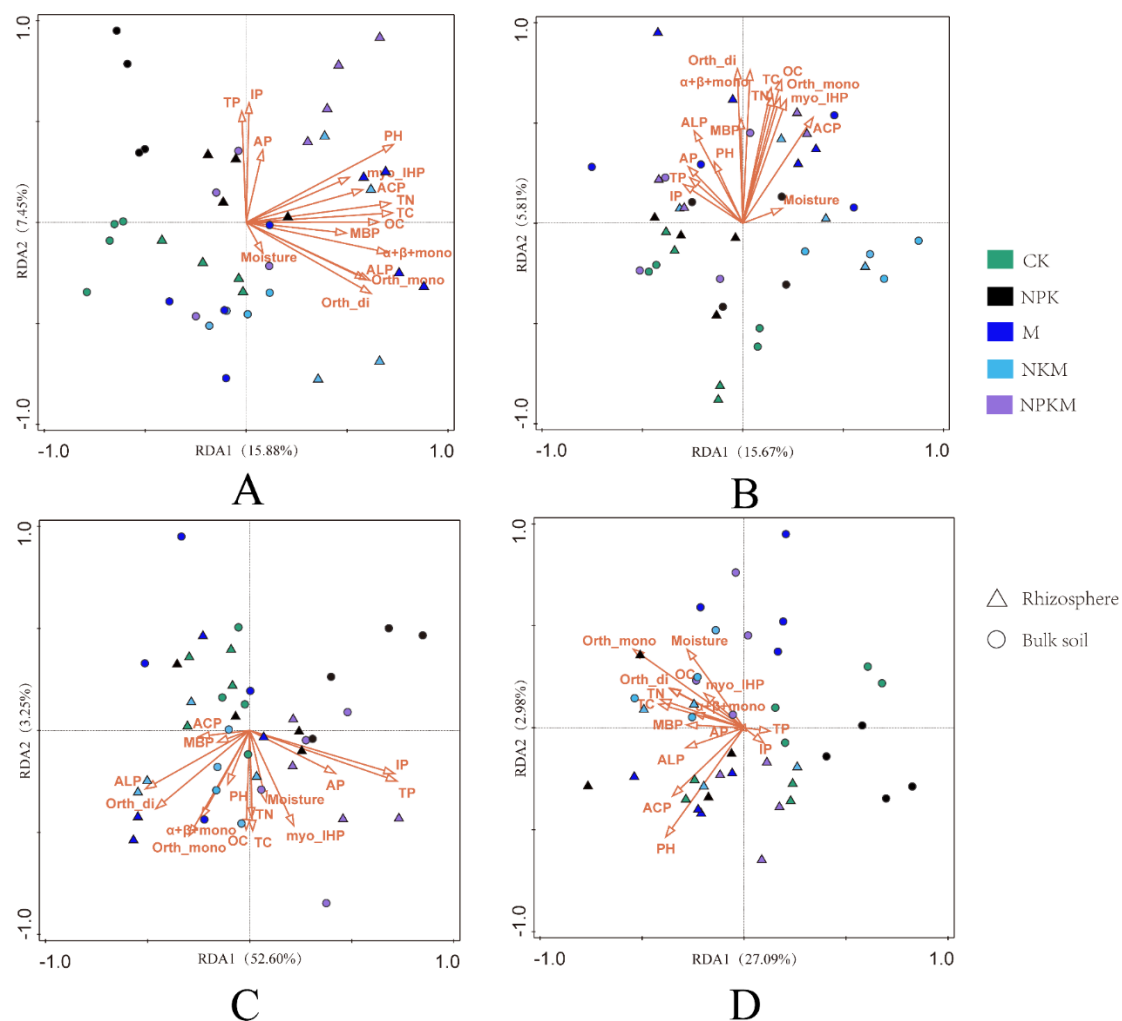
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789 Fig. 7 Correlations between soil properties and the community structure of total bacteria (A), total fungi (B),

790 phosphorus-solubilizing bacteria (C), and phosphorus-solubilizing fungi (D) as determined by redundancy analysis

791 (RDA). MBP, microbial biomass phosphorus; TP, total phosphorus; IP, inorganic phosphorus; AP, available

792 phosphorus; Orth-mono, orthophosphate monoester; Orth-di, orthophosphate diesters; Myo-IHP, myo-Inositol

793 hexakisphosphate; α+β+mono, α- and β-glycerophosphates and mononucleotides; ACP, activity of acidic

794 phosphatase; ALP, activity of alkaline phosphatase.
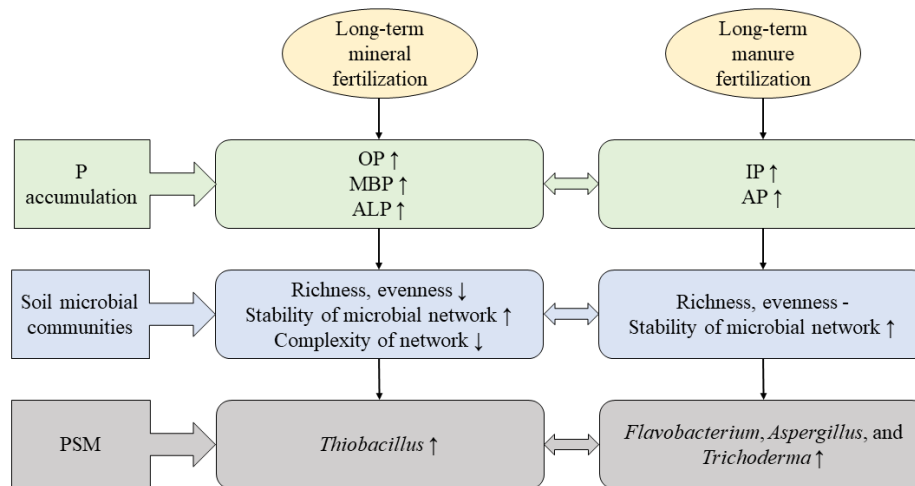
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799 Fig. 8 A diagrammatic sketch showing different responses of P accumulation, soil microbial communities and the

800 PSM after long-term mineral or manure fertilization. ↑, increase; -, no effect; ↓, decrease.

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817 Table 1

818 The soil properties in five treatments (CK, NPK, M, NPKM, NKM) and two sample types (Bulk and Rhizosphere

819 soil).

Soil properties	Sample type	CK	NPK	M	NPKM	NKM
Total C (g/kg)	Bulk soil	19.08±0.26 a**	23.38±0.56 b**	30.30±0.23 d**	34.08±0.22 e	28.80±0.87 c
	Rhizosphere	20.93±0.56 a	26.38±1.59 b	33.63±0.81 d	34.48±0.15 d	30.18±0.29 c
Organic C (g/kg)	Bulk soil	15.13±0.30 a	17.88±1.16 b	23.43±1.42 c	26.18±0.68 d	22.35±0.37 c
	Rhizosphere	16.38±0.66 a	18.90±1.00 b	25.33±1.69 d	25.88±1.14 d	22.23±0.52 c
Total N (g/kg)	Bulk soil	2.25±0.06 a	2.58±0.05 b**	3.28±0.10 c*	3.53±0.10 d	3.00±0.12 e*
	Rhizosphere	2.35±0.06 a	2.93±0.15 b	3.45±0.06 d	3.58±0.05 d	3.25±0.06 c
C/N	Bulk soil	8.48±0.10 a*	9.08±0.35 b	9.26±0.29 b	9.67±0.23 c	9.60±0.08 c
	Rhizosphere	8.90±0.06 a	9.02±0.19 ab	9.75±0.09 c	9.64±0.11 c	9.29±0.25 b
pH	Bulk soil	5.84±0.08 ab	5.85±0.02 ab	5.89±0.17 ab	5.89±0.01 b	5.76±0.05 a
	Rhizosphere	5.95±0.06 a	6.13±0.02 b	6.15±0.09 b	6.19±0.15 b	6.07±0.04 b
Gravimetric Moisture	Bulk soil	0.41±0.05 a	0.43±0.00 a	0.45±0.03 ab	0.49±0.03 b	0.48±0.02 b
	Rhizosphere	0.39±0.03 a	0.41±0.03 ab	0.43±0.02 ab	0.44±0.03 b	0.44±0.03 b
Nitrate-N (mg/kg)	Bulk soil	0.60±0.05 a	0.66±0.23 a**	1.30±0.90 a	0.99±0.26 a	1.45±1.13 a
	Rhizosphere	0.95±0.36 a	0.80±0.34 b	1.42±0.62 a	1.26±0.61 a	1.34±0.62 a
Ammonia-N (mg/kg)	Bulk soil	12.15±2.92 a	11.08±2.27 a	10.40±2.32 a	8.66±1.46 a	11.82±3.24 a
	Rhizosphere	10.07±2.59 a	17.23±1.02 a	11.79±1.60 a	11.38±2.21 a	11.66±3.90 a
Microbial biomass P (mg/kg)	Bulk soil	3.70±3.49 a	7.95±5.70 ab	15.56±8.42 ab	12.39±9.60 b	10.45±3.83 ab
	Rhizosphere	5.53±2.71 a	12.59±8.06 ab	17.90±4.27 b	21.40±8.59 b	17.77±11.14 b

820 Values are means ± standard error.

821 Significant differences between treatments are indicated by lowercase letters (p<0.05, n = 4).

822 Significant differences between rhizosphere and bulk soil are indicated by asterisks, where * p < 0.05, ** p < 0.01

823 (Duncan's test, n=4)

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833 Table 2

834 The phosphorus species in five treatments (CK, NPK, M, NPKM, NKM) and two sample types (Bulk and

835 Rhizosphere soil).

P Form or Compound Class	Sample type	CK	NPK	M	NPKM	NKM
NaOH-EDTA extracted phosphorus (mg/kg)	Bulk soil	253.86±34.05 a	560.13±22.78 b	361.80±2.00 a	738.70±40.05 c	304.31±1.66 a
	Rhizosphere	243.29±38.26 a	546.67±101.12 b	345.77±38.23 a	685.59±36.28 c	348.40±52.34 a
Orthophosphate (mg/kg)	Bulk soil	146.84±32.01 a	459.07±2.71 b	202.19±5.91 a	598.10±1.61 c	167.24±2.98 a
	Rhizosphere	148.23±33.88 a	409.37±88.42 b	187.37±20.00 a	540.29±40.60 c	186.66±25.94 a
Pyrophosphate (mg/kg)	Bulk soil	2.94±0.64 a	2.30±3.26 a	3.01±1.34 a	3.00±4.24 a	2.52±1.23 a
	Rhizosphere	2.73±1.42 a	1.73±2.45 a	2.74±1.02 a	2.56±3.62 a	2.89±1.71 a
Orthophosphate monoesters (mg/kg)	Bulk soil	63.87±0.75 a	64.31±13.36 a	93.87±7.26 b	92.73±21.40 b	91.09±4.29 b
	Rhizosphere	56.90±7.41 a	85.97±18.57 b	96.28±6.33 b	88.72±4.76 b	93.99±9.14 b
Myo-IHP (mg/kg)	Bulk soil	28.69±0.17 a**	36.73±0.22 b	40.40±1.68 bc	44.86±4.35 c*	39.29±0.48 bc
	Rhizosphere	19.03±2.31 a	38.58±5.51 b	40.21±2.98 b	51.18±0.04 c	44.43±0.95 b
Scyllo-IHP (mg/kg)	Bulk soil	5.03±0.08 a	6.90±3.29 a	10.15±3.16 a	8.98±4.25 a	7.52±1.05 a
	Rhizosphere	4.45±1.02 a	8.19±1.77 a	9.37±1.00 a	7.96±3.21 a	8.12±2.79 a
Other monoesters (mg/kg)	Bulk soil	30.16±0.49 ab	20.69±9.86 a	43.33±8.74 b	38.89±12.79 ab	44.29±2.76 b
	Rhizosphere	33.45±10.73 a	39.20±11.29 a	46.70±2.35 a	29.57±1.59 a	41.43±10.99 a
Orthophosphate diesters (mg/kg)	Bulk soil	40.21±0.66 a	34.44±3.45 a	62.72±4.69 b	44.87±12.81 ab	43.46±1.59 ab*
	Rhizosphere	35.43±13.20 a	49.61±3.42 ab	59.38±12.93 b	54.03±4.06 a	64.86±15.55 b
DNA (mg/kg)	Bulk soil	15.31±2.32 ab	6.90±3.29 a*	22.20±2.21 b	11.97±8.49 ab	13.38±0.24 ab
	Rhizosphere	12.34±6.90 a	21.58±3.82 a	18.15±8.52 a	13.65±4.84 a	20.06±9.32 a
α+β+mono (mg/kg)	Bulk soil	24.90±1.67 a	27.54±0.16 a	40.52±6.90 b	32.90±4.32 a	30.08±1.83 a**
	Rhizosphere	23.10±6.30 a	28.03±0.40 a	41.22±4.40 b	40.38±0.78 b	44.80±6.23 b

836 Myo-IHP: myo-Inositol hexakisphosphate; Scyllo-IHP: Scyllo-Inositol hexakisphosphate; α+β+mono, α- and β-

837 glycerophosphates and mononucleotides; Values are means ± standard error.

838 Significant differences between treatments are indicated by lowercase letters ($p<0.05$, n = 2). Significant differences

839 between rhizosphere and bulk soil are indicated by asterisks, where * $p < 0.05$, ** $p < 0.01$ (Duncan's test, n=2)

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