Application of mineral phosphorus fertilizer influences rhizosphere chemical and biological characteristics

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Supplemental material

Figure S1. Relative abundances of low-abundance bacterial genera in maize rhizosphere soil under different mineral phosphorus fertilizer application rates. P0, P60, P120, and P300 represent 0, 60, 120, and 300 kg P₂O₅ ha⁻¹ of phosphorus fertilizer application rates, respectively. Vertical bars represent standard deviations of the means ($n = 3$, * $P < 0.05$ by Kruskal-Wallis test).

Figure S2. Relative abundances of bacterial genera with significant responses to P0 (at 95% confidence intervals, Student's t-test, equal variance) in maize rhizosphere soil under different mineral phosphorus fertilizer application rates. P0, P60, P120, and P300 represent 0, 60, 120, and 300 kg P₂O₅ ha⁻¹ of phosphorus fertilizer application rates, respectively.

Figure S3. Path diagrams of structural equation modeling (SEM) for the relationship of soil bacterial community diversity and composition on maize yield. Ca₈-P, octacalcium bound phosphorus; O-P, occluded iron/aluminum bound phosphorus; LPo: labile organic phosphorus; MLPo: moderately labile organic phosphorus. Values above the line represent the path coefficients. Solid lines indicate positive path coefficients and dashed lines indicate negative path coefficients. The width of arrows indicates the strength of the standardized path coefficient (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Table S1. Soil properties in maize rhizosphere under different mineral phosphorus fertilizer application rates.

Treatment	pH	SOC	Olsen P	Ca ₂ -P	Ca ₈ -P	Al-P	Fe-P	O-P	Ca ₁₀ -P	LPo	MLPo	MRPo	HRPo
	(1:2.5)	(g/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
P0	6.51±0.39a	20.1±0.49a	31.9±0.27d	17.1±3.18c	8.24±1.27b	24.6±3.49b	52.9±5.04a	48.3±3.48b	69.1±4.22c	14.2±1.97b	46.0±6.09b	8.20±4.80c	15.5±3.31a
P60	6.25±0.32a	19.3±0.78ab	45.7±1.13c	20.0±3.05bc	9.76±2.85b	26.5±6.18b	54.4±3.59a	51.8±4.93b	75.3±9.15c	17.8±1.58b	63.6±7.45a	17.0±6.84bc	23.2±5.53a
P120	6.15±0.32a	18.8±0.48b	52.3±1.02b	21.3±1.69b	14.3±1.00a	33.2±4.50b	54.5±2.11a	51.2±3.74b	91.4±13.3b	23.3±2.22a	71.6±3.61a	27.3±10.4ab	26.1±14.6a
P300	6.24±0.43a	18.5±0.68b	76.7±1.30a	31.8±2.06a	14.5±4.06a	43.7±4.87a	60.6±13.9a	68.2±8.60a	107.7±12.2a	25.2±4.41a	74.5±16.3a	31.9±4.12a	27.0±5.26a

Values followed by different lowercase letters within the same column are significantly different at $P < 0.05$ determined by least significant difference (LSD).

P0, P60, P120, and P300 represent 0, 60, 120, and 300 kg P₂O₅ ha⁻¹ of phosphorus fertilizer application rates, respectively. SOC, soil organic carbon; Ca₂-P, dicalcium bound phosphorus; Ca₈-P, octacalcium bound phosphorus; Al-P, aluminum bound phosphorus; Fe-P, iron bound phosphorus; O-P, occluded iron/aluminum bound phosphorus; Ca₁₀-P: phosphorite bound phosphorus; LPo: labile organic phosphorus; MLPo: moderately labile organic phosphorus; MRPo: moderately resistant organic phosphorus; HRPo: highly resistant organic phosphorus.

Table S2. Phosphorus use efficiency, maize grain yield, and yield components under different mineral phosphorus fertilizer application rates.

Treatment	PFP (kg kg ⁻¹)	PAE (kg kg ⁻¹)	PAUE (%)	PUPE (kg kg ⁻¹)	EL (cm)	BTL (cm)	ERN	KNR	HKW (g)	Yield (g pot ⁻¹)
P0	—	—	—	—	14.7 ± 0.33 b	1.40 ± 0.73 a	16.3 ± 1.51 a	30.5 ± 2.43 b	27.8 ± 2.07 a	109.9 ± 7.15 b
P60	136.9 ± 3.00 a	17.8 ± 9.48 a	39.2 ± 14.9 a	1.40 ± 0.08 a	15.5 ± 0.42 a	0.87 ± 0.92 a	16.0 ± 1.79 a	32.0 ± 2.19 ab	29.8 ± 2.15 a	126.4 ± 2.77 a
P120	64.9 ± 4.29 b	5.39 ± 2.95 b	27.9 ± 4.62 a	0.78 ± 0.04 b	14.5 ± 0.61 b	0.90 ± 0.99 a	15.0 ± 1.10 ab	30.3 ± 1.75 b	29.6 ± 1.20 a	126.0 ± 5.74 a
P300	27.3 ± 1.77 c	3.49 ± 0.95 b	11.6 ± 1.80 b	0.32 ± 0.01 c	15.8 ± 0.67 a	0.42 ± 0.65 a	14.3 ± 0.82 b	34.5 ± 2.26 a	29.6 ± 2.97 a	119.9 ± 2.62 a

P0, P60, P120, and P300 represent 0, 60, 120, and 300 kg P₂O₅ ha⁻¹ of phosphorus fertilizer application rates, respectively. PFP: partial factor productivity; PAE: phosphorus agronomic efficiency; PAUE: phosphorus apparent utilization efficiency; PUPE: phosphorus uptake efficiency; EL, ear length; BTL, bald tip length; ERN, ear row number; KNR, kernel number per row; HKW, hundred kernel weight;.

Table S3. Illumina MiSeq sequenced bacterial data (at 97% sequence similarity) based on the 16S rRNA gene under different mineral phosphorus fertilizer application rates.

Treatment	Sequences	Bases (bp)	Average length (bp)	Maximum length (bp)	Minimum length (bp)
P0-1	137087	57054428	416	450	249
P0-2	97211	40375348	415	449	258
P0-3	42821	17788881	415	447	386
P60-1	99521	41395044	416	447	237
P60-2	45731	19060343	417	443	310
P60-3	96905	40466087	418	444	276
P120-1	53845	22392341	416	443	318
P120-2	110243	46006717	417	450	262
P120-3	49309	20495173	416	449	262
P300-1	37227	15478750	416	447	262
P300-2	116993	48644604	416	447	276
P300-3	76923	31950732	415	443	239

P0, P60, P120, and P300 represent 0, 60, 120, and 300 kg P₂O₅ ha⁻¹ of phosphorus fertilizer application rates, respectively.

Table S4. Relative abundance (%) at bacterial phyla level under different mineral phosphorus fertilizer application rates.

Treatment	Chloroflexi	Firmicutes	Nitrospirae	Cyanobacteria	Gemmatimonadetes	Bacteroidetes
P0	9.86	1.60	0.38	1.37	4.22	4.52
P60	10.5	1.33	0.43	1.01	5.40	3.85
P120	7.57	1.75	0.56	0.66	6.23	4.71
P300	9.48	1.51	0.55	0.65	5.14	3.73
<i>P</i> value						
P0 vs P60	ns	ns	ns	ns	0.034	5.41E-03
P0 vs P120	0.018	ns	ns	ns	1.74E-03	ns
P0 vs P300	0.029	ns	ns	ns	2.96E-03	0.014

P0, P60, P120, and P300 represent 0, 60, 120, and 300 kg P₂O₅ ha⁻¹ of phosphorus fertilizer application rates, respectively.

Table S5. Indicator species in maize rhizosphere soil under different mineral phosphorus fertilizer application rates.

Treatment	Indicator species ^a	A ^b	B ^b	stat ^b	IndVal ^b	p-value ^c	Taxonomy ^d	Identities (%)	Bit score	E-value	Accession No.
P0	OTU3501	1	1	1.000	1.0000	0.021	<i>g_Brachy bacterium</i>	100.00	409	0.0	KX645681.1
	OTU3097	0.9091	1	0.953	0.9091	0.035	<i>g_CandidatusMoeniiplasma</i>	91.10	309	8E-159	KP763348.1
	OTU1462	0.8182	1	0.905	0.8182	0.039	<i>g_Pseudomonas</i>	99.53	423	0.0	MN519642.1
	OTU580	0.7778	1	0.882	0.7778	0.021	<i>o_Cystobacterineae</i>	93.26	343	1E-177	KX367460.1
	OTU291	0.7500	1	0.866	0.75	0.021	<i>o_unculturedDesulfuromonadales</i>	97.67	400	0.0	LN555055.1
	OTU761	0.7321	1	0.856	0.7321	0.021	<i>s_Paraburkholderia</i> sp.	99.77	426	0.0	MH925968.1
	OTU4239	0.6667	1	0.816	0.6667	0.039	<i>p_candidatusSaccharibacteria</i>	93.28	321	2E-165	KJ191955.1
	OTU807	0.6081	1	0.780	0.6081	0.037	<i>c_unculturedGammaproteobacterium</i>	95.34	369	0.0	EF074577.1
	OTU2486	0.5811	1	0.762	0.5811	0.021	<i>s_Niastella</i> sp.	97.41	391	0.0	EU917053.1
	OTU4429	0.5455	1	0.739	0.5455	0.021	<i>o_unculturedMyxococcales</i>	98.27	384	0.0	KX367290.1
P60	OTU2173	1.0000	1	1.000	1.0000	0.019	<i>s_unculturedBlastopirellula</i> sp.	95.32	367	0.0	JN832629.1
	OTU5171	0.8049	1	0.897	0.8049	0.042	<i>s_Magnetospirillum</i> sp.	98.76	389	0.0	MH686102.1
	OTU3327	0.8571	1	0.926	0.8571	0.037	<i>s_Ornithinimicrobiumkibberense</i>	100.00	411	0.0	MK726118.1
	OTU2623	0.6923	1	0.832	0.6923	0.042	<i>o_unculturedMyxococcales</i>	97.64	394	0.0	FJ889280.1
	OTU4281	0.6923	1	0.832	0.6923	0.035	<i>p_unculturedGemmatimonadetes</i>	95.56	351	0.0	JN002780.1
	OTU3714	0.5778	1	0.760	0.5778	0.019	<i>p_unculturedChloroflexi</i>	95.82	354	0.0	KF183051.1
	OTU2275	0.5664	1	0.753	0.5664	0.019	<i>s_unculturedElusimicrobium</i> sp.	96.24	376	0.0	GQ375494.1
	OTU3296	0.5556	1	0.745	0.5556	0.019	<i>g_unculturedClostridia</i>	93.70	333	3E-172	JQ700615.1
	OTU3598	0.5517	1	0.743	0.5517	0.019	<i>s_unculturedTychonema</i> sp.	98.28	386	0.0	EU809762.1
	OTU84	0.5040	1	0.710	0.5040	0.019	<i>s_unculturedGeobacter</i> sp.	100.00	431	0.0	JN091600.1

Table S5. (Continue).

P120	OTU2267	0.7664	1	0.875	0.7664	0.044	s__uncultured <i>Croceibacter</i> sp.	92.24	324	3E-167	GU245904.1
	OTU855	0.6939	1	0.833	0.6939	0.015	c__unculturedGammaproteobacterium	92.79	336	8E-174	EF074555.1
	OTU803	0.5926	1	0.770	0.5926	0.026	s__ <i>Delftia</i> <i>suruhatensis</i>	100.00	429	0.0	MN393973.1
	OTU4027	0.5400	1	0.735	0.5400	0.044	s__Candidatus <i>Saccharimonas</i> sp.	97.77	377	0.0	KX028761.1
	OTU1758	0.5161	1	0.718	0.5161	0.026	c__unculturedGammaproteobacterium	97.90	401	0.0	GQ302552.1
P300	OTU1888	0.8750	1	0.935	0.8750	0.015	s__ <i>Paenibacillus</i> sp.	94.87	362	0.0	KY684255.2
	OTU4481	0.8235	1	0.907	0.8235	0.015	s__uncultured <i>Longilinea</i> sp.	95.31	347	5E-180	GQ421109.1
	OTU4315	0.8182	1	0.905	0.8182	0.015	p__unculturedCandidatus <i>Saccharibacteria</i>	94.10	333	3E-172	MF439312.1
	OTU1928	0.6364	1	0.798	0.6364	0.015	s__uncultured <i>Geobacter</i> sp.	97.20	391	0.0	AM941477.1
	OTU2747	0.6176	1	0.786	0.6176	0.048	s__uncultured <i>Sporocytophaga</i> sp.	94.81	357	0.0	MH174164.1
	OTU3584	0.6163	1	0.785	0.6163	0.015	s__uncultured <i>Phormidium</i> sp.	95.58	353	0.0	GQ421006.1
	OTU4151	0.5862	1	0.766	0.5862	0.015	p__unculturedCandidatus <i>Saccharibacteria</i>	95.33	348	2E-180	LC381003.1
	OTU4094	0.5706	1	0.755	0.5706	0.015	p__unculturedCandidatus <i>Saccharibacteria</i>	95.25	323	1E-166	KT271006.1
	OTU4879	0.5200	1	0.721	0.5200	0.015	s__uncultured <i>Acidobacterium</i> sp.	98.51	386	0.0	KF035826.1
	OTU2966	0.5075	1	0.712	0.5075	0.015	p__Gemmatimonadetes	96.44	376	0.0	AY673312.1

P0, P60, P120, and P300 represent 0, 60, 120, and 300 kg P₂O₅ ha⁻¹ of phosphorus fertilizer application rates, respectively.

^a OTUs with IndVal > 0.5 were selected.

^b A is called specificity or probable predictive value, i.e. if A=1 for species calpurp, then calpurp is a good indicator because it occurs only in that group B is called fidelity or sensitivity, i.e. if B=1 for species calpurp, then all sites within the group contain calpurp. Stat is the average of A and B. IndVal=A*B*, IndVal> 0.5 were selected for analyze.

^c *p*-value is based on 1000 Monte Carlo permutations.

^d Taxonomy of OTUs were identified by using blastn in NCBI: p, phylum; c, class; o, order; g, genus; s, species.

Table S6. Correlation coefficients between soil properties and bacterial community.

	pH	SOC	Olsen	Ca ₂ -P	Ca ₈ -P	Al-P	Fe-P	O-P	Ca ₁₀ -P	LPo	MLPo	MRPo	HRPo	Shannon	Chao	Comp1	Comp2
	P																
pH	1.00	0.69*	-0.58*	-0.42	-0.69*	-0.62*	-0.24	-0.65*	-0.37	–	-0.74**	-0.61*	-0.49	0.63*	0.57	0.27	–
										0.89***							0.91***
SOC		1.00	-0.61*	-0.58*	-0.56	-0.67*	-0.15	-0.77**	-0.69*	-0.68*	-0.67*	-0.69*	-0.43	0.52	0.39	0.39	-0.66*
Olsen P			1.00	0.91***	0.63*	0.91***	0.64*	0.90***	0.84***	0.74**	0.51	0.81**	0.55	-0.62*	-0.53	-0.60*	0.59*
Ca ₂ -P				1.00	0.50	0.87***	0.57	0.86**	0.85***	0.56	0.49	0.81**	0.49	-0.38	-0.31	-0.45	0.53
Ca ₈ -P					1.00	0.76**	0.30	0.78**	0.52	0.82**	0.78**	0.59*	0.24	-0.66*	-0.31	-0.69*	0.49
Al-P						1.00	0.48	0.93***	0.87***	0.77**	0.66*	0.88***	0.59*	-0.61*	-0.42	-0.59*	0.62*
Fe-P							1.00	0.46	0.39	0.35	0.06	0.40	0.50	0.01	0.03	-0.21	0.26
O-P								1.00	0.78**	0.77**	0.68*	0.83***	0.41	-0.58	-0.39	-0.61*	0.63*
Ca ₁₀ -P									1.00	0.56	0.57	0.75**	0.59*	-0.55	-0.41	-0.56	0.41
LPo										1.00	0.68*	0.75**	0.51	-0.77**	-0.67*	-0.65*	0.71*
MLPo											1.00	0.47	0.34	-0.51	-0.27	-0.27	0.69*
MRPo												1.00	0.46	-0.52	-0.40	-0.55	0.61*
HRPo													1.00	-0.25	-0.38	-0.09	0.54
Shannon														1.00	0.86***	0.74**	-0.44
Chao															1.00	0.52	-0.44
Comp1																1.00	< 0.001
Comp2																	1.00

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

SOC, soil organic carbon; Ca₂-P, dicalcium bound phosphorus; Ca₈-P, octacalcium bound phosphorus; Al-P, aluminum bound phosphorus; Fe-P, iron bound phosphorus; O-P, occluded iron/aluminum bound phosphorus; Ca₁₀-P: phosphorite bound phosphorus; LPo: labile organic phosphorus; MLPo: moderately labile organic phosphorus; MRPo: moderately resistant organic phosphorus; HRPo: highly resistant organic phosphorus; Comp: component.

Table S7. Variation portioning into soil bacterial community in maize rhizosphere soil under different mineral phosphorus fertilizer application rates explained by soil properties.

Soil properties	Contribution%	Pseudo-F	<i>P</i>
LPO	24.6	3.3	0.010
pH	12.1	1.7	0.136
Fe-P	11.1	1.7	0.132
Ca ₂ -P	9.8	1.9	0.124
Olsen P	9.2	1.5	0.176
O-P	6.9	1.1	0.418
MLPo	6.7	1.9	0.326
SOC	5.9	1.2	0.370
Ca ₈ -P	5.6	1.1	0.392
Ca ₁₀ -P	4.7	0.9	0.466
HRPo	3.4	< 0.1	1.000

LPO: labile organic phosphorus; Fe-P, iron bound phosphorus; Ca₂-P, dicalcium bound phosphorus; O-P, occluded iron/aluminum bound phosphorus; MLPo: moderately labile organic phosphorus; SOC, soil organic carbon; Ca₈-P, octacalcium bound phosphorus; Ca₁₀-P: phosphorite bound phosphorus; HRPo: highly resistant organic phosphorus.