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RESEARCH ARTICLE

Magnesium improves phosphorus availability in the rhizosphere soil of apple by regulating P-cycling microbial communities

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SUPPLEMENTARY MATERIALS

Text S1. Hedley sequential extraction procedure

H_2O-P: The soil residue and 30 mL of sterile water were mixed in a centrifuge tube, shaken for 16 h at 180 r·min^{-1} in a constant temperature incubator shaker, and centrifuged at 8000 r·min^{-1} for 5 min. The filtrate was oxidized and digested in an autoclave for 1 h (103.4 kPa, 121 °C). The digested solution was used to determine the H_2O-P content via the molybdenum-antimony anti-spectrophotometric method.

$NaHCO_3$-P: After washing the soil residue, 30 mL of 0.5 mol·L^{-1} $NaHCO_3$ solution was added and then shaken at 180 r·min^{-1} for 16 h. After centrifugation (8000 r·min^{-1}, 5 min), $NaHCO_3$-Pi was determined directly from the filtrate using the molybdenum-antimony ascorbic acid method. Another aliquot of the filtrate was oxidized and digested as described above to determine the total $NaHCO_3$-P content. The $NaHCO_3$-Po content was calculated as the difference between $NaHCO_3$-P and $NaHCO_3$-Pi.

NaOH-P: The soil residue was mixed with 30 mL of 0.1 mol·L^{-1} NaOH solution, shaken at 180 r·min^{-1} for 16 h, and centrifuged. The filtrate was used to determine the NaOH-Pi content. Another aliquot was digested to determine the total NaOH-P content. The NaOH-Po content was calculated as the difference between NaOH-P and NaOH-Pi.

HCl-P: The soil residue was mixed with 30 mL of 1.0 mol·L^{-1} HCl solution, shaken at 180 r·min^{-1} for 16 h at 23 °C, and centrifuged, and the filtrate was used to determine the HCl-P content via the molybdenum-antimony ascorbic acid method.

Residual-P: The soil residue after HCl extraction was transferred to a digestion tube and digested at 360 °C for 3 h. After cooling, the Residual-P content was determined using the molybdenum-antimony anti-spectrophotometric method.

Table S1 Summary of sequencing data from control and magnesium treatment groups

Sample	Raw data		Clean data		Effective ratio (read)	Q20 (%)	Q30 (%)	GC content (%)
	Read	Base (Gb)	Read	Base (Gb)				
CK-1	72237442	10.84	68965980	10.34	95.47	97.93	93.46	61.19
CK-2	73095690	10.96	69642250	10.45	95.28	97.95	93.56	62.16
CK-3	84534500	12.68	80208822	12.03	94.88	97.98	93.67	62.20
Mg50-1	66042174	9.91	62484952	9.37	94.61	97.72	92.98	61.74
Mg50-2	62511400	9.38	59452224	8.92	95.11	97.65	92.77	61.99
Mg50-3	63899824	9.58	61208324	9.18	95.79	98.07	93.92	62.26
Mg100-1	62055344	9.31	58917740	8.84	94.94	97.66	92.80	62.18
Mg100-2	60648914	9.10	57804236	8.67	95.31	97.92	93.47	61.70
Mg100-3	70493180	10.57	67006682	10.05	95.05	97.97	93.60	62.15
Mg150-1	85064246	12.76	80953504	12.14	95.17	97.98	93.67	62.14
Mg150-2	72662304	10.90	69341594	10.40	95.43	97.96	93.59	62.09
Mg150-3	63191900	9.48	60288812	9.04	95.41	97.77	93.21	62.35

Note: The treatment groups included: CK, no Mg; Mg50, pure MgO, 50 $mg \cdot kg^{-1}$; Mg100, pure MgO, 100 $mg \cdot kg^{-1}$; Mg150, pure MgO, 150$mg \cdot kg^{-1}$. Sample, sample name; Raw data/Read, number of raw sequencing reads (counted in units of four lines per sequence in FASTQ format); Raw data/Base, total raw bases, calculated as (number of sequences × read length), reported in gigabases (Gb); Clean data/Read , number of clean reads after preprocessing (counted in units of four lines per sequence); Clean data/Base, total clean bases after preprocessing, calculated as (number of clean sequences × read length), reported in gigabases (Gb); Effective ratio (read), percentage of clean reads relative to raw reads; Q20 (%), proportion of bases with a Phred quality score $\geqslant$ 20 in the clean data; Q30 (%), proportion of bases with a Phred quality score $\geqslant$ 30 in the clean data;GC content (%), GC content in the clean data.

Table S2 All the PCGs detected in this study with KO numbers corresponding genes

Metabolic processes	Gene	KO number
Two-component system	*phoU*	K02039
	phoR	K07636
	phoB	K07657
Pyruvate metabolism	*pps*	K01007
	ppdK	K01006
Transporters	*pstS*	K02040
	pstC	K02037
	pstA	K02038
	pstB	K02036
	ugpB	K05813
	ugpA	K05814
	ugpE	K05815
	ugpC	K05816
	pit	K03306
	phnD	K02044
	phnE	K02042
Phosphonate and phosphinate metabolism	*phnW*	K03430
	phnN	K05774
	phnI	K06164
	phnJ	K06163
	phnX	K05306
Pyrimidine metabolism	*nrdD*	K21636
	nrdA	K00525
	nrdE	K00525
	nrdB	K00526
	nrdF	K00526
Pentose phosphate pathway	*gcd*	K00117
	gnd	K00033
Oxidative phosphorylation	*ppk*	K00937
	ppa	K01507
Purine metabolism	*ppx*	K01524
	adk	K00939
Phosphotransferase system	*ptsI*	K08483
	ptsH	K02784, K11189
Organic phosphoester hydrolysis	*phoD*	K01113
	phy	K01083
	phoA	K01077
	opd	K07048
	ugpQ	K01126

Table S3 Topological properties of gene and microbial co-occurrence networks

Network type	Node	Edge	Average degree	Modularity	Average clustering coefficient	Average path length	Density
Gene-gene network	39	132	6.77	0.39	0.48	2.73	0.18
Microbe-microbe network	23	134	11.65	0.14	0.74	1.56	0.53