Diverse responses of *pqqC*- and *phoD*-harbouring bacterial communities to variation in soil properties of Moso bamboo forests

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Summary

Phosphate-mobilizing bacteria (PMB) play a critical role in the regulation of phosphorus availability in the soil. The microbial genes *pqqC* and *phoD* encode pyrroloquinoline quinone synthase and bacterial alkaline phosphatase, respectively, which regulate inorganic and organic phosphorus mobilization, and are therefore used as PMB markers. We examined the effects of soil properties in three Moso bamboo forest sites on the PMB communities that were profiled using high-throughput sequencing. We observed differentiated responses of *pqqC*- and *phoD*-harbouring PMB communities to various soil conditions. There was significant variation among the sites in the diversity and structure of the *phoD*-harbouring community, which correlated with variation in phosphorus levels and non-capillary porosity; soil organic carbon and soil water content also affected the structure of the *phoD*-harbouring community. However, no significant difference in the diversity of *pqqC*-harbouring community was observed among different sites, while the structure of the *pqqC*-harbouring bacteria community was affected by soil organic carbon and soil total nitrogen, but not soil phosphorus levels. Overall, changes in soil conditions affected the *phoD*-harbouring community more than the *pqqC*-harbouring community. These findings provide a new insight to explore the effects of soil conditions on microbial communities that solubilize inorganic phosphate and mineralize organic phosphate.

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Introduction

There is growing concern about the detrimental effects of phosphorus (P) deficiency on plant growth and biomass production (Niu *et al.*, 2013; Ha and Tran, 2014). Total P content in most soils ranges from 200 to 800 mg kg^{-1}, of which only 0.1% is directly available for plant growth in the form of free inorganic phosphate (Pi) (Saif *et al.*, 2014). Low P utilization by plants occurs as a result of P_i immobilization through precipitation and absorption (Gyaneshwar *et al.*, 2002), or due to the presence of larger amounts of unavailable organic P (Po), which can account for 30–65% (even up to 90%) of the total soil P.

Microbial populations are key components of the soil–plant continuum: they participate in the mobilization of nutrients that affect plant development (Vassilev *et al.*, 2006). Phosphate-mobilizing bacteria (PMB) play a critical role in the regulation of P acquisition (Nannipieri *et al.*, 2003): they solubilize Pi with organic acids or they mineralize recalcitrant Po with phosphatase including alkaline phosphatase and acid phosphatases. Their activity expands the pool of P available to plants (Richardson and Simpson, 2011). PMB are diverse and abundant, accounting for 1–50% of the entire soil microbial population (Jha *et al.*, 2014). Several bacteria, including *Pseudomonas*, *Azotobacter*, *Enterobacter*, *Burkholderia*, *Bacillus*, *Klebsiella*, *Aeromonas* and *Rhizobium* are able to convert insoluble P into available forms (Igual *et al.*, 2001; Jha *et al.*, 2013; Kour *et al.*, 2021). The rhizosphere contains abundant PMB (Ahemad and Kibret, 2014), but little is known about their diversity and community composition, in part because of the microbial complexity and the difficulties of characterization through culture-based methods (Hartmann *et al.*, 2015). High-throughput DNA sequencing using functional genes has opened up new possibilities for rapid analysis of PMB and other microbial communities in soil (Huang *et al.*, 2019).

According to previous studies, *pqq* and *pho* genes could be used as the potential bioindicator for Pi-solubilizing bacteria and Po-mineralizing bacteria respectively (Rodríguez *et al.*, 2006; Wagh *et al.*, 2014;

Hu *et al*., 2020). The *pqq* genes (*e.g. pqqA, B, C, D, E, F* and *G*) encode enzymes that synthesize pyrroloquinoline-quinone (PQQ), which is a cofactor involved in producing gluconic acid for Pi solubilization. The direct oxidation of glucose to gluconic acid has been demonstrated by Goldstein (1995) as a major mechanism for mineral phosphate solubilization in Gram-negative bacteria. Among the *pqq* genes, the *pqqC* gene, which encodes the best characterized enzyme and catalyses the final step in the production of pyrroloquinoline-quinone, has been accepted as a marker gene for tracking microbes able to solubilize inorganic phosphate (Kim *et al*., 2003; Meyer *et al*., 2011). Similarly, genes of the putative Pho regulon [*phoA* (COG1785), *phoD* (COG3540) and *phoX* (COG3211)] (Ragot *et al*., 2015) are associated with production of the alkaline phosphatase (ALP) that hydrolyses phosphomonoesters and phosphodiesters (Ragot *et al*., 2017), resulting in 90% of total P_o extracted from soil (Condron *et al*., 2005; Nannipieri *et al*., 2011), which may also be altered by climate conditions and other soil mineral properties (Margalef *et al*., 2017). Concomitantly, bacterial genera harbouring *pho* genes may also contain acid phosphatase genes, which may be presented to produce acid phosphatase and remobilize P sources in the soil, such as *Pseudomonas*, *Caulobacter* and *Lysobacter* (Rossolini *et al*., 1998; Neal *et al*., 2018). However, soil microorganisms (principally bacteria) are more likely to synthesize and secrete alkaline phosphatase than acid phosphatase, which is mostly derived by plant roots (Tiwari *et al*., 2015; Margalef *et al*., 2017). Hence, the *pho* genes have their potential to identify the bacterial communities in mineralizing organic P, particularly the *phoD* gene, which is more frequently selected as the key ALP gene than other *pho* genes (Gomez and Ingram, 1995; Tan *et al*., 2013).

The diversity and composition of microbial communities in forest soil depend on several properties of the soil, including physical properties, humidity and texture (Waymouth *et al*., 2020; Xia *et al*., 2020); vegetation (Carelli *et al*., 2000); agricultural practices (Potthoff *et al*., 2006), including fertilization (Tian *et al*., 2017) and climate (Puissant *et al*., 2015). Since PMB are a subset of the soil microbial community, they can also be influenced by changes in forest environment. Indeed, forest vegetation can affect the PMB community through root exudation and rhizodeposition, which can directly exchange material with soil microbes and even indirectly alter soil and microclimate conditions (Prashar *et al*., 2014). Thus, a detailed understanding of how soil conditions affect PMB growth and reproduction is essential, especially since microbes power many biochemical reactions that occur in the soil and participate in nutrient cycles and ecosystem processes (Bergkemper *et al*., 2016). While studies have provided solid evidence that soil properties have strong effects on soil microbial diversity and functional activities (Timmis and Ramos, 2021), as well as on *phoD*-harbouring PMB (Chen *et al*., 2017; Bi *et al*., 2020), how such bacteria respond to changes in soil properties is unclear. These changes may also be induced by the PMB themselves which can produce a series of extracellular enzymes to decay organic carbon (C), nitrogen (N) and P in the process of nutrient circulation. Exerting microbial functions associated with PMB activities can alter levels of extracellular enzymes, which in turn can affect the microbial community and its metabolic processes (Allison *et al*., 2010; Zechmeister-Boltenstern *et al*., 2015).

The objectives of this study were to assess how changes in forest soil affect *pqqC*- and *phoD*-harbouring PMB communities. We hypothesized that overall structure and key genera of *pqqC*- and *phoD*-harbouring bacterial communities would vary with soil properties, especially in P-limiting tropical and subtropical regions. To test these hypotheses, we focused on forests of Moso bamboo (*Phyllostachys edulis*), since they are economically important in tropical and subtropical regions and they contain abundant metal irons that strongly immobilize P (Terefe *et al*., 2019). How soil properties in these forests influence the diversity and community structure of PMB is still unsolved. We selected three Moso bamboo forest sites in Zhejiang province in China. After analysing soil properties, we used quantitative PCR (qPCR) and Illumina MiSeq sequencing to investigate *pqqC*- and *phoD*-harbouring bacterial communities at these sites.

Results

Physicochemical and biological soil properties

Soil chemical properties differed significantly among the three sites, except for the soil cation exchange capacity (Table S1). The following soil physical properties also differed significantly: soil water content (SWC), soil density, maximum water holding capacity, minimum water holding capacity, capillary porosity (CP) and non-capillary porosity (NCP) (Table S1). In contrast, soil capillary water holding capacity, total porosity and soil aeration varied to a smaller extent across the sites. Furthermore, there were significant differences among the sites in activities of the following extracellular enzymes: cellobiohydrolase, α-glucosidase, acid phosphatase, urease (URE) and ALP (Table 1).

Abundance of pqqC *and* phoD *genes and community diversity*

There were significant differences among the three sites in the abundance of *pqqC* and *phoD* genes (Fig. 1A and B, $P < 0.05$): The *pqqC* gene abundance ranged from 1.47×10^7 to 2.34×10^7 copies g^{-1}, and the *phoD* gene

Table 1. Extracellular enzyme activity of soil samples from three Moso bamboo forest sites.

Enzyme	Site			*F*(*P*)
	Site1_AJ	Site2_CX	Site3_LA	
Cellobiohyrolase (nmol h^{-1} g^{-1})	2.89 ± 0.48 a	0.96 ± 0.52 b	1.35 ± 0.30 b	10.67 (0.011)
α-glucosidase (nmol h^{-1} g^{-1})	2.47 ± 0.80 a	1.01 ± 0.13 b	1.11 ± 0.21 b	5.71 (0.041)
Leucine aminopeptidase (nmol h^{-1} g^{-1})	184.34 ± 9.83 a	162.97 ± 14.15 a	173.61 ± 17.78 a	1.12 (0.386)
β-1,4-N-acetylglucosaminidase (nmol h^{-1} g^{-1})	2.16 ± 0.13 a	1.70 ± 0.23 a	1.73 ± 0.44 a	1.48 (0.301)
β-1,4-glucosidase (nmol h^{-1} g^{-1})	3.75 ± 0.66 a	2.51 ± 0.35 a	2.77 ± 0.55 a	3.00 (0.125)
Acid phosphatase (μmol d^{-1} g^{-1})	41.00 ± 3.43 b	33.44 ± 0.82 c	48.16 ± 1.08 a	23.99 (0.001)
Alkaline phosphatase (μmol d^{-1} g^{-1})	7.98 ± 0.1 a	2.81 ± 0.23 b	7.62 ± 0.65 a	103.50 (< 0.001)
Urease (μg d^{-1} g^{-1})	1188.38 a	1004.55 c	1058.15 b	38.91 (< 0.001)

Values are mean ± standard error (n = 3). Values marked with different letters (a, b, c) in the same row differ significantly according to Duncan's test (α = 0.05).

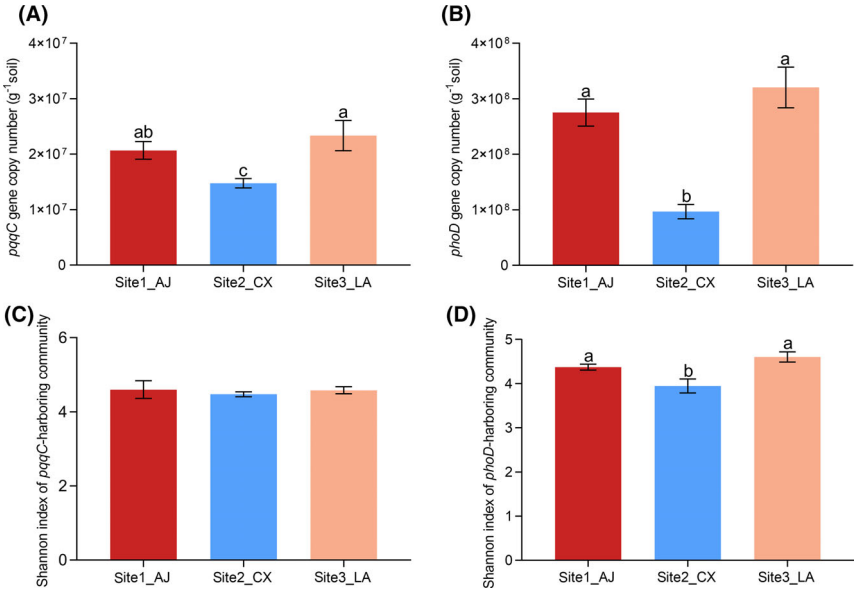

Fig. 1. Abundance and diversity of *pqqC* and *phoD* genes in the three Moso bamboo forest soils: (A) *pqqC* and (B) *phoD* gene copy numbers, as well as Shannon diversity index of (C) *pqqC*-harbouring community and (D) *phoD*-harbouring community. Bars marked with different letters differ significantly (Duncan's test, α = 0.05). Values are mean ± standard error (n = 3).

abundance ranged from 9.69×10^7 to 3.21×10^8 copies·g^{-1}. Site1_AJ and Site3_LA had significantly 0.4–0.5 fold and 1.8–2.3 fold higher abundances of *pqqC* and *phoD* genes in the soil than Site2_CX respectively. The soil at Site3_LA had the largest abundance of *pqqC* and *phoD* genes. Based on the Shannon index, there was significant variation among the sites in the diversity of *phoD*-harbouring bacterial communities, but not of *pqqC*-harbouring bacterial communities (Fig. 1C and D, $P < 0.05$).

Composition of pqqC- *and* phoD-*harbouring bacterial communities*

Principal component analysis (PCA) showed that the soil at each of the three sites contained significantly different compositions of *pqqC*- and *phoD*-harbouring bacteria at the level of operational taxonomic units (OTUs; Fig. 2): the first two axes (PC1 and PC2) explained 74.09% of the total variance in the *pqqC*-harbouring bacterial community and 94.94% of the total variance in the *phoD*-harbouring bacterial community. At the genus level, all three sites had similar dominant genera of *pqqC*- or *phoD*-harbouring bacteria (Fig. 3A and B). Nevertheless, the relative abundance of the dominant genera, that is the most dominant four genera of *pqqC*- or *phoD*-harbouring bacteria varied significantly across the three sites (Table S2; Fig. 3C and D).

The following genera (apart from 'unclassified' bacteria) were most abundant among *pqqC*-harbouring bacteria: *Burkholderia* (8.98–16.69%), *Azotobacter* (5.27–11.05%), *Pseudomonas* (3.31–9.28%) and

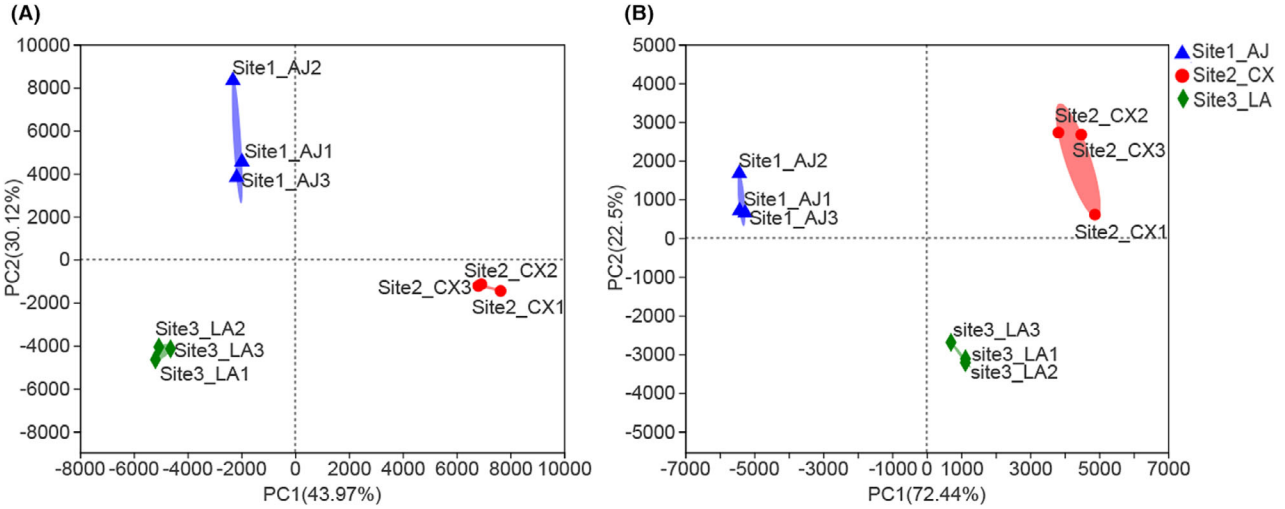

Fig. 2. Principal component analysis of the composition of (A) *pqqC*- or (B) *phoD*-harbouring bacterial communities at the level of operational taxonomic units (OTU).

Mycobacterium (1.25–9.8%; Fig. 3A and C). The relative abundances of *Burkholderia* and *Azotobacter* peaked at Site2_CX, while *Pseudomonas* and *Mycobacterium* had the highest relative abundances at Site1_AJ. The four most dominant genera among *phoD*-harbouring bacteria (except for 'unclassified' bacteria) were *Bradyrhizobium* (6.52–13.4%), *Aquabacterium* (0.01–2.44%), *Rhizobacter* (0.11–2.19%) and *Xanthomonas* (0.17–1.79%; Fig. 3B and D). *Aquabacterium* and *Rhizobacter* had the highest relative abundances at Site3_LA, while *Bradyrhizobium* and *Xanthomonas* had the highest relative abundances at Site2_CX. All four dominant genera showed significantly lower relative abundances at Site1_AJ than at the other sites.

Network structure and key taxa of pqqC- *or* phoD- *harbouring communities*

The network structure of the *pqqC*- or *phoD*-harbouring communities differed significantly among the three sites (Fig. 4). The *pqqC*-harbouring bacterial community at Site 3_LA showed the highest number of nodes (83), highest number of links (421 positive links) and lowest modularity (0.616; Fig. 4A–C). Based on its larger average degree index, the Site3_LA network exhibited higher complexity than the other sites, but the Site1_AJ network showed highest connectivity since it contained the smallest average-shortest-path-length (Table S3). The *phoD*-harbouring community at Site2_CX had the highest number of nodes (80), highest number of links (414 positive links) and lowest modularity (0.727; Fig. 4D–F). The Site2_CX network showed the highest complexity, but the Site1_AJ network showed highest connectivity (Table S3).

In the hub members, all the tested sites contained *Azotobacter* and *Pleomorphomonas* in the *pqqC*-harbouring network and *Afipia* and *Streptomyces* in the *phoD*-harbouring network (Tables S3, S4). Nevertheless, the hub members in the networks differed significantly among the sites, based on the higher degree, degree centrality and closeness centrality indexes (Table S3; Fig. 4). Around 70% of the hub genus in either the *pqqC* and *phoD* network were only observed in one particular site rather than cross presented. (Table S4).

Soil factors affecting phoD- *or* pqqC-*harbouring communities*

Soil properties had a strong effect on the diversity of the *phoD*-harbouring bacterial community, but not on the diversity of the *pqqC*-harbouring bacterial community (Table 2). In the *phoD*-harbouring microbial community, the Shannon index correlated positively with total iron (TI), total aluminium (TA), and NCP, and negatively with available phosphorus (AP), total phosphorus (TP), soil density (SD), CP and ACP.

Relationships between soil physicochemical properties and genera within the *pqqC*- or *phoD*-harbouring communities were evaluated using a correlation heatmap (Fig. 5A and D). In the *pqqC*-harbouring community, the relative abundance of 36% of the top 50 bacterial genera correlated significantly with total nitrogen (TN) and TA. In the *phoD*-harbouring community, in contrast, the relative abundance of 38% of the top 50 genera correlated significantly with soil organic carbon (SOC), SWC and NCP (Table S5). The relative abundance of a handful of genera in both types of communities correlated

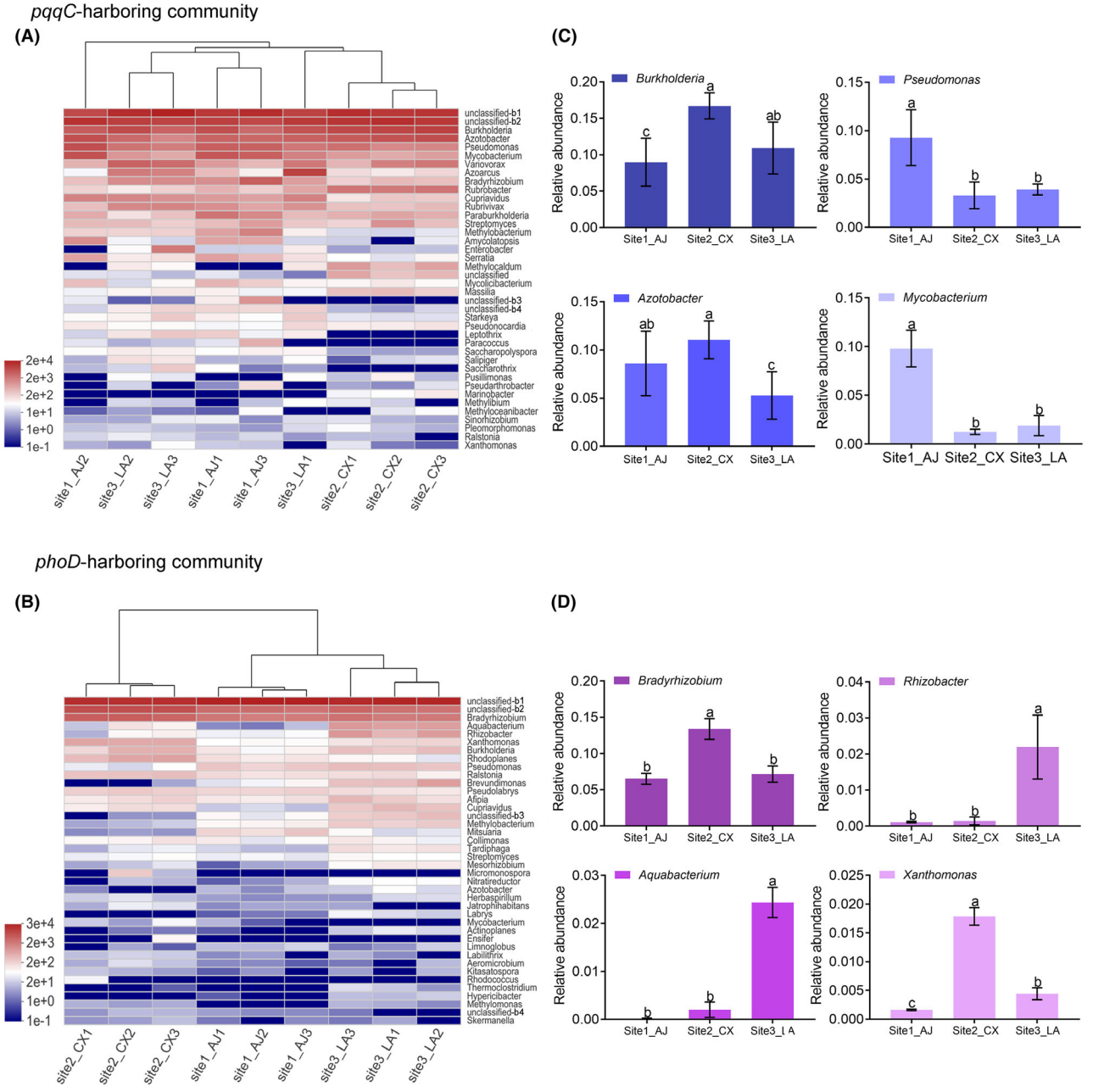

Fig. 3. Relative abundances of the top 40 dominant genera in the (A) *pqqC*- or (B) *phoD*-harbouring communities, as well as the four most abundant genera in the (C) *pqqC*- or (D) *phoD*-harbouring communities at the three sites. The columns in 4a and 4b were clustered based on Bray-Curtis distance. Each column is labelled based on the soil sample and replicate (three replicates for each site).

significantly with soil aeration, total porosity, as well as maximum and capillary water holding capacities.

Based on the first and second component of the redundancy analysis (RDA), the SOC content was the significant factor influencing the structure of the *pqqC*- and *phoD*-harbouring communities (Fig. 5B and E; Table S6). The structure of the *pqqC*-harbouring community also correlated positively with TN, while that of the *phoD*-harbouring community correlated with AP, TP, TI, TA, SWC and NCP. The community structure of both types of community correlated positively with URE and ALP, while the structure of the *phoD*-harbouring community also correlated positively with acid phosphatase activity (Fig. 5C and F).

Discussion

Although abundant genes in bacteria have been documented to associate with the mobilization of phosphorus in the soil, such as *gabY*, *gdh*, *gcd* genes associated

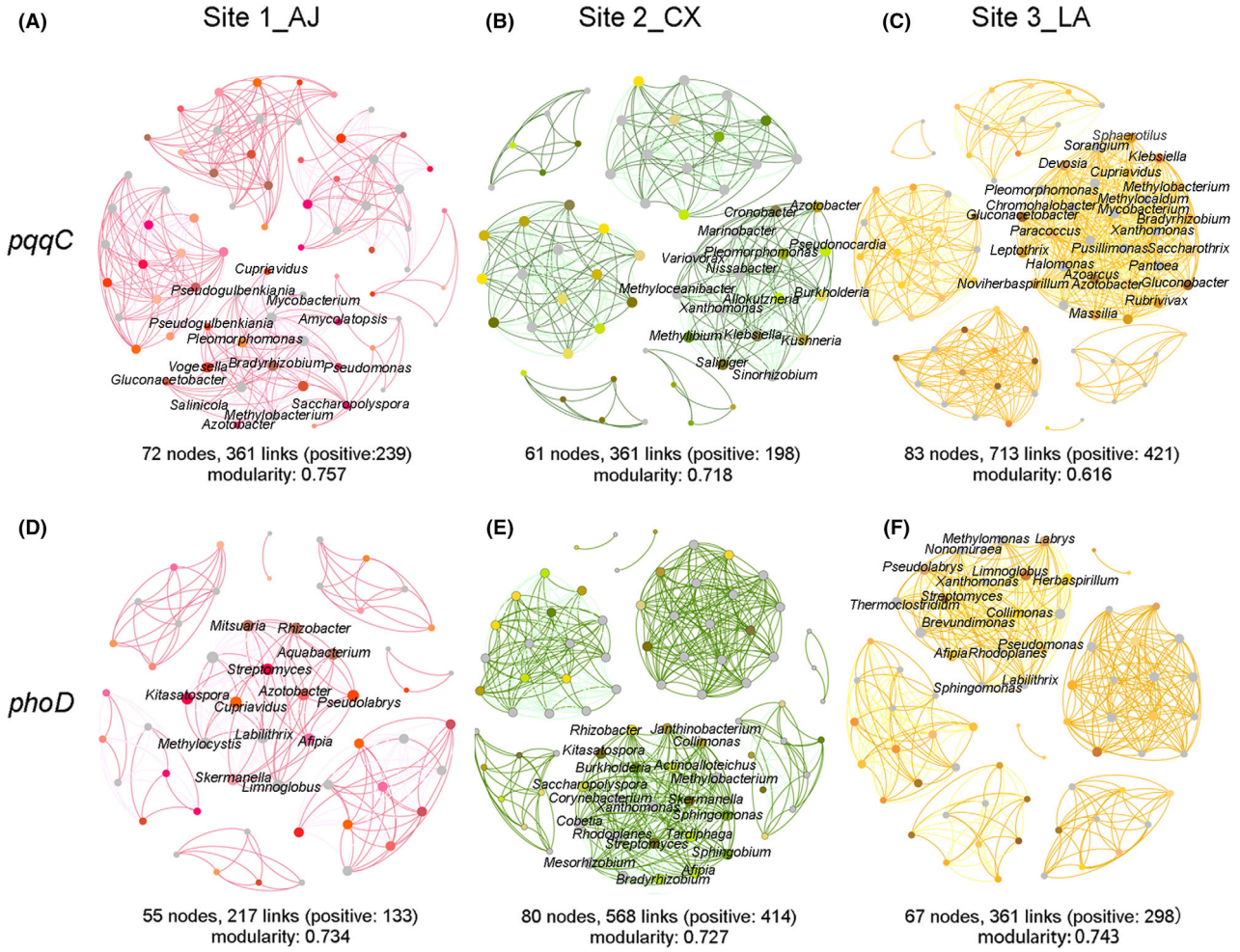

Fig. 4. Microbial association networks of *pqqC*- or *phoD*-harbouring communities at the three Moso bamboo forest sites, at the genus level. Site1_AJ: (A) *pqqC*- or (D) *phoD*-harbouring communities. Site2_CX: (B) *pqqC*- or (E) *phoD*-harbouring communities. Site3_LA: (C) *pqqC*- or (F) *phoD*-harbouring communities. Node colour corresponds to genus taxonomic classification. Edges with dark colour indicate positive interactions; those with light colour, negative interactions.

Table 2. Pearson's correlation analysis of the impact of soil properties on the diversity of *pqqC*- or *phoD*-harbouring bacterial communities. Significant correlations are labelled with asterisks (**$P < 0.01$, *$P < 0.05$).

	Shannon index			Shannon index			Shannon index	
Chemical properties	*pqqC*	*phoD*	Physical properties	*pqqC*	*phoD*	Biological properties	*pqqC*	*phoD*
pH	0.100	0.527	SWC	−0.175	0.634	CBH	0.398	0.146
AP	−0.191	−0.817**	SD	−0.109	−0.730*	αG	0.349	0.200
TP	−0.214	−0.707*	Max-WHC	−0.255	0.606	LAP	0.405	0.219
SOC	0.265	0.502	CWC	0.134	−0.109	NAG	0.427	0.349
TN	0.296	0.499	Min-WHC	−0.191	0.047	βG	0.615	0.213
TI	0.100	0.680*	CP	0.049	−0.832**	ACP	0.036	−0.759*
TA	0.333	0.878**	NCP	0.333	0.879**	URE	0.085	−0.061
CEC	−0.615	−0.476	TPO	−0.452	0.313	ALP	−0.109	−0.631
			SA	−0.067	0.516			

αG, α-glucosidase; βG, β-1,4-glucosidase; ACP, acid phosphatase; ALP, alkaline phosphatase; AP, available phosphorus; CBH, cellobiohyrolase; CEC, cation exchange capacity; CWC, capillary water holding capacity; CP, capillary porosity; LAP, leucine aminopeptidase; Max-WHC, maximum water-holding capacity; Min-WHC, Minimum water holding capacity; NAG, β-1,4-N-acetylglucosaminidase; NCP, non-capillary porosity; SA, soil aeration; SD, soil density; SOC, soil organic carbon; SWC, soil water content; TA, total aluminium; TI, total iron; TN, total nitrogen; TP, total phosphorus; TPO, total porosity; URE, urease.

with solubilization of Pi (Sashidhar and Podile, 2009; Kour *et al*., 2021) and *phoX*, *phoA* and non-specific acid phosphatases (NSAP) genes associated with mineralization of P_o (Lidbury *et al*., 2016; Neal *et al*., 2018), our findings show that the *pqqC* and *phoD* genes can be used as markers of PMB: using high-throughput sequencing, we identified 96 genera related to Pi-solubilizing bacteria and 97 genera related to Po-mineralizing bacteria. It was important to note that the typical *pqqC* and *phoD* genes, among multiple PMB biomarker genes, identified a relatively persuasive picture of the bacterial communities involved in P mobilization. In light of this, this study evaluated the effect of soil properties on PMB communities at three Moso bamboo forest sites in China, with a focus on bacterial communities harbouring the *pqqC* or *phoD* genes. We found significant variation in alpha diversity and gene abundance in PMB, especially *phoD*-harbouring bacteria, across the three sites. This likely reflects the substantial variation in soil conditions across the sites, reflecting the conclusion of Babar *et al*. (2016) that soil conditions can strongly influence soil microbial communities.

Soil microbes require diverse resources (Pivničková *et al*., 2010). Abundance of the *pqqC* or *phoD* gene correlated negatively with soil AP content (Table S7), suggesting that soil P deficiency can stimulate PMB proliferation and variation (Bargaz *et al*., 2017). Additionally, diversity of *phoD*-harbouring bacteria correlated significantly with soil porosity, as measured in CP and NCP (Table 2). In contrast, the diversity of *pqqC*-harbouring bacteria seemed unaffected by variations in most soil parameters. These results suggest that, under natural conditions, diversity of *pqqC*-harbouring microbial communities may tend to be stabler than that of *phoD*-harbouring communities. According to the study of Igwe and Vannette (2019), we suspected that the diversity of *pqqC*-harbouring communities is mainly influenced by vegetation rather than soil conditions.

PMB composition and microbial association networks, especially hub members in *pqq*C- or *phoD*-harbouring communities, varied significantly among the three sites. This partially supports our hypothesis that soil conditions play a critical role in shaping PMB communities. The dominant phylum of *pqqC*- and *phoD*-harbouring bacteria was Proteobacteria and Actinobacteria, as reported by Lagos Pailla *et al*. (2016) on the soil in a pasture in Chile and by Zheng *et al*. (2017) on the soil in an alkaline farmland in central China; soil samples from all three sites consisted of a similar set of dominant genera. This implies that the dominant types of *pqqC*- and *phoD*-harbouring bacteria may be similar across Moso bamboo forest soils (Lin *et al*., 2009). Nevertheless, we saw significant variation in the relative abundances of the dominant *pqqC*- and *phoD*-harbouring genera among the three sites (Fig. 3C and D). These results further support that soil properties can significantly affect PMB activity and abundance, thereby influencing the structure of *pqqC*- and *phoD*-harbouring bacterial communities (Nicolitch *et al*., 2016).

The build-up of phosphate-solubilizing bacterial communities is a result of series of interactions between plant, soil and bacteria, which is tightly related to the resource cycling involved in the interaction processes (Knights *et al*., 2021). Based on the RDA analysis, we found that SOC content played a significant role in determining the structure of *pqqC*- or *phoD*-harbouring bacterial communities (Fig. 5B and E; Table S6): these results are consistent with previous studies that were conducted in red orchard soil (Cui *et al*., 2015) and farmland soil in southern China (Wang *et al*., 2021). Soil C sources can provide nutrients and energy for microbes and affect their activities and biomass (Adamczyk *et al*., 2021), which in turn can stimulate the microbial production of C-mineralizing enzymes that decompose organic C in the soil (Brzostek *et al*., 2013). In this study, we observed that cellobiohydrolase and α-glucosidase activities were significant drivers of the structure of *phoD*-harbouring bacteria: these results indicate that soil C sources can strongly affect the structure of PMB communities (Wallenius *et al*., 2011).

We observed differences between how the structure of the *pqqC*- or *phoD*-harbouring bacterial communities responded to variations in certain soil conditions. The *pqqC*-harbouring community was less sensitive to soil properties, except for TN levels and C sources. This may indicate that N, as a source of nutrition, may stimulate the growth of microbes involved in soil N cycling, such as *Methylobacterium* (Kim *et al*., 2009), *Paracoccus* (Penton *et al*., 2013) and *Serrata* (Gyaneshwar *et al*., 2001) (Fig. 5A). Furthermore, soil urease activity can accelerate N mineralization in the soil, and a change in soil TN can alter soil urease activity (Du *et al*., 2018). Thus, the *pqqC*-harbouring community may regulate urease activity in response to changes in soil N content. At the same time, urease activity at our sites also significantly affected the structure of *phoD*-harbouring bacteria. In light of these results, we conclude that urease activity may be a more reliable soil marker than TN for assessing or predicting changes in PMB communities.

The structure of the *phoD*-harbouring bacterial communities at our sites varied to a greater extent than that of *pqqC*-harbouring communities in response to different soil conditions, especially soil P levels and physical properties. Similar to Gupta *et al*. (2014), we found that soil AP content correlated negatively with the amount of metal elements in the soil, such as TA and TI. These results reflect that soil metal irons immobilize free Pi and therefore reduce available P in the soil (Barroso and

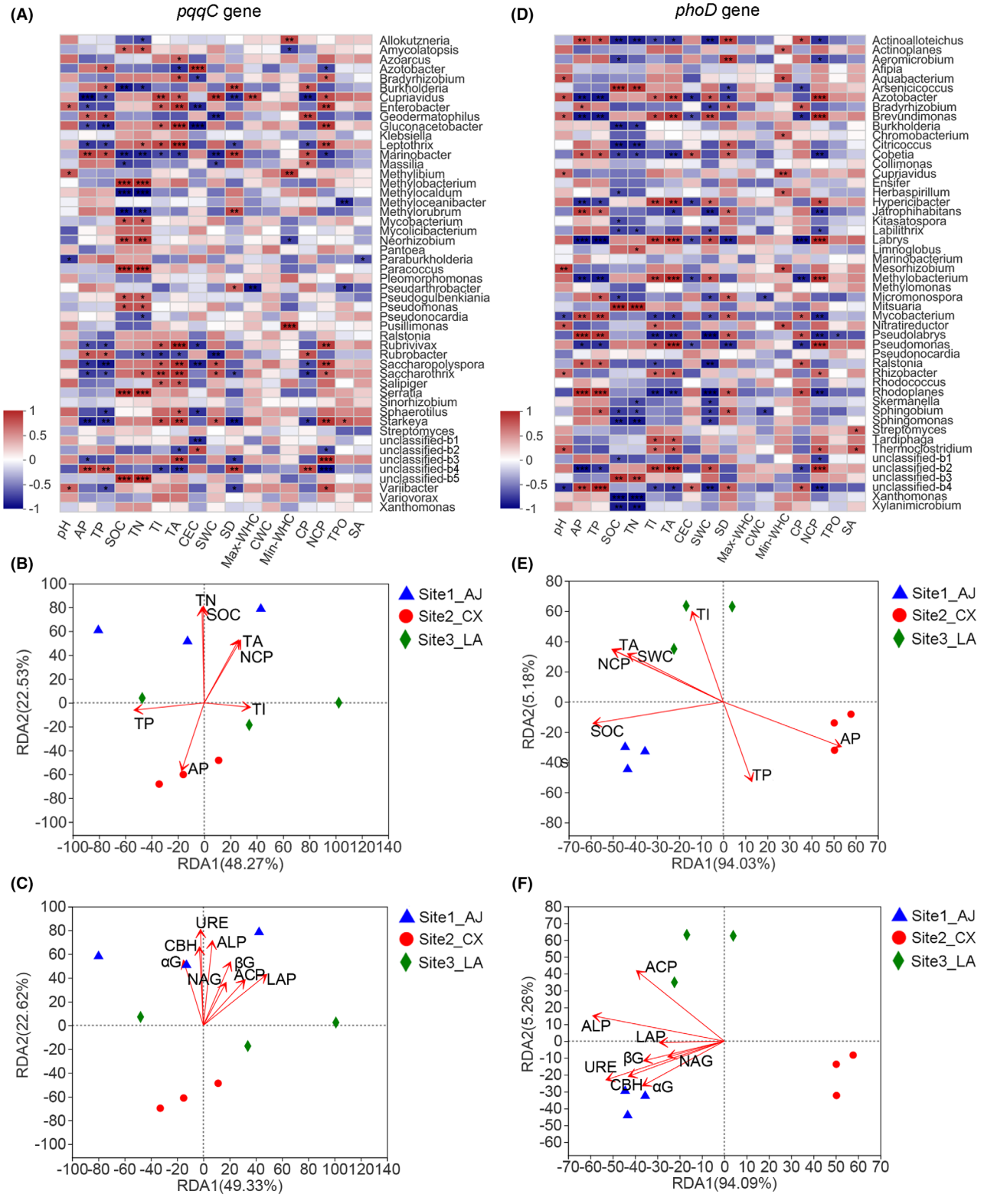

Fig. 5. Relationship between soil physicochemical properties and (A) *pqqC*- or (D) *phoD*-harbouring bacterial genera tested using Spearman's rank correlations analysis, as well as redundancy analysis of the genus-level community structure of (B) *pqqC*- or (E) *phoD*-harbouring bacteria and a subset of selected soil physicochemical properties. Redundancy analysis of the genus-level community structure of (C) *pqqC*- or (F) *phoD*-harbouring bacteria and a subset of soil extracellular enzyme activities. Red arrows indicate the lengths and angles between explanatory and response variables, reflecting their correlations. Coloured dots represent soil samples from the three Moso bamboo forest sites. αG, α-glucosidase; βG, β-1,4-glucosidase; AP, available phosphorus; ACP, acid phosphatase; ALP, alkaline phosphatase; CBH, cellobiohyrolase; LAP, Leucine aminopeptidase; NAG, β-1,4-N-acetylglucosaminidase; NCP, non-capillary porosity; SOC, soil organic carbon; SWC, soil water content; TA, total aluminium; TI, total iron; TN, total nitrogen; TP, total phosphorus. URE, urease.

Nahas, 2005). On the contrary, phosphatase play great roles in remobilizing P sources by dephosphorylating organic compounds in the soil, especially under low AP conditions; concomitantly, the activity of acid phosphatase universally indicated to enhance the interactions between plant, soil and microbes (Neal *et al*., 2018). These, in turn, affect the composition and relative abundance of soil bacteria, especially for those sensitive to soil conditions variations (*e.g. phoD*-harbouring bacteria). For example, we found that the dominant *phoD*-harbouring genus, *Bradyrhizobium*, had the highest relative abundance at Site2_CX, where soil AP content was highest (Table S1; Fig. 3). *Bradyrhizobium* is an N-fixing symbiotic bacterium that requires a phosphate transport system, and it can produce phosphatase to increase soil AP content (Ikoyi *et al*., 2018). Consistent with our findings, *Bradyrhizobium* is dominant in P-rich soil (Long *et al*., 2017) and soil P levels influence phosphatase activity (Hu *et al*., 2018), which correlated significantly with the structure of the *phoD*-harbouring bacterial communities in the present study (Table S6; Fig. 5C and F).

According to our RDA analysis, soil NCP and SWC showed significant positive correlations with the structure of the *phoD*-harbouring bacterial community, indicating that the air permeability and moisture conservation of the soil are important factors influencing *phoD*-harbouring bacterial community structure. This finding is consistent with the idea that the physical structure of the soil strongly influences microbial processes (Falconer *et al*., 2009; Trivedi *et al*., 2015). Reduced porosity and increased moisture in soil mean fewer air-filled pores and slower gas diffusion (Frey *et al*., 2009; Wu *et al*., 2017), which creates anoxic micro-environments that can strongly condition microbial habitats (Sitaula *et al*., 2000; Schnurr-Pütz *et al*., 2006). Our results suggest that there may be a larger ratio of anaerobic to aerobic bacteria in the *phoD*-harbouring bacterial community than in the *pqqC*-harbouring bacterial community, similar to the conclusion of Zhang *et al*. (2013) based on their studies of forest soils in southeastern China.

Our study suggests that soil C sources and P levels play important roles in determining the diversity and structure of *pqqC*- or *phoD*-harbouring bacterial communities. Of the two types of communities, *pqqC*-harbouring microbes at the Moso bamboo forest sites appear to be less sensitive to changes in soil properties. The *phoD*-harbouring communities were also structured by soil physical properties such as soil NCP and SWC, and the *pqqC*-harbouring community were only influenced by soil TN besides of SOC. The PMB communities at these sites strongly correlated with activities of various soil enzymes, especially urease and ALP. These suggests that the effects of PMB may be a set of actions orchestrated by different functions (Ossowicki *et al*., 2021), and in turn, the PMB communities can be affected by their functional attributes derived by various soil properties. Our findings extend the current understanding of how soil properties influence entire PMB communities, with implications for the biotechnological application of PMB to alleviate P limitation in agricultural and forest ecosystems. Future work should verify and extend our findings using other P-mobilizing genes (*e.g. phoX*, *gabY* and NSAP genes) as potential PMB markers. Such work should also aim for a more comprehensive analysis of how the PMB respond to the soil-plant system, and how microbial functions are affected by environmental factors such as soil P levels.

Experimental procedures

Soil sampling

Rhizosphere soil samples were collected in September 2020 from three Moso bamboo forest sites (Site1_AJ, Site2_CX and Site3_LA; Fig. S1). The three sites had a subtropical monsoon-type climate with a mean average annual temperature of 17.9°C (range, −8.6 to 41.8°C) and mean annual precipitation of 1865.4 mm for the period 2005 to 2020. The sites are located at an elevation of 102–240 m a.s.l, with slopes in the range of 0–5%. All three bamboo forest sites contain a single bamboo species, and no supplemental mineral elements or organic materials have been added to the soil since 2006.

After removing interposed stone and litter, we collected five replicated intact soil cores using cutting rings (100 cm^3) placed at a depth of 10–20 cm in order to measure soil physical properties. An additional 15 rhizosphere soil samples were collected at an upper depth of 5–10 cm at each site: five of these samples were thoroughly mixed and passed using a 2-mm sieve in order

to derive three replicates of mixed soil samples for each site. Each mixed soil sample was then divided into two groups, one of which was stored in liquid nitrogen and the other on ice. The samples stored in liquid nitrogen were then transferred to −80°C for subsequent DNA extraction and enzymatic determination, while the samples stored on ice were used for measurements of soil chemical properties and enzyme activity.

Analysis of physicochemical and biological soil properties

The pH of the soil samples was measured with a pH electrode (PHS-3CW, Hangzhou Secco Instrument Company, Hangzhou, China) using a suspension of air-dried soil and distilled water in a ratio of 1:2.5 (soil: water, w: v). Levels of AP and TP in the soil were determined using the molybdenum-blue colorimetric method (Murphy and Riley, 1962). Soil TN was determined by colorimetric analysis using the Kjeldahl method (Bremner, 1960), while SOC was measured using dichromate oxidation (Nelson and Sommers, 1982). TI and TA levels in the soil were determined using inductively coupled plasma-mass spectrometry (ICP-MS), while cation exchange capacity was measured using the $BaCl_2$-H_2SO_4 method (Zhang *et al*., 2010). Soil physical properties were determined using the cutting ring method (Institute of Soil Science, 1978). Activities of cellobiohydrolase, α-glucosidase, leucine aminopeptidase, β-1,4-N-acetylglucosaminidase and β-1,4-glucosidase were measured as described by Saiya-Cork *et al*. (2002). Acid phosphatase in soil was measured using the S-ACP Kit (Comin Biotech, Suzhou, China); ALP, using the S-ALP Kit (Comin Biotech); and urease, using the S-UE Kit (Comin Biotech).

DNA extraction and gene quantification

Microbial DNA was extracted from 0.5 g of soil samples stored in liquid nitrogen using a FastDNA® Spin Kit for Soil (MP Biomedicals, Burlingame, CA, USA) according to the manufacturer's protocols. The concentration of extracted DNA was analysed using NanoDrop2000 (Thermo Scientific, Wilmington, DE, USA), and DNA quality was assessed using 1% agarose gel electrophoresis. DNA was stored at −20°C until use. All qPCR reactions for *pqqC* and *phoD* genes were performed in triplicate using a ABI7300 real-time PCR system (Applied Biosystems, Carlsbad, California, USA) with the following primer pairs: Fw (AACCGCTTCTACTACCAG) and Rv (GCGAACAGCTCGGTCAG) for the *pqqC* gene (Zheng *et al*., 2017), or ALPS-F730 (CAGTGGGACGACCACGAGGT) and ALPS-R1101 (GAGGCCGATCGGCATGTCG) for the *phoD* gene (Sakurai *et al*., 2008). These *phoD* primers provide relatively accurate phylogenetic and taxonomic information, even if they have limited biases towards *Alphaproteobacteria* (Tan *et al*., 2013; Luo *et al*., 2019). The qPCR amplification of each sample was done in triplicate with 20-μl reactions under the following procedures: 5 min at 95°C, followed by 35 cycles for 30 s at 95°C, then 30 s at 58°C for annealing, and finally 1 min at 72°C. The qPCR reaction mixture included the primers (0.8 μl of each primer), diluted DNA solution (1 μl), ddH_2O (7.4 μl) and 2×Taq Plus Master Mix (10 μl). A plasmid standard harbouring the target genes was constructed from the pMD18-T Easy vector (Takara Bio, Beijing, China) and verified by sequencing. Standard curves were generated using 10-fold serial dilutions of the plasmid. The number of *pqqC* and *phoD* gene copies was calculated by measuring the concentration of the plasmid and the number of base pairs. Amplification efficiencies ranged from 96 to 100%, and we obtained a calibration R^2 value of 0.996 for *pqqC* and 0.998 for the *phoD* gene.

PCR amplification, high-throughput sequencing and data preprocessing

Partial fragments of the bacterial *pqqC* and *phoD* genes were amplified using the abovementioned primer pairs in a thermal cycler system (GeneAmp 9700, ABI, USA). Each 20-μl PCR reaction was performed in triplicate with 5× FastPfu Buffer (4 μl), 2.5 mM dNTPs (2 μl), 0.8 μl of each primer (5 μM), FastPfu Polymerase (0.4 μl), BSA (0.2 μl) and template DNA (10 ng). The PCR program for the *pqqC* gene and *phoD* gene included 3 min of denaturation at 95°C, 35 cycles of 30 s at 95°C, 30 s for annealing at 55°C and 45 s for elongation at 72°C, as well as a final extension for 10 min at 72°C. The PCR products were extracted from 2% agarose gels and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, Madison City, WI, USA) according to the manufacturer's protocol.

The purified PCR products were pooled in equimolar amounts, and paired-end sequenced on an Illumina MiSeq platform according to standard protocols outlined at Majorbio Bio-Pharm Technology (Shanghai, China). Raw fastq files were quality-filtered using Trimmomatic and merged using Flash 1.2.11 based on the following criteria: (i) reads were truncated at any site receiving an average quality score < 20 over a 50-bp sliding window; (ii) sequences overlapping by more than 10 bp were merged accordingly, while keeping the mismatch to no more than 2 bp; (iii) sequences of each sample were separated according to barcodes (exact matches) and primers (allowing for 2-nt mismatching); and (iv) reads containing ambiguous bases were removed.

We obtained a total of 1 292 434 raw sequences from the *pqqC* gene community and 1 137 922 raw sequences from the *phoD* gene community across all the three sites. There were a total of 646 217 effective sequences for the *pqqC* gene community, ranging from 69 172 to 74 260 across the sites; and 568 961 for the *phoD* gene community, ranging from 45 903 to 74 587 across the sites. A 97% similarity cut-off was used to cluster OTUs using Uparse 7.1 (http://drive5.com/uparse/). The taxonomy of each gene sequence was analysed by comparing the Ribosomal Database Project Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU128) database using a confidence threshold of 70%.

Construction of microbial association networks

The relationships among bacterial genera of the *pqqC*- or *phoD*-harbouring communities at each Moso bamboo forest site were analysed using Spearman's rank correlation coefficient ($|r| > 0.5$; $P < 0.05$): this analysis was performed using the *psych* package in R (R Core Team, 2019). Microbial association networks were generated using the bacterial genera as nodes, which were connected using lines ('edges') corresponding to positive or negative associations between the genera. The topology of the microbial association networks was determined using the *networkx* package in R (R Core Team, 2019). The topological properties (modularity, number of edges and number of nodes) show the relationships among the genera. Additionally, degree refers to the number of nodes directly connected with a node. Degree centrality is the most direct metric to describe node centrality, and closeness centrality is used to evaluate the distance from other nodes. The largest degree, degree centrality and closeness centrality can provide evidence on nodes of key microbial genera (hub members), which are part of essential network interactions. The networks were visualized using Gephi 0.9.2 (http://gephi.org).

Statistical analysis

Differences in abundance of *pqqC* and *phoD* genes, community diversity, and soil properties among the Moso bamboo forest sites were assessed for significance using one-way ANOVA in SPSS 22.0 (IBM, Chicago, IL, USA). Multiple comparison of means was conducted using a Duncan test at $\alpha = 0.05$. RDA of microbial structures was evaluated based on biological properties and seven physicochemical properties that correlated significantly with the relative abundance of many bacterial genera. PCA, RDA and community heatmaps were analysed using the *vegan* package in R (R Core Team, 2019). Correlation heatmaps were used to illustrate Spearman's correlations between soil properties and bacterial genera in the *pqqC* and *phoD* communities: these maps were generated individually using the *heatmap* package in R. All histograms were generated in GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA).

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Conflict of interest

The authors declare no conflicts of interest.

Data availability statement

Any other data not included in the manuscript but important for a detailed review can be found online in the Supporting Information section.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1: Distribution of soil sampling sites (Site1_AJ, Site2_CX, Site3_LA) in Zhejiang province, China.

Table S1: Physicochemical characteristics of soil samples from three Moso bamboo forest sites.

Table S2: Relative abundances of top 40 abundant bacterial genera in *pqqC*- and *phoD*-harboring bacterial community among three Moso bamboo forest sites.

Table S3: The topological construction of microbial association networks and microbial genera in *pqqC*- and *phoD*-

harboring bacterial community among three Moso bamboo forest sites.

Table S4: The hub genera in *pqqC*- and *phoD*-harboring bacterial community among three Moso bamboo forest sites.

Table S5: Spearman's correlations analysis of the impact of soil properties on the top 50 dominant genera in *pqqC*- and *phoD*-harboring bacterial community.

Table S6: Adonis tests of the effect of selected soil physico-chemical properties and enzyme activities on the *pqqC*- and *phoD*-harboring bacterial community structures.

Table S7: Pearson's correlations analysis of the impact of soil properties on the *pqqC* and *phoD* gene abundances.

