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Integrated microbiology and metabolomics analysis reveal responses of soil microorganisms and metabolic functions to phosphorus fertilizer on semiarid farm

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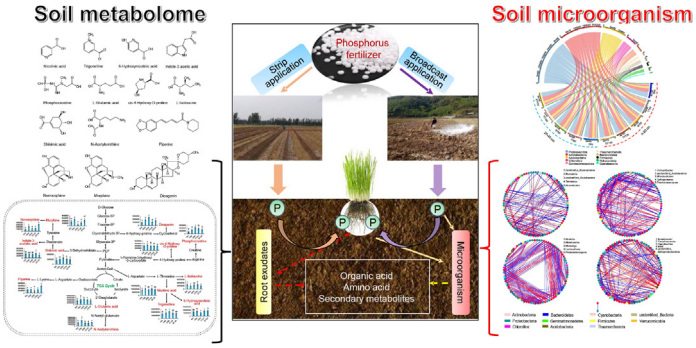
HIGHLIGHTS

- P fertilizer decreased the diversity of bacterial and fungal genes.
- P fertilizer significantly changed microbial community structures and compositions.
- P significantly altered soil metabolic compositions and their metabolic pathways.
- Accumulation of metabolites in soil was closely correlated with microbes.

GRAPHICAL ABSTRACT



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ABSTRACT

Localized fertilization of phosphorus has potential benefits in achieving higher crop productivity and nutrient use efficiency, but the underlying biological mechanisms of interactions between soil microorganisms and related metabolic cycle remain largely to be recognized. Here, we combined microbiology with non-target metabolomics to explore how P fertilizer levels and fertilization patterns affect wheat soil microbial communities and metabolic functions based on high-throughput sequencing and UPLC-MS/MS platforms. The results showed P fertilizer decreased the diversity of bacterial 16S rRNA genes and fungal ITS genes, and it did significantly change both soil bacterial and fungal overall community structures and compositions. The P levels and patterns also interfered with complexity of soil bacterial and fungal symbiosis networks. Moreover, metabolomics analysis showed that P fertilizer significantly changed soil metabolite spectrum, and the differential metabolites were significantly enriched to 7 main metabolic pathways, such as arginine and proline metabolism, biosynthesis of plant hormones, amino acids, plant secondary metabolites, and alkaloids derived from ornithine. Additionally, microbes also were closely related to the accumulation of metabolites through correlation analysis. Our results indicated that localized appropriate phosphorus fertilizer plays an important role in regulating soil microbial metabolism, and their interactions in soil providing valuable information for understanding how the changed phosphorus management practices affect the complex biological processes and the adaption capacity of plants to environments.

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1. Introduction

As the global population grows, global demand for food will continue to rise rapidly, including wheat, and a major cereal crop around the world (Long et al., 2015; Shi et al., 2020). An adequate phosphorus (P) supply is important for wheat production during the primary growth cycle of wheat (Shi et al., 2020). Therefore, this macronutrient is essential for improving crop production (Liu et al., 2020). Natural P sources are relatively few and nonrenewable, and a large proportion of P is immobilized in soil in a form of unusable organic or inorganic P (Rodriguez and Fraga, 1999; Tang et al., 2020), while available soil P is lost as run-off (Daverede et al., 2003; McDowell et al., 2001; Veneklaas et al., 2012). As a result, inadequate P is absorbed by most crops although Chinese soil contains up to 0.7 g P kg^{-1}, which severely limits their growth (Cordell and White, 2015; Dawson and Hilton, 2011; Li et al., 2020).

P fertilizer can improve plant growth and crop yield (Liu et al., 2016), and its global use has been increasing rapidly. However, its irresponsible application also harms the environment (Sharpley, 1995), such as when excess P runs off farmland, it will contaminate local water supplies (Dai et al., 2020). P fertilizer alters the N-to-P ratio and its other properties, such as organic matter and enzyme activity, which can further alter the structure, diversity and activity of soil microbial community (Fierer et al., 2007; Krashevska et al., 2014). This can also affect microbial functional characteristics responsible for C and N cycles (Leff et al., 2015). Indeed, soil microorganisms drive multiple ecosystem functions and maintain plant productivity (Chen et al., 2020; Lychuk et al., 2019). Thus, researches are needed about optimizing P fertilizer use on farmland to maximize crop production while also ensuring sustainable P use and causing minimal environmental pollution (Shi et al., 2020).

The availability of soil P to plants depends on numerous complex processes and on the soil chemical, biological, biochemical and physical characteristics (Chen et al., 2019; Wang et al., 2016). Soil microorganisms can encode enzymes and other proteins that contribute to the dissolution of inorganic P or the mineralization of organic P (Dai et al., 2020). For example, microbes produce glucosamine dehydrogenase that helps dissolve inorganic P (Elias et al., 2001) and alkaline phosphatases that mineralize organo-phosphorus compounds in soil (Rodriguez et al., 2006). These enzymes enable microorganisms to fix P in their biomass, so they may compete with plants for available P in agricultural ecosystems (Richardson and Simpson, 2011). P-solubilizing microorganisms play a key role in the P cycle (Zhu et al., 2018), so they are helpful to increase the availability of soil P in an inexpensive, and environmentally friendly way (Owen et al., 2015).

P fertilizer can alter the amount, diversity, abundance and composition of soil microorganisms, which include bacteria and fungi (Bunemann et al., 2008; Cruz et al., 2009; Spohn et al., 2015). In the case of bacteria, one study of grassland soil found that the diversity of bacterial community and the proto-nuclear alkaline phosphatase (*phoD*) genes that they expressed were increased by long-term application of P fertilizer (Tan et al., 2013). Another study found that P fertilizer significantly increased the diversity and abundance of most genes related to the P cycle, and it affected the species evenness and key genera in the community, such as increasing the abundances of Oxalobacteraceae (mainly *Massilia* and *Herbaspirillum*), *Klebsielle Burkholderia* sp. and *Bacillus* sp. (Su et al., 2015). Additionally, one study also concluded that P fertilizer significantly affected soil microbial community structure and a few rare microbial taxa (Li et al., 2020). P fertilizer also appears to substantially affect soil fungi, which in fact may be more sensitive to it than bacteria, which was confirmed by results of a study that P fertilizer increased soil fungal biomass faster than that of bacteria, and the ratio of fungi to bacteria increases (Li et al., 2015). These results highlight the significance for further research how P fertilizer affects microbial communities in soil. P fertilizer can sensitize soil microbial communities to changes in P level (Wang et al., 2018), altering bacterial diversity and composition (Cheng et al., 2020a). The effects of P fertilizer on wheat fields and on the soil microbial communities in those fields have rarely been studied.

However, the further results of microbial community change is the change of soil metabolic function, which will better reveal the influence mechanism of phosphorus fertilizer availability on soil microenvironment (Song et al., 2020; Wu et al., 2021). Thus, the key to exploring these issues is molecular analysis of soil metabolites (Seifert et al., 2016), which are important carbon sources for microorganisms (Swenson et al., 2018) and can alter soil microbial community structure and activity, which in turn influence the metabolite composition (Liang et al., 2017; Lucas et al., 2016). Soil is a highly complex mixture of minerals and organic substances (Traxler and Kolter, 2015), whose composition is affected not only by large-scale factors such as climate, temperature and humidity, but also local phenomena such as human activity (Nguyen et al., 2020). Soil composition plays a role in regulating many processes, such as plant growth and microbial biology (Rugova et al., 2017; Swenson et al., 2018). Thus, soil metabolomics, such as using liquid chromatography-tandem mass spectrometry (Nguyen et al., 2020), provides a window into microbial behavior (Rodriguez et al., 2020; Swenson et al., 2018).

Additionally, metabolomics technology can also help to obtain the changes of soil metabolites and metabolic pathways after soil fertilization. What's more, it can be combined with microbiome to further obtain potential impact biomarkers, and has proved to be a powerful tool to understand exogenous interference in biological systems (Wu et al., 2022). Soil metabolomics can also realize the coupling of organic/inorganic compounds and soil microbial community (Song et al., 2020). However, the effects of P fertilizer on soil microbial metabolites, metabolic pathways and functions are still lack of research. Therefore, combining analysis of the composition of microbial communities with the composition and functional metabolic pathways of soil metabolites helps us to have a deeper understanding of the complex biological processes in soil (Morton et al., 2019).

Therefore, the present study explored how P fertilizer alters soil microbial communities and metabolic functions in wheat fields, and analyze whether microorganisms are closely related to the synthesis of metabolites in soil. We studied the effects of P fertilizer at three levels (0, 60 and 120 kg ha^{-1}) and two patterns (strip and broadcast application) during wheat mature period. Quantitative PCR (qPCR) and Illumina NovaSeq sequencing of 16S rRNA and ITS genes were performed. The objective of this study was to investigate the response of soil microbial diversity and community structure, soil metabolic functions to the P fertilizer, which may help us identify the microbial taxa involved in P metabolism and guide P fertilizer application.

2. Materials and methods

2.1. Site description and experimental design

The study site was located at the Changwu Loess Plateau Agricultural Ecology Experimental Station of the Chinese Academy of Sciences (35°14′ N, 107°41′ E), which has a warm, temperate, semi-humid continental monsoon climate. The zonal soil is black loess, and the parent material is Malan loess. The soil exhibits a good "soil reservoir" effect because it is loose and permeable. The groundwater is too deep for crops to be irrigated, so plant growth is supported only by natural rainfall during the growing period (Cheng et al., 2020b).

Experiments were carried out in an area of 75 × 12 m (900 m^2) divided into plots of 7 m × 4 m. Wheat seeds (Changhan 58) were planted in October 2019 with a row spacing of 20 cm and a density of 3.5 million plants ha^{-1}. P fertilizer containing P_2O_5 (52% superphosphate) was applied in plots with 5 treatments, including three P levels (0, 60 and 120 kg ha^{-1}) and two patterns (strip application (applying at 5 cm below the wheat seeds) and broadcast application (applying spread evenly). All five treatments were performed in triplicate. The plots that received P fertilizer as well as the control also need to apply N and K fertilizer to meet other nutritional needs of plant growth. Urea containing 46.4% N was added to fields by three times: 60% of the total N fertilizer applied was

added initially as basic fertilizer, followed by 20% at the tillering stage and finally 20% at the jointing stage. K_2O with 50% potassium sulfate was applied once before sowing as a potassium source.

2.2. Soil sample collection

During the mature period, wheat soil samples of 0–20 cm (topsoil) and 20–40 cm (subsoil) below the surface were collected randomly in every plot after removing surface litter in May 2020. Soil samples from same treatment plots were evenly mixed, sieved, and divided into two portions. One portion was placed in the −20 °C refrigerator of Yangling Xinhua Ecological Technology Co., Ltd. for DNA extraction (three replicates), and the other was frozen for metabolite extraction (six replicates).

2.3. DNA extraction, Illumina NovaSeq sequencing and data analysis

Genomic DNA of each soil sample was extracted using the CTAB method, and its purity and concentration were assessed by agarose gel electrophoresis. The V3–V4 region of the bacterial 16S rRNA gene and the ITS1-5F region of the fungal ITS gene were amplified using the respective primer pairs 341F and 806R (Imparato et al., 2016) or pairs ITS5-1737F and ITS1-2043R (Li et al., 2016; Zhang et al., 2018). Sequencing libraries were constructed using the TruSeq® DNA PCR-Free Sample Preparation Kit according to the manufacturer's instructions. Finally, sequencing was performed using the NovaSeq6000 platform at Novogene Bioinformatics Technology Co. Ltd. (Beijing, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (accession codes PRJNA658959 and PRJNA659106).

Raw tags obtained through splicing by FLASH (version 1.2.7) (Magoc and Salzberg, 2011) were subjected to strict filtering to obtain clean tags (Bokulich et al., 2013), including tag interception and tags with length less than 75% filtering. Final effective tags were obtained after removing chimeric sequences. All effective tags of all soil samples were clustered using Uparse software (version 7.0.1001) (Haas et al., 2011), and sequences with ≥97% similarity were assigned to the same operational taxonomic unit (OTU) (Edgar, 2013). Each representative sequence was annotated with taxonomic information using the databases SILVA 138 (Quast et al., 2013) for bacteria or UNITE 7.2 for fungi. The number of OTUs and alpha diversity indexes, such as Shannon and Chao1, were calculated to assess microbial diversity and richness. The principal component analysis (PCA) was used to identify soil microbial community structure and estimate beta diversity.

2.4. Network analysis

Connections within and between bacterial and fungal taxa at the genera level were identified using network analysis in Cytoscape 3.7.1 and the *igraph* package in R (Shannon et al., 2003; Zheng et al., 2018). The bacterial and fungal genera were used to construct networks across the entire soil for 0 and 60 kg P ha^{-1} treatments (insufficient-P), and for 60 and 120 kg P ha^{-1} treatments (sufficient-P), respectively. Genera with average relative abundances higher than 0.1% (bacteria) or 0.01% (fungi) were subjected to Spearman's correlation analysis. Bacterial and fungal genera with Spearman's correlation >0.7 or <−0.7 and significance $P < 0.05$ were used to establish microbial networks. The Network Analyzer tool was used to estimate the network topological characteristics, such as topological coefficient, degree, clustering coefficient and average shortest path length. The minimal Common Oncology Data Elements (MCODE) application with standard parameters was used to analyze modular structure and highly interconnected nodes (Zheng et al., 2018), and the top five genera with the highest betweenness centrality scores were considered keystone species (Gonzalez et al., 2010; Vick-Majors et al., 2014), which will significantly influence the stability of microbial community.

2.5. Soil metabolite extraction

Topsoil samples (100 mg) were individually ground into homogenate with liquid nitrogen, then added into pre-chilled 80% methanol and 0.1% formic acid, and suspended by vortexing. Then the samples were incubated on ice for 5 min and centrifuged at 15,000*g* at 4 °C for 5 min. The obtained supernatants were diluted with LC/MS-grade water to the desired final concentration in 53% methanol. The diluted samples were subsequently transferred to a fresh tube and centrifuged again for 10 min. The final supernatants were used for metabolomics analysis, and an equal volume was taken aside for use as quality control (QC) samples.

2.6. UHPLC-MS/MS analysis and data analysis

A Vanquish UHPLC system (Thermo Fisher, Germany) coupled with an Orbi-trap Q Executive TM HF mass spectrometer (Thermo Fisher) (Novogene, Beijing, China) was used for an ultra-high performance liquid chromatography-tandem mass-spectrometry (UHPLC-MS/MS) analysis. The extracts were separated onto a Hypesil Gold column (100 × 2.1 mm, 1.9 μm) using a 17-min linear gradient at a flow rate of 0.2 mL min^{-1}. The mobile phase for the positive polarity mode was composed of 0.1% formic acid in MilliQ water (A) and methanol (B). Chromatographic gradient elution procedures were as follows: 0–1.5 min, 2% B; 1.5–12.0 min, 2–100% B; 12–14.0 min, 100% B; 14.0–14.1 min, 100–2% B; 14.1–17 min, 2% B. Mass spectrometry was performed using electrospray ionization and the following parameters: spray voltage, 3.2 kV; capillary temperature, 320 °C; sheath gas flow, 40 psi; auxiliary gas flow, 10 psi; and scan range, m/z 70–1050.

The raw data files generated by UHPLC-MS/MS were processed using the Compound Discoverer 3.1 (CD3.1, Thermo Fisher) for peak alignment, picking, and metabolite quantitation. The normalized data were used to predict molecular formulas based on additive ions, molecular ion peaks and fragment ions. Then peaks were matched using the mzCloud, mzVault and Mass. List database to obtain accurate qualitative and relative quantitative results. Metabolites were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa and Goto, 2000) and the human metabolome database (HMDB database, https://hmdb.ca/). PCA and partial least squares discriminant analysis (PLS-DA) were performed using metaX (www.metaxsoft.com/). Univariate analysis (*t*-test) was used to assess statistical significance (*P*-value). Metabolites that differed significantly between any two of the five treatments were defined as those with a Variable Importance in the Projection (VIP) > 1.0, *P*-value < 0.05 and fold-change (FC) ≥ 1.2 or ≤0.833. Correlations between differential metabolites (DMs) were analyzed using the Pearson method in R. The statistically significant correlations (*P*-value < 0.05) were identified using the *cor.mtest* in R. Correlation plots were generated using the *corrplot* package in R.

Metabolite function and metabolic pathways were enriched using the KEGG database. The metabolic pathways enriched in DMs were defined as those for which $P < 0.05$ and for which $x/n > y/N$, where N refers to the number of metabolites involved in the KEGG pathway; n, the number of DMs in N; y, the number of metabolites annotated to a KEGG pathway; and x, the number of DMs enriched in a KEGG pathway.

2.7. Correlation analysis of dominant microbial genera and differential metabolites

To identify candidate metabolites that could influence the bacterial and fungal communities associated with the P cycle. In addition, we determined correlations between the metabolic profiles (six independent replicates) and microbiome data (three independent replicates). We searched for correlations between the top 10 genera and obtained DMs.

2.8. Statistical analysis

All statistical analyses were conducted using SPSS 22.0. Differences in all P fertilizer treatments were assessed for significance using one-way

analysis of variance (ANOVA). The microbial diversity and richness were assessed by the number of OTUs, Shannon and Chao1 index. Metastats in Mothur 1.43.0 (https://mothur.org/) was used to analyze the differences of microbial community compositions among five treatments, and *P*-values less than 0.05 was considered that there were significant. The linear discriminant analysis (LDA) effect (LEfSe) method relying on the nonparametric factorial Kruskal-Wallis test ($\alpha = 0.05$) was to identify biomarker with statistical difference among all treatments (Segata et al., 2011). Multivariate statistical analysis, including PCA and PLS-DA, were conducted to reveal differences in functions among five treatments. Pearson correlation analysis and the *t*-test were performed to integrate and visualize sequencing and metabolomics data sets.

3. Results

3.1. Sequence data and microbial richness and diversity

The 16S rRNA and ITS gene sequencing yielded 1,473,243 and 2,486,782 effective tags after quality control. After OTU clustering at the 97% level, 2935 bacterial OTUs and 823 fungal OTUs were retained. Rarefaction analyses showed that the curve of bacterial 16S genes tended to flatten at 1,473,243 sequences (Fig. S1a), while the curve of fungal ITS genes tended to approach the saturation plateau at 2,486,782 randomly selected sequences (Fig. S1b). Alpha diversity indexes were calculated based on OTU level to quantify the diversity and richness of the microbial community (Table 1). Compared with the control, all P fertilizer treatments except 120 kg P ha^{-1} in broadcast application increased the number of observed OTUs and Chao1 index of 16S rRNA gene in topsoil, while significantly decreased the two indexes in subsoil except strip application at 60 kg P ha^{-1}. The Shannon index was higher only for strip application at 120 kg P ha^{-1} than for the control in topsoil and all P fertilizer treatments decreased the Shannon index in subsoil. In topsoil, only strip application at 60 kg P ha^{-1} significantly increased the number of OTUs as well as the diversity and richness indexes of fungi, such as Shannon and Chao1, and in subsoil, all P fertilizer treatments led to lower diversity and richness index values than the control. In short, the P levels and patterns will change the diversity and richness indexes of microbial community.

3.2. Community composition based on the 16S rRNA gene (bacteria) or ITS gene (fungi)

Variations in the microbial community across the five treatments were evaluated by PCA (Fig. S2). The first two principal components of bacterial and fungal communities explained the total variability based OTU level (Fig. S2a and b). The plots revealed clear differences in bacterial and fungal communities across the five treatments in both topsoil and subsoil. The three replicates of each treatment generally clustered together and showed separation from the other treatments. The larger distances across each treatment along the PC1 and PC2 showed that P fertilizer affected the community structure of bacteria more than that of fungi.

The bacterial sequences were distributed in 43 phyla, 209 families and 471 genera. The dominant phyla in the bacterial communities across all soil samples were Proteobacteria (31.81%), Actinobacteria (27.08%), Acidobacteria (15.12%), Chloroflexi (7.21%) and Gemmatimonadetes (4.61%), representing more than 80% of all sequences. The top 10 phyla also included Bacteroidetes (2.33%), Firmicutes (2.19%), Rokubacteria (1.90%) Thaumarchaeota (1.87%) and Cyanobacteria (0.51%), which was identified in all soil samples at <5% abundance at two soil layers (Fig. 1a). Among the top 10 phyla, the relative abundances of Chloroflexi and Thaumarchaeota were significantly increased by P fertilizer, which decreased the relative abundance of Proteobacteria, Gemmatimonadetes and Bacteroidetes in topsoil (Fig. 1b). In subsoil, all P fertilizer treatments reduced the relative abundances of Proteobacteria, Thaumarchaeota and Bacteroidetes, but increased those of Actinobacteria, Chloroflexi, and Firmicutes.

The top 10 genera of bacteria in all soil samples were *Arthrobacter* (3.59%), *unidentified Acidobacteria* (2.82%), *Sphingomonas* (2.67%), *Gaiella* (1.89%), *Blastococcus* (1.87%), *Dongia* (1.49%), *Skermanella* (1.41%), *Bacillus* (1.38%), *Streptomyces* (1.37%) and *Lysobacter* (0.10%) (Fig. 1a and b), which together represented less than 20% of all sequences. The significant differences in the relative abundances of the top 10 genera of two soil layers were observed in the all treatments. In topsoil, all P fertilizer treatments increased the relative abundances of *Arthrobacter*, *Gaiella*, and *Blastococcus*. The highest abundances were observed in the strip applications at 60 and 120 kg P ha^{-1}. Conversely, P fertilizer decreased those of *Bacillus* and *Dongia*. In subsoil, P fertilizer increased the abundances of *Arthrobacter*, *Blastococcus*, *Skermanella* and *Bacillus*, but decreased those of *Sphingomonas* and *Lysobacter*. Except for differences at the phylum and genus levels, the various treatments changed the abundances of bacterial taxa (Fig. S3). A total of 12 and 4 biomarkers, defined as those with a $\log_{10}$ LDA score > 4.0, were identified at the levels of phylum, family, class, order, genus or species in topsoil and subsoil, respectively, which represented differential species between all treatments (Fig. S3).

Fungal sequences were distributed in 14 phyla, 188 family and 307 genera. Ascomycota (55.67%), Mortierellomycota (10.18%), Basidiomycota (8.46%), Mucoromycota (0.62%), Chytridiomycota (0.49%), Glomeromycota (0.26%), Blastocladiomycota (0.13%), Olpidiomycota (0.02%), Aphelidiomycota (0.02%) and Kickxellomycota (0.02%) were the top 10 dominant fungal phyla in all soil samples, and represented approximately 75.87% of all sequences (Fig. 1c). At the phyla level in the topsoil, the abundances of Ascomycota, Mucoromycota, Glomeromycota and Olpidiomycota were increased by all P fertilizer treatments compared with the control. But the P fertilizer treatments decreased those of Basidiomycota and Chytridiomycota (Fig. 1c). In the subsoil, all P fertilizer treatments increased the relative abundances of Ascomycota and Olpidiomycota, while decreased that of Blastocladiomycota (Fig. 1c). At the genus level (Fig. 1d), the top 10 dominant genera were *Epicoccum* (23.50%), *Mortierella* (8.70%), *Gibberella* (4.54%), *Fusarium* (4.54%), *Solicoccozyma* (4.50%), *Alternaria* (3.31%), *Cladosporium* (2.20%), *Pseudombrophila* (1.94%), *Botrytis* (1.44%) and *Rhizoctonia* (0.33%), representing 55.0% of all sequences. In topsoil, all P fertilizer treatments significantly increased the relative abundances of *Epicoccum* and *Cladosporium* and decreased that of only the genus *Rhizoctonia*. In subsoil, P fertilizer increased the relative abundances of *Cladosporium*, *Alternaria* and *Rhizoctonia*, and decreased the relative abundances of *Epicoccum*, *Botrytis*, *Solicoccozyma* and *Rhizoctonia*. Except the differences of phylum and genus levels, some fungi were enriched in the five treatments (Fig. S4). The total of 8 and 20 biomarkers with a log10 LDA score > 4.0 were identified at the levels of phylum, family, class, order, genus or species, in topsoil and subsoil, respectively, which represented differential species differences between all treatments (Fig. S4).

3.3. Microbial networks

Microbial networks were established based on significant correlations (Spearman's correlation, $P < 0.05$) between the insufficient-P group and sufficient-P group after strip or broadcast application in topsoil or subsoil. For strip application, 86 nodes (insufficient-P) and 87 nodes (sufficient-P), as well as 200 links (insufficient-P) and 250 links (sufficient-P) made up the networks of bacteria in topsoil (Fig. 2a and b). For broadcast application, the networks consisted of 85 nodes (insufficient-P) and 80 nodes (sufficient-P), as well as 384 links (insufficient-P) and 255 links (sufficient-P) (Fig. 2c and d).

For strip application, the networks of ITS gene-based fungi in topsoil comprised 70 nodes (insufficient-P) and 68 nodes (sufficient-P) (Fig. 3a and b), as well as 285 links (insufficient-P) and 180 links (sufficient-P). For broadcast application, the networks comprised 69 nodes (insufficient-P) and 66 nodes (sufficient-P), as well as 248 links (insufficient-P) and 249 links (sufficient-P) (Fig. 3c and d). The corresponding data for subsoil are shown in Figs. S5 and S6. The topological properties of bacterial and

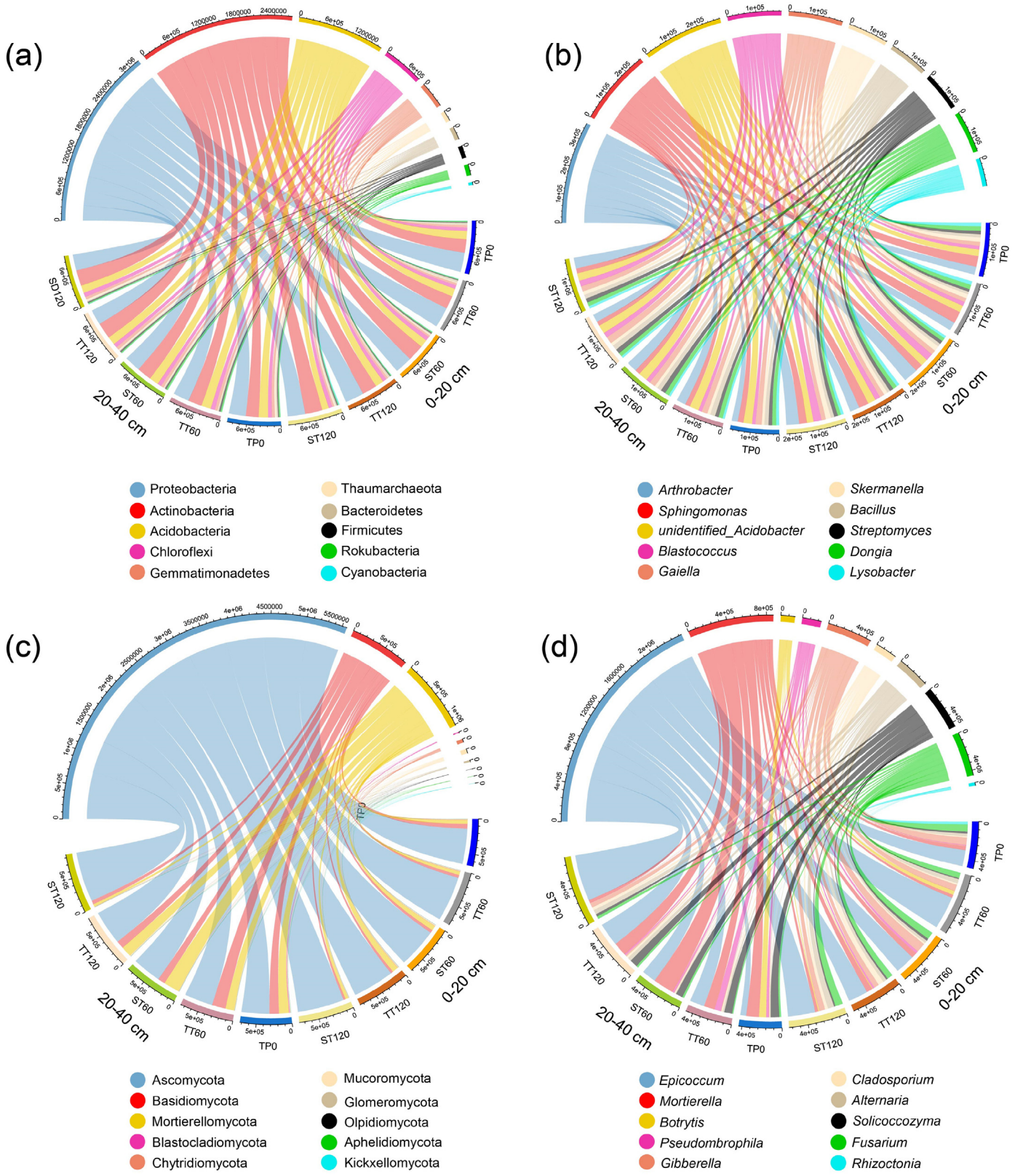

Fig. 1. Relative abundances of dominant bacterial phyla (a), bacterial genera (b), fungal phyla (c) and fungal genera (d) after P-fertilizer treatment. TP0: without P-fertilizer; TT60: strip application at 60 kg P ha^{-1}; ST60: broadcast application at 60 kg P ha^{-1}; TT120: strip application at 120 kg P ha^{-1}; ST120: broadcast application at 120 kg P ha^{-1}.

fungal networks were shown in Table 2. The values of the average clustering coefficient and degree of bacterial communities based on 16S rRNA genes in topsoil after strip application were higher in sufficient-P group than in insufficient-P group. The opposite results of the average shortest path length and topological coefficient were obtained. In the case of broadcast application, all these properties were lower in the sufficient-P group

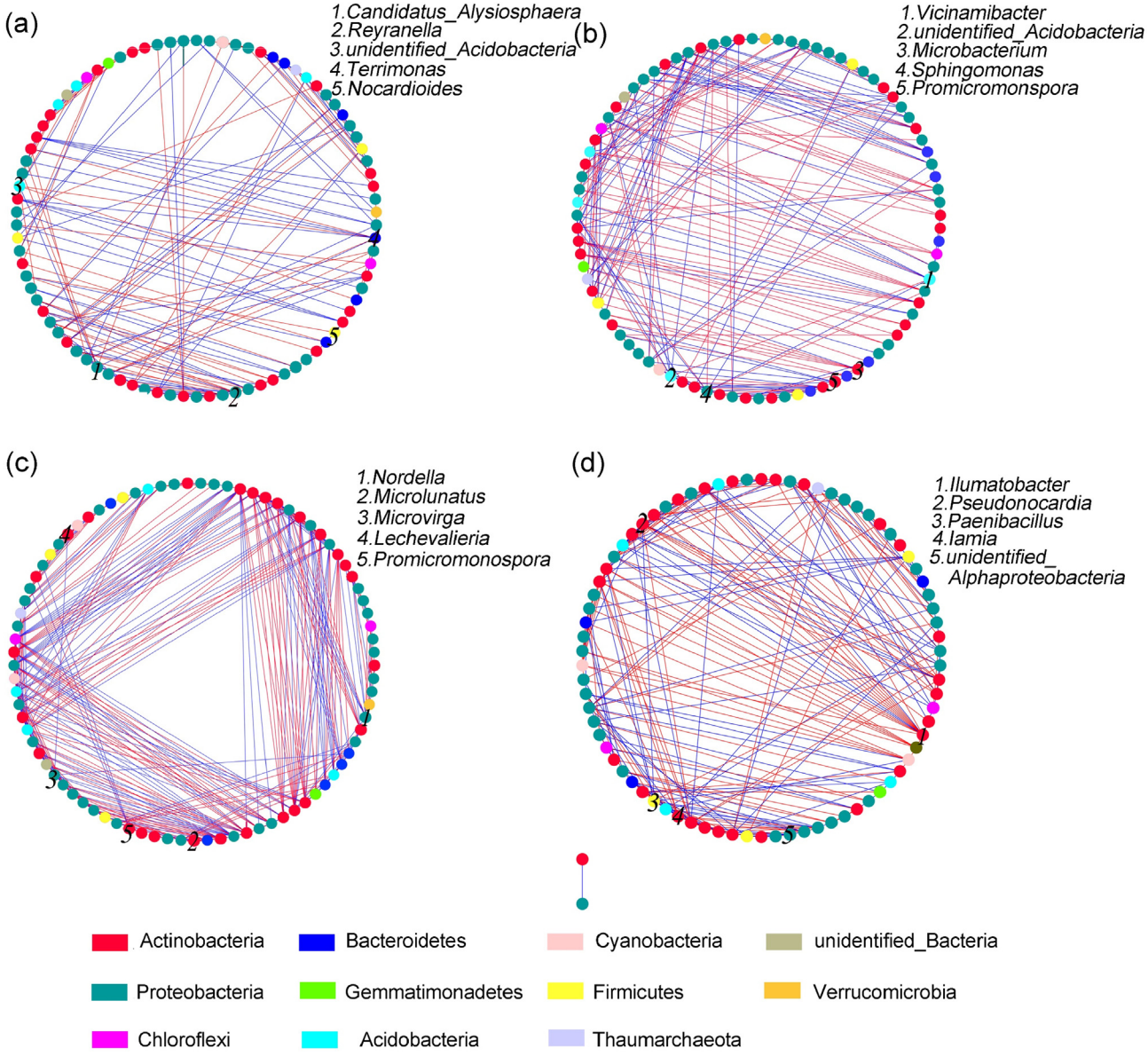

Fig. 2. Network analysis showing the connectedness among bacterial genera based on 16S rRNA gene sequences in topsoil (0–20 cm) after strip fertilization under (a) insufficient-P conditions or (b) sufficient-P conditions, or after broadcast fertilization under (c) insufficient-P conditions or (d) sufficient-P conditions. Nodes (colored dots) represent the genera involved in the networks, and links represent the relationships among the nodes. Red lines represent significant positive relationships (Spearman's correlation, $r > 0.7$ and $P < 0.05$), and blue lines denote negative relationships (Spearman's correlation, $r < -0.7$ and $P < 0.05$). The different colored dots represent the different phyla to which the genera belong. The numbers inside the nodes are the keystone genera in the network (top five based on the betweenness centrality score).

than in the insufficient-P group. In term of bacterial network of subsoil, only the property of degree was higher in sufficient-P group than in insufficient-P group after strip application, while all these properties were decreased by sufficient-P group compared with insufficient-P group after broadcast application. The degree and the average shortest path length based on ITS genes in topsoil fungi after strip application were higher in the sufficient-P group than in the insufficient-P group. The results of degree and clustering coefficient were increased and that of average shortest path length was decreased by broadcast application. In subsoil fungal network, only the degree was higher in the sufficient-P group than in the insufficient-P group whatever fertilization pattern was, while other properties have the opposite results. Additionally, significant differences of keystone taxa (genera) were observed in comparison group for the bacterial networks (Figs. 2 and S5) and fungal networks (Figs. 3 and S6), such as between insufficient-P and sufficient-P groups or strip and broadcast application.

3.4. Metabolic differences in wheat soil across P fertilizer treatments

To determine the response of soil metabolic activities to the P fertilizer, 30 soil samples were analyzed using metabolomics on the UPLC-MS/MS platform. After quality control, PCA was used to analyze differences among five treatment samples (Fig. 4a). The QC samples clustered together, suggesting that the method was stable and yielded high-quality data. Samples for the same treatments clustered and different treatment were separated from one another, indicating differences among five treatments. The dimensions P1, P2 and P3 were able to explain 41.29% of observed differences. Totally, we detected 582 known metabolites from 30 soil samples, including lipids and lipid-like molecules (14.1%), organoheterocyclic compounds (6.2%), organic acids and derivatives (6.0%), phenylpropanoids and polyketides (4.8%), benzenoids (3.1%), organic oxygen compounds (2.4%), nucleosides, nucleotides, and analogues (1.7%), organic nitrogen compounds (1.0%), alkaloids and

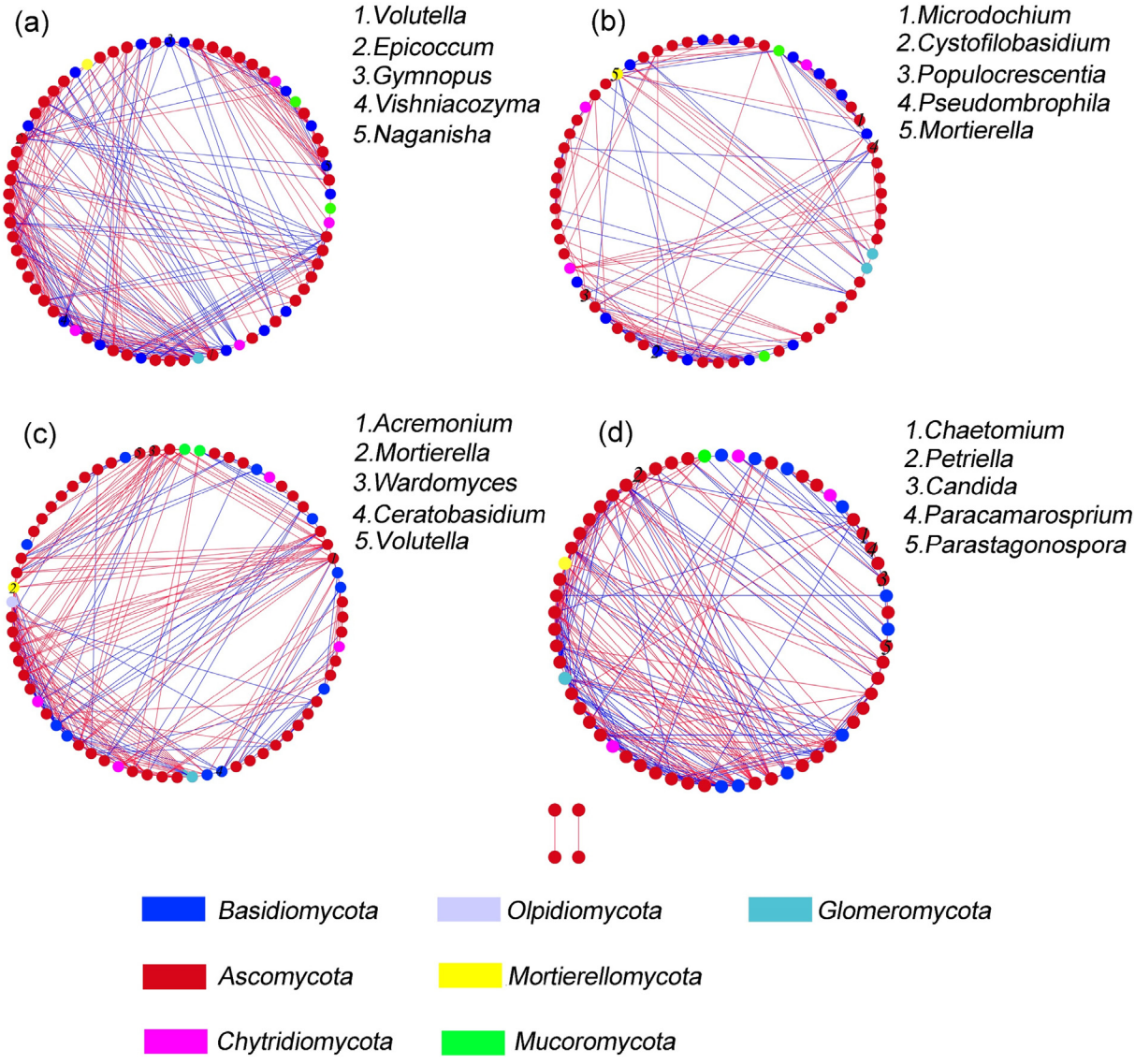

Fig. 3. Network analysis showing the connectedness among bacterial genera based on ITS gene sequences in topsoil (0–20 cm) after strip fertilization under (a) insufficient-P conditions or (b) sufficient-P conditions, or after broadcast fertilization under (c) insufficient-P conditions or (d) sufficient-P conditions. Nodes (colored dots) represent the genera involved in the networks, and links represent the relationships among the nodes. Red lines represent significant positive relationships (Spearman's correlation, $r > 0.7$ and $P < 0.05$), and blue lines denote negative relationships (Spearman's correlation, $r < -0.7$ and $P < 0.05$). The different colored dots represent the different phyla to which the genera belong. The numbers inside the nodes are the keystone genera in the network (top five based on the betweenness centrality score).

derivatives (0.9%), lignans, neolignans and related compounds (0.2%), organic compounds (0.2%), and organooxygen compounds (0.2%), and there were some metabolites were not classified.

Multivariate statistical methods were used to analyze the high degree of inter-group correlation. The differences within and between the control and treatment groups were analyzed using the supervised discriminant analysis statistical method PLS-DA model, so as to obtain the impacts of P levels and patterns on soil metabolites. According to PLS-DA (Fig. 4b), there was similar metabolite composition across all treatments, but there were differences between the two treatments of strip application at 60 kg P ha^{-1} and the control (e.g.). The PLS-DA model indicated that the model was good and could be used to screen for DMs ($R^2Y = 0.95$, $Q^2Y = 0.88$) (Fig. 4c), which differed among other treatment and control groups. DMs were screened through volcano plots (Fig. 4d).

Hierarchical cluster analysis was performed on all DMs among all the comparison pairs (Fig. S7). Different P fertilizer treatments had different effects on soil metabolism, as measured in terms of metabolites. Enrichment analysis of KEGG pathways indicated that all DMs were enriched in nicotinate and nicotinamide metabolism, arginine and proline metabolism, drug metabolism-cytochrome P450, biosynthesis of plant hormones, amino acids, plant secondary metabolites, and alkaloids derived from ornithine, lysine and nicotinic acid. These results indicate that P fertilizer significantly affected the synthesis of metabolites and their corresponding metabolic pathways (Fig. S8). The DMs involved in metabolic pathways included trigonelline ($C_7H_7NO_2$), phosphocreatine ($C_4H_{10}N_3O_5P$), normorphine ($C_{16}H_{17}NO_3$), shikimic acid ($C_7H_{10}O_5$), L-glutamic acid ($C_5H_9NO_4$), morphine ($C_{16}H_{17}NO_3$), 6-hydroxynicotinic acid ($C_6H_5NO_3$), indole-3-acetic acid ($C_{10}H_9NO_2$), L-isoleucine ($C_6H_{13}NO_2$), diosgenin ($C_{27}H_{42}O_3$), piperine ($C_{17}H_{19}NO_3$), nicotinic acid ($C_6H_5NO_2$), cis-4-hydroxy-D-proline ($C_5H_9NO_3$), and N-acetylornithine ($C_7H_{14}N_2O_3$) (Fig. 5a).

To further explore metabolism affected by P fertilizer in wheat soil, we generated a metabolic pathway involving 14 DMs by searching the KEGG pathway database (Fig. 5c). These metabolites were involved in sugar, amino acid, cytochrome P450, biosynthesis of plant secondary metabolism and biosynthesis of plant hormone, and so on. The contents of metabolites trigonelline, phosphocreatine, shikimic acid, L-glutamic acid and diosgenin

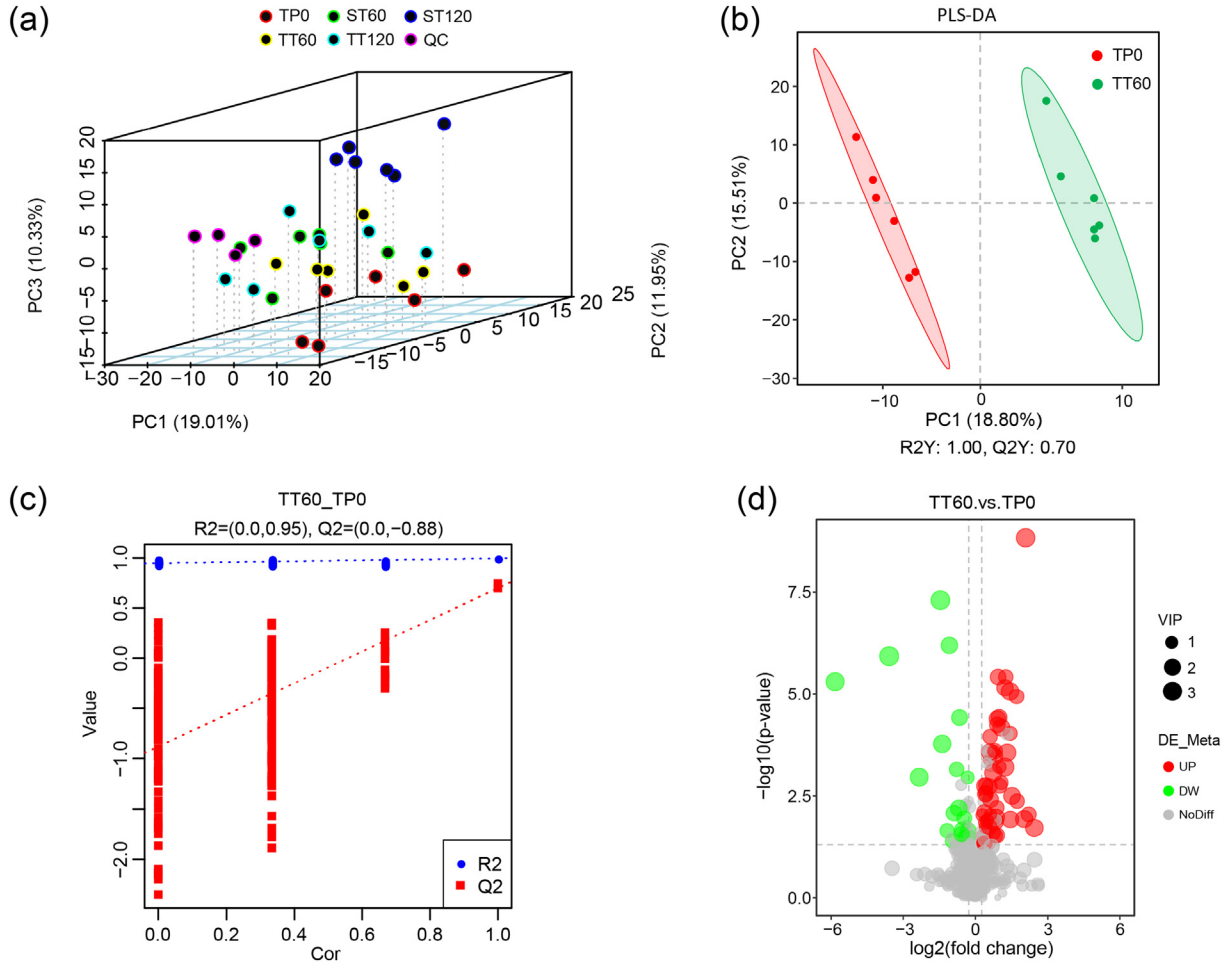

Fig. 4. Metabonomic analysis of soil treated with P-fertilizer. (a) Principal component analysis (PCA) of metabolites in the topsoil (0–20 cm). (b) PLS-DA analysis of metabolites between TT60 and TP0. (c) Cross-validation model of PLS-DA. (d) The expression volcano map of differential metabolites up and down regulate. Green dots represent down-regulated metabolites, red dots represent up-regulated metabolites, and gray dots represent no-differential metabolites. TP0: without P-fertilizer; TT60: strip application at 60 kg P ha^{-1}; ST60: broadcast application at 60 kg P ha^{-1}; TT120: strip application at 120 kg P ha^{-1}; ST120: broadcast application at 120 kg P ha^{-1}.

were present at maximum values after broadcast application at 60 kg P ha^{-1}, and all except L-glutamic acid significantly varied across the P fertilizer treatments. The levels of morphine, 6-hydroxynicotinic acid and piperine were least content after broadcast application at 60 kg P ha^{-1} and were even less than the control. All P fertilizer treatments significantly increased the levels of normorphine, which reached maximal value in strip application at 60 kg P ha^{-1} (2.74 times the control value) and in broadcast application at 120 kg P ha^{-1} (3.79 times the control value). Morphine levels were only 0.29 times and 0.73 times the control value after broadcast at 60 kg P ha^{-1} and strip application at 120 kg P ha^{-1}, while it significantly higher than TP0 after strip application at 60 kg P ha^{-1} or broadcast application at 120 kg P ha^{-1}. The levels of plant growth hormone indole-3-acetic acid differed significantly between all treatments, and it was significantly higher than the control value only after strip application at 120 kg P ha^{-1}. Piperine levels were not significantly affected by P fertilizer except after broadcast application at 120 kg P ha^{-1}, which increased the level of piperine. The levels of *N*-acetylornithine were significantly higher after strip application at 60 kg P ha^{-1} than other treatments (Fig. 5b and c). These results indicated P fertilizer can be indirectly affected the accumulation and composition of metabolites in soil.

3.5. The relationship between soil chemistry and microorganisms

Elucidating the relationship between microbes and their metabolites is a necessary step for optimizing P fertilizer for maximal plant growth. Therefore, we analyzed correlations between microorganisms and metabolites based on abundances. Significant ($P < 0.05$) and highly significant ($P < 0.01$) correlations were observed linking the top 10 bacterial (Fig. 6a) and fungal (Fig. 6b) genera with DMs. The metabolites normorphine, L-glutamic acid, 6-hydroxynicotinic acid, piperine, and nicotinic acid correlated significantly with the respective bacteria *Blastococcus* ($r = 0.557$, $P = 0.031$), *Streptomyces* ($r = -0.630$, $P = 0.012$), *Skermanella* ($r = -0.525$, $P = 0.044$), *unidentified_Acidobacteria* ($r = -0.532$, $P = 0.041$) and *Skermanella* ($r = -0.539$, $P = 0.038$) (Fig. 6a). Morphine correlated with *Sphingomonas* ($r = 0.650$, $P = 0.009$), *unidentified_Acidobacteria* ($r = -0.607$, $P = 0.016$), and *Gaiella* ($r = -0.756$, $P = 0.001$). Indole-3-acetic acid negatively correlated with the bacteria *Arthrobacter* ($r = -0.614$, $P = 0.015$), *Blastococcus* ($r = -0.600$, $P = 0.018$) and *Skermanella* ($r = -0.629$, $P = 0.012$). Diosgenin positively correlated with *Arthrobacter* ($r = 0.586$, $P = 0.022$) and *Blastococcus* ($r = 0.536$, $P = 0.040$). The metabolite cis-4-hydroxy-D-proline showed a positive correlation with *Sphingomonas* ($r = 0.618$, $P = 0.014$) and a highly negative correlation with *Gaiella* ($r = -0.652$, $P = 0.008$).

Relationships between fungi and metabolites are shown in Fig. 6b, which were less than those between bacteria and metabolites. Only metabolites trigonelline, phosphocreatine, normorphine, morphine, indole-3-acetic acid, L-isoleucine and N-acetylornithine correlated significantly with fungi. Normorphine correlated with *Epicoccum* ($r = 0.729$, $P = 0.002$), *Pseudombrophila* ($r = -0.632$, $P = 0.011$) and *Cladosporium* ($r = 0.568$,

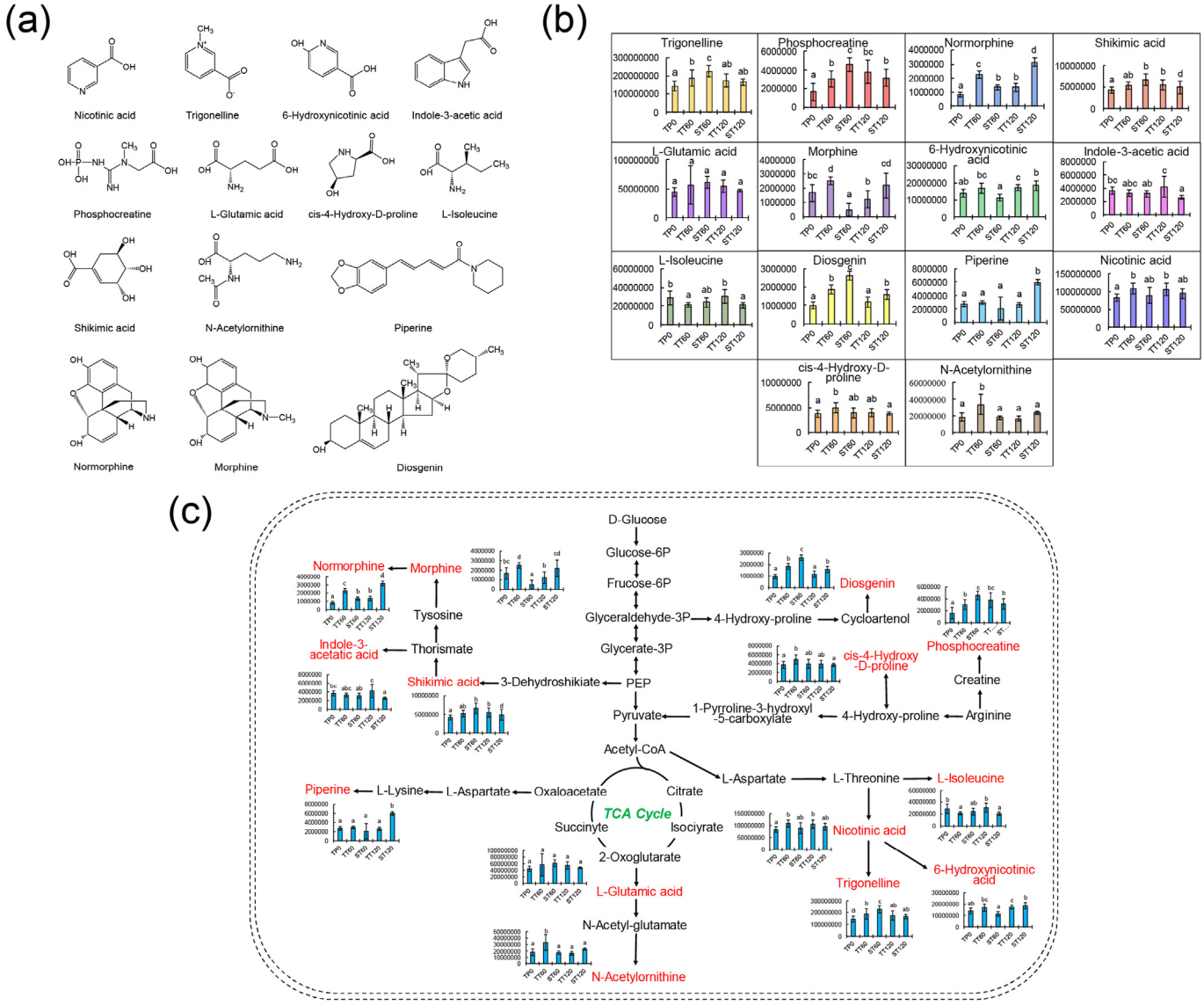

Fig. 5. Soil metabolomic analysis of wheat soil treated with P-fertilizer. (a) Structural formula of 14 differential metabolites (DMs). (b) Content changes of 14 DMs across the different treatments. (c) Metabolic pathway map of differential marker metabolites in wheat soil under different P levels and patterns. TP0: without P-fertilizer; TT60: strip application at 60 kg P ha^{-1}; ST60: broadcast application at 60 kg P ha^{-1}; TT120: strip application at 120 kg P ha^{-1}; ST120: broadcast application at 120 kg P ha^{-1}.

$P = 0.027$), while trigonelline, phosphocreatine, morphine, and indole-3-acetic acid positively correlated with *Epicoccum* ($r = 0.521$, $P = 0.046$), *Mortierella* ($r = 0.661$, $P = 0.007$), *Solicoccozyma* ($r = -0.614$, $P = 0.015$) and *Pseudombrophila* ($r = 0.525$, $P = 0.044$). *N*-acetylornithine negatively correlated with *Mortierella* ($r = -0.571$, $P = 0.026$) and *Solicoccozyma* ($r = -0.518$, $P = 0.048$). These results showed that the relative abundances of six bacterial genera (*Arthrobacter*, *Sphingomonas*, *unidentified_Acidobacteria*, *Blastococcus*, *Gailla*, and *Skermanella*) and four fungal genera (*Epicoccum*, *Mortierella*, *Pseudombrophila* and *Solicoccozyma*) had the strongest correlations with certain metabolites, which suggest that these microorganisms may be involved in the formation of most metabolites in soil.

4. Discussion

In this study, high-throughput sequencing and UHPLC-MS/MS were used to evaluate differences in bacterial and fungal communities as well as metabolomics in wheat soil as a result of different P fertilizer treatments. Different P levels and patterns altered the diversity, abundance index, community composition and metabolite content of bacteria and fungi. Soil microorganisms form complex microbial communities, regulate nutrient cycling, and affect soil properties, plant growth and ecosystem sustainability (Zhang et al., 2019), which play a key role in the P cycle and the availability of P for plants (Pantigoso et al., 2018; Li et al., 2020). Microbial communities are affected by fertilizer and other agricultural interventions affecting plant root growth (Bunemann et al., 2004; Ling et al., 2017). Addition of P fertilizer to grasslands significantly affected bacterial and fungal communities. In present study found that the effects of P fertilizer on microorganisms in wheat soil were different from that the effects on soil microorganisms of other plants. This reflects how plant species influence the response of fungi and bacteria to P addition (Marschner et al., 2001).

Bacteria are the most abundant and diverse species of soil microorganisms, and they play an important role in agricultural ecosystems by participating in the soil nutrient cycle, maintaining soil structure and promoting plant growth (Gans et al., 2005). Our results indicated that addition of P fertilizer changed the diversity of bacterial community in soil wheat, such as increasing the number of observed OTUs, while without significantly affecting diversity (Table 1). This study results is consistent with the findings of Liu et al. (2020). The soil bacterial community composition was influenced by application of P fertilizer (Beauregard et al., 2010; Ling et al., 2017), and

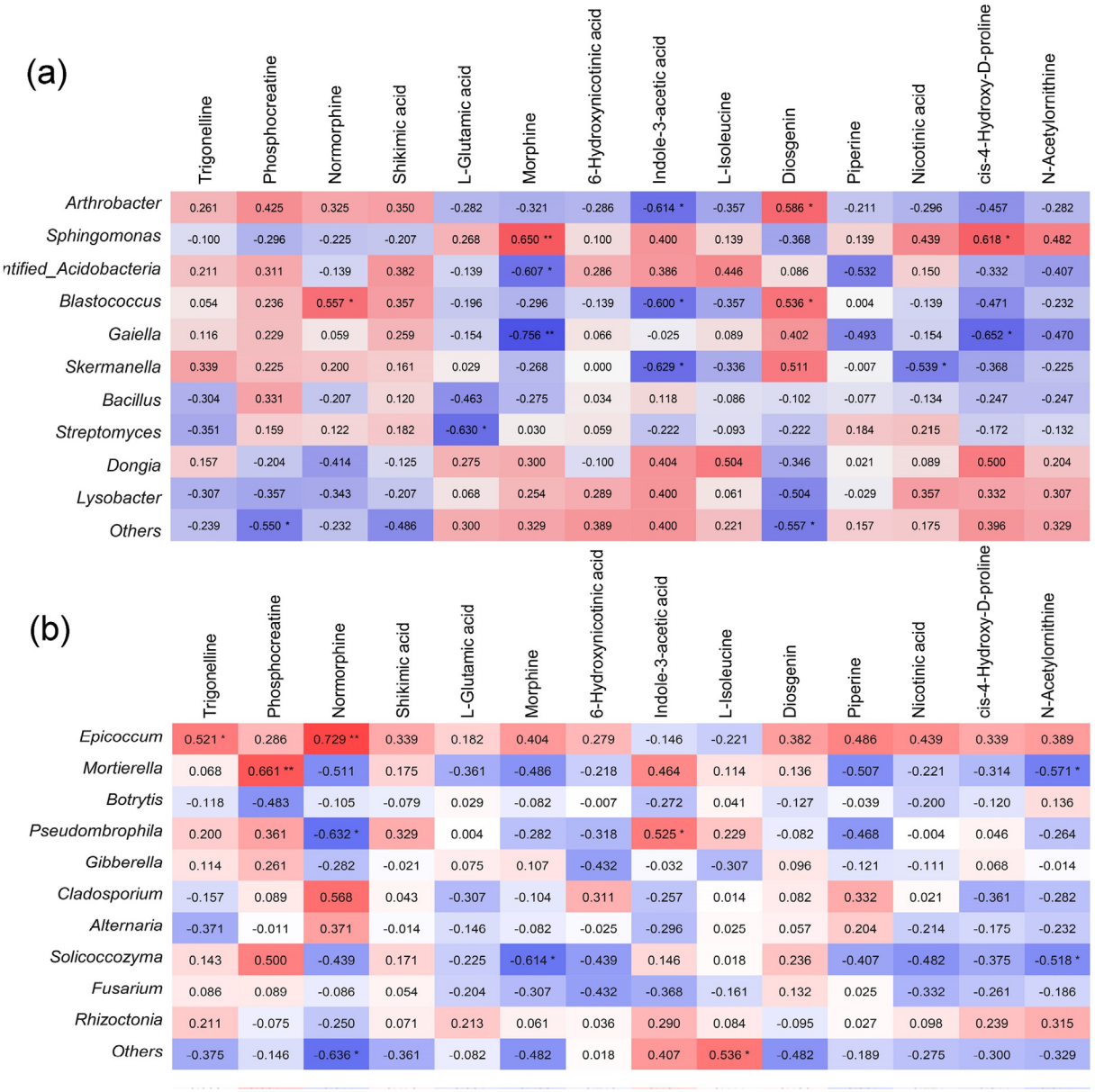

Fig. 6. Correlation of the top 10 (a) bacteria and (b) fungi, in terms of relative abundance, with the 14 differential metabolites. The vertical axis represents differential genera of bacteria (based on 16S rRNA gene sequences) and of fungi (based on ITS gene sequences). The horizontal axis represents the differential metabolites. Red and blue indicate positive and negative correlation, respectively. * and ** represent significance ($P < 0.05$) and high significance ($P < 0.01$).

Table 1
Diversity of the 16S rRNA gene-based bacterial communities and ITS gene-based fungal communities.

Soil layers	Treatments	Bacteria			Fungi		
		Observed OTUs	Shannon	Chao1	Observed OTUs	Shannon	Chao1
0–20 cm	TP0	2546 ± 42 a	9.53 ± 0.07 a	2821.91 ± 29.09 ab	757 ± 101 a	5.40 ± 0.28 b	842.74 ± 105.46 a
	TT60	2594 ± 56 a	9.51 ± 0.05 a	2902.90 ± 48.90 ab	675 ± 73 a	4.49 ± 0.69 a	791.94 ± 68.71 a
	ST60	2633 ± 110 a	9.41 ± 0.09 a	3060.23 ± 241.76 b	796 ± 28 a	5.44 ± 0.14 b	895.47 ± 43.72 a
	TT120	2814 ± 132 b	9.55 ± 0.07 a	3570.70 ± 174.31 c	669 ± 69 a	5.25 ± 0.16 b	754.95 ± 86.57 a
	ST120	2509 ± 60 a	9.41 ± 0.11 a	2710.54 ± 118.24 a	726 ± 92 a	4.97 ± 0.44 ab	825.98 ± 84.91 a
20–40 cm	TP0	2564 ± 87 b	9.52 ± 0.07 b	2881.05 ± 97.43 b	823 ± 28 c	5.73 ± 0.29 c	887.13 ± 54.95 c
	TT60	2585 ± 61 b	9.51 ± 0.06 b	2891.88 ± 53.57 b	706 ± 65 ab	5.12 ± 0.37 b	790.43 ± 76.22 abc
	ST60	2448 ± 41 a	9.41 ± 0.04 a	2765.83 ± 33.14 ab	764 ± 72 bc	5.13 ± 0.26 b	850.23 ± 73.15 bc
	TT120	2496 ± 26 ab	9.48 ± 0.03 ab	2808.04 ± 64.08 ab	703 ± 48 ab	5.06 ± 0.19 b	764.92 ± 55.08 ab
	ST120	2481 ± 27 ab	9.43 ± 0.44 ab	2749.57 ± 60.36 a	641 ± 17 a	3.85 ± 0.17 a	707.17 ± 18.32 a

Values are mean SD ($n = 3$). Different letters in the same column indicate a significant difference at $P < 0.05$ among the five treatments.
TP0: without P-fertilizer; TT60: strip application at 60 kg P ha^{-1}; ST60: broadcast application at 60 kg P ha^{-1}; TT120: strip application at 120 kg P ha^{-1}; ST120: broadcast application at 120 kg P ha^{-1}.

Table 2
Topological properties of bacterial and fungal co-occurrence networks obtained under P level and pattern treatments.

Soil layers	Treatments		Bacteria				Fungi			
			Degree	Clustering coefficient	Average shortest path length	Topological coefficient	Degree	Clustering coefficient	Average shortest path length	Topological coefficient
0–20 cm	Strip application	Insufficient-P group	4.65	0.38	4.05	0.40	8.14	0.47	3.11	0.40
		Sufficient-P group	5.75	0.40	3.51	0.36	5.29	0.46	3.49	0.40
	broadcast application	Insufficient-P group	9.04	0.52	4.10	0.47	7.19	0.40	3.42	0.39
		Sufficient-P group	6.38	0.36	3.25	0.33	7.55	0.47	2.95	0.37
20–40 cm	Strip application	Insufficient-P group	4.93	0.45	3.91	0.44	4.58	0.44	3.79	0.45
		Sufficient-P group	6.77	0.39	3.10	0.41	4.97	0.34	3.66	0.36
	broadcast application	Insufficient-P group	4.77	0.43	3.93	0.43	5.01	0.47	3.92	0.42
		Sufficient-P group	3.39	0.42	3.39	0.36	8.94	0.44	2.96	0.36

Insufficient-P indicated 0 and 60 kg P ha^{-1} treatments, and sufficient-P indicated 60 and 120 kg P ha^{-1} treatments.

the present results showed similar impacts (Fig. 1 and S3). Indeed, P status and the P cycle may be related to bacterial community composition in soil (Miller et al., 2010). Consistent with this idea, our results showed that P level and pattern treatments shifted bacterial community composition. For instance, the relative abundances of phyla Chloroflexi and Thaumarchaeota were increased by P fertilizer. But P fertilizer decreased that of Bacteroidetes, which may be due to changes in the environmental conditions for the growth of rhizosphere Bacteroidetes, or due to the decreases in competition from other microbes (Song et al., 2020). Some researchers found that the relative abundance of phylum Bacteroidota was increased by P fertilizer, which be related to different soil properties, fertilization rates and management methods (Beauregard et al., 2010; Li et al., 2019a). These changes may affect P metabolism in plants and soils.

Although, plant root exudates also have some soil enzyme activities, such as phosphatase and phytase, most organic P in soil is hydrolyzed by soil microorganisms (Zhalnina et al., 2018). The present study found that P fertilizer reduced fungal diversity (Shannon index) (Table 1) and significantly changed fungal community composition (Fig. 1c and d). Present study results are consistent with a previous study that analyzed the effects of 20-year P fertilizer (Lin et al., 2012). However, the present results contrast with a report that high-intensity P fertilizer has no significant effect on fungal diversity (Hijri et al., 2006; Tian et al., 2011). Soil microorganisms, for their part, can release a variety of soil enzymes to synthesize humus, mineralize and degrade organic matter, promote or inhibit plant growth, and cycle nutrients (Fan et al., 2019).

Symbiotic network is an important factor for the stability of microbial community in response to external interference (Jiao et al., 2019). Network analysis can be used to determine how some microbial species interact in a specific ecological environment and to identify key species that affect the community (Banerjee et al., 2016). Research showed that environmental change, agricultural management, or adding exogenous organic matter can change the complexity of soil microbial network. For example, glucose additions increased the complexity of *phoD*-harboring bacterial networks, and decreased that of fungal networks (Huang et al., 2021). Our research also showed that P levels and patterns influenced the complexity and stability of microbial network (Figs. 2 and 3). Key taxa play unique and key roles in microbial communities, and their change will substantially alter the structure and function of the whole microbial networks (Banerjee et al., 2018). In addition, the keystone taxa changed as microbial community composition and also altered network structure and function.

Soil metabolites come mainly from plant roots and microorganisms. The composition of root exudates varies with the plant species, genotype, and environmental stress (Luo et al., 2015; Strehmel et al., 2014; Zhang et al., 2014). The changes of soil metabolite composition and content can reveal the direct or past response of soil microorganisms to soil nutrients (Li et al., 2019b). Our study found that P levels and patterns significantly affected soil metabolite spectrum, including organic acids and derivatives, organic oxygen compounds, lipids and lipid-like molecules, alkaloids and derivatives, and then interfered with some metabolic pathways including those involving basic C, P and lipid metabolism. In addition, the metabolites involved in these metabolic pathways have also changed significantly, for example, P is involved in the synthesis of phosphocreatine, a high-energy P-compound. When hydrolyzed, phosphocreatine releases a lot of energy, regulating the activity of glycolytic enzymes and accelerating metabolic activity (Arkel et al., 2018).

Our results showed that P fertilizer promoted the synthesis of the compound and so accelerated metabolism in soil. Consistent with this, we found that the levels of trigonelline, a nutritional additive that can be extracted from legumes, increased after P fertilization. Shikimic acid, synthesized through glycolysis, is an important compound involved in plant growth and development, and it is a building block to synthesize phenylalanine and tyrosine (Malalgoda et al., 2020). Addition of P fertilizer increased the levels of this compound in our study. Similarly, P fertilizer increased the synthesis of L-glutamic acid and *N*-acetylcholine, which were not only precursors of glutamine, proline, arginine and lysine, but they also participate in the urea cycle (Wang et al., 2020). In addition, it indicates that P fertilizer can promote N-cycle. In our study, P fertilizer increased the levels of 6-hydroxynicotinic acid and piperine, which may increase plant resistance to pests since both compounds are intermediates in the production of plant-generated insecticides (Booth et al., 2019; Zhu et al., 2020). Indole-3-acetic acid, a typical auxin, can regulate the growth of plants (Fei et al., 2020), whereas L-isoleucine is an amino acid, which can be synthesized through sugar metabolism, amino acid metabolism, and microbial fermentation. P fertilizer also increased the level of diosgenin, which also exists widely in plants, and is used to synthesize secondary metabolites such as glycosides (Shan et al., 2020). All these results support the idea that P fertilizer promotes plant and microbial metabolism (Ashihara et al., 2012). The levels of cis-4-hydroxy-D-proline also increased by P fertilizer, which may promote stress resistance since the compound serves as an osmotic regulator, stabilizes biological macromolecule structure, reduces cell acidity, and regulates cell redox potential (Chen et al., 2016).

Soil microorganisms are important executors of soil metabolic activities, and which directly reflects the detectable biological reactions of soil microorganisms under various conditions (Song et al., 2020). More importantly, the changes of microbial species and abundance determine the changes of soil metabolites to some certain extent, and then determine the circulation and metabolism of exogenous nutrients in soil. Therefore, it is of great significance to reveal the correlation between soil metabolism and bacterial community. The research of Song et al. (2020) indicated starch and sucrose metabolism function showed a greatest relation with the differential bacterial members among the differential metabolite pathways, which may confirm that the different bacterial members lead to different metabolism with regulating of starch and sucrose metabolism function in rhizosphere. In addition, a study results also confirmed that different bacterial members lead to different metabolism by regulating the metabolic functions of starch and sucrose and citric acid cycle in soil (Song et al., 2020). Previous studies had found a significant correlation between metabolites and endophytic fungi (Cui et al., 2019). Our results of the research showed the fertilization-induced changes in several of these metabolites correlated with changes in the bacterial and fungal communities. This also supports the idea that

soil microorganisms can promote or inhibit the accumulation of soil metabolites. The relationship between soil metabolites and microbial community will guide plants to regulate plant root processes through soil improvement or biotechnology, so as to improve plant yield. It is worth noting that metabolomics analysis is the analysis of biological metabolites at a specific time under specific conditions (White et al., 2017). However, the dynamic changes of soil metabolite profile and its correlation with soil microbial community structure need to be further studied.

Although, our study results indicated that the effects of P fertilizer on wheat soil microbes and metabolism, it is only in the preliminary stage and still has defects. Many studies have suggested that soil properties are environmental factors driving microbial community structure, such as pH, total N, total C, total P, organic matter, and etc. (Zheng et al., 2016; Liu et al., 2020; Shi et al., 2020). Additionally, in soil, various species of fungi and bacteria live together to form a complex system of species interaction, rather than living alone (Freilich et al., 2010). Thus, it is important to understand how bacteria and fungi coexist and interact (Lu et al., 2013). While results confirmed that the symbiosis between bacteria and fungi, but the symbiosis between bacteria and fungi has not been revealed. More importantly, we only studied the soil at present. If we combine it with plants, it can be better to reveal the influence mechanism of P fertilizer. Therefore, further research needs us to continue.

5. Conclusion

To reveal the correlation between soil microbial community and metabolism, NovaSeq sequencing and UHPLC-MS/MS were used to evaluate changes in bacterial and fungal communities, as well as metabonomics in wheat soil as a result of different levels and patterns of P fertilizer. Based on Shannon index, P fertilizer reduced the bacterial diversity (except strip application at 120 kg P ha^{-1} in topsoil) compared with the control. The same results of fungal diversity obtained (except broadcast application at 60 kg P ha^{-1} in topsoil). What's more, both P levels and patterns significantly changed bacterial and fungal community structures and compositions, and interfered with the symbiosis networks of bacterial and fungal community and keystone taxa determining network stability. The results of metabolomics showed that P levels and patterns also significantly changed soil metabolic spectrum, as well as interfered with the metabolic pathways involved by the DMs, of which the levels were significantly increased by P fertilizer, and these changes significantly correlated with the changes in certain microbial taxa. The results of this study confirmed the close relationships between soil microorganisms and soil metabolism, which may help guide fertilization measures to sustainably improve soil quality and crop yield.

Abbreviations

P fertilizer Phosphate fertilizer
OTU Operational taxonomic unit
PCA Principal component analysis
UHPLC-MS Ultra-high performance liquid chromatography-tandem mass-spectrometry
KEGG Kyoto Encyclopedia of Genes and Genomes
HMDB Human metabolome database
PLS-DA Partial least squares discriminant analysis
VIP Variable Importance in the Projection
DMs Differential metabolites
ANOVA One-way analysis of variance
LDA Linear discriminant analysis

CRediT authorship contribution statement

Hongyan Cheng - He has conceptualization, design and supervision of review and also writing - original draft this article; **Minshu Yuan** - She has put some contribution to data correction and formal analysis; **Liang Tang** - He has put some contribution to data correction and formal analysis; **Yufang Shen** - He is supervision, validation, and visualization, as well as very help to improve the manuscript quality and English;

Qiang Yu - He has put some contribution to data correction and formal analysis; **Shiqing Li** - He has put some contribution to data correction and formal analysis,

Data accessibility

The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (accession codes PRJNA658959 and PRJNA659106).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.152878.

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