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Soil bacterial community functions and distribution after mining disturbance

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ABSTRACT

Mining disturbances alter soil edaphic factors, modifying soil biogeochemical processes and thus impacting the soil microbiome. The objectives of this study were (1) to identify the dominant edaphic factor influencing the soil bacterial functions after mining disturbance and (2) to investigate how the soil microbiome was distributed, relative to the dominant edaphic factor. We found that soil pH was the most important predictor explaining the distribution of microbial attributes, such as microbial diversity, taxonomic composition, and ecological clusters along a mining disturbance gradient. Our structural equation model (SEM) indicates that soil pH shaped the bacterial community indirectly, by altering soil nutrients and metal availability. Furthermore, the microbial functions responded to soil pH, as the soil microbiome was sensitive to changes in nutrient and metal(loid) availability. For example, the bacterial community was enriched in core functional genes associated with nutrient availability (including nitrogen and phosphorus) in soil with high pH, whereas there were more core functional genes involved in metal availability (including metal transport and resistance) in soil with low pH. We conclude that soil pH is a key controller of soil bacterial communities, due to its direct and indirect effects on the availability of nutrients and metal(loid)s, after mining disturbance.

1. Introduction

Soil microbiomes converge toward similar microbial attributes to improve their adaptation under identical soil environments. Environmental disturbances can influence certain soil biogeochemical processes and thus impact soil microbiomes (Xiao et al., 2016b; Chen et al., 2020). Mining disturbance changes soil edaphic factors such as soil pH, nutrients, and metal(loid) and thus influences the soil microbiome in terrestrial ecosystems (Hottenstein et al., 2019; Kane et al., 2020; Chen et al., 2020). Nevertheless, the dominant edaphic factor that drives the soil bacterial community after a mining disturbance and the mechanisms by which it regulates the distribution of the soil microbiome have not been clarified. Such information is important for understanding the distribution of the soil microbiome after mining disturbances to soil ecosystems.

Soil pH is the dominant edaphic factor that shapes the soil microbiome. This distributional pattern holds for both the overall bacterial community and individual bacterial groups (Krulwich et al., 2011; Lauber et al., 2009; Qi et al., 2018). These studies found that soil pH can affect the soil microbiome in two ways: (i) soil pH directly affects soil microorganisms because most proteins of living cells function with distinct ranges of soil pH (Krulwich et al., 2011), and (ii) soil pH can indirectly influence the bacterial community by altering metal and soil nutrient availability (Lauber et al., 2009; Qi et al., 2018). However, most of these studies were conducted on natural soil ecosystems, and few studies have focused on mining-disturbed soil ecosystems. Recent evidence has demonstrated that the distribution of the soil bacterial community and individual bacterial groups present different patterns between natural and mining-disturbed soil ecosystems (Xiao et al., 2016b; Sun et al., 2017). Whether soil pH dominantly regulates the distribution of the soil microbiome after accounting for other edaphic factors in mining-disturbed soil ecosystems and the mechanism by

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which soil pH regulates the distribution of the soil microbiome in mining-disturbed soil ecosystems have not been clarified. Empirical knowledge about the distribution of microbial functions could be important for a comprehensive understanding of the distribution of the soil microbiome after mining disturbance. Nevertheless, knowledge about how the edaphic factors, such as pH, metal, and nutrients, are affected by mining disturbance regulate soil functional traits is still ambiguous (Delgado-Baquerizo et al., 2018a). In this study, we used shotgun metagenomic sequencing to identify the functional genes and their responses to mining disturbance to complementarily investigate the pattern of the soil microbiome driven by dominant edaphic factors.

In the current study, we sought to investigate the distribution of soil bacterial communities and their functions after mining disturbance. To do so, we collected a total of 78 soil samples from similar vegetation types from a historical Hg–Tl mining area. The objectives of this study were as follows: (1) to identify the dominant edaphic factors driving the soil bacterial community in mining-disturbed soils and (2) to investigate the distribution of the soil microbiome driven by dominant edaphic factors. Therefore, this study offers new insights into the distribution of bacterial communities and functional attributes after environmental disturbance in soil ecosystems.

2. Materials and methods

2.1. Study area and sampling

The soil samples were obtained from a typical Tl–Hg mining area (0.2 km^2) in Guizhou Province in Southwest China. Ongoing weathering of sulfide ore has resulted in serious soil contamination in this region. At the sampling site, we applied a randomized field design and selected 78 sampling sites downstream of the mining area along a broad soil pH gradient. The texture and physical properties were similar across all soil samples. For each sampling site, 3 pseudoreplicates (0–15 cm depth) of ~20 g of soil were obtained and mixed as a composite soil sample. All samples were transported to the laboratory with ice packs (4 °C). Each sample was divided into two portions based on the intended use as follows: one portion for DNA extraction was stored at −40 °C, and the second portion for chemical analyses was stored at 4 °C.

2.2. Chemical analysis

Soil samples were freeze-dried and then passed through a 2-mm sieve to remove gravel, leaves, and plant roots. The samples were thoroughly ground before passing through a 200-mesh sieve. Soil pH was determined by using a calibrated HACH HQ30d pH meter (HACH, Loveland, USA) (Xiao et al., 2016b). Total sulfur (TS), total carbon (TC), and total organic carbon (TOC) were determined by an elemental analyzer (vario MACRO cube, Elementar, Hanau, Germany) following the procedure we previously proposed (Xiao et al., 2016b). To test the content of metal (loid)s, the soil was completely digested using concentrated HF and HNO_3 (1:5, v/v) (Xiao et al., 2016a). An ICP-MS system (Agilent, 7700x, California, USA) was employed to measure the concentrations of metal (loid)s. The Chinese GBW07310 soil reference and SLRS-5 (National Research Council, Canada) were used for the quality control of the sample treatments (Xiao et al., 2020).

2.3. Analysis of soil bacterial communities through Illumina MiSeq sequencing

Genomic DNA was extracted from soil (0.25 g) using the FastDNA® Spin Kit (MP Biomedicals, Santa Ana, USA) following the manufacturer's protocol. The V4–V5 hypervariable regions of the 16S rRNA were amplified using the 515f/907r primer pair. The 16S rRNA amplicons were sequenced on the Illumina MiSeq platform at Novogene Bioinformatics Company (Beijing, China). After filtering low-quality reads, we obtained clean reads. Detailed information on the filtering procedure is shown in the Supporting Information. Using UPARSE, we clustered OTUs with 97% similarity. Phylogenetic taxonomy for each OTU was obtained by blasting the RDP classifier and the Green Genes database (Wang et al., 2007).

2.4. Shotgun metagenomic sequencing and gene analysis

We selected 12 samples from the 78 samples for shotgun metagenomic analysis. These samples were selected because they contained a wide range of soil pH levels (ranging from 3.47 to 7.47). Shotgun metagenomic sequencing was then performed on the Illumina PE150 platform (Illumina Inc.) at Novogen Inc., Beijing, China. The clean data were obtained by preprocessing the raw data using Readfq (V8, https://github.com/cjfields/readfq). The assemblage of the clean data was obtained by following the procedure proposed by Nielsen et al. (2014). To obtain PE reads, we used Bowtie2.2.4 software to compare all samples' clean data with each scaffold. We then performed BLAST searches of the unigenes against the KEGG database (Version 201609, http://www.kegg.jp/kegg/) using DIAMOND software (V0.9.9) (Minoru et al., 2014). The gene number table for each taxonomic hierarchy was obtained from the functional annotation results and gene abundance table.

2.5. Statistical analysis

We selected 1073 OTUs to build a cooccurrence network across all soil samples by considering correlation coefficients that were strong ($\rho > 0.60$ or $\rho < -0.60$) and significant ($p < 0.01$) (Supplementary Dataset S1) (Delgado-Baquerizo et al., 2018b).

The network was performed on the interactive platform of Gephi. Finally, the ecological clusters (i.e., modules) of the cooccurrence network were obtained from the Gephi platform with the default parameters. The relative abundance of each ecological cluster was calculated by averaging the standardized relative abundances (z-score) (Delgado-Baquerizo et al., 2018b). Using linear regression models, we tested the correlations between soil pH and microbial attributes, including soil bacterial diversity, the dominant phyla, the relative abundance of ecological clusters, and keystone OTUs. Random forest analysis was employed to test the dominant predictor regulating microbial attributes when accounting for other environmental factors (Trivedi et al., 2016). The randomForest and rfPermute packages were used to conduct the random forest analysis in R statistical software, version 3.0.2 (http://cran.r-project.org/) (Breiman, 2001). Furthermore, we used similarity of percentage analysis (SIMPER) to identify the cumulative contribution of the top KEGG orthologs (KOs) to the dissimilarity along the soil pH category. SIMPER was performed using PAST software (Li et al., 2018). Furthermore, we employed structural equation modeling (SEM) to evaluate the direct and indirect effects of soil pH on microbial attributes. First, we built an a priori model based on current knowledge of the impact of soil pH and other key environmental factors on microbial attributes. Thereafter, we used the maximum-likelihood estimation method to fit the model. The criteria for each test followed a previous study (Delgado-Baquerizo et al., 2016). All SEM analyses were conducted in AMOS 21.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Variation in soil properties after mining disturbance

As shown in Table S1, we observed a typical pH gradient ranging from 3.47 (SB-13) to 7.47 (SB-15) among all samples. Nutrient and metal(loid) parameters showed similar distinct ranges relative to soil pH; for example, TC ranged from 1.78 to 15.5%; TOC ranged from 1.73 to 15.20%; total S ranged from 0.01 to 0.56%; Hg ranged from 0.4 to 845.0 mg/kg; Tl ranged from 1.1 to 730.0 mg/kg; and As ranged from 18.6 to 997.0 mg/kg. The nutrient (such as total C, organic C, and total

S) and metal(loid) (Hg, Tl, and As) contents decreased with soil pH (Fig. S1 and Table S2).

3.2. Soil pH altered soil microbial attributes after mining disturbance

Soil pH is a dominant predictor of bacterial diversity after accounting for nutrient and metal(loid) parameters according to random forest analysis (Fig. 1). The alpha diversity indices (including the Shannon and Chao1 indices) showed strong correlation patterns with soil pH (Fig. 1 and Table S3). Soil pH was a major predictor of the dominant phyla according to random forest analysis (Fig. 1 and S2). In addition, we found significant but different associations between soil pH and the relative abundance of some dominant bacterial phyla. For example, the relative abundance of *Proteobacteria* was positively related to soil pH, whereas the abundance of *Acidobacteria* was negatively correlated with soil pH (Fig. 1 and Table S4).

We demonstrated that soil pH shifted the distributional pattern of ecological clusters and keystone taxa. For example, the relative abundance of module #1 was negatively related to soil pH, while the relative abundance of modules #2, #3, and #4 was positively correlated with soil pH (Fig. S3). Notably, the bacterial composition (at the phylum level) of each cluster was different (Fig. 2b). In the current study, we identified 11 keystone OTUs with intermediate/low relative abundance. Interestingly, these keystone OTUs were significantly positively correlated with soil pH (Fig. S4). As expected, the random forest analysis suggested that soil pH was the most important predictor of ecological clusters (Fig. S3) and keystone OTUs (Fig. S4).

3.3. Structural equation modeling analysis revealed the role of soil pH in microbial attributes

We used SEM to clarify the role of soil pH in predicting bacterial diversity, the dominant phyla, the relative abundance of ecological clusters, and keystone OTUs (Fig. 3a). SEM showed a strong but differing direct impact of metal(loid)s and nutrients on these microbial attributes. Specifically, we found a negative effect of meta(loid)s on microbial diversity and keystone OTUs, while meta(loid)s showed a positive effect on the dominant phyla and module clusters. In addition, we found a negative effect of nutrients on the module clusters but a positive effect on microbial diversity. Interestingly, our SEM analysis showed no significant direct effects of soil pH on these microbial attributes. Additionally, the results showed that soil pH indirectly impacted these microbial attributes by altering metal(loid) and nutrient availability (Fig. 3b).

3.4. Functional genes related to variations in soil pH

To obtain insight into the physiological characteristics of the soil ecological system and the impacts of soil pH on functional attributes, we selected 12 typical samples from 3 pH categories (4 soil samples for each category) to perform shotgun metagenomic analysis. In total, we detected 7387 different bacterial KOs in all samples. After filtering out KOs with extremely low relative abundance (below 0.1%), we identified the majority of the functional attributes (1402 KOs) (Supplementary File Data 2). Using SIMIPER, we found that the top 54 KOs explained more than 20% of the cumulative contribution to the dissimilarity among the soil pH categories (Fig. S6 and Table S5). Notably, these KOs were all positively correlated with soil pH. Most of these genes are likely related to ABC transporters, DNA-binding proteins, and transcription regulators (Fig. 4). Furthermore, we found several genes encoding two-component systems (TCSs), RNA polymerase, carbon fixation pathways, methane metabolism, nitrogen metabolism, oxidative phosphorylation, and amino acid metabolism (Fig. S7). It is worth noting that the core microbial functional traits involved in nutrient (including nitrogen and phosphorus) (Figs. 5 and 6) and metal availability (including metal transport and resistance) (Fig. 7) were related to changes in soil pH.

4. Discussion

Environmental disturbances can influence certain soil chemical processes and thus impact the soil microbiomes. In the current study, of the factors examined, soil pH was identified as the most important

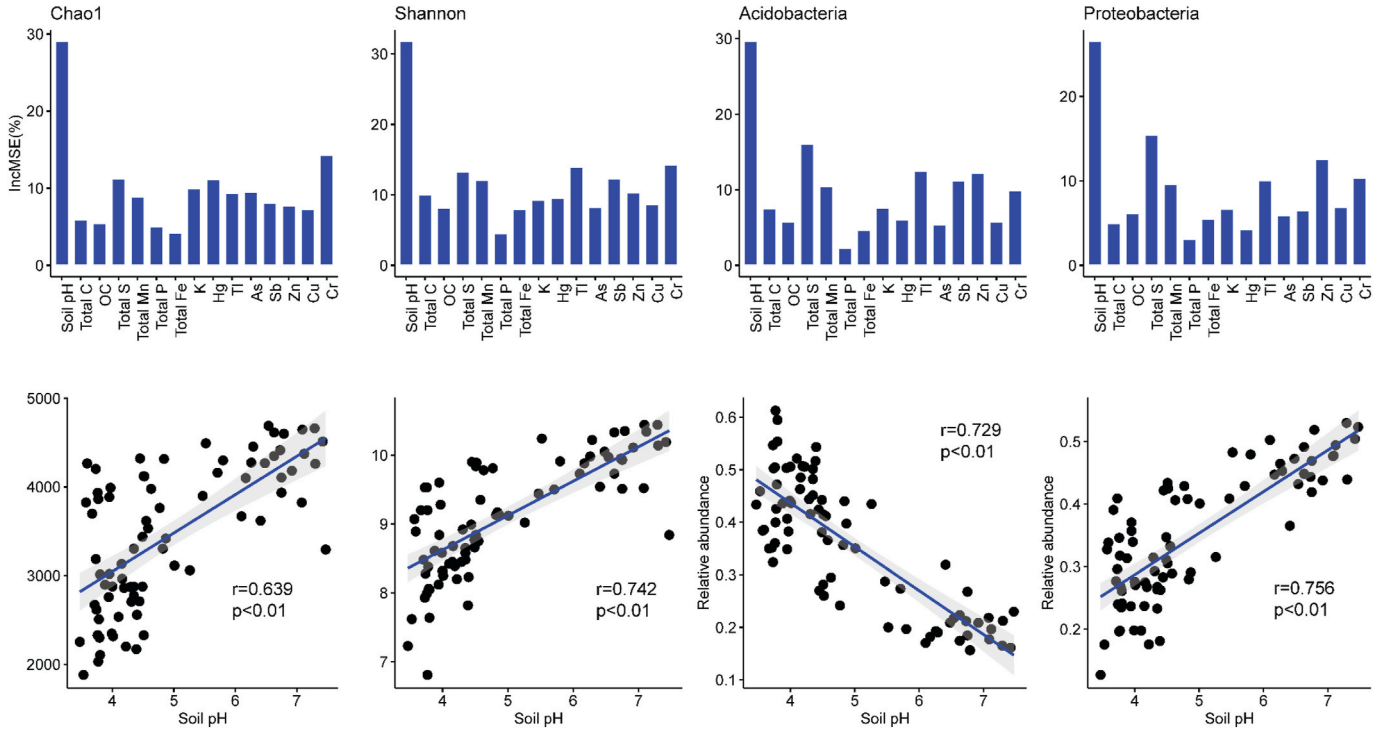


Fig. 1. RF analysis of microbial diversity indices (Chao1 and Shannon) and dominant phyla (*Acidobacteria* and *Protobacteria*). Correlations between soil pH and the relative abundance of all major bacterial phyla and microbial diversity indices are available in Table S1.

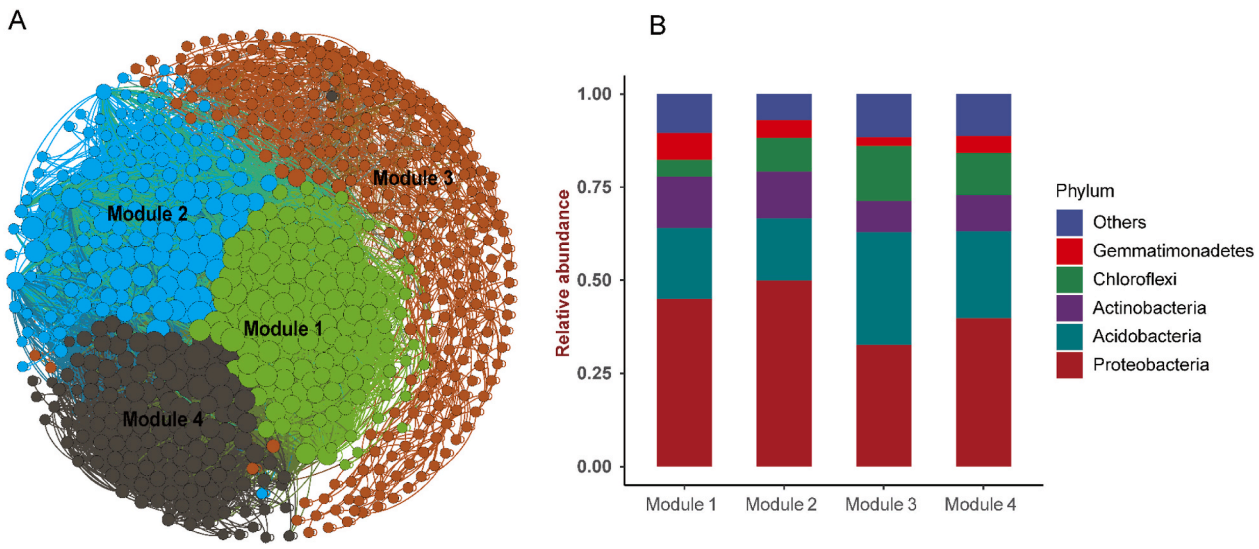
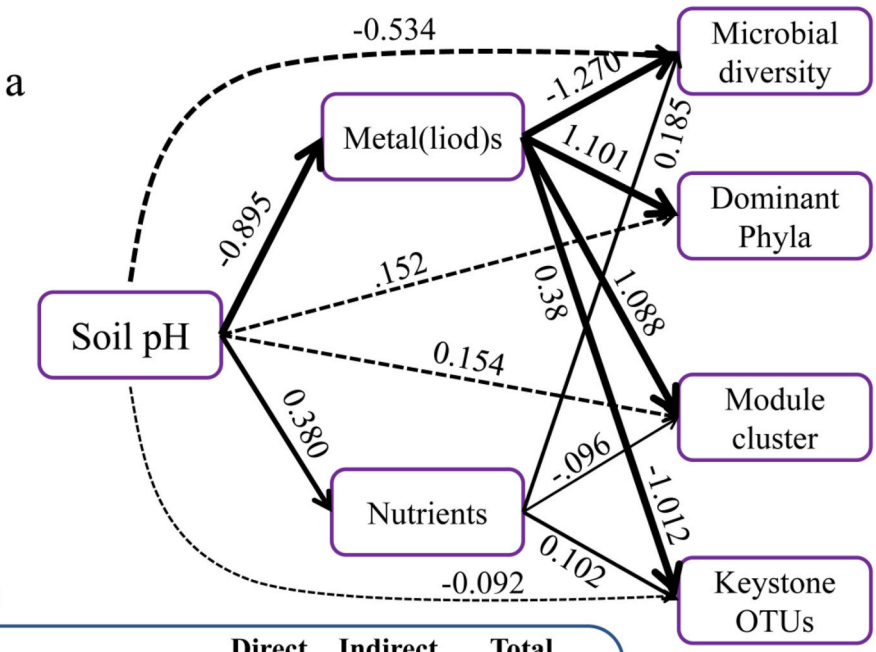


Fig. 2. Microbial co-occurrence network across all samples. (a) Network diagram with OTUs colored by each of the major four ecological clusters (modules); (b) microbial composition (phylum level) within four modules.



b

	Direct	Indirect	Total
Microbial diversity	**-0.534**	**-0.139**	**-0.673**
Phylum	**0.152**	**-0.985**	**-0.833**
Keystone OTUs	**-0.092**	**0.944**	**0.852**
Ecological Cluster	**0.154**	**-1.01**	**-0.856**

Fig. 3. Structural equation models are shown for the whole data set. (a) Direct and indirect effects of soil pH on microbial attributes (microbial diversity, dominant phyla, module cluster, and keystone OTUs). Continuous and dashed arrows indicate significant and not significant relationships, respectively. The width of arrows is proportional to the strength of path coefficients. (b) Standardized total, direct, and indirect effects were derived from the structural equation models depicted above.

predictor driving the distribution of bacterial diversity after accounting for metal(loid) and nutrient factors (Fig. 1). This result corroborates the findings of several previous studies that reported an obvious effect of soil pH on bacterial diversity across a variety of spatial scales (Lauber et al., 2009; Philippot et al., 2009; Sun et al., 2020; Xu et al., 2020) and land-use types (Lauber et al., 2008; Malik et al., 2018). Importantly, bacterial diversity and richness indices tend to increase under high pH conditions (Fig. 1). A possible explanation for this observation might be that a one-way evolutionary filter exists along pH gradients in which microorganisms can easily branch from environments with extreme pH levels to those with a more neutral pH (Tripathi et al., 2012). Such branching would result in greater microbial diversity in neutral-pH soil environments than in acidic soil environments, which broadly supports the idea that individual bacterial taxa grow within a relatively narrow

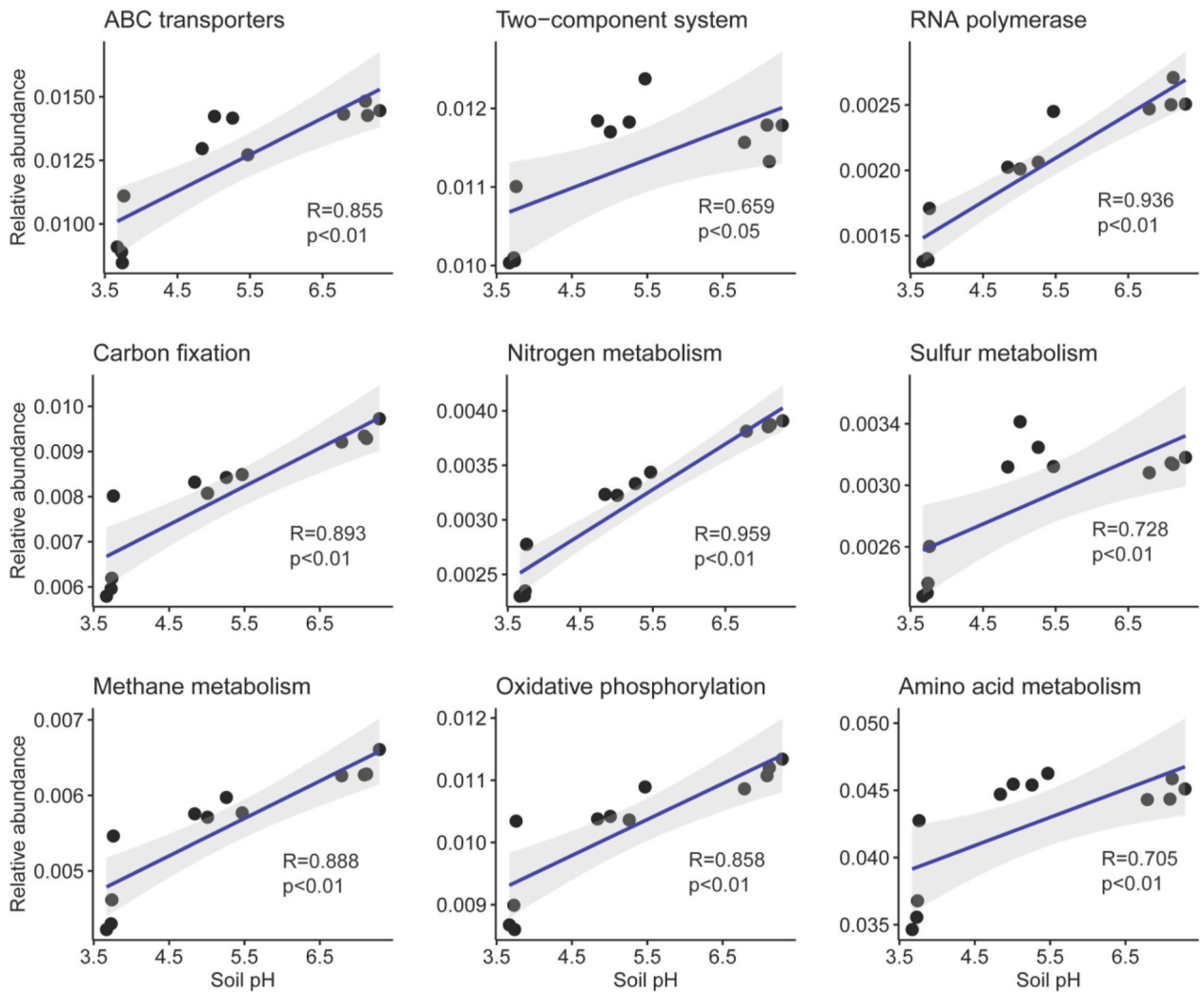


Fig. 4. Linear relationships between soil pH and the relative abundance of the dominant functional attributes.

range of soil pH levels (usually within 3–4 pH units) (Rosso et al., 1995). Furthermore, investigators have recently demonstrated that microorganisms have lower growth efficiency in acidic pH environments than in basic pH environments because of the increased energy investment required to alleviate soil pH stress (Tiemann and Billings, 2011). This scenario might be another possible explanation for the finding that soil pH is positively correlated with bacterial diversity. These observations demonstrated the critical role of soil pH in regulating soil microbial diversity.

We also demonstrated that soil pH played an important role in the cooccurrence pattern of soil bacterial communities. Thus, we proposed that changes in soil pH units led to changes in the module structures of the soil bacterial communities. Consistently, the random forest analysis demonstrated that soil pH was a significant predictor of ecological clusters after taking into account other environmental factors (Fig. S3). Moreover, the obvious patterns of the cooccurrence network were strongly related to the bacterial composition in each module (Fig. 2b). For example, module #1, which was negatively related to soil pH, contained several phylotypes from the phylum *Acidobacteria*. Module #2, module #3, and module #4, which were positively related to soil pH, belonged to the phylum *Proteobacteria*. Interestingly, we found that the responses of these taxonomic groups to soil pH were different from those in other studies. For example, our study found that the relative abundances of *Acidobacteria* tended to increase toward lower soil pH levels, whereas other studies have shown that the abundance of this phylum increases toward a higher soil pH in soil ecosystems (Yun et al., 2016). Similarly, we identified a significant positive correlation between soil pH and the relative abundances of *Proteobacteria*, while this pattern was not apparent in studies conducted in Changbai Mountain soils and tropical forest soils (Liu et al., 2014; Shen et al., 2013; Tripathi et al., 2014). These contradictory results may be due to the relationship of soil pH with other covarying soil factors influencing these phyla (Kim et al., 2016; Rousk et al., 2010). This assumption is largely based on empirical studies reporting that soil pH indirectly changes other environmental factors to influence the soil bacterial community. For example, investigators have recently demonstrated that soil pH alters the availability of metal(loid)s and nutrients, thereby indirectly influencing soil microorganisms (Qi et al., 2018). Another study conducted by Lauber et al. (2009) indicated that changes in soil pH alter soil edaphic factors, thereby indirectly affecting the distributional pattern of the soil bacterial community. Therefore, it is possible that in addition to direct impacts, the indirect effect of soil pH plays a crucial role in regulating the distributional pattern of the soil bacterial community, which explains the inconsistent impact of soil pH on the soil bacterial community observed in different environments. In the current study, it is worth noting that nutrient and metal parameters were also considered important predictors of microbial diversity (Fig. 1). Furthermore, the nutrient and metal contents were significantly correlated with the soil pH (Table S1). Based on these facts, we proposed that soil pH may indirectly change other environmental factors (such as nutrients and

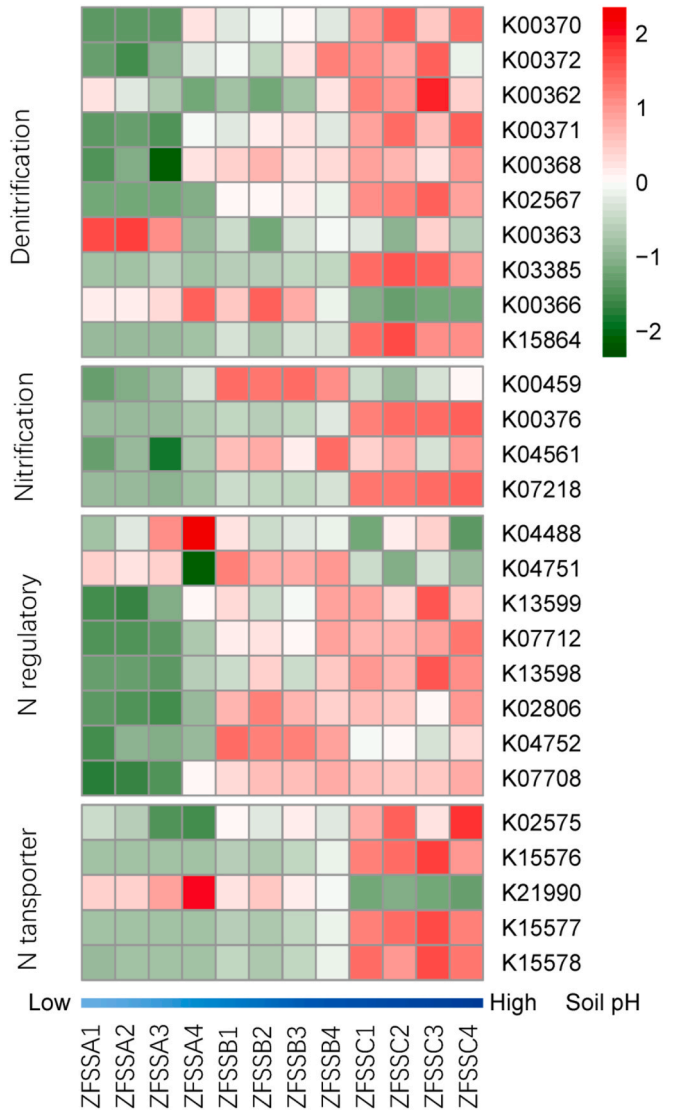


Fig. 5. Relative abundances of N related genes (the detail information was shown in Supplementary dataset S2). Scale, relative abundance of KOs at row normalization by removing the mean (centering) and dividing by the standard deviation (scaling). The color from green to red represents a relative abundance of each KO from low to high. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

metals) to influence the soil bacterial community.

Indeed, the SEM analysis demonstrated that soil pH indirectly shaped the community by altering metal(loid) availability (Fig. 3). In the current study, mining disturbance resulted in high contents of heavy metals in soil samples (Table S1). Notably, the soil pH was negatively correlated with the metal(loid) contents in this study. These facts are consistent with prior studies in which soil pH was found to strongly influence metal availability, thereby impacting the soil bacterial community (Lauber et al., 2009; Qi et al., 2018). Accordingly, we found that some KOs that included metal sensing were negatively correlated with soil pH ($p < 0.05$) (Fig. 7). Specifically, KO3088 (rpoE), which was annotated as a member of the ECF subfamily, was overrepresented in soils with low pH. A prior study demonstrated that rpoE could be triggered by several metal(loid)s (such as iron, nickel, cobalt, copper, zinc and cadmium) to maintain the metal response and homeostasis of bacteria (Moraleda-Muñoz et al., 2019). Furthermore, KO2529, which is significantly negatively correlated with soil pH, was annotated as LacI, which is considered a heavy metal ion responsive transcription regulator for synthetic biological heavy metal sensors (Jung and Lee., 2019). These observations suggest that decreasing soil pH could trigger microbial functionality of metal sensing in soil ecosystems. Importantly, our results also showed that the KOs involved in metal transport were negatively correlated with soil pH ($p < 0.05$). For example, the relative abundance of genes related to ABC transporters increased with decreasing soil pH, suggesting that decreasing soil pH may stimulate enzyme activities responsible for transportation. ABC transporters directly utilize the free energy released upon the hydrolysis of ATP to pump substrates against a concentration gradient of metal, which plays a critical role in transporting metal for bacterial metal(loid) homeostasis (Nies, 2003; Silver and Phung, 1996). Furthermore, amino acid metabolites are preferentially synthesized to bind heavy metals under soil heavy metal stress. For example, glutathione and phytochelatin react with metals to form complex metal cations at the cellular level and serve as long-distance, metal-chelating compounds (Dave et al., 2013; Richau et al., 2010). In general, the turnover of amino acid metabolism regulated by soil pH plays an important role in metal(loid) cycles. These metal(loid) transport-associated proteins that are overrepresented in soils with low pH can be logically associated with their growth because the soil microbiome needs to invest in metal transport to alleviate metal stress in low pH soils (Nies, 2003).

Additionally, the SEM analysis also demonstrated that soil pH indirectly shaped the community by altering nutrient availability (Fig. 3). This result is consistent with previous studies showing that soil pH strongly influences nutrient availability, thereby impacting the soil bacterial community. For example, Sapek (2000) found that increases in soil pH enhance soil nitrogen availability and typically increase soil bacterial diversity. Another study conducted by Malik et al. (2018) demonstrated that decreases in soil pH promote the breakdown of litter, increase the soil organic matter content, and promote the activity of soil microorganisms. Consistent with these findings, the KOs involved in nutrient availability were overrepresented in soils with high pH. For example, the functional attributes of carbon availability, including carbon fixation pathways and methane metabolism, increased with soil pH (Fig. 4). Such observations are consistent with evidence showing that an increasing soil pH decreases the availability of organic carbon, thereby activating specific physiological functions to maintain microorganism growth (Kamble and Baath, 2018; Kunito et al., 2012). Furthermore, we found that the pathways involved in nitrogen metabolism were also enriched in the high pH soils. Specifically, some KOs involved in nitrate/nitrite transport, nitrification, and denitrification were significantly enriched in soils with high pH (Fig. 5). This finding suggests that elevated soil pH could increase the nitrogen metabolism pathway. Notably, we identified various functional attributes of amino acid metabolism that were strongly positively correlated with soil pH. Amino acids are considered a major source of soil organic N compounds (Muruganandam et al., 2009) and serve as the main available N source for soil microorganisms (Dippold and Kuzyakov, 2013). We also found that many core KOs were involved in phosphorus acquisition processes, such as phosphate solubilization and phosphate transport, and phosphorus regulation. Similarly, all KOs involved in P availability were significantly enriched in high pH soils (Fig. 6). Collectively, our study provides evidence that increasing soil pH could increase microbial functional attributes involved in nutrient availability (carbon, nitrogen, and phosphorus). These findings were consistent with the fact that an increasing soil pH decreases the availability of nutrients, which means that soil microbiomes need to invest in nutrient availability in elevated pH soils (Kamble and Baath, 2018).

This study investigates the distribution of soil bacterial communities and their functions after mining disturbance. We found that soil pH indirectly impacts the distribution and functions of soil bacterial communities, including the alteration of metal(loid)s and nutrient availability. These findings have significant implications for understanding the distribution of the soil bacterial community after environmental disturbance in soil ecosystems. Nonetheless, this study is based on field

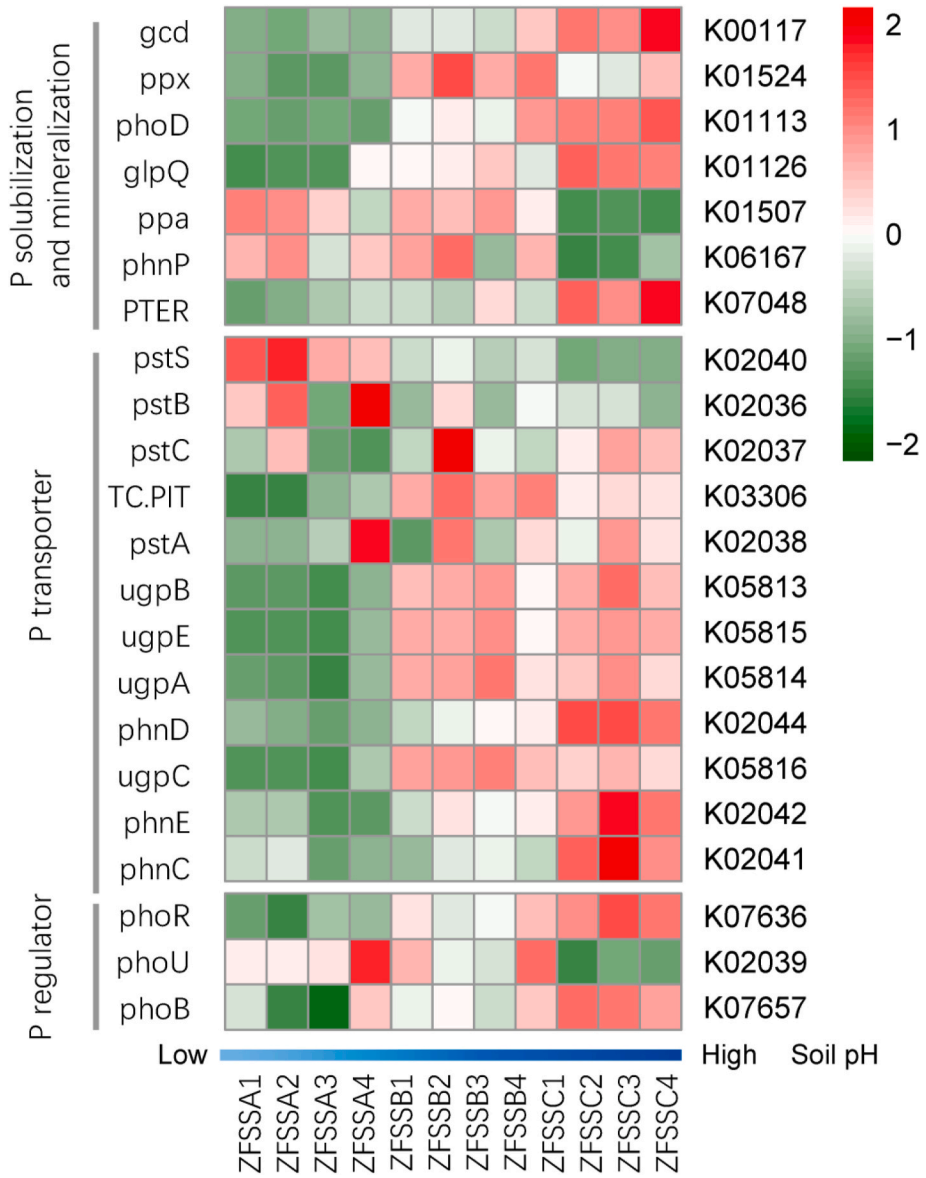


Fig. 6. Relative abundances of P related genes (the detail information was shown in Supplementary dataset S2). Scale, relative abundance of KOs at row normalization by removing the mean (centering) and dividing by the standard deviation (scaling). The color from green to red represents a relative abundance of each KO from low to high. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

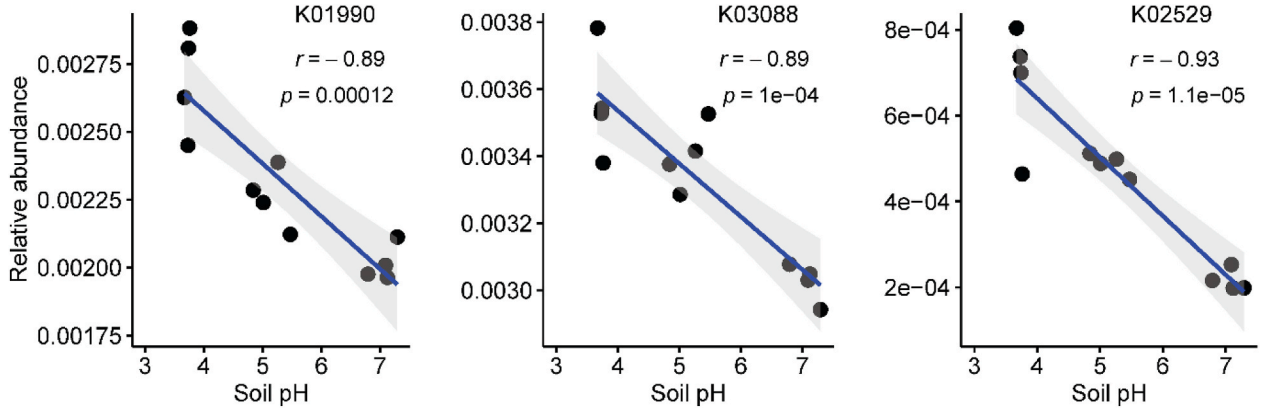


Fig. 7. Linear relationships between soil pH and the relative abundance of the metal related functional genes (the detail information was shown in Supplementary dataset S2). K01990: ABC-2 type transport system ATP-binding protein; K03088: rpoE, RNA polymerase sigma-70 factor, ECF subfamily; K02529: LacI family transcriptional regulator.

observations. Future laboratory-based studies are needed to verify the impact of edaphic factors on the distribution of the soil microbiome.

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.soilbio.2021.108232.

References

Breiman, L., 2001. Random forests. Machine Learning 45 (1), 5–32.
Chen, J., Nan, J., Xu, D.L., Mo, L., Zheng, Y.X., Chao, L.M., Qu, H.T., Guo, Y.Q., Li, F.S., Bao, Y.Y., 2020. Response differences between soil fungal and bacterial communities under opencast coal mining disturbance conditions. Catena 194, 104779.
Dave, R., Singh, P.K., Tripathi, P., Shri, M., Dixit, G., Dwivedi, S., Chakrabarty, D., Trivedi, P.K., Sharma, Y.K., Dhankher, O.P., 2013. Arsenite tolerance is related to proportional thiolic metabolite synthesis in Rice (*Oryza sativa L.*). Archives of Environmental Contamination and Toxicology 64 (2), 235–242.
Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D., Singh, B.K., 2016. Microbial diversity drives multifunctionality in terrestrial ecosystems. Nature Communications 7, 10541.
Delgado-Baquerizo, M., Oliverio, A.M., Brewer, T.E., Benavent-González, A., Eldridge, D. J., Bardgett, R.D., Maestre, F.T., Singh, B.K., Fierer, N., 2018a. A global atlas of the dominant bacteria found in soil. Science 359 (6373), 320–325.
Delgado-Baquerizo, M., Reith, F., Dennis, P.G., Hamonts, K., Powell, J.R., Young, A., Singh, B.K., Bissett, A., 2018b. Ecological drivers of soil microbial diversity and soil biological networks in the Southern Hemisphere. Ecology 7 (1), 583–596.
Dippold, M.A., Kuzyakov, Y., 2013. Biogeochemical transformations of amino acids in soil assessed by position-specific labelling. Plant and Soil 373 (1–2), 385–401.
Hottenstein, J.D., Neilson, J.W., Gil-Loaiza, J., Root, R.A., White, S.A., Chorover, J., Maier, R.M., 2019. Soil microbiome dynamics during pyritic mine tailing phytostabilization: understanding microbial bioindicators of soil acidification. Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2019.01211.
Jung, J., Lee, S.J., 2019. Biochemical and biodiversity insights into heavy metal ion-responsive transcription regulators for synthetic biological heavy metal sensors. Journal of Microbiology and Biotechnology 29 (10), 1522–1542.
Kamble, P.N., Baath, E., 2018. Carbon and nitrogen amendments lead to differential growth of bacterial and fungal communities in a high-pH soil. Pedosphere 28 (2), 255–260.
Kane, J.L., Morrissey, E.M., Skousen, J.G., Freedman, Z.B., 2020. Soil microbial succession following surface mining is governed primarily by deterministic factors. FEMS Microbiology Ecology. https://doi.org/10.1093/femsec/fiaa114.
Kim, J.M., Roh, A.S., Choi, S.C., Kim, E.J., Choi, M.T., Ahn, B.K., Kim, S.K., Lee, Y.H., Joa, J.H., Kang, S.S., 2016. Soil pH and electrical conductivity are key edaphic factors shaping bacterial communities of greenhouse soils in Korea. Journal of Microbiology 54 (12), 838–845.
Krulwich, T.A., Sachs, G., Padan, E., 2011. Molecular aspects of bacterial pH sensing and homeostasis. Nature Reviews Microbiology 9, 330–343.
Kunito, T., Tobitani, T., Moro, H., Toda, H., 2012. Phosphorus limitation in microorganisms leads to high phosphomonoesterase activity in acid forest soils. Pedobiologia 55 (5), 263–270.
Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75 (15), 5111–5120.
Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology and Biochemistry 40 (9), 2407–2415.
Li, B., Wu, W.-M., Watson, D.B., Cardenas, E., Chao, Y., Phillips, D.H., Mehlhorn, T., Lowe, K., Kelly, S.D., Li, P., Tao, H., Tiedje, J.M., Criddle, C.S., Zhang, T., 2018. Bacterial community shift and coexisting/coexcluding patterns revealed by network analysis in a uranium-contaminated site after bioreduction followed by reoxidation. Applied and Environmental Microbiology 84 (9), e02885-17.
Liu, J., Sui, Y., Yu, Z., Yu, S., Chu, H., Jian, J., Liu, X., Wang, G., 2014. High throughput sequencing analysis of biogeographical distribution of bacterial communities in the black soils of northeast China. Soil Biology and Biochemistry 70 (2), 113–122.
Malik, A.A., Puissant, J., Buckeridge, K.M., Goodall, T., Jehmlich, N., Chowdhury, S., Gweon, H.S., Peyton, J.M., Mason, K.E., van Agtmaal, M., Blaud, A., Clark, I.M., Whitaker, J., Pywell, R.F., Ostle, N., Gleixner, G., Griffiths, R.I., 2018. Land use driven change in soil pH affects microbial carbon cycling processes. Nature Communications 9 (1), 3591.
Moraleda-Munoz, A., Javier Marcos-Torres, F., Perez, J., Munoz-Dorado, J., 2019. Metal-responsive RNA polymerase extracytoplasmic function (ECF) sigma factors. Molecular Microbiology 112 (2), 385–398.
Minoru, K., Susumu, G., Yoko, S., Masayuki, K., Miho, F., Mao, T., 2014. Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Research 42, 199–205.
Muruganandam, S., Israel, D.W., Robarge, W.P., 2009. Activities of nitrogen-mineralization enzymes associated with soil aggregate size fractions of three tillage systems. Soil Science Society of America Journal 73, 751–759.
Nielsen, H.B., Almeida, M., Juncker, A.S., Rasmussen, S., Li, J., Sunagawa, S., Plichta, D. R., Gautier, L., Pedersen, A.G., Le Chatelier, E., Pelletier, E., Bonde, I., et al., 2014. Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. Nature Biotechnology 32, 822.
Nies, D.H., 2003. Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiology Reviews 27 (2–3), 313–339.
Philippot, L., Cuhel, J., Saby, N.P., Chèneby, D., Chronáková, A., Bru, D., Arrouays, D., Martin-Laurent, F., Simek, M., 2009. Mapping field-scale spatial patterns of size and activity of the denitrifier community. Environmental Microbiology 11 (6), 1518–1526.
Qi, D., Wieneke, X., Tao, J., Zhou, X., Desilva, U., 2018. Soil pH is the primary factor correlating with soil microbiome in Karst rocky desertification regions in the Wushan County, Chongqing, China. Frontiers in Microbiology 9, 1027.
Richau, K.H., Kozhevnikova, A.D., Seregin, I.V., Riet, V., Koevoets, P.L.M., Smith, J.A.C., Ivanov, V.B., Henk, S., 2010. Chelation by histidine inhibits the vacuolar sequestration of nickel in roots of the hyperaccumulator Thlaspi caerulescens. New Phytologist 183 (1), 106–116.
Rosso, L., Lobry, J.R., Bajard, S., Flandrois, J.P., 1995. Convenient model to describe the combined effects of temperature and pH on microbial growth. Applied and Environmental Microbiology 61 (2), 610–616.
Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4, 1340–1351.
Sapek, B., 2010. Study of the pH variability in permanent meadow soils resulting from nitrogen fertilization and after-effect of liming. Academic Radiology 16 (12), 1518–1530.
Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., Xinyu, L.I., Liang, W., Chu, H., 2013. Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. Soil Biology and Biochemistry 57, 204–211.
Silver, S., Phung, L.T., 1996. Bacterial heavy metal resistance: new surprises. Annual Reviews in Microbiology 50, 753–789.
Sun, W., Sun, X., Li, B., Xu, R., Young, L.Y., Dong, Y., Zhang, M., Kong, T., Xiao, E., Wang, Q., 2020. Bacterial response to sharp geochemical gradients caused by acid mine drainage intrusion in a terrace: relevance of C, N, and S cycling and metal resistance. Environment International 138, 105601.
Sun, W., Xiao, E., Xiao, T., Krumins, V., Wang, Q., Haggblom, M.M., Dong, Y., Tang, S., Hu, M., Li, B., 2017. Response of soil microbial communities to elevated antimony and arsenic contamination indicates the relationship between the innate microbiota and contaminant fractions. Environmental Science and Technology 51 (16), 9165–9175.
Tiemann, L.K., Billings, S.A., 2011. Changes in variability of soil moisture alter microbial community C and N resource use. Soil Biology and Biochemistry 43 (9), 1837–1847.
Tripathi, B.M., Kim, M., Singh, D., Lee-Cruz, L., Lai-Hoe, A., Ainuddin, A.N., Go, R., Rahim, R.A., Husni, M.H.A., Chun, J., Adams, J.M., 2012. Tropical soil bacterial communities in Malaysia: pH dominates in the equatorial tropics too. Microbial Ecology 64 (2), 474–484.
Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I.C., Jeffries, T.C., Zhou, J., Singh, B.K., 2016. Microbial regulation of the soil carbon cycle: evidence from gene–enzyme relationships. The ISME Journal 10, 2593–2604.
Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73 (16), 5261–5267.
Xiao, E., Krumins, V., Dong, Y., Xiao, T., Ning, Z., Xiao, Q., Sun, W., 2016a. Microbial diversity and community structure in an antimony-rich tailings dump. Applied Microbiology and Biotechnology 100, 7751–7763.
Xiao, E., Krumins, V., Xiao, T., Dong, Y., Tang, S., Ning, Z., Huang, Z., Sun, W., 2016b. Depth-resolved microbial community analyses in two contrasting soil cores contaminated by antimony and arsenic. Environmental Pollution 221, 244–255.
Xiao, E., Cui, J., Sun, W., Jiang, S., Huang, M., Kong, D., Wu, Q., Xiao, T., Sun, X., Ning, Z., 2020. Root microbiome assembly of As-hyperaccumulator Pteris vittata and its efficacy in arsenic requisition. Environmental Microbiology. https://doi.org/10.1111/1462-2920.15299.
Xu, R., Li, B., Xiao, E., Young, L.Y., Sun, X., Kong, T., Dong, Y., Wang, Q., Yang, Z., Chen, L., 2020. Uncovering microbial responses to sharp geochemical gradients in a terrace contaminated by acid mine drainage. Environmental Pollution 261, 114226.
Yun, Y., Wang, H., Man, B., Xiang, X., Zhou, J., Qiu, X., Duan, Y., Engel, A.S., 2016. The relationship between pH and bacterial communities in a single karst ecosystem and its implication for soil acidification. Frontiers in Microbiology 7 (485), 1955.