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Soil nutrient levels are associated with suppression of banana *Fusarium* wilt disease

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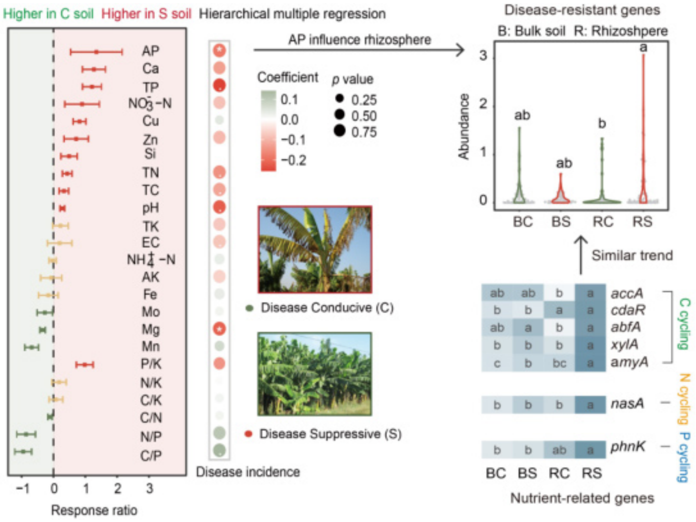
ABSTRACT

- Disease-suppressive soils exhibit enhanced soil nutrient status.
- Soil available phosphorus is a distinct feature of disease-suppressive soil.
- Rhizosphere hosts heightened microbial function for disease suppression.
- The soil microbial role in disease suppression is linked to nutrient cycling.

The role of soil nutrient status in disease suppression is of increasing interest for the control of soil-borne diseases. Here, we explored the soil chemical properties, composition, and functional traits of soil microbiomes in pair-located orchards that appeared suppressive or conducive to the occurrence of banana *Fusarium* wilt using mainly amplicon sequencing and metagenomic approaches. The enhancement of soil available phosphorus, succeeded by increments in soil nitrogen and carbon, played a pivotal role in the suppression of the disease. Additionally, in the rhizosphere of suppressive sites, there was an observed increase in the disease-suppressing function of the soil microbiome, which was found to be correlated with specific nutrient-related functions. Notably, this enhancement involved the presence of key microbes such as *Blastocatella* and *Bacillus*. Our results highlight the significant roles of soil nutrient status and soil microbiome in supporting the soil-related disease suppressiveness.



Keywords disease-suppressive soil, banana Panama disease, nutrient condition, soil microbiome, antagonistic genes

Fusarium wilt, caused by *Fusarium oxysporum*, presents a major threat to global agriculture, notably affecting the banana industry (banana Panama disease) by threatening widely cultivated varieties (Michielse and Rep, 2009; Butler, 2013). *F. oxysporum* induces rapid wilting by invading the plant's vascular system and impeding the transport of water and nutrients (Li et al., 2019). To counter this, enhancing soil-related resistance against pathogen invasion, including the promotion of beneficial microbial communities, has emerged as an essential, eco-friendly strategy for disease management due to a reduced application of chemical interventions (Expósito et al., 2017). The efficacy of disease-suppressive soil stems from the dynamic interplay of biotic and abiotic factors, with soil microorganisms and chemical properties being crucial (Berg and Koskella, 2018).

In our study, we analyzed soil chemistry and microbiomes in banana orchards having undergone long-term monocropping to determine the differences between soils that appeared either suppressive or conducive to the occurrence of *Fusarium* wilt (Fig. 1A, Fig. S1, Table S1). A total of

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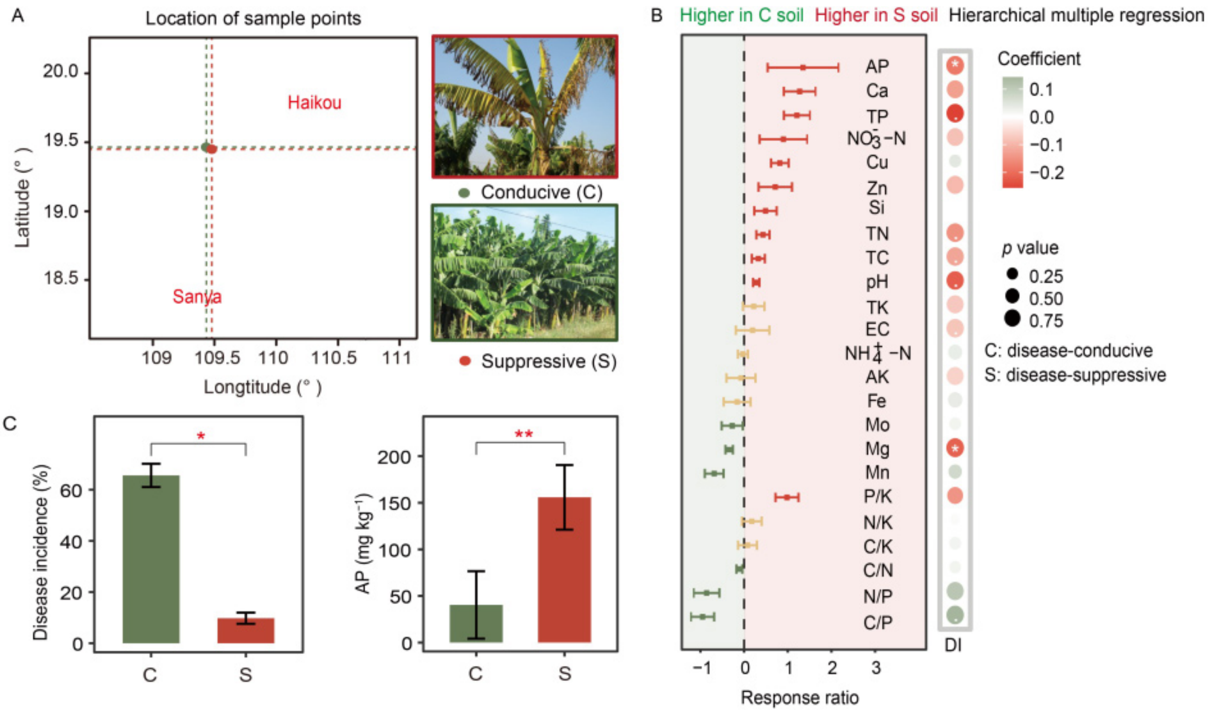


Fig. 1 Chemical properties of soils suppressive or conducive to *Fusarium* wilt. (A) A map of the location of soils suppressive or conducive to *Fusarium* wilt in a Hainan orchard. (B) Forest plots illustrating the response ratios (RR) of soil chemical properties, displayed with 95% confidence intervals (CI). The RRs marked in green and red represent factors that are significantly lower and higher, respectively, in the suppressive soil. The heatmap presents the coefficients from the hierarchical multiple regression analysis that correlate soil chemical properties with disease incidence. (C) A histogram depicting the disease incidence and soil available phosphorus content with standard deviations in both suppressive and conducive soils. AP, available phosphorus; Ca, calcium; TP, total phosphorus; NO_3^--N, nitrate; Cu, copper; Zn, zinc; Si, silicon; TN, total nitrogen; TC, total carbon; TK, total potassium; EC, electrical conductivity; NH_4^+-N, ammonium; AK, available potassium; Fe, iron; Mo, molybdenum; Mg, magnesium; Mn, manganese; P/K, ratio of total phosphorus and total potassium; N/K, ratio of total nitrogen and total potassium; C/K, ratio of total carbon and total potassium; C/N, ratio of total carbon and total nitrogen; N/P, ratio of total nitrogen and total phosphorus; C/P, ratio of total carbon and total phosphorus. The significance levels were determined using the Wilcoxon rank sum test. One and two asterisks indicate $p < 0.05$ and $p < 0.01$, respectively.

twenty soil samples, encompassing both bulk and rhizosphere soils from suppressive or conducive sites, were collected for further analysis in 2016. The detailed experimental design is described in the Method section in the Supplementary Materials. Our aims were to: 1) pinpoint key soil chemical properties that differentiate suppressive from conducive soils; 2) explore the variations in soil microbial functions related to disease suppression and nutrient cycling, as well as their interrelationships; and 3) identify the specific microbial groups involved in these processes. This understanding is vital for managing soil-borne diseases through nutrient and microbiome manipulation.

An elevated soil available phosphorus (AP) content was a prominent characteristic of disease-suppressive soils ($p < 0.01$) (Fig. 1B and 1C), and the total phosphorus ($p < 0.001$), total nitrogen ($p < 0.001$), total carbon ($p < 0.001$), and soil pH levels ($p < 0.001$) were also higher, to lesser extents. All the detailed information is listed in Tables S2 and S3. Consistent with our findings, existing literature suggests that increased soil AP levels enhance *Fusarium* wilt suppression in bananas (Shen et al., 2015) and strawberries (Li et al., 2019). This phenomenon may be ascribed to a trade-off between phosphorus acquisition and defense mechanisms in plants, wherein the response to phosphorus deficiency may compromise the overall defense response. For example, the master transcriptional regulators of phosphate stress responses in *Arabidopsis thaliana* have been reported to directly inhibit plant defense responses (Castrillo et al., 2017). Our stoichiometric analysis revealed that lower carbon/phosphorus and nitrogen/phosphorus ratios, coupled with a higher phosphorus/potassium ratio, favor wilt disease suppression (Fig. 1C), highlighting the importance of phosphorus adequacy and balanced nutrient availability in disease control. Soil nitrogen content was another crucial factor for disease suppression. Appropriate nitrogen fertilization rates (N: 90 kg ha^{-1}) are effective against *Fusarium* wilt (Lv et al., 2021), whereas excessive nitrogen usage increases disease incidence (N: 0.25 g $plant^{-1}$) (Segura-Mena et al., 2021). Intriguingly, the form of nitrogen was also associated with *Fusarium* wilt dynamics. Nitrate nitrogen (NO_3^--N) but not the ammonium nitrogen (NH_4^+-N), was characteristic of disease-suppressive soils (Fig. 1C). This supports previous findings that NO_3^- suppresses pathogen colonization, reduces fusaric acid production, and boosts plant resilience to pathogens. In contrast, NH_4^+ and organic nitrogen sources may heighten disease susceptibility by promoting pathogen growth and spore germination (Zhou et al., 2017; He et al., 2023). Therefore, it is crucial to manage soil nitrogen levels appropriately, prioritizing NO_3^--N to combat *Fusarium* wilt.

Enhanced soil nutrient status not only strengthens plant defense mechanisms but also fosters a resilient soil microbiome (Adiobo et al., 2007; Cao et al., 2022), which is another key driver in maintaining soil disease suppression. Our study found that the number of microbial genes associated with disease suppression, such as nonribosomal peptide synthetase NRPS, polyketide synthase PKS and bacteriocin, were more abundant in suppressive rhizosphere soils than in conducive ones, as evidenced by our metagenomic analysis (Fig. 2A). In soil, NRPS and PKS enzymes produce bioactive compounds that suppress soil-borne diseases by inhibiting the growth of pathogens like fungi, bacteria, and nematodes. These compounds contribute to plant defense. Bacteriocins, small antimicrobial peptides from bacteria, also target and inhibit pathogenic bacteria, aiding disease control. Together, NRPS, PKS, and Bacteriocins play crucial roles in promoting soil health (Raaijmakers et al., 2010; Le et al., 2014; Prieto, 2016). This highlights the vital roles of microbes in the rhizosphere. Furthermore, these genes were positively influenced by the soil AP content, as determined using the Boruta method (Fig. 2B). This emphasized the potential role of AP in maintaining soil microbiome properties linked to disease suppression. We also observed variations in nutrient cycling genes between suppressive and conducive soils (Fig. 2C), some of which paralleled the patterns of disease suppressive genes. This suggests a potential mechanism by which soil nutrient content impacts microbes related to disease suppression. Root exudates act as a selective mechanism to attract beneficial microbes to the rhizosphere (Vives-Peris et al., 2020), which are nutrient sources for microbes and can directly activate genes associated with nutrient responses and motility (Rolfe et al., 2019), thus shaping microbial communities with diverse nutrient cycling genes (Zhao et al., 2021). Specific microbes carrying genes associated with disease suppression could be selectively recruited through the mediation of the nutrient-related genes they concurrently possess. Microbial genes *phnK*, *nasA*, *amyA*, *xylA*, *abfA*, *accA*, and *cdaR*, identified in our study, are likely participants in these interactions. *phnK* is associated with organic phosphorus mineralization; *nasA* is involved in assimilatory nitrogen reduction; *amyA*, *xylA*, and *abfA* are implicated in carbon hydrolysis; *accA* and *cdaR* are linked to carbon fixation (Zheng et al., 2018). We also identified some important microbial groups involved in this process (Fig. 2D, details in Fig. S2). For example, the *Blastocatella*, known to thrive under NO_3^--N or organic carbon conditions, is associated with the suppression of apple replant disease (Peruzzi et al., 2017; Chen et al., 2022; Yu et al., 2023). *Bacillus* spp., renowned for their roles in combating *Fusarium* wilt (Yan et al., 2024), are also active participants in phosphorus, nitrogen and carbon cycling (Zeng et al., 2023; Salazar et al., 2023; Minnikova et al., 2023) through nutrient cycling genes, such as *nasA*, *xylA* and *CdaR* (Nakano et al., 1995; Cho and Choi, 1998; Gundlach et al., 2015). Consequently, they may be the disease-suppressive microbes influenced by nutrient levels, a characteristic shared with other microbial groups presented in Fig. 2D. However, further deep research on plant-microbiome interactions is needed to fully understand these relationships.

Our investigation into the soil's pivotal role in naturally suppressing banana *Fusarium* wilt has unveiled significant findings. Soils enriched in phosphorus, nitrogen and carbon are less susceptible to banana *Fusarium* wilt disease. In addition, an increased abundance of microbial genes associated with disease suppression was observed in the rhizosphere soil of the disease-suppressive site, a trend that was also promoted by the soil phosphorus levels. We identified key nutrient cycling genes (*accA*, *abfA*, *xylA*, *amyA*, *nasA* and *phnK*) and microbes (*Blastocatella*, *Bacillus* etc.) as integral components in the mechanism by which soil nutrient status influences disease suppression. These findings emphasize the critical role of soil nutrient status in the formation of disease-suppressive soils, which provides valuable insights for future research on the interactions among plants, soil nutrients and microbiomes.

Author contributions

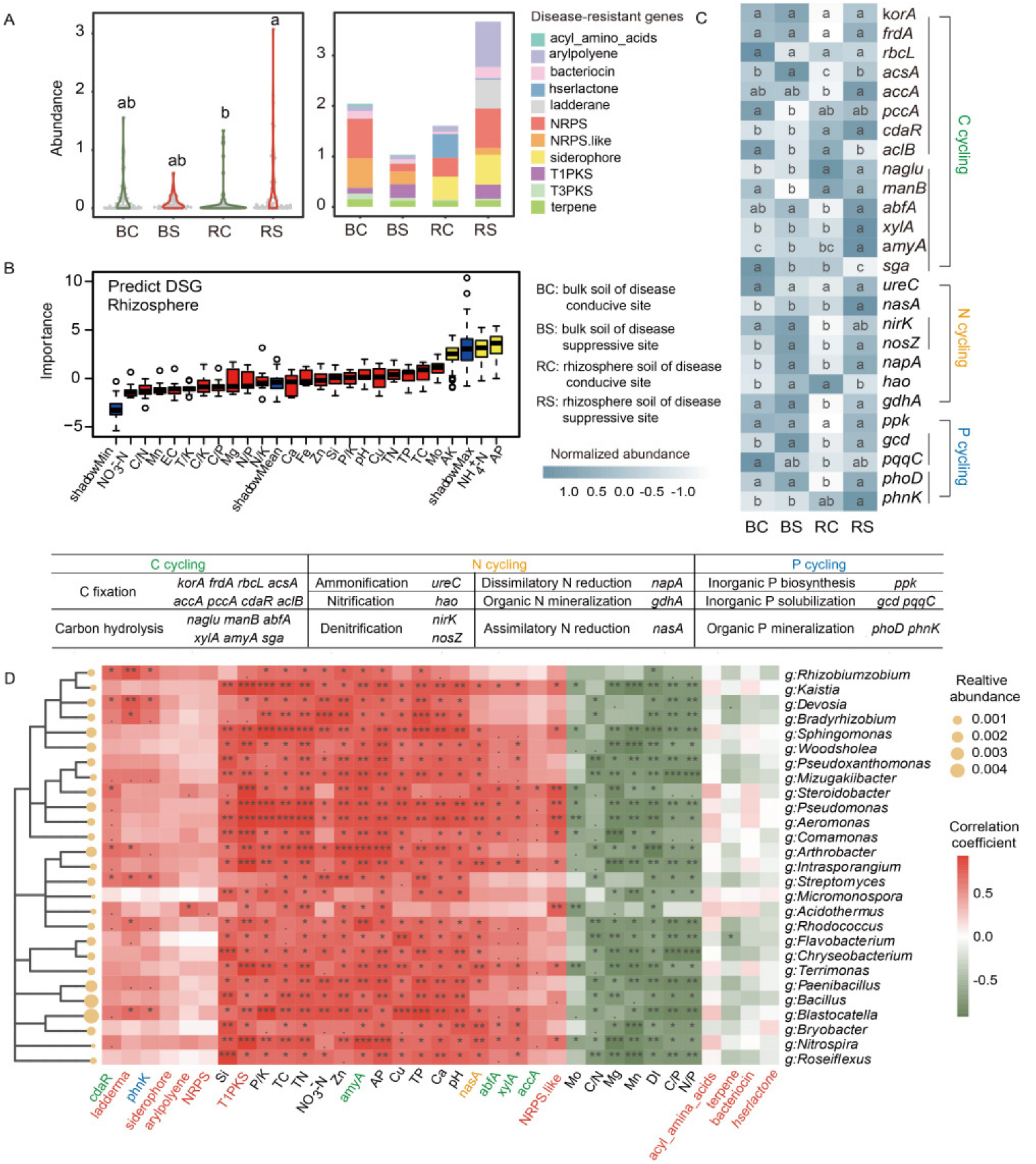
Y. Cao performed all experiments; Y. Cao, Z. Shen, R. Li, and Q. Shen designed the study and wrote the majority of the manuscript; Y. Cao, Y. Ou, and Z. Shen analyzed the data; Y. Cao, C. Tao, Na Lv, and H. Liu participated in the design of the study, provided comments, and edited the manuscript. The authors read and approved the final manuscript.

Declaration of interest statement

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.

Data availability

Raw amplicon sequencing data of bacteria and fungi was deposited at the National Center for Biotechnology Information (NCBI) under the accession numbers PRJNA1062112 and PRJNA1062117. Raw metagenomic sequencing data was



C cycling		N cycling				P cycling	
C fixation	*korA frdA rbcL acsA* *accA pccA cdaR aclB*	Ammonification	*ureC*	Dissimilatory N reduction	*napA*	Inorganic P biosynthesis	*ppk*
		Nitrification	*hao*	Organic N mineralization	*gdhA*	Inorganic P solubilization	*gcd pqqC*
Carbon hydrolysis	*naglu manB abfA* *xylA amyA sga*	Denitrification	*nirK* *nosZ*	Assimilatory N reduction	*nasA*	Organic P mineralization	*phoD phnK*

Fig. 2 Soil microbial functions and community dynamics in soils suppressive and conducive to *Fusarium* wilt. (A) A violin plot illustrates the abundance levels of microbial functional genes related to disease suppression complemented by a stacked histogram detailing their composition. (B) A boxplot showing the importance of soil properties as influencing factors on disease suppressive genes using the Boruta algorithm based on the Random Forest method. (C) A heatmap presenting the abundance levels of microbial functional genes related to soil nutrient cycling. The significance levels were established using the Kruskal–Wallis test. (D) A phylogenetic tree representing the microbial operational taxonomic unit (OTU)s that were enriched in suppressive soil compared with conducive soil ($p < 0.1$) and showed significant negative correlations with disease incidence ($p < 0.05$) in rhizosphere soil. OTUs in the same genus were pooled and presented together. Accompanying this is a heatmap delineating the Spearman correlations between these microbial groups and disease incidence, soil chemical properties and key soil microbial functional genes. NRPS, nonribosomal peptide synthetase; PKS, polyketide synthase. A dot and one, two and three asterisks indicate $p < 0.1$, $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

deposited at the NCBI under the accession number of PRJNA1043397.

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Electronic supplementary material

Supplementary material is available in the online version of this article at https://doi.org/10.1007/s42832-024-0247-1 and is accessible for authorized users.

References

Adiobo, A., Oumar, O., Perneel, M., Zok, S., Höfte, M., 2007. Variation of *Pythium*-induced cocoyam root rot severity in response to soil type. Soil Biology and Biochemistry 39, 2915–2925.

Bao, S.D., 2000. Soil and Agricultural Chemistry Analysis. 3rd ed. Beijing: China Agriculture Press.

Berg, M., Koskella, B., 2018. Nutrient- and dose-dependent microbiome-mediated protection against a plant pathogen. Current Biology 28, 2487–2492.e3.

Blin, K., Shaw, S., Steinke, K., Villebro, R., Ziemert, N., Lee, S.Y., Medema, M.H., Weber, T., 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Research 47, W81-W87.

Butler, D., 2013. Fungus threatens top banana. Nature 504, 195–196.

Cao, Y.F., Thomashow, L.S., Luo, Y., Hu, H.W., Deng, X.H., Liu, H. J., Shen, Z.Z., Li, R., Shen, Q.R., 2022. Resistance to bacterial wilt caused by *Ralstonia solanacearum* depends on the nutrient condition in soil and applied fertilizers: A meta-analysis. Agriculture, Ecosystems & Environment 329, 107874.

Castrillo, G., Teixeira, P.J.P.L., Paredes, S.H., Law, T.F., De Lorenzo, L., Feltcher, M.E., Finkel, O.M., Breakfield, N.W., Mieczkowski, P., Jones, C.D., Paz-Ares, J., Dangl, J.L., 2017. Root microbiota drive direct integration of phosphate stress and immunity. Nature 543, 513–518.

Chen, J., Song, D.L., Liu, D.H., Sun, J.W., Wang, X.B., Zhou, W., Liang, G.Q., 2022. Soil aggregation shaped the distribution and interaction of bacterial-fungal community based on a 38-year fertilization experiment in China. Frontiers in Microbiology 13, 824681.

Cho, S.G., Choi, Y.J., 1998. Catabolite repression of the *Bacillus stearothermophilus* β-xylosidase gene (*xylA*) in *Bacillus subtilis*. Journal of Microbiology and Biotechnology 8, 21–27.

Dita, M., Barquero, M., Heck, D., Mizubuti, E.S.G., Staver, C.P., 2018. Fusarium wilt of banana: current knowledge on epidemiology and research needs toward sustainable disease management. Frontiers in Plant Science 9, 1468.

Edgar, R.C., 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. BioRxiv, 081257.

Expósito, R.G., De Bruijn, I., Postma, J., Raaijmakers, J.M., 2017. Current insights into the role of rhizosphere bacteria in disease suppressive soils. Frontiers in Microbiology 8, 2529.

Gundlach, J., Mehne, F.M.P., Herzberg, C., Kampf, J., Valerius, O., Kaever, V., Stülke, J., 2015. An essential poison: synthesis and degradation of cyclic di-AMP in *Bacillus subtilis*. Journal of Bacteriology 197, 3265–3274.

He, C., Jia, Z.J., Fan, P.S., Ruan, Y.Z., Liang, Y., Ma, J.J., Li, J.K., 2023. ^{15}N tracing reveals preference for different nitrogen forms of *Fusarium oxysporum* f. sp. *cubense* tropical race 4. Frontiers in Microbiology 14.1102720.

Hedges, L.V., Gurevitch, J., Curtis, P.S., 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80, 1150–1156.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology 79, 5112–5120.

Kursa, M.B., Rudnicki, W.R., 2010. Feature selection with the Boruta package. Journal of Statistical Software 36, 1–13.

Le, T.H., Sivachidambaram, V., Yi, X.Z., Li, X.Z., Zhou, Z., 2014. Quantification of polyketide synthase genes in tropical urban soils using real-time PCR. Journal of Microbiological Methods 106, 135–142.

Li, N., Wang, C., Li, X.L., Liu, M.L., 2019. Effects of earthworms and arbuscular mycorrhizal fungi on preventing *Fusarium oxysporum* infection in the strawberry plant. Plant and Soil 443, 139–153.

Lv, J.X., Xiao, J.X., Guo, Z.P., Dong, K., Dong, Y., 2021. Nitrogen supply and intercropping control of *Fusarium* wilt in faba bean depend on organic acids exuded from the roots. Scientific Reports 11, 9589.

Michielse, C.B., Rep, M., 2009. Pathogen profile update: *Fusarium oxysporum*. Molecular Plant Pathology 10, 311–324.

Minnikova, T., Kolesnikov, S., Minin, N., Gorovtsov, A., Vasilchenko, N., Chistyakov, V., 2023. The influence of remediation with *Bacillus* and *Paenibacillus* strains and biochar on the biological activity of petroleum-hydrocarbon-contaminated Haplic Chernozem. Agriculture 13, 719.

Nakano, M.M., Yang, F., Hardin, P., Zuber, P., 1995. Nitrogen regulation of *nasA* and the *nasB* operon, which encode genes required for nitrate assimilation in *Bacillus subtilis*. Journal of Bacteriology 177, 573–579.

Peruzzi, E., Franke-Whittle, I.H., Kelderer, M., Ciavatta, C., Insam, H., 2017. Microbial indication of soil health in apple orchards affected by replant disease. Applied Soil Ecology 119, 115–127.

Prieto, C., 2016. Characterization of nonribosomal peptide synthetases with NRPSsp. Methods in Molecular Biology 1401, 273–278.

Raaijmakers, J.M., De Bruijn, I., Nybroe, O., Ongena, M., 2010. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. FEMS Microbiology Reviews 34, 1037–1062.

Rolfe, S.A., Griffiths, J., Ton, J., 2019. Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. Current Opinion in Microbiology 49, 73–82.

Salazar, J.M., Calle, J., Pereira, S., Cordero, P., Matovelle, C., 2023. Nitrite-oxidizing bacterial strains isolated from soils of Andean ecosystems and their potential use in nitrogen reduction. Sustainability 15, 9277.

Segura-Mena, R.A., Stoorvogel, J.J., García-Bastidas, F., Salacinas-Niez, M., Kema, G.H.J., Sandoval, J.A., 2021. Evaluating the potential of soil management to reduce the effect of *Fusarium oxysporum* f. sp. cubense in banana (Musa AAA). European Journal of Plant Pathology 160, 441–455.

Shen, Z.Z., Penton, C.R., Lv, N.N., Xue, C., Yuan, X.F., Ruan, Y.Z., Li, R., Shen, Q.R., 2018. Banana *Fusarium* wilt disease incidence is influenced by shifts of soil microbial communities under different monoculture spans. Microbial Ecology 75, 739–750.

Shen, Z.Z., Ruan, Y.Z., Xue, C., Zhong, S.T., Li, R., Shen, Q.R., 2015. Soils naturally suppressive to banana *Fusarium* wilt disease harbor unique bacterial communities. Plant and Soil 393, 21–33.

Vives-Peris, V., De Ollas, C., Gómez-Cadenas, A., Pérez-Clemente, R.M., 2020. Root exudates: from plant to rhizosphere and beyond. Plant Cell Reports 39, 3–17.

Yan, W.K., Liu, Y.T., Malacrinò, A., Zhang, J.Y., Cheng, X.L., Rensing, C., Zhang, Z.Y., Lin, W.X., Zhang, Z., Wu, H.M., 2024. Combination of biochar and PGPBs amendment suppresses soil-borne pathogens by modifying plant-associated microbiome. Applied Soil Ecology 193, 105162.

Yu, H.L., Shao, W., Xu, G.Y., Xie, N., Yang, X.J., Gao, D.T., Si, P., 2023. Soil amendment with sorbitol and mannitol changes the soil microbial community and its enzymatic activities. Journal of Soils and Sediments 23, 1857–1876.

Zeng, Q.W., Tang, L.S., Zhang, Y., Shao, Y., Wu, W.T., Wang, J.C., Ding, X.L., Han, X.J., Bilal, M., 2023. Isolation and characterization of phosphate-solubilizing bacteria from rhizosphere of poplar on road verge and their antagonistic potential against various phytopathogens. BMC Microbiology 23, 221.

Zhao, M.L., Zhao, J., Yuan, J., Hale, L., Wen, T., Huang, Q.W., Vivanco, J.M., Zhou, J.Z., Kowalchuk, G.A., Shen, Q.R., 2021. Root exudates drive soil-microbe-nutrient feedbacks in response to plant growth. Plant, Cell & Environment 44, 613–628.

Zheng, B.X., Zhu, Y.G., Sardans, J., Peñuelas, J., Su, J.Q., 2018. QMEC: a tool for high-throughput quantitative assessment of microbial functional potential in C, N, P, and S biogeochemical cycling. Science China Life Sciences 61, 1451–1462.

Zhou, J.Y., Wang, M., Sun, Y.M., Gu, Z.C., Wang, R.R., Saydin, A., Shen, Q.R., Guo, S.W., 2017. Nitrate increased cucumber tolerance to *Fusarium* wilt by regulating fungal toxin production and distribution. Toxins 9, 100.